PHOTOSYNTHESIS

JOHN WHITMARSH, *Agricultural Research Service/USDA, Department of Plant Biology, University of Illinois, Urbana, Illinois, U.S.A.*

GOVINDJEE, *Department of Plant Biology, University of Illinois, Urbana, Illinois, U.S.A.*

INTRODUCTION

Photosynthesis is the physicochemical process by which plants, algae, and photosynthetic bacteria use light energy to drive the synthesis of organic compounds. In plants, algae, and certain types of bacteria, the photosynthetic process results in the release of molecular oxygen and the removal from the atmosphere of carbon dioxide, which is used to synthesize carbohydrates. Other types of bacteria use light energy to create organic compounds but do not produce oxygen. Photosynthesis provides the energy and reduced carbon required for the survival of virtually all life on our planet, as well as the molecular oxygen necessary for the survival of oxygen-consuming organisms. In addition, the fossil fuels currently being burned to provide energy for human activity were produced by ancient photosynthetic organisms. Although photosynthesis occurs in cells or organelles that are typically only a few microns across, the process has a profound impact on the earth's atmosphere and climate. Each year more than 10% of the total atmospheric carbon dioxide is reduced to carbohydrate by photosynthetic organisms. Most, if not all, of

3-527-28135-5/95/$5.00 + .50

the reduced carbon is returned to the atmosphere as carbon dioxide by microbial, plant, and animal metabolism, and by biomass combustion. In turn, the performance of photosynthetic organisms depends on the earth's atmosphere and climate. Over the next century, the large increase in the amount of atmospheric carbon dioxide created by human activity is certain to have a profound impact on the performance and competition of photosynthetic organisms. Knowledge of the physicochemical process of photosynthesis is essential for understanding the relationship between living organisms and the atmosphere and the balance of life on earth.

1. BRIEF HISTORY

In the 1770s, Joseph Priestley, an English chemist and clergyman, performed experiments showing that plants release a type of air that allows combustion. He demonstrated this by burning a candle in a closed vessel until the flame went out. He placed a sprig of mint in the chamber and after several days showed that the candle could burn again. Although Priestley did not know about molecular oxygen, his work showed that plants release oxygen into the atmosphere. It is noteworthy that over 200 years later, investigating the mechanism by which plants produce oxygen is one of the most active areas of photosynthetic research. Building on the work of Priestley, Jan Ingenhousz, a Dutch physician, demonstrated that sunlight was necessary for photosynthesis and that only the green parts of plants could release oxygen. During this period, Jean Senebier, a Swiss botanist and naturalist, discovered that CO_2 is required for photosynthetic growth, and Nicolas-Théodore de Saussure, a Swiss chemist and plant physiologist, showed that water is required. It was not until 1845 that Julius Robert von Mayer, a German physician and physicist, proposed that photosynthetic organisms convert light energy into chemical free energy.

By the middle of the nineteenth century, the key features of plant photosynthesis were known, namely, that plants could use light energy to make carbohydrates from CO_2 and water. The empirical equation representing the net reaction of photosynthesis for oxygen-evolving organisms is

$$CO_2 + 2H_2O + \text{light energy} \rightarrow [CH_2O] + O_2 + H_2O, \quad (1)$$

where $[CH_2O]$ represents a carbohydrate (e.g., glucose, a six-carbon sugar). The synthesis of carbohydrate from carbon and water requires a large input of light energy. The standard free energy for the reduction of one mole of CO_2 to the level of glucose is +478 kJ/mol. Because glucose, a six-carbon sugar, is often an intermediate product of photosynthesis, the net equation of photosynthesis is frequently written as

$$6CO_2 + 12H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O. \quad (2)$$

The standard free energy for the synthesis of glucose is +2870 kJ/mol.

Not surprisingly, early scientists studying photosynthesis concluded that the O_2 released by plants came from CO_2, which was thought to be split by light energy. In the 1930s, comparison of bacterial and plant photosynthesis lead Cornelis van Niel to propose the general equation of photosynthesis that applies to plants, algae, and photosynthetic bacteria (discussed by Wraight, 1982). Van Niel was aware that some photosynthetic bacteria could use hydrogen sulfide (H_2S) instead of water for photosynthesis and that these organisms released sulfur instead of oxygen. Van Niel, among others, concluded that photosynthesis depends on electron donation and acceptor reactions and that the O_2 released during photosynthesis comes from the oxidation of water. Van Niel's generalized equation is

$$CO_2 + 2H_2A + \text{light energy} \rightarrow [CH_2O] + 2A + H_2O. \quad (3)$$

In oxygenic photosynthesis, $2A$ is O_2, whereas in anoxygenic photosynthesis, which occurs in some photosynthetic bacteria, the electron donor can be an inorganic hydrogen donor, such as H_2S in which case A is elemental sulfur, or an organic hydrogen donor such as succinate in which case A is fumarate.

The biochemical conversion of CO_2 to carbohydrate is a reduction reaction that involves the rearrangement of covalent bonds between carbon, hydrogen, and oxygen. The

energy for the reduction of carbon is provided by energy-rich molecules that are produced by the light-driven electron-transfer reactions. Carbon reduction can occur in the dark and involves a series of biochemical reactions that were elucidated by Melvin Calvin, Andrew Benson, and James Bassham in the late 1940s and 1950s. Using the radioisotope carbon-14, most of the intermediate steps that result in the production of carbohydrate were identified. Calvin was awarded the Nobel Prize for Chemistry in 1961 for this work.

In 1954, Daniel Arnon and co-workers discovered that plants, and A. Frenkel discovered that photosynthetic bacteria, use light energy to produce ATP, an organic molecule that serves as an energy source for many biochemical reactions. During the same period, L. N. M. Duysens showed that the primary photochemical reaction of photosynthesis is an oxidation/reduction reaction that occurs in a protein complex (the reaction center). Over the next few years, the work of several groups, including those of Robert Emerson, Bessel Kok, L. N. M. Duysens, Robert Hill, and Horst Witt, combined to prove that plant, algae, and cyanobacteria require two reaction centers, photosystem II and photosystem I, operating in series.

In 1961, Peter Mitchell suggested that cells can store energy by creating an electric field or a proton gradient across a membrane. Mitchell's proposal that energy is stored as an electrochemical gradient across a vesicular membrane opened the door for understanding energy transformation by membrane systems. He was awarded the Nobel Prize in Chemistry in 1978 for his theory of chemiosmotic energy transduction.

Most of the proteins required for the conversion of light energy and electron transfer reactions of photosynthesis are located in membranes. Despite decades of work, efforts to determine the structure of membrane-bound proteins had little success. This changed in the 1980s when Johann Deisenhofer, Hartmut Michel, and Robert Huber determined the structure of the reaction center of the purple bacterium *Rhodospeudomonas viridis*. They were awarded the Nobel Prize for Chemistry in 1988 for their work, which provided insight into the relationship between structure and function in membrane-bound proteins.

A key element in photosynthetic energy conversion is electron transfer within and between protein complexes and simple organic molecules. The electron-transfer reactions are rapid (as fast as a few picoseconds) and highly specific. Much of our current understanding of the physical principles that guide electron transfer is based on the pioneering work of Rudolph A. Marcus, who received the Nobel Prize in Chemistry in 1992 for his contributions to the theory of electron-transfer reaction in chemical systems.

The overall equation for photosynthesis is deceptively simple. In fact, a complex set of physical and chemical reactions must occur in a coordinated manner for the synthesis of carbohydrates. To produce a sugar molecule such as sucrose, plants require nearly 30 distinct proteins that work within a complicated membrane structure. Research into the mechanism of photosynthesis centers on understanding the structure of the photosynthetic components and the molecular processes that use radiant energy to drive carbohydrate synthesis. The research involves several disciplines, including physics, chemistry, structural biology, biochemistry, molecular biology, and physiology, and serves as an outstanding example of the success of multidisciplinary research. As such, photosynthesis presents a special challenge in understanding several interrelated molecular processes. From a physicist's viewpoint, the reactions that transform energy are of particular interest, and it is on these processes that this description will focus.

2. CLASSIFICATION OF PHOTOSYNTHETIC ORGANISMS

All life can be divided into three domains, Archaea, Bacteria, and Eucarya, which originated from a common ancestor (Woese *et al.*, 1990). Historically, the term photosynthesis has been applied to organisms that depend on chlorophyll (or bacteriochlorophyll) for the conversion of light energy into chemical free energy (Gest, 1993). These include organisms in the domains Bacteria (photosynthetic bacteria) and Eucarya (algae and higher plants). The most primitive domain, Archaea, includes organisms known as halobacteria, which convert light energy into chemical free energy. However, the mechanism by which halobacteria convert light is fundamentally

different from that of higher organisms because there is no oxidation/reduction chemistry and halobacteria cannot use CO_2 as their carbon source. Consequently, some biologists do not consider halobacteria as photosynthetic (Gest, 1993). This article will follow the historical definition of photosynthesis and omit halobacteria.

2.1 Oxygenic Photosynthetic Organisms

The photosynthetic process in all plants and algae, as well as in certain types of photosynthetic bacteria, involves the reduction of CO_2 to carbohydrate and the removal of electrons from H_2O, which results in the release of O_2. In this process, known as oxygenic photosynthesis, water is oxidized by the photosystem II reaction center, a multisubunit protein located in the photosynthetic membrane. Years of research have shown that structure and function of photosystem II is similar in plants, algae, and certain bacteria, so that knowledge gained in one species can be applied to others. This homology is a common feature of proteins that perform the same reaction in different species. The importance of this homology at the molecular level is shown by the fact that there are an estimated 300 000–500 000 species of plants. If different species had evolved diverse mechanisms for oxidizing water, research aimed at a general understanding of photosynthetic water oxidation would be hopeless.

2.2 Anoxygenic Photosynthetic Organisms

Some photosynthetic bacteria can use light energy to extract electrons from molecules other than water. These organisms are of ancient origin, presumed to have evolved before oxygenic photosynthetic organisms. Anoxygenic photosynthetic organisms occur in the domain Bacteria and have representatives in four phyla: Purple Bacteria, Green Sulfur Bacteria, Green Gliding Bacteria, and Gram Positive Bacteria.

3. GENERAL PRINCIPLES OF PHOTOSYNTHETIC ENERGY TRANSFORMATION IN PLANTS

The energy that drives photosynthesis originates in the center of the sun, where mass is converted to heat by the fusion of hydrogen. Over time, the heat energy reaches the sun's surface, where some of it is converted to light by blackbody radiation that reaches the earth. A small fraction of the visible light incident on the earth is absorbed by plants. Through a series of energy-transducing reactions, plants are able to transform light energy into chemical free energy in a stable form that can last for hundreds of millions of years (e.g., fossil fuels). A simplified scheme describing how plants transform energy is presented in this section. The focus is on the structural and functional features essential for the energy-transforming reactions. For clarity, mechanistic and structural details are omitted. A more highly resolved description of oxygenic and anoxygenic photosynthesis is given in the remaining sections.

The photosynthetic process in plants and algae occurs in small organelles, known as chloroplasts, that are located inside cells. The photosynthetic reactions are traditionally divided into two types: the "light reactions," which consist of electron- and proton-transfer reactions, and the "dark reactions," which consist of the biosynthesis of carbohydrates from CO_2. The light reactions occur in a complex membrane system (the photosynthetic membrane) that is made up of protein complexes, electron carriers, and lipid molecules. The photosynthetic membrane is surrounded by water and can be thought of as a two-dimensional surface that defines a closed space, with an inner and an outer water phase. A molecule or ion must pass through the photosynthetic membrane to go from the inner space to the outer space. The protein complexes embedded in the photosynthetic membrane have a unique orientation with respect to the inner and outer phases. The asymmetrical arrangement of the protein complexes allows some of the energy released during electron transport to create an electrochemical gradient of protons across the photosynthetic membrane.

Photosynthetic electron transport consists of a series of individual electron-transfer steps from one electron carrier to another. The electron carriers are metal ion complexes and aromatic groups. The metal ion complexes and most of the aromatic groups are bound within proteins. Most of the proteins involved in photosynthetic electron transport are composed of numerous polypeptide chains that lace through the membrane, providing

a scaffolding for metal ions and aromatic groups. An electron enters a protein complex at a specific site, is transferred within the protein from one carrier to another, and exits from the protein at a different site. The protein controls the pathway of electrons between the carriers by determining the location and environment of the metal ion complexes and aromatic groups. By setting the distance between electron carriers and controlling the electronic environment surrounding a metal ion complex or an aromatic group, the protein controls pairwise electron transfer reactions. Between proteins, electron transfer is controlled by distance and free energy, as for intraprotein transfer, and by the probability that the two proteins are in close contact. Protein association is controlled by a number of factors, including the structure of the two proteins, their surface electrical and chemical properties, and the probability that they collide with one another. Not all electron carriers are bound to proteins. The reduced forms of plastoquinone and nicotinamide adenine dinucleotide phosphate (NADPH) act as mobile electron carriers operating between protein complexes. For electron transfer to occur, these small molecules must bind to special pockets in the proteins known as binding sites. The binding sites are highly specific and are a critical factor in controlling the rate and pathway of electron transfer.

The light reactions convert energy into several forms (Fig. 1). The first step is the conversion of a photon to an excited electronic state of an antenna pigment molecule located in the antenna system. The antenna system consists of hundreds of pigment molecules (mainly chlorophyll and carotenoids) that are anchored to proteins within the photosynthetic membrane and serve a specialized protein complex known as a reaction center. The electronic excited state is transferred over the antenna molecules as an exciton. Some excitons are converted back into photons and emitted as fluorescence, some are converted to heat, and some are trapped by a reaction-center protein. Excitons trapped by a reaction center provide the energy for the primary photochemical reaction of photosynthesis—the transfer of an electron from a donor molecule to an acceptor molecule. Both the donor and acceptor molecules are attached to the reaction-center protein complex. Once primary charge separation occurs, the subsequent electron-transfer reactions are energetically downhill. In oxygenic photosynthetic organisms, two different reaction centers work in series: photosystem II and photosystem I. Electrons are transferred from photosystem II to the photosystem I reaction center by intermediate carriers. The net reaction is the transfer of electrons from a water molecule to $NADP^+$, producing the reduced form, NADPH. In the photosynthetic process, much of the energy initially provided by light energy is stored as redox free energy (a form of chemical free energy) in NADPH, to be used later in the reduction of carbon. In addition, the electron-transfer reactions concentrate protons inside the membrane vesicle and create an electric field across the photosynthetic membrane. In this process, the electron-transfer reactions convert redox free energy into an electrochemical po-

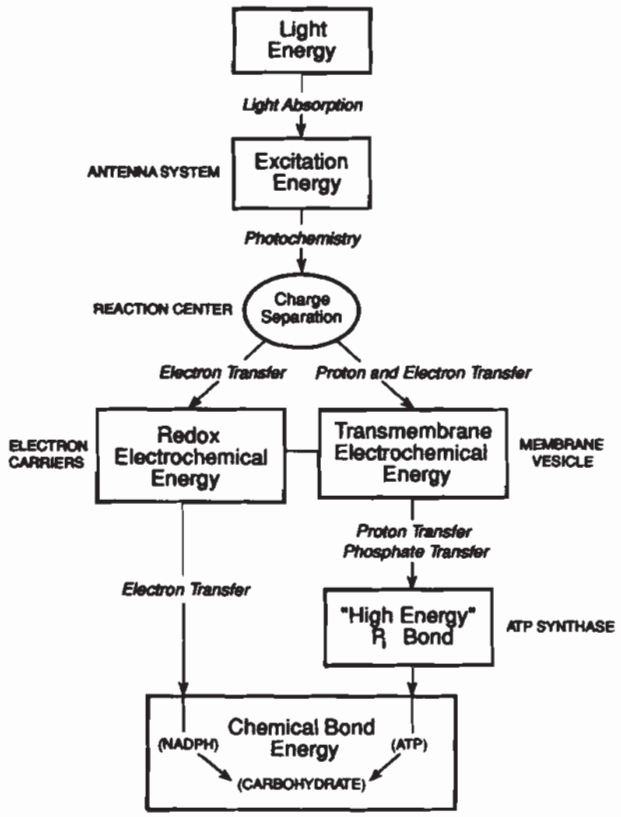


FIG. 1. Photosynthesis shown as a series of reactions that transform energy from one form to another. The different forms of energy are shown in boxes, and the direction of energy transformation is shown by the arrows. The energy-transforming reaction is shown by italics in the arrows. The site at which the energy is stored is shown in capital letters outside the boxes. The primary photochemical reaction, charge separation, is shown in the oval. Details of these reactions are given in the text.

tential of protons. The energy stored in the proton electrochemical potential is used by a membrane-bound protein complex (ATP synthase) to attach a phosphate group covalently to adenosine diphosphate (ADP), forming adenosine triphosphate (ATP). Protons pass through the ATP-synthase protein complex that transforms electrochemical free energy into a type of chemical free energy known as phosphate group-transfer potential (or a high-energy phosphate bond) (Klotz, 1967). The energy stored in ATP can be transferred to another molecule by transferring the phosphate group. The net effect of the light reactions is to convert radiant energy into redox free energy in the form of NADPH and phosphate group-transfer energy in the form of ATP. In the light reactions, the transfer of a single electron from water to $NADP^+$ involves about 30 metal ions and seven aromatic groups. The metal ions include 19 Fe, 5 Mg, 4 Mn, and 1 Cu. The aromatics include quinones, pheophytin, NADPH, tyrosine, and a flavoprotein.

The NADPH and ATP formed by the light reactions provide the energy for the dark reactions of photosynthesis, known as the Calvin cycle or the photosynthetic carbon reduction cycle. The reduction of atmospheric CO_2 to carbohydrate occurs in the aqueous phase of the chloroplast and involves a series of enzymatic reactions. The first step is catalyzed by the protein Rubisco (D-ribulose 1,5-bisphosphate carboxylase/oxygenase), which attaches CO_2 to a five-carbon compound. The reaction produces two molecules of a three-carbon compound. Subsequent biochemical reactions involve several enzymes that reduce carbon by hydrogen transfer and rearrange the carbon compounds to synthesize carbohydrates. The carbon reduction cycle involves the transfer and rearrangement of chemical bond energy.

4. OXYGENIC PHOTOSYNTHESIS IN PLANTS

4.1 Chloroplasts—Structure and Organization

Photosynthesis occurs inside chloroplasts, which are small organelles found in plant cells. Chloroplasts provide the energy and reduced carbon needed for plant growth and development, while the plant provides the chloroplast with CO_2, water, nitrogen, organic molecules, and minerals necessary for the chloroplast biogenesis. Most chloroplasts are located in specialized leaf cells, which often contain 50 or more chloroplasts per cell. Each chloroplast is defined by an inner and an outer envelope membrane and is shaped like a meniscus convex lens that is 5–10 μm in diameter (Fig. 2), although many different shapes and sizes can be found in plants. The inner envelope membrane acts as a barrier, controlling the flux of organic and charged molecules in and out of the chloroplast. Water passes freely through the envelope membranes, as do other small neutral molecules like CO_2 and O_2. There is convincing evidence that chloroplasts were once free-living bacteria that invaded a nonphotosynthetic cell long ago. They have retained some of the DNA necessary for their assembly, but much of the DNA necessary for their biosynthesis is located in the cell nucleus. This enables a cell to control the biosynthesis of chloroplasts within its domain.

Inside the chloroplast is a complicated membrane system, known as the photosynthetic membrane (or thylakoid membrane), that contains most of the proteins required for the light reactions. The proteins required for the fixation and reduction of CO_2 are located outside the photosynthetic membrane in the surrounding aqueous phase. The photosynthetic membrane is composed mainly of glycerol lipids and protein. The glycerol lipids are a family of molecules characterized by a polar head group that is hydrophilic and two fatty acid side chains that are hydrophobic. In membranes, the lipid mol-

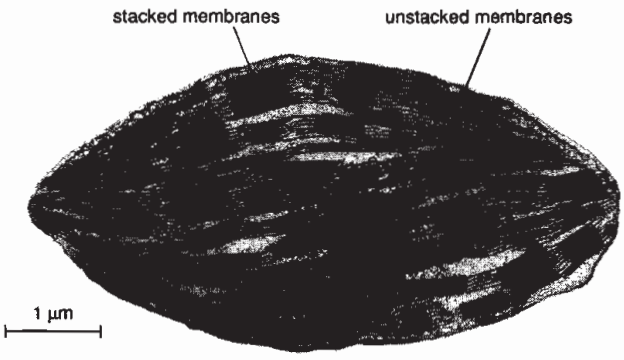


FIG. 2. An electron micrograph of a plant chloroplast. Micrograph by A. D. Greenwood, courtesy of J. Barber.

ecules arrange themselves in a bilayer, with the polar head toward the water phase and the fatty acid chains aligned inside the membrane forming a hydrophobic core (Fig. 3). The photosynthetic membrane is vesicular, defining a closed space with an outer water space (stroma) and an inner water space (lumen). The organization of the photosynthetic membrane can be described as groups of stacked membranes (like stacks of pita bread, with the inner pocket representing the inner aqueous space), interconnected by non-stacked membranes that protrude from the edges of the stacks (Staehelin, 1986). Experiments indicate that the inner aqueous space of the photosynthetic membrane is likely continuous inside of the chloroplast. It is not known why the photosynthetic membrane forms such a convoluted structure. To understand the energetics of photosynthesis, the complicated structure can be ignored and the photosynthetic membrane can be viewed as a simple vesicle.

4.2 Light Absorption—The Antenna System

Plant photosynthesis is driven primarily by visible light (wavelengths from 400 to 700 nm) that is absorbed by pigment molecules (mainly chlorophyll *a* and *b* and carotenoids). Plants appear green because of chlorophyll, which is so plentiful that regions of the earth appear green from space. The absorption spectra of chlorophyll *a* and *b* and of a chloroplast are shown in the article Excitons. Light is collected by 200–300 pigment molecules that are bound to light-harvesting protein complexes located in the photosynthetic membrane. The three-dimensional structure of the light-harvesting complex (Kühlbrandt *et al.*, 1994) shows that the protein determines the position and orientation of the antenna pigments. The light-harvesting complexes surround the reaction centers and serve as antennae. Photosynthesis is initiated by the absorption of a photon by an antenna molecule, which occurs in

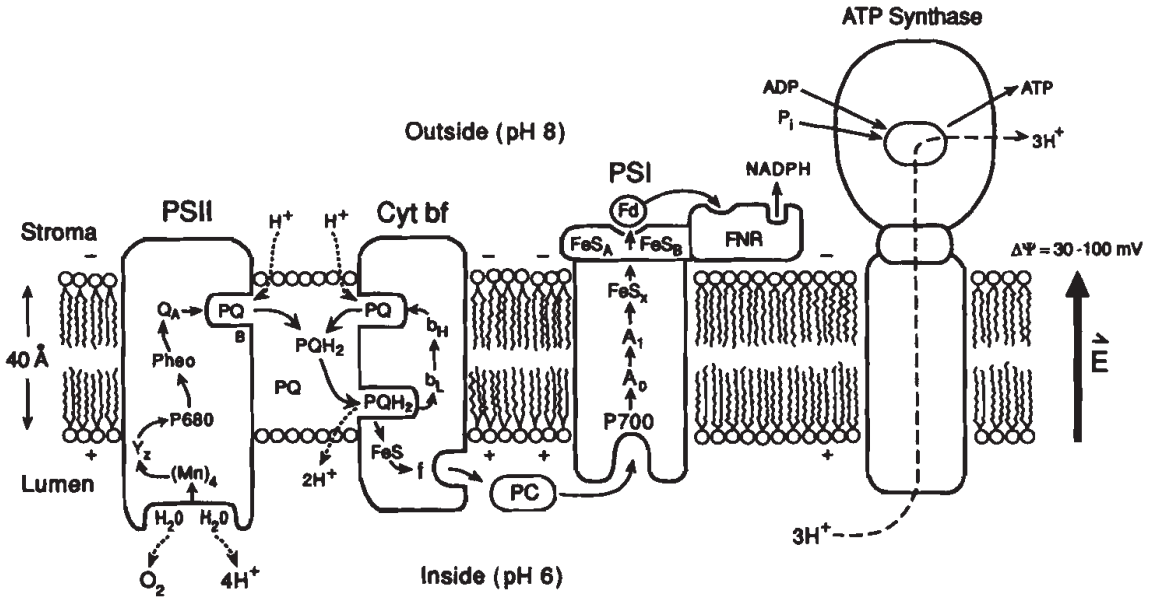


FIG. 3. Model of the photosynthetic membrane of plants showing the electron-transport components and the ATP-synthase enzyme (cross-sectional view). The complete membrane forms a vesicle. The pathways of electrons are shown by solid arrows. The membrane-bound electron-transport protein complexes involved in transferring electrons are the photosystem II and I reaction centers (PSII and PSI) and the cytochrome *bf* complex (Cyt *bf*). Abbreviations: Y_z, tyrosine; P680 and P700, the reaction-center chlorophyll of photosystem II and photosystem I, respectively; Pheo, pheophytin; Q_A, bound plastoquinone; PQ, free plastoquinone (oxidized form), PQH_2, free plastoquinone (reduced form); b_L and b_H, different forms of *b*-type cytochromes; FeS, iron-sulfur centers; *f*, cytochrome *f*; PC, plastocyanin; A_0, chlorophyll; A_1, phylloquinone; Fd, ferredoxin; FNR, ferredoxin/$NADP^+$ oxidoreductase; NADPH, nicotinomide adenine dinucleotide phosphate (reduced form); ADP, adenosine diphosphate; ATP, adenosine triphosphate; P_i, inorganic phosphate; H^+, protons; $\Delta\Psi$, the light-induced electrical potential across the membrane. The light-harvesting protein complexes are not shown. Details are given in the text.

10^{-15} s and causes a transition from the electronic ground state to an excited state. Within 10^{-13} s, the excited state decays by vibrational relaxation to the first excited singlet state. The fate of the excited-state energy is guided by the structure of the protein. Because of the proximity of other antenna molecules with the same or similar energy states, the excited-state energy has a high probability of being transferred by resonance energy transfer to a near neighbor. Exciton energy transfer between antenna molecules is due to the interaction of the transition dipole moment of the molecules. The probability of transfer is dependent on the distance between the transition dipoles of the donor and acceptor molecules ($1/R^6$), the relative orientation of the transition dipoles, and the overlap of the emission spectrum of the donor molecule with the absorption spectrum of the acceptor molecule (for a discussion of resonance energy transfer, see EXCITONS). Photosynthetic antenna systems are very efficient at this process. Under optimum conditions, over 90% of the absorbed quanta are transferred within a few hundred picoseconds from the antenna system to the reaction center, which acts as a trap for the excitons.

4.3 Primary Photochemistry—Photosystem II and Photosystem I Reaction Centers

Photosystem II uses light energy to drive two chemical reactions: the oxidation of water and the reduction of plastoquinone. The photosystem II complex is composed of more than 15 polypeptides, and at least nine different redox components (chlorophyll, pheophytin, plastoquinone, tyrosine, Mn, Fe, cytochrome b559, carotenoid, and histidine) have been shown to undergo light-induced electron transfer (Debus, 1992). However, only five of these redox components are known to be involved in transferring electrons from H_2O to the plastoquinone pool—the water-oxidizing manganese cluster $(Mn)_4$, the amino acid tyrosine, the reaction center chlorophyll (P680), pheophytin, and the plastoquinone molecules, Q_A and Q_B. Of these essential redox components, tyrosine, P680, pheophytin, Q_A, and Q_B have been shown to be bound to two key polypeptides that form the heterodimeric reaction center core of photosystem II (*D*1 and *D*2). Recent work indicates that the *D*1 and *D*2 polypeptides also provide ligands for the $(Mn)_4$ cluster. The three-dimensional structure of photosystem II is not known. Our knowledge of its structure is guided by the known structure of the reaction center in purple bacteria and biochemical and spectroscopic data. Figure 4 shows a schematic view of photosystem II that is consistent with current data.

Photochemistry in photosystem II is initiated by charge separation between P680 and pheophytin, creating $P680^+/Pheo^-$. Primary charge separation takes about 3 ps (Fig. 5). Subsequent electron-transfer steps have been designed through evolution to prevent the primary charge separation from recombining. This is accomplished by transferring the electron within 200 ps from pheophytin to a plastoquinone molecule (Q_A) that is permanently bound to photosystem II. Although plastoquinone normally acts as a two-electron acceptor, it works as a one-electron acceptor at the Q_A site. The electron on Q_A- is then transferred to another plastoquinone molecule that is loosely bound at the Q_B site. Plastoquinone at the Q_B site differs from Q_A in that it works as a two-electron acceptor, becoming fully reduced and protonated after two photochemical turnovers of the reaction center. The full reduction of plastoquinone requires the addition of two electrons and two

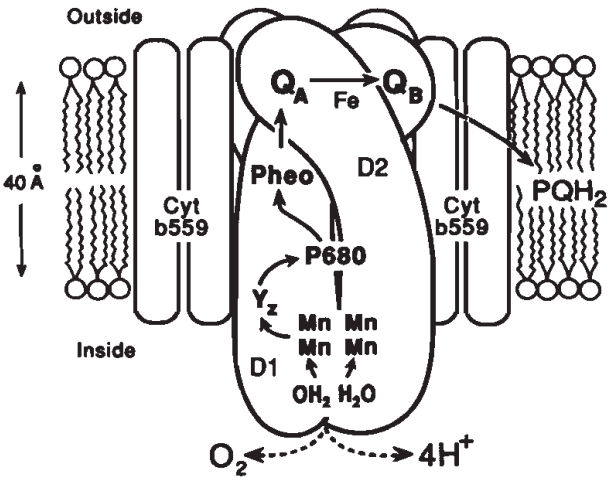


FIG. 4. Schematic drawing of photosystem II. Photosystem II is composed of numerous polypeptides, but only two of them, *D*1 and *D*2, bind the electron carriers involved in transferring electrons from Y_z to plastoquinone. Abbreviations: Y_z, tyrosine; P680, reaction-center chlorophyll (primary electron donor); Pheo, pheophytin; Q_A and Q_B, bound plastoquinone; PQH_2, reduced plastoquinone; Cyt *b*559, *b*-type cytochrome. Details are given in the text.

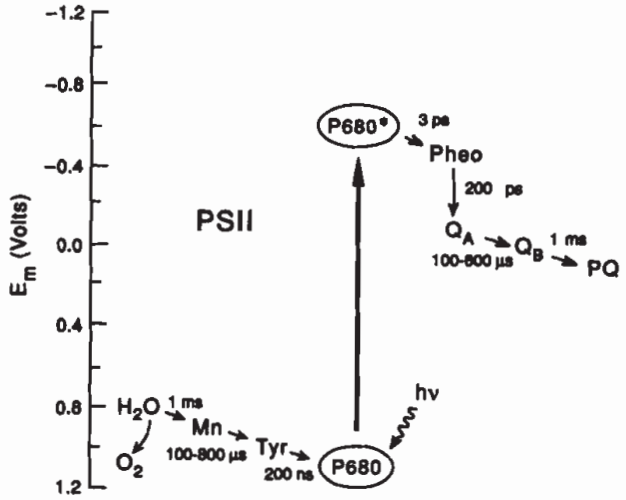


FIG. 5. Photosystem II electron-transport pathways and rates. The vertical axis shows the midpoint potential of the electron carriers. The heavy vertical arrow shows light absorption. P680* is the electronically excited state of P680. The abbreviations are given in the legend of Fig. 4.

protons, i.e., the addition of two hydrogen atoms (Fig. 6). The reduced plastoquinone then debinds from the reaction center and diffuses into the hydrophobic core of the membrane, then an oxidized plastoquinone molecule finds its way to the Q_B-binding site, and the process is repeated. Because the Q_B site is near the outer aqueous phase, the protons added to plastoquinone during its reduction are taken from the outside of the membrane.

Photosystem II is the only known protein complex that can oxidize water, resulting in the release of O_2 into the atmosphere. Despite years of research, little is known about the molecular events that lead to water oxidation. Energetically, water is a poor electron donor. The oxidation-reduction midpoint potential ($E_{m,7}$) of water is +0.82 V (*p*H 7). In photosystem II, this reaction is driven by the oxidized reaction center, $P680^+$ (the midpoint potential of $P680/P680^+$ is estimated to be +1.2 V at *p*H 7). How electrons are transferred from water to $P680^+$ remains a mystery (Govindjee and Coleman, 1990). It is known that $P680^+$ oxidizes a tyrosine on the *D*1 protein and that Mn plays a key role in water oxidation. Four Mn ions are present in the water-oxidizing complex. X-ray absorption spectroscopy shows that Mn undergoes light-induced oxidation. Water oxidation requires two molecules of water and involves four sequential turnovers of the reaction center. Each photochemical reaction creates an oxidant that removes one electron. The net reaction results in the release of one O_2 molecule, the deposition of four protons into the inner water phase, and the transfer of four electrons to the Q_B site (producing two reduced plastoquinone molecules).

The photosystem I complex catalyzes the oxidation of plastocyanin, a small soluble Cu protein, and the reduction of ferredoxin, a small FeS protein (Fig. 7). Photosystem I is

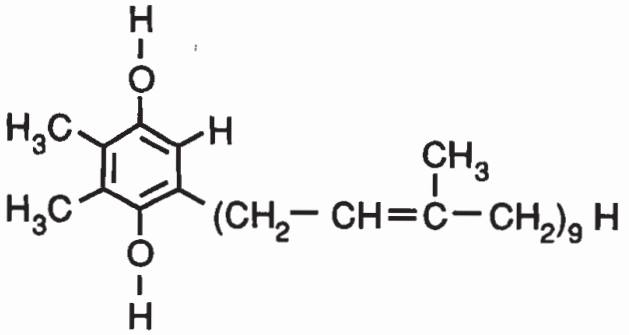


FIG. 6. Structure of plastoquinone (reduced form), an aromatic molecule that carries electrons and protons in photosynthetic electron transport.

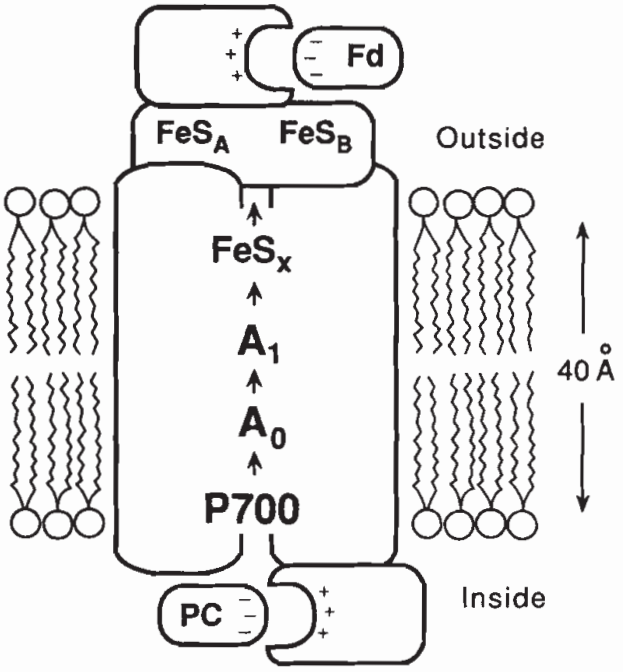


FIG. 7. Schematic drawing of photosystem I. Photosystem I is composed of numerous polypeptides, but only three of them bind the electron carriers. Abbreviations: PC, plastocyanin; P700, reaction-center chlorophyll (primary electron donor); A_0, chlorophyll; A_1, phylloquinone; FeS, FeS centers; Fd, ferredoxin. Details are given in the text.

composed of a heterodimer of proteins that act as ligands for most of the electron carriers (Krauss *et al.*, 1993). The reaction center is served by an antenna system that consists of about two hundred chlorophyll molecules (mainly chlorophyll *a*), and primary photochemistry is initiated by a chlorophyll *a* dimer, P700. In contrast to photosystem II, many of the antenna chlorophyll molecules in photosystem I are bound to the reaction-center proteins. Also, FeS centers serve as electron carriers in photosystem I, and so far as is known, photosystem I electron transfer is not coupled to proton translocation. Primary charge separation occurs between a primary donor, P700, a chlorophyll dimer, and a chlorophyll monomer (A_0). The subsequent electron transfer events and rates are shown in Fig. 8.

4.4 Electron Transport

Electron transport from water to $NADP^+$ requires three membrane-bound protein complexes operating in series: photosystem II, the cytochrome bf complex, and photosystem I (Fig. 3). Electrons are transferred between these large protein complexes by small mobile molecules. Because these small molecules carry electrons (or hydrogen atoms) over relatively long distances, they play a unique role in photosynthetic energy conversion. This is illustrated by plastoquinone (PQ), which serves two key functions. Plastoquinone transfers electrons from the photosystem II reaction center to the cytochrome *bf* complex and carries protons across the photosynthetic membrane. It does this by shuttling hydrogen atoms across the membrane from photosystem II to the cytochrome *bf* complex. Because plastoquinone is hydrophobic, its movement is restricted to the hydrophobic core of the photosynthetic membrane. Plastoquinone operates by diffusing through the membrane until, as a result of random collisions, it becomes bound to a specific site on the photosystem II complex. The photosystem II reaction center reduces plastoquinone at the Q_B site by adding two electrons and two protons, creating PQH_2. The reduced plastoqinone molecule debinds from photosystem II and diffuses randomly in the photosynthetic membrane until it encounters a specific binding site on the cytochrome *bf* complex. The cytochrome *bf* complex is a membrane-bound protein complex that contains four electron carriers, three cytochromes, and an FeS center. In a complicated reaction sequence that is not fully understood, the cytochrome *bf* complex removes the electrons from reduced plastoquinone and facilitates the release of the protons into the inner aqueous space. The electrons are eventually transferred to the photosystem I reaction center. The protons released into the inner aqueous space contribute to the proton chemical free energy across the membrane.

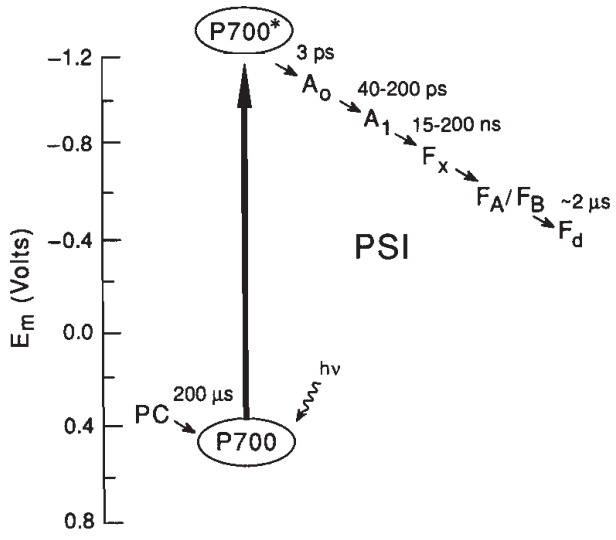


FIG. 8. Photosystem I electron transport pathways and rates. The vertical axis shows the midpoint potential of the electron carriers. Abbreviations are given in the legend of Fig. 7.

Electron transfer from the cytochrome *bf* complex to photosystem I is mediated by a small Cu protein, plastocyanin (PC). Plastocyanin is water soluble and operates in the inner water space of the photosynthetic membrane. Electron transfer from photosystem I to $NADP^+$ requires ferredoxin, a small FeS protein, and ferredoxin-NADP oxidoreductase, a peripheral flavoprotein that operates on the outer surface of the photosynthetic membrane. Ferredoxin and $NADP^+$ are water soluble and are found in the outer aqueous phase.

As discussed in Sec. 3, the pathway of electrons is largely determined by the energetics of the reaction and the distance between the carriers. The electron affinity of the carriers is represented in Fig. 9 by their midpoint potentials, which show the free energy available for electron-transfer reactions under equilibrium conditions. (It should be kept

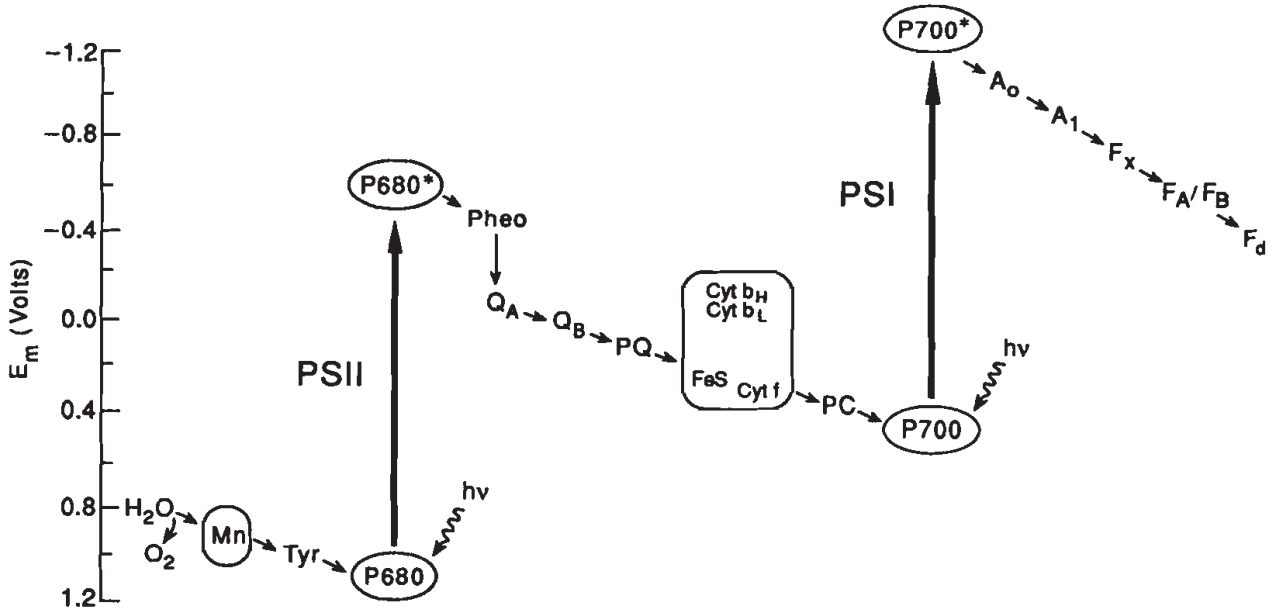


FIG. 9. The electron transport pathway of plants (oxygenic photosynthesis). Abbreviations are given the legend of Fig. 3. Details are given in the text.

in mind that reaction conditions during photosynthesis are not in equilibrium.) Subsequent to primary charge separation, electron transport is energetically downhill (from a lower to a higher redox potential). It is the downhill flow of electrons that provides free energy for the creation of a proton chemical gradient.

Photosynthetic membranes effectively limit electron transport to two dimensions. For mobile electron carriers, limiting diffusion to two dimensions increases the number of random encounters (Whitmarsh, 1986). Furthermore, because plastocyanin is mobile, any one cytochrome *bf* complex can interact with a number of photosystem I complexes. The same is true for plastoquinone, which commonly operates at a stoichiometry of about six molecules per photosystem II complex.

4.5 Creation of a Proton Electrochemical Potential

Electron transport creates the proton electrochemical potential of the photosynthetic membrane by two types of reactions.

1. The release of protons during the oxidation of water by photosystem II and the translocation of protons from the outer aqueous phase to the inner aqueous phase by the coupled reactions of photosystem II and the cytochrome *bf* complex in reducing and oxidizing plastoquinone on opposite sides of the membrane creates a concentration difference of protons across the membranes ($\Delta pH = pH_{in} - pH_{out}$).
2. Primary charge separation at a reaction center drives an electron across the photosynthetic membrane, which creates an electric potential across the membrane ($\Delta\Psi = \Psi_{in} - \Psi_{out}$).

Together, these two forms of energy make up the proton electrochemical potential across the photosynthetic membrane ($\Delta\mu_{H^+}$), which is related to the *p*H difference across the membrane and the electrical potential difference across the membrane by the following equation:

$$\Delta\mu_{H^+} = F\Delta\Psi - 2.3RT\,\Delta pH,$$

where F is the Faraday constant, R is the gas constant, and T is the temperature in kelvins. Although the value of $\Delta\Psi$ across the photosynthetic membrane in chloroplasts can be as large as 100 mV, under normal conditions the proton gradient dominates. For example, during photosynthesis, the outer *p*H is typically near 8 and the inner *p*H is typically near 6, giving a *p*H difference of 2 across the membrane that is equivalent to 120 mV. Under these conditions, the free energy for proton transfer from the inner to the outer aqueous phase is −12 kJ/mol of protons.

4.6 Synthesis of ATP by the ATP-Synthase Enzyme

The conversion of proton electrochemical energy into chemical free energy is accomplished by a single protein complex known as ATP synthase. This enzyme catalyzes a phosphorylation reaction, which is the formation of ATP by the addition of inorganic phosphate (P_i) to ADP:

$$ADP^{-3} + P_i^{-2} + H^+ \rightarrow ATP^{-4} + H_2O.$$

The reaction is energetically uphill (ΔG = +32 kJ/mol) and is driven by proton transfer through the ATP-synthase protein. The ATP-synthase complex is composed of two major subunits, CF_0 and CF_1 (Fig. 10). The CF_0 subunit spans the photosynthetic membrane and forms a proton channel through the membrane. The CF_1 subunit is attached to the top of the CF_0 on the outside of the membrane and is located in the aqueous space. The CF_1 subunit is composed of several different protein subunits, referred to as α, β, γ, δ, and ϵ. The top portion of the CF_1 subunit is composed of three $\alpha\beta$ dimers that contain the catalytic sites for ATP synthesis. The molecular processes that couple proton transfer through the protein to the chemical addition of phosphate to ADP are poorly understood. It is known that phosphorylation can be driven by a *p*H gradient, a transmembrane electric field, or a combination of the two. Experiments indicate that three protons must pass through the ATP-synthase complex for the synthesis of one molecule of ATP. However, the protons are not involved in the chemistry of adding phosphate to ADP. Paul Boyer and co-workers have proposed an alternating binding site mechanism for ATP synthesis (Boyer, 1993). One model based on their proposal is that there are three catalytic sites on each CF_1 that cycle among three different states. The states differ in their affinity for ADP, P_i, and ATP. At any one time, each site is in a different state. Initially, one catalytic site on CF_1 binds one ADP and one inorganic phosphate molecule relatively loosely. Through a conformational change of the protein, the site becomes a tight binding site, which stabilizes ATP. Next, proton transfer induces an alteration in protein conformation that causes the site to release the ATP molecule into the aqueous phase. In this model, the energy from the proton electrochemical gradient is used to lower the affinity of the site for ATP, allowing its release to the water phase. The three sites on CF_1 act cooperatively; i.e., the conformational states of the sites are linked. It has been proposed that protons affect the conformational change by driving the rotation of the top part (the three $\alpha\beta$ dimers) of CF_1. This revolving-site mechanism would require rates as high as 100 rev/s. It is worth noting that flagella that propel some bacteria are driven by a proton pump and can rotate at 60 rev/s.

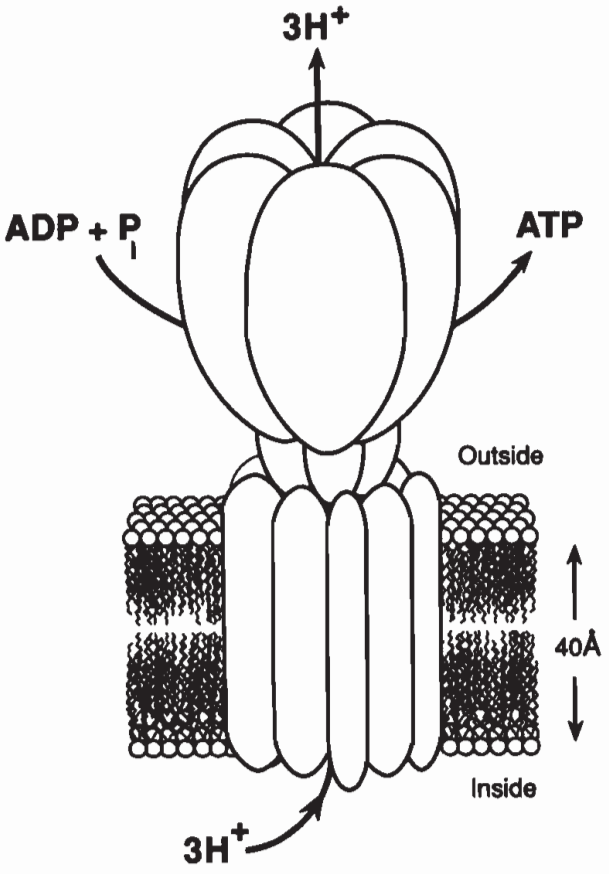


FIG. 10. Schematic drawing of the ATP-synthase enzyme embedded in the membrane. Proton transfer through the ATP synthase provides the energy for the creation of ATP from ADP and P_i. Abbreviations are given in the legend of Fig. 3. Details are given in the text.

4.7 Synthesis of Carbohydrates from Atmospheric CO_2 by the Calvin-Cycle Enzymes

All plants and algae remove CO_2 from the environment and reduce it to carbohydrate by the Calvin cycle. The process is a sequence of biochemical reactions that reduce carbon and rearrange bonds to produce car-

bohydrate from CO_2 molecules. The first step is the addition of CO_2 to a five-carbon compound (ribulose 1,5-bisphosphate) (Fig. 11). The six-carbon compound is split, giving two molecules of a three-carbon compound (3-phosphoglycerate). This key reaction is catalyzed by Rubisco, a large water-soluble protein complex. The carboxylation reaction is energetically downhill. The main energy input in the Calvin cycle is the phosphorylation by ATP and subsequent reduction by NADPH of the initial three-carbon compound forming a three-carbon sugar, triose phosphate. Some of the triose phosphate is exported from the chloroplast and provides the building block for synthesizing more complex molecules. In a process known as regeneration, the Calvin cycle uses some of the triose phosphate molecules to synthesize the energy-rich ribulose 1,5-bisphosphate needed for the initial carboxylation reaction. This reaction requires the input of energy in the form of one ATP. Overall, 13 enzymes are required to catalyze the reactions in the Calvin cycle. The energy-conversion efficiency of the Calvin cycle is approximately 90%. The reactions do not involve energy transduction but rather the rearrangement of chemical energy. Each molecule of CO_2 reduced to a sugar $[CH_2O]_n$ requires two molecules of NADPH and three molecules of ATP.

Rubisco is a bifunctional enzyme that, in addition to binding CO_2 to ribulose bisphosphate, can also bind O_2. This oxygenation reaction produces the 3-phosphoglycerate that is used in the Calvin cycle and a two-carbon compound (2-phosphoglycolate) that is not useful for the plant. In response, a complicated set of reactions (known as photorespiration) are initiated that serve to recover reduced carbon and to remove phosphoglycolate. The Rubisco oxygenation reaction appears to serve no useful purpose for the plant. Some plants have evolved specialized structures and biochemical pathways that concentrate CO_2 near Rubisco, which serves to decrease the fraction of oxygenation reactions.

4.8 Photosynthetic Quantum Yield and Energy Conversion Efficiency

The theoretical minimum quantum requirement for photosynthesis is eight quanta for each molecule of oxygen evolved (four quanta required by photosystem II and four by photosystem I). Measurements in leaves under optimal conditions (e.g., low light) give

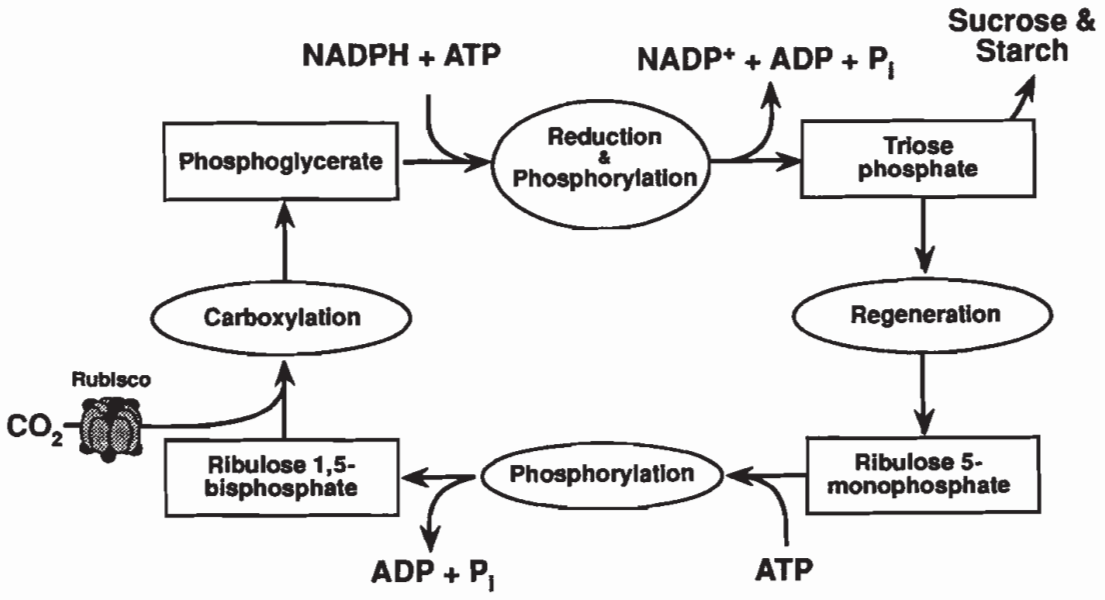


FIG. 11. An abbreviated scheme showing reduction of carbon dioxide by the Calvin cycle. The first step is carboxylation, in which ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes the addition of CO_2 to the five-carbon compound ribulose 1,5-bisphosphate, which is subsequently split into two molecules of the three-carbon compound 3-phosphoglycerate. Next are reduction and phosphorylation reactions that form the carbohydrate triose phosphate. Some of the triose phosphate molecules are used to form the products of photosynthesis, sucrose and starch, while the rest is used to regenerate ribulose 1,5-bisphosphate needed for the continuation of the cycle. Details are given in the text.

quantum requirements of 8–10 photons per oxygen molecule released. These quantum yield measurements show that the quantum yields of photosystem II and photosystem I reaction centers under optimal conditions are near 100%. These values can be used to calculate the theoretical energy conversion efficiency of photosynthesis (free energy stored as carbohydrate/light energy absorbed). If eight red quanta are absorbed (8 mol of red photons are equivalent to 1400 kJ) for each CO_2 molecule reduced (480 kJ/mol), the theoretical maximum energy efficiency for carbon reduction is 34%. Under optimal conditions, plants can achieve energy conversion efficiencies within 90% of the theoretical maximum. However, under normal growing conditions, the actual performance of the plant is far below these theoretical values. The factors that conspire to lower the quantum yield of photosynthesis include limitations imposed by biochemical reactions in the plant and environmental conditions that limit photosynthetic performance. One of the most efficient crop plants is sugar cane, which has been shown to store up to 1% of the incident visible radiation over a period of 1 year. However, most crops are less productive. The annual conversion efficiency of corn, wheat, rice, potatoes, and soybeans typically ranges from 0.1% to 0.4% (Odum, 1971).

5. OXYGENIC PHOTOSYNTHESIS IN ALGAE

Algae are photosynthetic eukaryotic organisms that evolve O_2 and reduce CO_2. They represent a diverse group that include dinoflagellates, euglenoids, yellow-green algae, golden-brown algae, diatoms, red algae, brown algae, and green algae. The photosynthetic apparatus and biochemical pathways of carbon reduction of algae are similar to plants. Photosynthesis occurs in chloroplasts that contain photosystems II and I, the cytochrome bf complex, the Calvin-cycle enzymes, and pigment-protein complexes containing chlorophyll *a* and other antenna pigments (*e.g.*, chlorophyll *b* in green algae, chlorophyll *c* and fucoxanthol in brown algae and diatoms, and phycobilins in red algae). Green algae are thought to be the ancestral group from which land plants evolved. Algae are abundant and widespread on the earth, living mainly in fresh and sea water. Some algae live as single-celled organisms, while others form multicellular organisms, some of which can grow quite large, such as kelp and seaweed. Phytoplankton in the ocean is made up of algae and oxygenic photosynthetic bacteria. Most photosynthesis in the ocean is due to phytoplankton, which is an important source of food for marine life.

6. OXYGENIC PHOTOSYNTHESIS IN BACTERIA

6.1 Cyanobacteria

Cyanobacteria are photosynthetic prokaryotic organisms that evolve O_2. Fossil evidence indicates that cyanobacteria existed over 3 billion years ago, and it is thought that they were the first oxygen-evolving organisms on earth. Cyanobacteria are presumed to have evolved in water in an atmosphere that lacked O_2. Initially, the O_2 released by cyanobacteria reacted with ferrous iron in the oceans and was not released into the atmosphere. Geological evidence indicates that the ferrous Fe was depleted around 2 billion years ago, and earth's atmosphere became aerobic. The release of O_2 into the atmosphere by cyanobacteria has had a profound affect on how life evolved.

The photosynthetic apparatus of cyanobacteria is similar to that of chloroplasts. The main difference lies in the antenna system. Cyanobacteria depend on chlorophyll *a* and specialized protein complexes called phycobilisomes that gather light energy. They do not contain chlorophyll *b*. As in chloroplasts, the chlorophyll *a* is located in membrane-bound proteins. The phycobilisomes are bound to the outer side of the photosynthetic membrane and act to funnel exciton energy to the photosystem II reaction center. They are composed of phycobiliproteins, protein subunits that contain covalently attached open ring structures known as bilins that are the light-absorbing pigments. Primary photochemistry, electron transport, phosphorylation, and carbon reduction occur much as they do in chloroplasts. Cyanobacteria have a simpler genetic system than plants and algae, which enables them to be easily modified genetically. Because of this, cyanobacteria have been used as a model to understand

photosynthesis in plants. By genetically altering photosynthetic proteins, researchers can investigate the relationship between molecular structure and function.

6.2 Prochlorophytes

Over the past three decades, several types of oxygenic bacteria known as prochlorophytes (or oxychlorobacteria) have been discovered that have light-harvesting protein complexes that contain chlorophyll *a* and *b* but do not contain phycobilisomes (Palenik and Haselkorn, 1992). Because prochlorophytes have chlorophyll *a*/*b* light-harvesting proteins like chloroplasts, they are being investigated as models for plant photosynthesis.

7. ANOXYGENIC PHOTOSYNTHESIS IN BACTERIA

Anoxygenic photosynthetic bacteria differ from oxygenic organisms in that each species has only one type of reaction center. In some photosynthetic bacteria, the reaction center is similar to photosystem II, and in others, it is similar to photosystem I. However, neither of these two types of bacterial reaction center is capable of extracting electrons from water, and so they do not evolve O_2. Many species can only survive in environments that have a low concentration of O_2. To provide electrons for the reduction of CO_2, anoxygenic photosynthetic bacteria must oxidize inorganic or organic molecules available in their environment. For example, the purple bacterium *Rhodobacter sphaeroides* can use succinate to reduce NAD^+ by a membrane-linked reverse electron transfer that is driven by a transmembrane electrochemical potential. Although many photosynthetic bacteria depend on Rubisco and the Calvin cycle for the reduction of CO_2, some are able to fix atmospheric CO_2 by other biochemical pathways.

Despite these differences, the general principles of energy transduction are the same in anoxygenic and oxygenic photosynthesis. Anoxygenic photosynthetic bacteria depend on bacteriochlorophyll, a family of molecules similar to chlorophyll that absorb strongly in the infrared between 700 and 1000 nm. The antenna system consists of bacteriochlorophyll and carotenoids that serve a reaction center where primary charge separation occurs. The electron carriers include quinones (e.g., ubiquinone, menaquinone) and the cytochrome *bc* complex, which is similar to the cytochrome *bf* complex of oxygenic photosynthetic apparatus. As in oxygenic photosynthesis, electron transfer is coupled to the generation of an electrochemical potential that drives phosphorylation by ATP synthase. The energy required for the reduction of CO_2 is provided by ATP and NADH, a molecule similar to NADPH.

7.1 Purple Bacteria

There are two divisions of photosynthetic purple bacteria: the nonsulfur purple bacteria (e.g., *Rhodobacter sphaeroides* and *Rhodospeudomonas viridis*) and the sulfur purple bacteria (e.g., *Chromatium vinosum*). Nonsulfur purple bacteria typically use an organic electron donor, such as succinate or malate, but they can also use hydrogen gas. The sulfur bacteria use an inorganic sulfur compound, such as hydrogen sulfide, as the electron donor. The only pathway for carbon fixation by purple bacteria is the Calvin cycle. Sulfur purple bacteria must fix CO_2 to live, whereas nonsulfur purple bacteria can grow aerobically in the dark by respiration on an organic carbon source.

The determination of the three-dimensional structures of the reaction center of the nonsulfur purple bacteria, *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides,* provides an unprecedented opportunity to understand the structure and function of photosynthetic reaction centers (Deisenhofer *et al.*, 1984, 1985; Feher *et al.*, 1989). The positions of the electron-transfer components in the reaction center of *Rhodobacter sphaeroides* are shown in Fig. 12 (Norris and van Brakel, 1986). The reaction center contains four bacteriochlorophyll and two bacteriopheophytin molecules. Two of the bacteriochlorophyll molecules form the primary donor (P870). At present, there is controversy over whether a bacteriochlorophyll molecule is an intermediate in electron transfer from the P870 to bacteriopheophytin. However, there is agreement that the remaining steps involve two quinone molecules (Q_A and Q_B) and that two turnovers of the reaction center result in the release of reduced quinone (QH_2)

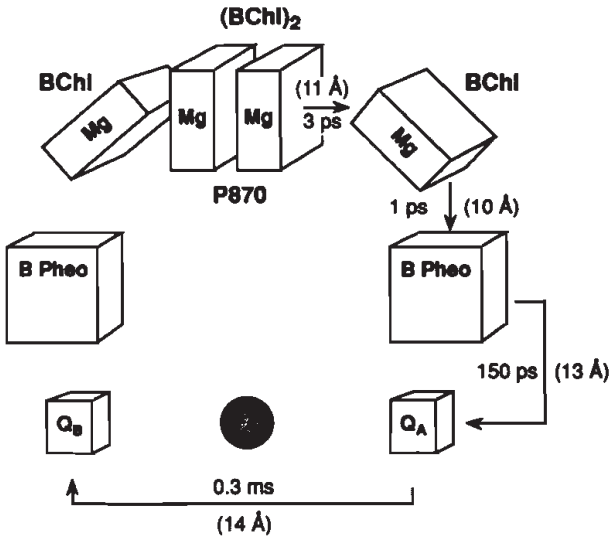


FIG. 12. Relative positions of the chromophores of the reaction center of *Rhodobacter sphaeroides* (from Norris and van Brakel, 1986). Abbreviations: P870, reaction center bacteriochlorophyll (primary electron donor); B Pheo, bacteriopheophytin; Q_A and Q_B, bound ubiquinone. Details are given in the text.

into the photosynthetic membrane. Although there is a nonheme Fe between the two quinone molecules, there is convincing evidence that this Fe is not involved directly in transferring an electron from Q_A to Q_B. Because the primary donor (P870), bacteriopheophytin, and quinone acceptors of the purple bacterial reaction center are similar to the photosystem II reaction center, the bacterial reaction center is used as a guide to understand the structure and function of photosystem II.

Light-driven electron transfer is cyclic in *Rhodobacter sphaeroides* and other purple bacteria (Fig. 13). The reaction center produces reduced quinone, which is oxidized by the cytochrome *bc* complex. Electrons from the cytochrome *bc* complex are transferred to a soluble electron carrier, cytochrome c_2, which reduces the oxidized primary donor $P870^+$. The product of the light-driven electron transfer reactions is ATP. The electrons for the reduction of carbon are extracted from an organic donor, such as succinate or malate, or from hydrogen gas, but not by the reaction center. The energy needed to reduce NAD^+ is provided by light-driven cyclic electron transport in the form of ATP. The energy transformation pathway is complicated. Succinate is oxidized by the membrane-bound enzyme succinate dehydrogenase that transfers the electrons to quinone—the source of electrons for the reduction of NAD^+. However, electron transfer from reduced quinone to NAD^+ is energetically uphill. By a mech-

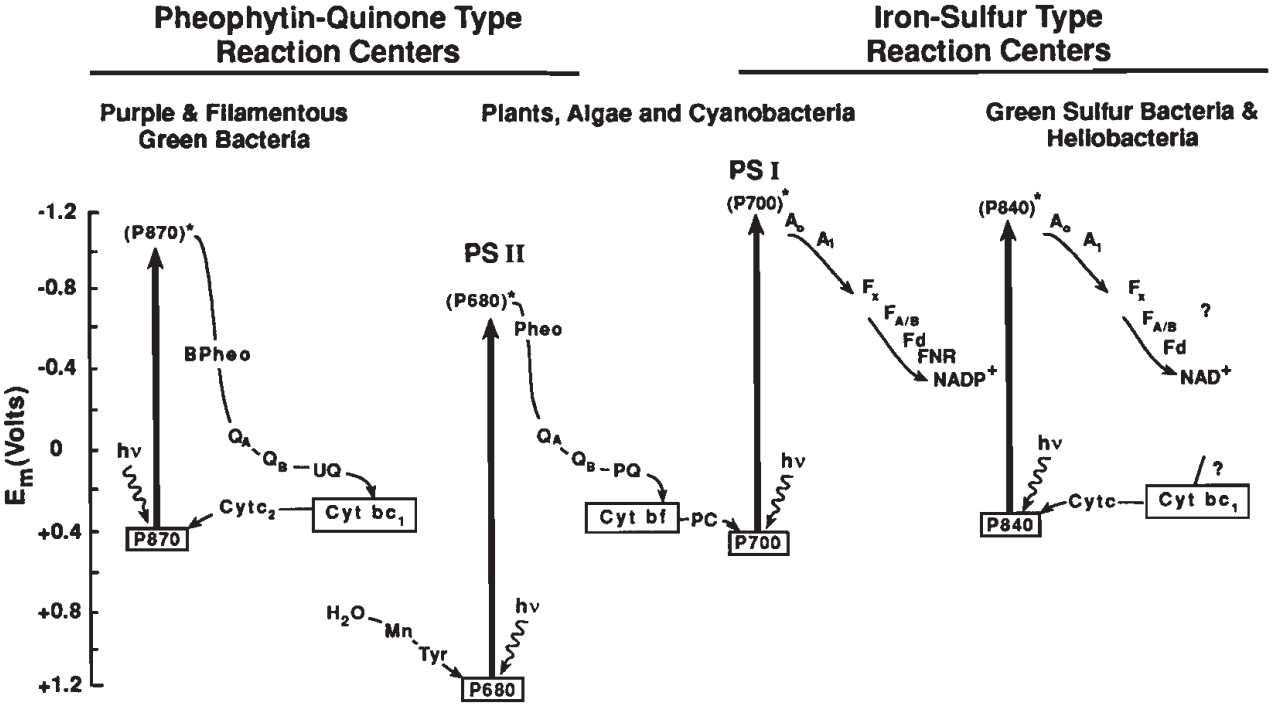


FIG. 13. Comparison of electron-transport pathways in oxygenic and anoxygenic organisms (from Blankenship, 1992). Abbreviations: Cyt bc_1, cytochrome *bc* complex; P840, reaction center bacteriochlorophyll; other abbreviations are given in the legends of Figs. 3 and 12.

anism that is poorly understood, a membrane-bound enzyme is able to use energy stored in the proton electrochemical potential to drive electrons from reduced quinone to NAD^+.

7.2 Green Sulfur Bacteria

Green sulfur bacteria (e.g., *Chlorobium thiosulfatophilum* and *Chlorobium vibrioforme)* can use sulfur compounds as the electron donor as well as organic hydrogen donors. As shown in Fig. 13, the reaction center of green sulfur bacteria is similar to the photosystem I reaction center of oxygenic organisms. The FeS centers in the reaction center can reduce NAD^+ (or $NADP^+$) by ferredoxin and the ferredoxin-$NAD(P)^+$ oxidoreductase enzyme; therefore green sulfur bacteria are not necessarily dependent on reverse electron flow for carbon reduction. The antenna system of the green sulfur bacteria is composed of bacteriochlorophyll and carotenoids and is contained in complexes known as chlorosomes that are attached to the surface of the photosynthetic membrane. This antenna arrangement is similar to the phycobilisomes of cyanobacteria. Green sulfur bacteria can fix CO_2 without Rubisco. It has been proposed that they accomplish this by using the respiratory chain that normally oxidizes carbon (known as the Krebs cycle), resulting in the release of CO_2. With the input of energy, this process can be run in the reverse direction, resulting in the uptake and reduction of CO_2.

7.3 Green Gliding Bacteria

Green gliding bacteria (e.g., *Chloroflexus aurantiacus*), also known as green filamentous bacteria, can grow photosynthetically under anaerobic conditions or in the dark by respiration under aerobic conditions. Like the green sulfur bacteria, green gliding bacteria harvest light using chlorosomes. The green gliding bacteria appear to have reaction centers similar to those of the purple bacteria (Fig. 13), but there are several notable differences. For example, instead of two monomer bacteriochlorophyll molecules, *C. aurantiacus* has one bacteriochlorophyll and one bacteriopheophytin, and the metal between the two quinones is Mn rather than Fe. *C. aurantiacus* appears to fix CO_2 by a scheme that does not involve the Calvin cycle or the reverse Krebs cycle (Ivanovsky *et al.*, 1993).

7.4 Heliobacteria

Heliobacteria (e.g., *Heliobacterium chlorum* and *Heliobacillus mobilis*) are in the phylum Gram Positive Bacteria that are strict anaerobes. Although the heliobacterial reaction center is similar to photosystem I in that it can reduce NAD^+ (or $NADP^+$), it contains a different type of chlorophyll known as bacteriochlorophyll *g*.

8. CONTROL OF INTRAPROTEIN ELECTRON TRANSFER

The three-dimensional structure of the reaction center of *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides* reveals the distances between the electron donors and acceptors (Deisenhofer *et al.*, 1984,1985; Norris and van Brakel, 1986; Feher *et al.*, 1989) and has had an important influence on biophysical and molecular genetics studies designed to identify the factors that control the rate of electron transfer within proteins. There is currently a controversy concerning the importance of the amino acid composition of the protein on the rate of intraprotein electron transfer. In part, the disagreement centers on whether the protein between the donor and acceptor molecules can be treated as a uniform material or whether the specific amino acid composition of the protein significantly alters the rate. For example, it has been proposed that aromatic amino acids may provide a particular pathway that facilitates electron transfer between a donor and acceptor pair. This is the case in the photosystem II reaction center, where a tyrosine residue on one of the reaction center core proteins donates an electron to the primary donor chlorophyll, $P680^+$. However, in other cases, replacement of an aromatic residue by another nonaromatic residue results in relatively minor changes in the rate of electron transfer. L. Dutton and co-workers (Moser *et al.*, 1992) have analyzed electron-transfer reactions in biological and chemical systems in terms of electron tunneling theory developed by R. Marcus and others (DeVault, 1984). Dutton and co-workers argue that protein provides a uniform electronic barrier to elec-

tron tunneling and a uniform nuclear characteristic frequency. They suggest that the specific amino acid residues between an electron transfer pair are generally of less importance than the distance in determining the rate of pairwise electron transfer. In their view, protein controls the rate of electron transfer mainly through the distance between the donor and acceptor molecules, the free energy, and the reorganization energy of the reaction. The importance of distance is demonstrated by electron-transfer data from biological and synthetic systems showing that the dependence of the electron transport rate on the edge-to-edge distance is exponential over 12 orders of magnitude when the free energy is optimized (Moser *et al.*, 1992). Increasing the distance between two carriers by 1.7 Å slows the rate of electron transfer tenfold. The extent to which this view is generally applicable for intraprotein electron transfer remains to be established (Williams, 1992). One of the challenges in understanding pairwise electron-transfer rates from first principles is illustrated by the reaction center of *Rhodobacter sphaeroides*, in which the redox components are arranged along a twofold axis of symmetry that extends from the primary donor (P870) to the Fe. Despite the fact that the reaction center presents two spatially similar pathways for electron transfer from P870 to quinone, nearly all electrons are transferred down the right arm of the reaction center, as shown in Fig. 12. The same holds true for the reaction center of *Rhodopseudomonas viridis*, in which it is estimated that electron transfer down the left arm is less than 1:100 (Kellogg *et al.*, 1989). The challenge to theorists is to explain the surprisingly high probability of electron flow down the right arm. Since the distances are similar, it has been suggested that electron transfer down the left arm is less probable because of an endothermic free-energy change (Parson *et al.*, 1990) or to an unfavorable rearrangement energy for the reaction (Moser *et al.*, 1992).

9. GLOBAL PHOTOSYNTHESIS AND THE ATMOSPHERE

The amount of CO_2 removed from the atmosphere each year by oxygenic photosynthetic organisms is massive. It is estimated that photosynthetic organisms remove 100×10^{15} g/yr of carbon (Houghton and Woodwell, 1989). This is equivalent to 4×10^{18} kJ of free energy stored in reduced carbon, which is roughly 0.1% of the incident visible radiant energy incident on the earth per year. Each year the photosynthetically reduced carbon is oxidized, either by living organisms for their survival or by combustion. The result is that more CO_2 is released into the atmosphere from the biota than is taken up by photosynthesis. The amount of carbon released by the biota is estimated to be $(1–2) \times 10^{15}$ g/yr of carbon. Added to this amount is carbon released by the burning of fossil fuels, which amounts to 5×10^{15} g/yr of carbon. The oceans mitigate this increase by acting as a sink for atmospheric CO_2. It is estimated that the oceans remove about 2×10^{15} g/yr of carbon from the atmosphere. This carbon is eventually stored on the ocean floor. Although these estimates of sources and sinks are uncertain, the net global CO_2 concentration is increasing. Direct measurements show that each year the atmospheric carbon content is currently increasing by about 3×10^{15} grams. Over the past 200 years, CO_2 in the atmosphere has increased from about 280 parts per million (ppm) to its current level of 360 ppm. On the basis of predicted fossil fuel use and land management, it is estimated that the amount of CO_2 in the atmosphere will reach 700 ppm within the next century. The consequences of this rapid change in our atmosphere are unknown. Because CO_2 acts as a greenhouse gas, some climate models predict that the temperature of the earth's atmosphere may increase by 2–8 °C. Such a large temperature increase would lead to significant changes in rainfall patterns. Little is known about the impact of such drastic atmospheric and climatic changes on plant communities and crops. Current research is directed at understanding the interaction between global climate change and photosynthetic organisms.

GLOSSARY

ATP: Adenosine triphosphate, a small water-soluble molecule that acts as an energy currency in cells.

ATP Synthase: A membrane-bound protein complex that uses the energy stored

across the photosynthetic membrane to add inorganic phosphate to ADP, thus creating ATP. (Also known as coupling factor.)

Calvin Cycle: The biochemical reactions, initiated by Rubisco, that result in the reduction of CO_2 to a carbohydrate (also known as the photosynthetic carbon reduction cycle).

Cytochrome: Heme-containing protein.

Cytochrome *bc* Complex: A membrane-bound electron-transfer protein complex, found in all anoxygenic photosynthetic organisms, that oxidizes reduced quinone and reduces a *c*-type cytochrome. The complex contains a *c*-type cytochrome, two *b*-type cytochromes, and an FeS center.

Cytochrome *bf* Complex: A membrane-bound electron-transfer protein complex, found in all oxygenic photosynthetic organisms, that oxidizes reduced plastoquinone and reduces plastocyanin (or cytochrome *c*). The complex contains a *c*-type cytochrome, two *b*-type cytochromes, and an FeS center.

Free Energy: The amount of energy in a reaction available to do work. Because most biochemical reactions occur at a constant temperature and pressure, the free energy is frequently the Gibbs energy.

Light-Harvesting Complex: A protein complex that harvests light energy and converts it to exciton energy that can migrate to a reaction center. The light is absorbed by pigment molecules (e.g., chlorophyll, bacteriochlorophyll, carotenoids, phycobilin) that are attached to the protein.

NADPH: Reduced form of nicotinamide adenine dinucleotide phosphate, a small water-soluble molecule that acts as a hydrogen carrier in biochemical reactions.

$NADP^+$: Oxidized form of nicotinamide adenine dinucleotide phosphate.

Oxidation: The removal of one or more electrons from an atom or molecule. In the case of a molecule, protons may be involved as well, resulting in hydrogen being removed.

Phosphorylation: The covalent attachment of a phosphate group to a molecule.

Photorespiration: The removal of O_2 from the atmosphere by Rubisco and the subsequent biochemical reactions that serve to recycle some of the reduced carbon.

Photosynthesis: The physical-chemical process by which certain chlorophyll- (or bacteriochlorophyll-) containing organisms use light energy for the biosynthesis of organic molecules.

Photosynthetic Membrane: A bilayer of lipid molecules in which are embedded proteins that transform light energy into chemical free energy. (Also known as the thylakoid membrane.)

Photosystem I: A protein complex located in the photosynthetic membrane. Photosystem I is one of two types of reaction centers found in higher plants, algae, and cyanobacteria. The photosystem I reaction center uses light energy to transfer an electron from a mobile electron-transfer protein (plastocyanin or cytochrome *c*) on one side of the photosynthetic membrane to a mobile electron-transfer protein (ferredoxin) on the opposite side of the photosynthetic membrane.

Photosystem II: A protein complex found in the photosynthetic membrane. Photosystem II is one of two types of reaction centers found in higher plants, algae, and cyanobacteria. The photosystem II reaction center uses light energy to transfer electrons from water to plastoquinone. Photosystem II is the source of the molecular oxygen in the atmosphere.

Plastoquinone: A small organic molecule involved in electron and proton transfer in photosynthesis.

Protein: A chemical structure composed of one or more polypeptides. In photosynthesis, proteins serve as the scaffolding that hold the cofactors that gather light energy, transfer electrons, and catalyze biochemical reactions.

Reaction Center: A protein complex that uses light energy to create a stable charge separation by transferring a single electron energetically uphill from a donor molecule to an acceptor molecule, both of which are located in the reaction center.

Reduction: The addition of one or more electrons to an atom or molecule. In the case of a molecule, protons may be involved as well, resulting in hydrogen being added.

Rubisco (D-ribulose 1,5-bisphosphate carboxylase/oxygenase): A water-soluble protein complex responsible for the removal of CO_2 from the atmosphere. The enzyme works by attaching CO_2 to a five-carbon compound (1,5 ribulose bisphosphate) that is split into two identical three-carbon compounds (phosphoglycerate). In addition to catalyzing the removal of CO_2 from the atmosphere, Rubisco also catalyzes the removal of O_2 from

the atmosphere (less efficiently). The removal of O_2 is thought to be a consequence of poor design and leads to a complex set of compensatory reactions known as photorespiration.

Works Cited

Blankenship, R. (1992), *Photosynth. Res.* **33,** 91–111.

Boyer, P. D. (1993), *Biochim. Biophys. Acta* **1140,** 215–250.

Debus, R. (1992), *Biochim. Biophys. Acta* **1102,** 269–352.

Deisenhofer, J., Epp, O., Miki, K., Huber, R., Michel, H. (1984), *J. Mol. Biol.* **180,** 385–398.

Deisenhofer, J., Epp, O., Miki, K., Huber, R., Michel, H. (1985), *Nature* **318,** 618–624.

DeVault, D. (1984), *Quantum Mechanical Tunnelling in Biological Systems,* Cambridge, U.K.: Cambridge University Press.

Feher, G., Allen, J. P., Okamura, M. Y., Rees, D. C. (1989), *Nature* **339,** 111–116.

Gest, H. (1993), *FEMS Microbiol. Lett.* **112,** 1–6.

Govindjee and Coleman, W. J. (1990), *Sci. Am.* **262** (2), 50–58.

Houghton, R. A., Woodwell, G. M. (1989), *Sci. Am.* **260** (4), 36–44.

Ivanovsky, R. N., Krasilnikova, E. N, Fal, Y. I. (1993), *Arch. Microbiol.* **159,** 257–264.

Kellogg, E. C., Kolaczkowski, S., Wassielewski, M. R., Tiede, D. M. (1989), *Photosynth. Res.* **22,** 47–59.

Klotz, I. M. (1967), *Energy Changes in Biochemical Reactions,* New York: Academic Press, Chap. 6.

Krauss, N., Hinrichs, W., Witt, I., Fromme, P. Pritzkow, W., Dauter, Z. Betzel, C., Wilson, K. S., Witt, H. T., Saenger, W. (1993), *Nature* **361,** 326–331.

Kühlbrandt, W., Wang, D. N., Fujiyoshi, Y. (1994), *Nature* **367,** 614–621.

Moser, C. C., Keske, J. M., Warncke, K., Farid, R. S., Dutton, P. L. (1992), *Nature* **355,** 796–802.

Norris, J. F., van Brakel, G. (1986), in: Govindjee, J. Amesz, D. C. Fork (Eds.), *Light Emission by Plants and Bacteria,* Orlando: Academic Press, pp. 35–36.

Odum, E. P. (1971), *Fundamentals of Ecology,* Philadelphia: W.B. Saunders Co.

Palenik, B., Haselkorn, R. (1992), *Nature* **355,** 265–267.

Parson, W. W., Chu, Z. T., Warshel, A. (1990), *Biochim. Biophys. Acta* **1017,** 251–272.

Staehelin, L. A. (1986), *Encyclopedia Plant Physiol.* **19,** 1–84.

Whitmarsh, J. (1986), *Encyclopedia Plant Physiol.* **19,** 508–527.

Williams, R. J. P. (1992), *Nature* **355,** 770.

Woese, C. R., Kandler, O., Wheeler, M. L. (1990), *Proc. Natl. Acad. Sci. USA* **87,** 4576–4579.

Wraight, C. (1982), in: Govindjee (Ed.), *Photosynthesis,* New York: Academic Press, pp. 17–61

Further Reading

Cramer, W. A., Knaff, D. B. (1991), *Energy Transduction in Biological Membranes,* New York: Springer-Verlag.

Barber, J. (Ed.) (1992), *The Photosystems: Structure, Function and Molecular Biology,* New York: Elsevier.

Huzisige, H., Ke, B. (1993), "Dynamics of the History of Photosynthesis," in: *Photosynthesis Res.* **38,** 185–209.

Lawlor, D. W. (1993), *Photosynthesis: Molecular, Physiological and Environmental Processes,* Essex, U.K.: Longman Scientific & Technical.

Taiz, L., Zeiger, E. (1991), *Plant Physiology,* Redwood City, CA: Benjamin/Cummings Publishing Co.

Walker, D. (1992), *Energy, Plants and Man,* East Sussex, U.K.: Oxygraphics Limited.

Photosystem II

Secondary article

John Whitmarsh, *University of Illinois, Urbana, Illinois, USA*

Govindjee, *University of Illinois, Urbana, Illinois, USA*

Photosystem II is a specialized protein complex that uses light energy to oxidize water, resulting in the release of molecular oxygen into the atmosphere, and to reduce plastoquinone, which is released into the hydrophobic core of the photosynthetic membrane. All oxygenic photosynthetic organisms, which include plants, algae and some bacteria, depend on photosystem II to extract electrons from water that are eventually used to reduce carbon dioxide in the carbon reduction cycle. The complex is composed of a central reaction centre in which electron transport occurs, and a peripheral antenna system which contains chlorophyll and other pigment molecules that absorb light.

Article Contents

Introduction

Photosynthetic organisms use light energy to produce organic molecules (Ort and Whitmarsh, 2001). In plants, algae and some types of bacteria, the photosynthetic process depends on photosystem II, a membrane-bound protein complex that removes electrons from water and transfers them to plastoquinone, a specialized organic molecule. Because the removal of electrons from water results in the release of molecular oxygen into the atmosphere, this photosystem II-dependent process is known as oxygenic photosynthesis. Photosystem II is the only protein complex known to oxidize water and release molecular oxygen. A more ancient form of photosynthesis occurs in some bacteria that are unable to oxidize water and therefore do not release oxygen. There is fossil evidence that photosystem II-containing organisms evolved about three billion years ago and that oxygenic photosynthesis converted the earth's atmosphere from a highly reducing anaerobic state to the oxygen-rich air surrounding us today (Des Marais, 2000). By releasing oxygen into the atmosphere, photosystem II enabled the evolution of cellular respiration and thus profoundly affected the diversity of life.

Oxygenic photosynthesis depends on two reaction centre protein complexes, photosystem II and photosystem I, which are linked by the cytochrome *bf* complex and small mobile electron carriers (Whitmarsh and Govindjee, 1999) (**Figure 1**). Photosystem II, the cytochrome *bf* complex and photosystem I are embedded in the thylakoid membrane and operate in series to transfer electrons from water to nicotinamide–adenine dinucleotide phosphate ($NADP^+$). The energy necessary to move electrons from water to $NADP^+$ is provided by light, which is captured by the photosystem II and photosystem I antenna systems. In plants and algae the thylakoid membranes are located inside chloroplasts, which are subcellular organelles. In oxygenic bacteria the thylakoids are located inside the plasma membrane.

Chloroplasts originated from photosynthetic bacteria invading a nonphotosynthetic cell. In both chloroplasts and bacteria, thylakoid membranes form vesicles that define an inner and outer aqueous space. Light-driven electron transfer through the photosystem II and photosystem I reaction centres provides the energy for creating a proton electrochemical potential across the thylakoid membrane. The energy stored in the proton electrochemical gradient drives a membrane-bound adenosine triphosphate (ATP) synthase that produces ATP. Overall, the light-driven electron transport reactions, occurring through the thylakoid membrane, provide NADPH and ATP for the production of carbohydrates, the final product of oxygenic photosynthesis.

Photosystem II uses light energy to drive two chemical reactions: the oxidation of water and the reduction of plastoquinone (Govindjee and Coleman, 1990; Nugent, 2001). The primary photochemical reaction of photosystem II results in separating a positive and a negative charge within the reaction centre and is governed by Einstein's law of photochemistry: one absorbed photon drives the transfer of one electron. This means that four photochemical reactions are needed to remove four electrons from water, which results in the release of one molecule of dioxygen and four protons, and in the reduction of two molecules of plastoquinone:

$$2H_2O + 2PQ + 4H^+ + (4h\nu) \rightarrow O_2 + 2PQH_2 + 4H^+$$

This chapter focuses on the composition, structure and operation of photosystem II. For brevity we describe what we know without explaining the experimental results that underlie our knowledge. The references given at the end of

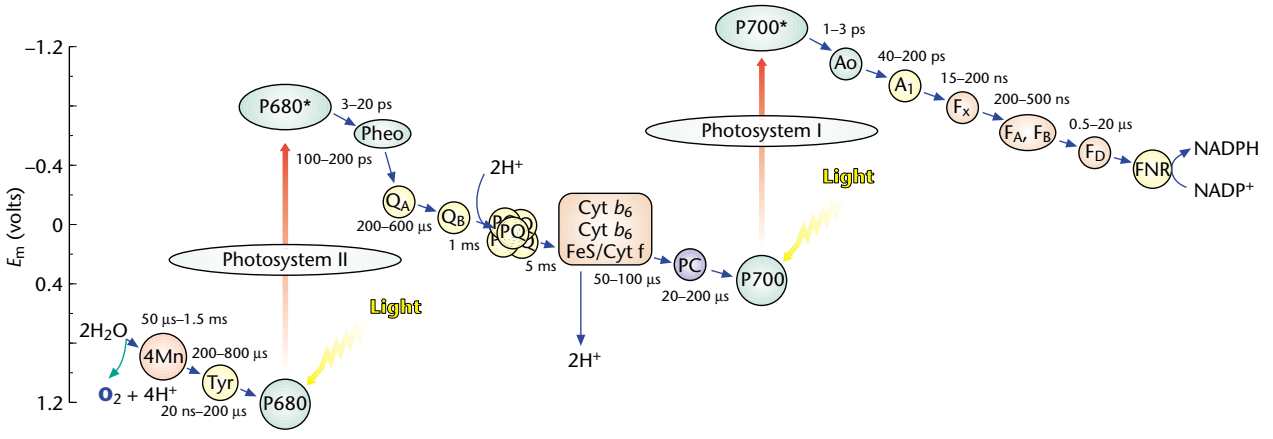


Figure 1 The Z scheme showing the pathway of oxygenic photosynthetic electron transport. The vertical scale shows the equilibrium midpoint potential (E_m) of the electron transport components. Approximate electron transfer times are shown for several reactions. Mn, tetranuclear manganese cluster; Tyr tyrosine-161 on D1 protein (Y_z); P680, reaction centre chlorophyll *a* of photosystem II; P680*, excited electronic state of P680; Pheo, pheophytin; Q_A, a bound plastoquinone; Q_B, a plastoquinone that binds and unbinds from photosystem II; PQ, a pool of mobile plastoquinone molecules; the brown box is a protein complex containing cytochrome b_6 (Cyt b_6), iron–sulfur protein (FeS) and cytochrome *f* (Cyt *f*); PC, plastocyanin; P700, reaction centre chlorophyll *a* of photosystem I; P700*, excited electronic state of P700; Ao, a special chlorophyll *a* molecule; A_1, vitamin K; F_X, F_A, F_B, iron sulfur centres; F_D, ferredoxin; FNR, ferredoxin–NADP reductase; $NADP^+$, nicotinamide–adenine dinucleotide phosphate. For details see Whitmarsh and Govindjee (1999).

the article are an entryway into the vast literature on photosystem II that extends back more than half a century.

Organization, Composition and Structure

Photosystem II is embedded in the thylakoid membrane, with the oxygen-evolving site near the inner aqueous phase and the plastoquinone reduction site near the outer aqueous phase (**Figure 2**), an orientation that enables the oxidation–reduction chemistry of the reaction centre to contribute to the proton electrochemical gradient across the thylakoid membrane. In chloroplasts, the photosystem II and photosystem I complexes are distributed in different regions of the thylakoid membrane. Most of the photosystem II complexes are located in the stacked membranes (grana), whereas the photosystem I complexes are located in the stomal membranes. It is not clear why two reaction centres that operate in series are spatially separated in eukaryotic organisms. In prokaryotes, the thylakoid membranes do not form grana and the photosystem II and I complexes appear to be intermixed. In eukaryotes the photosystem II complex is densely packed in the thylakoid membrane, with average centre to centre distances of a few hundred angstroms. One square centimetre of a typical leaf contains about 30 trillion photosystem II complexes.

Although photosystem II is found in prokaryotic cyanobacteria (e.g. *Synechocystis* spp.) and prochlorophytes (e.g. *Prochloron* spp.), in eukaryotic green algae (e.g. *Chlamydomonas* spp.), red algae (e.g. *Porphyridium* spp.), yellow-green algae (e.g. *Vaucheria*) and brown algae

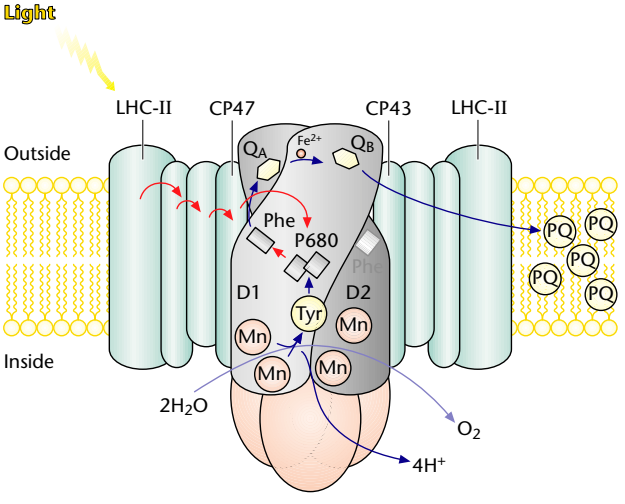


Figure 2 Schematic drawing of the photosystem II antenna system and reaction centre in the thylakoid membrane. D1 and D2 are the core proteins of photosystem II reaction centre. Photosystem II uses light energy to remove electrons from water, resulting in the release of oxygen and protons. The electrons from water are transferred via redox cofactors in the protein complex to form reduced plastoquinone. $(Mn)_4$, manganese cluster involved in removing electrons from water; P680, reaction centre chlorophyll of photosystem II; Pheo, pheophytin; Q_A, a bound plastoquinone; Q_B, plastoquinone that binds and unbinds from photosystem II; Tyr, a tyrosine residue in photosystem II (Y_z); PQ, a pool of mobile plastoquinone molecules. The antenna complexes are: LHC-II, peripheral light-harvesting complex of photosystem II; CP47 and CP43, chlorophyll–protein complexes of 47 and 43 kDa, respectively.

(e.g. *Fucus* spp.) and all higher plants (e.g. *Arabidopsis* spp.), extensive research indicates that its structure and function are remarkably similar in these diverse organisms.

Photosystem II is arranged as a central reaction centre core surrounded by a light-harvesting antenna system. The reaction centre is the site of primary charge separation and of the subsequent electron transfer reactions that oxidize water and reduce plastoquinone. The antenna system consists of protein complexes that contain light-harvesting molecules (chlorophyll and other accessory pigments) which serve to capture light energy and deliver it to the reaction centre.

In eukaryotic organisms the light-harvesting protein complexes are organized into an inner antenna system located close to the reaction centre and a peripheral antenna system composed of pigment proteins known as light-harvesting complex II (LHC-II). Excluding the peripheral light-harvesting complexes, the reaction centre complex contains more than 20 different polypeptides. A list of the genes encoding the known polypeptides, together with the molecular weights and putative functional roles of the proteins, is shown in **Table 1**. Most of the photosystem II polypeptides are membrane-bound integral proteins. The exceptions are a few peripheral membrane proteins that are located in the lumen.

Photosystem II contains at least nine different redox components (chlorophyll, pheophytin, plastoquinone, tyrosine, manganese, iron, cytochrome *b*559, carotenoid and histidine) which have been shown to undergo light-induced electron transfer. However, only five of these redox components are known to be involved in transferring electrons from H_2O to the plastoquinone pool: the water-oxidizing manganese cluster $(Mn)_4$, the amino acid tyrosine (Y_Z), the reaction centre chlorophyll (P680), pheophytin and two plastoquinone molecules, Q_A and Q_B (**Figures 1** and **2**). At the heart of photosystem II is a heterodimer protein complex composed of the D1 and D2 polypeptides; this complex coordinates the key redox components of photosystem II (including P680 plus four additional chlorophyll molecules, two pheophytin molecules, Q_A and Q_B) and provides ligands for the $(Mn)_4$ cluster. In addition to these components, the photosystem II reaction centre core together with the inner antenna proteins (CP43 and CP47) binds about 45 molecules of chlorophyll *a*, five or six carotenoids, one nonhaem iron, one calcium, one or more chloride, and one or two bicarbonate ions. Cyanobacteria have an additional redox component, cytochrome *c*550, located on the lumenal side of photosystem II. All photosystem II complexes contain cytochrome *b*559, which is a *b*-haem composed of two polypeptides. Despite numerous studies, there is disagreement in the literature concerning whether there is one or two *b*-haems in each photosystem II reaction centre (Whitmarsh and Pakrasi, 1996).

After decades of effort, the inner core of photosystem II has finally been crystallized and its three-dimensional structure determined to 3.8-Å resolution by Witt, Saenger and coworkers (Zouni *et al*., 2001) (**Figure 3**). While this resolution does not provide the position of most of the atoms that make up the reaction centre, an atomic resolution of photosystem II should be available in the near future. Current research is guided by photosystem II structural models that were developed using the atomic structure of the reaction centre in purple bacteria, together with biochemical and spectroscopic data (**Figure 4a**) (e.g. Xiong *et al*., 1998). These homology-based models all suffer from the fact that reaction centres of purple bacteria do not oxidize water.

As revealed by Zouni *et al*. (2001), the photosystem II reaction centre core is 100 Å across (in the plane of the membrane) and extends about 10 Å into the stromal aqueous phase and about 55 Å into the lumen. At the centre of the reaction centre are the D1 and D2 polypeptides, which combine to provide the scaffolding for the electron carriers, locating them at precise distances from one another (**Figure 4b**). The rate of electron transfer from one redox site to another is controlled by several factors, including the distance between the components (Moser *et al*., 1992). In addition to positioning the electron carriers, the photosystem II polypeptides provide multiple pathways for proton transfer from the outer water phase to the Q_B site. Note that the D1 and D2 polypeptides form a symmetrical central structure which appears to provide two potential electron transport pathways through the reaction centrety. However, as shown in **Figure 2**, only one pathway is active.

Light Capture: The Antenna System

Oxygenic photosynthesis is driven mainly by visible light (wavelength from 400 to 700 nm), which is absorbed by chlorophyll and other pigments (e.g. carotenoids) that are anchored in the thylakoid membrane to the light-harvesting proteins (**Table 2**). Chlorophyll absorbs light mainly in the blue and red regions of the spectrum due to a cyclic tetrapyrrole in which the nitrogens of the pyrroles are coordinated to a central magnesium ion. Plants (and many types of algae) contain two types of chlorophyll, *a* and *b*, which differ by a single group on one of the pyrrole rings. Typically about 200–250 chlorophyll and 40–60 carotenoid molecules serve a single reaction centre. Carotenoids are linear polyenes that serve as accessory pigments in the antenna system, absorbing light in the blue and green spectral region. In addition, carotenoids protect the photosynthetic apparatus from damage caused by excess light in a process known as downregulation (see below), as well as quenching relatively stable, excited states of chlorophyll known as triplet states which lead to oxidative damage of components in the thylakoid membrane (Frank *et al*., 1999).

The light-harvesting protein complexes (LHC-II complexes) are made up of a family of related proteins that bind chlorophyll *a*, chlorophyll *b* and carotenoids. The structure

Table 1 Photosystem II genes, proteins and putative roles (excluding antenna light-harvesting complex II)[a]

Gene[b]	Protein	Mass (kDa)[c]	Integral or peripheral[d]	Comments
*psb*A (c)	D1	39	I (5)	D1 (and D2) form the reaction centre core that binds most of the PS-II electron transport components; Q_B binds to D1
*psb*B (c)	CP47	56	I (6)	Binds antenna chlorophyll *a*
*psb*C (c)	CP43	47	I (6)	Binds antenna chlorophyll *a*
*psb*D (c)	D2	39	I (5)	D2 (and D1) form the reaction centre core that binds most of the PS-II electron transport components; Q_A binds to D2
*psb*E (c)	α subunit Cyt *b*559	9.3	I (1)	Binds *b*-haem; involved in photoprotection
*psb*F (c)	β subunit Cyt *b*559	4.5	I (1)	Binds *b*-haem; may be involved in photoprotection
*psb*H (c)	PsbH	7.8	I (1)	Unknown function
*psb*I (c)	PsbI	4.2	I (1)	Unknown function
*psb*J (c)	PsbJ	4.2	I (0)	Unknown function
*psb*K (c)	PsbK	4.3	I (1)	Unknown function
*psb*L (c)	PsbL	4.5	I (0)	Unknown function
*psb*M (c)	PsbM	4	I	Unknown function
*psb*N (c)	PsbN	4.7	I (1)	Unknown function
*psb*O (n)	PsbO (MSP)	27	P (0)	Involved in regulating oxygen evolution
*psb*P (n)	PsbP	20	P (0)	Involved in oxygen evolution; eukaryote specific
*psb*Q (n)	PsbQ	17	P (0)	Involved in oxygen evolution; eukaryote specific
*psb*R (n)	PsbR	10	I (0 or 1)	Unknown function; eukaryote specific
*psb*S (n)	PsbS	22	I (4)	Binds chlorophyll; involved in downregulation
*psb*T (c)	PsbT	3.8	P (1)	Unknown function; eukaryote specific
*psb*U	PsbU	10	P	Unknown function; prokaryote specific
*psb*V	Cyt *c*550	12	P (0)	Binds *c*-haem; prokaryote specific
*psb*W (n)	PsbW	6	I (1)	Involved in PS-II dimerization; eukaryote specific
*psb*X (c)	PsbX	4	I (1)	Unknown function
*psb*Y (c)	PsbY	3	I	Unknown function
*psb*Z (c)	PsbZ	11	I	Unknown function

[a]The authors thank Drs H.P. Pakrasi and K.-H. Rhee (Rhee, 2001) for information contributing to this table.
[b]For eukaryotic organisms, the letter in parentheses indicates whether nuclear (n) or chloroplast (c) gene is encoded.
[c]Mass calculated from amino acid sequence.
[d]Number of α helices is given in parentheses.
I, integral; P, peripheral; Cyt, cytochrome; PS, photosystem; MSP, manganese-stabilizing protein.

of one of the LHC-II complexes has been determined by electron crystallography (Kühlbrandt *et al*., 1994). The complex forms a trimer in the membranes, with each subunit binding seven molecules of chlorophyll *a*, five molecules of chlorophyll *b* and two carotene molecules.

Photosynthesis starts with the absorption of a photon by an antenna molecule, which causes a rapid (10^{-15} s) transition from the electronic ground state to an excited state. Within 10^{-13} s, the electronic excited state decays by vibrational relaxation to the first excited singlet state. These excited electron states are short lived, and the fate of the excitation energy in the antenna system is guided by the structure of the protein–pigment complexes. Because of the proximity of other antenna molecules with the same or similar electron energy levels, the excited state energy has a

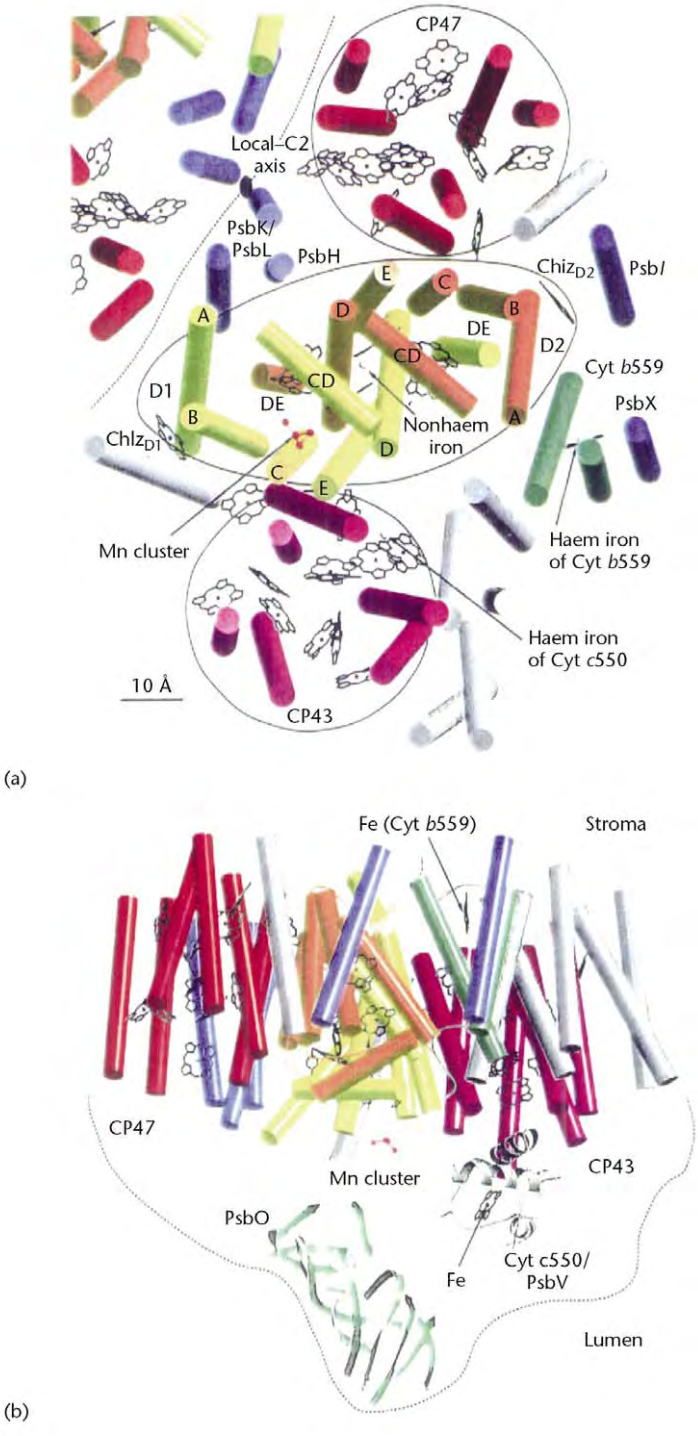


Figure 3 Organization of *Synechococcus elongatus* photosystem II α helices determined by X-ray crystallography. (a) Viewed from above the thylakoid membrane. (b) Viewed in the plane of the membrane. Abbreviations are as in **Figure 1** and **Table 1**. From Zouni *et al.* (2001); reproduced with permission from H. T. Witt.

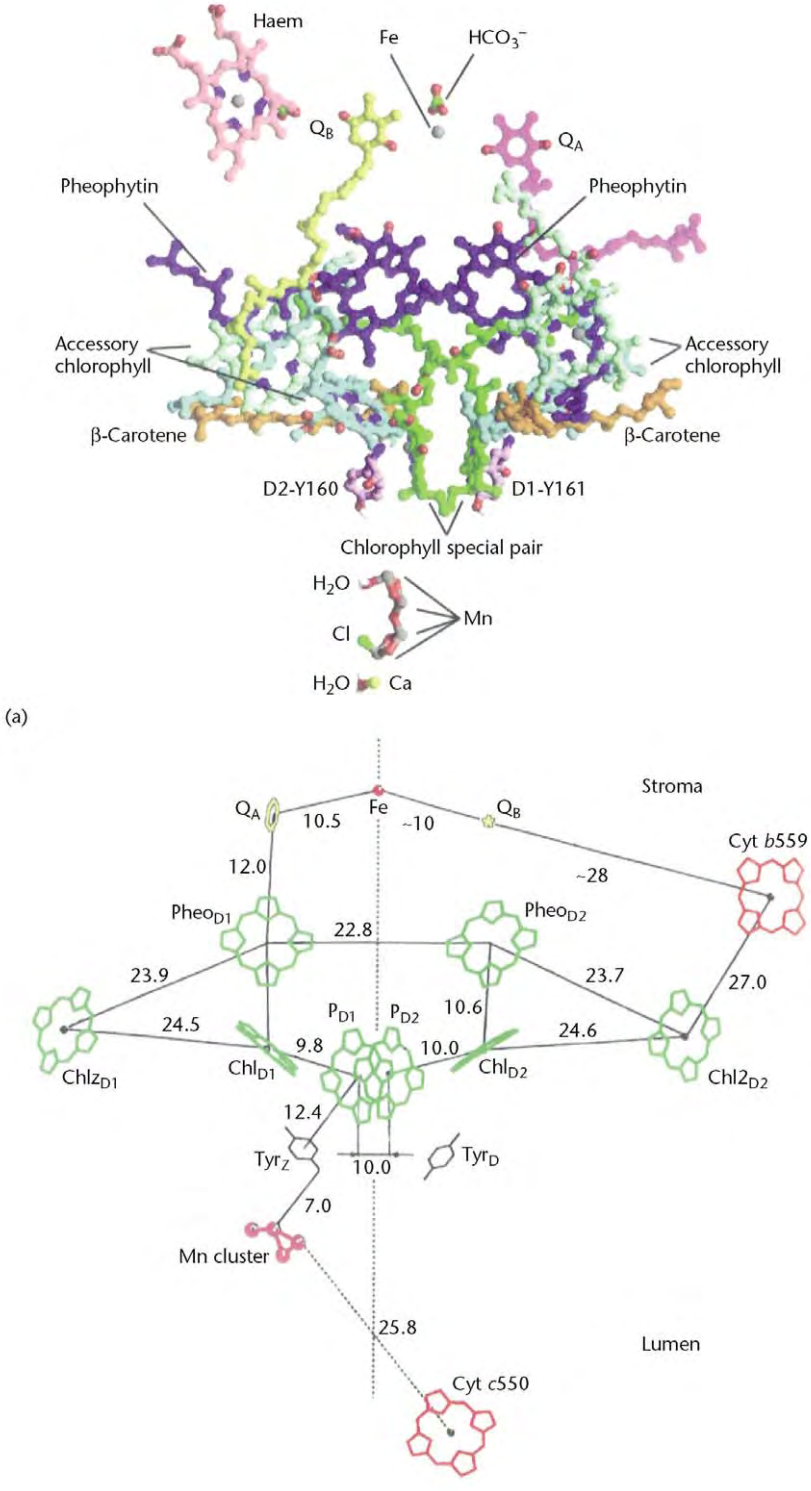


Figure 4 Organization of photosystem II cofactors. (a) Determined by modelling. Abbreviations are as in **Figure 1**, except D1-Y161 is Tyr (Y_Z) and D2-Y160 is a tyrosine in D2 (Y_D). From Xiong *et al.* (1998). (b) Organization of *Synechococcus elongatus* photosystem II cofactors determined by X-ray crystallography. The numbers represent centre to centre distances in angstroms. From Zouni *et al.* (2001); reproduced with permission from H. T. Witt.

Table 2 Distribution of chlorophyll in photosystem II

Protein	No. of molecules
Reaction centre proteins (D1/D2)	6 Chl *a*
Inner antenna proteins	
CP47	13 Chl *a*
CP43	13 Chl *a*
CP24 + CP26 + CP29	21 Chl *a* + 6 Chl *b*
Outer antenna proteins	
One tightly bound + one medium-bound LH-IIb trimer	42 Chl *a* + 30 Chl *b*
Loosely bound LH-IIb + other LHs	Approx. 100 Chl (*a* + *b*)
Photosystem II (reaction centre + antenna system)	Approx. 230 Chl (*a* + *b*)

Chl, chlorophyll; CP, chlorophyll-binding protein; LH, light-harvesting complex.

high probability of being transferred to a neighbouring molecule by a process known as Förster resonance energy transfer (Lakowicz, 1999).

The transfer of excitation energy between chlorophyll molecules is due to interaction between the transition dipole moments of the donor and acceptor molecules. The probability of transfer falls off quickly as the distance between the pigments increases (the rate is proportional to R^{-6}, where R is the distance between the transition dipoles), and depends strongly on the overlap of the emission spectrum of the donor molecule and the absorption spectrum of the acceptor molecule, as well as the relative orientation of the donor and acceptor chromophores.

As shown in **Figure 5**, resonance energy transfer enables excitation energy to migrate over the antenna system. Because the first excited singlet state of chlorophyll *a* is lower than that of chlorophyll *b* or the carotenoids, excitation energy is rapidly localized on the chlorophyll *a* molecules. As a consequence, the energy that escapes the antenna system as fluorescence comes almost entirely from chlorophyll *a*.

During the migration process, the excitation energy is either trapped by a reaction centre, converted into heat or released as photons. Photosynthetic antenna systems are designed to be very efficient at getting the excited state energy to a reaction centre. Measurements of photosynthesis at low light intensities show that over 90% of absorbed photons can be trapped by a reaction centre and promote primary charge separation. However, environmental conditions may impose limitations on photosynthesis that significantly slow the rate of electron transport, which greatly increases the fraction of absorbed light energy that goes into heat and fluorescence. Measurements of chlorophyll fluorescence provide a noninvasive method for monitoring photosynthetic performance *in vivo* (see below).

Primary Photochemistry: The Reaction Centre

The primary photochemical reaction of photosystem II depends on electron transfer from P680 to pheophytin (Pheo), creating the charge separated state: $P680^+/Pheo^-$ (Dekker and van Grondelle, 2000). This primary photochemical reaction differs from subsequent electron transfer reactions in that the equilibrium redox midpoint potential of the primary donor (P680) is lower in energy than that of the primary electron acceptor (Pheo). As a consequence, electron transfer from P680 to Pheo can occur only if P680 is in an excited electronic state (denoted by $P680^*$), which is created either by excitation energy from the antenna system or by direct absorption of a photon by the reaction centre.

Subsequent electron transfer steps prevent the primary charge separation from recombining by transferring the electron within 200 ps from $Pheo^-$ to Q_A (**Figures 1**, **2** and **4**). From Q_A^-, the electron is transferred to another plastoquinone molecule bound at the Q_B site. After two photochemical turnovers, Q_B becomes fully reduced (PQH_2), after which it unbinds from photosystem II and is released into the thylakoid membrane. While the electron removed from P680 is rapidly sent away, an electron from a tyrosine residue (Y_Z) on the D1 polypeptide reduces $P680^+$. Electrons for the reduction of Y_Z are extracted from the water-oxidizing complex, which includes the $(Mn)_4$ cluster. The rate of electron transfer from Y_Z to $P680^+$ ranges from 20 ns to 200 μs, depending on the redox state of components involved in water oxidation.

Although photochemistry in photosystem II leads to charge separation between P680 and Pheo, the steps leading to this relatively stable state are complicated and not well understood. As shown in **Figure 4b**, P680 is

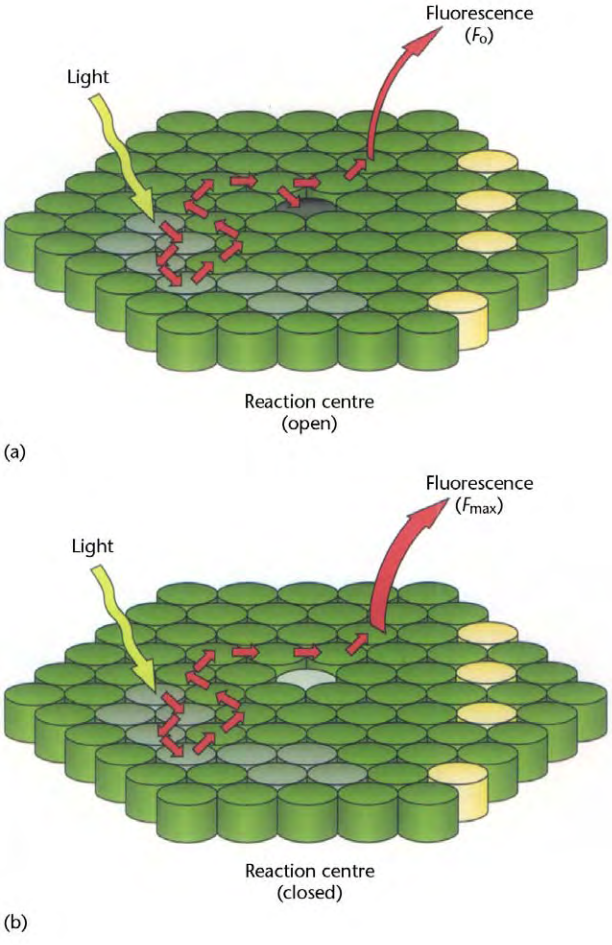


Figure 5 Schematic drawing showing excitation energy transfer from one chlorophyll molecule to another in the antenna system. Green cylinders represent chlorophylls *a* and *b*, and yellow cylinders represent carotenoids; the dark cylinder in (a) represents an open reaction centre and the grey cylinder in (b) represents a closed reaction centre.

surrounded by four chlorophyll and two pheophytin molecules. Owing to the close proximity of these molecules, and the fact that the electronic energy levels of the six chlorophyll molecules are quite similar, excitation energy within the reaction centre equilibrates between the chlorophyll and pheophytin molecules before charge separation. The result appears to be that charge separation can occur between different chromophores in the reaction centre (Dekker and van Grondelle, 2000). Despite uncertainty in the early events of the photochemical reaction, ultrafast spectroscopic measurements indicate that $P680^+/Pheo^-$ is created within 8 ps (Greenfield *et al.*, 1997).

Oxidation of Water: The Source of Molecular Oxygen

In 1969, Pierre Joliot and coworkers published a paper in which they measured the amount of oxygen during successive singe-turnover light flashes (Joliot *et al.*, 1969). Using algae that were adapted to darkness, they found that the yield of oxygen plotted as a function of the flash number exhibited a periodicity of 4 (**Figure 6a**). This is a classical experiment because it demonstrated that each photosystem II complex operates independently, and that four photochemical reactions are required for the release of one molecule of oxygen (Joliot and Kok, 1975). The periodicity of 4 was readily explained by the chemistry of water oxidation, but the observation that the maximum oxygen yield occurred on the third rather than the fourth flash, and that the modulation disappeared after several cycles, revealed an unexpected level of complexity in the mechanism of water oxidation.

Based on the results of the Joliot *et al.* experiments, Kok and coworkers (1970) developed an elegant model of water oxidation by photosystem II. In this model, the oxygen-evolving complex of the reaction centre can exist in five states, labelled S_0, S_1, S_2, S_3 and S_4 (**Figure 6b**). A photochemical reaction, which removes one electron, advances the oxygen-evolving complex to the next higher S state. The result is the creation of four oxidizing equivalents in the water-oxidizing complex. Substrate water is bound in separate sites in the complex through the S_3 state and does not involve the formation of symmetrical peroxo intermediates (Hillier and Wydrzyndski, 2000). Then, either through sequential steps or through a concerted reaction in the S_3–S_4–S_0 transition, the oxidation of water results. The net reaction results in the release of one oxygen molecule, the release of four protons into the lumen, and the sequential transfer of four electrons through the reaction centre to the plastoquinone pool.

To account for the maximum oxygen yield on the third flash, Kok *et al.* (1970) suggested that in the dark-adapted algae most of the oxygen-evolving complexes are in the S_1 state, rather than S_0. Thus, after three flashes the S_4 state is reached and oxygen is released. Noting that some oxygen was released on the fourth flash, the Kok model assumes that in the dark-adapted sample the ratio of $S_1 : S_0$ is 3 : 1. To explain the loss of periodicity as the flash number increased, the model assumes that in some photosystem II complexes the light flash fails to advance the S state (misses), while in others the light flash promotes an advance of two states (double hits). This remarkably insightful model successfully explained the flash dependence of oxygen evolution, and continues to guide research into the mechanism of water oxidation and oxygen release by photosystem II.

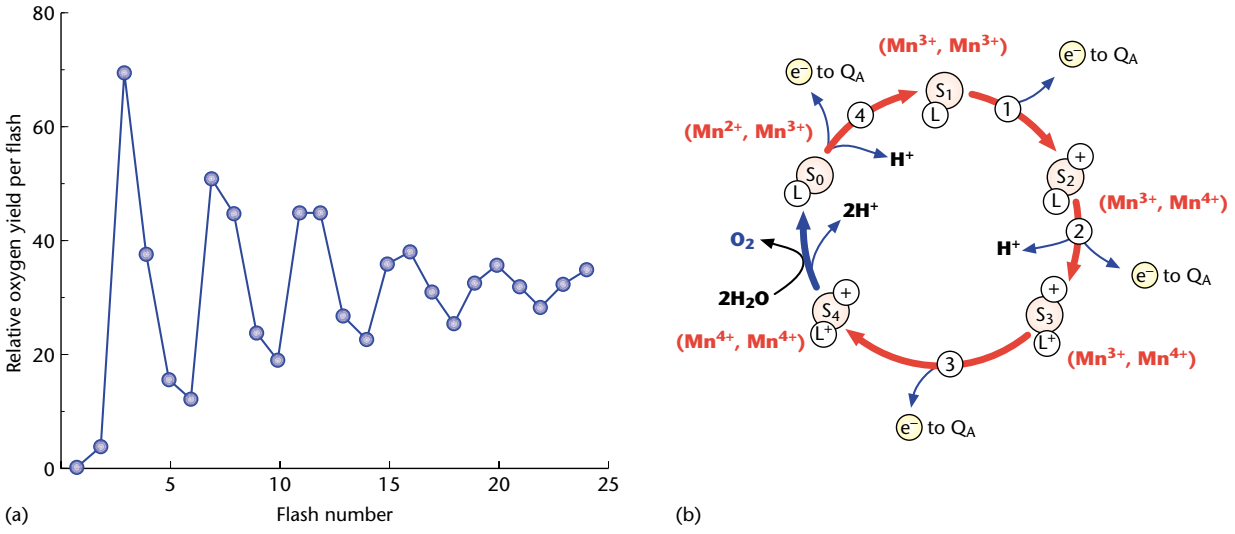


Figure 6 The oxygen cycle. (a) Oxygen yield from photosystem II as a function of flash number (oxygen cycle) (see Joliot and Kok, 1975). (b) One of the current models of the steps in oxygen evolution in photosystem II. See text or Joliot and Kok (1975) for details.

Oxygen evolution occurs on the lumenal side of the D1 and D2 proteins, and involves four manganese ions, several extrinsic polypeptides, including a 33-kDa protein known as the manganese-stabilizing protein (MSP), a 23-kDa protein (PsbP) and a 17-kDa protein (PsbQ), as well as calcium, chloride and bicarbonate ions (van Rensen *et al.*, 1999). In addition to these components, numerous studies have shown a dependence of oxygen evolution on cytochrome *b*559 and CP47. In cyanobacteria the 23-and 17-kDa polypeptides are absent, while a *c*-type cytochrome (cytochrome *c*550) and a 10-kDa polypeptide are present on the luminal side of the reaction centre.

A cluster of four manganese ions lies at the heart of the oxygen-evolving site of photosystem II (**Figure 4b**). Although it appears that all four manganese ions are needed for efficient oxygen evolution, there is a suggestion that only two of them undergo redox changes associated with water oxidation. Extended X-ray absorption fine-structure analysis indicates that in the transition from the S_2 to S_3 state manganese does not change oxidation state, leading to the suggestion that a redox active ligand is oxidized during this transition ('L' in **Figure 6b**). There is evidence that the ligand may be a histidine on the D1 polypeptide. Proton release during water oxidation has been measured by monitoring pH changes in the lumen. As shown in **Figure 6b**, protons are released during the advancement of the S states in a 1 : 0 : 1 : 2 pattern during the S_0–S_1, S_1–S_2, S_2–S_3 and S_3–(S_4)–S_0 transitions, respectively (Fowler, 1977). The observation that the protons released into the lumen appear to come from amino acids near the water oxidation site indicates that this pattern may not reveal proton release associated with the catalytic steps involved in water oxidation.

Although Cl^- and Ca^{2+} are needed for water oxidation, their role is not known. One possibility is that the negative charge of the Cl^- ions serves to stabilize the water-oxidizing complex during the accumulation of positive charge. Based on the close proximity of Cl^- and Ca^{2+} to the Mn cluster, both ions have been proposed to stabilize the $(Mn)_4$ cluster for efficient water oxidation. Ca^{2+} may play a role in gating the access of water to the catalytic site. In addition, bicarbonate has been recently shown to play an important role on the water oxidation process, although the molecular mechanism remains unknown (Klimov and Baranov, 2001).

Reduction of Plastoquinone: The Two-electron Gate

Plastoquinone plays a key role in photosynthesis by linking electron transport to proton transfer across the photosynthetic membrane. In the photosystem II complex, two plastoquinone molecules work in tandem, with one molecule permanently bound at the Q_A site and another molecule bound at the Q_B site. Once plastoquinone at the Q_B site has been fully reduced by the addition of two electrons and two protons, it unbinds from the reaction centre and is released into the thylakoid membrane. The reduction of plastoquinone at the Q_B site is known as the two-electron gate, because two photochemical reactions are needed for the formation and release of plastoquinol (Velthuys and Amesz, 1974) (**Figure 7a**). Numerous compounds have been discovered that inhibit photosynthetic electron transport by binding at or near the Q_B site, thereby preventing access of plastoquinone to the site

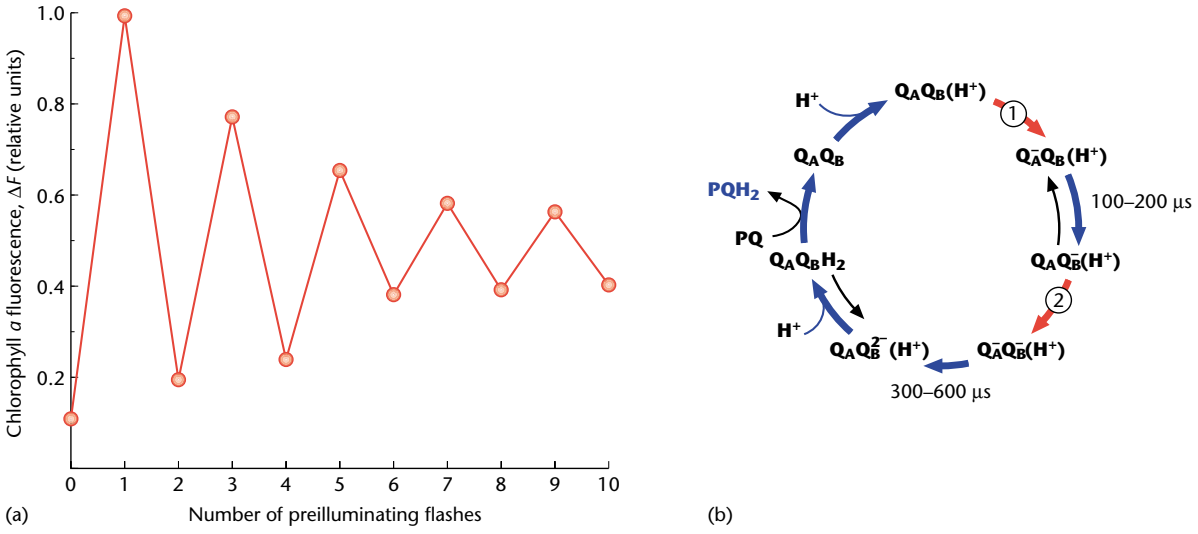


Figure 7 The two-electron gate. (a) Chlorophyll *a* fluorescence from photosystem II as a function of flash number showing the two-flash dependence. (b) Steps in the two-electron reduction of plastoquinone at the Q_B site of photosystem II. See text for details.

(Wraight, 1981). A few of these compounds (e.g. atrazine) are used as commercial herbicides.

The pathway of electrons from P680 to Q_B is shown in **Figures 1** and **2**. The reduction of plastoquinone requires two photochemical reactions. In the first reaction an electron is transferred from Q_A^- to Q_B within 100–200 μs, producing the state Q_A/Q_B^- (**Figure 7b**). In the second reaction an electron is transferred from Q_A^- to Q_B^- within 400–600 μs, producing the state Q_A/Q_B^{2-}, which rapidly takes up protons, producing PQH_2. Protons involved in the reduction of plastoquinone come from the outer water phase and are delivered by branched pathways through the protein that include amino acids near the Q_B site. There is evidence that bicarbonate ions also play a role by binding near the Q_B site. Reduced plastoquinone unbinds from the Q_B site and enters the hydrophobic core of the membrane, which allows an oxidized plastoquinone molecule to bind to the site so that the cycle can be repeated.

Photosystem II Contributes to a Proton Electrochemical Potential that Drives ATPase

The production of ATP in photosynthesis depends on the conversion of redox free energy into a proton electrical chemical potential, which is made up of a pH difference (ΔpH) and an electrical potential difference (ΔΨ) across the thylakoid membrane, and is given by the following equation:

$$\Delta\mu_H^+ = F\Delta\Psi - 2.3RT\Delta pH$$

where F is the Faraday constant, R is the gas constant, and T is the temperature in kelvin. Photosystem II contributes to this proton potential energy by: (1) the release of protons during the oxidation of water by photosystem II into the lumen; (2) the uptake of protons from the stromal phase associated with the reduction of plastoquinone at the Q_B site – this reaction is the first half of a proton-transporting mechanism that is completed by the oxidation of plastoquinol by the cytochrome *bf* complex, which releases the protons initially taken up by photosystem II into the lumen; and (3) the creation of an electrical potential across the membrane due to electron transfer through the photosystem II reaction centre from water to plastoquinone.

Downregulation: Energy is Diverted Away from the Reaction Centre when there is Excess Light

Although photosynthesis can be very efficient, environmental conditions typically impose severe limitations on both rate and efficiency. One of the most common stress situations for a photosynthetic organism is the absorption of more light than they can use for carbon reduction. Excess light can drive inopportune electron transfer reactions, which may cause both long-term and short-term damage to photosystem II, impairing photosynthetic productivity.

Photosynthetic organisms have evolved different strategies to avoid injury due to excess light. One of the dominant

protective mechanisms in plants and algae is known as downregulation or nonphotochemical quenching: this is a dynamic regulation of excitation energy transfer pathways within the antenna system that diverts excitation energy into heat before it reaches the reaction centre (Demmig-Adams *et al*., 1996). It is not unusual for half of the absorbed quanta to be directed from the antenna array and converted into heat. Under such conditions the efficiency of photosynthetic light energy conversion can be reduced by more than 50%. It is not known why plants respond to excess light by downregulating light capture rather than increasing photosynthetic capacity.

Secondary Electron Transfer Reactions in Photosystem II Protect Against Photodamage

Photosystem II is susceptible to damage by excess light. This is not surprising in view of the fact that photosystem II must switch between various high-energy states that involve powerful oxidants required for the oxidation of water, and strong reductants for the reduction of plastoquinone. In saturating light, a single reaction centre can have an energy throughput of $600\,eV\,s^{-1}$, which is equivalent to 60 000 kW per mole of photosystem II. To avoid damage, photosystem II contains redox components that appear to serve as safety valves by accepting or donating electrons at opportune times. For example, it has been proposed that cytochrome *b*559 serves to deactivate a rarely formed, but highly damaging, redox state of photosystem II, by accepting an electron from pheophytin when forward electron transfer is overloaded (Whitmarsh and Pakrasi, 1996).

Inactive Photosystem II: A Significant Proportion of Reaction Centres do not Work *In Vivo*

Although most photosystem II reaction complexes work efficiently to oxidize water and reduce plastoquinone, a number of *in vivo* assays have shown that a significant proportion are unable to transfer electrons to the plastoquinone pool at physiologically significant rates. Experiments using higher plants, algae and cyanobacteria indicate that photosynthetically inactive photosystem II complexes are a common feature of oxygenic organisms. For example, in healthy spinach leaves, *in vivo* measurements show that 30% of the photosystem II complexes are inactive (Chylla and Whitmarsh, 1989). Inactive photosystem II centres are impaired at the Q_A site, which is reoxidized approximately 1000 times slower than in active centres. In addition, the antenna system serving inactive photosystem II complexes is approximately half the size of that serving each active complex. Membrane fractionation studies indicate that stromal membranes are enriched in inactive photosystem II centres, but they are also present in granal membranes. These differences, between the antenna size and membrane distribution of active and inactive centres, are shared by photosystems II_α and II_β, which are defined by their relative antenna size. It is not known why plants contain photosystem II reaction centre complexes that do not contribute to energy transduction, but it has been estimated that inactive centres could reduce the quantum efficiency of photosynthesis by as much as 10%.

Fluorescence: Monitoring Photosystem II Activity *In Vivo*

Measurements of chlorophyll fluorescence provide a noninvasive technique for monitoring photosynthetic processes in plants, algae and cyanobacteria. One of the primary applications is determining the activity of photosystem II reaction centres *in vivo*. The technique relies on the observation that the yield of chlorophyll fluorescence depends in large part on the capacity of photosystem II to carry out a stable charge separation between P680, the primary donor, and Q_A, the primary quinone acceptor of the reaction centre. When Q_A is oxidized, the reaction centre is able to utilize the light energy harvested by the antenna system for charge separation and the fraction of excitation lost to fluorescence is low, giving rise to low fluorescence yields (**Figure 5**). In contrast, when Q_A is reduced, the reaction centre is unable to undergo stable charge separation and the fraction of excitation lost to fluorescence is high, giving rise to the maximum fluorescence yield.

Measurements of the chlorophyll fluorescence emission from leaves provide data for calculating photochemical yields under physiological conditions (Schreiber *et al*., 1998). The recent introduction of highly sensitive charge-coupled device cameras has enabled instrumentation that images chlorophyll fluorescence in cells, leaves and plants (Nedbal *et al*., 2000; Holub *et al*., 2000). The next stage in development is to use remote sensing to measure chlorophyll fluorescence dynamics for crops, forests, grasslands and aquatic photosynthesis.

Summary

Photosystem II is a multiunit chlorophyll–protein complex that uses light energy to transfer electrons from water to plastoquinone. It is located in the thylakoid membrane and is made up of an antenna system, which captures light, and

a reaction centre core, which uses the light energy to drive electron and proton transfer. The antenna system is composed of protein complexes that contain pigment molecules, chlorophyll *a* and *b* plus accessory molecules, which capture light by converting photon energy to excitation energy. The reaction centre contains carriers that create a pathway for electrons from water to plastoquinone. These carriers include a cluster of four manganese ions, a tyrosine (Y_Z) residue, a specialized pair of chlorophyll molecules (P680), pheophytin, a permanently bound plastoquinone (Q_A) and a plastoquinone that binds reversibly to photosystem II at the Q_B site.

The primary photochemical reaction leads to the formation of a charge-separated state in which P680, a specialized pair of chlorophyll molecules, is oxidized and a pheophytin molecule is reduced. Oxidized P680 serves to remove electrons from water, while reduced pheophytin provides the electrons for the reduction of plastoquinone. Four consecutive photochemical reactions lead to the oxidation of two water molecules, which results in the release of one dioxygen molecule.

The structure of the photosystem II reaction centre is just becoming available, which means that the long-time goal of understanding the molecular mechanism of water oxidation is finally within reach.

References

Chylla RA and Whitmarsh J (1989) Inactive photosystem II complexes in leaves: turnover rate and quantitation. *Plant Physiology* **90**: 765–772.

Dekker JP and van Grondelle R (2000) Primary charge separation in photosystem II. *Photosynthesis Research* **63**: 195–208.

Demmig-Adams B, Gilmore A and Adams WW III (1996) *In vivo* functions of carotenoids in plants. *FASEB Journal* **10**: 403–412.

Des Marais DJ (2000) When did photosynthesis emerge on earth? *Science* **289**: 1703–1704.

Fowler CF (1977) Proton evolution from photosystem II. Stoichiometry and mechanistic considerations. *Biochimica et Biophysica Acta* **462**: 414–421.

Frank HA, Young AJ, Britton G and Cogdell RJ (eds) (1999) The photochemistry of carotenoids, vol. 8. In: Govindjee (series ed.) *Advances in Photosynthesis*. Dordrecht: Kluwer Academic.

Govindjee and Coleman W (1990) How does photosynthesis make oxygen? *Scientific American* **262**: 50–58.

Greenfield SR, Seibert M, Govindjee and Wasielewski MR (1997) Direct measurement of the effective rate constant for primary charge separation in isolated photosystem II reaction centers. *Journal of Physical Chemistry* **B101**: 2251–2255.

Hillier W and Wydrzyndki T (2000) The affinities for the two substrate water binding sites in the O_2 evolving complex of Photosystem III vary independently during the S-state turnover. *Biochemistry* **39**: 4399–4405.

Holub O, Seufferheld MJ, Gohlke C, Govindgee and Clegg RM (2000) Fluorescence lifetime imaging (FLI) in real time – a new technique in photosynthesis research. *Photosynthica* **38**: 581–599.

Joliot P and Kok B (1975) Oxygen evolution in photosynthesis. In: Govindjee (ed.) *Bioenergetics in Photosynthesis*, pp. 387–412. New York: Academic Press.

Joliot P, Barbieri G and Chabaud R (1969) Un nouveau modèle des centres photochimiques du système II. *Photochemistry and Photobiology* **10**: 309–329.

Klimov VV and Baranov SV (2001) Bicarbonate requirement for the water-oxidizing complex of photosystem II. *Biochimica et Biophysica Acta* **1503**: 187–196.

Kok B, Forbush B and McGloin M (1970) Cooperation of charges in photosynthetic oxygen evolution. *Photochemistry and Photobiology* **11**: 457–475.

Kühlbrandt W, Wang DN and Fujiyoshi Y (1994) Atomic model of plant light harvesting complex by electron crystallography. *Nature* **367**: 614–621.

Lakowicz JR (1999) *Principles of Fluorescence Spectroscopy*, 2nd edn. New York: Kluwer Academic–Plenum.

Moser CC, Keske JM, Warncke K, Farid RS and Dutton PL (1992) Nature of biological electron transfer. *Nature* **355**: 796–802.

Nedbal L, Soukupová J, Whitmarsh J and Trilele M (2000) Postharvest imaging of chlorophyll fluorescence from lemons can be used to predict fruit quality. *Photosynthetica* **38**: 571–579.

Nugent J (ed.) (2001) Photosynthetic water oxidation. *Biochimica et Biophysica Acta* **1503**: 1–259.

Ort D and Whitmarsh J (2001) Photosynthesis. *Encyclopedia of Life Sciences*. London: Macmillan.

Rhee K-H (2001) Photosystem II: the solid structural era. *Annual Review of Biophysical and Biomolecular Structure* **30**: 307–328.

Schreiber U, Bilger W, Hormann H and Neubauer C (1998) Chlorophyll fluorescence as a diagnostic tool: basics and some aspects of practical relevance. In: Raghavendra AS (ed.) *Photosynthesis: A Comprehensive Guide*, pp. 320–336. Cambridge.

van Rensen JJS, Xu C and Govindjee (1999) Role of bicarbonate in photosystem II, the water-plastoquinone oxido-reductase of plant photosynthesis. *Physiol. Plantarum* **105**: 585–592.

Velthuys BR and Amesz J (1974) Charge accumulation at the reducing side of system 2 of photosynthesis. *Biochimica et Biophysica Acta* **325**: 277–281.

Whitmarsh J and Pakrasi H (1996) Form and function of cytochrome *b*559. In: Ort DR and Yocum CF (eds) *Oxygenic Photosynthesis: The Light Reactions*, pp. 249–264. Dordrecht: Kluwer Academic.

Whitmarsh J and Govindjee (1999) The photosynthetic process. In: Singhal GS, Renger G, Sopory SK, Irrgang K-D and Govindjee (eds) *Concepts in Photobiology: Photosynthesis and Photomorphogenesis*, pp. 11–51. Dordrecht: Kluwer Academic.

Wraight C (1981) Oxidation–reduction physical chemistry of the acceptor complex in bacterial photosynthetic reaction centers: evidence for a new model of herbicide activity. *Israel Journal of Chemistry* **21**: 348–354.

Xiong J, Subramaniam S and Govindjee (1998) A knowledge-based three dimensional model of the photosystem II reaction center of *Chlamydomonas reinhardtii*. *Photosynthesis Research* **56**: 229–254.

Zouni A, Witt HT, Kern J *et al*. (2001) Crystal structure of photosystem II from *Synechococcus elongatus* at 3.8 Angstrom resolution. *Nature* **409**: 739–743.

Further Reading

Blankenship RE (2002) *Mechanisms of Photosynthesis*. Oxford: Blackwell Science.

Falkowski PG and Raven JA (1997) *Aquatic Photosynthesis*. Oxford: Blackwell Science.

Govindjee (1999) Milestones in photosynthesis research. In: Yunus M, Pathre U and Mohanty P (eds) *Probing Photosynthesis: Mechanisms, Regulation and Adaptation*, pp. 9–39. London: Taylor and Francis.

Ke B (2001) *Photosynthesis: Photobiochemistry and Photobiophysics*. Dordrecht: Kluwer Academic.

Ort D and Yocum CF (eds) (1996) Oxygenic photosynthesis: the light reactions. In: Govindjee (ed.) *Advances in Photosynthesis*. Dordrecht: Kluwer Academic.

Raghavendra AS (ed.) (1998) *Photosynthesis: A Comprehensive Treatise*. Cambridge: Cambridge University Press.

van Amerongen H, Valkunas L and van Grondelle R (2000) *Photosynthetic Excitons*. Singapore: World Scientific.

The Light Reactions of Photosynthesis

John Whitmarsh and Govindjee

Department of Biochemistry
University of Illinois at Urbana-Champaign

In: *Plant Sciences for Students* (2001)
Editor: R. Robinson.
Macmillan Press, New York, NY

Photosynthesis, Light Reactions and

Life requires a continuous input of energy. On Earth, the main source of energy is sunlight, which is transformed by photosynthesis into a form of chemical energy that can be used by photosynthetic and nonphotosynthetic organisms alike. Photosynthesis is the molecular process by which plants, algae, and certain bacteria use light energy to build molecules of sugar from carbon dioxide (CO_2) and water (H_2O). The sugar molecules produced by photosynthetic organisms provide the energy as well as chemical building blocks needed for their growth and reproduction. In plants and algae the photosynthetic process removes CO_2 from the atmosphere while releasing molecular oxygen (O_2) as a by-product. Some photosynthetic bacteria function like plants and algae, giving off O_2; other types of photosynthetic bacteria, however, use light energy to create organic **compounds** without producing O_2. The type of photosynthesis that releases O_2 emerged early in

compound a substance formed from two or more elements

Earth's history, more than three billion years ago, and is the source of the O_2 in our atmosphere. Thus photosynthetic organisms not only provide the food we eat, but also the air we breathe. In addition, ancient photosynthesis produced the building blocks for the oil, coal, and natural gas that we currently depend on for our survival.

The overall photosynthetic process can be written as:

Carbon Dioxide + Water + Light → Carbohydrate + Oxygen

and can be summarized by the following chemical equation:

$$6\ CO_2 + 6\ H_2O + \text{Light Energy} \rightarrow (CH_2O)_6 + 6\ O_2$$

However, this simple chemical equation does not reveal all the reactions that must occur inside a plant to produce carbohydrate. If you shine light on a mixture of CO_2 and H_2O, you end with what you started, CO_2 and H_2O. Add a plant, however, and you get sugar. Plants create this sugar in a series of molecular steps using a complicated machinery made up of proteins and other organic molecules.

This article describes the photosynthetic process in plants, focusing on the first stage of photosynthesis, known as the light reactions. The light reactions capture light energy and store it within two chemicals, **NADPH** (nicotinamide adenine dinucleotide phosphate) and **ATP** (adenosine triphosphate). These two molecules provide the energy needed to drive the second stage of photosynthesis, known as the Calvin-Benson cycle, in which carbohydrates (sugars) are made from CO_2 and H_2O.

NADPH reduced form of nicotinomide adenine dinucleotide phosphate, a small, water-soluble molecule that acts as a hydrogen carrier in biochemical reactions

ATP adenosine triphosphate, a small, water-soluble molecule that acts as an energy currency in cells

ions charged particles

To perform photosynthesis a plant must gather light energy, transport electrons between molecules, transfer protons across a membrane, and finally rearrange chemical bonds to create carbohydrates. To understand the light reactions it is helpful to focus on the path of three critical elements: energy, electrons, and protons (hydrogen **ions**). However, before considering the series of individual reactions that make up the light reactions, the molecular machinery that does all the work must be examined.

Chloroplasts

In plants and algae, photosynthesis occurs in chloroplasts, which are small **organelles** located inside cells. The chloroplast can be thought of as a factory, providing the plant with food and energy. A typical cell in a leaf contains many chloroplasts. Fortunately chloroplasts from different plants are more similar than different. This means that if you understand how photosynthesis works in one plant, you will have a general understanding of photosynthesis in all plants. The chloroplast contains a membrane system, known as the photosynthetic membrane (or thylakoid membrane), that contains most of the proteins required for the light reactions. The Calvin-Benson cycle **enzymes** that capture CO_2 and produce carbohydrate are located in the water phase of the chloroplast outside the photosynthetic membrane. The photosynthetic membrane, like other cellular membranes, is composed mainly of lipid molecules arranged in a bi-layer. As will be explained, a critical feature of the photosynthetic membrane is that it forms a **vesicle** that defines an inner and an outer water space. The photosynthetic membrane is organized into stacked membranes that are interconnected by nonstacked

organelle a membrane-bound structure within a cell

enzyme a protein that controls a reaction in a cell

vesicle a membrane-bound cell structure with specialized contents

membranes. Researchers are uncertain as to why the photosynthetic membrane is organized in such a complicated structure. Fortunately, to understand the photosynthetic light reactions we can represent the shape of the photosynthetic membrane as a simple vesicle.

Gathering Sunlight: The Antenna System

Plants capture sunlight by using pigment molecules that absorb visible light (wavelengths from 400 to 700 **nanometers**). The main light-absorbing molecule is chlorophyll, which gives plants their green color. Chlorophyll is green because it is efficient at absorbing blue light and red light, but not very efficient at absorbing green light. The chlorophyll and other light-absorbing molecules (for example, **carotenoids**, which are yellow) are bound to protein complexes embedded in the photosynthetic membrane that make up an **antenna system**. This antenna system is designed to absorb light energy and funnel it to a protein complex called a **reaction center**. The reaction center can use the energy to drive an electron uphill from one site to another within the reaction center. Each reaction center is located at the center of the antenna system, which contains two hundred to three hundred chlorophyll molecules. Before the first chemical step can take place, the light energy captured by the antenna system must be transferred to the reaction center.

To understand light absorption it is best to think of light as packets of energy known as photons. The job of the antenna system is to capture photons and change the light energy into another form of energy known as excitation energy, which is a type of electronic energy. The excitation energy can be thought of as a packet of energy that jumps from antenna molecule to antenna molecule until it is trapped by a reaction center. The antenna system is very efficient. Under optimum conditions more than 90 percent of the photons gathered by the antenna system are transferred to the reaction center. The migration of excitation energy in the antenna system is also very fast. A photon is absorbed, transferred around the antenna system, and trapped by a reaction center within a few trillionths of a second (10^{-12} s).

nanometer one-billionth of a meter

carotenoid a yellow-colored molecule made by plants

antenna system a collection of protein complexes that harvests light energy and converts it to excitation energy that can migrate to a reaction center. The light is absorbed by pigment molecules (e.g., chlorophyll, carotenoids, phycobilin) that are attached to the protein

reaction center a protein complex that uses light energy to create a stable charge separation by transferring a single electron energetically uphill from a donor molecule to an acceptor molecule, both of which are located in the reaction center

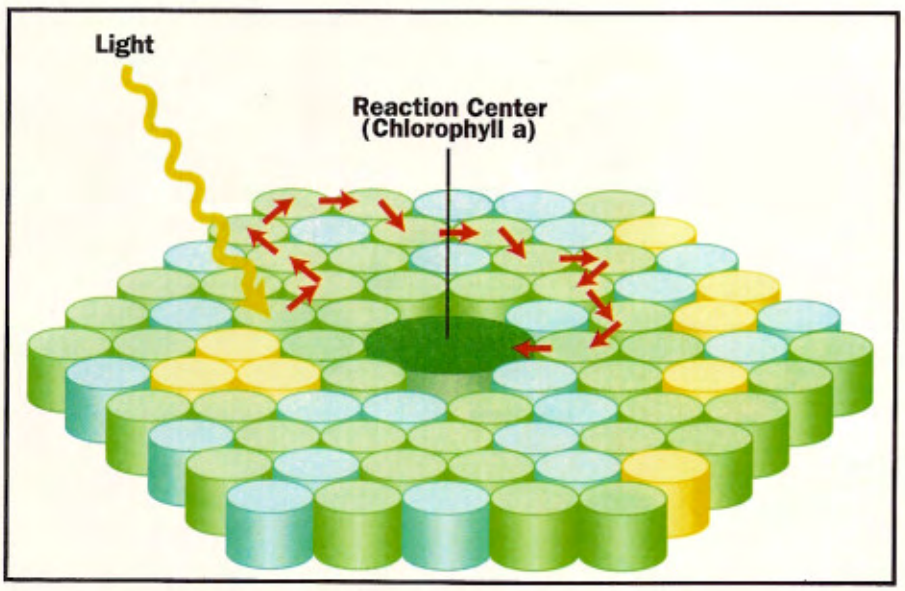


Figure 1: Antenna system with a reaction center (middle). The arrows indicate the pathway of excitation energy migration. Redrawn from Starr and Taggart, 1998, Figure 7.9.

Electron Transport

NADP$^+$ oxidized form of nicotinomide adenine dinucleotide phosphate

The excitation energy trapped by a reaction center provides the energy needed for electron transfer, which is the next step in the photosynthetic light reactions. During electron transfer, individual electrons are removed from water molecules and transferred, by an electron transport chain, to **NADP$^+$**. Electron transport in photosynthesis is like electron flow in an electric circuit driven by a battery. The voltage difference across the battery pushes electrons through the circuit, and the electron current can be used to do work. In photosynthesis, light energy pushes electrons up an energy hill in the reaction centers. Subsequent electron flow in the electron transport chain is energetically downhill and can be used to do work. Figure 2 shows the electron carriers that make up the photosynthetic electron transport chain in a way that reveals the relative electronic energy on the vertical scale. This is known as the Z-scheme. Note that a negative voltage corresponds to a higher energy, so that downhill electron flow is from the top to the bottom of the figure.

The electron transport pathway includes electron transfer from one site to another within a protein, as well as electron transfer from one molecule to another (Figure 3). Most of the electron carriers are located in the photosynthetic membrane, but a few (for example, NADP$^+$) are located in the water phase surrounding the membrane. It is important to keep in mind that the electron transport chain shown in the figure is repeated many times in each chloroplast. A typical chloroplast will contain more than a million electron transport chains.

Electron transfer from one molecule to another is possible because certain types of molecules can easily give up or receive electrons. Some electron carriers can give up and receive a single electron (e.g., plastocyanin), while others can accept or donate more than one electron (e.g., NADP$^+$

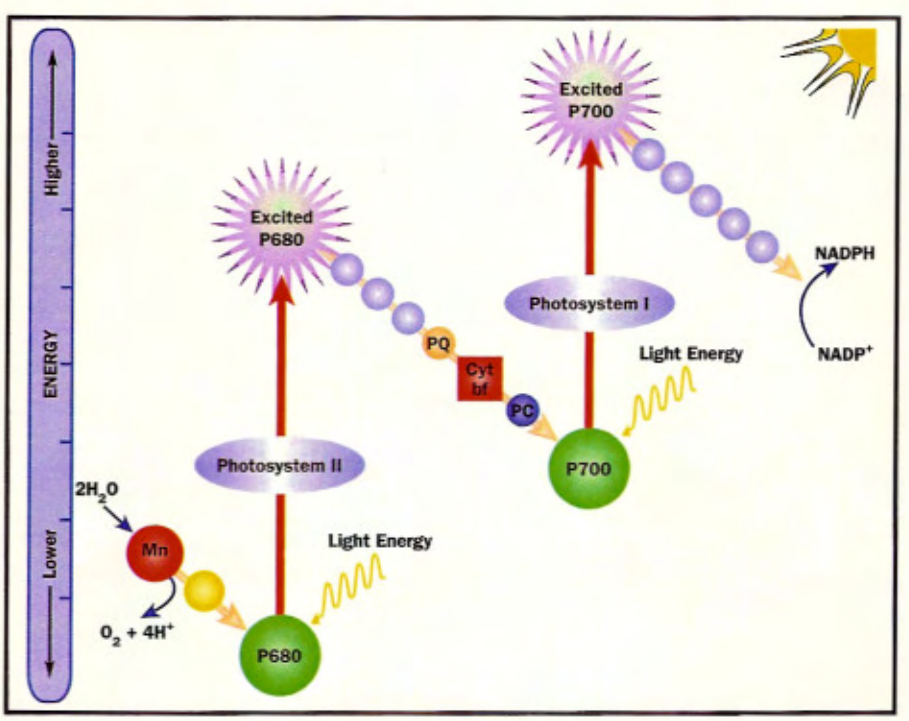


Figure 2: Z-scheme showing the pathway of electrons from water to NADP* producing oxygen and the reducing power (NADPH). Redrawn from www.life.uiuc.edu/govindjee/ZschemeG.html Mn = manganese; P680 = reaction center chlorophyll *a* of Photosystem II; PQ = plastoquinone; Cyt bf = cytochrome bf complex; PC = plastocyanin; P700 = reaction center chlorophyll of Photosystem I.

can accept two electrons). In addition, some electron carriers can take up a proton along with an electron (plastoquinone can accept two electrons and two protons), making them hydrogen (H) carriers.

When a compound gains an electron it is said to be *reduced* (**reduction**), whereas when it gives up an electron it is said to be *oxidized* (**oxidation**). In biological electron transport pathways, the electrons are always bound to a molecule (they are too reactive to hang around free), which means that an oxidation reaction is always coupled to a reduction reaction. Electrons spontaneously jump from one molecule to another because some molecules hold onto their electrons more tightly than others. This is another way of saying that energetically, electrons flow downhill. If two molecules, A and B, are close enough together, and if A is reduced and B is oxidized, an electron will jump from A to B if it is energetically downhill.

reduction the addition of one or more electrons to an atom or molecule. In the case of a molecule, protons may be involved as well, resulting in hydrogen being added

oxidation The removal of one or more electrons from an atom or molecule. In the case of a molecule, a proton may be involved as well, resulting in hydrogen being removed

NADPH Production

Moving an electron from water to $NADP^+$ requires an input of energy. This job is done by reaction centers, which use the light energy gathered by the antenna system to move an electron energetically uphill. As shown in Figure 3 the electron transport chain in chloroplasts uses two different types of reactions centers: Photosystem II and Photosystem I. (For historical reasons the reaction centers are not numbered according to their order in the electron transport chain, i.e., Photosystem II sends electrons to photosystem I.)

Photosystem II catalyzes two different chemical reactions. One is the oxidation of water and the other is the reduction of plastoquinone. Water oxidation is a critical reaction in photosynthesis because the electrons removed from H_2O are ultimately used to reduce CO_2 to carbohydrate. Photosystem II performs this reaction by binding two H_2O molecules and removing one electron at a time. The energy for the removal of a single electron is provided by a single photon. For Photosystem II to completely oxidize two H_2O molecules and reduce two molecules of plastoquinone, it requires four photons. (Note that electron transport from H_2O all the way to $NADP^+$ requires two light reactions: Photosystem II and Photosystem I. Thus eight photons are required for the release of one O_2 molecule.) This process creates O_2, which is released, and H^+ ions, which are used in ATP synthesis (see below).

As shown in Figure 3, electron transfer from water to $NADP^+$ requires three membrane-bound protein complexes: Photosystem II, the cytochrome *bf* complex (Cyt bf), and Photosystem I. Electrons are transferred between these large protein complexes by small mobile molecules. Because these small molecules carry electrons (or hydrogen atoms) over relatively long distances, they play a critical role in photosynthesis. This is illustrated by plastoquinone (PQ), which transfers electrons from the Photosystem II reaction center to the cytochrome *bf* complex and at the same time carries protons across the photosynthetic membrane.

Plastoquinone operates by diffusing in the photosynthetic membrane until it becomes bound to a pocket on the Photosystem II complex. The photosystem II reaction center reduces plastoquinone by adding two electrons taken from H_2O and two protons taken from the outer water phase, creating PQH_2. The reduced plastoqinone molecule unbinds from Photosystem II and diffuses in the photosynthetic membrane until it encounters a binding site on the cytochrome *bf* complex. In a reaction sequence that is

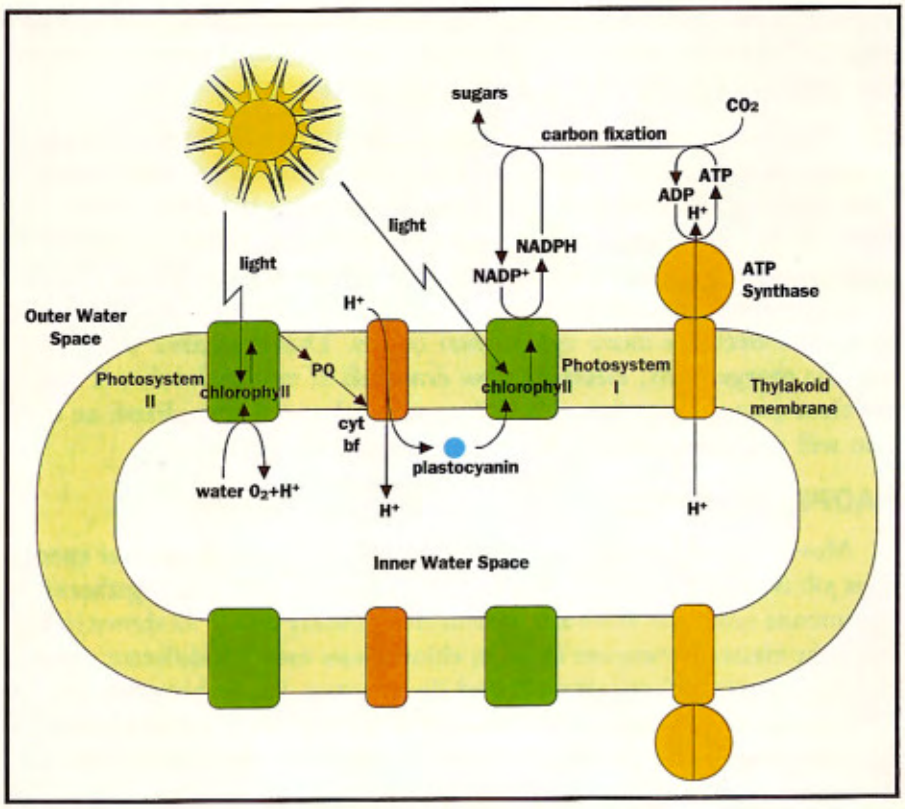


Figure 3: The electron transport chain showing the carriers in a membrane that forms a vesicle. Modified from photoscience.la.asu.edu/photosyn/education/photointro.html. See text for abbreviations used.

not completely understood, the cytochrome *bf* complex removes the electrons from reduced plastoquinone and releases protons into the inner water space of the photosynthetic vesicle. The cytochrome *bf* complex then gives up the electrons to another small molecule, plastocyanin (PC). The electrons are transferred to the Photosystem I reaction center by plastocyanin. The proton gradient, produced by water oxidation and oxidation of reduced plastoquinone, is used to create ATP (see below).

The Photosystem I reaction center is like Photosystem II in that it is served by a chlorophyll-containing antenna system and uses light energy to move an electron energetically uphill, but Photosystem I catalyzes different reactions: it oxidizes plastocyanin and reduces ferredoxin. Ferredoxin itself becomes oxidized, losing its electrons to another acceptor. The last step in the photosynthetic electron transport chain is reduction of $NADP^+$, producing NADPH.

ATP Production

redox oxidation and reduction

In plants essentially all electron flow from water follows the pathway shown in Figure 3, at least up to ferredoxin. However, once an electron reaches ferredoxin the electron pathway becomes branched, enabling a fraction of the **redox** free energy to enter other pathways, including cycling through the Photosystem I reaction center. Photosystem I cyclic electron transport provides additional energy for ATP production, which allows plants to adjust the energy flow according to their metabolic needs.

Most of the energy from the electron transfer reactions is stored as redox energy in NADPH as described above. However, some of the energy

is stored across the membrane of the photosynthetic vesicle in the form of a **pH** gradient (or protein gradient) and an electric potential (positive inside). As previously noted, the electron transport chain concentrates protons in the inner water phase of the vesicle by the release of protons during the oxidation of water by Photosystem II and by transporting protons from the outer water phase to the inner water phase via plastoquinone (Figure 3). In addition, electron transport creates a net positive charge on the inner side and a net negative charge on the outer side of the vesicle, which gives rise to an electric potential across the membrane. The energy stored in the pH gradient and electric potential is known as the transmembrane proton electrochemical potential or the proton motive force.

pH a measure of acidity or alkalinity; the pH scale ranges from 0 to 14, with 7 being neutral; low pH numbers indicate high acidity; high numbers indicate alkalinity

The conversion of proton electrochemical energy into the chemical-free energy of ATP is accomplished by a single protein complex known as ATP synthase, which catalyzes the formation of ATP by the addition of inorganic phosphate (P_i) to ADP:

$$ADP + P_i \rightarrow ATP + H_2O$$

The reaction is energetically uphill and is driven by the transmembrane proton electrochemical gradient. The ATP synthase enzyme is a molecular rotary motor. Protons move through a channel in the ATP synthase pro-

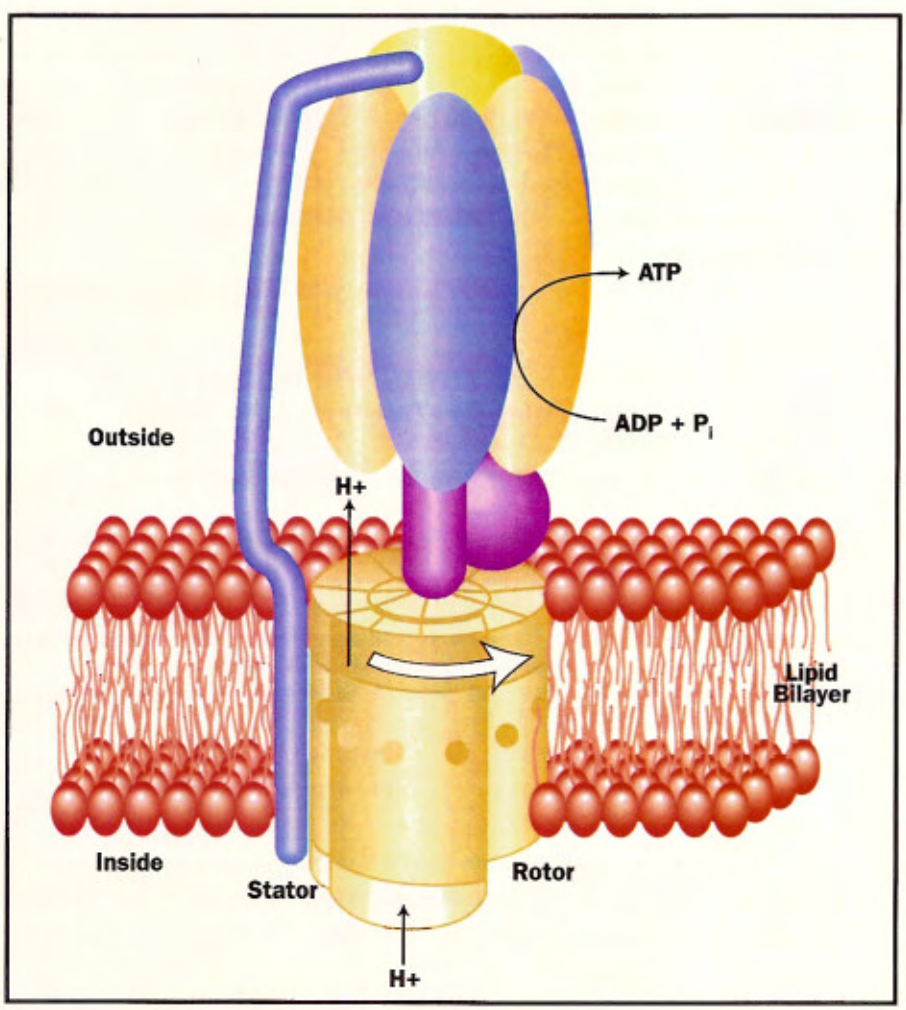


Figure 4: Rotary model of how ATP synthase catalyzes ATP. Redrawn from Fillingame, 1999, pp. 1687–88.

tein (from the inner water phase to the outer water phase of the vesicle) providing the energy for ATP synthesis. However, the protons are not involved in the chemistry of adding phosphate to ADP at the catalytic site. Although it has not been proven, it appears that proton flow drives the rotation part of the ATP synthase at rates as high as one hundred revolutions per second (Figure 4). The rotation of ATP synthase can be thought of as pushing ADP and P_i together to form ATP and water.

From the Light Reactions to the Calvin-Benson Cycle

The job of the photosynthetic light reactions is to provide energy in the form NADPH and ATP for the Calvin-Benson cycle. Although all plants depend on the Calvin-Benson cycle to make carbohydrates, the way they get the carbon dioxide to the cycle varies. The most efficient plants (soybean, for example) require two molecules of NADPH and three molecules of ATP for each molecule of CO_2 that is taken up, while some other types of plants (corn, for example) must use more energy to fix a single CO_2 molecule. During brief periods photosynthesis in plants can store nearly 30 percent of the light energy they absorb as chemical energy. However, under normal, day-to-day growing conditions the actual performance of the plant is less than one-tenth of the maximum efficiency. The factors that conspire to lower photosynthesis include limitations imposed by molecular reactions and environmental conditions that limit plant performance such as low soil moisture or high temperature. Our increasing understanding of plant **genomes** opens the door for improving plant performance under diverse environmental conditions (for example, enabling farmers to grow crops on marginal lands). A crucial step in this direction is understanding the molecular processes involved in photosynthesis. SEE ALSO CHLOROPHYLL; CHLOROPLASTS; INGENHOUSZ, JAN; PHOTOSYNTHESIS, CARBON FIXATION AND; WATER MOVEMENT.

genome the genetic material of an organism

John Whitmarsh and Govindjee

Bibliography

Fillingame, R. H. "Molecular Rotary Motors." *Science* 286 (1999): 1687–88.

Govindjee, and W. Coleman. "How Does Photosynthesis Make Oxygen?" *Scientific American* 262 (1990): 50–58.

Hall, D. O., and K. K. Rao. *Photosynthesis*, 6th ed. Cambridge: Cambridge University Press, 1999.

Starr, Cecie, and Ralph Taggart. *Biology: The Unity and Diversity of Life*. Belmont: CA: Wadsworth Publishing Co., 1998.

Walker, D. A. *Energy, Plants and Man*. East Sussex, U.K.: Oxygraphics Limited, 1992.

Whitmarsh, J., and Govindjee. "The Photosynthetic Process." In *Concepts in Photobiology: Photosynthesis and Photomorphogenesis*, ed. G. S. Singhal, G. Renger, K-D. Irrgang, S. Sopory, and Govindjee. New Delhi/Dordrecht: Narosa Publishers/ Kluwer Academic Publishers, 1999.

Phyllotaxis

Phyllotaxis is the study of the patterns on plants. The word itself comes from the Greek *phullon*, meaning "leaf," and *taxis*, meaning "arrangement." Phyllotaxis, in the restricted sense, is the study of the relative arrangement of what is called the primordia of plants. A primordium is, for example, what

Concepts in Photobiology: Photosynthesis and Photomorphogenesis
G.S. Singhal, R. Renger, S.K. Sopory, K-D. Irrgang and Govindjee (Eds)

2. The Photosynthetic Process

John Whitmarsh[†,*] and Govindjee*

[†]Photosynthesis Research Unit, Agricultural Research Service/USDA
*Department of Biochemistry and Center of Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, IL, USA

Summary

The primary source of energy for nearly all life is the Sun. The energy in sunlight is introduced into the biosphere by a process known as photosynthesis, which occurs in plants, algae and some types of bacteria. Photosynthesis can be defined as the physico-chemical process by which photosynthetic organisms use light energy to drive the synthesis of organic compounds. The photosynthetic process depends on a set of complex protein molecules that are located in and around a highly organized membrane. Through a series of energy transducing reactions, the photosynthetic machinery transforms light energy into a stable form that can last for hundreds of millions of years. This introductory chapter focuses on the structure of the photosynthetic machinery and the reactions essential for transforming light energy into chemical energy.

1. Introduction

Photosynthesis is the physico-chemical process by which plants, algae and photosynthetic bacteria use light energy to drive the synthesis of organic compounds. In plants, algae and certain types of bacteria, the photosynthetic process results in the release of molecular oxygen and the removal of carbon dioxide from the atmosphere that is used to synthesize carbohydrates (oxygenic photosynthesis). Other types of photosynthetic bacteria use light energy to create organic compounds but do not produce oxygen (anoxygenic photosynthesis). Photosynthesis provides the energy and reduced carbon required for the survival of virtually all life on our planet, as well as the molecular oxygen necessary for the survival of oxygen consuming organisms[1]. In addition, the fossil fuels currently being burned to provide energy for human activity were produced by ancient photosynthetic organisms. Although photosynthesis occurs in cells or organelles that are typically only a few microns across, the process has a profound impact on the earth's atmosphere and climate. Each year more than 10% of the total atmospheric carbon

[1]Some organisms derive their energy from electron donating inorganic molecules such as hydrogen gas or sulfur compounds and are not dependent on current or past photosynthesis for their survival. Examples include the bacterium *Methanobacterium thermoautotrophicum*, which grows in sewage sludge living on hydrogen gas and carbon dioxide and the bacterium *Methanococcoccus jannaschii*, which grows in the ocean near hot vents.

dioxide is reduced to carbohydrate by photosynthetic organisms. Most, if not all, of the reduced carbon is returned to the atmosphere as carbon dioxide by microbial, plant and animal metabolism, and by biomass combustion. In turn, the performance of photosynthetic organisms depends on the earth's atmosphere and climate. Over the next century, the large increase in the amount of atmospheric carbon dioxide created by human activity is certain to have a profound impact on the performance and competition of photosynthetic organisms. Knowledge of the physico-chemical process of photosynthesis is essential for understanding the relationship between living organisms and the atmosphere and the balance of life on earth. Several books on photosynthesis are available for the uninitiated (Hall and Rao, 1994; Lawlor, 1993; Walker, 1992; Falkowski and Raven, 1997; Wild and Ball, 1997) or advanced student (Govindjee, 1982; Amesz, 1987; Briggs, 1989; Barber, 1992; Scheer, 1991; Bryant, 1994; Blankenship et al., 1995; Amesz and Hoff, 1996, Baker, 1996; Ort and Yocum, 1996; Raghavendra, 1998; Siegenthaler and Murata, 1998; Rochaix et al., 1998). Taiz and Zeiger (1991) place the photosynthetic process in the context of over all plant physiology, Heldt (1997) places it in the context of plant biochemistry, and Cramer and Knaff (1991) describe the bioenergetic foundation of photosynthesis.

The overall equation for photosynthesis is deceptively simple. In fact, a complex set of physical and chemical reactions must occur in a coordinated manner for the synthesis of carbohydrates. To produce a sugar molecule such as sucrose, plants require nearly 30 distinct proteins that work within a complicated membrane structure. Research into the mechanism of photosynthesis centers on understanding the structure of the photosynthetic components and the molecular processes that use radiant energy to drive carbohydrate synthesis. The research involves several disciplines, including physics, biophysics, chemistry, structural biology, biochemistry, molecular biology and physiology, and serves as an outstanding example of the success of multidisciplinary research. As such, photosynthesis presents a special challenge in understanding several interrelated molecular processes.

2. Conceptual Developments in Photosynthesis

In the 1770s Joseph Priestley, an English chemist and clergyman, performed experiments showing that plants release a type of air that allows combustion. He demonstrated this by burning a candle in a closed vessel until the flame went out. He placed a sprig of mint in the chamber and after several days showed that the candle could burn again. Although Priestley did not know about molecular oxygen, his work showed that plants release oxygen into the atmosphere. It is noteworthy that over 200 years later, investigating the mechanism by which plants produce oxygen is one of the most active areas of photosynthetic research. Building on the work of Priestley, Jan Ingenhousz, a Dutch physician, demonstrated that sunlight was necessary for photo-

synthesis and that only the green parts of plants could release oxygen. During this period Jean Senebier, a Swiss botanist and naturalist, discovered that CO_2 is required for photosynthetic growth and Nicolas-Théodore de Saussure, a Swiss chemist and plant physiologist, showed that water is required. It was not until 1845 that Julius Robert von Mayer, a German physician and physicist, proposed that photosynthetic organisms convert light energy into chemical free energy. An interesting time line of the history of photosynthesis has been presented by Huzisige and Ke (1993).

By the middle of the nineteenth century the key features of plant photosynthesis were known, namely, that plants could use light energy to make carbohydrates from CO_2 and water. The empirical equation representing the net reaction of photosynthesis for oxygen evolving organisms is:

$$CO_2 + 2H_2O + \text{Light Energy} \rightarrow [CH_2O] + O_2 + H_2O, \qquad (1)$$

where $[CH_2O]$ represents a carbohydrate (e.g., glucose, a six-carbon sugar). The synthesis of carbohydrate from carbon and water requires a large input of light energy. The standard free energy for the reduction of one mole of CO_2 to the level of glucose is +478 kJ/mol. Because glucose, a six carbon sugar, is often an intermediate product of photosynthesis, the net equation of photosynthesis is frequently written as:

$$6CO_2 + 12H_2O + \text{Light Energy} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O. \qquad (2)$$

The standard free energy for the synthesis of glucose is +2,870 kJ/mol.

Not surprisingly, early scientists studying photosynthesis concluded incorrectly that the O_2 released by plants came from CO_2, which was thought to be split by light energy. In the 1930s comparison of bacterial and plant photosynthesis lead Cornelis van Niel to propose the general equation of photosynthesis that applies to plants, algae and photosynthetic bacteria (discussed by Wraight, 1982). Van Niel was aware that some photosynthetic bacteria could use hydrogen sulfide (H_2S) instead of water for photosynthesis and that these organisms released sulfur instead of oxygen. Van Niel, among others, concluded that photosynthesis depends on electron donation and acceptor reactions and that the O_2 released during photosynthesis comes from the oxidation of water. Van Niel's generalized equation is:

$$CO_2 + 2H_2A + \text{Light Energy} \rightarrow [CH_2O] + 2A + H_2O. \qquad (3)$$

In oxygenic photosynthesis, 2A is O_2, whereas in anoxygenic photosynthesis, which occurs in some photosynthetic bacteria, the electron donor can be an inorganic hydrogen donor, such as H_2S (in which case A is elemental sulfur) or an organic hydrogen donor such as succinate (in which case, A is fumarate). Experimental evidence that molecular oxygen came from water was provided by Hill and Scarisbrick (1940) who demonstrated oxygen evolution in the absence of CO_2 in illuminated chloroplasts and by Ruben et al. (1941) who used ^{18}O enriched water.

The biochemical conversion of CO_2 to carbohydrate is a reduction that involves the rearrangement of covalent bonds between carbon, hydrogen and oxygen. The energy for the reduction of carbon is provided by energy rich molecules that are produced by the light driven electron transfer reactions. Carbon reduction can occur in the dark and involves a series of biochemical reactions that were elucidated by Melvin Calvin, Andrew Benson and James Bassham in the late 1940s and 1950s. Using the radioisotope ^{14}C, most of the intermediate steps that result in the production of carbohydrate were identified. Calvin was awarded the Nobel Prize for Chemistry in 1961 for this work (see Calvin, 1989).

In 1954 Daniel Arnon and coworkers discovered that plants, and A. Frenkel discovered that photosynthetic bacteria, use light energy to produce ATP, an organic molecule that serves as an energy source for many biochemical reactions (discussed by Frenkel, 1995). During the same period L.N.M. Duysens showed that the primary photochemical reaction of photosynthesis is an oxidation/reduction reaction that occurs in a protein complex (the reaction center). Over the next few years the work of several groups, including those of Robert Emerson, Bessel Kok, L.N.M. Duysens, Robert Hill and Horst Witt, combined to prove that plants, algae and cyanobacteria require two reaction centers, photosystem II and photosystem I, operating in series (Duysens, 1989; Witt, 1991).

In 1961 Peter Mitchell suggested that cells can store energy by creating an electric field or a proton gradient across a membrane. Mitchell's proposal that energy is stored as an electrochemical gradient across a vesicular membrane opened the door for understanding energy transformation by membrane systems. He was awarded the Nobel Prize in Chemistry in 1978 for his theory of chemiosmotic energy transduction (Mitchell, 1961).

Most of the proteins required for the conversion of light energy and electron transfer reactions of photosynthesis are located in membranes. Despite decades of work, efforts to determine the structure of membrane bound proteins had little success. This changed in the 1980s when Johann Deisenhofer, Hartmut Michel, Robert Huber and co-workers determined the structure of the reaction center of the purple bacterium *Rhodospeudomonas viridis* (Deisenhofer et al., 1984, 1985; Deisenhofer and Michel, 1993). They were awarded the Nobel Prize for Chemistry in 1988 for their work, which has provided insight into the relationship between structure and function in membrane-bound proteins. In 1997, J.E. Walker (see Abrahams et al., 1994; Junge et al., 1997) and P. Boyer (see Boyer, 1997) were awarded the Nobel Prize for Chemistry for providing the atomic level structure of ATP-synthase, and for the mechanism by which this enzyme synthesizes ATP, respectively.

A key element in photosynthetic energy conversion is electron transfer within and between protein complex and simple organic molecules. The electron transfer reactions are rapid (as fast as a few picoseconds) and

highly specific. Much of our current understanding of the physical priciples that guide electron transfer is based on the pioneering work of Rudolph A. Marcus (Marcus and Sutin, 1985), who received the Nobel Prize in Chemistry in 1992 for his contributions to the theory of electron transfer reactions in chemical systems.

3. Classification of Photosynthetic Organisms

All life can be divided into three domains, Archaea, Bacteria and Eucarya, which originated from a common ancestor (Woese et al., 1990). Historically, the term *photosynthesis* has been applied to organisms that depend on chlorophyll (or bacteriochlorophyll) for the conversion of light energy into chemical free energy (Gest, 1993). These include organisms in the domains Bacteria (photosynthetic bacteria) and Eucarya (algae and higher plants). The most primitive domain, Archaea, includes organisms known as halobacteria, that convert light energy into chemical free energy. However, the mechanism by which halobacteria convert light is fundamentally different from that of higher organisms because there is no oxidation/reduction chemistry and halobacteria cannot use CO_2 as their carbon source. Consequently some biologists do not consider halobacteria as photosynthetic (Gest 1993). This chapter will follow the historical definition of photosynthesis and omit halobacteria.

3.1 Oxygenic Photosynthetic Organisms

The photosynthetic process in all plants and algae as well as in certain types of photosynthetic bacteria involves the reduction of CO_2 to carbohydrate and removal of electrons from H_2O, which results in the release of O_2. In this process, known as oxygenic photosynthesis, water is oxidized by the photosystem II reaction center, a multisubunit protein located in the photosynthetic membrane. Years of research have shown that the structure and function of photosystem II is similar in plants, algae and certain bacteria, so that knowledge gained in one species can be applied to others. This homology is a common feature of proteins that perform the same reaction in different species. This homology at the molecular level is important because there are estimated to be 300,000–500,000 species of plants. If different species had evolved diverse mechanisms for oxidizing water, research aimed at a general understanding of photosynthetic water oxidation would be hopeless.

3.2 Anoxygenic Photosynthetic Organisms

Some photosynthetic bacteria can use light energy to extract electrons from molecules other than water. These organisms are of ancient origin, presumed to have evolved before oxygenic photosynthetic organisms. Anoxygenic photosynthetic organisms occur in the domain Bacteria and have representatives in four phyla—Purple Bacteria, Green Sulfur Bacteria, Green Gliding Bacteria, and Gram Positive Bacteria.

4. Principles of Photosynthetic Energy Transformation

The energy that drives photosynthesis originates in the center of the sun, where mass is converted to heat by the fusion of hydrogen. Over time, the heat energy reaches the sun's surface, where some of it is converted to light by black body radiation that reaches the earth. A small fraction of the visible light incident on the earth is absorbed by plants. Through a series of energy transducing reactions, photosynthetic organisms are able to transform light energy into chemical free energy in a stable form that can last for hundreds of millions of years (e.g., fossil fuels). A simplified scheme describing how energy is transformed in the photosynthetic process is presented in this section. The focus is on the structural and functional features essential for the energy transforming reactions. For clarity, mechanistic and structural details are omitted. A more highly resolved description of oxygenic and anoxygenic photosynthesis is given in the remaining sections.

The photosynthetic process in plants and algae occurs in small organelles known as chloroplasts that are located inside cells. The more primitive photosynthetic organisms, for example oxygenic cyanobacteria, prochlorophytes and anoxygenic photosynthetic bacteria, lack organelles. The photosynthetic reactions are traditionally divided into two stages—the "light reactions," which consist of electron and proton transfer reactions and the "dark reactions," which consist of the biosynthesis of carbohydrates from CO_2. The light reactions occur in a complex membrane system (the photosynthetic membrane) that is made up of protein complexes, electron carriers, and lipid molecules. The photosynthetic membrane is surrounded by water and can be thought of as a two-dimensional surface that defines a closed space, with an inner and outer water phase. A molecule or ion must pass through the photosynthetic membrane to go from the inner space to the outer space. The protein complexes embedded in the photosynthetic membrane have a unique orientation with respect to the inner and outer phase. The asymmetrical arrangement of the protein complexes allows some of the energy released during electron transport to create an electrochemical gradient of protons across the photosynthetic membrane.

Photosynthetic electron transport consists of a series of individual electron transfer steps from one electron carrier to another. The electron carriers are metal ion complexes and aromatic groups. The metal ion complexes and most of the aromatic groups are bound within proteins. Most of the proteins involved in photosynthetic electron transport are composed of numerous polypeptide chains that lace through the membrane, providing a scaffolding for the metal ions and aromatic groups. An electron enters a protein complex at a specific site, is transferred within the protein from one carrier to another, and exits the protein at a different site. The protein controls the pathway of electrons between the carriers by determining the location and environment of the metal ion complexes and aromatic groups. By setting the distance between electron carriers and controlling the electronic environment

surrounding a metal ion complex or aromatic group, the protein controls pairwise electron transfer reactions. Between proteins, electron transfer is controlled by distance and free energy, as for intraprotein transfer, and by the probability that the two proteins are in close contact. Protein association is controlled by a number of factors, including the structure of the two proteins, their surface electrical and chemical properties and the probability that they collide with one another. Not all electron carriers are bound to proteins. The reduced forms of plastoquinone or ubiquinone and nicotinamide adenine dinucleotide phosphate (NADPH) or NADH act as mobile electron carriers operating between protein complexes. For electron transfer to occur, these small molecules must bind to special pockets in the proteins known as binding sites. The binding sites are highly specific and are a critical factor in controlling electron transfer.

The light reactions convert energy into several forms (Fig. 1). The first step is the conversion of a photon to an excited electronic state of an antenna pigment molecule located in the antenna system. The antenna system consists of hundreds of pigment molecules (mainly chlorophyll or bacteriochlorophyll and carotenoids) that are anchored to proteins within the photosynthetic membrane and serve a specialized protein complex known as a reaction center. The electronic excited state is transferred over the antenna molecules as an exciton. Some excitons are converted back into photons and emitted as fluorescence, some are converted to heat, and some are trapped by a reaction center protein. (For a discussion of the use of fluorescence as a probe of photosynthesis, see e.g., Govindjee et al., 1986 and Krause and Weis, 1991). Excitons trapped by a reaction center provide the energy for the primary photochemical reaction of photosynthesis—the transfer of an electron from a donor molecule to an acceptor molecule. Both the donor and acceptor molecules are attached to the reaction center protein complex. Once primary charge separation occurs, the subsequent electron transfer reactions are energetically downhill.

In oxygenic photosynthetic organisms (see section 5), two different reaction centers, known as photosystem II and photosystem I, work in series. In the light, photosystem II feeds electrons to photosystem I. The electrons are transferred from photosystem II to photosystem I by intermediate carriers. The net reaction is the transfer of electrons from a water molecule to $NADP^+$, producing the reduced form, NADPH. In the photosynthetic process, much of the energy initially provided by light is stored as redox free energy (a form of chemical free energy) in NADPH, to be used later in the reduction of carbon. In addition, the electron transfer reactions concentrate protons inside the membrane vesicle and create an electric field across the photosynthetic membrane. In this process the electron transfer reactions convert redox free energy into an electrochemical potential of protons. The energy stored in the proton electrochemical potential is used by a membrane bound protein complex (ATP-synthase) to covalently attach a phosphate

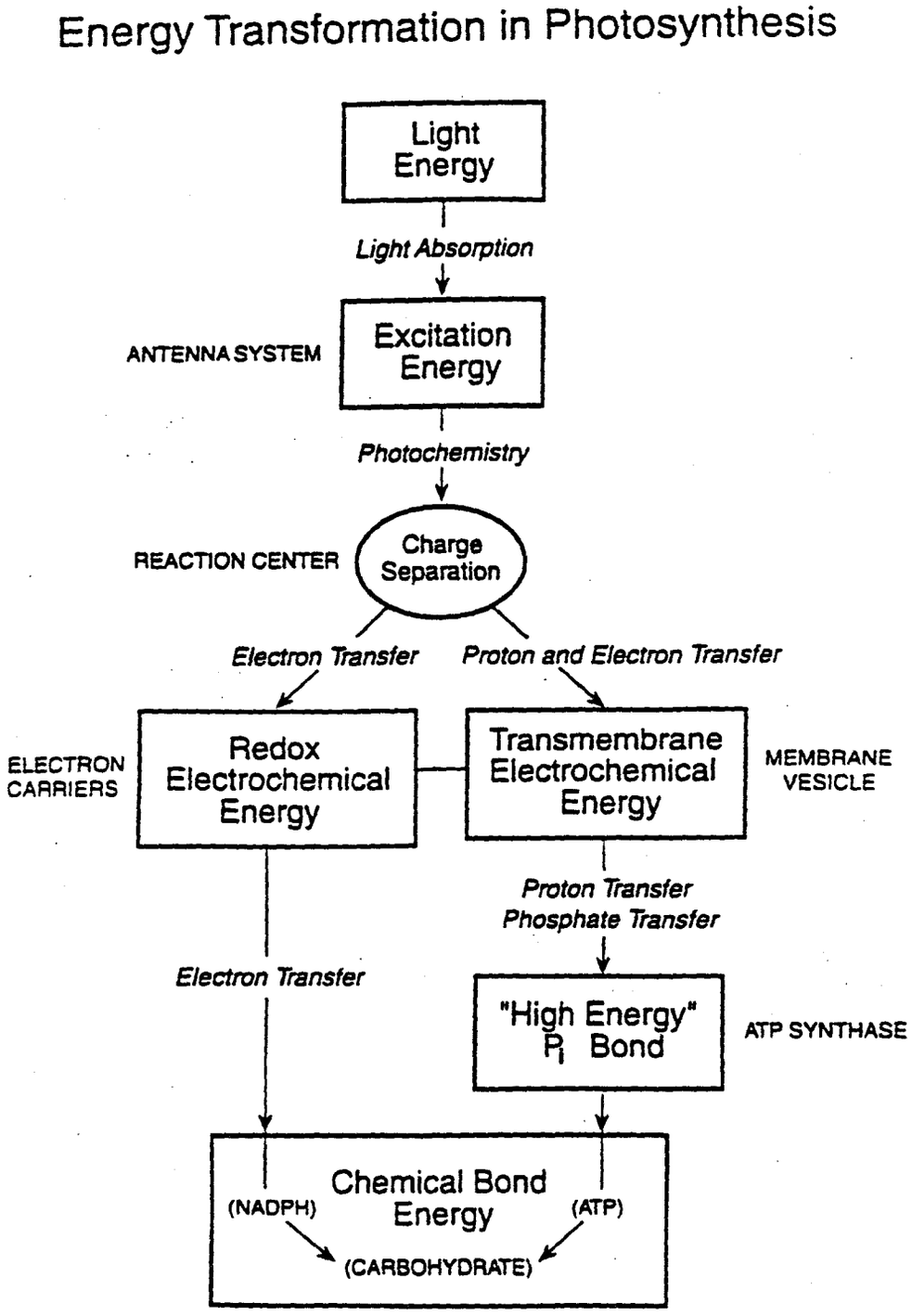


Fig. 1 **Photosynthesis is shown as a series of reactions that transform energy from one form to another. The different forms of energy are shown in boxes and the direction of energy transformation is shown by the arrows. The energy-transforming reaction is shown by italics in the arrows. The site at which the energy is stored is shown in capital letters outside the boxes. The primary photochemical reaction, charge separation, is shown in the oval. Details of these reactions are given in the text.**

group to adenosine diphosphate (ADP), forming adenosine triphosphate (ATP). Protons pass through the ATP-synthase protein complex that transforms electrochemical free energy into a type of chemical free energy known as phosphate group-transfer potential (or a high-energy phosphate bond) (Klotz, 1967). The energy stored in ATP can be transferred to another molecule by transferring the phosphate group. The net effect of the light reactions is to convert radiant energy into redox free energy in the form of NADPH and phosphate group-transfer energy in the form of ATP. In the light reactions, the transfer of a single electron from water to $NADP^+$ involves about 30 metal ions and 7 aromatic groups. The metal ions include 19 Fe, 5 Mg, 4 Mn, and 1 Cu. The aromatics include quinones, pheophytin, NADPH, tyrosine and a flavoprotein. The NADPH and ATP formed by the light reactions provide the energy for the dark reactions of photosynthesis, known as the Calvin cycle or the photosynthetic carbon reduction cycle. The reduction of atmospheric CO_2 to carbohydrate occurs in the aqueous phase of the chloroplast and involves a series of enzymatic reactions. The first step is catalyzed by the protein Rubisco (D-ribulose 1,5-bisphosphate carboxylase/oxygenase), which attaches CO_2 to a five-carbon compound. The reaction produces two molecules of a three-carbon compound. Subsequent biochemical reactions involve several enzymes that reduce carbon by hydrogen transfer and rearrange the carbon compounds to synthesize carbohydrates. The carbon reduction cycle involves the transfer and rearrangement of chemical bond energy.

In anoxygenic photosynthetic organisms (see section 6) water is not used as the electron donor. Electron flow is cyclic and is driven by a single photosystem, producing a proton electrochemical gradient that is used to provide energy for the reduction of NAD^+ by an external H-atom or e-donor (e.g., H_2S or an organic acid) in a process known as "reverse electron flow". The fixation of CO_2 occurs via different pathways in different organisms.

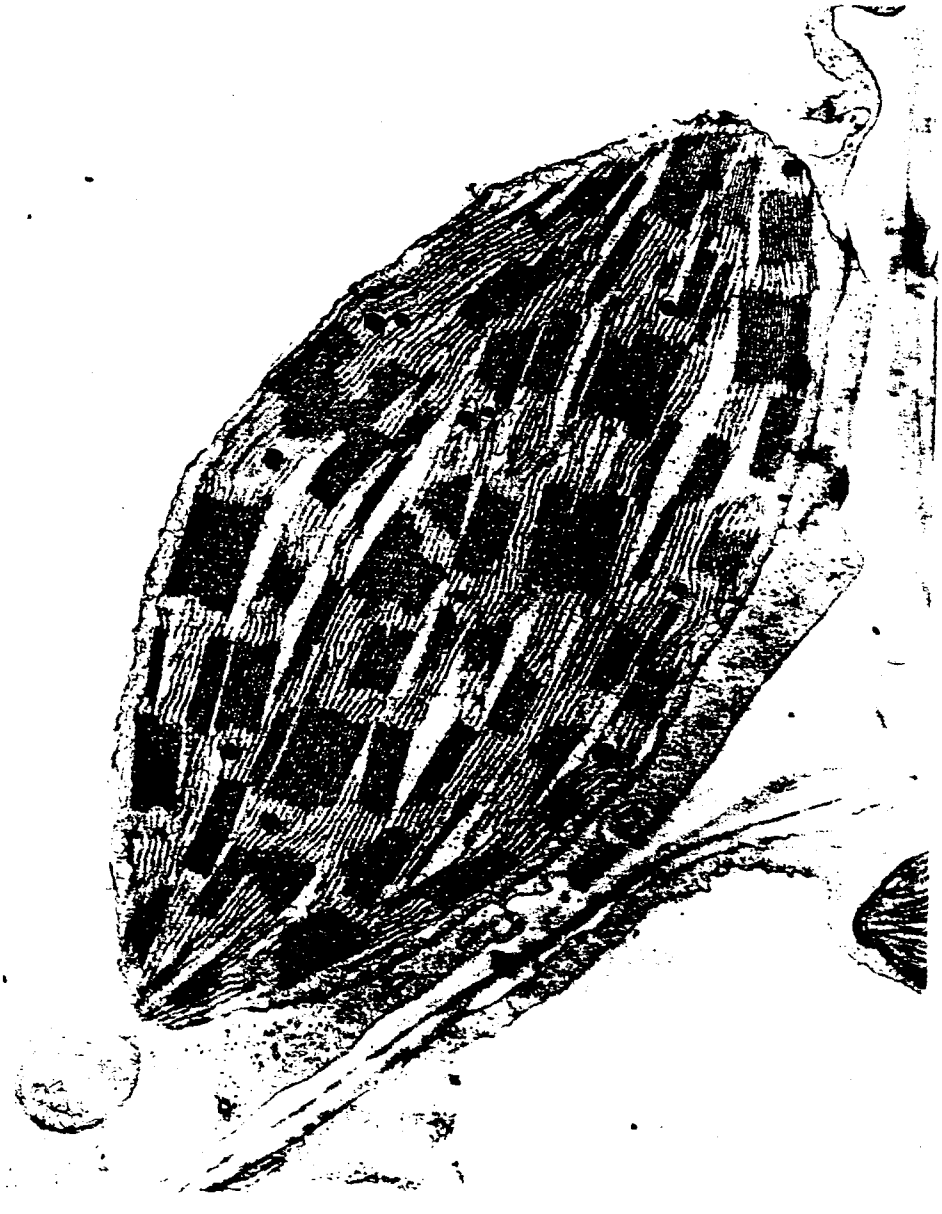
5. Oxygenic Photosynthesis

5.1 Chloroplast Structure and Organization

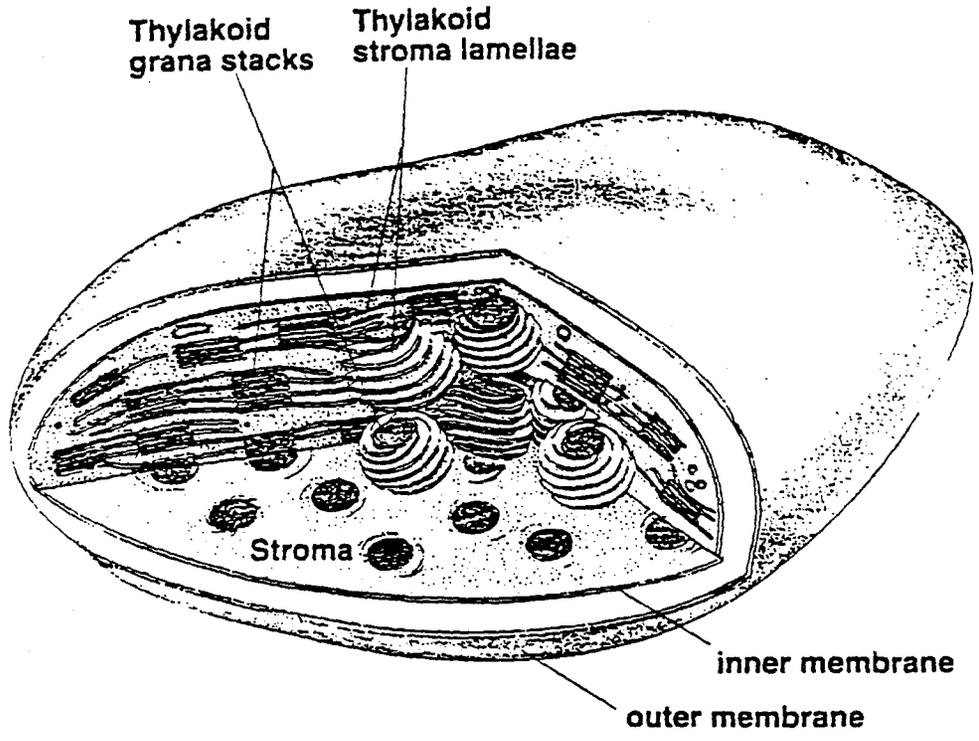
In plants the photosynthetic process occurs inside chloroplasts, which are organelles found in certain cells. Chloroplasts provide the energy and reduced carbon needed for plant growth and development, while the plant provides the chloroplast with CO_2, water, nitrogen, organic molecules and minerals necessary for chloroplast biogenesis. Most chloroplasts are located in specialized leaf cells, which often contain 50 or more chloroplasts per cell. Chloroplasts are generally defined by an inner and an outer envelope membrane and are shaped like a meniscus convex lens 5–10 microns in diameter (Fig. 2), although many different shapes and sizes can be found in plants. For details of chloroplast structure, see Staehlin (1986). The inner envelope membrane acts as a barrier, controlling the flux of organic and

charged molecules in and out of the chloroplast. Water passes freely through the envelope membranes, as do other small neutral molecules like CO_2 and O_2. There is evidence that chloroplasts were once free living bacteria that invaded a non-photosynthetic cell long ago. They have retained some of the DNA necessary for their assembly, while much of the DNA necessary for their biosynthesis is located in the cell nucleus. This enables a cell to control the biosynthesis of chloroplasts within its domain.

Inside the chloroplast is a complicated membrane system, known as the photosynthetic membrane (or thylakoid membrane), that contains most of the proteins required for the light reactions. The proteins required for the fixation and reduction of CO_2 are located outside the photosynthetic membrane



(a)



(b)

Fig. 2 (a): Electron micrograph of a plant chloroplast (Micrograph by A.D. Greenwood, courtesy of J. Barber). Chloroplast is about 6μm long. Inside the chloroplast is the photosynthetic membrane, which is organized into stacked and unstacked regions. It is not known why the photosynthetic membrane forms such a complicated architecture. The stacked regions are linked by unstacked membranes. (b): Model of the chloroplast (Ort, 1994) showing the photosynthetic membrane.

in the surrounding aqueous phase. The photosynthetic membrane is composed mainly of glycerol lipids and protein. The glycerol lipids are a family of molecules characterized by a polar head group that is hydrophilic and two fatty acid side chains that are hydrophobic. In membranes. the lipid molecules arrange themselves in a bilayer, with the polar head toward the water phase and the fatty acid chains aligned inside the membrane forming a hydrophobic core (Fig. 3). The photosynthetic membrane is vesicular, defining a closed space with an outer water space (stromal phase) and an inner water space (lumen). The organization of the photosynthetic membrane can be described as groups of stacked membranes (like stacks of pita bread with the inner pocket representing the inner aqueous space), interconnected by non-stacked membranes that protrude from the edges of the stacks (Fig. 2). Experiments indicate that the inner aqueous space of the photosynthetic membrane is likely continuous within the chloroplast. It is not known why the photosynthetic membrane forms such a convoluted structure. To understand the energetics of photosynthesis the complicated structure can be ignored and the photosynthetic membrane can be viewed as a simple vesicle.

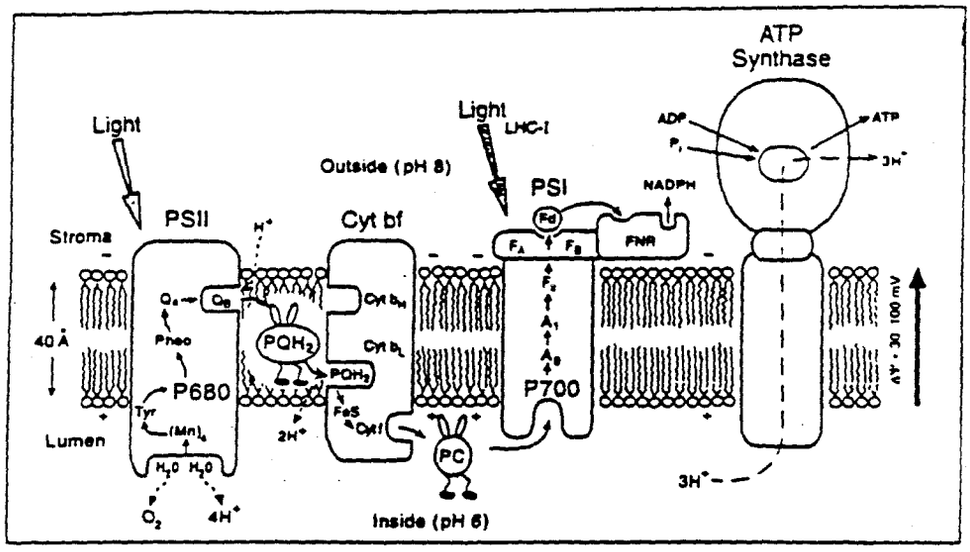


Fig. 3 Model of the photosynthetic membrane of plants showing the electron transport components and the ATP synthase enzyme (cross sectional view). Complete membrane forms a vesicle. Pathways of electrons are shown by solid arrows. Membrane bound electron transport protein complexes involved in transferring electrons are the photosystem II and I reaction centers (PSII and PSI) and the cytochrome bf complex (Cyt bf). Tyr, a specific tyrosine on the D1 protein; P680 and P 700, the reaction center chlorophyll of photosystem II and photosystem I, respectively; Pheo, pheophytin; Q_A, and Q_B bound plastoquinones; PQH_2, reduced plastoquinone; Cyt b_L and Cyt b_H, different forms of b-type cytochromes; FeS, iron-sulfur centers; Cyt f, cytochome f; PC, plastocyanin; A_0, chlorophyll; A_1, phylloquinone; F_X, F_A and F_B, iron sulfur centers; Fd ferredoxin; FNR, ferredoxin/NADP⁺ oxidoreductase; NADPH, nicotinamide adenine dinucleotide phosphate (reduced form); ADP, adenosine diphosphate; ATP, adenosine triphosphate; P_i, inorganic phosphate; H⁺, protons; $\Delta\psi$, light-induced electrical potential across the membrane. Plastoquinone (PQ, PQH_2) and plastocyanin (PC) are shown with feet to indicate that they are mobile. The light-harvesting protein complexes are not shown.

5.2 Light Absorption—The Antenna System

Plant photosynthesis is driven primarily by visible light (wavelengths from 400 to 700 nm) that is absorbed by pigment molecules (mainly chlorophyll *a* and *b* and carotenoids). The chemical structure of chlorophyll *a* molecules is shown in Fig. 4. In chlorophyll *b*, CH_3 in ring II is replaced by CHO group. Plants appear green because of chlorophyll, which is so plentiful that regions of the earth appear green from space. The absorption spectrum of chlorophyll *a* chlorophyll *b* and carotenoids, along with the action spectrum of photosynthesis of a chloroplast is shown in Fig. 5. Light is collected by 200–300 pigment molecules that are bound to light-harvesting protein complexes located in the photosynthetic membrane. The light-harvesting complexes surrounding the reaction centers serve as an antenna. The three-dimensional structure of a light-harvesting complex, labeled as LHCIIb

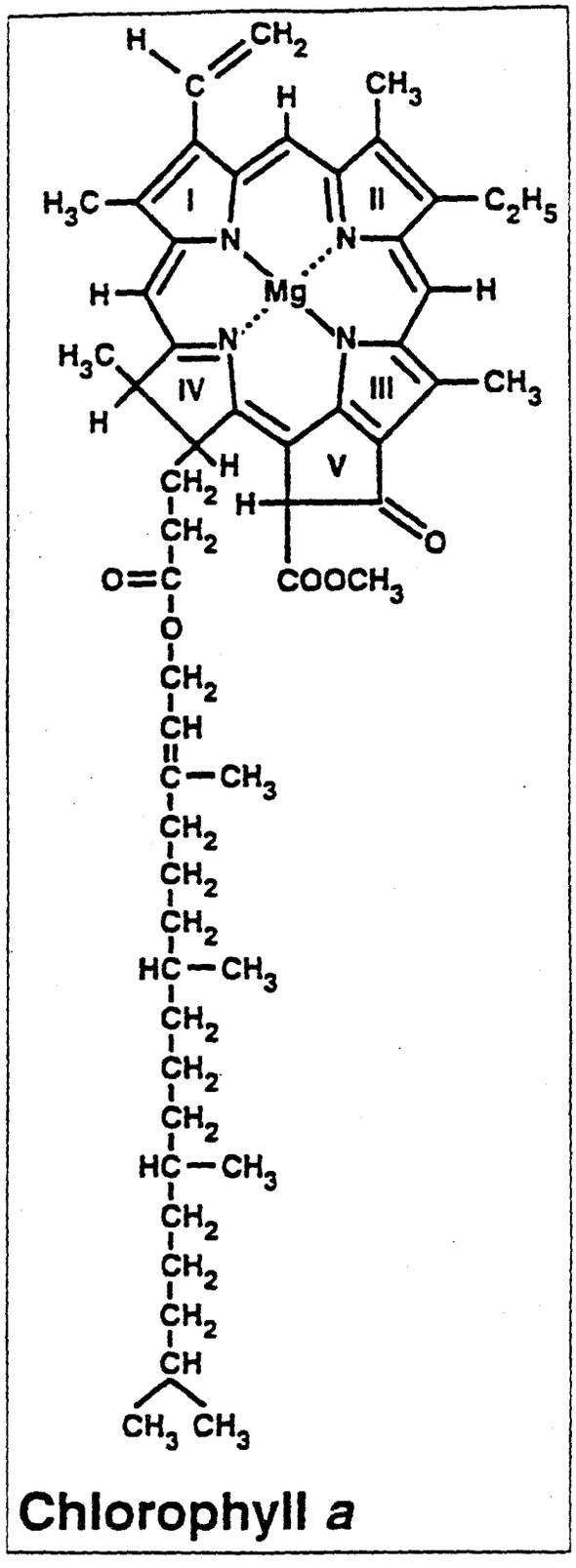


Fig. 4 Chemical structure of chlorophyll *a* molecule.

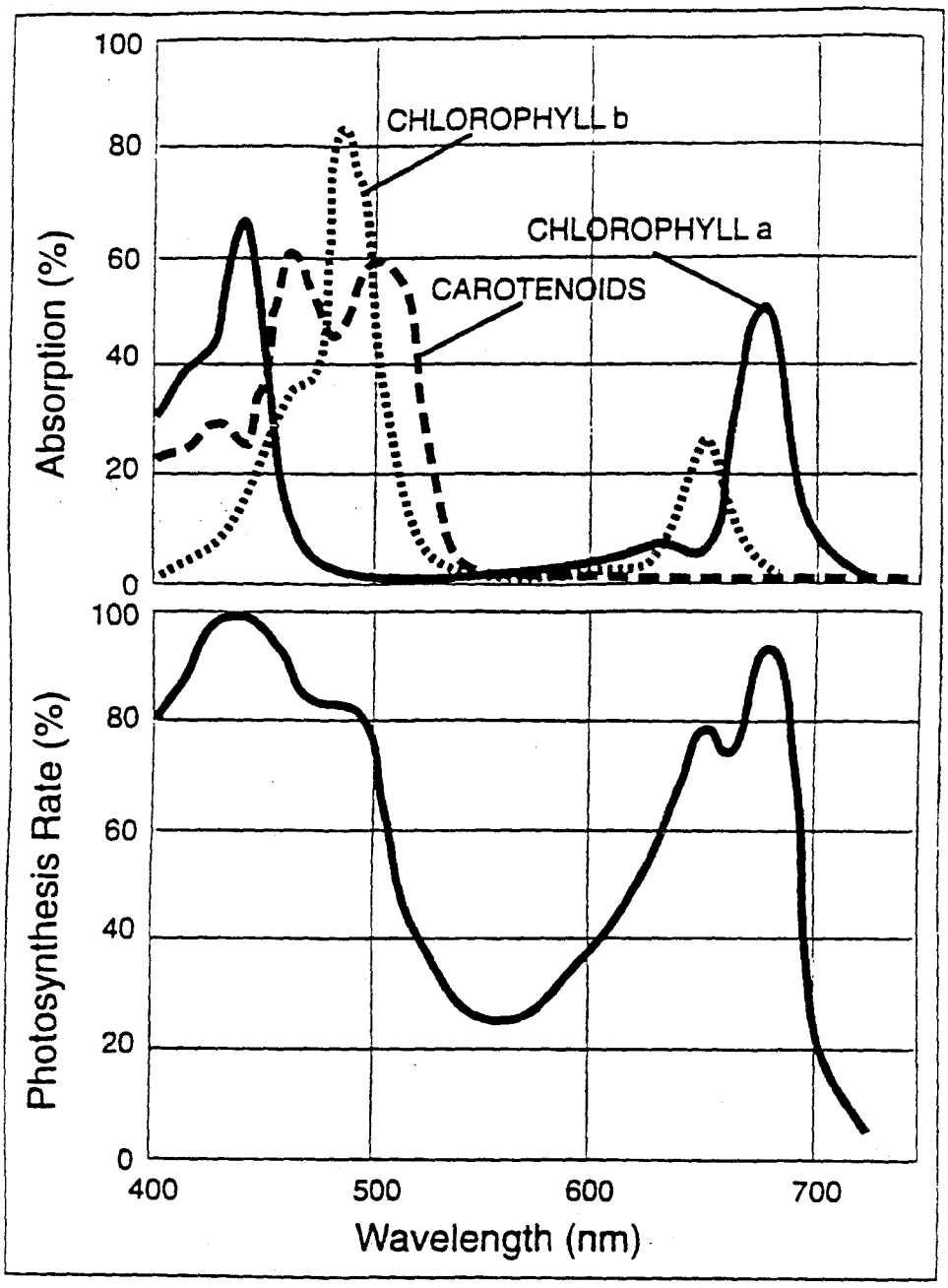


Fig. 5 **Estimated absorption spectra of chlorophyll *a*, chlorophyll *b* and carotenoids in chloroplasts (top). Action spectrum of photosynthesis (oxygen evolution/ incident photon) shows peaks at wavelengths where chlorophylls *a* and *b* have absorption peaks, proving that light absorbed by these pigments leads to photosynthesis (Govindjee unpublished data, 1961) (bottom).**

(Kühlbrandt et al., 1994) shows that the protein determines the position and orientation of the antenna pigments.

Photosynthesis is initiated by the absorption of a photon by an antenna molecule, which occurs in about a femtosecond (10^{-15} s) and causes a transition from the electronic ground state to an excited state. Within

10^{-13} s the excited state decays by vibrational relaxation to the first excited singlet state. The fate of the excited state energy is guided by the structure of the protein. Because of the proximity of other antenna molecules with the same or similar energy states, the excited state energy has a high probability of being transferred by resonance energy transfer to a near neighbor. Exciton energy transfer between antenna molecules is due to the interaction of the transition dipole moment of the molecules. The probability of transfer is dependent on the distance between the transition dipole of the donor and acceptor molecules ($1/R^6$), the relative orientation of the transition dipoles, and the overlap of the emission spectrum of the donor molecule with the absorption spectrum of the acceptor molecule (see van Grondelle and Amesz, 1986). Photosynthetic antenna systems are very efficient at this transfer process. Under optimum conditions over 90% of the absorbed quanta

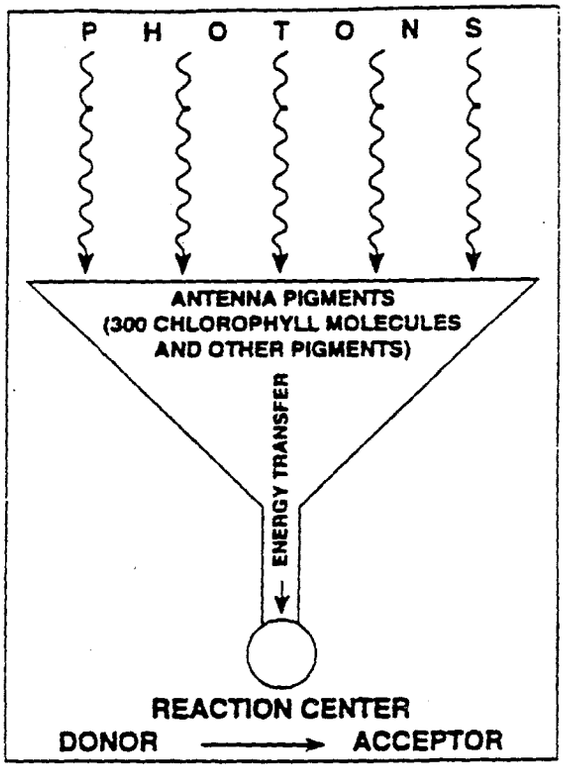


Fig. 6 A simplified scheme showing light absorption in antenna pigments followed by excitation energy transfer to a reaction center chlorophyll. The antenna and reaction center chlorophyll molecules are physically located in different proteins. Primary photochemistry (electron transfer from the primary electron donor to the primary electron acceptor) takes place in the reaction center.

are transferred within a few hundred picoseconds from the antenna system to the reaction center which acts as a trap for the exciton. A simple model of the antenna and its reaction center is shown in (Fig. 6).

5.3 Primary Photochemistry—Photosystem II and Photosystem I Reaction Centers

Photosystem II uses light energy to drive two chemical reactions—the oxidation of water and the reduction of plastoquinone. The photosystem II complex is composed of more than fifteen polypeptides and at least nine different redox components (chlorophyll, pheophytin, plastoquinone, tyrosine Y_z, Mn, Fe, cytochrome b559, carotenoid and histidine) have been shown to undergo light-induced electron transfer (Debus, 1992). However, only five of these redox components are known to be involved in transferring electrons from H_2O to the plastoquinone pool—the water oxidizing manganese cluster $(Mn)_4$, a specific amino acid tyrosine (# 161 on D1 protein), the reaction center chlorophyll (P680), pheophytin, and two plastoquinone molecules, Q_A and Q_B. Of these essential redox components, tyrosine Y_z, P680, pheophytin, Q_A and Q_B have been shown to be bound to two key polypeptides that form the heterodimeric reaction center core of photosystem II (D1 and D2). Recent work indicates that the D1 and D2 polypeptides also provide

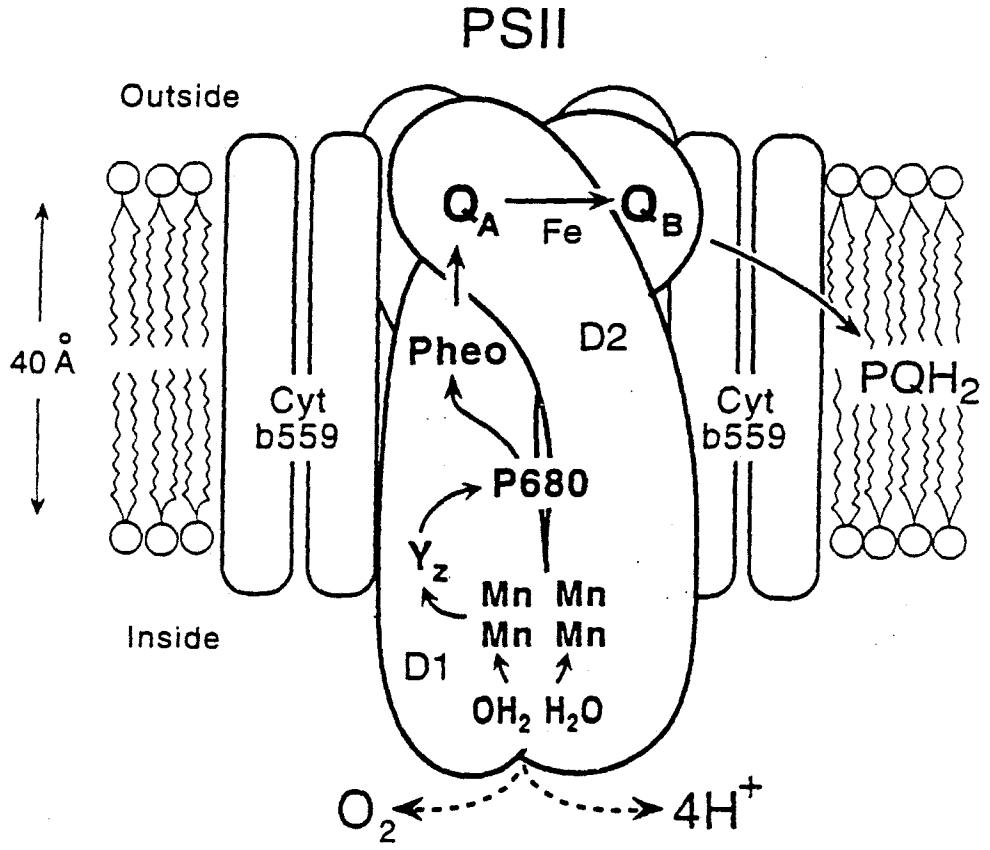


Fig. 7 Schematic drawing of photosystem II. Photosystem II is composed of numerous polypeptides, but only two of them, D1 and D2, bind the electron carriers involved in transferring electrons from Y_z to plastoquinone. Y_z, tyrosine; P680, reaction center chlorophyll (primary electron donor); Pheo, pheophytin; Q_A and Q_B, bound plastoquinone; PQH_2, reduced plastoquinone, Cyt b 559, b-type cytochrome. Details are given in the text.

ligands for the $(Mn)_4$ cluster. The three-dimensional structure of photosystem II is not known. Our knowledge of its structure is guided by the known structure of the reaction center in purple bacteria (see e.g.; Xiong et al., 1996) and biochemical and spectroscopic data. Fig. 7 shows a schematic view of photosystem II that is consistent with current data.

Photochemistry in photosystem II is initiated by charge separation between P680 and pheophytin, creating $P680^+/Pheo^-$. Primary charge separation takes a few picoseconds (Fig. 8). Subsequent electron transfer steps have been designed through evolution to prevent the primary charge separation from recombining. This is accomplished by transferring the electron within 200 picoseconds from pheophytin to a plastoquinone molecule (Q_A) that is permanently bound to photosystem II. Although plastoquinone normally acts as a two-electron acceptor, it works as a one-electron acceptor at the Q_A-site. The electron on Q_A is then transferred to another plastoquinone molecule that is loosely bound at the Q_B-site. Plastoquinone at the Q_B-site differs from Q_A in that it works as a two-electron acceptor, becoming fully reduced and protonated after two photochemical turnovers of the reaction center. The full reduction of plastoquinone requires the addition of two electrons and two protons, i.e., the addition of two hydrogen atoms. The

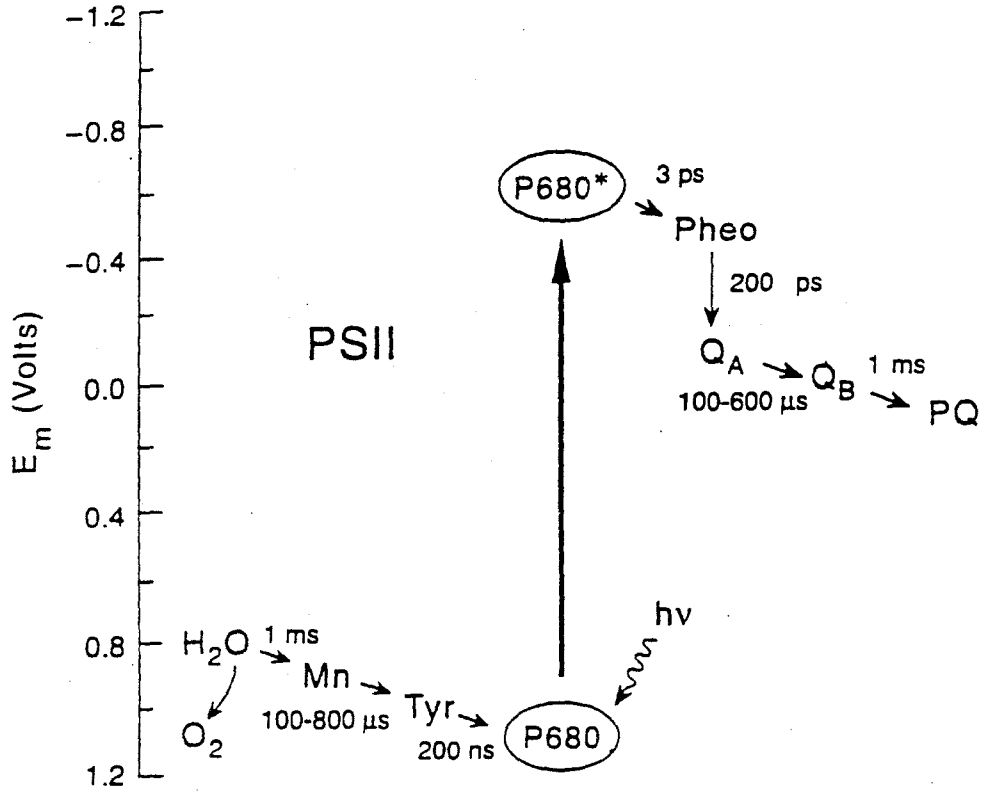


Fig. 8 Photosystem II electron transport pathways and transfer times. The vertical axis shows the midpoint potential of the electron carriers. The heavy vertical arrow shows the result of light absorption. P680* is the electronically excited state of P680. The abbreviations are given in the legend of Fig. 3.

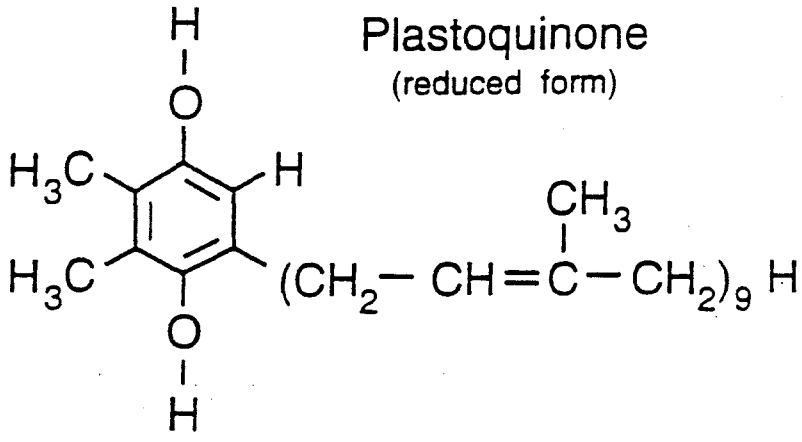


Fig. 9 Structure of plastoquinone (reduced from), an aromatic molecule that carries electrons and protons in photosynthetic electron transport.

reduced plastoquinone (Fig. 9) then debinds from the reaction center and diffuses into the hydrophobic core of the membrane. After which, an oxidized plastoquinone molecule finds its way to the Q_B-binding site and the process is repeated. Because the Q_B-site is near the outer aqueous phase, the protons added to plastoquinone during its reduction are taken from the outside of the membrane.

Photosystem II is the only known protein complex that can oxidize water, resulting in the release of O_2 into the atmosphere. Despite years of research, little is known about the molecular events that lead to water oxidation. Energically, water is a poor electron donor. The oxidation-reduction midpoint potential ($E_{m,7}$) of water is +0.82 V (pH 7). In photosystem II this reaction is driven by the oxidized reaction center, $P680^+$ (the midpoint potential of $P680/P680^+$ is estimated to be +1.2 V at pH 7). How electrons are transferred from water to $P680^+$ remains a mystery (Govindjee and Coleman, 1990). It is known that $P680^+$ oxidizes a tyrosine on the D1 protein and that Mn plays a key role in water oxidation. Four Mn ions are present in the water oxidizing complex. X-ray absorption spectroscopy shows that Mn undergoes light-induced oxidation. Water oxidation requires two molecules of water and involves four sequential turnovers of the reaction center. This was shown by an experiment demonstrating that oxygen release by photosystem II occurs with a four flash dependence (Fig. 10; Joliot et al., 1969; Joliot and Kok, 1975). Each photochemical reaction creates an oxidant that removes one electron. The net reaction results in the release of one O_2 molecule, the deposition of four protons into the inner water phase, and the transfer of four electrons through the Q_B-site (producing two reduced plastoquinone molecules) (reviewed by Renger, 1993; Klein et al., 1993; and Lavergne and Junge, 1996).

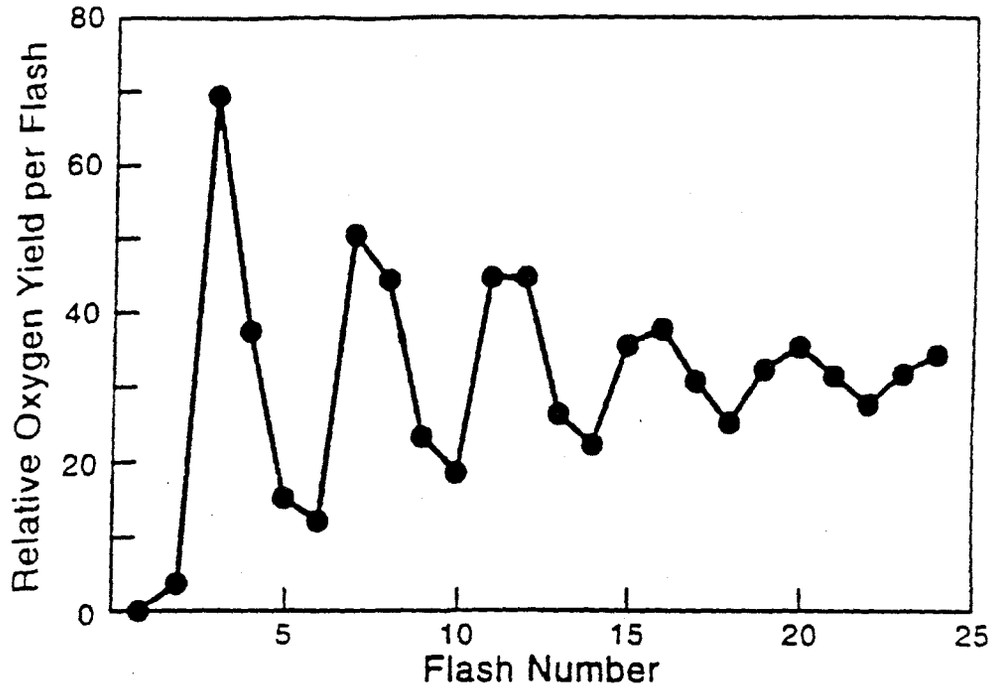


Fig. 10 **Yield of oxygen from photosynthetic membranes exposed to a series of brief flashes as a function of flash number. Maximum oxygen yield exhibits a four-flash periodicity. Yield is highest after the third flash and peaks again four flashes later. The four flash dependence of the amplitude gradually decreases as the number of flashes increases due to misses and double hits. Occurrence of the peaks every 4th flash is due to the chemistry of water oxidation (4 electrons must be removed from two water molecules to yield one oxygen molecule) and the machinery of photosystem II (each reaction center works independently, binding two water molecules and releasing one molecule of oxygen every four flashes). Water oxidizing machinery works as a cyclic process that supplies electrons to the oxidized primary donor, $P680^+$. After one flash of light, $P680^+$ is formed, and an electron is transferred via the tyrosine Y_Z from a manganese complex (4 Mn atoms). After a second flash, this process is repeated and a second oxidation occurs at the Mn complex; after a third flash, a third oxidation occurs; and after a fourth flash, a fourth oxidation occurs, i.e., the Mn complex accumulates 4 positive (+) charges. This enables the Mn complex to oxidize $2H_2O$, release molecular oxygen and 4 protons (H^+s). This is the process known as the oxygen clock.**

Photosystem II reaction centers contain a number of redox components with no known function. An example is cytochrome b559, a heme protein, that is an essential component of all photosystem II reaction centers (discussed by Whitmarsh and Pakrasi, 1996). If the cytochrome is not present in the membrane, a stable photosystem II reaction center cannot be formed. Although the structure and function of Cyt b559 remain to be discovered, it is known that the cytochrome is not involved in the primary enzymatic activity of photosystem II, which is the transfer of electrons from water to plastoquinone. Why photosystem II reaction centers contain redox components that are not

involved in the primary enzymatic reactions is a puzzling question. The answer may be found in the unusual chemical reactions occurring in photosystem II and the fact that the reaction center operates at a very high power level. Photosystem II is an energy transforming enzyme that must switch between various high energy states that involve the creation of the powerful oxidants required for removing electrons from water and the complex chemistry of plastoquinone reduction which is strongly influenced by protons. In saturating light a single reaction center can have an energy throughput of 600 eV/s (equivalent to 60,000 kW per mole of photosystem II). Operating at such a high power level causes damage to the reaction center. It may be that some of the "extra" redox components in photosystem II serve to protect the reaction center.

Photosystem II has another perplexing feature. Many plants and algae have been shown to have a significant number of photosystem II reaction centers that do not contribute to photosynthetic electron transport (e.g., Chylla and Whitmarsh, 1989). Why plants devote resources for the synthesis of reaction centers that apparently do not contribute to energy conversion is unknown (for reviews of photosystem II heterogeneity see Ort and Whitmarsh, 1990; Guenther and Melis, 1990; Govindjee, 1990; Melis, 1991; Lavergne and Briantais, 1996; Oxborough et al., 1996). Further, photosystem II is unique among the photosystems in showing a bicarbonate-reversible formate inhibition (Govindjee and Van Rensen, 1993).

The photosystem I complex catalyzes the oxidation of plastocyanin, a small soluble Cu-protein, and the reduction of ferredoxin, a small FeS protein (Fig. 11). Photosystem I is composed of a heterodimer of proteins that act as ligands for most of the electron carriers (Krauss et al., 1993). The reaction center is served by an antenna system that consists of about two hundred chlorophyll molecules (mainly chlorophyll *a*) and primary photochemistry is initiated by a chlorophyll *a* dimer, P700. In contrast to photosystem II, many of the antenna chlorophyll molecules in photosystem I are bound to the reaction center proteins. (For an atomic structure at 4Å resolution, see Krauss et al., 1996). Also, FeS centers serve as electron carriers in photosystem I and, so far as is known, photosystem I electron transfer is not coupled to proton translocation. Primary charge separation occurs between a primary donor, P700, a chlorophyll dimer, and a chlorophyll monomer (A_0). The subsequent electron transfer events and transfer times are shown in Fig. 12 (see Golbeck, 1994).

5.4 Electron Transport

Electron transport from water to $NADP^+$ requires three membrane bound protein complexes operating in series—photosystem II, the cytochrome bf complex and photosystem I (Fig. 3). Electrons are transferred between these large protein complexes by small mobile molecules (plastoquinone and plastocyanin in plants). Because these small molecules carry electrons

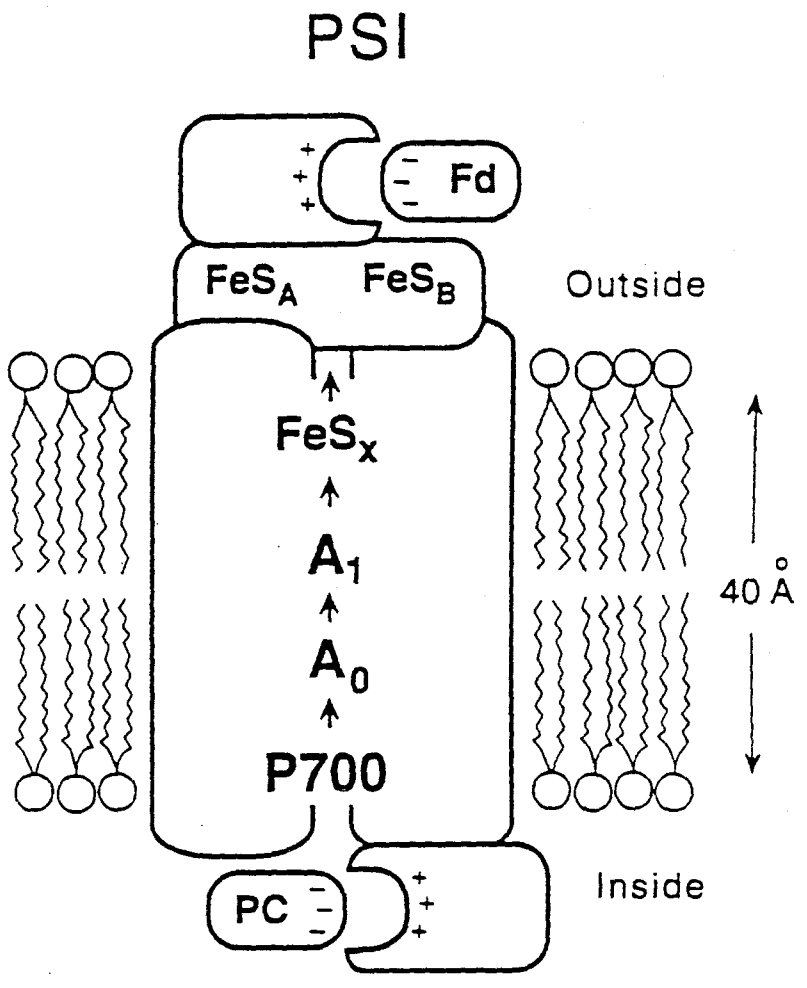


Fig. 11. Schematic drawing of photosystem I. Photosystem I is composed of numerous polypeptides, but only three of them bind the electron carriers. PC, plastocyanin; P700, reaction center chlorophyll (primary electron donor); A_0, chlorophyll; A_1, phylloquinone; FeS, FeS centers; Fd, ferredoxin. Details are given in the text.

(or hydrogen atoms) over relatively long distances, they play a unique role in photosynthetic energy conversion. This is illustrated by plastoquinone (PQ), which serves two key functions. Plastoquinone transfers electrons from the photosystem II reaction center to the cytochrome bf complex and carries protons across the photosynthetic membrane (see Kallas, 1994). It does this by shuttling hydrogen atoms across the membrane from photosystem II to the cytochrome bf complex. Because plastoquinone is hydrophobic, its movement is restricted to the hydrophobic core of the photosynthetic membrane. Plastoquinone operates by diffusing through the membrane until, due to random collisions, it becomes bound to a specific site on the photosystem II complex. The photosystem II reaction center reduces plastoquinone at the Q_B-site by adding two electrons and two protons creating PQH_2. The reduced plastoquinone molecule debinds from photosystem II and diffuses randomly in the photosynthetic membrane until it encounters

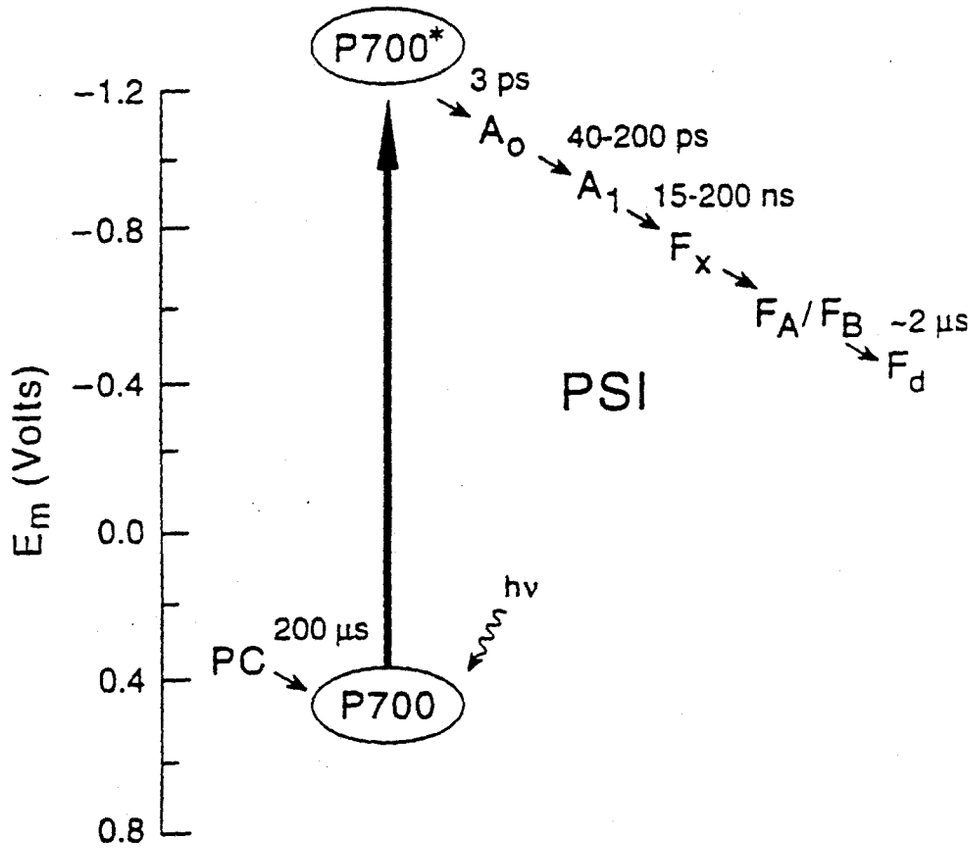


Fig. 12 **Photosystem I electron transport pathways and transfer times. Vertical axis shows the midpoint potential of the electron carriers. Abbreviations: Same as in Fig. 11 (F_A and F_B are equivalent names for FeS_A and FeS_B).**

a specific binding site on the cytochrome bf complex. The cytochrome bf complex is a membrane bound protein complex that contains four electrons carriers, three hemes and an FeS center. The crystal structure has been solved for cytochrome f from turnip (Martinez et al., 1994) and the FeS center from bovine heart mitochondria (Iwata et al., 1996). Further, the atomic structure of cyt b/c complex is now available from beef heart mitochondria (Xia et al., 1997). In a complicated reaction sequence that is not fully understood, the cytochrome bf complex removes electrons from reduced plastoquinone and facilitates the release of the protons into the inner aqueous space. The electrons are eventually transferred to the photosystem I reaction center. The protons released into the inner aqueous space contribute to the proton chemical free energy across the membrane.

Electron transfer from the cytochrome bf complex to photosystem I is mediated by a small Cu-protein, plastocyanin (PC). Plastocyanin is water soluble and operates in the inner water space of the photosynthetic membrane. Electron transfer from photosystem I to $NADP^+$ requires ferredoxin, a small FeS protein, and ferredoxin-NADP oxidoreductase, a peripheral flavoprotein that operates on the outer surface of the photosynthetic membrane. Ferredoxin and $NADP^+$ are water soluble and are found in the outer aqueous phase.

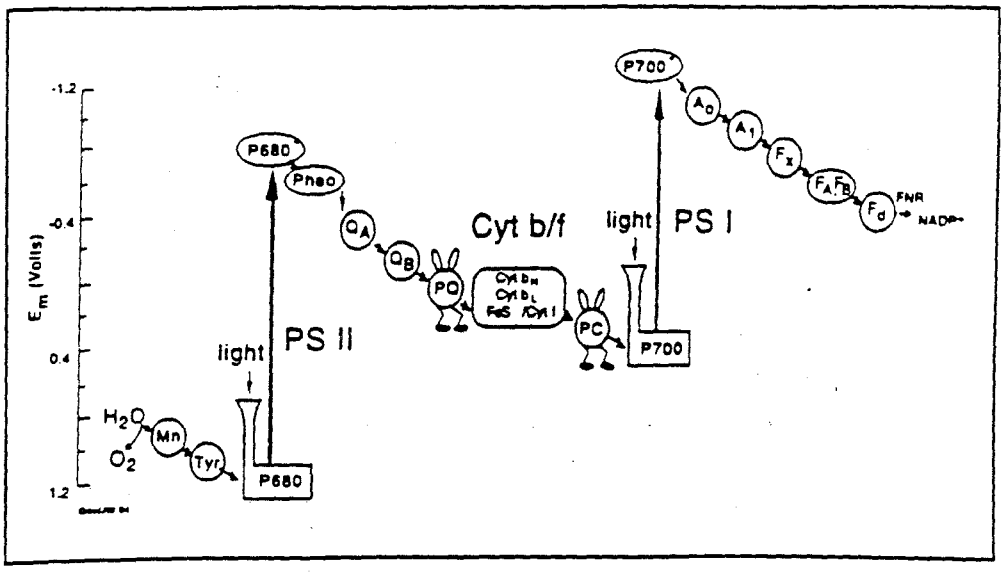


Fig. 13 Electron transport pathway of plants (oxygenic photosynthesis). Abbreviations: Same as in Fig. 3.

The pathway of electrons is largely determined by the energetics of the reaction and the distance between the carriers. The electron affinity of the carriers is represented in Fig. 13 by their midpoint potentials, which show the free energy available for electron transfer reactions under equilibrium conditions. (It should be kept in mind that reaction conditions during photosynthesis are not in equilibrium.) Subsequent to primary charge separation, electron transport is energetically downhill [from a lower (more negative) to a higher (more positive) redox potential]. It is the downhill flow of electrons that provides free energy for the creation of a proton chemical gradient.

Photosynthetic membrane effectively limits electron transport to two dimensions. For mobile electron carriers, limiting diffusion to two dimensions increases the number of random encounters (Whitmarsh, 1986). Furthermore, because plastocyanin is mobile, any one cytochrome bf complex can interact with a number of photosystem I complexes. The same is true for plastoquinone, which commonly operates at a stoichiometry of about six molecules per photosystem II complex.

5.5 Creation of a Proton Electrochemical Potential

Electron transport creates the proton electrochemical potential of the photosynthetic membrane by two types of reactions. (1) The release of protons during the oxidation of water by photosystem II and the translocation of protons from the outer aqueous phase to the inner aqueous phase by the coupled reactions of photosystem II and the cytochrome bf complex in reducing and oxidizing plastoquinone on opposite sides of the membrane. This creates a concentration difference of protons across the membranes ($\Delta pH = pH_{in} - pH_{out}$). (2) Primary charge separation at the reaction center

drives an electron across the photosynthetic membrane, which creates an electric potential across the membrane ($\Delta\Psi = \Psi_{in} - \Psi_{out}$). Together, these two forms of energy make up the proton electrochemical potential across the photosynthetic membrane ($\Delta\mu_H+$) which is related to the pH difference across the membrane and the electrical potential difference across the membrane by the following equation:

$$\Delta\mu_{H+} = F\Delta\Psi - 2.3\ RT\ \Delta\text{pH}, \tag{4}$$

where F is the Faraday constant, R the gas constant and T the temperature in Kelvin. Although the value of $\Delta\Psi$ across the photosynthetic membrane in chloroplast can be as large as 100 mV, under normal conditions the proton gradient dominates. For example, during photosynthesis the outer pH is typically near 8 and the inner pH is typically near 6, giving a pH difference of 2 across the membrane that is equivalent to 120 mV. Under these conditions the free energy for proton transfer from the inner to the outer aqueous phase is – 12 kJ/mol of proton.

5.6 Synthesis of ATP by the ATP Synthase Enzyme

The conversion of proton electrochemical energy into chemical free energy is accomplished by a single protein complex known as ATP synthase. This enzyme catalyzes a phosphorylation reaction, which is the formation of ATP by the addition of inorganic phosphate (P_i) to ADP

$$ADP^{-3} + P_i^{-2} + H^+ \rightarrow ATP^{-4} + H_2O. \tag{5}$$

The reaction is energetically uphill (ΔG = +32 kJ/mol) and is driven by proton transfer through the ATP synthase protein. The ATP synthase complex is composed of two major subunits, CF_0 and CF_1 (Fig. 14). The CF_0 subunit spans the photosynthetic membrane and forms a proton channel through the membrane. The CF_1 subunit is attached to the top of the CF_0 on the outside of the membrane and is located in the aqueous space. CF_1 is composed of several different protein subunits referred to as α, β, γ, δ and ε. The top portion of the CF_1 subunit is composed of three $\alpha\beta$-dimers that contain the catalytic sites for ATP synthesis. A recent major breakthrough has been the elucidation of the structure of ATPase of beef heart mitochondria by Abrahams et al. (1994). The molecular processes that couple proton transfer through the protein to the chemical addition of phosphate to ADP are poorly understood. It is known that phosphorylation can be driven by a pH gradient, a transmembrane electric field, or a combination of the two. Experiments indicate that three protons must pass through the ATP synthase complex for the synthesis of one molecule of ATP. However, the protons are not involved in the chemistry of adding phosphate to ADP. Paul Boyer and coworkers have proposed an alternating binding site mechanism for ATP synthesis (Boyer, 1993). One model based on their proposal is that there are three catalytic sites on each CF_1 that cycle among three different states (Fig. 15).

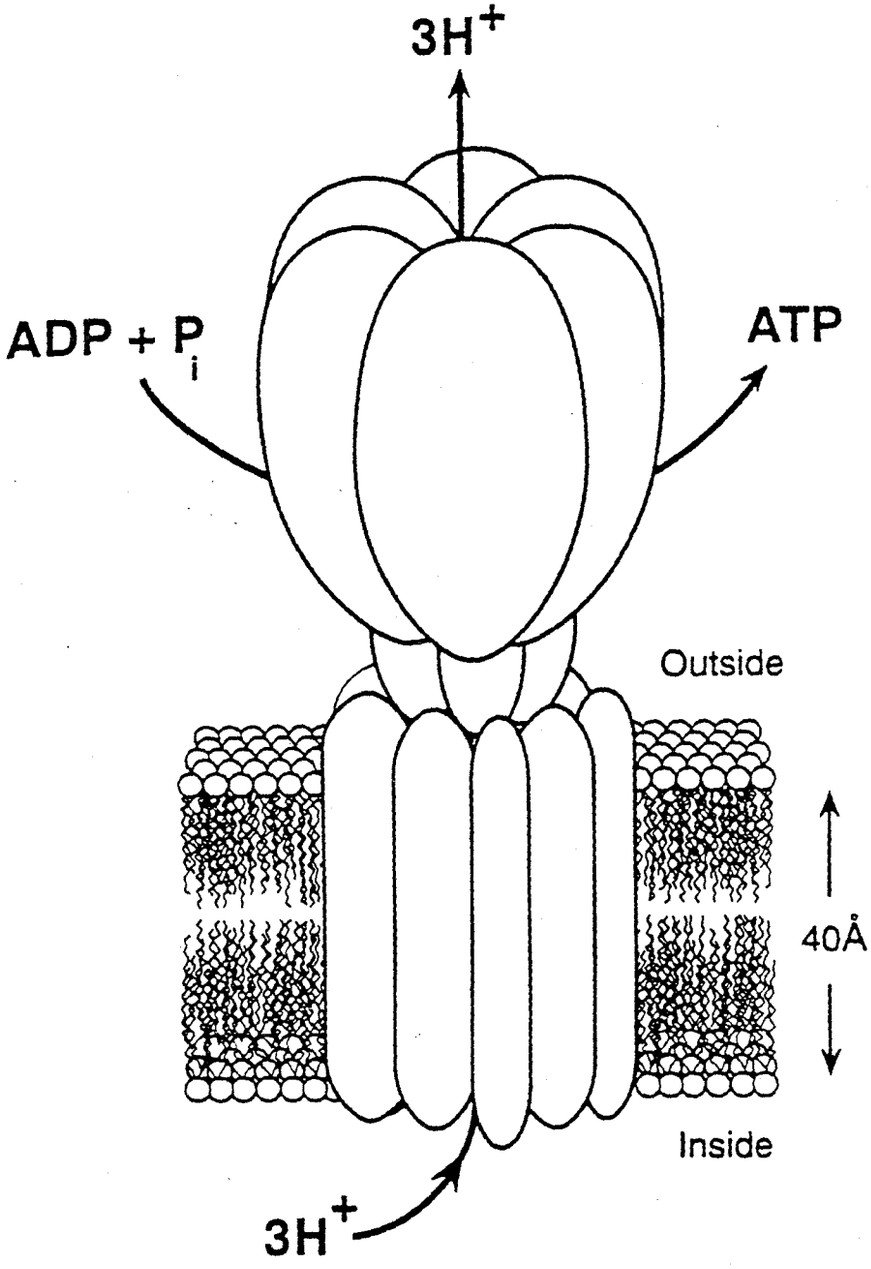


Fig. 14 Schematic drawing of the ATP synthase enzyme embedded in the membrane. Proton transfer through the ATP synthase provides the energy for the creation of ATP from ADP and P_i. Abberviations: Same as in Fig. 3.

The states differ in their affinity for ADP, P_i and ATP. At any one time, each site is in a different state. This model is supported by the structure of ATPase elucidated by Abrahams et al. (1994). Initially, one catalytic site on CF_1 binds one ADP and one inorganic phosphate molecule relatively loosely. Due to a conformational change of the protein, the site becomes a tight binding site, that stabilizes ATP. Next, proton transfer induces an alternation

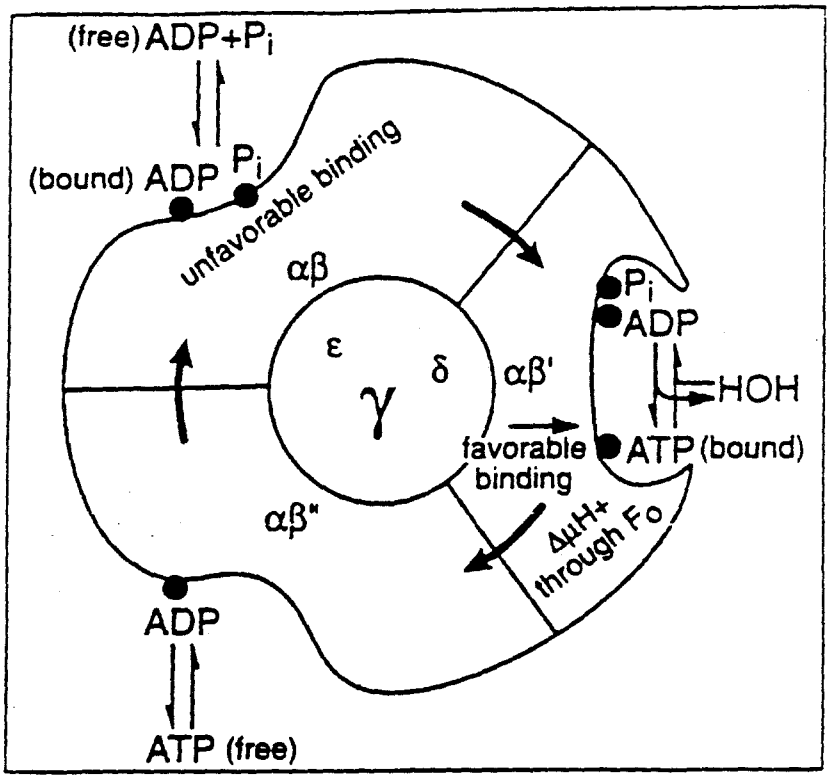


Fig. 15 **The ATP synthase consists of a membrane portion and an water exposed portion (see Fig. 14). Water exposed portion, which looks like a door knob, has five subunits (3α, 3β, 1γ, 1δ, 1ε). The 3α, 3β combine as $3\alpha/\beta$ pairs. Catalytic sites of enzyme are on the β-subunits. The γ subunit sort of connects the exposed part to the membrane part (F_o). Diagram shows a model of top of the ATP synthase (Boyer, 1993). With three alternate binding sites: at one site ADP and Pi bind; at two ADP and Pi produce bound ATP; and at third the bound ATP is released. In this model, most energy is used to release bound ATP. Each of the three sites perform all three steps, but at different times. Thus, the activity apparently rotates on the α/β pairs. Energy of the proton gradient is converted, in this model, to conformational energy of the γ protein that rotates and transfers the energy to the α/β pairs for the simultaneous binding of ADP and P_i and the release of ATP. (Evidence for such a scheme has been found by Abrahams et al. (1994) in beef-heart mitochondria and by Sabbert et al. (1996) in chloroplast.)**

in protein conformation that causes the site to release the ATP molecule into the aqueous phase. In this model, the energy from the proton electrochemical gradient is used mainly to lower the affinity of the site for ATP, allowing its release to the water phase. The three sites on CF_1 act cooperatively, i.e., the conformational states of the sites are linked. It has been proposed that protons affect the conformational change of part of CF_1. It is the γ subunit, that protrudes into the space between the α-β pairs, does

the rotating. Such a rotating model has recently been supported by recording of a rotation of the gamma subunit relative to the alpha-beta subunits by Sabbert et al. (1996). This revolving site mechanism would require rates as high as 100 revolutions per second to account for steady state rates of ATP synthesis. It is worth noting that flagella that propel some bacteria are driven by a proton pump and can rotate at 60 revolutions per second.

5.7 Synthesis of Carbohydrates

All plants and algae remove CO_2 from the environment and reduce it to carbohydrate. The process is a sequence of biochemical reactions that reduce carbon and rearrange bonds to produce carbohydrate from CO_2 molecules. In the Calvin cycle, the first step is the addition of CO_2 to a five-carbon compound (ribulose 1,5-bisphosphate) (Fig. 16). The six-carbon compound is spilt, giving two molecules of a three-carbon compound (3-phosphoglycerate). This key reaction is catalyzed by Rubisco, a large water soluble protein complex. The 3-dimensional structure has been determined by X-ray analysis for Rubisco isolated from tobacco (Schreuder et al., 1993), from a cyanobacterium (*Synechococcus)* (Newman and Gutteridge, 1993), and from a purple bacterium (*Rhodospirillum rubrum*) (Schneider et al., 1990). The carboxylation reaction is energetically downhill. The main

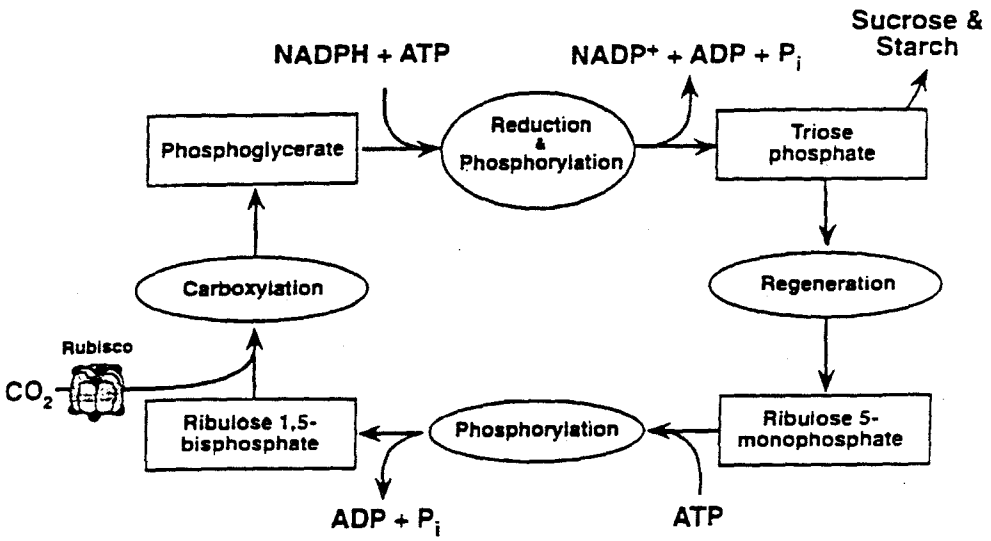


Fig. 16 **An abbreviated scheme showing reduction of CO_2 by the Calvin Cycle. First step is carboxylation, in which Ribulose 1,5-bisphosphate carboxylase/ oxygenase (Rubisco) catalyzes the addition of CO_2 to the five-carbon compound, ribulose, 1,5-bisphosphate, which is subsequently split into two molecules of the three-carbon compound, 3-phosphoglycerate. Next are reduction and phosphorylation reactions that form the carbohydrate, triose phosphate. Some of the triose phosphate molecules are used to form the products of photosynthesis, sucrose and starch, while the rest is used to regenerate ribulose 1,5-bisphosphate neded for the continuation of the cycle.**

energy input in the Calvin cycle is the phosphorylation by ATP and subsequent reduction by NADPH of the initial three-carbon compound forming a three-carbon sugar, triosephosphate. Some of the triosephosphate is exported from the chloroplast and provides the building block for synthesizing more complex molecules. In a process known as regeneration, the Calvin cycle uses some of the triosephosphate molecules to synthesize the energy rich ribulose 1,5-bisphosphate needed for the initial carboxylation reaction. This reaction requires the input of energy in the form of one ATP. Overall, thirteen enzymes are required to catalyze the reactions in the Calvin cycle. The energy conversion efficiency of the Calvin cycle is approximately 90%. The reactions do not involve energy transduction, but rather the rearrangement of chemical energy. Each molecule of CO_2 reduced to a sugar $[CH_2O]_n$ requires 2 molecules of NADPH and 3 molecules of ATP.

Rubisco is a bifunctional enzyme that in addition to binding CO_2 to ribulose bisphosphate, can also bind O_2. This oxygenation reaction produces the 3-phosphoglycerate that is used in the Calvin cycle and a two-carbon compound (2-phosphoglycolate) that is not useful for the plant. In response, a complicated set of reactions (known as photorespiration) are initiated that serve to recover reduced carbon and to remove phosphoglycolate. The Rubisco oxygenation reaction appears to serve no useful purpose for the plant. Some plants have evolved specialized structures and biochemical pathways that concentrate CO_2 near Rubisco. These pathways (C_4 and CAM), serve to decrease the fraction of oxygenation reactions.

5.8 Photosynthetic Quantum Yield and Energy Conversion Efficiency

The theoretical minimum quantum requirement for photosynthesis is 8 quanta for each molecule of oxygen evolved (four quanta required by photosystem II and four by photosystem I). Measurements in algal cells and leaves under optimal conditions (e.g., low light) give quantum requirements of 8-10 photons per oxygen molecule released (see Emerson, 1958). These quantum yield measurements show that the quantum yields of photosystem II and photosystem I reaction centers under optimal conditions are near 100%. These values can be used to calculate the theoretical energy conversion efficiency of photosynthesis (free energy stored as carbohydrate/light energy absorbed). If 8 red quanta are absorbed (8 mol of red photons are equivalent to 1,400 kJ) for each CO_2 molecule reduced (480 kJ/mol), the theoretical maximum energy efficiency for carbon reduction is 34%. Under optimal conditions, plants can achieve energy conversion efficiencies within 90% of the theoretical maximum. However, under normal growing conditions the actual performance of the plant is far below these theoretical values. The factors that conspire to lower the quantum yield of photosynthesis include limitations imposed by biochemical reactions in the plant and environmental conditions that limit photosynthetic performance. One of the most efficient crop plants is sugar cane, which has been shown to store up

to 1% of the incident visible radiation over a period of one year. However, most crops are less productive. The annual conversion efficiency of corn, wheat, rice, potatoes, and soybeans typically ranges from 0.1% to 0.4% (Odum, 1971).

5.9 Oxygenic Photosynthesis in Algae

Algae are photosynthetic eukaryotic organisms that, like plants, evolve O_2 and reduce CO_2. They represent a diverse group that include the dino-flagellates, the euglenoids, yellow-green algae, golden-brown algae, diatoms, red algae, brown algae, and green algae. The photosynthetic apparatus and biochemical pathways of carbon reduction of algae are similar to plants. Photosynthesis occurs in chloroplasts that contain photosystems II and I, the cytochrome bf complex, the Calvin cycle enzymes and pigment-protein complexes containing chlorophyll *a*, and other antenna pigments (e.g., chlorophyll *b* in green algae, chlorophyll *c* and fucoxanthol in brown algae and diatoms, and phycobilins in red algae). Green algae are thought to be the ancestral group from which land plants evolved (see Douglas, 1994). Algae are abundant and widespread on the earth, living mainly in fresh and sea water. Some algae live as single celled organisms, while others form multicellular organisms some of which can grow quite large, like kelp and seaweed. Phytoplankton in the ocean is made up of algae and oxygenic photosynthetic bacteria. Most photosynthesis in the ocean is due to phytoplankton, which is an important source of food for marine life.

5.10 Oxygenic Photosynthesis in Bacteria

Cyanobacteria are photosynthetic prokaryotic organisms that evolve O_2 (Bryant, 1994). Fossil evidence indicates that cyanobacteria existed over 3 billion years ago and it is thought that they were the first oxygen evolving organisms on Earth (Wilmotte, 1994). Cyanobacteria are presumed to have evolved in water in an atmosphere that lacked O_2. Initially, the O_2 released by cyanobacteria reacted with ferrous iron in the oceans and was not released into the atmosphere. Geological evidence indicates that the ferrous Fe was depleted around 2 billion years ago, and earth's atmosphere became aerobic. The release of O_2 into the atmosphere by cyanobacteria has had a profound effect on the evolution of life.

The photosynthetic apparatus of cyanobacteria is similar to that of chloroplasts. The main difference is in the antenna system. Cyanobacteria depend on chlorophyll *a* and specialized protein complexes (phycobilisomes) to gather light energy (Sidler, 1994). They do not contain chlorophyll *b*. As in chloroplasts, the chlorophyll *a* is located in membrane bound proteins. The phycobilisomes are bound to the outer side of the photosynthetic membrane and act to funnel exciton energy to the photosystem II reaction center. They are composed of phycobiliproteins, protein subunits that contain covalently attached open ring structures known as bilins that are the light

absorbing pigments. Primary photochemistry, electron transport, phosphorylation and carbon reduction occur much as they do in chloroplasts. Cyanobacteria have a simpler genetic system than plants and algae that enable them to be easily modified genetically. Because of this cyanobacteria have been used as a model to understand photosynthesis in plants. By genetically altering photosynthetic proteins, researchers can investigate the relationship between molecular structure and mechanism (Barry et al., 1994).

Over the past three decades several types of oxygenic bacteria known as prochlorophytes (or oxychlorobacteria) have been discovered that have light harvesting protein complexes that contain chlorophyll *a* and *b*, but do not contain phycobilisomes* (Palenik and Haselkorn, 1992; Urbach et al., 1992; Matthijs et al., 1994). Because prochlorophytes have Chlorophyll *a/b* light harvesting proteins like chloroplasts, they are being investigated as models for plant photosynthesis.

6. Anoxygenic Photosynthesis

Anoxygenic photosynthetic bacteria differ from oxygenic organisms in that each species has only one type of reaction center (Blankenship et al., 1995). In some photosynthetic bacteria the reaction center is similar to photosystem II and in others it is similar to photosystem I. However, neither of these two types of bacterial reaction center is capable of extracting electrons from water, so they do not evolve O_2. Many species can only survive in environments that have a low concentration of O_2. To provide electrons for the reduction of CO_2, anoxygenic photosynthetic bacteria must oxidize inorganic or organic molecules available in their environment. For example, the purple bacterium *Rhodobacter sphaeroides* can use succinate to reduce NAD^+ by a membrane-linked reverse electron transfer that is driven by a transmembrane electrochemical potential. Although many photosynthetic bacteria depend on Rubisco and the Calvin cycle for the reduction of CO_2, some are able to fix atmospheric CO_2 by other biochemical pathways.

Despite these differences, the general principles of energy transduction are the same in anoxygenic and oxygenic photosynthesis. Anoxygenic photosynthetic bacteria depend on bacteriochlorophyll, a family of molecules that are similar to the chlorophyll, that absorb strongly in the infrared between 700 and 1000 nm. The antenna system consists of bacteriochlorophyll and carotenoids that serve a reaction center where primary charge separation occurs. The electron carriers include quinone (e.g., ubiquinone, menaquinone) and the cytochrome bc complex, which is similar to the cytochrome bf complex of oxygenic photosynthetic apparatus. As in oxygenic photosynthesis, electron transfer is coupled to the generation of an electrochemical potential

*In a recent paper, however, Hess et al. (1996) have shown that at least in one prochlorophyte, *Prochlorococcus marinus*, both phycobiliproteins and chlorophyll *a/b* coexist.

that drives phosphorylation by ATP synthase and the energy required for the reduction of CO_2 is provided by ATP and NADH, a molecule similar to NADPH.

6.1 Purple Bacteria

There are two divisions of photosynthetic purple bacteria, the non-sulfur purple bacteria (e.g., *Rhodobacter sphaeroides* and *Rhodospeudomonas viridis)* and the sulfur purple bacteria (e.g., *Chromatium vinosum)* (Blankenship et al., 1995). Non-sulfur purple bacteria typically use an organic electron donor, such as succinate or malate, but they can also use hydrogen gas. The sulfur bacteria use an inorganic sulfur compound, such as hydrogen sulfide as the electron donor. The only pathway for carbon fixation by purple bacteria is the Calvin cycle. Sulfur purple bacteria must fix CO_2 to live, whereas non-sulfur purple bacteria can grow aerobically in the dark by respiration on an organic carbon source.

The determination of the three-dimensional structure of the reaction centers of the non-sulfur purple bacteria, *Rhodopseudomonas viridis* and *Rhodobacter*

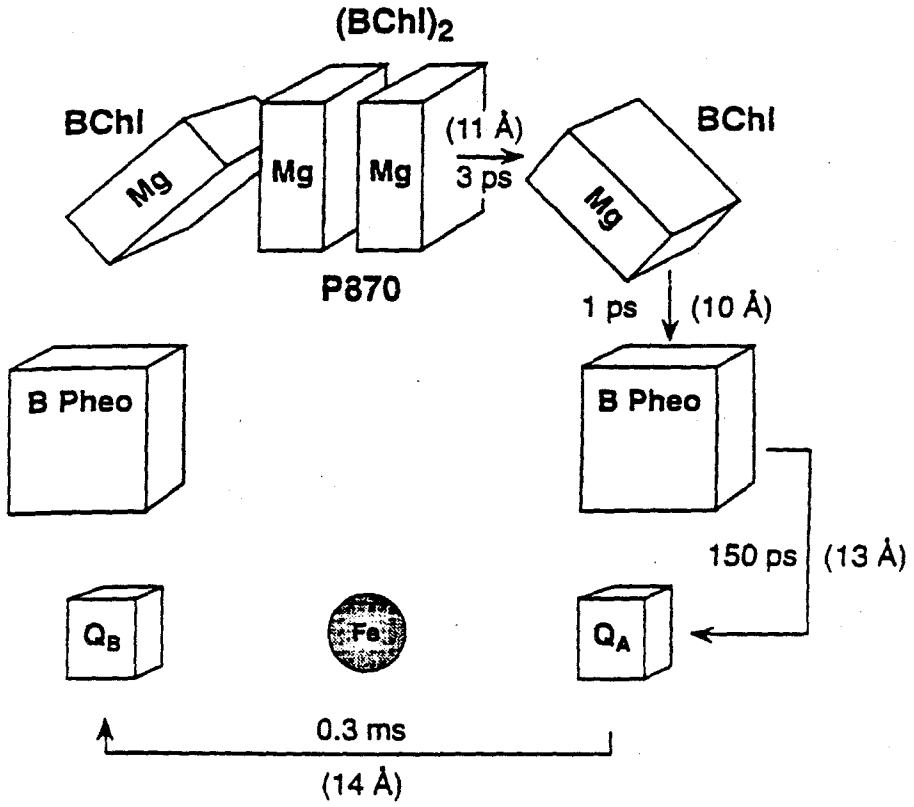


Fig. 17 **Relative positions of the chromophores of the reaction center of *Rhodobacter sphaeroides* (from Norris and van Brakel, 1986). It shows center to center distances and times for the electron transfers. P870, reaction center bacteriochlorophyll (primary electron donor); BChl, bacteriochlorophyll; B Pheo, bacteriopheophytin; Q_A and Q_B, bound ubiquinones. Fe is non-heme iron.**

sphaeroides, has provided an unprecedented opportunity to understand the structure and function of photosynthetic reaction centers (Deisenhofer et al., 1984, 1985; Feher et al., 1989; Lancaster et al., 1995). The positions of the electron transfer components in the reaction center of *Rhodobacter sphaeroides* are shown in Fig. 17 (Norris and van Brakel, 1986), and those of the three protein subunits L, M, and H, in Fig. 18. The reaction center contains four bacteriochlorophyll and two bacteriopheophytin molecules. Two of the bacteriochlorophyll molecules form the primary donor (P870). At present, there is controversy over whether a bacteriochlorophyll molecule is an intermediate in electron transfer from the P870 to bacteriopheophytin. However, there is agreement that the remaining steps involve two quinone molecules (Q_A and Q_B) and that two turnovers of the reaction center result in the release of reduced quinone (QH_2) into the photosynthetic membrane. Although there is a non-heme Fe between the two quinone molecules, there is convincing evidence that this Fe is not involved directly in transferring an electron from Q_A to Q_B. Because the primary donor (P870), bacteriopheophytin and quinone acceptors of the purple bacterial reaction center are similar to the photosystem II reaction center, the bacterial reaction

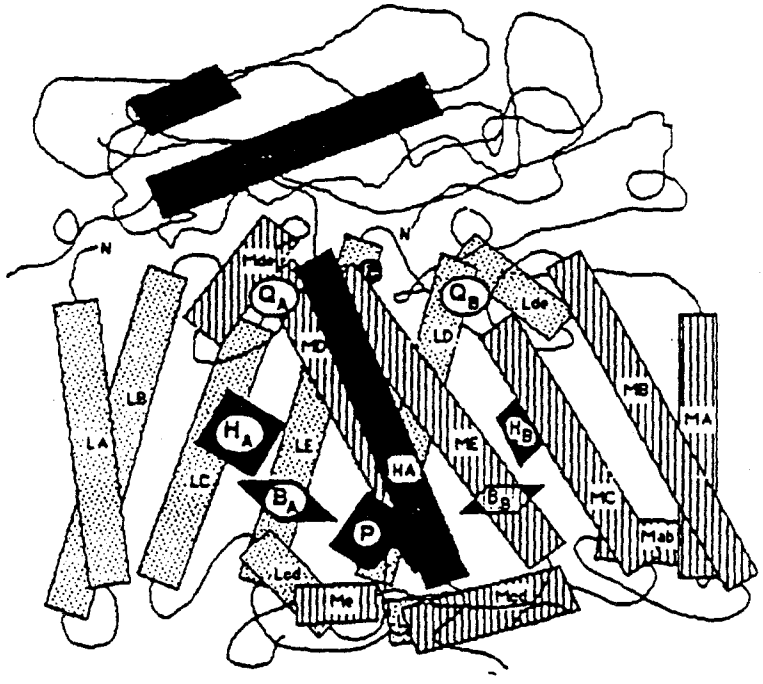


Fig. 18 Structure of the bacterial reaction center by H. Michel, J. Deisenhofer and R. Huber and co-workers containing three proteins: H(black), L (dotted) and M (hatched bars). Both L and M have 5 helices each (LA, LB, etc.) and H shown on the very top of the molecule having one helix (HA) that goes through the membrane. P, photoactive dimer of bacteriochlorophyll; B, monomeric bacteriochlorophyll; H, bacteriopheophytin-like bacteriochlorophyll, but without Mg^{2+}; Q_A and Q_B, quinone molecules. Courtesy: Colin Wraight.

center is used as guide to understand the structure and function of photosystem II.

Light driven electron transfer is cyclic in *Rhodobacter sphaeroides* and other purple bacteria (Fig. 19). The reaction center produces reduced quinone, which is oxidized by the cytochrome bc complex. Electrons from the cytochrome bc complex are transferred to a soluble electron carrier, cytochrome c_2, which reduces the oxidized primary donor $P870^+$. The product of the light driven electron transfer reactions is ATP. The electrons for the reduction of carbon are extracted from an organic donor, such as succinate or malate or from hydrogen gas, but not by the reaction center. The energy needed to reduce NAD^+ is provided by light driven cyclic electron transport in the form of ATP. The energy transformation pathway is complicated. Succinate is oxidized by a membrane bound enzyme (succinate dehydrogenase) that transfers the electrons to quinone, which is the source of electrons for the reduction of NAD^+. However, electron transfer from reduced quinone to NAD^+ is energetically uphill. By a mechanism that is poorly understood, a membrane bound enzyme is able to use energy stored in the proton electrochemical potential to drive electrons from reduced quinone to NAD^+.

6.2 Green Sulfur Bacteria

Green sulfur bacteria (e.g., *Chlorobium thiosulfatophilum* and *Chlorobium vibrioforme*) can use sulfur compounds as the electron donor as well as

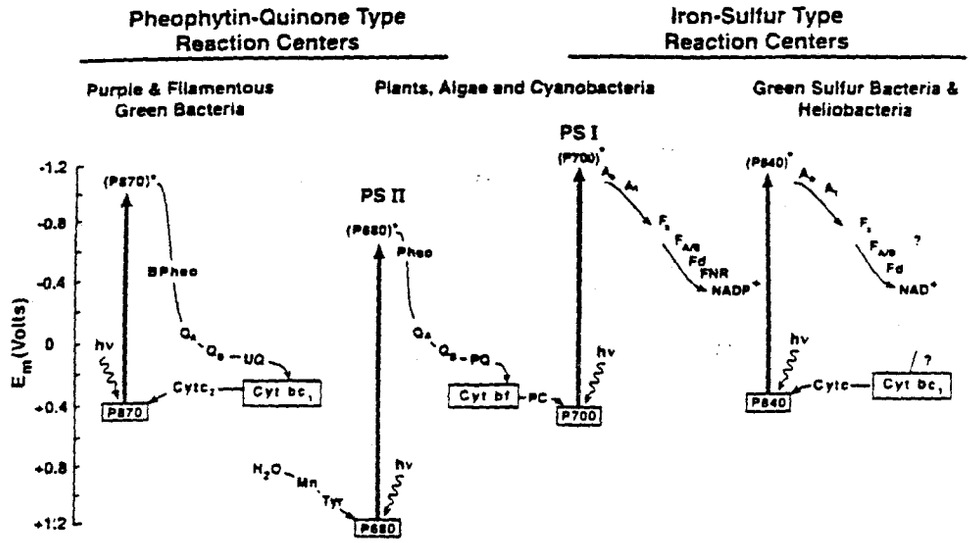


Fig. 19 Comparison of electron transport pathways in oxygenic and anoxygenic organisms (Blankenship, 1992). Cyt bc_1, cytochrome bc_1 complex; P840, reaction center bacteriochlorophyll; other abbreviations same as in Figs. 3 and 17.

organic hydrogen donors (Blankenship et al., 1995). As shown in Fig. 19 the reaction center of green sulfur bacteria is similar to the photosystem I

reaction center of oxygenic organisms (Feiler and Hauska, 1995). The FeS centers in the reaction center can reduce NAD^+ (or $NADP^+$) by ferredoxin and the ferredoxin-$NAD(P)^+$ oxidoreductase enzyme; therefore, green sulfur bacteria are not necessarily dependent on reverse electron flow for carbon reduction. The antenna system of the green sulfur bacteria is composed of bacteriochlorophyll and carotenoids and is contained in complexes known as a chlorosomes that are attached to the surface of the photosynthetic membrane. This antenna arrangement is similar to the phycobilisomes of cyanobacteria. Green sulfur bacteria can fix CO_2 without Rubisco. It has been proposed that they accomplish this by using the respiratory chain that normally oxidizes carbon (known as the Krebs cycle), resulting in the release of CO_2. With the input of energy this process can be run in the reverse direction, resulting in the uptake and reduction of CO_2.

6.3 Green Gliding Bacteria

Green gliding bacteria (e.g., *Chloroflexus aurantiacus*), also known as green filamentous bacteria, can grow photosynthetically under anaerobic conditions or in the dark by respiration under aerobic conditions. Like the green sulfur bacteria, green gliding bacteria harvest light using chlorosomes. The green gliding bacteria appear to have reaction centers similar to those of the purple bacteria (Fig. 19), but there are several notable differences. For example, instead of two monomer bacteriochlorophyll molecules, *C. aurantiacus* has one bacteriochlorophyll and one bacteriopheophytin and the metal between the two quinones is Mn rather than Fe (Feick et al., 1995). *C. aurantiacus* appears to fix CO_2 by a scheme that does not involve the Calvin cycle or the reverse Krebs cycle (Ivanovsky et al., 1993).

6.4 Heliobacteria

Heliobacteria (e.g., *Heliobacterium chlorum* and *Heliobacillus mobilis*) are in the phylum Gram Positive Bacteria that are strict anaerobes. Although the heliobacterial reaction center is similar to photosystem I in that it can reduce NAD^+ (or $NADP^+$), it contains a different type of chlorophyll known as bacteriochlorophyll g (Amesz, 1995).

7. Control of Intraprotein Electron Transfer

The three-dimensional structure of the reaction center of *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides* reveals the distances between the electron donors and acceptors (Deisenhofer et al., 1984, 1985; Norris and van Brakel, 1986; Feher et al., 1989) and has had an important influence on biophysical and molecular genetics studies designed to identify the factors that control the rate of electron transfer within proteins. There is currently a controversy concerning the importance of specific amino acid composition of the protein on the rate of intraprotein electron transfer. In part, the disagreement centers on whether the protein betweem the donor and acceptor molecules can be

treated as a uniform material, or whether the specific amino acid composition of the protein significantly alters the rate. For example, it has been proposed that aromatic amino acids may provide a particular pathway that facilitates electron transfer between a donor and acceptor pair. This is the case in the photosystem II reaction center, where a tyrosine residue on one of the reaction center core proteins (precisely, Tyr 161 on the D1 protein) donates an electron to the primary donor chlorophyll, $P680^+$. However, in other cases, replacement of an aromatic by another non-aromatic residue has resulted in relatively minor changes in the rate of electron transfer. L. Dutton and coworkers (Moser et al., 1992) have analyzed electron transfer reactions in biological and chemical systems in terms of electron tunneling theory developed by R. Marcus and others (De Vault, 1984). Dutton and coworkers argue that protein provides a uniform electronic barrier to electron tunneling and a uniform nuclear characteristic frequency. They suggest that the specific amino acid residues between an electron transfer pair is generally of less importance than the distance in determining the rate of pairwise electron transfer. In their view, protein controls the rate of electron transfer mainly through the distance between the donor and acceptor molecules, the free energy, and the reorganization energy of the reaction. The importance of distance is demonstrated by electron transfer data from biological and synthetic systems showing that the dependence of the electron transport rate on the edge to edge distance is exponential over 12-orders of magnitude when the free energy is optimized (Moser et al., 1992). Increasing the distance between two carriers by 1.7 Å slows the rate of electron transfer 10-fold. The extent to which this view is generally applicable for intraprotein transfer remains to be established (Williams, 1992). One of the challenges in understanding pairwise electron transfer rates from first principles is illustrated by the reaction centers of *Rhodobacter sphaeroides* in which the redox components are arranged along two-fold axis of symmetry that extends from the primary donor (P870) to the non-heme Fe. Despite the fact that the reaction center presents two spatially similar pathways for electron transfer from P870 to quinone, nearly all electrons are transferred down the right-arm of the reaction center as shown in Fig. 17. The same is true for the reaction center of *Rhodopseudomonas viridis*, in which it is estimated that electron transfer down the left-arm is less than 1:100 (Kellogg et al., 1989). The challenge to theorists is to explain the surprisingly high probability that electron flow goes down the right-arm. Since the distances are similar, it has been suggested that electron transfer down the left-arm is less probable due to an endothermic free energy change (Parson et al., 1990) or to an unfavorable rearrangement energy for the reaction (Moser et al., 1992).

8. Global Photosynthesis and the Atmosphere

The amount of CO_2 removed from the atmosphere each year by oxygenic photosynthetic organisms is massive. It is estimated that photosynthetic

organisms remove 100×10^{15} grams of carbon (C)/year (Houghton and Woodwell, 1990). This is equivalent to 4×10^{18} kJ of free energy stored in reduced carbon, which is roughly 0.1% of the incident visible radiant energy incident on the earth/year. Each year the photosynthetically reduced carbon is oxidized, either by living organisms for their survival, or by combustion. The result is that more CO_2 is released into the atmosphere from the biota than is taken up by photosynthesis. The net amount of carbon released by the biota is estimated to be $1–2 \times 10^{15}$ grams of carbon/year. Added to this is carbon released by the burning of fossil fuels, which amounts to 5×10^{15} g of carbon/year. The oceans mitigate this increase by acting as a sink for atmospheric CO_2. It is estimated that the oceans remove about 2×10^{15} g of carbon/year from the atmosphere. This carbon is eventually stored on the ocean floor. Although these estimates of sources and sinks are uncertain, the net global CO_2 concentration is increasing. Direct measurements show that each year the atmospheric carbon content is currently increasing by about 3×10^{15} g. Over the past two hundred years, CO_2 in the atmosphere has increased from about 280 parts per million (ppm) to its current level of 360 ppm. Based on predicted fossil fuel use and land management, it is estimated that the amount of CO_2 in the atmosphere will reach 700 ppm within the next century. The consequences of this rapid change in our atmosphere are unknown. Because CO_2 acts as a greenhouse gas, some climate models predict that the temperature of the earth's atmosphere may increase by 2–8°C. Such a large temperature increase would lead to significant changes in rainfall patterns. Little is known about the impact of such drastic atmospheric and climatic changes on plant communities and crops. (However, see Culotta, 1995.) Current research is directed at understanding the interaction between global climate change and photosynthetic organisms.

Acknowledgement

This text is a revised and modified version of "Photosynthesis" by J. Whitmarsh and Govindjee (1995), published in Encyclopedia of Applied Physics (Vol. 13, pp. 513–532) by VCH Publishers, Inc. It is published here with full permission from the Managing Editor Dr. E.H. Immergut.

References

Abrahams, J.P., Leslie, A.G.W., Lutter, R. and Walker, J.E. 1994. Structure at 2.8 Å resolution of F1-ATPase from bovine heart mitochondria. *Nature* **370**: 621–628.

Amesz, J. 1987. Photosynthesis. Elsevier, Amsterdam.

Amesz, J. 1995. The antenna-reaction center complex of heliobacteria. In: D. Bryant (ed.) The Molecular Biology of Cyanobacteria, pp. 687–697. Kluwer Academic, The Netherlands.

Amesz, J. and Hoff, A. (eds.) 1996. Biophysical Techniques in Photosynthesis. Kluwer Academic, The Netherlands.

Baker, N. (ed.) 1996. Photosynthesis and the Environment. Kluwer Academic, The Netherlands.

Baker, J. (ed.) 1992. The photosystems: Structure, Function and Molecular Biology. Elsevier, Amsterdam.

Barry, B.A., Boerner, R.J. and dePaula, J.C. 1994. The use of cyanobacteria in the study of the structure and function of photosystem II. In: D. Bryant (ed.) The Molecular Biology of Cyanobacteria, pp. 217–257. Kluwer Academic, The Netherlands.

Blankenship, R. 1992. Origin and early evolution of photosynthesis. *Photosynth. Res.* **33:** 91–111.

Blankenship, R., Madigan, M.T. and Bauer, C. (eds.) 1995. Anoxygenic Photosynthetic Bacteria. Kluwer Academic, The Netherlands.

Boyer, P.D. 1993. The binding change mechanism of ATP synthase—some probabilities and possibilities. *Biochim. Biophys. Acta* **114:** 215–250.

Boyer, P.D. 1997. The ATP synthase—a splendid molecular machine. *Ann. Rev. Biochem.* **66:** 717–749.

Briggs, W. (ed.) 1989. *Photosynthesis.* Alan Liss., N.Y.

Bryant, D. (ed.) 1994. The Molecular Biology of Cyanobacteria. Kluwer Academic, The Netharlands.

Calvin, M. 1989. Forty years of photosynthesis and related activities. *Photosynth. Res.* **21:** 3–16.

Chylla, R.A. and Whitmarsh, J. 1989. Inactive photosystem II complexes in leaves: Turnover rate and quantitation. *Plant Physiol.* **90:** 765–772.

Cramer, W.A. and Knaff, D.B. 1991. Energy transduction in Biological Membranes, Springer-Verlag, Berlin.

Culotta, E. 1995. Will plants profit from high CO_2? Science **268:** 654–656.

Debus, R. 1992. The manganese and calcium ions of photosynthetic oxygen evolution. *Biochim. Biophys. Acta* **1102:** 269–352.

Deisenhofer, J. and Michel, H. 1993 Three-dimensional structure of the reaction center of *Rhodopsuedomonas viridis.* In: Deisenhofer, J. and Norris, J.R. (eds.) The Photosynthetic Reaction Center. Vol. II, pp. 541–574, Academic Press, San Diego.

Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. 1985a. X-ray structure analysis of a membrane protein complex. Electron density map at 3A resolution and a model of the chromophores of the photosynthetic reaction center from *Rhodopseudomonas viridis. J. Mol. Biol.* **180:** 385–398.

Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. 1985b. Structure of the protein subunits in the photosynthetic reaction centre of *Rhodopseudomonas viridis* at 3 Å resolution. *Nature* **318:** 618–624.

DeVault, D. 1984. Quantum Mechanical Tennelling in Biological Systems. Cambridge University Press, Cambridge, UK.

Douglas, S.E. 1994. Chloroplast origins and evolution. In: Bryant, D. (ed.) The Molecular Biology of Cyanobacteria, pp. 91–118. Kluwer Academic, The Netherlands.

Duysens, L.N.M. 1989. The discovery of the two photosynthetic systems: a personal accpount. *Photosynth. Res.* **21:** 61–79.

Emerson, R. 1958. The quantum yield of photosynthesis. *Ann. Rev. Plant Physiol.* **9:** 1–24.

Falkowski, P.G. and Raven, J.A. 1997. Aquatic Photosynthesis. Blackwell Science, Oxford.

Feick, R., Shiozawa, J.A. and Ertlmaier, A. 1995. Biochemical and spectroscopic properties of the reaction center of the green filamentous bacterium *Chloroflexus aurantiacus.* In: Blankership, R., Madigan, M.T. and Bauer, C. (eds.) Anoxygenic Photosynthetic Bacteria, pp. 699–708. Kluwer Academic, The Netherlands.

Feiler, U. and Hauska, G. 1995. The reaction center from green sulfur bacteria. In: Blankenship, R., Madigan, M.T. and Bauer, C. (eds.) Anoxygenic Photosynthetic Bacteria, pp. 665–685. Kluwer Academic, The Netherlands.

Frenkel, A.W. 1995. Photosynthetic phosphorylation. *Photosynth. Res.* **46:** 73–77.

Feher, G., Allen, J.P., Okamura, M.Y. and Rees, D.C. 1989. Structure and function of bacterial photosynthetic reaction centers. *Nature* **339:** 111–116.

Gest, H. 1193. Photosynthetic and quasi-photosynthetic bacteria. *FEMS Microbiology Letters* **112:** 1–6.

Golbeck, J.H. 1994. Photosystem I in Cyanobacteria. In: Bryant, D. (ed.) The Molecular Biology of Cyanobacteria, pp. 319–360. Kluwer Academic, The Netherlands.

Govindjee 1990. Photosystem II heterogeneity: the acceptor side. *Photosysnth. Res.* **25:** 151–160.

Govindjee, (ed.) 1982. Photosynthesis. Vol. 1 and Vol. 2. Academic Press, N.Y.

Govindjee and Coleman, W.J. 1990. How plants make oxygen. *Scientific American* **262:** 50–58.

Govindjee and van Rensen, J.J.S. 1993. Photosystem II reaction centers and bicarbonate. In: Deisenhofer, J. and Norris, J.R. (eds.) Photosynthetic Reaction centers. vol. I, pp. 357–389, Academic Press, San Diego.

Govindjee, Amesz, J. and Fork, D.C. (eds.) 1986. Light Emission by Plants and Bacteria. Academic Press, Orlando.

Guenther, J.E. and Melis, A. 1990. The physiological significance of photosystem II heterogeneity in chloroplasts. *Photosynth. Res.* **23:** 105–109.

Hall, D.O. and Rao, K.K. 1994. Photosynthesis. Cambridge University Press, Boca Raton, FL.

Heldt, H-W. 1997. Plant Biochemistry and Molecular Biology. Oxford University Press, Oxford.

Hess, W., Partensky, F., Vander Staay, G.W.M., Garcia-Martinez, J.M., Boerner, T. and Vanlot, D. 1996. Coexistence of phycoerythrin and a chlorophyll a/b antenna in a marine prokaryote. *Proc. Nat. Acad. Sci USA* **93:** 1126–1130.

Hill, R. and Scarisbrick, R. 1940. Production of oxygen by illuminated chloroplasts. *Nature* **146:** 61–62.

Houghton, R.A. and Woodwell, G.M. 1989. Global climatic change. *Scientific American* **260:** 36–44.

Huzisige, H. and Ke, B. 1993. Dynamics of the history of photosynthesis. *Photosynth. Res.* **38:** 185–209.

Ivanovsky, R.N., Krasilnikova, E.N. and Fal, Y.I. 1993. A pathway of the autotrophic CO_2 fixation in Chloroflexus aurantiacus. *Arch. Microbiol.* **159:** 257–264.

Iwata, S., Saynovits, M., Link, T.A. and Michel, H. 1996. Structure of a water soluble fragment of the 'Rieske' iron-sulfur protein of the bovine heart mitochondrial cytochrome bc 1 complex determined by MAD phasing at 1.5 Å resolution. Structure **4:** 567–579.

Joliot, P. and Kok, B. 1975. Oxygen evolution in photosynthesis. In: Govindjee (ed.) Bioenergetics of Photosynthesis, pp. 387–412. Academic Press, N.Y.

Joliot, P., Barbieri, G. and Chabaud, R. 1969. Un nouveau modele des centre photochimique du systeme II. Photochem. Photobiol. **10:** 309–329.

Junge, W., Lill, H. and Engelbrecht, S. 1997. ATP synthase: an electrochemical transducer with rotatory mechanics. *Trends Biochem. Sci.* **22:** 420–423.

Kallas, T. 1994. The Cytochrome b_6f complex. In: Bryant, D. (ed.) The Molecular Biology of Cyanobacteria, pp. 259–317. Kluwer Academic, The Netherlands.

Kellogg, E.C., Koplaczkowoski, S., Wasielewski, M.R. and Tiede, D.M. 1989. Measurement of the extent of electron transfer to the bacteriopheophytin in the M-subunit in reaction centers of Rhodopseudomonas viridis. *Photosynth. Res.* **22:** 47–59.

Klein, M.P., Sauer, K. and Yachandra, Y.K. 1993. Perspectives on the structure of the photosynthetic oxygen evolving manganese complex and its relation to the Kok's cycle. *Photosynth. Res.* **38:** 265–277.

Klotz, I.M. 1967. Energy changes in Biochemical Reactions, Chapter 6, Academic Press, New York, NY.

Krause, G.H. and Weis, E. 1991. Chlorophyll fluorescence and photosynthesis: The basics. Annu. Rev. Plant Physiol. *Plant Mol. Biol.* **42:** 313–359.

Krauss, N., Hinrichs, W., Witt, I., Fromme, P., Pritzkow, W., Dauter, Z., Betzel, C., Wilson, K.S., Witt, H.T. and Saenger, W. 1993. Three-dimensional structure of system I of photosynthesis at 6 A resolution. *Nature* **361:** 326–331.

Krauss, N., Schubert, W.-D., Klukas, O., Fromme, P., Witt, H.T. and Saenger, W. 1996 Photosystem I at 4 Å resolution represents the first structural model of a joint photosynthetic reaction center and core antenna system. *Nature Struct. Bio.* **3:** 965–973.

Kuehlbrandt, D.N., Wang, and Fujiyoshi, 1994. Atomic model of plant harvesting complex by electron crystallography. *Nature* **367:** 614–621.

Lancaster, C.R.D., Ermler, U. and Michel, H. 1995. The structures of photosynthetic reaction centers from purple bacteria as revealed by X–ray crystallography. In: Blankenship, R., Madigan, M.T. and Bauer, C. (eds.) Anoxygenic Photosynthetic Bacteria, pp. 503–526. Kluwer Academic, The Netherlands.

Lavergne, J. and Briantais, J.-M. 1996. Photosystem II heterogeneity. In: Advances in Photosynthesis, Vol. 4, Oxygenic Photosynthesis: The Light Reactions, eds. Ort,. D.R. and Yocum, C.F., pp. 265–287, Kluwer Academic, The Netherlands.

Lavergne, J. and Junge, W. 1995. Proton release during the redox cycle of the water oxidase. *Photosynth. Res.* **38:** 289–296.

Lawlor, D.W. 1993. Photosynthesis: Molecular, Physiological and Environmental Processes. Longman Scientific & Technical, Essex, UK.

Marcus, R.A., and Sutin, N. 1985. Electron transfers in chemistry and biology. *Biochim. Biophys. Acta* **811**: 265–322.

Martinez, S.E., Huang, D., Szczepaniak, A., Cramer, W.A. and Smith, J.L. 1994. Crystal structure of chloroplast cytochrome of reveals a novel cytochrome fold and unexpected heme ligation. *Structure* **2:** 95–105.

Matthijs, H.C.P., van der Staay, G.W.M. and Mur, L.R. 1994. Prochlorophytes: the "other" cyanobacteria?, pp. 49–64. Kluwer Academic, The Netherlands.

Melis, A. 1991. Dynamics of photosynthetic membrane composition and function. *Biochim. Biophys. Acta* **1058:** 87–106.

Mitchell, P. 1961. Coupling of phosphorylation to electron and proton transfer by a chemiosmotic type of mechanism. *Nature* **191**: 144–148.

Moser, C.C., Keske, J.M., Warncke, K., Farid, R.S. and Dutton, P.L. 1992. Nature of biological electron transfer. *Nature* **355**: 796–802.

Newman, J. and Gutteridge, S. 1993. The X-ray structure of Synechococcus ribulose-bisphosphate carboxylase/oxygenase-activated quaternary complex at 2.2 Å resolution. *J. Biol. Chem.* **268:** 25876–25886.

Norris, J.F. and van Brakel G. 1986. Energy trapping in photosynthesis of purple bacteria. In: Govindjee, Amesz, J. and Fork, D.C. (eds.) Light Emission by Plants and Bacteria, pp. 35–56, Academic Press, Orlando.

Odum, E.P. 1971. Fundamentals of Ecology. Saunders, W.B. Co. Philadelphia.

Ort, D.R. 1994. Photosynthesis. Encyclopedia of Agricultural Sciences **3:** 187–195.

Ort, D.R. and Whitmarsh, J. 1990. Inactive photosystem II centers: A resolution of discrepancies in photosystem II quantitation? *Photosynth. Res.* **23**: 101–104.

Ort, D.R. and Yocum, C.F. (editions) 1996. Oxygenic photosynthesis: the Light Reactions. Kluwer Academic, The Netherlands.

Oxborough, K., Nedbal, L., Chylla, R.A. and Whitmarsh, J. 1996. Light-dependent modification of photosystem II in spinach leaves. *Photosynth. Res.* **48:** 247–254.

Palenik, B. and Haselkorn, R. 1992. Multiple evolutionary origins of prochlorophytes, the chlorophyll b- containing prokaryotes. *Nature* **355:** 265–267.

Parson, W.W., Chu, Z.T. and Warshel, A. 1990. Electrostatic control of charge separation in bacterial photosynthesis. *Biochim. Biophys. Acta* **1017**: 251–272.

Raghavendra, A.S. (editor) 1998. Photosynthesis: A comprehensive Treatise. Cambridge University Press, Cambridge, U.K.

Renger, G. 1993. Water cleavage by solar radiation- an inspiring challenge to photosynthesis research. *Photosynth. Res.* **38:** 229–287.

Ruben, S., Randall, M., Kamen, M. and Hyde, J.L. 1941. Heavy oxygen (^{18}O) as a tracer in the study of photosynthesis. *J. Am. Chem. Soc.* **63:** 877–879.

Rochaix, J-D., Goldschmidt-Clermont, M. and Merchant, S. 1998. (editors). The Molecular Biology of Chloroplasts and Mitochondria in Chlamydomonas. Kluwer Academic, Dordrecht, The Netherlands.

Sabbert, D., Engelbrecht, S. and Junge, W. 1996. Intersubunit rotation in active F-ATPase. *Nature.* **381:** 623–625.

Scheer, H. (ed.) 1991. Chlorophylls, CRC Press, Boca Raton, FL.

Schneider, G., Lindqvist, Y. and Lundqvist, T. 1990. Crystallographic refinement and structure of ribulose-1, 5-Bisphosphate carboxylase from Rhodospirillum rubrum at 1.7 A resolution. *J. Mol. Biol.* **211:** 989–1008.

Schreuder, H.A., Knight, S., Curmi, P.M.G., Andersson, I., Cascio, D., Sweet, R.M., Branden, C.I. and Eisenberg, D. 1993. Crystal structure of activated tobacco rubisco complexes with the reaction-intermediate analogue 2-carboxy-arabinitol 1.5-bisphosphate. *Protein Structure* **2:** 1136–1146.

Sidler, W.A. 1994. Phycobilisome and phycobiliprotein structures. In: Bryant, D. (ed.) The Molecular Biology of Cyanobacteria, pp. 139–216. Kluwer Academic, The Netherlands.

Siegenthaler, P.A. and Murata N. (eds). 1998. Lipids in Photosynthesis: Structure, Function and Genetics. Kluwer Academic, Dordrecht, The Netherlands.

Staehelin, L.A. 1986. Chloroplast structure and supermolecular organization of photosynthetic membranes. Encyclopedia of Plant Physiology New Series **19:** 1–84.

Taiz, L. and Zeiger, E. 1991. Plant Physiology, Benjamin/Cummings Publishing Co., Redwood City, CA.

Urbach, E., Robertson, D.L. and Chisholm, S.W. 1992. Multiple evolutionary origins of prochlorophytes within the cyanobacterial radiation. *Nature* **355:** 267–270.

Van Grondele, R. and Amesz, J. 1986. Excitation energy transfer in photosynthetic systems. In: Govindjee, Amesz, J. and Fork, D.C. (eds.) Light Emission by Plants and Bacteria, pp. 191–223. Kluwer Academic, The Netherlands.

Walker, D. 1992. Energy, Plants and Man. Oxygraphics Limited, East Sussex, UK.

Whitmarch, J. 1986. Mobile electron carriers in thylakoids. Encyclopedia of Plant Physiology New Series **19:** 508–527.

Whitmarsh, J. and Pakrasi, H.B. 1996. Form and Function of cytochrome b 559. In: Advances in Photosynthesis, vol. 4, Oxygenic Photosynthesis: The Light Reactions, Ort, D.R. and Yocum, C.F. (eds). pp. 249–269, Kluwer Academic, The Netherlands.

Wild, A. and Ball, R. 1997. Photosynthetic unit and Photosystems: History of Research and Current view (Relationship of Structure and Function). Backhuys Publishers, Leiden, The Netherlands.

Williams, R.J.P. 1992. Biology's wiring circuits. Nature **355:** 770–771.

Wilmott, A. 1994. Molecular evolution and taxonomy of the cyuanobacteria. In: Bryant, D. (ed.) The Molecular Biolgy of Cyanobacteria, pp. 1–25. Kluwer Academic, The Netherlands.

Witt, H.T. 1991. Functional mechanism of water splitting photosynthesis. *Photosynth. Res.* **29:** 55–77.

Woese, C.R., Kandler, O. and Wheeler, M.L. 1990. Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria and Eucarya. *Proc. Natl. Acad. Sci. USA* **87:** 4576–4579.

Wraight, C. 1982. Reaction centers, electron flow and energy transduction. In: Govindjee (ed.) Photosynthesis, pp. 17–61, Academic Press, New York, NY.

Xia, D., Yu, C.-A., Kim, H., Xia, J.-Z., Kachurin, A.M., Zhang, L., Yu and Deisenhofer, J. 1997. Crystal structure of the cytochrome bc_1 complex from bovine heart mitochondria *Science* **277:** 60–66.

Xiong, J., Subramaniam, S. and Govindjee. 1996. Modeling of the D1/D2 proteins and cofactors of the photosystem II reaction center: Implications for herbicide and bicarbonate binding. *Protein Science* **5:** 2054–2073.

the reduced carbon is returned to the atmosphere as carbon dioxide by microbial, plant, and animal metabolism, and by biomass combustion. In turn, the performance of photosynthetic organisms depends on the earth's atmosphere and climate. Over the next century, the large increase in the amount of atmospheric carbon dioxide created by human activity is certain to have a profound impact on the performance and competition of photosynthetic organisms. Knowledge of the physicochemical process of photosynthesis is essential for understanding the relationship between living organisms and the atmosphere and the balance of life on earth.

1. BRIEF HISTORY

In the 1770s, Joseph Priestley, an English chemist and clergyman, performed experiments showing that plants release a type of air that allows combustion. He demonstrated this by burning a candle in a closed vessel until the flame went out. He placed a sprig of mint in the chamber and after several days showed that the candle could burn again. Although Priestley did not know about molecular oxygen, his work showed that plants release oxygen into the atmosphere. It is noteworthy that over 200 years later, investigating the mechanism by which plants produce oxygen is one of the most active areas of photosynthetic research. Building on the work of Priestley, Jan Ingenhousz, a Dutch physician, demonstrated that sunlight was necessary for photosynthesis and that only the green parts of plants could release oxygen. During this period, Jean Senebier, a Swiss botanist and naturalist, discovered that CO_2 is required for photosynthetic growth, and Nicolas-Théodore de Saussure, a Swiss chemist and plant physiologist, showed that water is required. It was not until 1845 that Julius Robert von Mayer, a German physician and physicist, proposed that photosynthetic organisms convert light energy into chemical free energy.

By the middle of the nineteenth century, the key features of plant photosynthesis were known, namely, that plants could use light energy to make carbohydrates from CO_2 and water. The empirical equation representing the net reaction of photosynthesis for oxygen-evolving organisms is

$$CO_2 + 2H_2O + \text{light energy} \rightarrow [CH_2O] + O_2 + H_2O, \quad (1)$$

where $[CH_2O]$ represents a carbohydrate (e.g., glucose, a six-carbon sugar). The synthesis of carbohydrate from carbon and water requires a large input of light energy. The standard free energy for the reduction of one mole of CO_2 to the level of glucose is +478 kJ/mol. Because glucose, a six-carbon sugar, is often an intermediate product of photosynthesis, the net equation of photosynthesis is frequently written as

$$6CO_2 + 12H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O. \quad (2)$$

The standard free energy for the synthesis of glucose is +2870 kJ/mol.

Not surprisingly, early scientists studying photosynthesis concluded that the O_2 released by plants came from CO_2, which was thought to be split by light energy. In the 1930s, comparison of bacterial and plant photosynthesis lead Cornelis van Niel to propose the general equation of photosynthesis that applies to plants, algae, and photosynthetic bacteria (discussed by Wraight, 1982). Van Niel was aware that some photosynthetic bacteria could use hydrogen sulfide (H_2S) instead of water for photosynthesis and that these organisms released sulfur instead of oxygen. Van Niel, among others, concluded that photosynthesis depends on electron donation and acceptor reactions and that the O_2 released during photosynthesis comes from the oxidation of water. Van Niel's generalized equation is

$$CO_2 + 2H_2A + \text{light energy} \rightarrow [CH_2O] + 2A + H_2O. \quad (3)$$

In oxygenic photosynthesis, $2A$ is O_2, whereas in anoxygenic photosynthesis, which occurs in some photosynthetic bacteria, the electron donor can be an inorganic hydrogen donor, such as H_2S in which case A is elemental sulfur, or an organic hydrogen donor such as succinate in which case A is fumarate.

The biochemical conversion of CO_2 to carbohydrate is a reduction reaction that involves the rearrangement of covalent bonds between carbon, hydrogen, and oxygen. The

energy for the reduction of carbon is provided by energy-rich molecules that are produced by the light-driven electron-transfer reactions. Carbon reduction can occur in the dark and involves a series of biochemical reactions that were elucidated by Melvin Calvin, Andrew Benson, and James Bassham in the late 1940s and 1950s. Using the radioisotope carbon-14, most of the intermediate steps that result in the production of carbohydrate were identified. Calvin was awarded the Nobel Prize for Chemistry in 1961 for this work.

In 1954, Daniel Arnon and co-workers discovered that plants, and A. Frenkel discovered that photosynthetic bacteria, use light energy to produce ATP, an organic molecule that serves as an energy source for many biochemical reactions. During the same period, L. N. M. Duysens showed that the primary photochemical reaction of photosynthesis is an oxidation/reduction reaction that occurs in a protein complex (the reaction center). Over the next few years, the work of several groups, including those of Robert Emerson, Bessel Kok, L. N. M. Duysens, Robert Hill, and Horst Witt, combined to prove that plant, algae, and cyanobacteria require two reaction centers, photosystem II and photosystem I, operating in series.

In 1961, Peter Mitchell suggested that cells can store energy by creating an electric field or a proton gradient across a membrane. Mitchell's proposal that energy is stored as an electrochemical gradient across a vesicular membrane opened the door for understanding energy transformation by membrane systems. He was awarded the Nobel Prize in Chemistry in 1978 for his theory of chemiosmotic energy transduction.

Most of the proteins required for the conversion of light energy and electron transfer reactions of photosynthesis are located in membranes. Despite decades of work, efforts to determine the structure of membrane-bound proteins had little success. This changed in the 1980s when Johann Deisenhofer, Hartmut Michel, and Robert Huber determined the structure of the reaction center of the purple bacterium *Rhodospeudomonas viridis*. They were awarded the Nobel Prize for Chemistry in 1988 for their work, which provided insight into the relationship between structure and function in membrane-bound proteins.

A key element in photosynthetic energy conversion is electron transfer within and between protein complexes and simple organic molecules. The electron-transfer reactions are rapid (as fast as a few picoseconds) and highly specific. Much of our current understanding of the physical principles that guide electron transfer is based on the pioneering work of Rudolph A. Marcus, who received the Nobel Prize in Chemistry in 1992 for his contributions to the theory of electron-transfer reaction in chemical systems.

The overall equation for photosynthesis is deceptively simple. In fact, a complex set of physical and chemical reactions must occur in a coordinated manner for the synthesis of carbohydrates. To produce a sugar molecule such as sucrose, plants require nearly 30 distinct proteins that work within a complicated membrane structure. Research into the mechanism of photosynthesis centers on understanding the structure of the photosynthetic components and the molecular processes that use radiant energy to drive carbohydrate synthesis. The research involves several disciplines, including physics, chemistry, structural biology, biochemistry, molecular biology, and physiology, and serves as an outstanding example of the success of multidisciplinary research. As such, photosynthesis presents a special challenge in understanding several interrelated molecular processes. From a physicist's viewpoint, the reactions that transform energy are of particular interest, and it is on these processes that this description will focus.

2. CLASSIFICATION OF PHOTOSYNTHETIC ORGANISMS

All life can be divided into three domains, Archaea, Bacteria, and Eucarya, which originated from a common ancestor (Woese *et al.*, 1990). Historically, the term photosynthesis has been applied to organisms that depend on chlorophyll (or bacteriochlorophyll) for the conversion of light energy into chemical free energy (Gest, 1993). These include organisms in the domains Bacteria (photosynthetic bacteria) and Eucarya (algae and higher plants). The most primitive domain, Archaea, includes organisms known as halobacteria, which convert light energy into chemical free energy. However, the mechanism by which halobacteria convert light is fundamentally

different from that of higher organisms because there is no oxidation/reduction chemistry and halobacteria cannot use CO_2 as their carbon source. Consequently, some biologists do not consider halobacteria as photosynthetic (Gest, 1993). This article will follow the historical definition of photosynthesis and omit halobacteria.

2.1 Oxygenic Photosynthetic Organisms

The photosynthetic process in all plants and algae, as well as in certain types of photosynthetic bacteria, involves the reduction of CO_2 to carbohydrate and the removal of electrons from H_2O, which results in the release of O_2. In this process, known as oxygenic photosynthesis, water is oxidized by the photosystem II reaction center, a multisubunit protein located in the photosynthetic membrane. Years of research have shown that structure and function of photosystem II is similar in plants, algae, and certain bacteria, so that knowledge gained in one species can be applied to others. This homology is a common feature of proteins that perform the same reaction in different species. The importance of this homology at the molecular level is shown by the fact that there are an estimated 300 000–500 000 species of plants. If different species had evolved diverse mechanisms for oxidizing water, research aimed at a general understanding of photosynthetic water oxidation would be hopeless.

2.2 Anoxygenic Photosynthetic Organisms

Some photosynthetic bacteria can use light energy to extract electrons from molecules other than water. These organisms are of ancient origin, presumed to have evolved before oxygenic photosynthetic organisms. Anoxygenic photosynthetic organisms occur in the domain Bacteria and have representatives in four phyla: Purple Bacteria, Green Sulfur Bacteria, Green Gliding Bacteria, and Gram Positive Bacteria.

3. GENERAL PRINCIPLES OF PHOTOSYNTHETIC ENERGY TRANSFORMATION IN PLANTS

The energy that drives photosynthesis originates in the center of the sun, where mass is converted to heat by the fusion of hydrogen. Over time, the heat energy reaches the sun's surface, where some of it is converted to light by blackbody radiation that reaches the earth. A small fraction of the visible light incident on the earth is absorbed by plants. Through a series of energy-transducing reactions, plants are able to transform light energy into chemical free energy in a stable form that can last for hundreds of millions of years (e.g., fossil fuels). A simplified scheme describing how plants transform energy is presented in this section. The focus is on the structural and functional features essential for the energy-transforming reactions. For clarity, mechanistic and structural details are omitted. A more highly resolved description of oxygenic and anoxygenic photosynthesis is given in the remaining sections.

The photosynthetic process in plants and algae occurs in small organelles, known as chloroplasts, that are located inside cells. The photosynthetic reactions are traditionally divided into two types: the "light reactions," which consist of electron- and proton-transfer reactions, and the "dark reactions," which consist of the biosynthesis of carbohydrates from CO_2. The light reactions occur in a complex membrane system (the photosynthetic membrane) that is made up of protein complexes, electron carriers, and lipid molecules. The photosynthetic membrane is surrounded by water and can be thought of as a two-dimensional surface that defines a closed space, with an inner and an outer water phase. A molecule or ion must pass through the photosynthetic membrane to go from the inner space to the outer space. The protein complexes embedded in the photosynthetic membrane have a unique orientation with respect to the inner and outer phases. The asymmetrical arrangement of the protein complexes allows some of the energy released during electron transport to create an electrochemical gradient of protons across the photosynthetic membrane.

Photosynthetic electron transport consists of a series of individual electron-transfer steps from one electron carrier to another. The electron carriers are metal ion complexes and aromatic groups. The metal ion complexes and most of the aromatic groups are bound within proteins. Most of the proteins involved in photosynthetic electron transport are composed of numerous polypeptide chains that lace through the membrane, providing

a scaffolding for metal ions and aromatic groups. An electron enters a protein complex at a specific site, is transferred within the protein from one carrier to another, and exits from the protein at a different site. The protein controls the pathway of electrons between the carriers by determining the location and environment of the metal ion complexes and aromatic groups. By setting the distance between electron carriers and controlling the electronic environment surrounding a metal ion complex or an aromatic group, the protein controls pairwise electron transfer reactions. Between proteins, electron transfer is controlled by distance and free energy, as for intraprotein transfer, and by the probability that the two proteins are in close contact. Protein association is controlled by a number of factors, including the structure of the two proteins, their surface electrical and chemical properties, and the probability that they collide with one another. Not all electron carriers are bound to proteins. The reduced forms of plastoquinone and nicotinamide adenine dinucleotide phosphate (NADPH) act as mobile electron carriers operating between protein complexes. For electron transfer to occur, these small molecules must bind to special pockets in the proteins known as binding sites. The binding sites are highly specific and are a critical factor in controlling the rate and pathway of electron transfer.

The light reactions convert energy into several forms (Fig. 1). The first step is the conversion of a photon to an excited electronic state of an antenna pigment molecule located in the antenna system. The antenna system consists of hundreds of pigment molecules (mainly chlorophyll and carotenoids) that are anchored to proteins within the photosynthetic membrane and serve a specialized protein complex known as a reaction center. The electronic excited state is transferred over the antenna molecules as an exciton. Some excitons are converted back into photons and emitted as fluorescence, some are converted to heat, and some are trapped by a reaction-center protein. Excitons trapped by a reaction center provide the energy for the primary photochemical reaction of photosynthesis—the transfer of an electron from a donor molecule to an acceptor molecule. Both the donor and acceptor molecules are attached to the reaction-center protein complex. Once primary charge separation occurs, the subsequent electron-transfer reactions are energetically downhill. In oxygenic photosynthetic organisms, two different reaction centers work in series: photosystem II and photosystem I. Electrons are transferred from photosystem II to the photosystem I reaction center by intermediate carriers. The net reaction is the transfer of electrons from a water molecule to $NADP^+$, producing the reduced form, NADPH. In the photosynthetic process, much of the energy initially provided by light energy is stored as redox free energy (a form of chemical free energy) in NADPH, to be used later in the reduction of carbon. In addition, the electron-transfer reactions concentrate protons inside the membrane vesicle and create an electric field across the photosynthetic membrane. In this process, the electron-transfer reactions convert redox free energy into an electrochemical po-

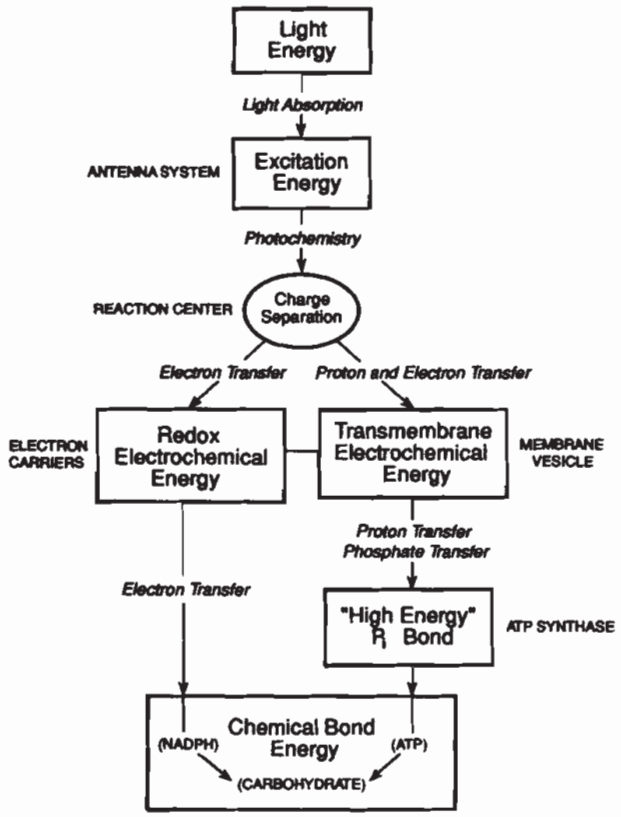


FIG. 1. Photosynthesis shown as a series of reactions that transform energy from one form to another. The different forms of energy are shown in boxes, and the direction of energy transformation is shown by the arrows. The energy-transforming reaction is shown by italics in the arrows. The site at which the energy is stored is shown in capital letters outside the boxes. The primary photochemical reaction, charge separation, is shown in the oval. Details of these reactions are given in the text.

tential of protons. The energy stored in the proton electrochemical potential is used by a membrane-bound protein complex (ATP synthase) to attach a phosphate group covalently to adenosine diphosphate (ADP), forming adenosine triphosphate (ATP). Protons pass through the ATP-synthase protein complex that transforms electrochemical free energy into a type of chemical free energy known as phosphate group-transfer potential (or a high-energy phosphate bond) (Klotz, 1967). The energy stored in ATP can be transferred to another molecule by transferring the phosphate group. The net effect of the light reactions is to convert radiant energy into redox free energy in the form of NADPH and phosphate group-transfer energy in the form of ATP. In the light reactions, the transfer of a single electron from water to $NADP^+$ involves about 30 metal ions and seven aromatic groups. The metal ions include 19 Fe, 5 Mg, 4 Mn, and 1 Cu. The aromatics include quinones, pheophytin, NADPH, tyrosine, and a flavoprotein.

The NADPH and ATP formed by the light reactions provide the energy for the dark reactions of photosynthesis, known as the Calvin cycle or the photosynthetic carbon reduction cycle. The reduction of atmospheric CO_2 to carbohydrate occurs in the aqueous phase of the chloroplast and involves a series of enzymatic reactions. The first step is catalyzed by the protein Rubisco (D-ribulose 1,5-bisphosphate carboxylase/oxygenase), which attaches CO_2 to a five-carbon compound. The reaction produces two molecules of a three-carbon compound. Subsequent biochemical reactions involve several enzymes that reduce carbon by hydrogen transfer and rearrange the carbon compounds to synthesize carbohydrates. The carbon reduction cycle involves the transfer and rearrangement of chemical bond energy.

4. OXYGENIC PHOTOSYNTHESIS IN PLANTS

4.1 Chloroplasts—Structure and Organization

Photosynthesis occurs inside chloroplasts, which are small organelles found in plant cells. Chloroplasts provide the energy and reduced carbon needed for plant growth and development, while the plant provides the chloroplast with CO_2, water, nitrogen, organic molecules, and minerals necessary for the chloroplast biogenesis. Most chloroplasts are located in specialized leaf cells, which often contain 50 or more chloroplasts per cell. Each chloroplast is defined by an inner and an outer envelope membrane and is shaped like a meniscus convex lens that is 5–10 μm in diameter (Fig. 2), although many different shapes and sizes can be found in plants. The inner envelope membrane acts as a barrier, controlling the flux of organic and charged molecules in and out of the chloroplast. Water passes freely through the envelope membranes, as do other small neutral molecules like CO_2 and O_2. There is convincing evidence that chloroplasts were once free-living bacteria that invaded a nonphotosynthetic cell long ago. They have retained some of the DNA necessary for their assembly, but much of the DNA necessary for their biosynthesis is located in the cell nucleus. This enables a cell to control the biosynthesis of chloroplasts within its domain.

Inside the chloroplast is a complicated membrane system, known as the photosynthetic membrane (or thylakoid membrane), that contains most of the proteins required for the light reactions. The proteins required for the fixation and reduction of CO_2 are located outside the photosynthetic membrane in the surrounding aqueous phase. The photosynthetic membrane is composed mainly of glycerol lipids and protein. The glycerol lipids are a family of molecules characterized by a polar head group that is hydrophilic and two fatty acid side chains that are hydrophobic. In membranes, the lipid mol-

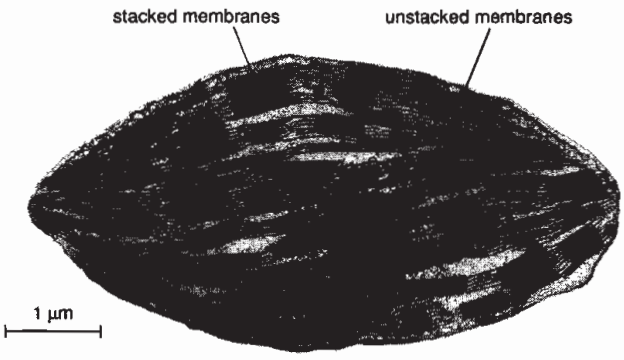


FIG. 2. An electron micrograph of a plant chloroplast. Micrograph by A. D. Greenwood, courtesy of J. Barber.

ecules arrange themselves in a bilayer, with the polar head toward the water phase and the fatty acid chains aligned inside the membrane forming a hydrophobic core (Fig. 3). The photosynthetic membrane is vesicular, defining a closed space with an outer water space (stroma) and an inner water space (lumen). The organization of the photosynthetic membrane can be described as groups of stacked membranes (like stacks of pita bread, with the inner pocket representing the inner aqueous space), interconnected by non-stacked membranes that protrude from the edges of the stacks (Staehelin, 1986). Experiments indicate that the inner aqueous space of the photosynthetic membrane is likely continuous inside of the chloroplast. It is not known why the photosynthetic membrane forms such a convoluted structure. To understand the energetics of photosynthesis, the complicated structure can be ignored and the photosynthetic membrane can be viewed as a simple vesicle.

4.2 Light Absorption—The Antenna System

Plant photosynthesis is driven primarily by visible light (wavelengths from 400 to 700 nm) that is absorbed by pigment molecules (mainly chlorophyll *a* and *b* and carotenoids). Plants appear green because of chlorophyll, which is so plentiful that regions of the earth appear green from space. The absorption spectra of chlorophyll *a* and *b* and of a chloroplast are shown in the article Excitons. Light is collected by 200–300 pigment molecules that are bound to light-harvesting protein complexes located in the photosynthetic membrane. The three-dimensional structure of the light-harvesting complex (Kühlbrandt *et al.*, 1994) shows that the protein determines the position and orientation of the antenna pigments. The light-harvesting complexes surround the reaction centers and serve as antennae. Photosynthesis is initiated by the absorption of a photon by an antenna molecule, which occurs in

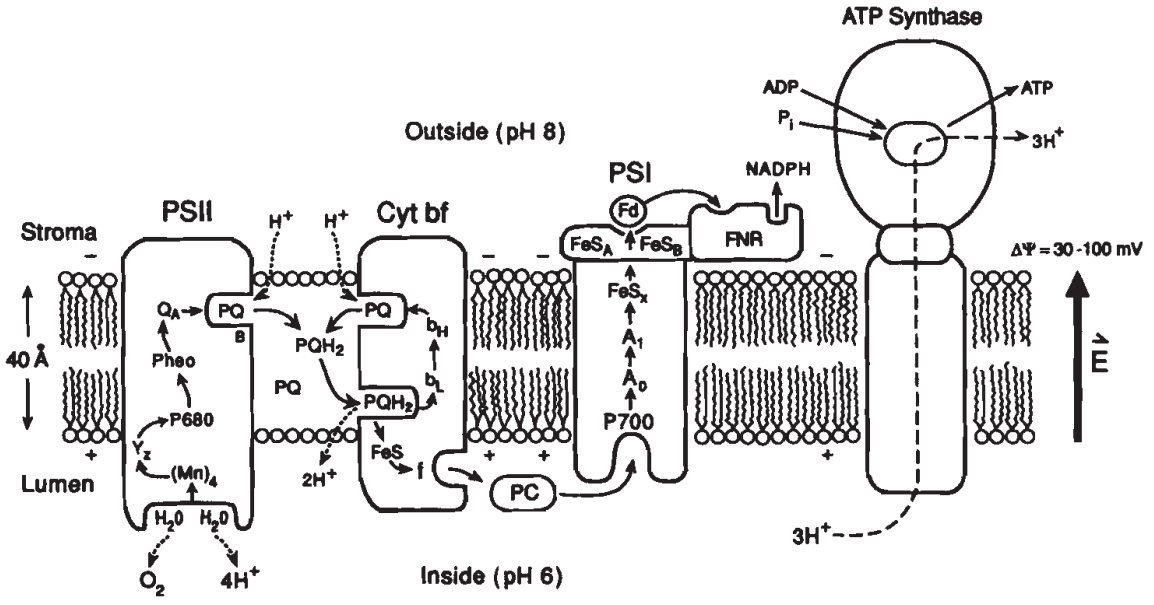


FIG. 3. Model of the photosynthetic membrane of plants showing the electron-transport components and the ATP-synthase enzyme (cross-sectional view). The complete membrane forms a vesicle. The pathways of electrons are shown by solid arrows. The membrane-bound electron-transport protein complexes involved in transferring electrons are the photosystem II and I reaction centers (PSII and PSI) and the cytochrome *bf* complex (Cyt *bf*). Abbreviations: Y_z, tyrosine; P680 and P700, the reaction-center chlorophyll of photosystem II and photosystem I, respectively; Pheo, pheophytin; Q_A, bound plastoquinone; PQ, free plastoquinone (oxidized form), PQH_2, free plastoquinone (reduced form); b_L and b_H, different forms of *b*-type cytochromes; FeS, iron-sulfur centers; *f*, cytochrome *f*; PC, plastocyanin; A_0, chlorophyll; A_1, phylloquinone; Fd, ferredoxin; FNR, ferredoxin/$NADP^+$ oxidoreductase; NADPH, nicotinomide adenine dinucleotide phosphate (reduced form); ADP, adenosine diphosphate; ATP, adenosine triphosphate; P_i, inorganic phosphate; H^+, protons; $\Delta\Psi$, the light-induced electrical potential across the membrane. The light-harvesting protein complexes are not shown. Details are given in the text.

10^{-15} s and causes a transition from the electronic ground state to an excited state. Within 10^{-13} s, the excited state decays by vibrational relaxation to the first excited singlet state. The fate of the excited-state energy is guided by the structure of the protein. Because of the proximity of other antenna molecules with the same or similar energy states, the excited-state energy has a high probability of being transferred by resonance energy transfer to a near neighbor. Exciton energy transfer between antenna molecules is due to the interaction of the transition dipole moment of the molecules. The probability of transfer is dependent on the distance between the transition dipoles of the donor and acceptor molecules ($1/R^6$), the relative orientation of the transition dipoles, and the overlap of the emission spectrum of the donor molecule with the absorption spectrum of the acceptor molecule (for a discussion of resonance energy transfer, see EXCITONS). Photosynthetic antenna systems are very efficient at this process. Under optimum conditions, over 90% of the absorbed quanta are transferred within a few hundred picoseconds from the antenna system to the reaction center, which acts as a trap for the excitons.

4.3 Primary Photochemistry—Photosystem II and Photosystem I Reaction Centers

Photosystem II uses light energy to drive two chemical reactions: the oxidation of water and the reduction of plastoquinone. The photosystem II complex is composed of more than 15 polypeptides, and at least nine different redox components (chlorophyll, pheophytin, plastoquinone, tyrosine, Mn, Fe, cytochrome b559, carotenoid, and histidine) have been shown to undergo light-induced electron transfer (Debus, 1992). However, only five of these redox components are known to be involved in transferring electrons from H_2O to the plastoquinone pool—the water-oxidizing manganese cluster $(Mn)_4$, the amino acid tyrosine, the reaction center chlorophyll (P680), pheophytin, and the plastoquinone molecules, Q_A and Q_B. Of these essential redox components, tyrosine, P680, pheophytin, Q_A, and Q_B have been shown to be bound to two key polypeptides that form the heterodimeric reaction center core of photosystem II (*D*1 and *D*2). Recent work indicates that the *D*1 and *D*2 polypeptides also provide ligands for the $(Mn)_4$ cluster. The three-dimensional structure of photosystem II is not known. Our knowledge of its structure is guided by the known structure of the reaction center in purple bacteria and biochemical and spectroscopic data. Figure 4 shows a schematic view of photosystem II that is consistent with current data.

Photochemistry in photosystem II is initiated by charge separation between P680 and pheophytin, creating $P680^+/Pheo^-$. Primary charge separation takes about 3 ps (Fig. 5). Subsequent electron-transfer steps have been designed through evolution to prevent the primary charge separation from recombining. This is accomplished by transferring the electron within 200 ps from pheophytin to a plastoquinone molecule (Q_A) that is permanently bound to photosystem II. Although plastoquinone normally acts as a two-electron acceptor, it works as a one-electron acceptor at the Q_A site. The electron on Q_A- is then transferred to another plastoquinone molecule that is loosely bound at the Q_B site. Plastoquinone at the Q_B site differs from Q_A in that it works as a two-electron acceptor, becoming fully reduced and protonated after two photochemical turnovers of the reaction center. The full reduction of plastoquinone requires the addition of two electrons and two

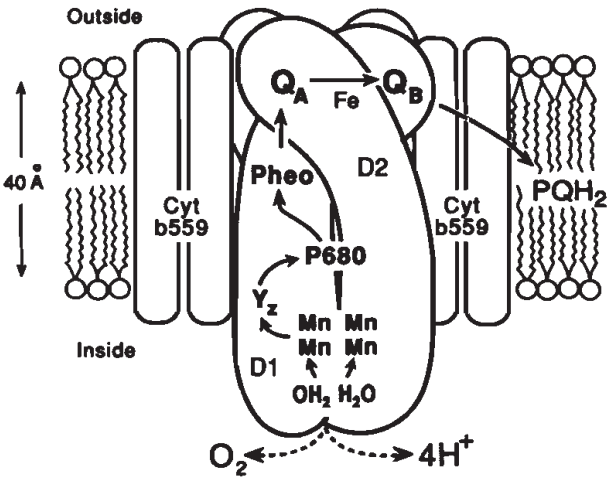


FIG. 4. Schematic drawing of photosystem II. Photosystem II is composed of numerous polypeptides, but only two of them, *D*1 and *D*2, bind the electron carriers involved in transferring electrons from Y_z to plastoquinone. Abbreviations: Y_z, tyrosine; P680, reaction-center chlorophyll (primary electron donor); Pheo, pheophytin; Q_A and Q_B, bound plastoquinone; PQH_2, reduced plastoquinone; Cyt *b*559, *b*-type cytochrome. Details are given in the text.

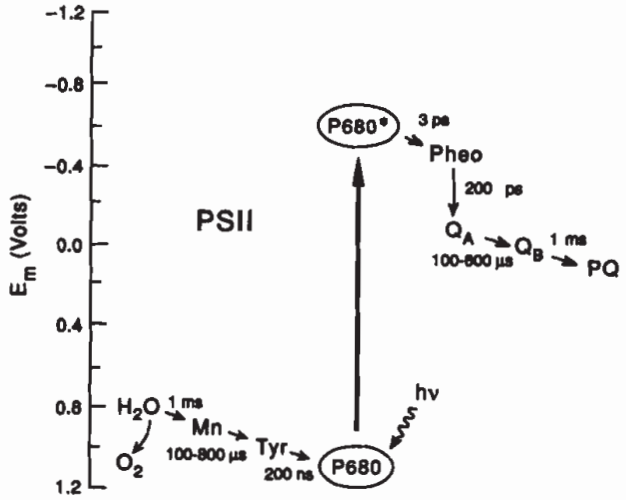


FIG. 5. Photosystem II electron-transport pathways and rates. The vertical axis shows the midpoint potential of the electron carriers. The heavy vertical arrow shows light absorption. P680* is the electronically excited state of P680. The abbreviations are given in the legend of Fig. 4.

protons, i.e., the addition of two hydrogen atoms (Fig. 6). The reduced plastoquinone then debinds from the reaction center and diffuses into the hydrophobic core of the membrane, then an oxidized plastoquinone molecule finds its way to the Q_B-binding site, and the process is repeated. Because the Q_B site is near the outer aqueous phase, the protons added to plastoquinone during its reduction are taken from the outside of the membrane.

Photosystem II is the only known protein complex that can oxidize water, resulting in the release of O_2 into the atmosphere. Despite years of research, little is known about the molecular events that lead to water oxidation. Energetically, water is a poor electron donor. The oxidation-reduction midpoint potential ($E_{m,7}$) of water is +0.82 V (*p*H 7). In photosystem II, this reaction is driven by the oxidized reaction center, $P680^+$ (the midpoint potential of $P680/P680^+$ is estimated to be +1.2 V at *p*H 7). How electrons are transferred from water to $P680^+$ remains a mystery (Govindjee and Coleman, 1990). It is known that $P680^+$ oxidizes a tyrosine on the *D*1 protein and that Mn plays a key role in water oxidation. Four Mn ions are present in the water-oxidizing complex. X-ray absorption spectroscopy shows that Mn undergoes light-induced oxidation. Water oxidation requires two molecules of water and involves four sequential turnovers of the reaction center. Each photochemical reaction creates an oxidant that removes one electron. The net reaction results in the release of one O_2 molecule, the deposition of four protons into the inner water phase, and the transfer of four electrons to the Q_B site (producing two reduced plastoquinone molecules).

The photosystem I complex catalyzes the oxidation of plastocyanin, a small soluble Cu protein, and the reduction of ferredoxin, a small FeS protein (Fig. 7). Photosystem I is

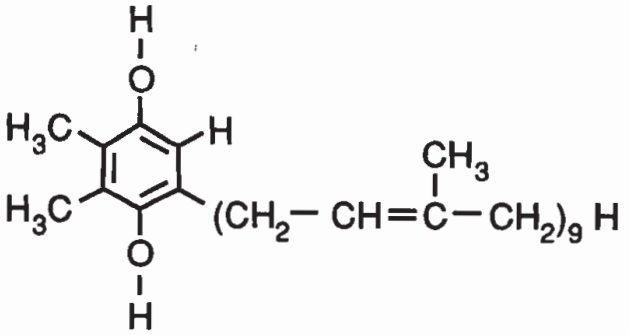


FIG. 6. Structure of plastoquinone (reduced form), an aromatic molecule that carries electrons and protons in photosynthetic electron transport.

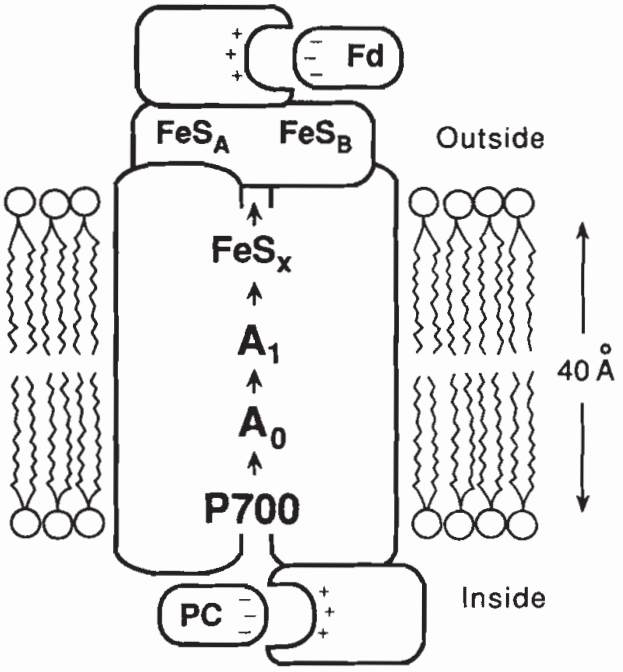


FIG. 7. Schematic drawing of photosystem I. Photosystem I is composed of numerous polypeptides, but only three of them bind the electron carriers. Abbreviations: PC, plastocyanin; P700, reaction-center chlorophyll (primary electron donor); A_0, chlorophyll; A_1, phylloquinone; FeS, FeS centers; Fd, ferredoxin. Details are given in the text.

composed of a heterodimer of proteins that act as ligands for most of the electron carriers (Krauss *et al.*, 1993). The reaction center is served by an antenna system that consists of about two hundred chlorophyll molecules (mainly chlorophyll *a*), and primary photochemistry is initiated by a chlorophyll *a* dimer, P700. In contrast to photosystem II, many of the antenna chlorophyll molecules in photosystem I are bound to the reaction-center proteins. Also, FeS centers serve as electron carriers in photosystem I, and so far as is known, photosystem I electron transfer is not coupled to proton translocation. Primary charge separation occurs between a primary donor, P700, a chlorophyll dimer, and a chlorophyll monomer (A_0). The subsequent electron transfer events and rates are shown in Fig. 8.

4.4 Electron Transport

Electron transport from water to $NADP^+$ requires three membrane-bound protein complexes operating in series: photosystem II, the cytochrome bf complex, and photosystem I (Fig. 3). Electrons are transferred between these large protein complexes by small mobile molecules. Because these small molecules carry electrons (or hydrogen atoms) over relatively long distances, they play a unique role in photosynthetic energy conversion. This is illustrated by plastoquinone (PQ), which serves two key functions. Plastoquinone transfers electrons from the photosystem II reaction center to the cytochrome *bf* complex and carries protons across the photosynthetic membrane. It does this by shuttling hydrogen atoms across the membrane from photosystem II to the cytochrome *bf* complex. Because plastoquinone is hydrophobic, its movement is restricted to the hydrophobic core of the photosynthetic membrane. Plastoquinone operates by diffusing through the membrane until, as a result of random collisions, it becomes bound to a specific site on the photosystem II complex. The photosystem II reaction center reduces plastoquinone at the Q_B site by adding two electrons and two protons, creating PQH_2. The reduced plastoqinone molecule debinds from photosystem II and diffuses randomly in the photosynthetic membrane until it encounters a specific binding site on the cytochrome *bf* complex. The cytochrome *bf* complex is a membrane-bound protein complex that contains four electron carriers, three cytochromes, and an FeS center. In a complicated reaction sequence that is not fully understood, the cytochrome *bf* complex removes the electrons from reduced plastoquinone and facilitates the release of the protons into the inner aqueous space. The electrons are eventually transferred to the photosystem I reaction center. The protons released into the inner aqueous space contribute to the proton chemical free energy across the membrane.

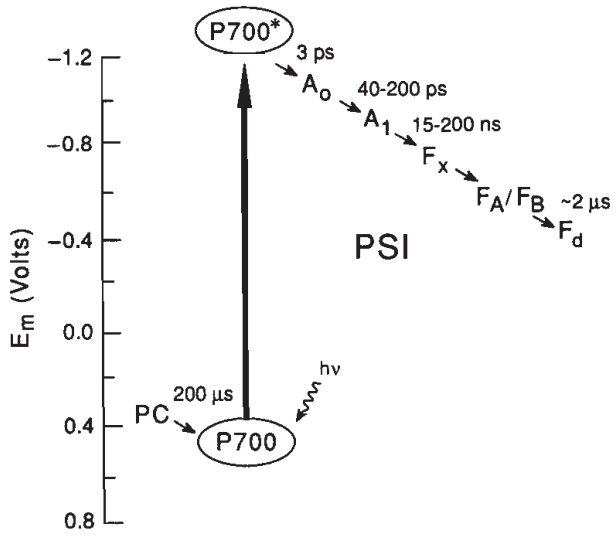


FIG. 8. Photosystem I electron transport pathways and rates. The vertical axis shows the midpoint potential of the electron carriers. Abbreviations are given in the legend of Fig. 7.

Electron transfer from the cytochrome *bf* complex to photosystem I is mediated by a small Cu protein, plastocyanin (PC). Plastocyanin is water soluble and operates in the inner water space of the photosynthetic membrane. Electron transfer from photosystem I to $NADP^+$ requires ferredoxin, a small FeS protein, and ferredoxin-NADP oxidoreductase, a peripheral flavoprotein that operates on the outer surface of the photosynthetic membrane. Ferredoxin and $NADP^+$ are water soluble and are found in the outer aqueous phase.

As discussed in Sec. 3, the pathway of electrons is largely determined by the energetics of the reaction and the distance between the carriers. The electron affinity of the carriers is represented in Fig. 9 by their midpoint potentials, which show the free energy available for electron-transfer reactions under equilibrium conditions. (It should be kept

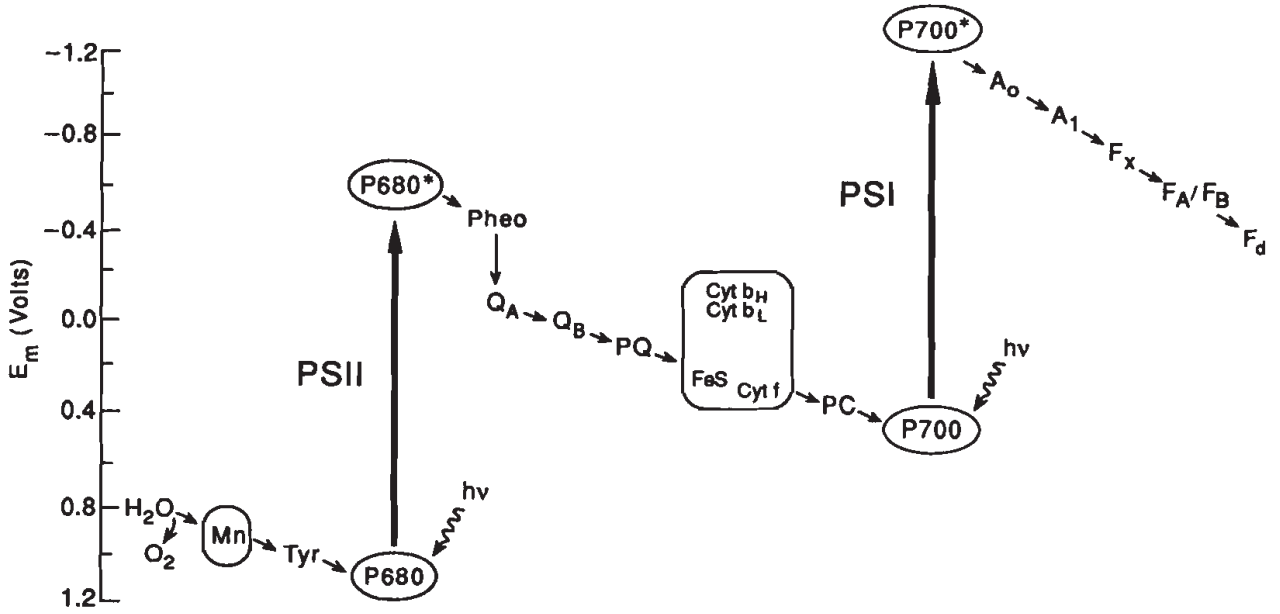


FIG. 9. The electron transport pathway of plants (oxygenic photosynthesis). Abbreviations are given the legend of Fig. 3. Details are given in the text.

in mind that reaction conditions during photosynthesis are not in equilibrium.) Subsequent to primary charge separation, electron transport is energetically downhill (from a lower to a higher redox potential). It is the downhill flow of electrons that provides free energy for the creation of a proton chemical gradient.

Photosynthetic membranes effectively limit electron transport to two dimensions. For mobile electron carriers, limiting diffusion to two dimensions increases the number of random encounters (Whitmarsh, 1986). Furthermore, because plastocyanin is mobile, any one cytochrome *bf* complex can interact with a number of photosystem I complexes. The same is true for plastoquinone, which commonly operates at a stoichiometry of about six molecules per photosystem II complex.

4.5 Creation of a Proton Electrochemical Potential

Electron transport creates the proton electrochemical potential of the photosynthetic membrane by two types of reactions.

1. The release of protons during the oxidation of water by photosystem II and the translocation of protons from the outer aqueous phase to the inner aqueous phase by the coupled reactions of photosystem II and the cytochrome *bf* complex in reducing and oxidizing plastoquinone on opposite sides of the membrane creates a concentration difference of protons across the membranes ($\Delta pH = pH_{in} - pH_{out}$).
2. Primary charge separation at a reaction center drives an electron across the photosynthetic membrane, which creates an electric potential across the membrane ($\Delta\Psi = \Psi_{in} - \Psi_{out}$).

Together, these two forms of energy make up the proton electrochemical potential across the photosynthetic membrane ($\Delta\mu_{H^+}$), which is related to the *p*H difference across the membrane and the electrical potential difference across the membrane by the following equation:

$$\Delta\mu_{H^+} = F\Delta\Psi - 2.3RT\,\Delta pH,$$

where F is the Faraday constant, R is the gas constant, and T is the temperature in kelvins. Although the value of $\Delta\Psi$ across the photosynthetic membrane in chloroplasts can be as large as 100 mV, under normal conditions the proton gradient dominates. For example, during photosynthesis, the outer *p*H is typically near 8 and the inner *p*H is typically near 6, giving a *p*H difference of 2 across the membrane that is equivalent to 120 mV. Under these conditions, the free energy for proton transfer from the inner to the outer aqueous phase is −12 kJ/mol of protons.

4.6 Synthesis of ATP by the ATP-Synthase Enzyme

The conversion of proton electrochemical energy into chemical free energy is accomplished by a single protein complex known as ATP synthase. This enzyme catalyzes a phosphorylation reaction, which is the formation of ATP by the addition of inorganic phosphate (P_i) to ADP:

$$ADP^{-3} + P_i^{-2} + H^+ \rightarrow ATP^{-4} + H_2O.$$

The reaction is energetically uphill (ΔG = +32 kJ/mol) and is driven by proton transfer through the ATP-synthase protein. The ATP-synthase complex is composed of two major subunits, CF_0 and CF_1 (Fig. 10). The CF_0 subunit spans the photosynthetic membrane and forms a proton channel through the membrane. The CF_1 subunit is attached to the top of the CF_0 on the outside of the membrane and is located in the aqueous space. The CF_1 subunit is composed of several different protein subunits, referred to as α, β, γ, δ, and ϵ. The top portion of the CF_1 subunit is composed of three $\alpha\beta$ dimers that contain the catalytic sites for ATP synthesis. The molecular processes that couple proton transfer through the protein to the chemical addition of phosphate to ADP are poorly understood. It is known that phosphorylation can be driven by a *p*H gradient, a transmembrane electric field, or a combination of the two. Experiments indicate that three protons must pass through the ATP-synthase complex for the synthesis of one molecule of ATP. However, the protons are not involved in the chemistry of adding phosphate to ADP. Paul Boyer and co-workers have proposed an alternating binding site mechanism for ATP synthesis (Boyer, 1993). One model based on their proposal is that there are three catalytic sites on each CF_1 that cycle among three different states. The states differ in their affinity for ADP, P_i, and ATP. At any one time, each site is in a different state. Initially, one catalytic site on CF_1 binds one ADP and one inorganic phosphate molecule relatively loosely. Through a conformational change of the protein, the site becomes a tight binding site, which stabilizes ATP. Next, proton transfer induces an alteration in protein conformation that causes the site to release the ATP molecule into the aqueous phase. In this model, the energy from the proton electrochemical gradient is used to lower the affinity of the site for ATP, allowing its release to the water phase. The three sites on CF_1 act cooperatively; i.e., the conformational states of the sites are linked. It has been proposed that protons affect the conformational change by driving the rotation of the top part (the three $\alpha\beta$ dimers) of CF_1. This revolving-site mechanism would require rates as high as 100 rev/s. It is worth noting that flagella that propel some bacteria are driven by a proton pump and can rotate at 60 rev/s.

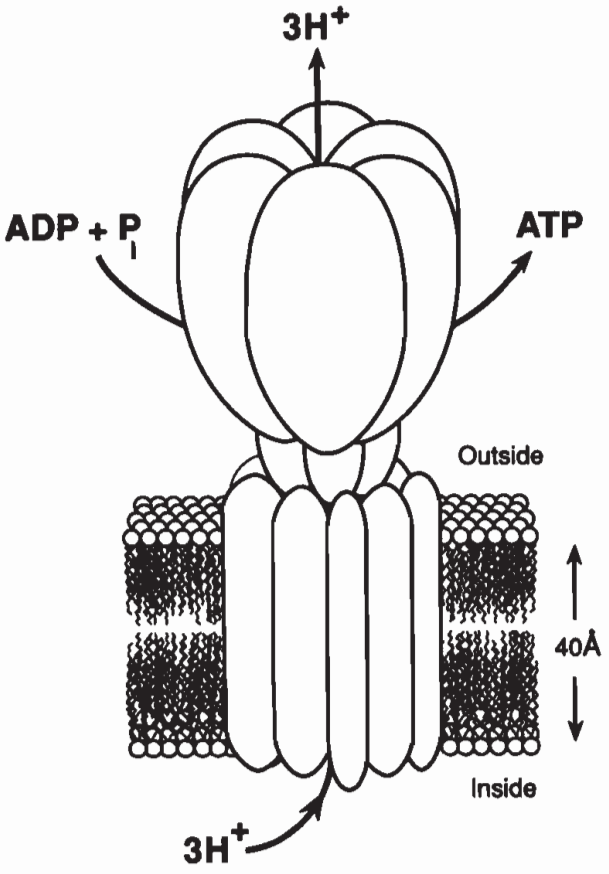


FIG. 10. Schematic drawing of the ATP-synthase enzyme embedded in the membrane. Proton transfer through the ATP synthase provides the energy for the creation of ATP from ADP and P_i. Abbreviations are given in the legend of Fig. 3. Details are given in the text.

4.7 Synthesis of Carbohydrates from Atmospheric CO_2 by the Calvin-Cycle Enzymes

All plants and algae remove CO_2 from the environment and reduce it to carbohydrate by the Calvin cycle. The process is a sequence of biochemical reactions that reduce carbon and rearrange bonds to produce car-

bohydrate from CO_2 molecules. The first step is the addition of CO_2 to a five-carbon compound (ribulose 1,5-bisphosphate) (Fig. 11). The six-carbon compound is split, giving two molecules of a three-carbon compound (3-phosphoglycerate). This key reaction is catalyzed by Rubisco, a large water-soluble protein complex. The carboxylation reaction is energetically downhill. The main energy input in the Calvin cycle is the phosphorylation by ATP and subsequent reduction by NADPH of the initial three-carbon compound forming a three-carbon sugar, triose phosphate. Some of the triose phosphate is exported from the chloroplast and provides the building block for synthesizing more complex molecules. In a process known as regeneration, the Calvin cycle uses some of the triose phosphate molecules to synthesize the energy-rich ribulose 1,5-bisphosphate needed for the initial carboxylation reaction. This reaction requires the input of energy in the form of one ATP. Overall, 13 enzymes are required to catalyze the reactions in the Calvin cycle. The energy-conversion efficiency of the Calvin cycle is approximately 90%. The reactions do not involve energy transduction but rather the rearrangement of chemical energy. Each molecule of CO_2 reduced to a sugar $[CH_2O]_n$ requires two molecules of NADPH and three molecules of ATP.

Rubisco is a bifunctional enzyme that, in addition to binding CO_2 to ribulose bisphosphate, can also bind O_2. This oxygenation reaction produces the 3-phosphoglycerate that is used in the Calvin cycle and a two-carbon compound (2-phosphoglycolate) that is not useful for the plant. In response, a complicated set of reactions (known as photorespiration) are initiated that serve to recover reduced carbon and to remove phosphoglycolate. The Rubisco oxygenation reaction appears to serve no useful purpose for the plant. Some plants have evolved specialized structures and biochemical pathways that concentrate CO_2 near Rubisco, which serves to decrease the fraction of oxygenation reactions.

4.8 Photosynthetic Quantum Yield and Energy Conversion Efficiency

The theoretical minimum quantum requirement for photosynthesis is eight quanta for each molecule of oxygen evolved (four quanta required by photosystem II and four by photosystem I). Measurements in leaves under optimal conditions (e.g., low light) give

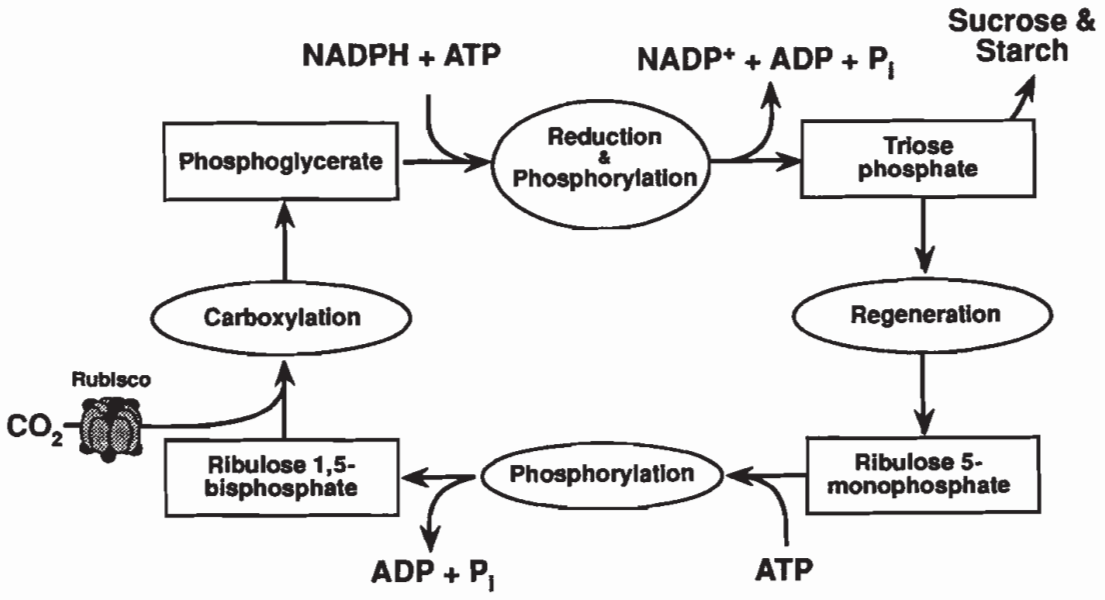


FIG. 11. An abbreviated scheme showing reduction of carbon dioxide by the Calvin cycle. The first step is carboxylation, in which ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes the addition of CO_2 to the five-carbon compound ribulose 1,5-bisphosphate, which is subsequently split into two molecules of the three-carbon compound 3-phosphoglycerate. Next are reduction and phosphorylation reactions that form the carbohydrate triose phosphate. Some of the triose phosphate molecules are used to form the products of photosynthesis, sucrose and starch, while the rest is used to regenerate ribulose 1,5-bisphosphate needed for the continuation of the cycle. Details are given in the text.

quantum requirements of 8–10 photons per oxygen molecule released. These quantum yield measurements show that the quantum yields of photosystem II and photosystem I reaction centers under optimal conditions are near 100%. These values can be used to calculate the theoretical energy conversion efficiency of photosynthesis (free energy stored as carbohydrate/light energy absorbed). If eight red quanta are absorbed (8 mol of red photons are equivalent to 1400 kJ) for each CO_2 molecule reduced (480 kJ/mol), the theoretical maximum energy efficiency for carbon reduction is 34%. Under optimal conditions, plants can achieve energy conversion efficiencies within 90% of the theoretical maximum. However, under normal growing conditions, the actual performance of the plant is far below these theoretical values. The factors that conspire to lower the quantum yield of photosynthesis include limitations imposed by biochemical reactions in the plant and environmental conditions that limit photosynthetic performance. One of the most efficient crop plants is sugar cane, which has been shown to store up to 1% of the incident visible radiation over a period of 1 year. However, most crops are less productive. The annual conversion efficiency of corn, wheat, rice, potatoes, and soybeans typically ranges from 0.1% to 0.4% (Odum, 1971).

5. OXYGENIC PHOTOSYNTHESIS IN ALGAE

Algae are photosynthetic eukaryotic organisms that evolve O_2 and reduce CO_2. They represent a diverse group that include dinoflagellates, euglenoids, yellow-green algae, golden-brown algae, diatoms, red algae, brown algae, and green algae. The photosynthetic apparatus and biochemical pathways of carbon reduction of algae are similar to plants. Photosynthesis occurs in chloroplasts that contain photosystems II and I, the cytochrome bf complex, the Calvin-cycle enzymes, and pigment-protein complexes containing chlorophyll *a* and other antenna pigments (*e.g.*, chlorophyll *b* in green algae, chlorophyll *c* and fucoxanthol in brown algae and diatoms, and phycobilins in red algae). Green algae are thought to be the ancestral group from which land plants evolved. Algae are abundant and widespread on the earth, living mainly in fresh and sea water. Some algae live as single-celled organisms, while others form multicellular organisms, some of which can grow quite large, such as kelp and seaweed. Phytoplankton in the ocean is made up of algae and oxygenic photosynthetic bacteria. Most photosynthesis in the ocean is due to phytoplankton, which is an important source of food for marine life.

6. OXYGENIC PHOTOSYNTHESIS IN BACTERIA

6.1 Cyanobacteria

Cyanobacteria are photosynthetic prokaryotic organisms that evolve O_2. Fossil evidence indicates that cyanobacteria existed over 3 billion years ago, and it is thought that they were the first oxygen-evolving organisms on earth. Cyanobacteria are presumed to have evolved in water in an atmosphere that lacked O_2. Initially, the O_2 released by cyanobacteria reacted with ferrous iron in the oceans and was not released into the atmosphere. Geological evidence indicates that the ferrous Fe was depleted around 2 billion years ago, and earth's atmosphere became aerobic. The release of O_2 into the atmosphere by cyanobacteria has had a profound affect on how life evolved.

The photosynthetic apparatus of cyanobacteria is similar to that of chloroplasts. The main difference lies in the antenna system. Cyanobacteria depend on chlorophyll *a* and specialized protein complexes called phycobilisomes that gather light energy. They do not contain chlorophyll *b*. As in chloroplasts, the chlorophyll *a* is located in membrane-bound proteins. The phycobilisomes are bound to the outer side of the photosynthetic membrane and act to funnel exciton energy to the photosystem II reaction center. They are composed of phycobiliproteins, protein subunits that contain covalently attached open ring structures known as bilins that are the light-absorbing pigments. Primary photochemistry, electron transport, phosphorylation, and carbon reduction occur much as they do in chloroplasts. Cyanobacteria have a simpler genetic system than plants and algae, which enables them to be easily modified genetically. Because of this, cyanobacteria have been used as a model to understand

photosynthesis in plants. By genetically altering photosynthetic proteins, researchers can investigate the relationship between molecular structure and function.

6.2 Prochlorophytes

Over the past three decades, several types of oxygenic bacteria known as prochlorophytes (or oxychlorobacteria) have been discovered that have light-harvesting protein complexes that contain chlorophyll *a* and *b* but do not contain phycobilisomes (Palenik and Haselkorn, 1992). Because prochlorophytes have chlorophyll *a*/*b* light-harvesting proteins like chloroplasts, they are being investigated as models for plant photosynthesis.

7. ANOXYGENIC PHOTOSYNTHESIS IN BACTERIA

Anoxygenic photosynthetic bacteria differ from oxygenic organisms in that each species has only one type of reaction center. In some photosynthetic bacteria, the reaction center is similar to photosystem II, and in others, it is similar to photosystem I. However, neither of these two types of bacterial reaction center is capable of extracting electrons from water, and so they do not evolve O_2. Many species can only survive in environments that have a low concentration of O_2. To provide electrons for the reduction of CO_2, anoxygenic photosynthetic bacteria must oxidize inorganic or organic molecules available in their environment. For example, the purple bacterium *Rhodobacter sphaeroides* can use succinate to reduce NAD^+ by a membrane-linked reverse electron transfer that is driven by a transmembrane electrochemical potential. Although many photosynthetic bacteria depend on Rubisco and the Calvin cycle for the reduction of CO_2, some are able to fix atmospheric CO_2 by other biochemical pathways.

Despite these differences, the general principles of energy transduction are the same in anoxygenic and oxygenic photosynthesis. Anoxygenic photosynthetic bacteria depend on bacteriochlorophyll, a family of molecules similar to chlorophyll that absorb strongly in the infrared between 700 and 1000 nm. The antenna system consists of bacteriochlorophyll and carotenoids that serve a reaction center where primary charge separation occurs. The electron carriers include quinones (e.g., ubiquinone, menaquinone) and the cytochrome *bc* complex, which is similar to the cytochrome *bf* complex of oxygenic photosynthetic apparatus. As in oxygenic photosynthesis, electron transfer is coupled to the generation of an electrochemical potential that drives phosphorylation by ATP synthase. The energy required for the reduction of CO_2 is provided by ATP and NADH, a molecule similar to NADPH.

7.1 Purple Bacteria

There are two divisions of photosynthetic purple bacteria: the nonsulfur purple bacteria (e.g., *Rhodobacter sphaeroides* and *Rhodospeudomonas viridis*) and the sulfur purple bacteria (e.g., *Chromatium vinosum*). Nonsulfur purple bacteria typically use an organic electron donor, such as succinate or malate, but they can also use hydrogen gas. The sulfur bacteria use an inorganic sulfur compound, such as hydrogen sulfide, as the electron donor. The only pathway for carbon fixation by purple bacteria is the Calvin cycle. Sulfur purple bacteria must fix CO_2 to live, whereas nonsulfur purple bacteria can grow aerobically in the dark by respiration on an organic carbon source.

The determination of the three-dimensional structures of the reaction center of the nonsulfur purple bacteria, *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides,* provides an unprecedented opportunity to understand the structure and function of photosynthetic reaction centers (Deisenhofer *et al.*, 1984, 1985; Feher *et al.*, 1989). The positions of the electron-transfer components in the reaction center of *Rhodobacter sphaeroides* are shown in Fig. 12 (Norris and van Brakel, 1986). The reaction center contains four bacteriochlorophyll and two bacteriopheophytin molecules. Two of the bacteriochlorophyll molecules form the primary donor (P870). At present, there is controversy over whether a bacteriochlorophyll molecule is an intermediate in electron transfer from the P870 to bacteriopheophytin. However, there is agreement that the remaining steps involve two quinone molecules (Q_A and Q_B) and that two turnovers of the reaction center result in the release of reduced quinone (QH_2)

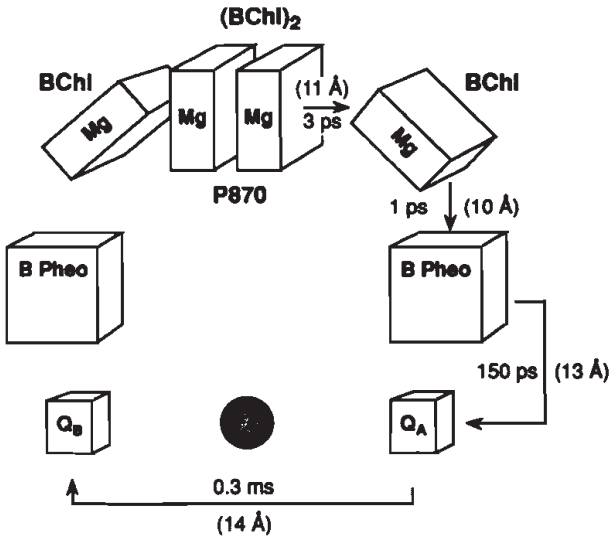


FIG. 12. Relative positions of the chromophores of the reaction center of *Rhodobacter sphaeroides* (from Norris and van Brakel, 1986). Abbreviations: P870, reaction center bacteriochlorophyll (primary electron donor); B Pheo, bacteriopheophytin; Q_A and Q_B, bound ubiquinone. Details are given in the text.

into the photosynthetic membrane. Although there is a nonheme Fe between the two quinone molecules, there is convincing evidence that this Fe is not involved directly in transferring an electron from Q_A to Q_B. Because the primary donor (P870), bacteriopheophytin, and quinone acceptors of the purple bacterial reaction center are similar to the photosystem II reaction center, the bacterial reaction center is used as a guide to understand the structure and function of photosystem II.

Light-driven electron transfer is cyclic in *Rhodobacter sphaeroides* and other purple bacteria (Fig. 13). The reaction center produces reduced quinone, which is oxidized by the cytochrome *bc* complex. Electrons from the cytochrome *bc* complex are transferred to a soluble electron carrier, cytochrome c_2, which reduces the oxidized primary donor $P870^+$. The product of the light-driven electron transfer reactions is ATP. The electrons for the reduction of carbon are extracted from an organic donor, such as succinate or malate, or from hydrogen gas, but not by the reaction center. The energy needed to reduce NAD^+ is provided by light-driven cyclic electron transport in the form of ATP. The energy transformation pathway is complicated. Succinate is oxidized by the membrane-bound enzyme succinate dehydrogenase that transfers the electrons to quinone—the source of electrons for the reduction of NAD^+. However, electron transfer from reduced quinone to NAD^+ is energetically uphill. By a mech-

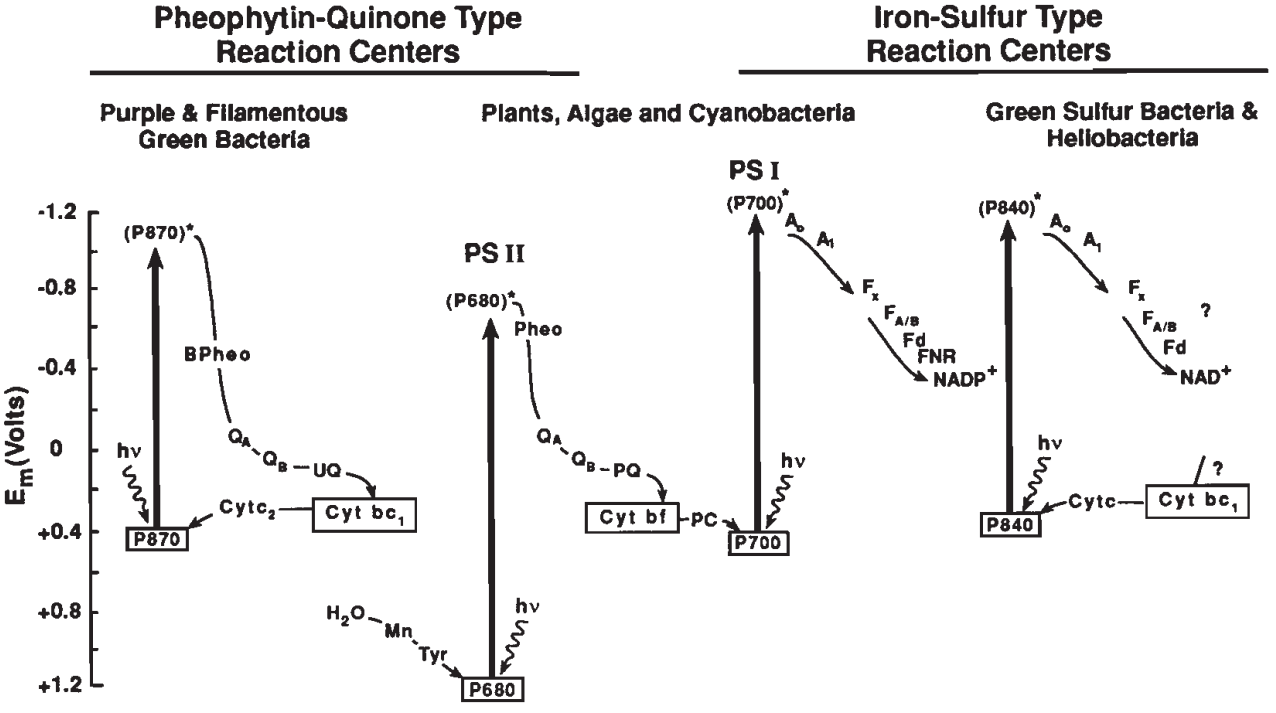


FIG. 13. Comparison of electron-transport pathways in oxygenic and anoxygenic organisms (from Blankenship, 1992). Abbreviations: Cyt bc_1, cytochrome *bc* complex; P840, reaction center bacteriochlorophyll; other abbreviations are given in the legends of Figs. 3 and 12.

anism that is poorly understood, a membrane-bound enzyme is able to use energy stored in the proton electrochemical potential to drive electrons from reduced quinone to NAD^+.

7.2 Green Sulfur Bacteria

Green sulfur bacteria (e.g., *Chlorobium thiosulfatophilum* and *Chlorobium vibrioforme)* can use sulfur compounds as the electron donor as well as organic hydrogen donors. As shown in Fig. 13, the reaction center of green sulfur bacteria is similar to the photosystem I reaction center of oxygenic organisms. The FeS centers in the reaction center can reduce NAD^+ (or $NADP^+$) by ferredoxin and the ferredoxin-$NAD(P)^+$ oxidoreductase enzyme; therefore green sulfur bacteria are not necessarily dependent on reverse electron flow for carbon reduction. The antenna system of the green sulfur bacteria is composed of bacteriochlorophyll and carotenoids and is contained in complexes known as chlorosomes that are attached to the surface of the photosynthetic membrane. This antenna arrangement is similar to the phycobilisomes of cyanobacteria. Green sulfur bacteria can fix CO_2 without Rubisco. It has been proposed that they accomplish this by using the respiratory chain that normally oxidizes carbon (known as the Krebs cycle), resulting in the release of CO_2. With the input of energy, this process can be run in the reverse direction, resulting in the uptake and reduction of CO_2.

7.3 Green Gliding Bacteria

Green gliding bacteria (e.g., *Chloroflexus aurantiacus*), also known as green filamentous bacteria, can grow photosynthetically under anaerobic conditions or in the dark by respiration under aerobic conditions. Like the green sulfur bacteria, green gliding bacteria harvest light using chlorosomes. The green gliding bacteria appear to have reaction centers similar to those of the purple bacteria (Fig. 13), but there are several notable differences. For example, instead of two monomer bacteriochlorophyll molecules, *C. aurantiacus* has one bacteriochlorophyll and one bacteriopheophytin, and the metal between the two quinones is Mn rather than Fe. *C. aurantiacus* appears to fix CO_2 by a scheme that does not involve the Calvin cycle or the reverse Krebs cycle (Ivanovsky *et al.*, 1993).

7.4 Heliobacteria

Heliobacteria (e.g., *Heliobacterium chlorum* and *Heliobacillus mobilis*) are in the phylum Gram Positive Bacteria that are strict anaerobes. Although the heliobacterial reaction center is similar to photosystem I in that it can reduce NAD^+ (or $NADP^+$), it contains a different type of chlorophyll known as bacteriochlorophyll *g*.

8. CONTROL OF INTRAPROTEIN ELECTRON TRANSFER

The three-dimensional structure of the reaction center of *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides* reveals the distances between the electron donors and acceptors (Deisenhofer *et al.*, 1984,1985; Norris and van Brakel, 1986; Feher *et al.*, 1989) and has had an important influence on biophysical and molecular genetics studies designed to identify the factors that control the rate of electron transfer within proteins. There is currently a controversy concerning the importance of the amino acid composition of the protein on the rate of intraprotein electron transfer. In part, the disagreement centers on whether the protein between the donor and acceptor molecules can be treated as a uniform material or whether the specific amino acid composition of the protein significantly alters the rate. For example, it has been proposed that aromatic amino acids may provide a particular pathway that facilitates electron transfer between a donor and acceptor pair. This is the case in the photosystem II reaction center, where a tyrosine residue on one of the reaction center core proteins donates an electron to the primary donor chlorophyll, $P680^+$. However, in other cases, replacement of an aromatic residue by another nonaromatic residue results in relatively minor changes in the rate of electron transfer. L. Dutton and co-workers (Moser *et al.*, 1992) have analyzed electron-transfer reactions in biological and chemical systems in terms of electron tunneling theory developed by R. Marcus and others (DeVault, 1984). Dutton and co-workers argue that protein provides a uniform electronic barrier to elec-

tron tunneling and a uniform nuclear characteristic frequency. They suggest that the specific amino acid residues between an electron transfer pair are generally of less importance than the distance in determining the rate of pairwise electron transfer. In their view, protein controls the rate of electron transfer mainly through the distance between the donor and acceptor molecules, the free energy, and the reorganization energy of the reaction. The importance of distance is demonstrated by electron-transfer data from biological and synthetic systems showing that the dependence of the electron transport rate on the edge-to-edge distance is exponential over 12 orders of magnitude when the free energy is optimized (Moser *et al.*, 1992). Increasing the distance between two carriers by 1.7 Å slows the rate of electron transfer tenfold. The extent to which this view is generally applicable for intraprotein electron transfer remains to be established (Williams, 1992). One of the challenges in understanding pairwise electron-transfer rates from first principles is illustrated by the reaction center of *Rhodobacter sphaeroides*, in which the redox components are arranged along a twofold axis of symmetry that extends from the primary donor (P870) to the Fe. Despite the fact that the reaction center presents two spatially similar pathways for electron transfer from P870 to quinone, nearly all electrons are transferred down the right arm of the reaction center, as shown in Fig. 12. The same holds true for the reaction center of *Rhodopseudomonas viridis*, in which it is estimated that electron transfer down the left arm is less than 1:100 (Kellogg *et al.*, 1989). The challenge to theorists is to explain the surprisingly high probability of electron flow down the right arm. Since the distances are similar, it has been suggested that electron transfer down the left arm is less probable because of an endothermic free-energy change (Parson *et al.*, 1990) or to an unfavorable rearrangement energy for the reaction (Moser *et al.*, 1992).

9. GLOBAL PHOTOSYNTHESIS AND THE ATMOSPHERE

The amount of CO_2 removed from the atmosphere each year by oxygenic photosynthetic organisms is massive. It is estimated that photosynthetic organisms remove 100×10^{15} g/yr of carbon (Houghton and Woodwell, 1989). This is equivalent to 4×10^{18} kJ of free energy stored in reduced carbon, which is roughly 0.1% of the incident visible radiant energy incident on the earth per year. Each year the photosynthetically reduced carbon is oxidized, either by living organisms for their survival or by combustion. The result is that more CO_2 is released into the atmosphere from the biota than is taken up by photosynthesis. The amount of carbon released by the biota is estimated to be $(1–2) \times 10^{15}$ g/yr of carbon. Added to this amount is carbon released by the burning of fossil fuels, which amounts to 5×10^{15} g/yr of carbon. The oceans mitigate this increase by acting as a sink for atmospheric CO_2. It is estimated that the oceans remove about 2×10^{15} g/yr of carbon from the atmosphere. This carbon is eventually stored on the ocean floor. Although these estimates of sources and sinks are uncertain, the net global CO_2 concentration is increasing. Direct measurements show that each year the atmospheric carbon content is currently increasing by about 3×10^{15} grams. Over the past 200 years, CO_2 in the atmosphere has increased from about 280 parts per million (ppm) to its current level of 360 ppm. On the basis of predicted fossil fuel use and land management, it is estimated that the amount of CO_2 in the atmosphere will reach 700 ppm within the next century. The consequences of this rapid change in our atmosphere are unknown. Because CO_2 acts as a greenhouse gas, some climate models predict that the temperature of the earth's atmosphere may increase by 2–8 °C. Such a large temperature increase would lead to significant changes in rainfall patterns. Little is known about the impact of such drastic atmospheric and climatic changes on plant communities and crops. Current research is directed at understanding the interaction between global climate change and photosynthetic organisms.

GLOSSARY

ATP: Adenosine triphosphate, a small water-soluble molecule that acts as an energy currency in cells.

ATP Synthase: A membrane-bound protein complex that uses the energy stored

across the photosynthetic membrane to add inorganic phosphate to ADP, thus creating ATP. (Also known as coupling factor.)

Calvin Cycle: The biochemical reactions, initiated by Rubisco, that result in the reduction of CO_2 to a carbohydrate (also known as the photosynthetic carbon reduction cycle).

Cytochrome: Heme-containing protein.

Cytochrome *bc* Complex: A membrane-bound electron-transfer protein complex, found in all anoxygenic photosynthetic organisms, that oxidizes reduced quinone and reduces a *c*-type cytochrome. The complex contains a *c*-type cytochrome, two *b*-type cytochromes, and an FeS center.

Cytochrome *bf* Complex: A membrane-bound electron-transfer protein complex, found in all oxygenic photosynthetic organisms, that oxidizes reduced plastoquinone and reduces plastocyanin (or cytochrome *c*). The complex contains a *c*-type cytochrome, two *b*-type cytochromes, and an FeS center.

Free Energy: The amount of energy in a reaction available to do work. Because most biochemical reactions occur at a constant temperature and pressure, the free energy is frequently the Gibbs energy.

Light-Harvesting Complex: A protein complex that harvests light energy and converts it to exciton energy that can migrate to a reaction center. The light is absorbed by pigment molecules (e.g., chlorophyll, bacteriochlorophyll, carotenoids, phycobilin) that are attached to the protein.

NADPH: Reduced form of nicotinamide adenine dinucleotide phosphate, a small water-soluble molecule that acts as a hydrogen carrier in biochemical reactions.

$NADP^+$: Oxidized form of nicotinamide adenine dinucleotide phosphate.

Oxidation: The removal of one or more electrons from an atom or molecule. In the case of a molecule, protons may be involved as well, resulting in hydrogen being removed.

Phosphorylation: The covalent attachment of a phosphate group to a molecule.

Photorespiration: The removal of O_2 from the atmosphere by Rubisco and the subsequent biochemical reactions that serve to recycle some of the reduced carbon.

Photosynthesis: The physical-chemical process by which certain chlorophyll- (or bacteriochlorophyll-) containing organisms use light energy for the biosynthesis of organic molecules.

Photosynthetic Membrane: A bilayer of lipid molecules in which are embedded proteins that transform light energy into chemical free energy. (Also known as the thylakoid membrane.)

Photosystem I: A protein complex located in the photosynthetic membrane. Photosystem I is one of two types of reaction centers found in higher plants, algae, and cyanobacteria. The photosystem I reaction center uses light energy to transfer an electron from a mobile electron-transfer protein (plastocyanin or cytochrome *c*) on one side of the photosynthetic membrane to a mobile electron-transfer protein (ferredoxin) on the opposite side of the photosynthetic membrane.

Photosystem II: A protein complex found in the photosynthetic membrane. Photosystem II is one of two types of reaction centers found in higher plants, algae, and cyanobacteria. The photosystem II reaction center uses light energy to transfer electrons from water to plastoquinone. Photosystem II is the source of the molecular oxygen in the atmosphere.

Plastoquinone: A small organic molecule involved in electron and proton transfer in photosynthesis.

Protein: A chemical structure composed of one or more polypeptides. In photosynthesis, proteins serve as the scaffolding that hold the cofactors that gather light energy, transfer electrons, and catalyze biochemical reactions.

Reaction Center: A protein complex that uses light energy to create a stable charge separation by transferring a single electron energetically uphill from a donor molecule to an acceptor molecule, both of which are located in the reaction center.

Reduction: The addition of one or more electrons to an atom or molecule. In the case of a molecule, protons may be involved as well, resulting in hydrogen being added.

Rubisco (D-ribulose 1,5-bisphosphate carboxylase/oxygenase): A water-soluble protein complex responsible for the removal of CO_2 from the atmosphere. The enzyme works by attaching CO_2 to a five-carbon compound (1,5 ribulose bisphosphate) that is split into two identical three-carbon compounds (phosphoglycerate). In addition to catalyzing the removal of CO_2 from the atmosphere, Rubisco also catalyzes the removal of O_2 from

the atmosphere (less efficiently). The removal of O_2 is thought to be a consequence of poor design and leads to a complex set of compensatory reactions known as photorespiration.

Works Cited

Blankenship, R. (1992), *Photosynth. Res.* **33,** 91–111.

Boyer, P. D. (1993), *Biochim. Biophys. Acta* **1140,** 215–250.

Debus, R. (1992), *Biochim. Biophys. Acta* **1102,** 269–352.

Deisenhofer, J., Epp, O., Miki, K., Huber, R., Michel, H. (1984), *J. Mol. Biol.* **180,** 385–398.

Deisenhofer, J., Epp, O., Miki, K., Huber, R., Michel, H. (1985), *Nature* **318,** 618–624.

DeVault, D. (1984), *Quantum Mechanical Tunnelling in Biological Systems,* Cambridge, U.K.: Cambridge University Press.

Feher, G., Allen, J. P., Okamura, M. Y., Rees, D. C. (1989), *Nature* **339,** 111–116.

Gest, H. (1993), *FEMS Microbiol. Lett.* **112,** 1–6.

Govindjee and Coleman, W. J. (1990), *Sci. Am.* **262** (2), 50–58.

Houghton, R. A., Woodwell, G. M. (1989), *Sci. Am.* **260** (4), 36–44.

Ivanovsky, R. N., Krasilnikova, E. N, Fal, Y. I. (1993), *Arch. Microbiol.* **159,** 257–264.

Kellogg, E. C., Kolaczkowski, S., Wassielewski, M. R., Tiede, D. M. (1989), *Photosynth. Res.* **22,** 47–59.

Klotz, I. M. (1967), *Energy Changes in Biochemical Reactions,* New York: Academic Press, Chap. 6.

Krauss, N., Hinrichs, W., Witt, I., Fromme, P. Pritzkow, W., Dauter, Z. Betzel, C., Wilson, K. S., Witt, H. T., Saenger, W. (1993), *Nature* **361,** 326–331.

Kühlbrandt, W., Wang, D. N., Fujiyoshi, Y. (1994), *Nature* **367,** 614–621.

Moser, C. C., Keske, J. M., Warncke, K., Farid, R. S., Dutton, P. L. (1992), *Nature* **355,** 796–802.

Norris, J. F., van Brakel, G. (1986), in: Govindjee, J. Amesz, D. C. Fork (Eds.), *Light Emission by Plants and Bacteria,* Orlando: Academic Press, pp. 35–36.

Odum, E. P. (1971), *Fundamentals of Ecology,* Philadelphia: W.B. Saunders Co.

Palenik, B., Haselkorn, R. (1992), *Nature* **355,** 265–267.

Parson, W. W., Chu, Z. T., Warshel, A. (1990), *Biochim. Biophys. Acta* **1017,** 251–272.

Staehelin, L. A. (1986), *Encyclopedia Plant Physiol.* **19,** 1–84.

Whitmarsh, J. (1986), *Encyclopedia Plant Physiol.* **19,** 508–527.

Williams, R. J. P. (1992), *Nature* **355,** 770.

Woese, C. R., Kandler, O., Wheeler, M. L. (1990), *Proc. Natl. Acad. Sci. USA* **87,** 4576–4579.

Wraight, C. (1982), in: Govindjee (Ed.), *Photosynthesis,* New York: Academic Press, pp. 17–61

Further Reading

Cramer, W. A., Knaff, D. B. (1991), *Energy Transduction in Biological Membranes,* New York: Springer-Verlag.

Barber, J. (Ed.) (1992), *The Photosystems: Structure, Function and Molecular Biology,* New York: Elsevier.

Huzisige, H., Ke, B. (1993), "Dynamics of the History of Photosynthesis," in: *Photosynthesis Res.* **38,** 185–209.

Lawlor, D. W. (1993), *Photosynthesis: Molecular, Physiological and Environmental Processes,* Essex, U.K.: Longman Scientific & Technical.

Taiz, L., Zeiger, E. (1991), *Plant Physiology,* Redwood City, CA: Benjamin/Cummings Publishing Co.

Walker, D. (1992), *Energy, Plants and Man,* East Sussex, U.K.: Oxygraphics Limited.