2. The Photosynthetic Process

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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

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[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 *Methanococcus 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:

$$CO_2 + 2H_2O + \text{Light Energy} \rightarrow [CH_2O] + O_2 + H_2O, \qquad (1)$$
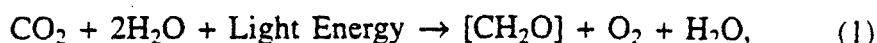
where $[CH_2O]$ represents a carbohydrate (e.g., glucose, a six-carbon sugar). The synthesis of carbohydrate from carbon and water requires a large input of light energy. The standard free energy for the reduction of one mole of CO_2 to the level of glucose is +478 kJ/mol. Because glucose, a six carbon sugar, is often an intermediate product of photosynthesis, the net equation of photosynthesis is frequently written as:

$$6CO_2 + 12H_2O + \text{Light Energy} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O. \qquad (2)$$
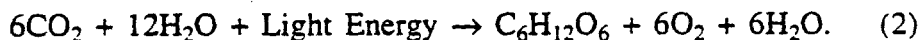
The standard free energy for the synthesis of glucose is +2.870 kJ/mol.

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:

$$CO_2 + 2H_2A + \text{Light Energy} \rightarrow [CH_2O] + 2A + H_2O. \qquad (3)$$
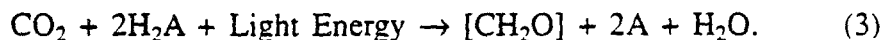
In oxygenic photosynthesis. 2A is O_2. whereas in anoxygenic photosynthesis, which occurs in some photosynthetic bacteria. the electron donor can be an inorganic hydrogen donor, such as H_2S (in which case A is elemental sulfur) or an organic hydrogen donor such as succinate (in which case. A is fumarate). 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
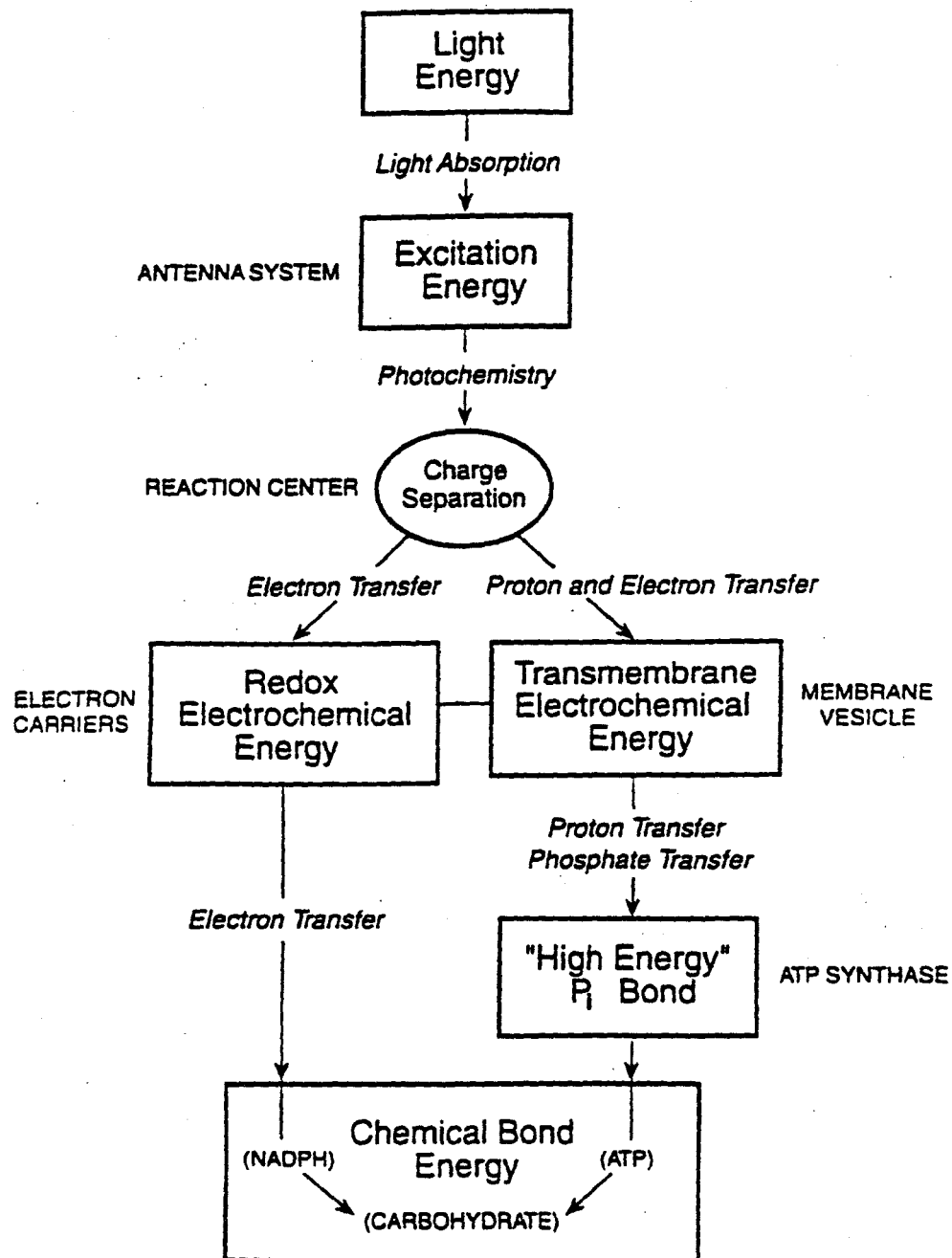
Energy Transformation in Photosynthesis

Light Energy

Light Absorption

ANTENNA SYSTEM — Excitation Energy

Photochemistry

REACTION CENTER — Charge Separation

Electron Transfer   *Proton and Electron Transfer*

ELECTRON CARRIERS — Redox Electrochemical Energy — Transmembrane Electrochemical Energy — MEMBRANE VESICLE

Proton Transfer
Phosphate Transfer

Electron Transfer

"High Energy" P_i Bond — ATP SYNTHASE

Chemical Bond Energy

(NADPH)   (ATP)

(CARBOHYDRATE)

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
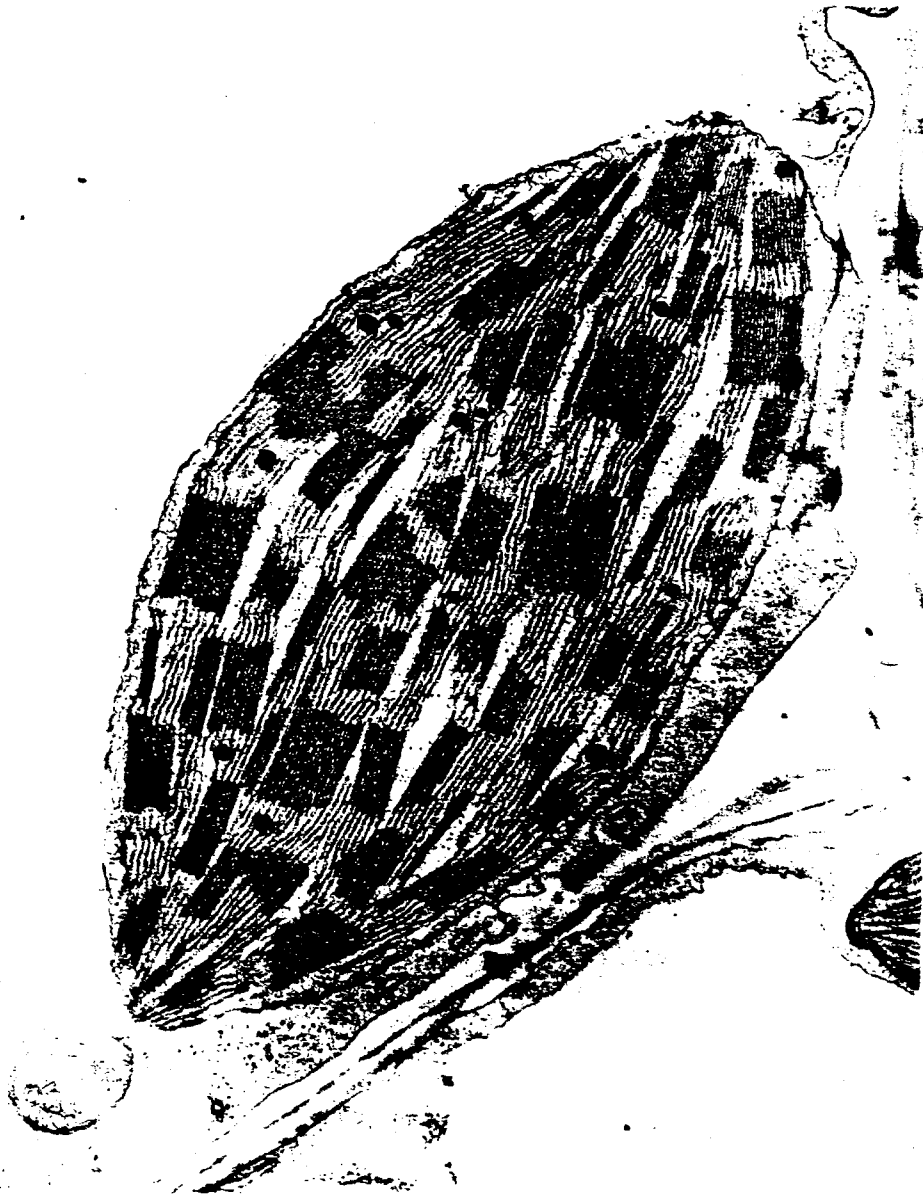
(a)
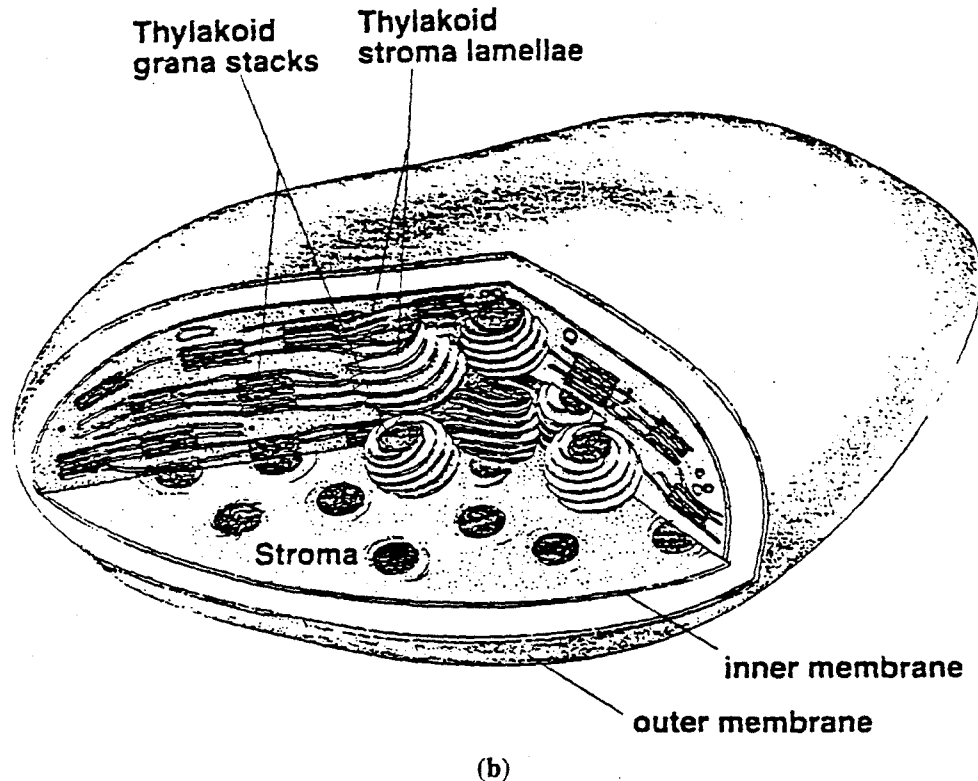
(b)

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.
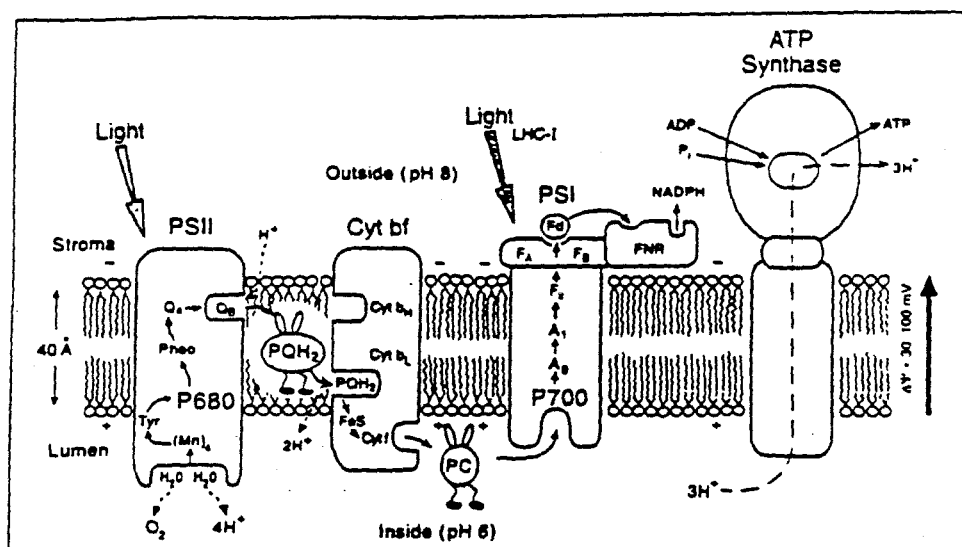
Fig. 3   Model of the photosynthetic membrane of plants showing the electron transport components and the ATP synthase enzyme (cross sectional view). Complete membrane forms a vesicle. Pathways of electrons are shown by solid arrows. Membrane bound electron transport protein complexes involved in transferring 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
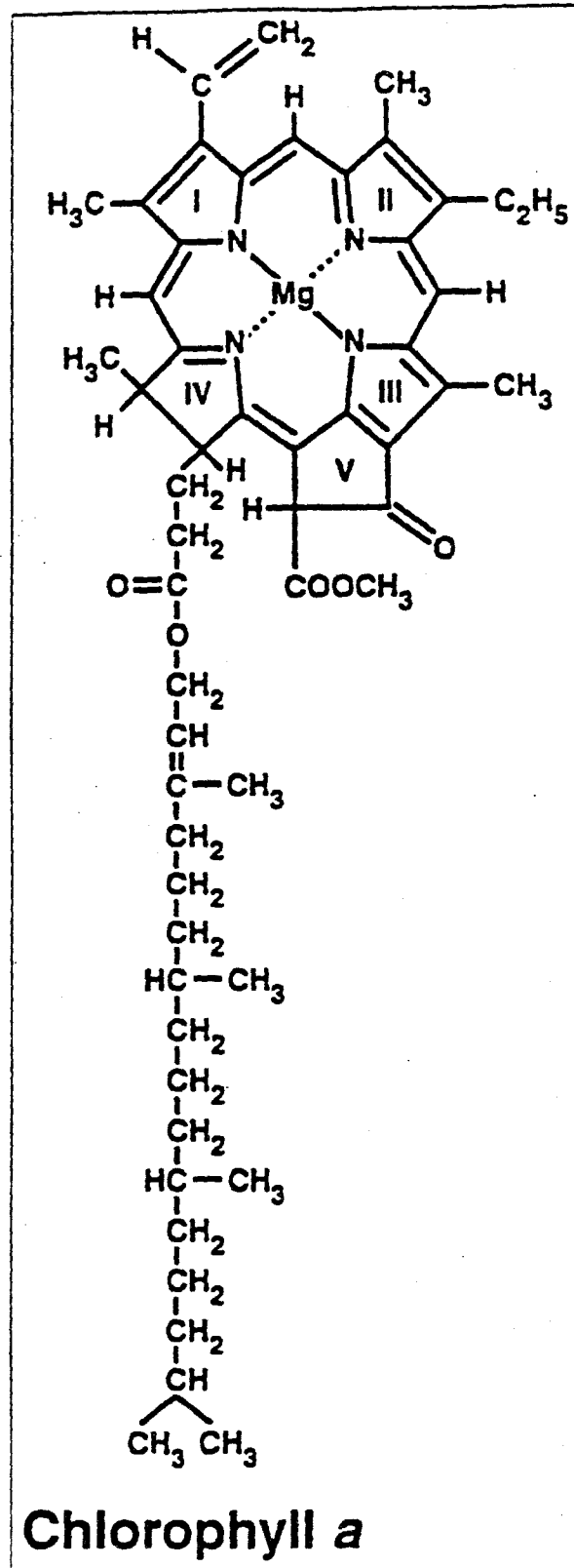
Chlorophyll *a*

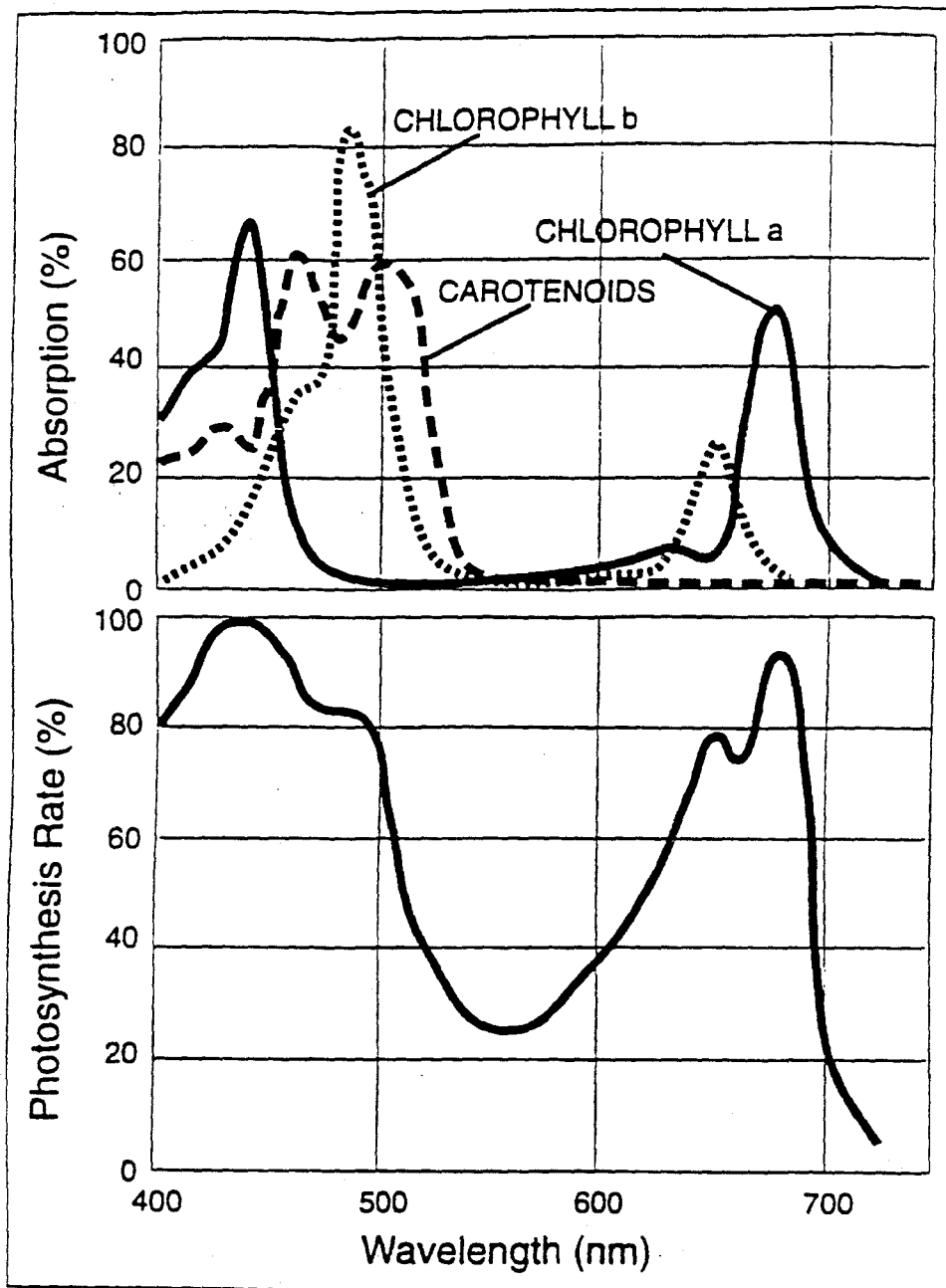Fig. 4   Chemical structure of chlorophyll *a* molecule.

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

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        P   H   O   T   O   N   S

                ANTENNA PIGMENTS
              (300 CHLOROPHYLL MOLECULES
                 AND OTHER PIGMENTS)

                 ENERGY TRANSFER

                  REACTION CENTER
            DONOR ——————▶ ACCEPTOR
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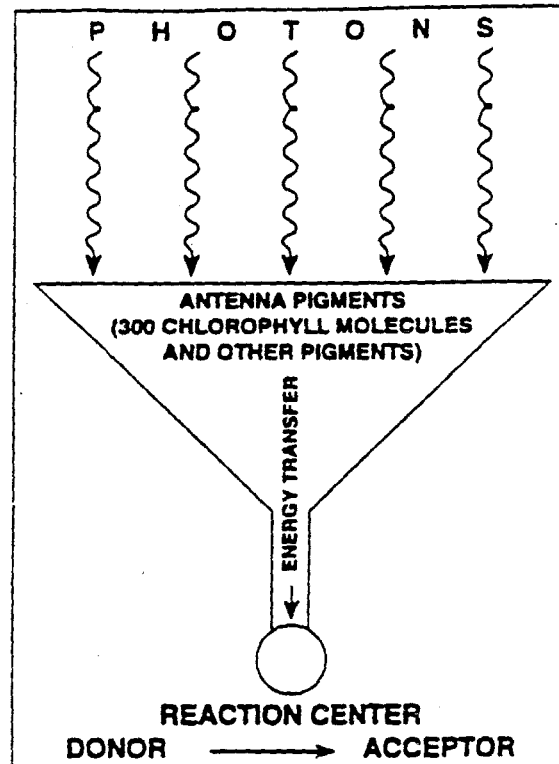
Fig. 6 A simplified scheme showing light absorption in antenna pigments followed by excitation energy transfer to a reaction center chlorophyll. The antenna and reaction center chlorophyll molecules are physically located in different proteins. Primary photochemistry (electron transfer from the primary 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
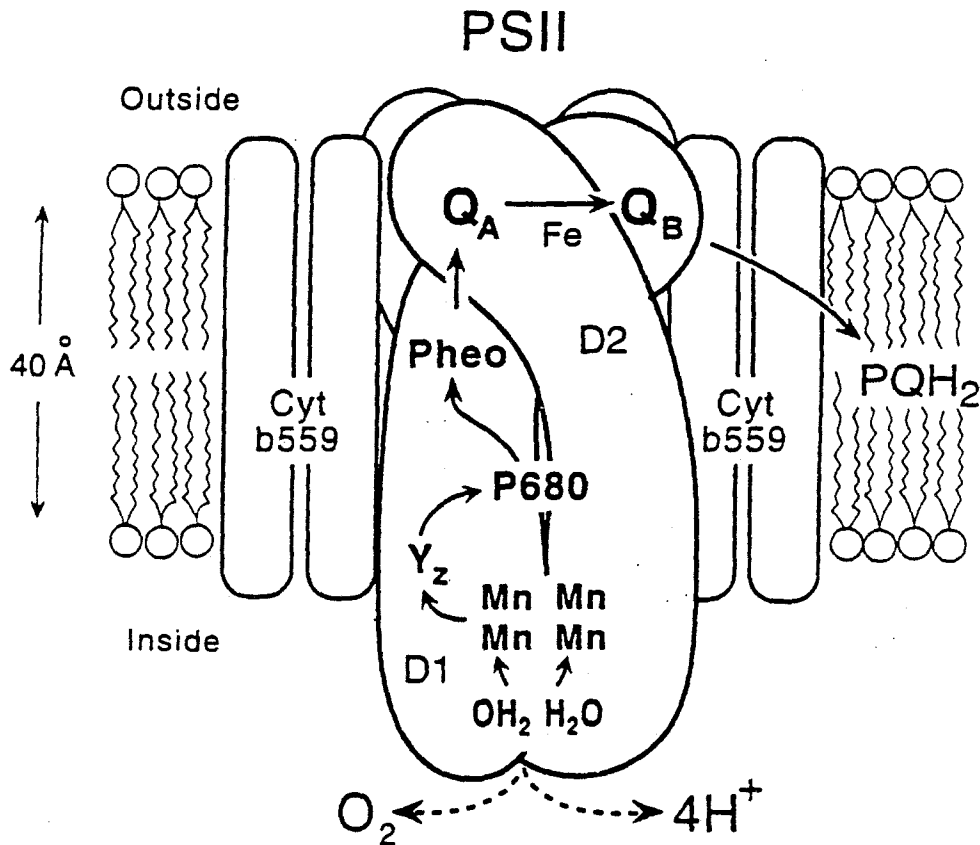

Fig. 7 Schematic drawing of photosystem II. Photosystem II is composed of numerous polypeptides, but only two of them, D1 and D2, bind the electron carriers involved in transferring electrons from Y_z to plastoquinone. Y_z, tyrosine; P680, reaction center chlorophyll (primary electron donor); Pheo, pheophytin; 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
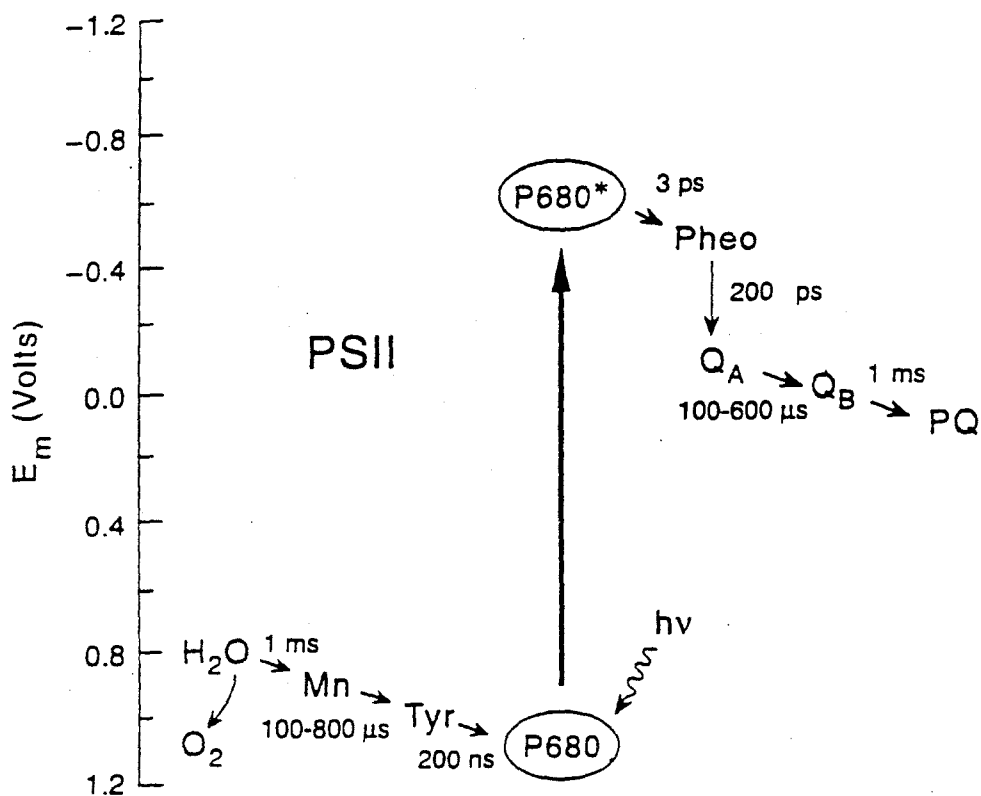

Fig. 8   Photosystem II electron transport pathways and transfer times. The vertical axis shows the midpoint potential of the electron carriers. The heavy vertical arrow shows the result of light absorption. P680* is the electronically excited state of P680. The abbreviations are given in the legend of Fig. 3.
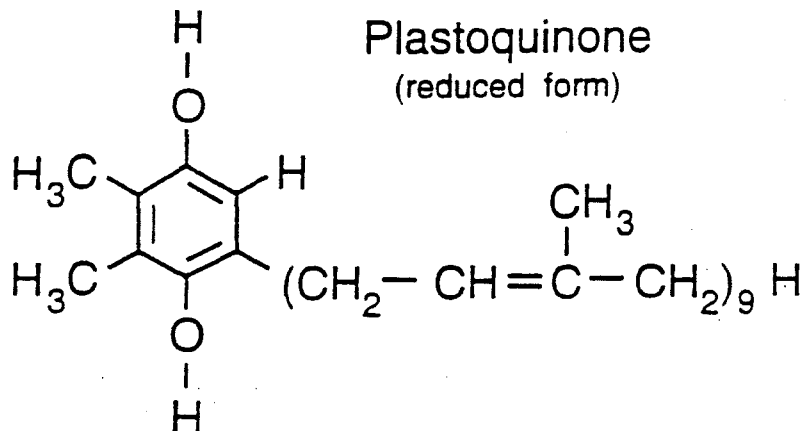
Fig. 9   Structure of plastoquinone (reduced from), an aromatic molecule that carries electrons and protons in photosynthetic electron transport.

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).
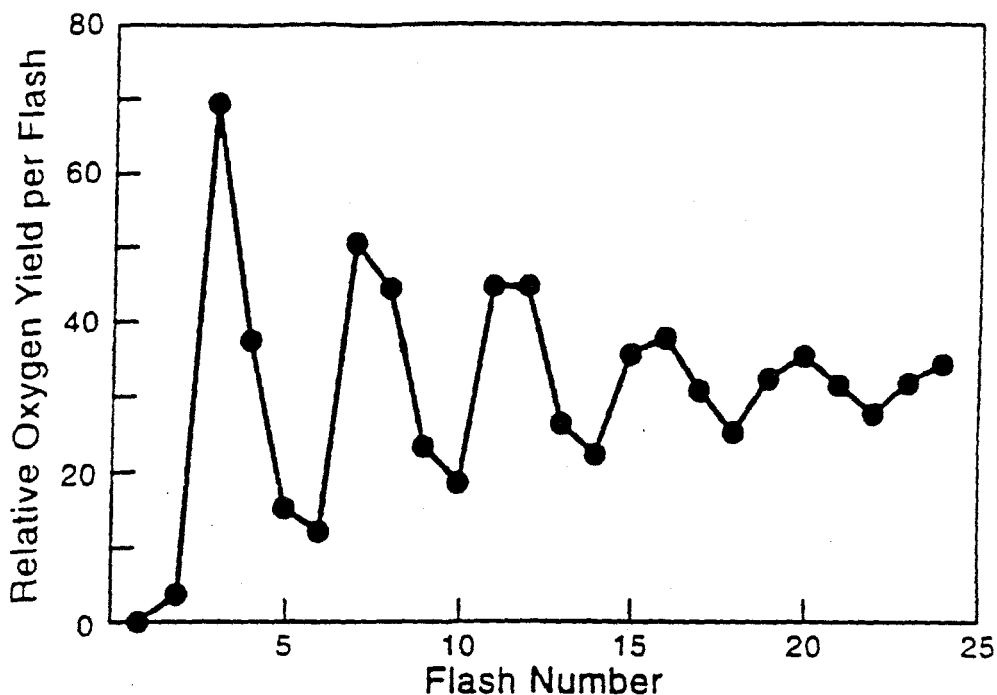
Fig. 10   Yield of oxygen from photosynthetic membranes exposed to a series of brief flashes as a function of flash number. Maximum oxygen yield exhibits a four-flash periodicity. Yield is highest after the third flash and peaks again four flashes later. The four flash dependence of the amplitude gradually 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
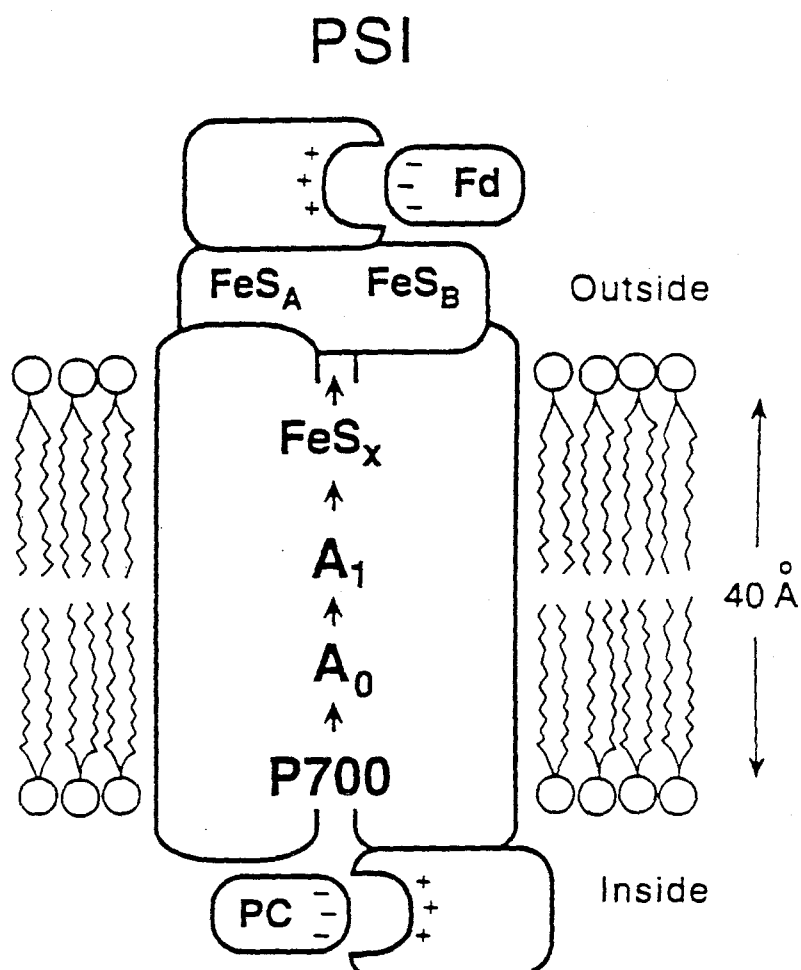
PSI

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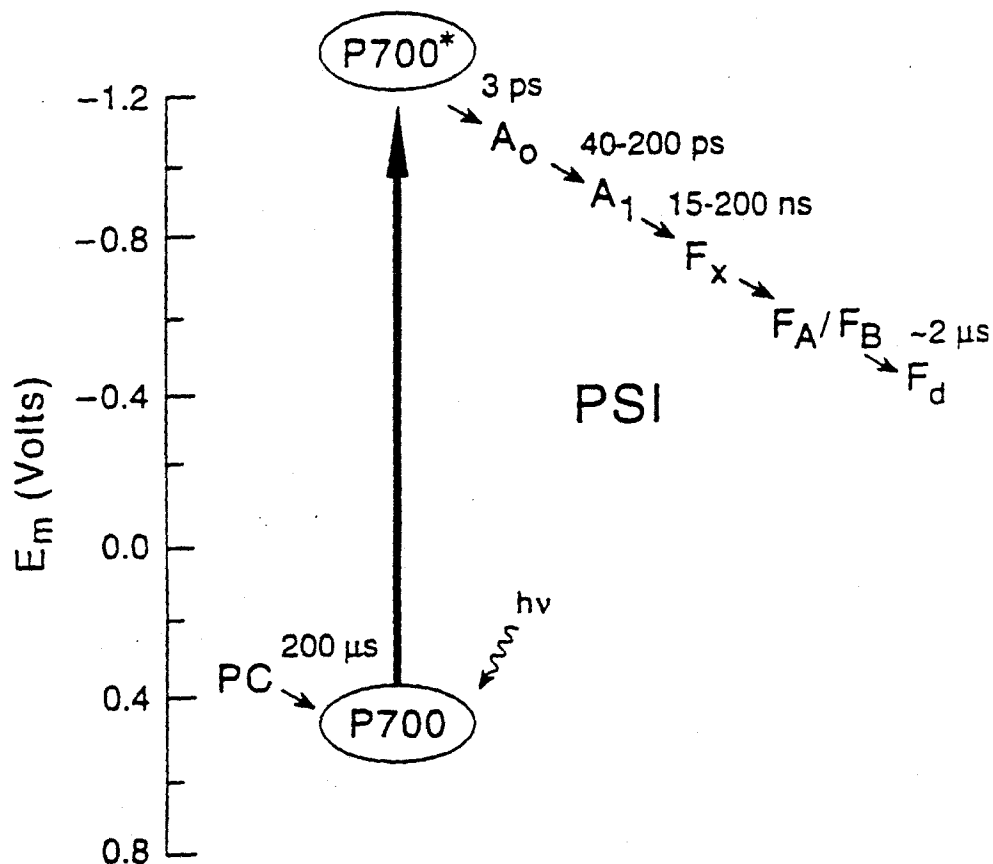
Fig. 12   Photosystem I electron transport pathways and transfer times. Vertical
axis shows the midpoint potential of the electron carriers. Abbreviations:
Same as in Fig. 11 (F_A and F_B are equivalent names for FeS_A and FeS_B).

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
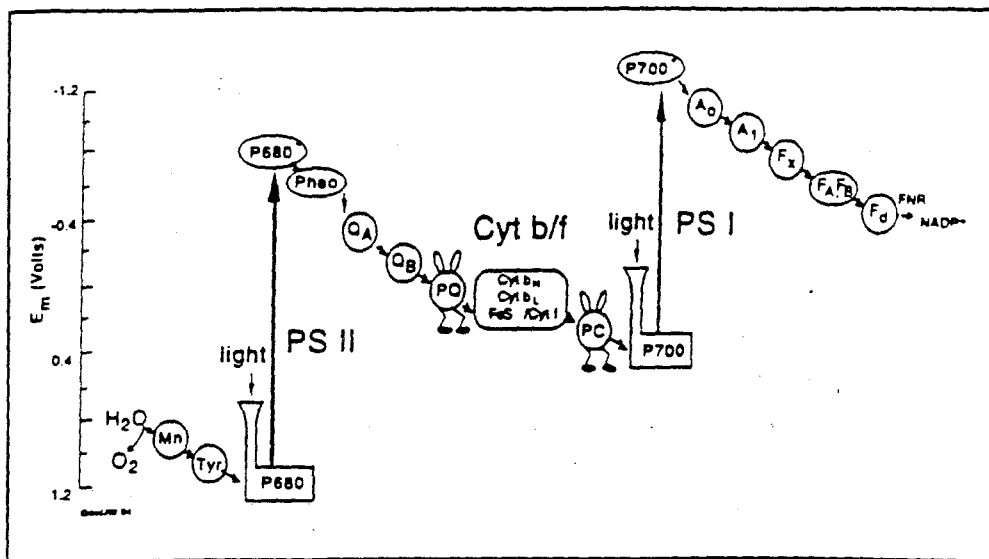and $NADP^+$ are water soluble and are found in the outer aqueous phase.

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:

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

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

$$ADP^{-3} + P_i^{-2} + H^+ \rightarrow ATP^{-4} + H_2O. \tag{5}$$
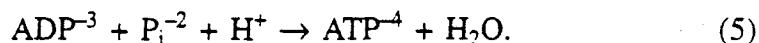
The reaction is energetically uphill ($\Delta G = +32$ kJ/mol) and is driven by proton transfer through the ATP synthase protein. The ATP synthase complex is composed of two major subunits, CF_0 and CF_1 (Fig. 14). The CF_0 subunit spans the photosynthetic membrane and forms a proton channel through 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).
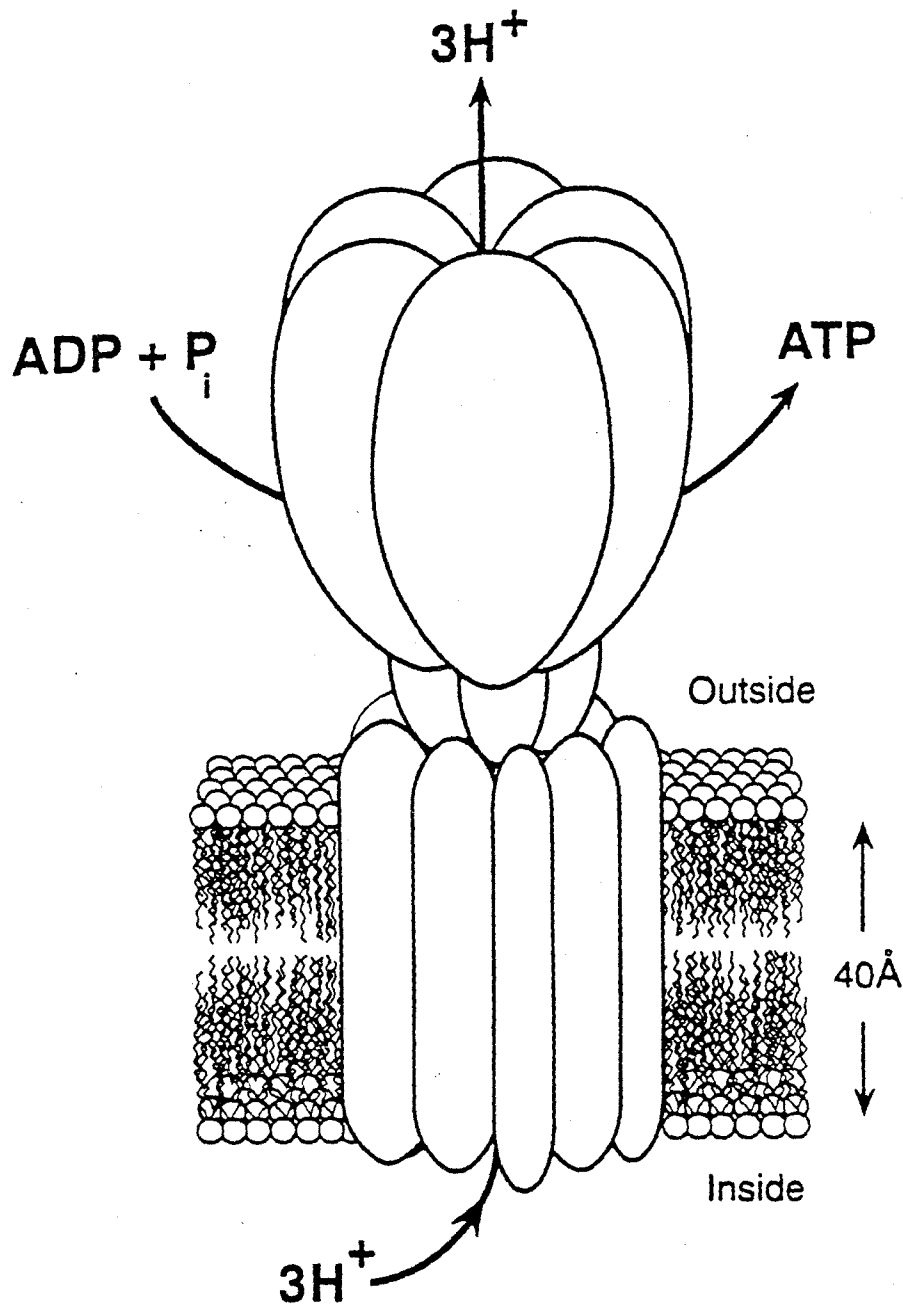
Fig. 14   Schematic drawing of the ATP synthase enzyme embedded in the membrane. Proton transfer through the ATP synthase provides the energy for the creation of ATP from ADP and P_i. Abberviations: Same as in Fig. 3.

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
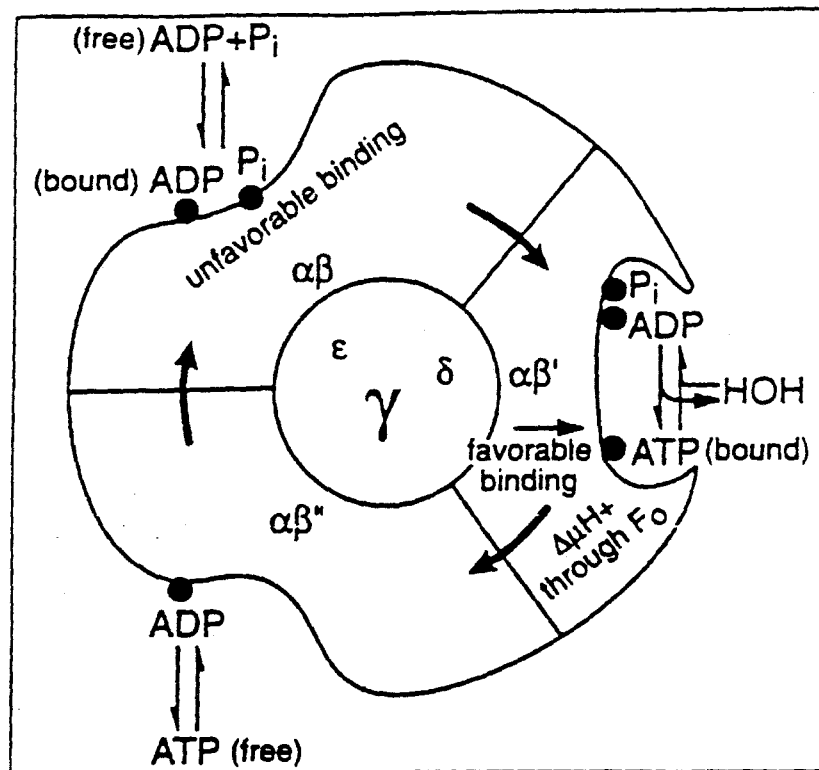
Fig. 15   The ATP synthase consists of a membrane portion and an water exposed portion (see Fig. 14). Water exposed portion, which looks like a door knob, has five subunits (3α, 3β, 1γ, 1δ, 1ε). The 3α, 3β combine as $3\alpha/\beta$ pairs. Catalytic sites of enzyme are on the β-subunits. The γ subunit 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
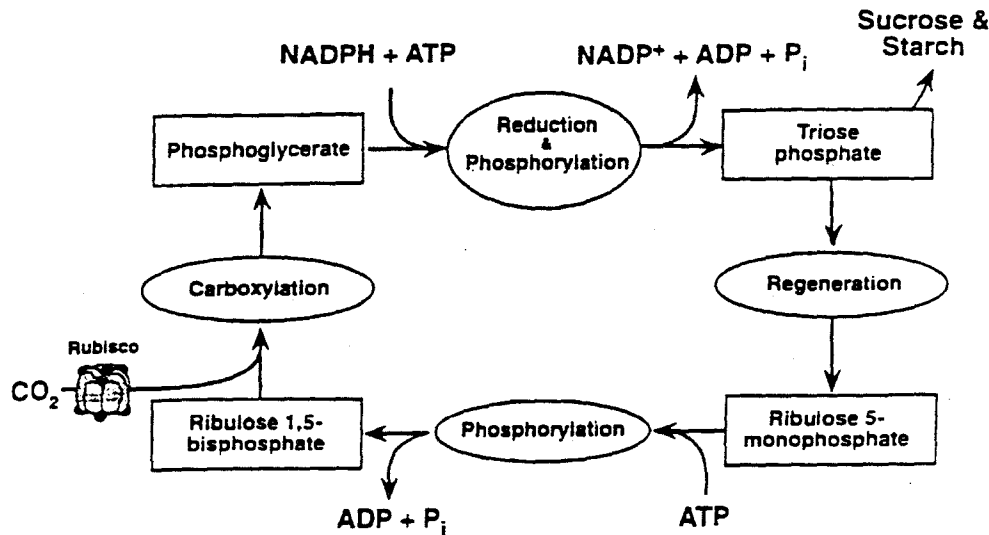

Fig. 16   An abbreviated scheme showing reduction of CO_2 by the Calvin Cycle. First step is carboxylation, in which Ribulose 1,5-bisphosphate carboxylase/ oxygenase (Rubisco) catalyzes the addition of CO_2 to the five-carbon compound, ribulose, 1,5-bisphosphate, which is subsequently split into two 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

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*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*
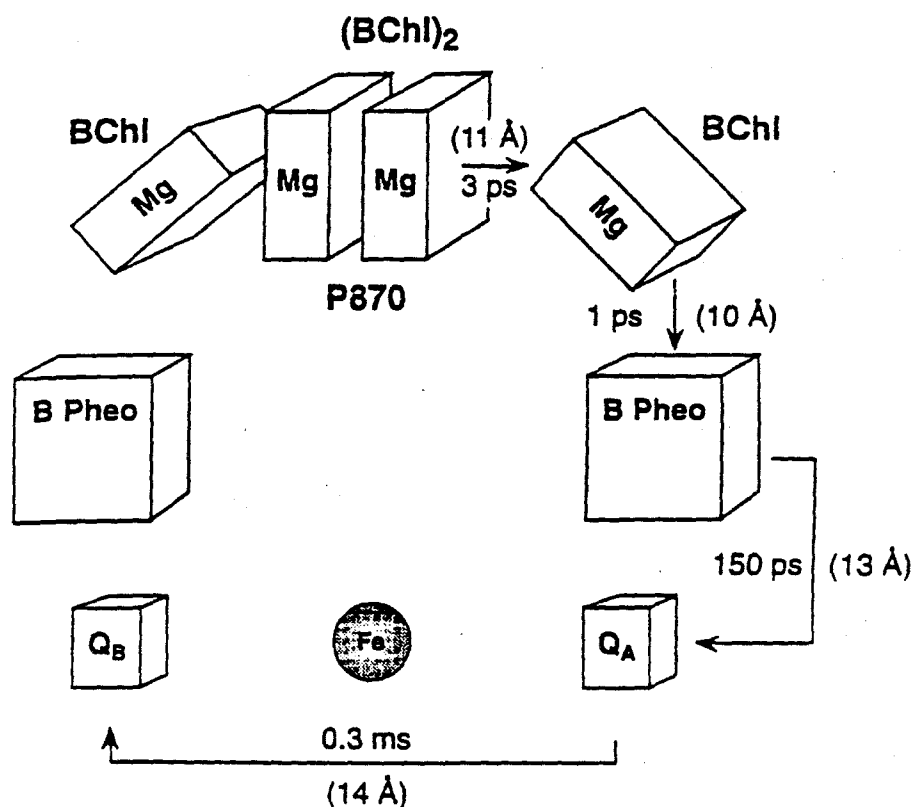

Fig. 17   Relative positions of the chromophores of the reaction center of *Rhodobacter sphaeroides* (from Norris and van Brakel, 1986). It shows center to center distances and times for the electron transfers. P870, reaction center bacteriochlorophyll (primary electron donor); BChl, bacteriochlorophyll; 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
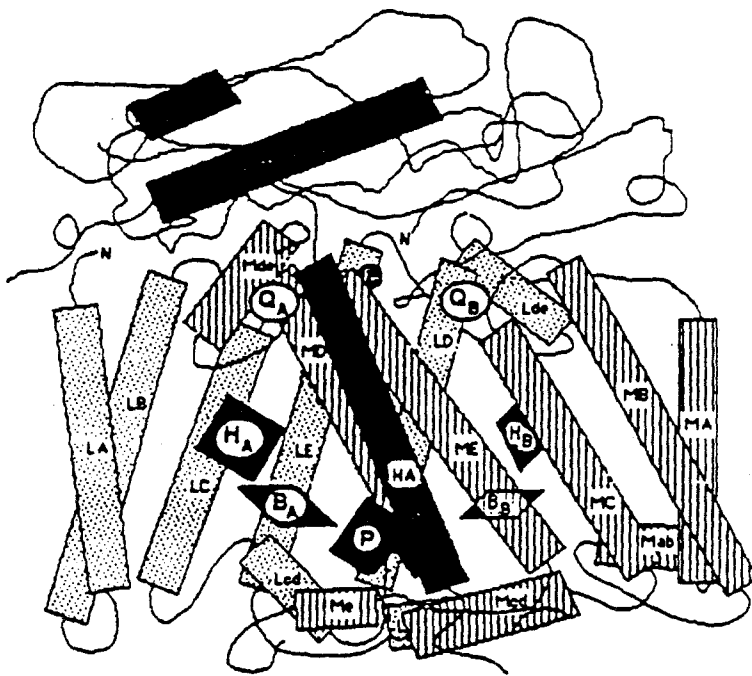

Fig. 18   Structure of the bacterial reaction center by H. Michel, J. Deisenhofer and R. Huber and co-workers containing three proteins: H(black), L (dotted) and M (hatched bars). Both L and M have 5 helices each (LA, LB, etc.) and H shown on the very top of the molecule having one helix (HA) that goes 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
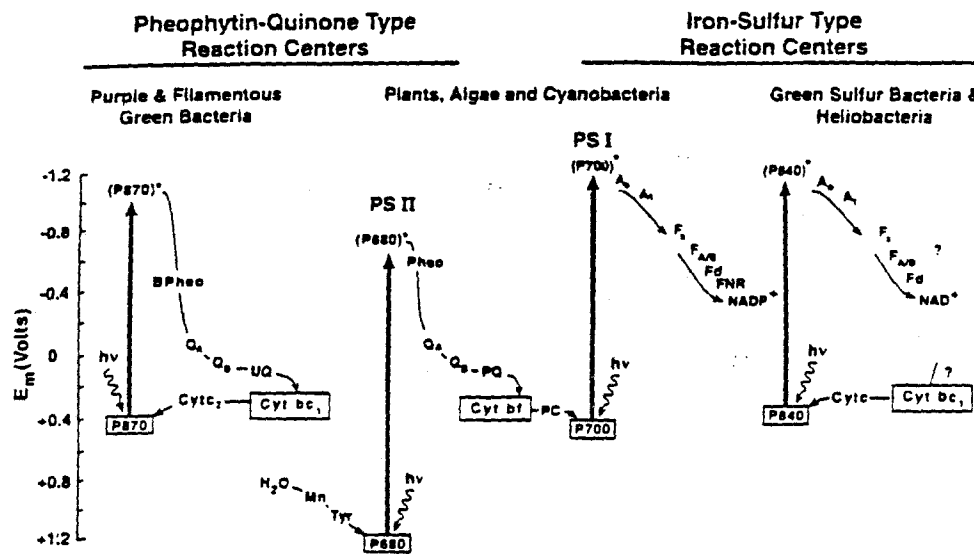

Fig. 19   Comparison of electron transport pathways in oxygenic and anoxygenic organisms (Blankenship, 1992). Cyt bc_1, cytochrome bc_1 complex; P840, reaction center bacteriochlorophyll; other abbreviations same as in Figs. 3 and 17.

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

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.