PHOTOSYNTHESIS

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INTRODUCTION

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

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

1. BRIEF HISTORY

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

2. CLASSIFICATION OF PHOTOSYNTHETIC ORGANISMS

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

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

2.1 Oxygenic Photosynthetic Organisms

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

2.2 Anoxygenic Photosynthetic Organisms

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

3. GENERAL PRINCIPLES OF PHOTOSYNTHETIC ENERGY TRANSFORMATION IN PLANTS

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

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

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

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

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

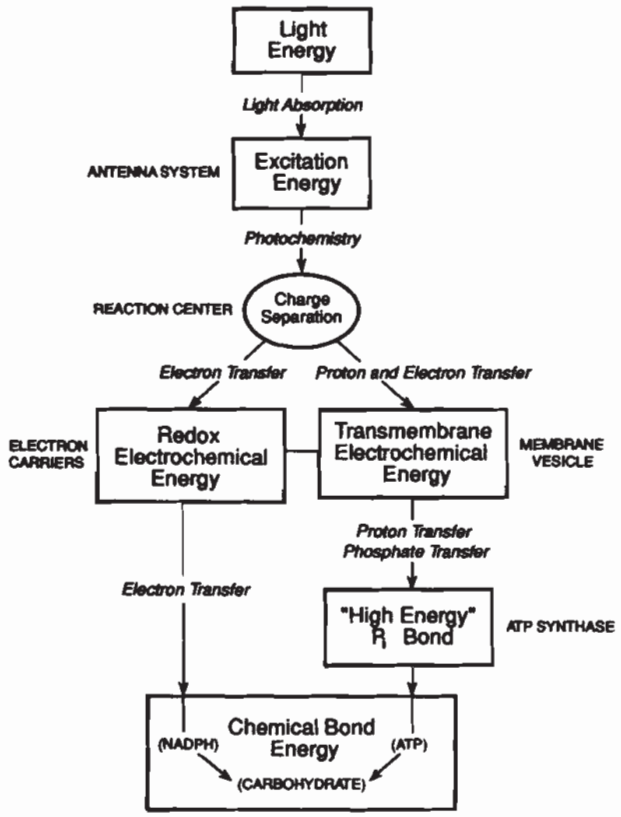

FIG. 1. Photosynthesis shown as a series of reactions that transform energy from one form to another. The different forms of energy are shown in boxes, and the direction of energy transformation is shown by the arrows. The energy-transforming reaction is shown by italics in the arrows. The site at which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

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

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

4. OXYGENIC PHOTOSYNTHESIS IN PLANTS

4.1 Chloroplasts—Structure and Organization

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

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

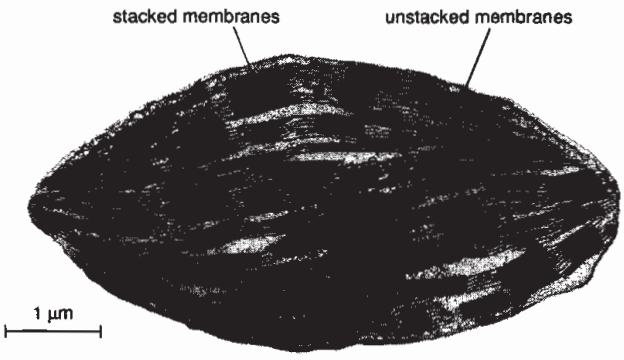

FIG. 2. An electron micrograph of a plant chloroplast. Micrograph by A. D. Greenwood, courtesy of J. Barber.

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

4.2 Light Absorption—The Antenna System

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

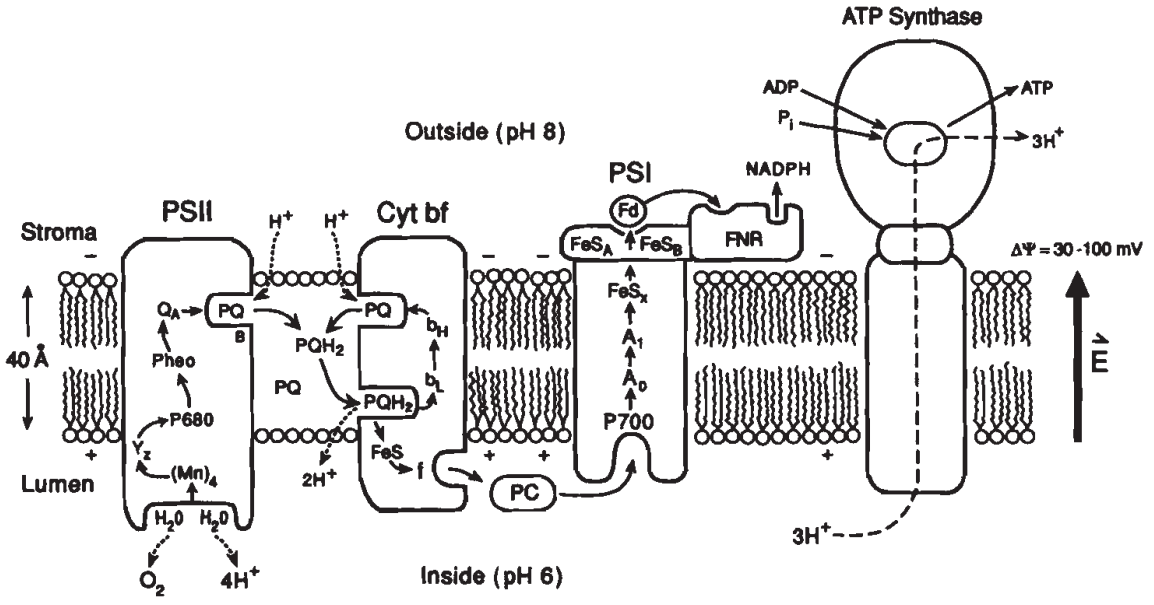

FIG. 3. Model of the photosynthetic membrane of plants showing the electron-transport components and the ATP-synthase enzyme (cross-sectional view). The complete membrane forms a vesicle. The pathways of electrons are shown by solid arrows. The membrane-bound electron-transport protein complexes involved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

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

4.3 Primary Photochemistry—Photosystem II and Photosystem I Reaction Centers

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

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

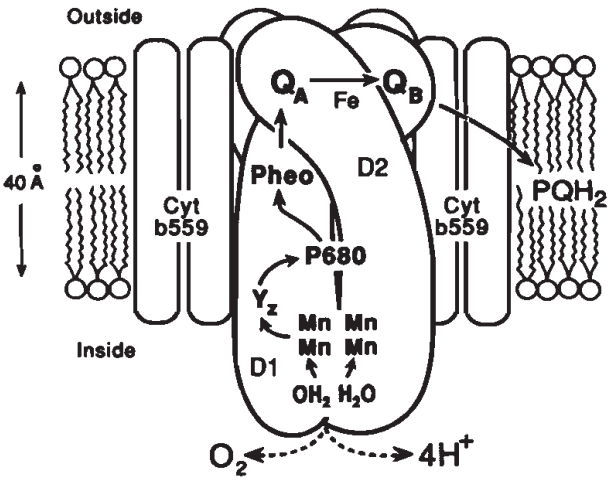

FIG. 4. Schematic drawing of photosystem II. Photosystem II is composed of numerous polypeptides, but only two of them, *D*1 and *D*2, bind the electron carriers involved in transferring electrons from Y_z to plastoquinone. Abbreviations: Y_z, tyrosine; P680, reaction-center chlorophyll (primary electron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

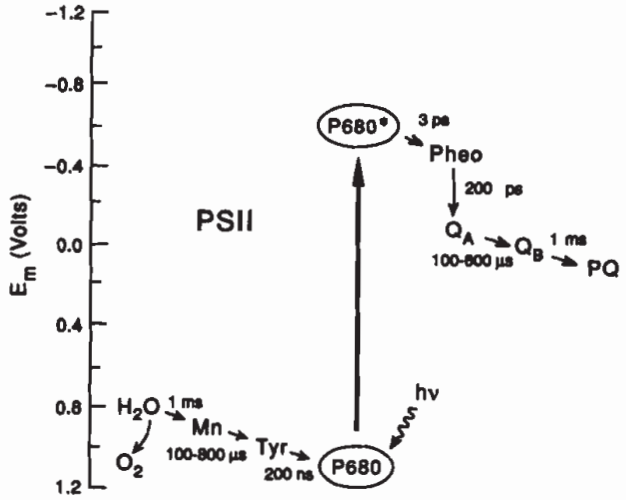

FIG. 5. Photosystem II electron-transport pathways and rates. The vertical axis shows the midpoint potential of the electron carriers. The heavy vertical arrow shows light absorption. P680* is the electronically excited state of P680. The abbreviations are given in the legend of Fig. 4.

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

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

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

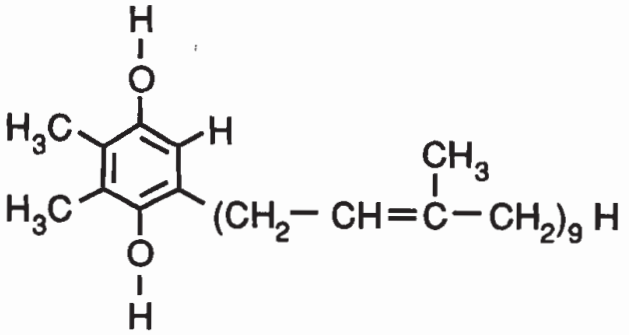

FIG. 6. Structure of plastoquinone (reduced form), an aromatic molecule that carries electrons and protons in photosynthetic electron transport.

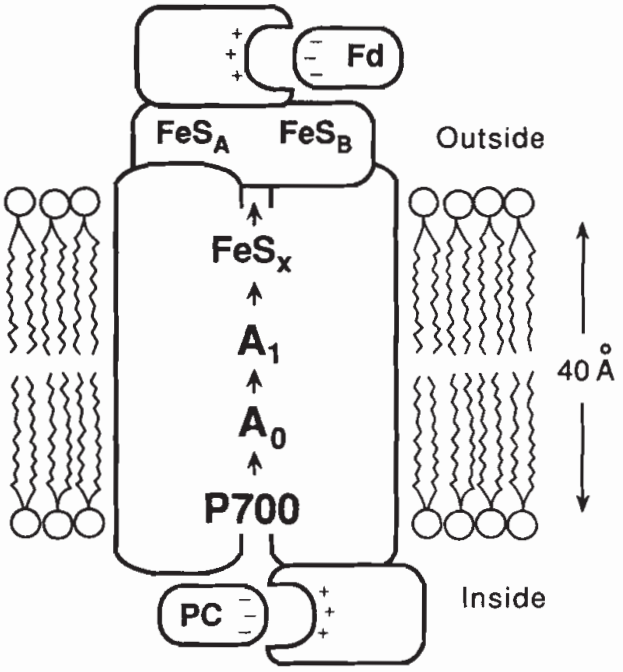

FIG. 7. Schematic drawing of photosystem I. Photosystem I is composed of numerous polypeptides, but only three of them bind the electron carriers. Abbreviations: PC, plastocyanin; P700, reaction-center chlorophyll (primary electron donor); A_0, chlorophyll; A_1, phylloquinone; FeS, FeS centers; Fd, ferredoxin.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

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

4.4 Electron Transport

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

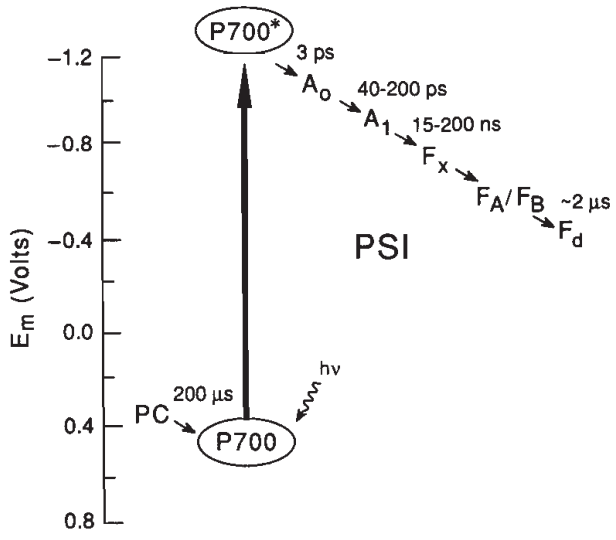

FIG. 8. Photosystem I electron transport pathways and rates. The vertical axis shows the midpoint potential of the electron carriers. Abbreviations are given in the legend of Fig. 7.

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

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

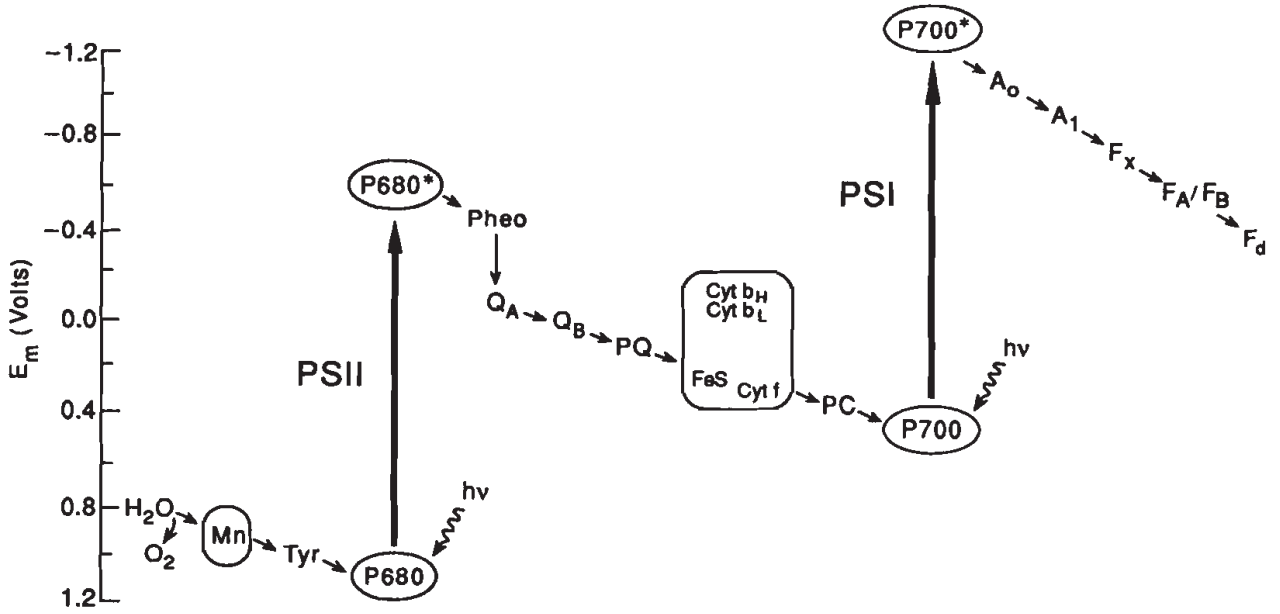

FIG. 9. The electron transport pathway of plants (oxygenic photosynthesis). Abbreviations are given the legend of Fig. 3. Details are given in the text.

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

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

4.5 Creation of a Proton Electrochemical Potential

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

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

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

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

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

4.6 Synthesis of ATP by the ATP-Synthase Enzyme

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

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

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

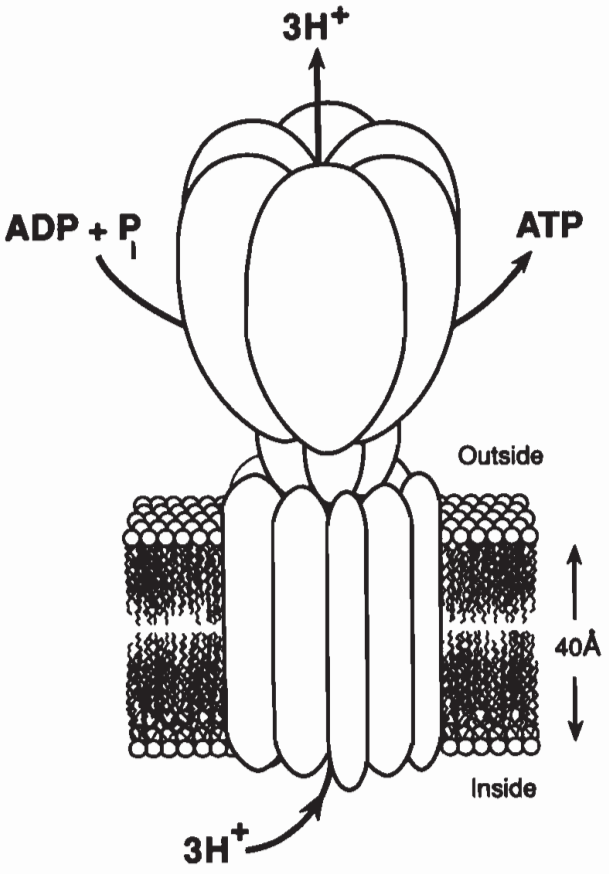

FIG. 10. Schematic drawing of the ATP-synthase enzyme embedded in the membrane. Proton transfer through the ATP synthase provides the energy for the creation of ATP from ADP and P_i. Abbreviations are given in the legend of Fig. 3. Details are given in the text.

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

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

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

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

4.8 Photosynthetic Quantum Yield and Energy Conversion Efficiency

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

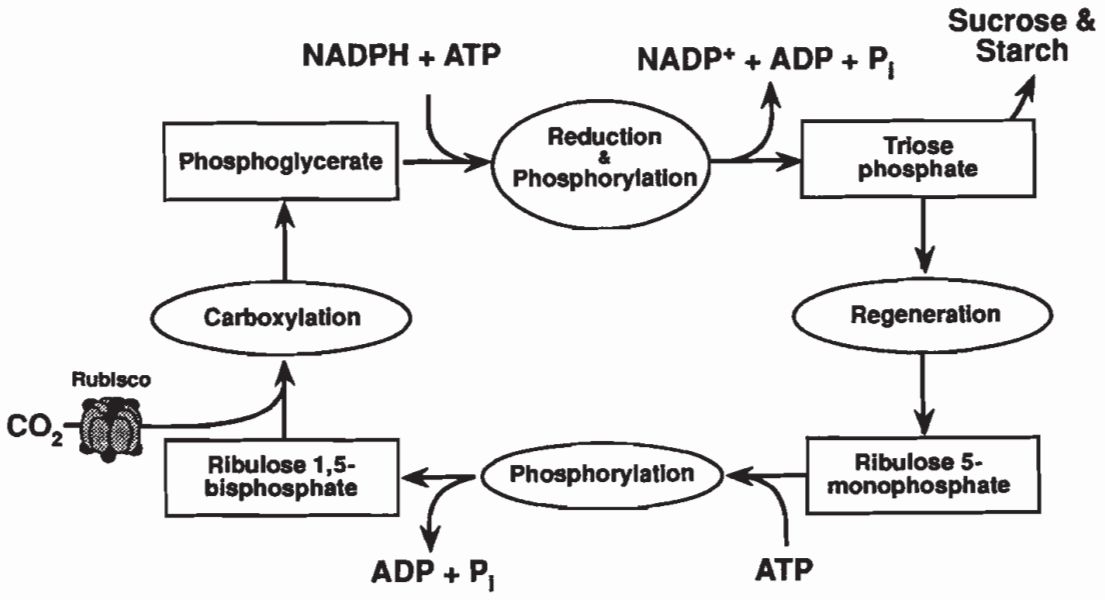

FIG. 11. An abbreviated scheme showing reduction of carbon dioxide by the Calvin cycle. The first step is carboxylation, in which ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes the addition of CO_2 to the five-carbon compound ribulose 1,5-bisphosphate, which is subsequently split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

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

5. OXYGENIC PHOTOSYNTHESIS IN ALGAE

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

6. OXYGENIC PHOTOSYNTHESIS IN BACTERIA

6.1 Cyanobacteria

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

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

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

6.2 Prochlorophytes

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

7. ANOXYGENIC PHOTOSYNTHESIS IN BACTERIA

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

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

7.1 Purple Bacteria

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

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

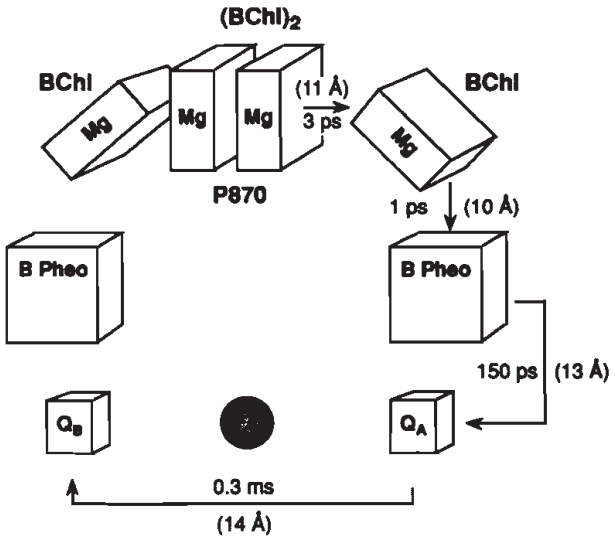

FIG. 12. Relative positions of the chromophores of the reaction center of *Rhodobacter sphaeroides* (from Norris and van Brakel, 1986). Abbreviations: P870, reaction center bacteriochlorophyll (primary electron donor); B Pheo, bacteriopheophytin; Q_A and Q_B, bound ubiquinone. Details are given in the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

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

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

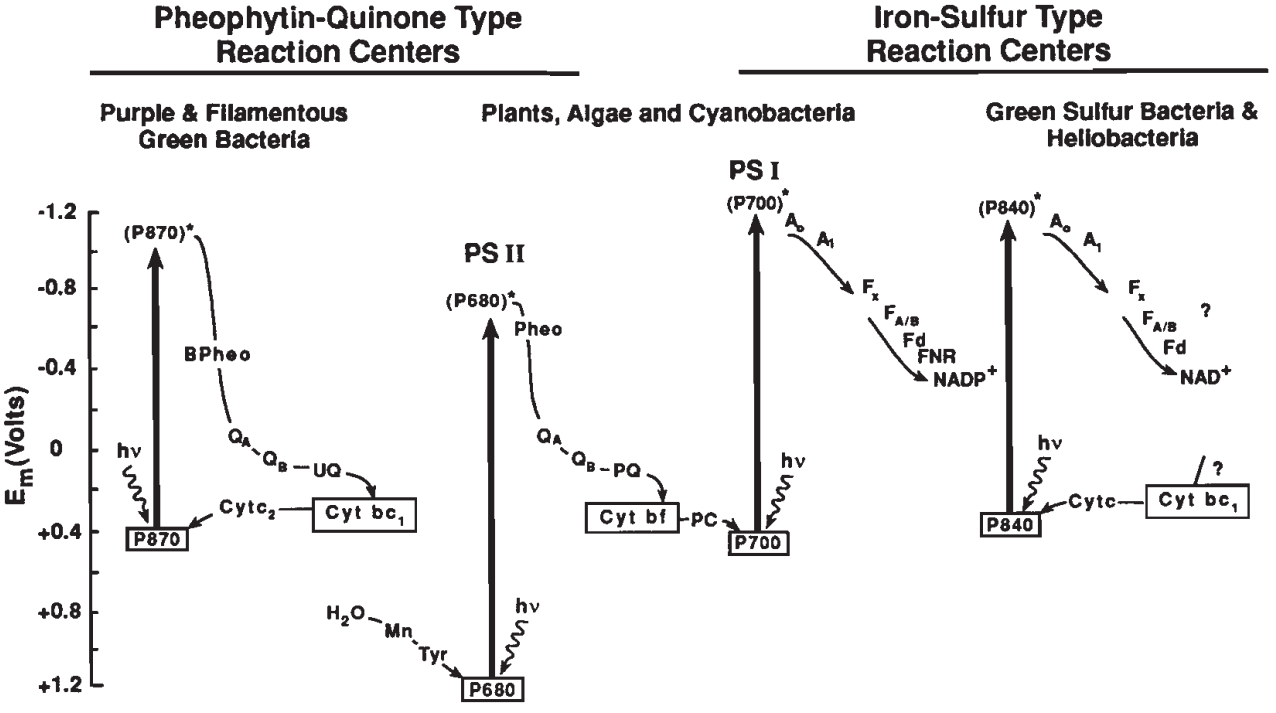

FIG. 13. Comparison of electron-transport pathways in oxygenic and anoxygenic organisms (from Blankenship, 1992). Abbreviations: Cyt bc_1, cytochrome *bc* complex; P840, reaction center bacteriochlorophyll; other abbreviations are given in the legends of Figs. 3 and 12.

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

7.2 Green Sulfur Bacteria

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

7.3 Green Gliding Bacteria

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

7.4 Heliobacteria

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

8. CONTROL OF INTRAPROTEIN ELECTRON TRANSFER

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

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

9. GLOBAL PHOTOSYNTHESIS AND THE ATMOSPHERE

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

GLOSSARY

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

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

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

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

Cytochrome: Heme-containing protein.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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