Photosynthesis

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All forms of life in this universe require energy for growth and maintenance. Plants and some forms of bacteria capture light energy directly from solar radiation and utilize it for synthesis of food materials besides producing basic raw materials from which other cellular biomolecules are produced. The term **photosynthesis** describes the process by which green plants synthesize organic compounds from inorganic raw materials using light. Photosynthesis is the source of all biological energy, viz., food, biological fuels, and biomass, and is also most important for availability of free oxygen. Whatever free oxygen is there in the atmosphere is the result of photosynthesis. Since heterotrophic organisms including animals cannot use sunlight as direct source of energy, they consume plants as the source of energy. Photosynthesis is the means for solar energy to enter into the global ecosystem, and it alone is the essential biological process by which solar energy is transformed into metabolic form of energy for all forms of life on earth. An understanding of the fundamental and applied aspects of the process comes from a wide range of studies including agriculture, forestry, plant biochemistry, plant molecular biology, tissue culture, and metabolic engineering.

Primitive life is believed to have existed in anaerobic conditions which utilized energy stored in chemical compounds for the biosynthesis of the biomolecules required for their growth. However, hundreds of millions of years ago, with the depletion of these compounds, photoautotrophs might have originated which utilized solar energy to produce reduced organic compounds, which either oxidized water and released oxygen (**oxygenic photosynthesis**), ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) (e.g., purple photosynthetic bacteria), or used H_2S as source of electrons (e.g., green sulfur bacteria). In the latter case, sulfur got deposited by the organisms (**anoxygenic** photosynthesis). However, in due course of time almost 3.5×10^9 years ago, because of free availability, water is believed to have been utilized by cyanobacteria in place of H_2S. It was almost 2.7×10^9 years ago when oxygen is presumed to have been released as a waste product which started to accumulate on the earth surface resulting

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in an oxygenic environment. Accumulation of oxygen shielded living organisms from damaging effects of UV irradiations.

The site of photosynthesis in eukaryotes (algae and higher plants) are the cells that contain few to numerous (about 1–1000) **chloroplasts** which vary in size and shape. Chloroplasts are unique double-membrane-bound organelles that originated through an endosymbiotic association between free-living oxygen-evolving photosynthetic bacteria which might have been incorporated into the growing eukaryotic cells as chloroplast**.** Outer chloroplast membrane is relatively freely permeable, while the inner membrane exhibits more selective permeability. The sites of light reactions in the chloroplast are the saclike structures, known as **chloroplast lamellae** or **thylakoids**. The space within the chloroplasts is divided into two compartments, viz., one enclosed within the thylakoids called **lumen** and the other outside the thylakoids, which is called **stroma**. Stroma, the matrix around the thylakoid, is the site where CO_2 is assimilated, leading to the synthesis of sugars. Thylakoids exist either as stacks called **grana** or are unstacked and are interconnected to form **stroma lamellae**. Each chloroplast contains 10–100 grana. Light is captured by various pigments which includes chlorophyll molecules as the photoreceptors for photosynthesis. These exist as the chlorophyll-protein complexes which are involved in harvesting light energy and transporting electrons, resulting in generation of reductant and synthesis of ATP. In cyanobacteria, photosynthetic machinery required for light reactions exists in plasma membrane which forms invaginations or folded structures resembling grana of chloroplasts in eukaryotic cells.

Photosynthesis is an oxidation-reduction process in which oxidation of water (electrons being removed from water) is coupled with the release of oxygen and reduction of carbon dioxide leads to synthesis of carbohydrates. It is a two-stage process. During stage I, known as light reaction, photolysis of water takes place:

$$2H_2O \xrightarrow{\text{Light}} O_2 + 4[H^+] + 4e^- \qquad \text{(Stage I)}$$

Electrons removed from water are used to reduce CO_2 in the subsequent stage II, known as CO_2 assimilation:

$$4e^- + 4[H^+] + CO_2 \rightarrow (CH_2O) + H_2O \qquad \text{(Stage II)}$$

Thus, light energy is converted into chemical energy and is conserved in the form of carbohydrates. Stage I is photochemical, while stage II is a purely chemical reaction. At present the molecular mechanism involved in photosynthesis is fairly understood.

5.1 General Concepts

Learning basic concepts of photosynthesis is necessary before understanding its mechanism. This includes properties of light, photosynthetic pigments, mechanism of light absorption, and emission.

5.1.1 Properties of Light

Human eye is sensitive to a narrow range of light spectrum from i.e. 400 to 700 nm, which is called as visible light (Fig. 5.1). The wavelengths shorter than 400 nm (UV light) have very high energy, and they are hazardous for biomolecules, while wavelengths longer than 700 nm (infrared) have much less energy. It is the light with wavelengths ranging from 400 to 700 nm that is significant for most of the photobiological processes. Light and all other electromagnetic radiations are transmitted in form of waves, while absorption and emission of light occur in the form of particles. Parameters such as wavelength (λ) or frequency (υ) characterize the wave aspect of the light. When light having a particular wavelength (λ) passes across an observer at a velocity (c), the number of waves passing per second is the frequency (υ), and the relationship between these parameters is represented as

$$\upsilon = c/\lambda$$

where c is the velocity of light, i.e., 2.99×10^8 m.s^{-1}.

Light beam can be imagined as a stream of particles or photons. Associated unit of energy of each photon is called a **quantum**. The energy value of a quantum (E) is related to the frequency (υ) of the light which is represented by the equation known as **Planck's equation**,

$$E = h\upsilon$$

where h is Planck's constant (6.62×10^{-34} J.s). By replacing υ by c/λ from the previous equation, it can be understood that the energy of a photon is inversely related to the wavelength of the light. But at the time of absorption and emission of light, single photon is rarely dealt with, and in biochemical processes, conversion of light energy to chemical energy is usually expressed on molar basis, i.e., energy of a mole (6.02×10^{23}) of photons (which is an Avogadro's number, N_A). A mole of photons is called one **Einstein**. Energy of a mole of photon amounts to

$$E = h\upsilon.N_{\mathrm{A}}$$

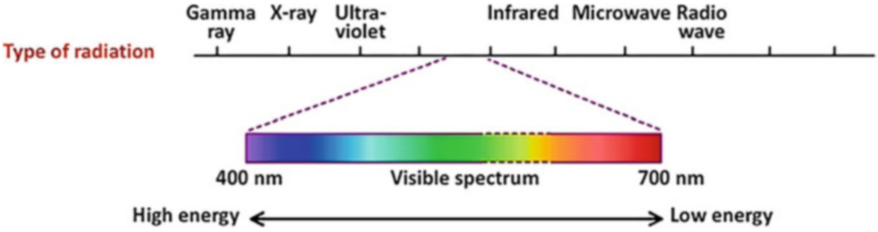


Fig. 5.1 Electromagnetic spectrum of sunlight

Not all colors of light have equal energy. The energy content of light is inversely proportional to its wavelength, e.g., one mole of photon (an Einstein) of 490 nm blue light will have energy of 240 kJ, whereas 700 nm red light will have only 170 kJ. For any photochemical reaction energy of a photon should be at least as much as that required for the reaction (ΔG).

$$\Delta G = E = h\upsilon . N_{\mathrm{A}}$$

It is more useful to state electric potential (ΔE) of irradiance instead of energy (ΔG), when photosynthetic reactions are compared with redox reactions. This can be calculated by the following equation:

$$\Delta E = -\Delta G / F$$

F is Faraday's constant which refers to number of charges per mole (96,480 Amp.s.mol^{-1}).

The solar energy, which is radiating toward earth, is nearly 13 × 10^{23} calories per year. Out of this 30% is reflected back straight away into outer space, 20% is absorbed by the atmosphere, and the remaining almost 50% is absorbed by earth which is converted to heat. Plants convert, utilize, and store less than 1% of the solar energy which is responsible for all chemical, mechanical, and electrical energy driving all organisms on earth. Oxygenic photosynthetic organisms use visible light with wavelength of 400–700 nm, whereas anoxygenic photosynthetic organisms can harness less energetic wavelengths in the infrared region at wavelengths greater than 700 nm.

5.1.2 Mechanism of Light Absorption and Emission

For light energy to be used by plants, it has to be absorbed. The absorption of photons by a pigment molecule results in bringing the pigment from its lowest ground energy state (E_{g}) to an excited state (E_{e}), which causes difference in distribution of electrons in the excited molecule. According to the law of quantum mechanics, a given molecule can absorb photons of only certain wavelengths, so that that the energy difference between the two states of the molecule ($E_{\mathrm{e}} - E_{\mathrm{g}}$) must match exactly the energy of the absorbed photons. The molecules can exist in two type of excited states, **singlet state** which contains electrons with opposite (antiparallel) spins and is relatively short lived (10^{-9} s) and the more longer-lived **triplet state** (10^{-3} s) with electron spins that are aligned (parallel). Triplet state is achieved from singlet state after losing some energy to the surroundings. Electron from the triplet state may come back to ground level, and the energy is released in the form of light known as **phosphorescence**. Rarely transition from triplet state to the singlet state occurs after the electron acquires energy from the surroundings, which is followed by release of energy in the form of light wavelength known as delayed **fluorescence** (Fig. 5.2). In the singlet state, the excited chlorophyll molecules have

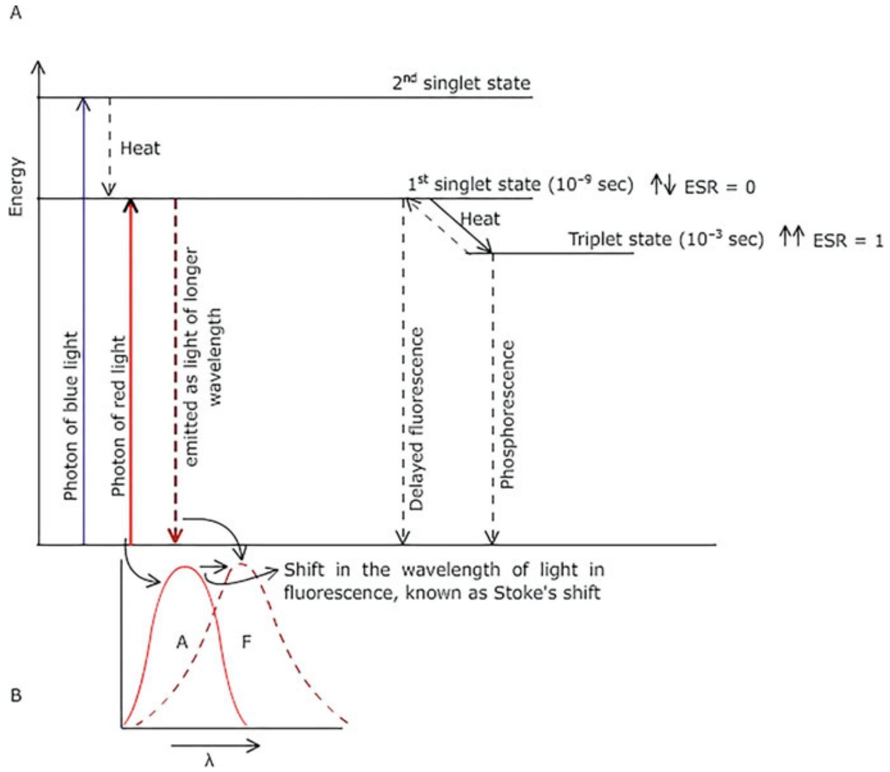


Fig. 5.2 (**a**) Mechanism of light absorption and emission. Electron is excited to higher energy level (second singlet) by absorption of blue-light photon than when photon of red light is absorbed since blue light has higher energy. In this excited state, chlorophyll molecule is extremely unstable, and some energy is lost as heat resulting in electron attaining new lower energy level, first singlet state which has natural lifetime of 10^{-9} s after which the electron may come to the ground level after losing the energy as light. (**b**) Some of the absorbed excitation energy is lost due to vibrations and rotations; as a result energy of the emitted light is lesser than that of absorbed light and wavelengths of emitted light are longer, a phenomenon known as fluorescence. *ESR* electron spin resonance, *F* fluorescence

alternative pathways for dissipation of their available energy. Energy may be lost by internal conversions, which refers to non-radiative decay and is common mode of energy loss from the excited molecule in singlet state. The electronic energy is converted to the kinetic energy of molecular motion, i.e., in the form of heat. Chlorophyll molecules usually relax and attain ground state by this common mode of energy release as heat without any emission of photon. Alternatively, an excited pigment molecule may decay to its ground state by emitting a photon (fluorescence). A fluorescently emitted photon generally has a longer wavelength (lower energy) than the initially absorbed wavelength since some of the excitation energy has been lost as heat. Fluorescence accounts for dissipation of only 3–6% of the light energy absorbed by living plants. The excited molecules may directly transfer their excitation energy to nearby unexcited molecules with similar electronic properties by resonance energy transfer. Because of the inherent instability of the excited singlet state of chlorophyll molecule, any process that captures its energy must be extremely rapid. The process with the faster rate will be favored over others and will predominate. Photochemical reactions of photosynthesis are the fastest chemical reactions. This extreme speed is necessary for photochemistry to compete with the three other alternate ways by which the excited state loses its energy described above. In photochemical reaction excited pigment may lose an electron to an acceptor molecule triggering a charge separation event in which excited molecule is oxidized on losing the electron and the acceptor molecule is reduced on accepting the electron.

$$\text{Pigment} + h\upsilon \rightarrow \text{Pigment}^{+} + \text{e}^{-}$$

$$\text{Acceptor} + \text{e}^{-} \rightarrow \text{Acceptor}^{-}$$

For many pigments in the photosynthetic apparatus, fluorescence occurs in nanoseconds (10^{-9} s), whereas photochemical reactions (in photosynthetic organisms) occur more rapidly, in picoseconds (10^{-12} s). When a faster (thousand-fold) pathway of photochemistry is available, minimal fluorescence is observed and photosynthesis proceeds with high efficiency. Efficiency of photosynthesis is also measured by fluorescence. The higher is the efficiency of photosynthesis, the lesser will be the fluorescence and vice versa.

The efficiency of photochemistry in any system can be estimated by determining the **quantum yield** (phi, ϕ) of the photochemical event.

$$\phi = \frac{\text{Number of products formed photochemically}}{\text{Number of photon absorbed}}$$

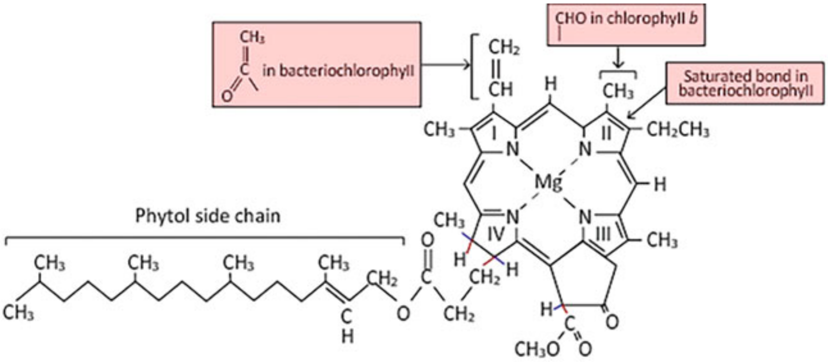
According to this equation, a quantum yield of 1 would indicate that every absorbed photon is converted into a chemical product. Quantum yield less than 1 would indicate that other decays are responsible for decreasing the efficiency of the photochemical reactions. Under optimum conditions, the measured quantum yield of photochemical reaction in a photosynthetic system is approximately 1 which is indicative of highly efficient photosynthetic process. This indicates that almost all absorbed photons are utilized for photochemical charge separation and other significant decay routes do not occur. Quantum requirement is reverse of ϕ and refers to number of quanta required for the production of one photochemical product.

5.1.3 Photosynthetic Pigments

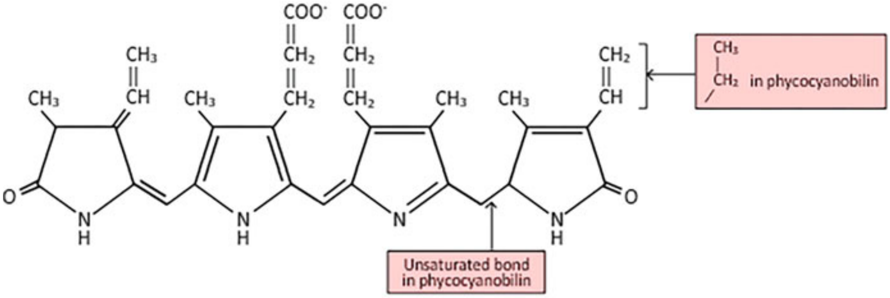
In order for light to be absorbed, plants must possess light-absorbing molecules, which occur as complexes bound with proteins. These complexes are called

pigments. The pigments consist of **chromophores** (Greek, carrier of color)—the light-absorbing component, and the associated proteins. Absorption of light by the chromophore-protein complex differs from that of free chromophores. On the basis of structure of the chromophore, photosynthetic pigments are classified as follows:

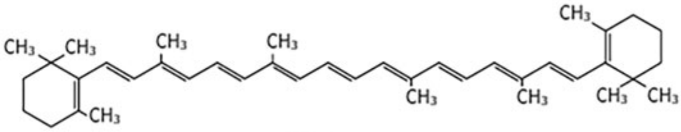
Chlorophylls The principal photoreceptor in photosynthesis is chlorophyll which has cyclic tetrapyrrole ring structure termed as porphyrins or chlorin. Structural formula of the green leaf pigment chlorophyll was given by Richard Willstatter and his collaborators as a result of work conducted during 1905–1913 in Zurich and Berlin. He was awarded the Nobel Prize in Chemistry for the same work in 1915. The structure shows similarity with porphyrin ring of hemoglobin. However, chlorophyll molecules have Mg^{2+} instead of Fe^{2+}, which occupies the central position. The hydrophobic alcohol, phytol, is derived from four isoprenoid units. The presence of phytol tail facilitates its location along with membrane proteins in thylakoids due to hydrophobic interactions, and it makes the chlorophyll molecules soluble in organic solvents. The five-ring heterocyclic structure surrounding Mg^{2+} has an extended polyene structure with alternating single and double bonds which is responsible for absorption in the visible region of light spectrum. The major forms of chlorophylls in higher plants and green algae are chlorophyll a (Chl a) and chlorophyll b (Chl b) which generally are present in a ratio of 3:1. Chl a is universally present in all organisms which carry out oxygenic photosynthesis. The major forms of chlorophylls in purple photosynthetic bacteria are bacteriochlorophyll a (BChl a) and bacteriochlorophyll b (BChl b). The difference between Chl a and Chl b lies in the substitution of methyl group by an aldehyde group in ring b of the porphyrin ring of Chl b (Fig. 5.3). The difference between Chl a and BChl a is the presence of double bond in ring b of the porphyrin ring of Chl a, while it is saturated in case of BChl a. This lack of alternating double and single bond in the ring b of porphyrin ring of BChl a causes a significant difference in the absorption of light by BChl a as compared to Chl a and b. Phytol tail is identical in all forms of chlorophylls. In angiosperms chlorophyll does not accumulate in dark. However, in gymnosperms and some algae, it can be synthesized in dark. Chl b is synthesized from Chl a through the action of an oxygenase enzyme that converts methyl group present in ring b to formyl side group. These small changes in chemical structure of the chlorophyll molecules and non-covalent interactions with membrane proteins in thylakoids significantly alter the absorption properties of different chlorophyll species. Pigments are also named after the wavelength of their absorption maximum; e.g., chlorophyll a_{700} refers to chlorophyll a molecules which has absorption maximum at 700 nm. Chl c is a member of chlorophyll family largely associated with marine photosynthetic organisms, especially golden-brown eukaryotic algae. They are associated with Chl a and carotenoids to harvest light energy to perform photosynthesis in these algae. Chl c differs from other chlorophylls by having **Mg-phytoporphyrins** (ring d is unsaturated) instead of Mg-phytochlorins. Chl c also possesses trans-acrylic (propionic) acid at C-17 region instead of propionic acid side chains present in chlorophylls a and b. However, chlorophyll c also differs from other forms of chlorophylls in not having phytol tail. Chl c absorbs moderately in red



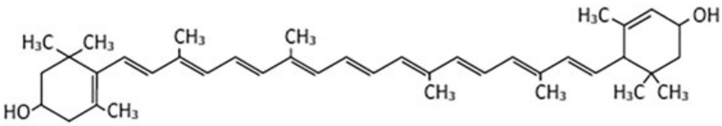
(a) Chlorophyll *a*



(b) Phycoerythrobilin



(c) β-Carotene



(d) Lutein (xanthophyll)

Fig. 5.3 Structures of chlorophylls, phycobilins, and carotenoid region, i.e., around 620 nm, but approximately tenfold more strongly in 400–450 nm. Chl d has formyl group present in place of vinyl group found in Chl a. It shows absorption peak in infrared region of light spectrum (700 nm instead of 665 nm). It is present in cyanobacteria which grow as epiphytes under the fronds of red algae.

Carotenoids Another group of photosynthetic pigments present along with chlorophylls are carotenoids. Carotenoids are linear polyenes which can be yellow, purple, or red. These absorb blue and green light and serve both as antenna pigments as well as photoprotective agents. Carotenoids include **carotenes** (it includes β-carotene and lutein) and **xanthophylls**. These are tetraterpenes consisting of eight isoprene units ($C_{40}H_{64}$). They comprise conjugated double bond between carbon and hydrogen, while xanthophylls additionally contain one oxygen atom in each of their terminal rings. These are primarily responsible for the orange-yellow coloration of senescent leaves of the plants. Carotenoids serve as accessary pigments and have a secondary role in photosynthesis. They act as antenna pigments which absorb light between 400 and 500 nm and transfer it to chlorophyll molecules. Fucoxanthin, a type of xanthophyll, is particularly efficient in harvesting blue and green light in brown seaweeds. These have an additional function to give structural stability to the assembly of light-harvesting complexes (LHCs). Xanthophylls also protect the photosynthetic apparatus from photooxidative damage (**Xanthophyll cycle,** Fig. 5.4). In the presence of light and oxygen, mutants of carotenoid biosynthesis result in generation of reactive oxygen species (ROS), which are responsible for causing damage to the photosynthetic apparatus.

Phycobilins Phycobilins are the chromophores consisting of linear tetrapyrroles derived from the chlorophyll biosynthetic pathway. These are group of accessory pigments which are found in algae. These are water soluble since the phytol tail is absent and structurally resemble bile pigments. These are present as complex protein-containing structures called **phycobilisomes**. Phycobilins are covalently linked via thioester bond to specific proteins which are known as phycobiliproteins.

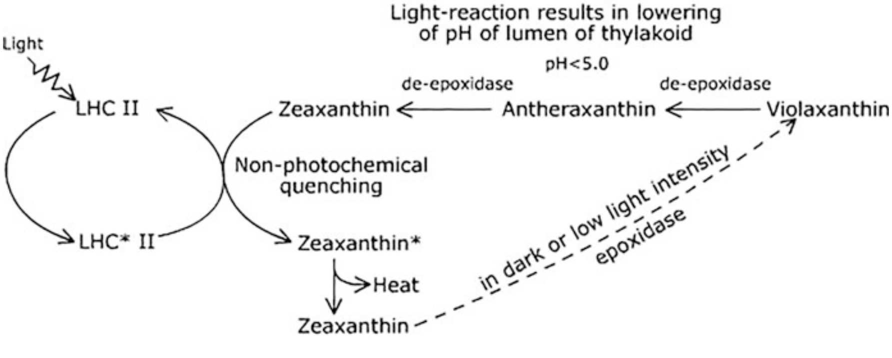


Fig. 5.4 Xanthophyll cycle

The cysteine residue in the protein and vinyl side chain of the phycobilins forms this bond. Basic structure of phycobiliproteins is a heterodimer (α- and β-proteins). Each of the protein subunit contains one to four phycobilins as the chromophores. The prefix, *phyco*, designates algal origin. Three of the phycobilins, i.e., phycoerythrin, phycocyanin, and allophycocyanin, serve as photosynthetic pigments in algae, while the fourth phytochromobilin is an important photoreceptor (phytochrome) in plant growth and development. The chromophores of phycoerythrin and phycocyanin are known as phycoerythrobilin and phycocyanobilin. The presence of phycobilins enables red algae and cyanobacteria to absorb light and carry out photosynthesis even in dim light. Cyanobacteria and red algae are able to survive in deep water because of the presence of phycobilisomes which absorb green light. Light reaching to the bottom of the ocean is rich in green light because it is not absorbed by the chlorophylls of green algae **(green window)** which are growing in the upper regions. Algae growing in deeper water are able to mainly get dim green light. Red algae appear black in daylight because of absorption of almost all the wavelengths of visible light spectrum. All oxygen-evolving photosynthetic organisms contain Chl a. These organisms also contain other forms of chlorophyll, like chlorophyll b (higher plants), chlorophyll c (diatoms), or chlorophyll d (red algae). However, most prokaryotic cyanobacteria usually contain only Chl a. In addition, photosynthetic organisms contain carotenoids. Some of them contain phycobilins as well (Table 5.1). Therefore, the range of wavelengths that can be absorbed by the organisms is broadened by the presence of accessory pigments. This results in more effective utilization of visible light energy than could be achieved with only one pigment. In organisms which are present in submerged aquatic niches where penetration of red light is limited, a variety of light-absorbing pigments makes it possible for maximum absorption of light.

High light intensities allow plants to absorb more light energy than that which can actually be used for photosynthesis. This leads to excessive excitation of chlorophyll molecules resulting in generation of more of “triplet state” of chlorophyll and singlet state of oxygen. High levels of singlet oxygen can decrease the efficiency of photosynthesis by a process called **photoinhibition**. In plants which have lower carotenoid level (either by inhibiting their biosynthesis or by mutation under experimental conditions), when exposed with high light intensity, there is an increase in the level of singlet oxygen which is lethal to photosynthetic apparatus. Carotenoids help in accepting the high excitation energy from chlorophyll molecules in triplet state and prevent singlet oxygen formation. Therefore carotenoids, besides broadening the range of spectrum of light absorption, also protect photosynthetic apparatus photodamage (xanthophyll cycle).

5.1.4 Action Spectrum Relates to Absorption Spectra

A photobiological response is the outcome of the light absorbed by a particular photoreceptor. In order to find out which photoreceptor is responsible for carrying

Table 5.1 Photosynthetic pigments

Name of the pigment	Unique features of the structure	Solubility	Absorption maxima in their respective solvents (nm)	Distribution in oxygenic photosynthetic organisms, examples	Role in photosynthesis
Chlorophylls	Porphyrin structure with long hydrocarbon phytol tail	Organic solvents, petroleum ether	435, 670–680	All organisms carrying out oxygenic photosynthesis, higher plants, green algae, diatoms, brown algae, cyanobacteria	Primary photosynthetic pigment, responsible for carrying out photochemical reaction
Chl a	Porphyrin structure with long hydrocarbon phytol tail, presence of -CHO group in place of CH_3 in ring B	Methyl alcohol	480, 650	Higher plants green algae	Accessory pigment helps in harvesting and funneling light energy to reaction center
Chl b	Phytol tail is absent	Soluble in ether, acetone, methanol; insoluble in water and petroleum ether	445, 625	Diatoms, brown algae	Accessory pigments of PSII
Chl c	Presence of formyl group in place of vinyl group found in porphyrin ring of Chl a	Soluble in ether and acetone, very slightly soluble in petroleum ether	450, 456, 696	Red algae, in some cyanobacterium discovered lately, *Acaryochloris marina*	Major chlorophyll present in cyanobacterium *Acaryochloris marina* in place of Chl a
Carotenoids	Linear molecules with multiple conjugated double bonds, oxygen-free hydrocarbons	Organic solvents	420–525	In all photosynthesizing organisms	Accessory pigment helps in harvesting and funneling light energy to reaction center

(continued)

Table 5.1 (continued)

Name of the pigment	Unique features of the structure	Solubility	Absorption maxima in their respective solvents (nm)	Distribution in oxygenic photosynthetic organisms, examples	Role in photosynthesis
Carotenes	Oxygenated derivatives of carotenes	Organic solvents	500–650	In all photosynthesizing organisms	Accessory pigment helps in harvesting and funneling light energy to reaction, also protects photosynthetic apparatus from photooxidation
Xanthophyll	Open tetrapyrrole ring chromophore covalently bound to protein	Water soluble	Phycoerythrin $\lambda_{max} = 565$ Phycocyanin, $\lambda_{max} = 620–638$	Present in red algae and cyanobacteria	Accessory pigment helps in harvesting and funneling light energy to reaction center

out a particular photobiological response, absorption spectrum of the photoreceptor should match with the action spectrum of that process. **Absorption spectrum** of a pigment is obtained when relative absorbance is plotted as a function of wavelengths. Height of absorption of any wavelength in the given absorption spectrum reflects the probability by which light of that energy level will be absorbed. The absorption spectrum of a pigment is unique and is used for identification of that molecule (Fig. 5.5). An **action spectrum** shows the scale of response of a biological system as a function of wavelength. The action spectrum of photosynthesis is obtained by plotting the rate of oxygen evolution by a plant under different wavelengths of light. Studying action spectrum has been crucial in understanding the photosynthetic light reaction besides establishing the relationship of chlorophyll pigments with photosynthetic process. Theodore W. Englemann gave the first action

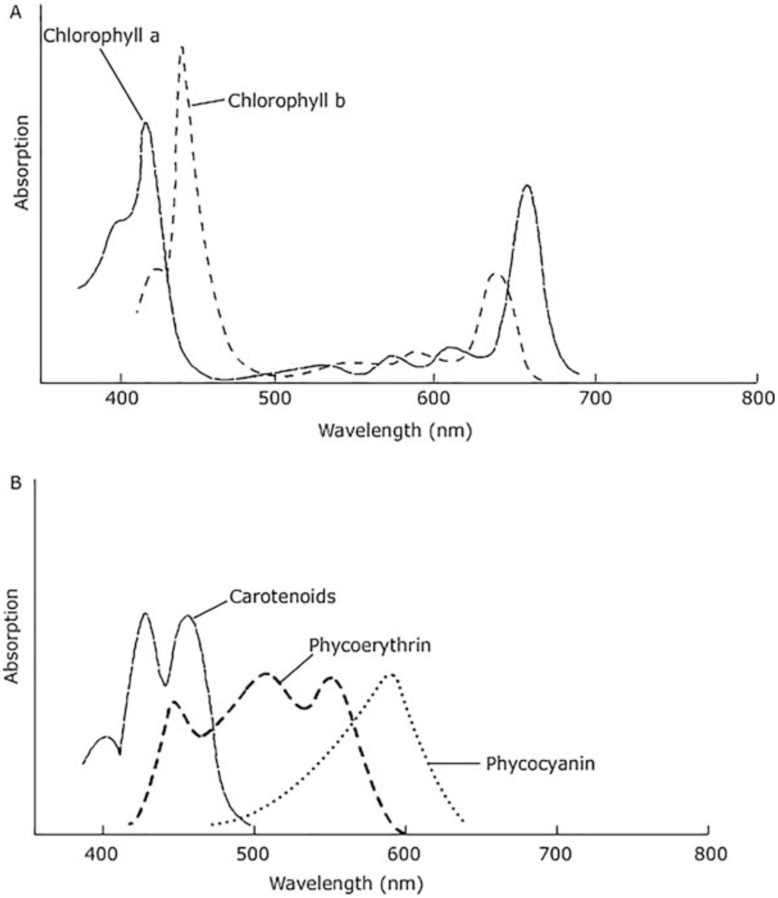


Fig. 5.5 Absorption spectrum (**a**) of chlorophylls a and b and (**b**) carotenoids and phycobiliproteins

spectrum of photosynthesis in *Cladophora*. He had taken aerophilic bacteria along with the alga, which was exposed to a spectrum of visible light. Maximum accumulation of bacteria occurred in the regions of alga exposed to blue and red light which indicated maximum evolution of oxygen occurred in blue and red region of the light spectrum. This indicated rate of photosynthesis being maximum in blue and red light. Chlorophylls absorb maximally in the blue and red region of light spectrum which indicates the role of chlorophylls in photosynthesis.

5.2 Phases of Photosynthesis

In 1905, F.F. Blackman, a British plant physiologist, interpreted the light curve of photosynthesis as an evidence of it being a two-step process. According to Blackman, initial part of the light curve, which shows increase in photosynthesis with increasing light intensity, corresponds to light-limited phase of photosynthesis. He proposed that the intermediates produced during light reaction need to be supplied for further conversion during dark. Horizontal bending of the curve observed due to light saturation of photosynthesis provided the evidence that chemical apparatus of the plant is being overtaxed and is incapable of taking care of the intermediates as quickly as these are produced during light reaction. Initially dark reaction was also called as “Blackman reaction.” This observation was further validated by using flashlight in experiments conducted by Robert Emerson and William Arnold in 1932. They exposed *Chlorella* suspension to brief light flashes lasting 10^{-5} s. They measured O_2 evolution and duration of dark intervals in between the flashes in relation to the energy of light flashes. Light saturation, measured as O_2 yield, was observed when only one of the 2500 chlorophyll molecules had received a photon. However, presently it is known that 8 photons are required for the release of one O_2 molecule (quantum requirement is 8). Thus, it is the 300 chlorophyll molecules (2500/8) which are responsible for absorption of one photon. They are termed as **photosynthetic unit**. Duration of dark interval following the light flash is determined by the rate-limiting activity of enzymes. It was observed that yield of O_2 per flash was the function of dark interval which was found to increase at low temperature. In the presence of potassium cyanide, flash yield was not influenced. However, required dark interval was increased. T. Thunberg proposed in 1923 photosynthesis to be a redox system in which CO_2 is reduced and H_2O is oxidized. Cornelis B. van Niel (1897–1985), a Dutch-American microbiologist in 1931, made a comparative study of anoxygenic photosynthesis in bacteria with oxygenic photosynthesis in plants. He proposed photosynthesis to be the result of transfer of hydrogen atoms from H_2A to CO_2 (an oxidation-reduction reaction):

$$CO_2 + 2H_2A \rightarrow CH_2O + H_2O + 2A$$

$$CO_2 + 2H_2O \rightarrow CH_2O + H_2O + O_2$$

In green plants H_2A is water, while in purple sulfur bacteria (anoxygenic photosynthesis) it is H_2S. In case of bacteria, accumulation of sulfur reinforced the reduction-oxidation concept of photosynthesis and photolysis of water. This observation also gave evidence in support of H_2O being the source of released O_2 rather than CO_2. Using water ($H_2{}^{18}O$) labeled with heavy isotope of oxygen (^{18}O), Sam Ruben also demonstrated in 1941 that oxygen released during photosynthesis originated from water. Role of photochemical light reaction has been developed from many experiments. Pioneer among them was a study in 1939 by Robert Hill at the University of Cambridge. He made the crucial observation that isolated chloroplasts (when illuminated) could promote reduction of artificial electron acceptors. Ferric salts (Fe^{3+}), used in the experiment, were reduced to ferrous form (Fe^{2+}). The experiment was conducted in the absence of CO_2.

$$4Fe^{3+} + 2H_2O \xrightarrow[\substack{\text{Isolated chloroplasts;} \\ \text{Light energy}}]{} 4Fe^{2+} + 4H^+ + O_2$$

Robert Hill observed that a variety of compounds can act as electron acceptors. This reduction of artificial electron acceptors and release of oxygen by isolated chloroplasts in presence of light and absence of CO_2 is called **Hill's reaction**. The reaction has been demonstrated in the laboratory using different oxidants such as the dye, DCPIP (2,6-dichlorophenolindophenol), which acts as artificial electron acceptors. The dye changes from its blue color to a colorless state on being reduced.

$$H_2O \rightarrow 2H^+ + ½O_2 + 2e^-$$

$$\underset{\text{Blue}}{\text{DCPIP (oxidized)} + 2e^- + 2H^+} \longrightarrow \underset{\text{Colorless}}{\text{DCPIPH}_2\text{ (reduced)}}$$

This demonstrates that the photosynthetic systems can oxidize water to O_2 without any involvement of CO_2. Dye reduction could be measured spectrophotometrically at 620 nm. Hill's landmark experiments lead to the following findings: (i) evolution of oxygen in photosynthesis is independent of reduction of carbon dioxide, (ii) released oxygen originates from water and not from carbon dioxide since no CO_2 was used in Hill's experiment, and (iii) fragments of isolated chloroplasts are capable of performing partial reactions of photosynthesis and some intermediates are reduced prior to reduction of CO_2. The above observations gave clear indications that light and dark reactions are separate processes in photosynthesis. It was observed that spinach grana had the ability to reduce NADP under the influence of light. In 1951, three scientists, Wolf Vishniac, S. Ochoa, and Dan Arnon, independently reported the role of pyridine nucleotide ($NADP^+$) then called TPN as the natural electron acceptor in vivo for the light reactions in photosynthesis yielding NADPH. An overview of photosynthesis is given in Fig. 5.6.

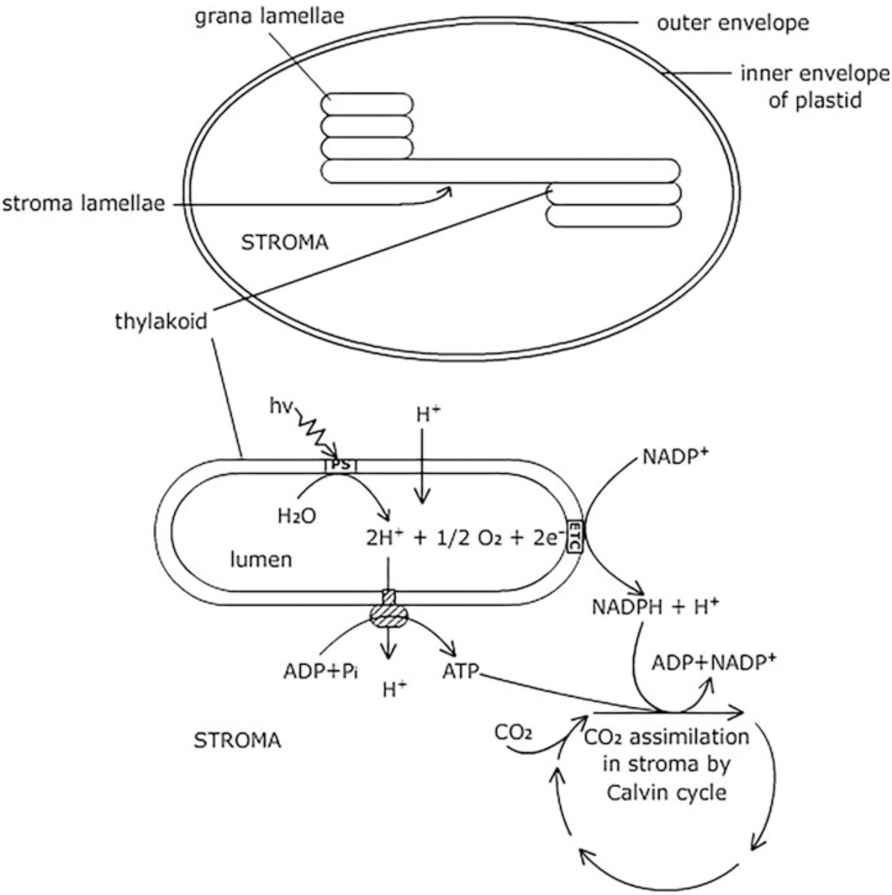


Fig. 5.6 Overview of two steps of photosynthesis, light reaction which occurs in thylakoids and CO_2 assimilation which occurs in the stroma of chloroplasts

5.3 Light Reactions in Photosynthesis

In 1943, Robert Emerson and Charlton Lewis explained the action spectrum for photosynthesis in the visible region of the light spectrum, while performing experiment with the green alga *Chlorella pyrenoidosa*. It was visualized that if light is absorbed by chlorophyll molecules, its energy should be utilized for O_2 evolution. Since absorption of 8 photons is required for release of one oxygen, they assumed that quantum yield of 0.11 should be fairly constant for light absorbed by the chlorophyll molecule. (Theoretically quantum requirement was 8, but practically the value was calculated to be 10). They reported that value of 0.1 (quantum yield) was remarkably constant over most of the spectrum. This indicates that any photon absorbed by chlorophyll is more or less equally effective in driving photosynthesis.

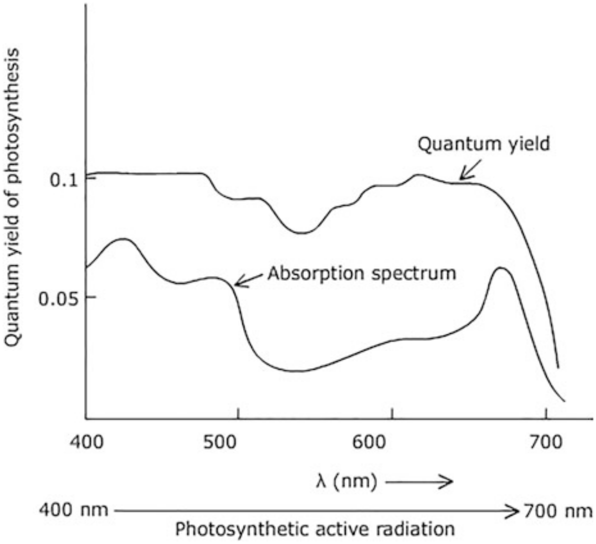


Fig. 5.7 Graph showing effect of visible light on quantum yield of photosynthesis—"red drop phenomenon." There is sudden decline in quantum yield of oxygen evolution in plants irradiated with light of longer wavelength greater than 680 nm, which is still absorbed by chlorophylls, as is depicted in their absorption spectrum

However, a sudden drop in the quantum yield at wavelengths greater than 680 nm was observed, even though chlorophyll still absorbed in that range. This puzzling drop in quantum yield in the red region of the spectrum is called the **red drop phenomenon** (Fig. 5.7). This red drop effect was a strange observation since chlorophylls show appreciable absorbance at 700 nm when quantum yield declines. It showed that the energy was just not being used as efficiently above 680 nm. Interestingly, it was observed by Emerson in 1960 that simultaneous illumination with lower wavelengths of red light (650–680 nm) along with far-red (700–720 nm) light produced a marked two to three times increase in rate of photosynthesis as compared to when both the wavelengths were given separately and value of rate of photosynthesis was added (rate of photosynthesis was measured as the rate of O_2 evolution). Therefore, it was predicted that action of both wavelengths (680 nm and 700 nm) must be required simultaneously for photosynthesis to proceed with maximum efficiency. This phenomenon of enhancement of photosynthetic efficiency under simultaneous irradiation was termed as the **Emerson enhancement effect** (E), which can be expressed as the ratio of rate of oxygen evolution (ΔO_2) in presence of far-red light as the supplementary beam and the same in absence of it.

$$E = \frac{\Delta O_2 \text{ (in combined beam)} - \Delta O_2 \text{ (short beam alone)}}{\Delta O_2 \text{ (long wave beam alone)}}$$

The enhancement effect suggested that photosynthesis must involve two photochemical events or systems, one driven by short wavelengths of light ($\leq$680 nm) and another driven by long wavelengths of light (>680 nm). For optimal photosynthesis both wavelength ranges must be utilized by the photosynthetic apparatus simultaneously or in rapid succession. Initially, observations were made by Louis Duysens in *Rhodospirillum rubrum* in 1952, and later on Bessel Kok made similar observations while working with chloroplasts that bleaching of chlorophyll by longer wavelengths or shorter wavelengths occurred which was reversed when these were placed in dark. Bleaching could be attributed to primary reaction of photosynthesis, and the concept of existence of two **pigment systems I and II** was held responsible for catalyzing two light reactions. Light reaction I refers to reduction of NADP$^+$, while light reaction II refers to photolysis of water. Pigment system I was found to be responsible for light reaction I and pigment system II was for light reaction II. These were later on called as photosystem I and II (PSI and PSII), respectively. Photosystem I and Photosystem II were so called according to the order of their discovery. In 1960, Robin Hill and Fay Bendall proposed role of cytochrome b and f as the intermediates in the electron transfer. They demonstrated coupling of the two light reactions involving two photosystems in a linear electron transfer chain which formed the basis of Z-scheme. The excited PSI was oxidized when exposed to long wavelengths (700 nm), because an electron was lost to an electron acceptor. The oxidized PSI replenished its electron obtaining it from cytochrome resulting in their oxidation. PSII gets oxidized on receiving shorter wavelength (680 nm) and is responsible for reducing cytochromes. In this way cytochrome mediates electron transport and the two photosystems operated in a linear manner. This observation was the basis of Z-scheme. The components of electron transport chain were identified because of changes in their redox potentials. When these components were placed according their redox potential, the arrangement appeared in the shape of Z. Consequently, this scheme of electron transport in the light reaction of photosynthesis was called **Z-scheme** (Fig. 5.8)**.**

5.3.1 Organization of Photosynthetic Apparatus into Photosystems

The structure and composition of photosynthetic apparatus are responsible for its functional characteristics. In eukaryotic photoautotrophs, photosynthesis takes place in the subcellular organelles known as chloroplasts. Its extensive system of internal membranes, known as thylakoids, contains all the chlorophyll molecules where the light reactions of photosynthesis take place. A variety of proteins essential to photosynthesis are embedded in the thylakoid membranes. These integral membrane proteins contain a large portion of hydrophobic amino acids which are, therefore, much more stable in a nonaqueous hydrophobic region of the membrane. For maximum optimization of energy transfer in antenna complexes and for electron transfer in the reaction centers, chlorophylls and light-harvesting pigments in the thylakoid membranes are always associated with proteins through non-covalent but highly specific bindings, forming pigment-protein complexes within the thylakoid

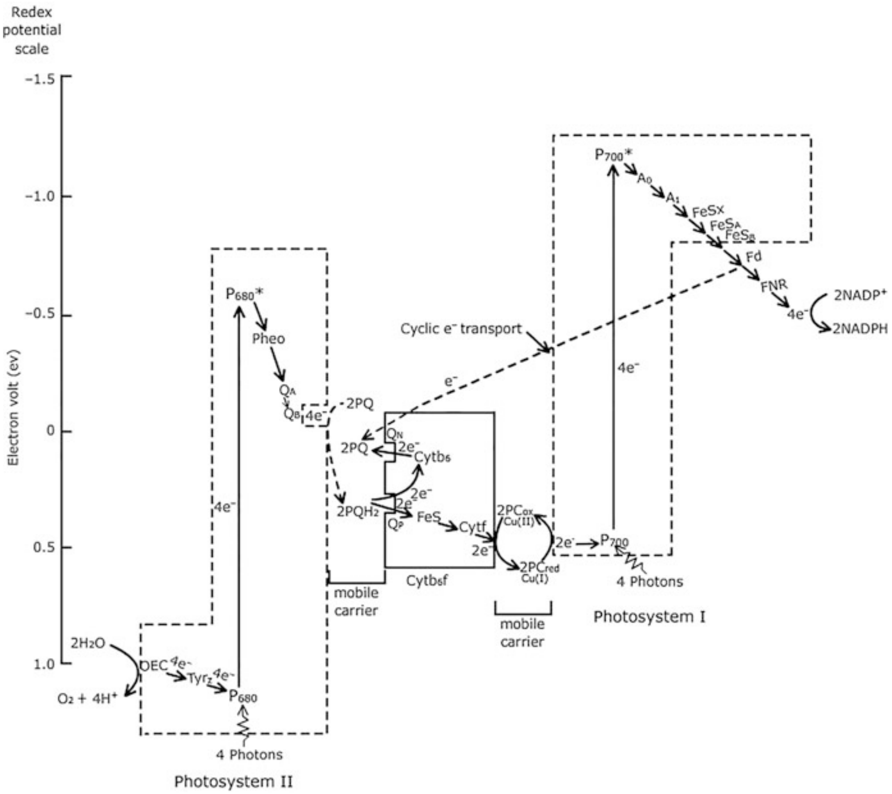


Fig. 5.8 Detailed Z-scheme consisting of photosystem II, photosystem I, cytochrome b_6f, and two mobile carriers plastoquinone and plastocyanin for O_2 evolution. Out of the four electrons removed from two molecules of water, two electrons are passed to plastocyanin (PC). Electrons are replenished in oxidized P_{700} from reduced PC. In the absence of oxidized $NADP^+$, electrons are passed from reduced ferredoxin to PQ which are cycled back through P_{700} (cyclic electron transport). *OEC* oxygen evolving complex

membranes. In 1960, Govindjee and Rabinowitch suggested two spectroscopically different forms of Chl a in vivo, which had different photochemical functions, one absorbing shorter wavelength of light, i.e., 680 nm, while the other form of Chl a absorbs longer wavelength of light, i.e., 700 nm. These two forms are primarily responsible for carrying out the two light-driven photochemical reactions. These two pigment systems are now known as photosystems II and photosystem I, respectively.

Both photosystems contain a reaction center where the photosynthetic electron transfer begins with the removal of an electron. As a result, the electron donor pigment is oxidized resulting in its positive charge (Chl a^+). The primary electron acceptor (A) is reduced on receiving the electron thus carries a negative charge (A^-).

This process is the charge separation event and is known as photochemical reaction. The rest of the components of the reaction center are involved in stabilizing this charge separation.

$$\text{Chl a} \xrightarrow{h\nu} \text{Chl a}^{+} + e^{-}$$

$$A + e^{-} \rightarrow A^{-}$$

Besides primary pigment, which is a pair of chlorophyll a molecules, other components of the electron transport are associated with specific proteins in the photosystems. The reaction center of plants shares a conserved core structure with that of purple photosynthetic bacteria. The second component of photosystems is an array of antenna pigments forming **light-harvesting complex (LHC)**. LHCs associated with photosystem II and photosystem I are called LHCII and LHCI, respectively. Their proteins are encoded by nuclear genes belonging to LHCA and LHCB gene families. The electrophoretic analysis shows a spectrum of individual chlorophyll-protein complexes in which each protein retains its natural chlorophyll array. These antenna pigments function to absorb light energy and transfer it to the reaction center where this energy is then conserved as chemical form of energy. The antenna pigments involved in light harvesting are of two types, peripheral and core antenna pigments, which are associated with proteins. In most plants, 250 chlorophyll molecules are associated with each reaction center. The transfer of excitation energy from one pigment molecule to another (by a mechanism known as Foster energy transfer or resonance) does not require emission and reabsorption of photons. The proximity of the donor and acceptor molecules within the antennae pigments is critical because the efficiency of energy transfer is inversely proportional to the sixth power of the distance separating the two molecules. For two pigments separated by approximately 10 Å, an energy transfer time of less than 1 picosecond has been estimated. The relative orientation of the pigments in LHC is also significant, and the absorbance spectra of a pigment must overlap with the fluorescence spectrum of another one for efficient energy transfer. The sequence of pigments within the antenna molecules which funnel the absorbed energy toward the reaction center has an absorption maxima that is progressively shifted toward red wavelength. This red shift in the absorption maxima means that the energy of the excited state is somewhat lower nearer the reaction center than in the more peripheral portions of the antenna system. Because of this arrangement, when excitation energy is transferred from one chlorophyll molecule to another, the difference in energy between the two excited chlorophylls is lost to the environment as heat. Chlorophyll a is found in all reaction center complexes as well as in the antenna, whereas chlorophyll b is found only in the antenna complexes and thus have role in light harvesting. Approximately 15 different chlorophyll-binding proteins (CP) have been identified. Some are associated with PSI and others with PSII. They are all encoded in nucleus and, therefore, must be imported into the chloroplasts before binding with chlorophylls and associating with their proper photosystems. In addition to the chlorophyll pigments, carotenoids are also commonly found in the antenna complexes. The antenna complexes in most plants typically have a ratio of 0.5 for carotenoid/total chloroplast pigments. Association with specific proteins causes a shift in peak absorption wavelength by chloroplasts toward the red (lower energy) wavelength because the reaction center complex absorbs longer wavelength than the antenna. The reaction center chlorophyll (Chl a) acts as an energy trap, promoting transfer of energy from the antenna toward the reaction center complex. Photosystems have their own complement of chlorophyll-binding antenna proteins. Properties of these pigments protein complexes are optimal for the particular reaction center chlorophyll in the photosystem. Therefore, the antenna chlorophylls of PSI with a reaction center Chl a absorbing maximally at 700 nm would be expected to absorb wavelengths longer than the antenna of PSII, which has a reaction center Chl a that absorbs maximally at 680 nm (P_{680}).

5.3.2 Organization of Chlorophylls and Other Pigments in LHCII and LHCI

The major pigment-binding protein of LHCII represents about half of the total protein in the thylakoid membranes. It is the second most abundant protein after Rubisco. It is a trimeric complex. The three polypeptides are encoded by nuclear family of genes Lhcb1, Lhcb2, and Lhcb3. Each monomeric unit consists of 230–250 amino acid residues, has a molecular weight of 24–29 kDa, and consists of three transmembrane helices. It is bound non-covalently to eight Chl a, six Chl b, and four xanthophyll molecules. Two lutein molecules serve as scaffolding, while a neoxanthin and violaxanthin are bound at the periphery. Besides these proteins of LHCII, other polypeptides Lhcb4, Lhcb5, and Lhcb6 are also present which facilitate forming bridges between PSII and major LHCII components. Core proteins of LHCII include CP43 and CP47 where CP refers to chlorophyll proteins and number refers to their molecular weight (Fig. 5.9). Small proteins such as cytochrome$_{b559}$ encoded by plastid gene *psb*E and *psb*F are required for correct assembly of PSII. The detergent fractionation of thylakoid membranes yields intact PSI and PSII complexes with full array of pigment molecules. Proteins associated with PSI are called LHCI proteins. The structure of the LHCI proteins is generally similar to that of the LHCII proteins. All these proteins have a significant sequence similarity and, therefore, reflect common ancestral proteins. The LHCI consists of four peripheral polypeptide subunits Lhca1–4, which belong to a family of 25 kDa proteins. These are arranged in two dimers. Each Lhca protein is associated with 13 chlorophylls and 2–3 carotenoid molecules. The amount of Lhca proteins is variable depending on light intensity and nutrient availability.

5.3.3 Photochemical Reaction Centers

There are two reaction centers identified on the basis of the primary electron donor, Chl a molecule. These reaction centers are the components of the two photosystems

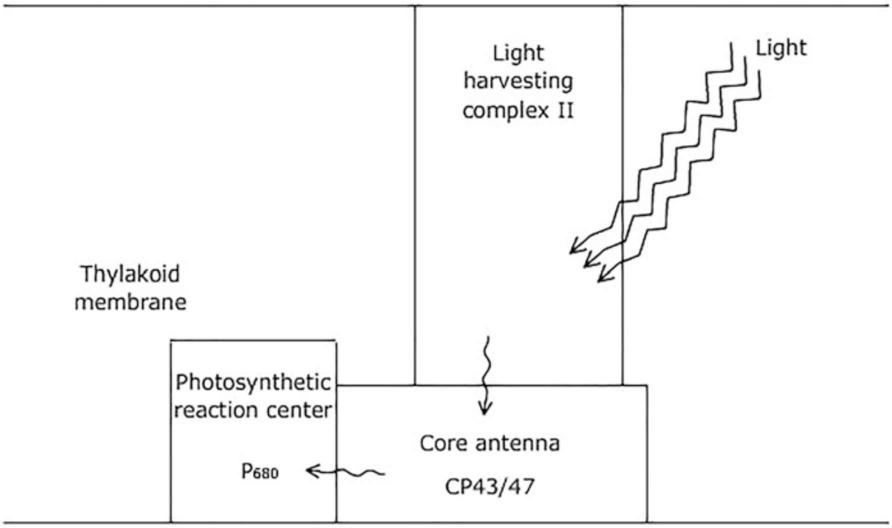


Fig. 5.9 Basic scheme of light-harvesting complex

in plants. On the basis of electron acceptor, there are two types of reaction centers. Reaction center which reduces a quinone is a type II reaction center, while type I ultimately reduces Fe-S cluster. The organisms which carry out oxygenic photosynthesis contain both type II and type I reaction centers. Unlike plants there is only one reaction center in the bacteria which carry anoxygenic photosynthesis. Green sulfur bacteria contain type I reaction center, while purple bacteria have only type II. Electron carriers of type II reaction center in plants resemble the type present in purple bacteria. There is similarity of electron carriers of type I in the reaction center of oxygenic organisms with those present in green sulfur bacteria. PSII is present in all the organisms which carry out oxygenic photosynthesis. It is also called as **water-plastoquinone oxidoreductase.** Reaction center of PSII is associated with the oxygen-evolving complex (OEC) which is present toward the lumen side of thylakoids. The two proteins associated with reaction center of PSII are D1 and D2 (Fig. 5.10). D1 is a hydrophobic 32 kDa protein which is encoded by the plastid gene. Another protein which is 34 kDa protein is also encoded in the plastid genome. Since D1 is exposed to extremely oxidizing environment created by excited P_{680}, it leads to high light-dependent turnover of the protein. On the contrary D2 represents inactive branch of the reaction center. There is a similarity of these proteins with L and M proteins of reaction center complex present in *Rhodopseudomonas viridis*. Hartmut Michel, Johann Deisenhofer, and Robert Huber carried out the X-ray crystallography studies of the reaction center in these bacteria and elucidated for the first time the three-dimensional structure of a membrane protein. These three scientists were awarded Noble Prize in chemistry in 1988 for their work. Chl a dimer is associated with the heterodimeric proteins in plants (D1 and D2) and is called P_{680} because of its characteristic absorption maxima. Besides dimeric P_{680}, the other

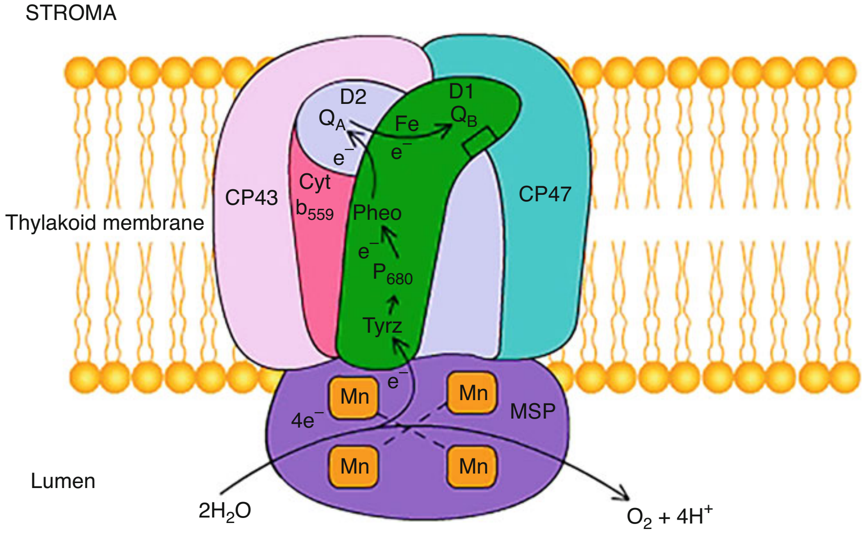


Fig. 5.10 Molecular structure of photosystem II, MSP (manganese-stabilizing protein), CP43 and CP47 are part of inner core of light-harvesting complex

components of PSII which are involved in charge separation and electron transport are two pheophytin molecules (chlorophyll lacking Mg^{2+} ions), a non-heme iron, and two plastoquinones designated as Q_A and Q_B which act as the terminal electron acceptor. The site for binding Q_A is present in D2 and for Q_B is present in D1. P_{680}, pheophytin, and plastoquinone are the prosthetic groups of the reaction center proteins. The reaction center in **PSI** (also known as **plastocyanin-ferredoxin oxidoreductase**) contains dimeric P_{700} Chl a molecule which is associated with the proteins PsaA and PsaB (Fig. 5.11). The reaction center chlorophyll P_{700} absorbs a photon and subsequently transfers an electron through a series of acceptors which includes an acceptor Chl a molecule A_0, a phylloquinone A_1, and the proteins having iron-sulfur (Fe-S) center. Comparison between PSII and PSI is given in Table 5.2. The reaction center subunits of PSI are encoded in plastid genome referred to as *psa*, while the genes encoding the components of PSII are referred to as *psb*. D1, a hydrophobic 32 kDa protein, is encoded by *psb*A in plastid genome, while D2, a 34 kDa protein, is encoded by the gene *psb*B in plastid genome.

5.3.4 Cytochrome b_6f (Plastoquinol-Plastocyanin Oxidoreductase)

Another component which mediates transfer of electrons during light reaction between PSII and PSI is cytochrome b_6f which functions as plastoquinol-plastocyanin oxidoreductase. It is similar to mitochondrial cytochrome bc_1. Both complexes are dimers with a molecular mass of 220 kDa, each monomeric unit

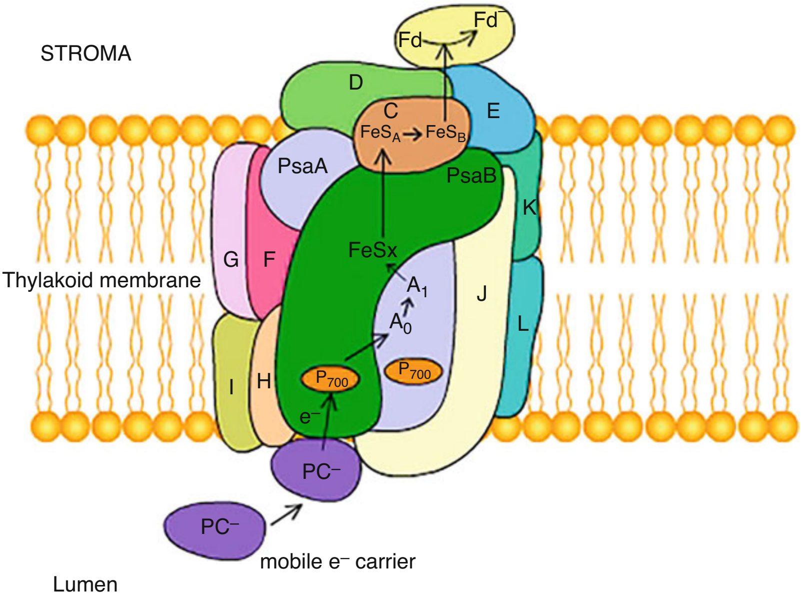


Fig. 5.11 Molecular structure of photosystem I, psa A and psa B, are major proteins associated with PSI. Proteins labeled from C to L are minor proteins. A_0 is a chlorophyll (a molecule which is a primary electron acceptor from P_{700}); A_1 is a phylloquinone; Fe-S_x, Fe-S_A, and Fe-S_B are Fe-S centers; Fd is a soluble iron-sulfur protein ferredoxin

Table 5.2 Comparison of PSII and PSI

Characteristics	PSII	PSI
Chlorophyll a present in reaction center	P_{680}	P_{700}
Location in thylakoids	Appressed regions of thylakoids	Non-appressed region of thylakoid
Primary electron acceptor (A_o)	Pheophytin-a	Chlorophyll a
A_1	Plastoquinone (Q_A)	Phylloquinone
A_2	Plastoquinone (Q_B)	F_X (Fe-S center)
Primary function	Photolysis of water	$NADP^+$ reduction

containing a conserved core of four electron carriers which contains two **cytochromes** (the proteins which have heme as the prosthetic group) belonging to b type cytochromes (cyt b_6 in chloroplasts or cyt b in mitochondria) and c type of cytochromes (cyt f in chloroplasts or cyt c_1 in mitochondria). These are linked by a protein having **Rieske Fe-S center** which constitutes the third redox component of the complex. Fourth component of the complex is a small 17 kDa peripheral protein which has got two binding sites for quinone, Q_p, site for binding with quinol (QH_2), which is present toward the lumen of the thylakoid, while another site Q_n is present toward the stroma side; significance of these two sites will be discussed later on. Q_p and Q_n refer to quinone-binding sites toward the positive and negative sides of the thylakoids, respectively.

5.3.5 Two Mobile Electron Carriers

There are two mobile electron carriers—plastoquinone and plastocyanin—which facilitate electron transfer between PSII and cytochrome b_6f and cytochrome b_6f and PSI, respectively. Plastoquinone facilitates two-electron transfer from PSII and is highly lipophilic in reduced plastoquinone form, which enables its lateral diffusion within the lipid bilayer of thylakoid. Plastocyanin is a small copper-containing protein (11 kDa) which is located in the aqueous phase of thylakoid lumen. Almost 50% of the copper is utilized for plastocyanin synthesis in a photosynthetic cell. Unlike plastoquinone, plastocyanin facilitates the transfer of single electron at a time. The genes for plastocyanin are encoded in nuclear genome.

5.3.6 Electron Transport Pathway During Light Reaction of Photosynthesis

The primary step in light reaction involves transfer of electrons (excited by light) from the reaction center P_{680} or P_{700} (in PSII or PSI, respectively) into an electron transport chain. The ultimate source of the electrons is the water molecule, which is photolyzed to release electrons, protons, and O_2, while the final destination is $NADP^+$, which is thereby reduced to NADPH. Energy derived during electron transport is coupled to transfer of protons from stroma into the lumen, resulting in pH gradient which drives ATP synthesis similar to ATP synthesis in mitochondria. Products of the light reactions, ATP and NADPH, are utilized subsequently during CO_2 assimilation.

5.3.7 Photosystem II (Splitting of Water)

As a result of electron transport during light reaction, an organized series of oxidation-reduction reactions occur. On losing an electron, a molecule is oxidized and attains positive charge (+). On the contrary, molecules on gaining electrons are reduced and become negatively charged (−). In a series of electron transport chain, electrons move from molecules having less positive redox potential (act as reductant) to molecules having more positive redox potential (act as oxidant) spontaneously. LHCII acts like an antenna, which on receiving the energy of photons, transfers it to chlorophylls bound to CP43/47—the core proteins of LHCII. Similar to cyanobacteria, X-ray crystallographic studies of PSII in plants have shown it to be a heterodimeric structure. However, its molecular structure in plants has not been determined. Reaction center of PSII consists of dimeric P_{680}, one on each polypeptide D1 and D2. There are other proteins bound with PSII toward the lumen of the thylakoid which are involved in water oxidation (oxygen-evolving complex, OEC). Various prosthetic groups which are bound with proteins in PSII participate in electron transport. Wavelengths of light absorbed by LHCII are slightly shorter (650–670 nm). These are funneled to the Chl a (P_{680}) at the reaction center by fluorescence. The absorbed photons raise the molecule, i.e., P_{680}, from the ground state to an excited state. The excited P_{680}^{*} molecule becomes an excellent reducing agent which is now capable of transferring an electron quickly to the primary electron acceptor, pheophytin "a" (Pheo), which carries lower redox potential than excited P_{680}^{*}. Pheophytin is identical to chlorophylls except that two protons substitute for the centrally bound magnesium ion. The electron is then transferred quickly to plastoquinone. There are quinones which are bound to the two binding sites: Q_A is bound tightly to D2, while Q_B is loosely bound to D1. $Pheo^{-}$ immediately transfers the electron to Q_A located in D2 to form semiquinone (PQ^{-}), which transfers electron to Q_B which is located in D1. Q_A^{-} is returned to Q_A form and is ready to receive another electron from $Pheo^{-}$. Transferring of electron to $Q_{A,}$ which is located in D2 at a site different from Pheo, must be to stop short circuiting since Q_B is located at a different site, i.e., in D1. Unlike Q_A, Q_B requires transfer of two electrons from Q_A which occurs in two steps and is fully reduced to PQ^{2-} on receiving two electrons. This transfer of electron from Q_A to Q_B is believed to involve non-heme iron and HCO_3^{-}. After receiving two electrons at Q_B site, PQ^{2-} picks up two protons from stroma and is converted to plastoquinol (PQH_2), which is lipid soluble in the lipid bilayer of the thylakoid membrane**.** After losing electrons P_{680} becomes a strong oxidant P_{680}^{+} (redox potential $E'^0 = -1.0$ V). It gains electron from the closely placed tyrosine residue (Tyr161) located on the luminal side of D1. On becoming deficient, tyrosine residue collects electron from water. However, first of all electrons are removed from oxygen-evolving complex (OEC), a protein, which is present toward the luminal side of thylakoid. OEC contains a cluster of four oxygen-bridged manganese atoms. Electrons are removed from OEC one at a time. As a result, there is accumulation of positive charge. OEC is always positive (+) in dark and will require three photochemical events for accumulation of four positive charges. This results into the strongest biological oxidant having redox potential +1.2 eV. It was first observed by P. Joliot and coworkers in 1969 and was interpreted by B. Kok and coworkers in 1970 as the S-cycle. The metal cluster in OEC can exist in a series of oxidation states (S_0–S_4) depending upon number of electrons removed. On removal of four electrons, four positive charges are accumulated on OEC (S_4) (Fig. 5.12). It is now capable of oxidizing water by removing electrons, resulting in photolysis of water with simultaneous release of O_2 and $4H^{+}$ in the thylakoid lumen. Electrons are removed one by one, resulting in accumulation of charges on OEC. If electrons are removed directly from water one by one, this would result in formation of free radicals. OEC consists of three extrinsic proteins bound to D1 and D2 proteins of PSII projecting toward the luminal side of thylakoid. Core of OEC consists of four manganese atoms, a calcium atom, and a chlorine atom. It is the manganese atoms of OEC which provide electrons to Tyr_z, while calcium and

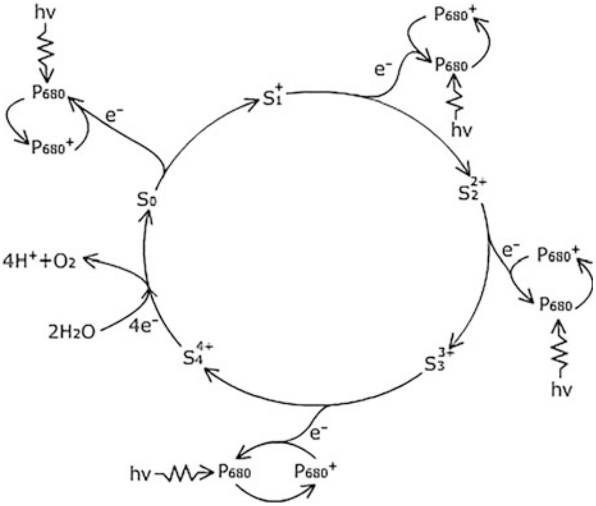


Fig. 5.12 The S-state cycle of oxygen-evolving complex (OEC). P_{680} is oxidized by a photon of 680 nm wavelength of light. It derives the electron from OEC, which accumulates positive charge and becomes an oxidant. After four electrons have been removed, OEC becomes a strong oxidant because of the accumulation of four positive charges and is able to remove electrons from water

chlorine are thought to facilitate combining of OEC with oxygen atoms of water molecule. Released oxygen diffuses out of the chloroplasts. The four protons produced from photolysis of $2H_2O$ add to pH gradient between the lumen and stroma of the chloroplast. Photolysis of water by thylakoids is summarized as:

$$2H_2O \rightarrow 4H^+ + 4e^- + O_2$$

5.3.8 Q-Cycle Results in Pumping of Protons

Plastoquinol (PQH_2) is lipophilic and is released in the membrane lipid bilayer from PQ_B which is present toward stroma on D1 of PSII. It interacts with thylakoid-bound cytochrome b_6f complex. The major components of this complex are two cytochromes (b_6 and f) and an iron-sulfur protein (ISF). Cytochrome b_6f complex is a dimer having two plastoquinone binding sites facing each other. The sites are called Q_P and Q_N sites toward lumen and stroma, respectively. These are called as Q_P and Q_N since the lumen side of thylakoids is positive because of accumulation of protons, while the side toward the stroma is negative because of proton movement from the stroma to lumen during light reaction (Fig. 5.13). Cytochrome b_6f complex carries out three functions; it regenerates PQ from PQH_2, catalyzes transfer of electron to PSI via plastocyanin, and transports protons from the stroma to lumen of the thylakoid. Energy for transport of two electrons received from PQH_2 is utilized for transport of 4 H^+, which occurs through Q-cycle, thus contributing to proton gradient. Q-cycle occurs in two steps. In the first step, one of the electrons

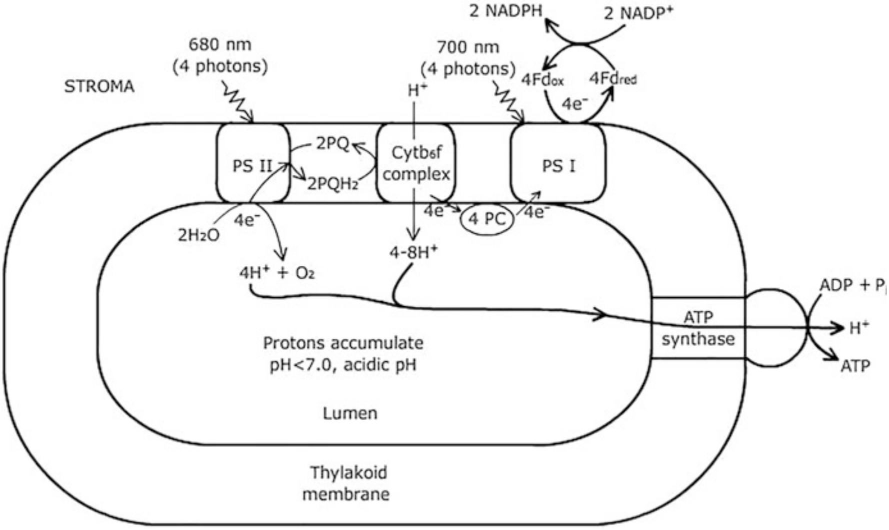


Fig. 5.13 Figure showing positioning of the photosynthetic electron transport complexes in thylakoids and proton accumulation in lumen

received from PQH_2 is transferred via Fe-S protein and Cyt f to a small copper-containing protein, plastocyanin (PC), which is present toward the luminal side. Cu (II) of plastocyanin is reduced to Cu (I) which further is oxidized by PSI. When PQH_2 is oxidized, protons ($2H^+$) are released in the lumen of thylakoid. Complex b_6f serves two purposes. First, one of the two electrons of PQH_2 is transferred to PS I through plastocyanin. At the same time, it pumps two protons from stroma into thylakoid lumen. The order of transfer of one of the electron is mentioned below.

$$\text{Plastoquinol} \rightarrow \text{ISF} \rightarrow \text{Cytochrome f} \rightarrow \text{Plastocyanin}$$

The other electron is cycled back via cyt b_6 to plastoquinone bound to Q_N site of the complex. This transfer of electron is facilitated by two hemes of cyto b_6 (b_l and b_h), which refers to low-potential and high-potential hemes, respectively. PQ, on receiving the electron at Q_N site of the complex, is reduced to semiquinone (PQ^-), which, on receiving another electron in second cycle, is further reduced to PQ^{2-}. PQ^{2-} picks up two protons from stroma and is reduced to PQH_2 followed by similar cycle (Fig. 5.14). Net result is translocation of $4H^+$ across thylakoid coupled with transport of two electrons to plastocyanin. The cytochrome b_6f complex plays a role analogous to that of the cytochrome oxidoreductase ($cytbc_1$) complex in mitochondria. In this process, copper in plastocyanin is first reduced to Cu(I) on receiving electron and then reoxidized to Cu (II) when electron is given to PSI. Reactions of the cyt b_6f are rate limiting in the electron transport since quinol oxidation requires 10–20 ms, while PQH_2 is formed in 100 μs.

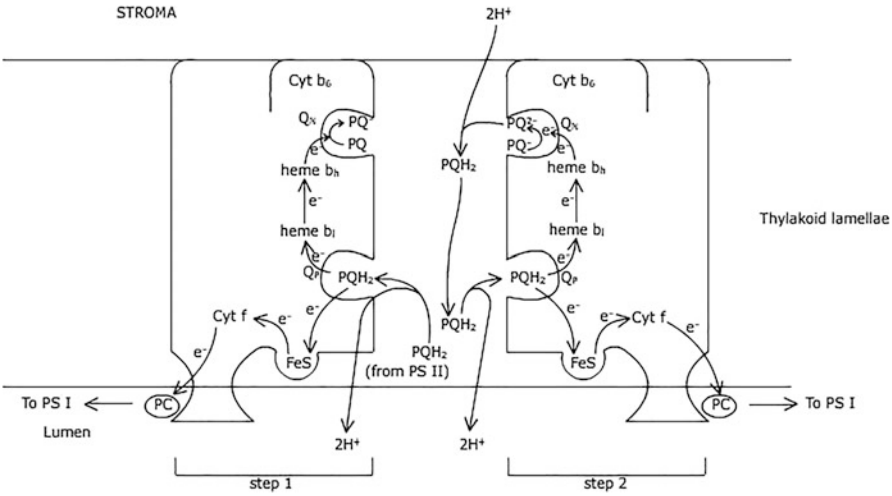


Fig. 5.14 Q-cycle. Cyt b_6f dimer in thylakoid having quinone-binding sites Q_P and Q_N, which face each other. PQ^- is a semiquinone, PQ^{2-} is a fully reduced plastoquinone, PQH^{2-} is a plastquinol, heme b_l and b_h are cytochrome b with heme moiety having low and high midpoint potential, respectively

5.3.9 Photosystem I (Production of NADPH)

Through X-ray diffraction techniques, the structure of photosystem I complex in plants has been shown to be a supercomplex with monomeric reaction center unlike in cyanobacterium where PSI is trimer of reaction centers. PS I is a multiprotein complex containing at least 11 polypeptide chains. It contains many antenna chlorophylls (LHCI) and reaction center chlorophyll P_{700} which can absorb light of 700 nm. The number of Chl a molecules is double of Chl b. In plants central heterodimer of the reaction center is bound with major electron carriers, P_{700} and accessory pigments, chlorophyll A_0 acceptor molecule, phylloquinone (A_1), and the bound Fe-S centers F_A and F_B. Excitation by a photon, absorbed and transferred by antenna chlorophylls, raises electrons in P_{700} from ground state to an excited state (−1.5 eV). Electrons are released from the excited reaction center (P_{700}). As a result, P_{700} is oxidized to P_{700}^+ (redox potential +0.5 eV). Excited electron then passes through an electron transport chain. It is first accepted by Chl a-like molecule A_0, which is reduced to A_0^- on receiving the electron. Electron is transferred from A_0^- to phylloquinone and then is passed through a series of proteins (F_x, F_B, and F_A) which contain iron-sulfur clusters. Finally, the electron is accepted by 2Fe-2S soluble protein ferredoxin (Fd), which is present in the stroma of chloroplast. Electron from ferredoxin is not directly transferred to $NADP^+$. The transfer occurs through the activity of an intermediate enzyme ferredoxin-NADP reductase (FNR). There is complex electrostatic interaction between ferredoxin and FNR. FNR is an FAD-containing enzyme, which is reduced by two single-electron steps. The enzyme FNR catalyzes transfer of electrons to $NADP^+$ reducing it to NADPH after ferredoxin has been reduced by photosystem I. Reduction of $NADP^+$ to NADPH requires transfer of two electrons. However, only one proton is covalently bound, while the other H^+ remains free in the medium.

$$2Fd^- \rightarrow 2Fd^+ + 2e^-$$

$$NADP^+ + 2e^- + 2H^+ \rightarrow NADPH + H^+$$

It is ferredoxin, rather than $NADP^+$, which can be considered as the ultimate recipient of electrons during light reaction. Much of the reduced ferredoxin is used to reduce $NADP^+$, but some of it is also used for other reductive reactions. Reduced ferredoxin is a source of low-potential electrons for many reductive processes such as NO_2^- assimilation, sulfur assimilation, and lipid biosynthesis. Daniel Arnon had observed in 1951 for the first time that isolated chloroplasts could reduce $NADP^+$ to NADPH and found out in 1954 that it is coupled with ATP synthesis. In 1962, Arnon discovered that $NADP^+$ reduction requires two electrons and a proton. The NADPH produced by ferredoxin oxidation is released into the stroma where it is utilized in CO_2 reduction.

5.3.10 Non-cyclic and Cyclic Electron Transport

When both the photosystems PSII and PSI are working, electrons for $NADP^+$ reduction are ultimately replenished from H_2O. This movement of electrons, which requires participation of both photosystems, occurs through open chain and is known as non-cyclic electron transport, since it is a one-way transport of electrons. There are inhibitors which interfere electron transport on binding with specific sites of the complexes in the electron transport chain. This includes atrazine and DCMU (3–3-4-dichlorophenyl)-1,1-dimethyl urea) which bind with Q_B site of PSII, blocking electron flow from Q_A to $Q_{B.}$ As a result, non-cyclic electron transport is blocked, and there are no photolysis of water and no $NADP^+$ reduction to NADPH. DCMU is commercially known as diuron. DBMIB (dibromothymoquinone) blocks electron flow to cyt b_6f at the Q_P site of the complex. The herbicide, methyl viologen (commercially known as paraquat), accepts electrons instead of $NADP^+$ resulting in generation of oxygen radicals which is responsible for destroying the photosynthetic apparatus. In the absence of CO_2 reduction or if it is slow, NADPH will not be oxidized. In the absence of availability of oxidized $NADP^+$, electrons are transferred to PQ bound to Q_N, resulting in cycling back of the electrons through cyt b_6f, PC, and PSI. This closed cycle of electron transport is known as cyclic electron transport. Cyclic electron transport is operational when only long wavelength of light (700 nm) is available. There is no photolysis of water, resulting in zero quantum yield in the absence of functional PSII. As a result, red drop phenomenon is observed (Fig. 5.7).

5.3.11 ATP Generation During Electron Transport in Light Reaction

ATP synthesis during light reaction of photosynthesis is known as **photophosphorylation** (see Chap. 8). Daniel Arnon observed in 1954 that along with reduction of $NADP^+$ to NADPH, ATP is produced by isolated chloroplasts in light. When exposed to shorter wavelengths, P_{680} is oxidized to $P_{680}{}^+$ making it a very strong oxidant with redox potential of +1.2 eV, and a weak relatively stable reductant $Q_A{}^-$, plastosemiquinone, is generated. $Q_A{}^-$ donates electrons to PSI via Q_B, cyt b_6f, and PC. When excited by the long wavelengths (700 nm), a strong reductant (reduced Fe-S center) having redox potential of −0.73 eV and a weak oxidant (i.e., $P_{700}{}^+$) with the redox potential of +0.49 eV are produced. Energy released during the flow of electrons downhill from the weak reductant ($Q_A{}^-$) to the weak oxidant ($P_{700}{}^+$) is conserved as a gradient of protons across thylakoid membranes with H^+ getting accumulated in the lumen. Protons flow back in response to the gradient through ATP synthase resulting in ATP synthesis (Fig. 5.13). Proton gradient between the lumen of thylakoid and stroma is the result of (1) photolysis of water in the lumen, (2) transport of H^+ from stroma to the lumen during Q-cycle, (3) and utilization of H^+ for reduction of $NADP^+$ to NADPH at the stroma side.

Six protons accumulate in the lumen coupled with transport of two electrons through non-cyclic electron transport. $2H^+$ are released during photolysis of a water molecule and $4H^+$ are transported through Q-cycle. Release of an O_2 molecule requires non-cyclic electron transport of four electrons. Accumulation of protons makes the lumen more acidic than the stroma. This light-triggered proton gradient results into pH difference of 3–4 units. Unlike oxidative phosphorylation in mitochondria, a pH gradient is the main contributing factor for generation of proton motive force (PMF) in chloroplasts. In chloroplasts, H^+ accumulates in the lumen of thylakoid, which is an isolated compartment. In lumen lowering of pH to as low as 4 will not affect any enzyme activity as most of them are present in stroma. On the contrary, in case of mitochondria PMF is mainly contributed by the membrane potential (ΔE). H^+ accumulation in the intermembrane space will adversely affect cytosolic enzymes by lowering the pH of the cytosol. In chloroplasts ATP synthase complexes are called $\mathbf{CF_0\text{-}CF_1}$ which are similar to $\mathbf{F_0\text{-}F_1}$ complexes of mitochondria. In chloroplasts most of the evidence indicates the stoichiometry of number of protons translocated for synthesis of one ATP to be 4.67 ($H^+/ATP = 4.67$). The pH gradients across the membrane correspond to a ΔG of about 20 $kJ.mol^{-1}$ for the passage of one proton. Photosystems I and II, the cytochrome b_6f complex, and ATP synthase (CF_0–CF_1) are all separate entities embedded in the thylakoid membrane but are not contiguous. The mobile components that link the photosystems and the b_6f complex are plastoquinone in the lipid phase of the membrane and plastocyanin in the thylakoid lumen. Therefore, it seems that electrons can be transported over long distances in this system. Such a range of transport is necessary because two photosystems are separated from each other. The entire process of electrons being displaced from PSI due to excitation by light, getting replaced from photosystem II, and PSII obtaining the electrons then from water is called **non-cyclic electron flow**. ATP production during this process is called **non-cyclic photophosphorylation.**

Cyt b_6f complex and $NADP^+$ are the competitors for the electrons from reduced ferredoxin. In the limited availability of $NADP^+$, electrons pass from reduced ferredoxin to the cytochrome b_6f complex via PQ instead of $NADP^+$ and are cycled back to P_{700} via plastocyanin. This results in pumping of protons across thylakoid membrane, creating H^+ gradient which leads to ATP generation. This process is called **cyclic photophosphorylation**. No O_2 is released since there is no photolysis of water and no $NADP^+$ is reduced. Cyclic electron flow generally serves to generate ATP when availability of oxidized $NADP^+$ is limiting or if plants are irradiated only with long wavelengths (700 nm). Requirement for ATP in the CO_2 reduction is large, and only non-cyclic electron flow may not be able to generate sufficient ATP to meet the requirement. Since assimilation of each CO_2 molecule requires 2NADPH and 3ATP molecules, requirement of ATP cannot be met only through non-cyclic photophosphorylation. Cyclic electron transport produces ATP and no NADPH, which helps to maintain the necessary balance between ATP and NADPH production**.** In bundle sheath chloroplasts of C4 plants cyclic electron transport meets ATP requirement for CO_2 assimilation.

5.3.12 Balancing Distribution of the Light Energy in Between the Two Photosystems

Ideally there should be even distribution of energy between the two photosystems for maximum utilization of light energy for photosynthetic process. However, it does not happen since photons are preferentially transferred to pigments requiring the least energy for excitation. Since PSI requires lesser energy, there is a chance of excitation of PSI more in comparison with PSII. One of the ways by which this problem is handled by plants is by spatial separation of the two photosystems. PSI and PSII are not distributed randomly throughout the thylakoid membrane. PSI, along with ATP synthase, is primarily present in the unstacked stroma membranes, whereas PSII is mostly present in the stacked grana membranes. PSI and ATP synthase are present in the outer membrane of the stacked lamellae also and are thus exposed to stroma. This phenomenon is called **lateral heterogeneity**, indicating that the photosystems which participate in transferring electrons from H_2O to $NADP^+$ in the photosynthetic electron transport chain are spatially separated from each other. One to one stoichiometry of the two photosystems is also not required. Most commonly the ratio of PSII to PSI is 1.5:1 which can change depending upon the light conditions in which plants are grown. ATP synthase, which is involved in ATP synthesis during photophosphorylation, is located almost entirely in the exposed membranes in stroma (Fig. 5.15). Not all membrane-binding proteins, which participate in photosynthetic electron transport, are distributed unequally in the thylakoid. Cytochrome b_6f complex, which facilitates electron transfer between the two photosystems, is distributed evenly in both types of lamellae. Excitation of PSII

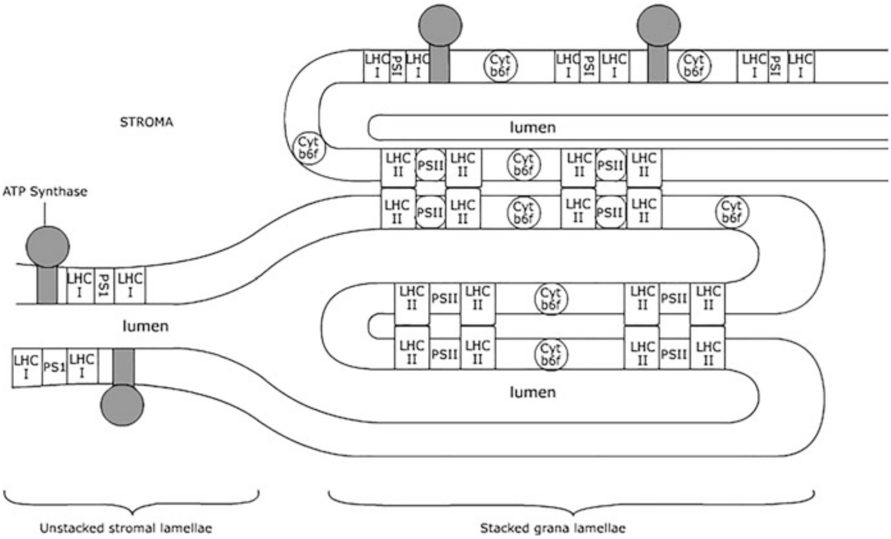


Fig. 5.15 Lateral heterogeneity. LHCII has hydrophobic region protruding out from thylakoid, which is responsible for stacking of thylakoids resulting in grana lamellae. In case PQ is present as PQH_2, a protein kinase phosphorylates a specific threonine of LHCII, resulting in its detachment from PSII, and it gets associated with PSI. As a result, there is a balance in distribution of energy in between the two photosystems

reduces the common intermediate pool of lipid soluble mobile carrier, plastoquinone, which is oxidized by the intermediates of electron transport. Oxidized PSI (P_{700}^{+}) obtains electrons from the common pool of reduced intermediates. Absence of direct energy transfer from PSII to PSI prevents over-excitation of PSI. Thus, spatial separation of photosystems prevents light energy from being preferably transferred to PSI, and PSII also functions effectively. Another mechanism involved in the regulation of energy distribution between the two photosystems is the participation of LHCII. Less functional PSI in comparison of PSII will lead to accumulation of PQH_2 because of which a protein kinase is activated which phosphorylates hydroxyl residue of threonine residue of peripheral LHCII. As a result, LHCII get dissociated from PSII and bind with PSI because of the changed conformation, resulting in PSI getting more light energy. Reverse happens when PQ is oxidized (PSI being more active than PSII) due to activation of a protein phosphatase. Thus accumulation of reduced PQ regulates energy distribution in between the two photosystems so that a balance is maintained.

5.3.13 Elimination of Excess Light Energy as Heat

At times NADPH and ATP accumulate because of much faster photochemical reaction that can be consumed by CO_2 assimilation, especially when high light intensity is coupled with low temperature, which lowers the metabolic rate of CO_2 assimilation. This results in ATP accumulation and shortage of oxidized $NADP^+$, which causes reduction in the availability of oxidized pheophytin (Pheo). In the absence of oxidized Pheo, there will be no acceptor of excited electron of P_{680}* at the reaction center of PSII. As a result excited chlorophyll molecules close to P_{680}* are not able to lose the energy. This results in triplet state of the chlorophyll molecules which has much longer life (from microseconds to milliseconds). Triplet state of chlorophyll molecules generates **singlet O_2 molecule** which is highly reactive and causes damage of the adjacent photosynthetic machinery, especially to the D1 protein of PSII. Following this, DI is targeted for degradation by proteolysis since it becomes vulnerable to breakdown by the action of proteases due to alteration in its conformation by the singlet O_2 or by the superoxide anion radicals. It is replaced by new D1 which is synthesized on the chloroplast ribosomes and is inserted in the thylakoid membrane followed by the reassembly of PSII. D1 has a high turnover. Excess rate of D1 damage than its synthesis reduces the rate of photosynthesis. This is called **photoinhibition**. Plants have devised mechanism to control the energy loss of singlet O_2 by a process known as **non-photochemical quenching (NPQ)**, which refers to molecular mechanisms for removing this trapped energy (quenching the excited state). Carotenoids present adjacent to P_{680} in the core of the reaction center of PSII play an important role in protecting PSII apparatus. Because of movement of H^+ from the stroma to lumen of thylakoids in high light intensity conditions, there is a decrease in pH in lumen which increases the quenching process. Decrease in pH results in the activation of violaxanthin de-epoxidase which converts violaxanthin to antheraxanthin and then to zeaxanthin. Zeaxanthin accepts energy from excited chlorophyll molecules and, on returning to ground state, loses energy as heat. Antiquenching becomes necessary at low light levels or in dark. It occurs due to activation of the enzyme epoxidase with rise in luminal pH under low light conditions. The process is reversed and zeaxanthin is converted back to antheraxanthin and violaxanthin. There is a possible involvement of a very hydrophobic transmembrane protein which undergoes protonation in low pH of lumen and facilitates quenching of excited PSII by zeaxanthin (Fig. 5.4).

5.4 Photosynthetic Carbon Dioxide Assimilation

Photosynthesis consists of reactions involving two redox phases. The first phase includes generation of NADPH and ATP in thylakoids during light reaction, while the second set of redox reactions occurs in the stroma. The second set of redox phase includes CO_2 assimilation which uses chemical energy, NADPH and ATP generated during the first phase. CO_2 reduction was earlier known as dark reactions. However, it is a misnomer since activity of many enzymes of CO_2 assimilation is light-regulated. Instead, CO_2 assimilation or photosynthetic carbon reduction are the terms which are now used. It is because of these reactions that carbon is incorporated into living beings. In green plants, chloroplasts contain unique enzymatic machinery which catalyzes CO_2 assimilation. Plants convert simple products of photosynthesis into more complex biomolecules, including various sugars, polysaccharides, and other metabolites, which are derived from them through various associated metabolic pathways.

5.4.1 Calvin-Benson Cycle

CO_2 assimilation makes up a cyclic pathway in which key intermediates are constantly regenerated. This pathway was elucidated by Melvin Calvin, James Bassham, and Andrew Benson (1946–1953) for which Melvin Calvin was awarded the Nobel Prize for chemistry in 1961. The cycle is often called the *Calvin-Benson cycle* **or** *reductive pentose phosphate pathway*. In 1940, Samuel Ruben and Martin Kamen discovered a new radioisotope of carbon, ^{14}C, which has a very long half-life (>5000 years) in comparison with ^{11}C (half-life of 20 min). They conducted a series of experiments to trace the metabolic fate of the labeled $^{14}CO_2$ during CO_2 fixation**.** Liquid cultures of *Chlorella* were exposed to $^{14}CO_2$ for varying time periods, and plants were exposed to different illumination conditions. Cells were then dropped into the boiling alcohol so as to stop the metabolic pathway by killing them while preserving the labeling pattern. The radioactive products were subsequently identified using two-dimensional paper chromatography coupled with autoradiography. They observed that a three-carbon compound, 3-phosphoglycerate, was first to get the labeled carbon, followed by a series of other compounds. By following the pattern of getting labeled compounds and relating it to the time of exposure of plants to $^{14}CO_2$ as well as by studying the distribution pattern of radioactivity within the compound, path of $^{14}CO_2$ assimilation was traced. Interconversion of various intermediates during assimilation of $^{14}CO_2$ could also be understood. Intramolecular labeling studies were carried out chiefly by Andrew Benson. Since the first compound identified was a three-carbon compound, it was envisaged that the primary acceptor for $^{14}CO_2$ could be either a two-carbon compound or a five-carbon compound. A five-carbon compound, ribulose 1,5-bisphosphate, was identified as the acceptor molecule. Convincing evidence for RuBP as the acceptor molecule for ^{14} CO_2 came from studies involving transferring algae from light to dark, which were conducted by James Bassham when increase in PGA pool was coupled with decrease in RuBP (Fig. 5.16). Since RuBP also gets the labeling, CO_2 assimilation was considered as a cyclic process, and it was called as Calvin-Benson cycle.

Calvin-Benson cycle can be studied in three stages—the first stage involves condensation of CO_2 with ribulose 1,5-bisphosphate to generate 3-phosphoglycerate. In the second stage, 3-phosphoglycerate is reduced to yield glyceraldehyde 3-phosphate (a triose phosphate) at the expense of ATP and NADPH produced during light reaction. In the third stage, known as the regeneration phase, 5/6 of glyceraldehyde 3-phosphate is used for regeneration of ribulose 1,5-bisphosphate (Fig. 5.17), while 1/6 of glyceraldehyde 3-phosphate is either transported out of the chloroplasts through the transporters located in the inner envelope of chloroplasts or is stored as transient starch during the daytime. The transported glyceraldehyde 3-phosphate is used for sucrose synthesis in cytosol

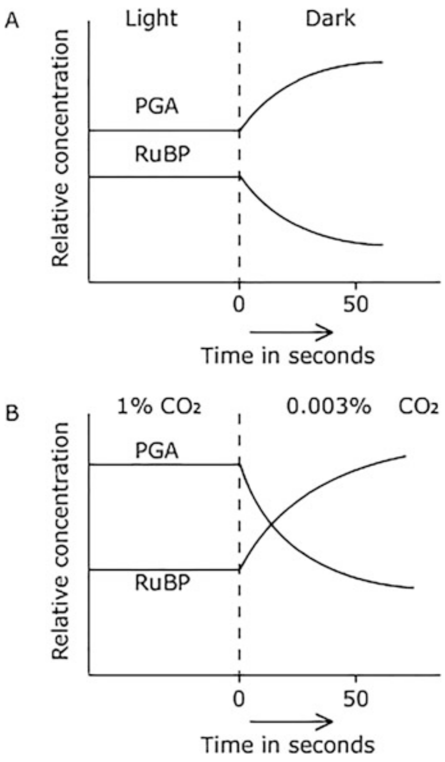


Fig. 5.16 Experimental results demonstrating that RuBP is the acceptor of CO_2. Concentrations of RuBP and PGA change in reverse order when plants growing (**a**) in light are transferred to dark. (**b**) Plants transferred from 1% CO_2 to 0.03% CO_2

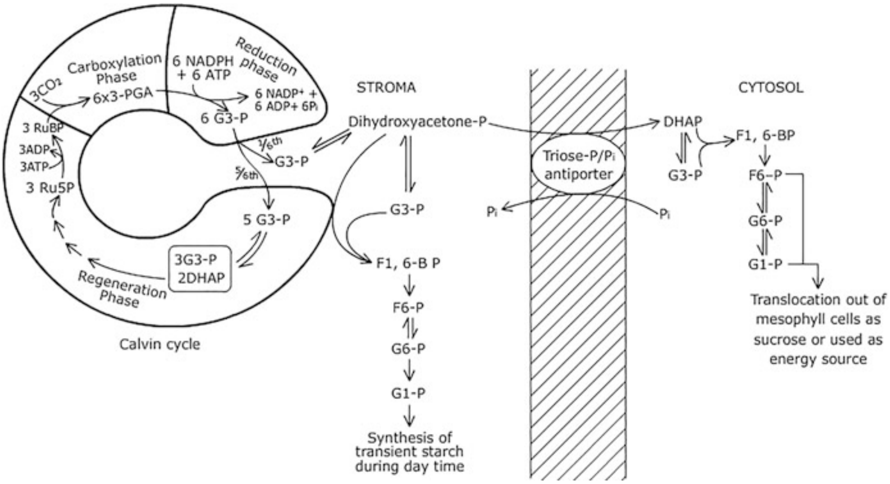


Fig. 5.17 Triose P/P_i antiporter localized on the inner membrane of plastid facilitates the exchange of triose-P between stroma of the plastid and cytosol

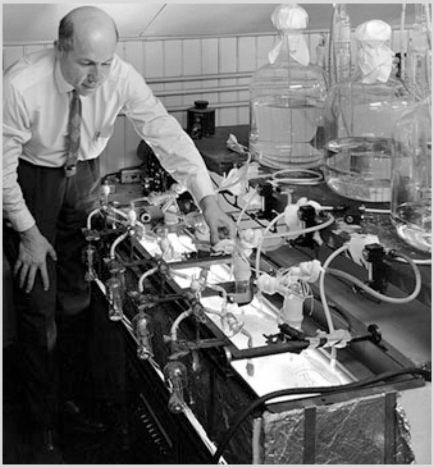
which is then transported out to other parts of the plant. Thus, the cyclic process allows continuous conversion of CO_2 into triose phosphates, hexose phosphates, and other intermediates besides generating acceptor molecules of CO_2. Calvin-Benson cycle involves 13 enzyme-catalyzed reactions which occur in stroma of chloroplasts.

5.4.2 Carboxylation Phase

Melvin Calvin and his associates (Box 5.1) observed that in the algae when exposed to $^{14}CO_2$ for a few seconds only, first stable radioactively labeled compound was

Box 5.1: Melvin Ellis Calvin (1911–1997)

M.E. Calvin was born to Jewish immigrant parents in 1911. His parents owned a grocery store in Detroit where Calvin had his school education. He did his graduation in chemistry major and obtained his Ph.D. with George C. Glockler in 1935 from University of Minnesota. He joined the chemistry faculty at the University of California in 1937 as an instructor where he remained for the rest of his life. Calvin in 1947 begun his Nobel Prize winning work on photosynthesis using alga *Chlorella pyrenoidosa.* After illuminated algal cells were exposed to $^{14}CO_2$, its growth was stopped at different stages starting from 5 s. He used paper chromatography to isolate and identify the minute quantities of radioactive intermediates. Calvin supported interdisciplinary research. He has written 7 books and almost 600 research articles. He was the sole recipient of Noble Prize in Chemistry in 1961 for his work on Calvin cycle, which is also known as Calvin-Benson cycle. In 1963 he was given an additional title of Professor in Molecular Biology. The search of NASA for extraterrestrial life was greatly influenced by Professor Calvin. Later on his research area of interest included artificial photosynthesis and plants, producing hydrocarbons as substitute for fuels.



identified to be 3-phosphoglycerate (3-PGA). Initially 3-PGA was labeled on its carboxyl (-COOH) group. This suggested 3-PGA to be an early intermediate generated during CO_2 fixation. Experiments were conducted in plants which were either shifted from light to dark or from high to low CO_2 concentration. Increase or decrease in 3-PGA was found to be coupled with decrease or increase in five-carbon compound, ribulose-I,5-biphosphate (RuBP) (Fig. 5.16). This indicated formation of 3-PGA occurred at the expanse of RuBP. Carboxylation of RuBP is catalyzed by the enzyme *ribulose-1,5-bisphosphate carboxylase/oxygenase*, for which the acronym Rubisco was proposed by David Eisenberg in 1979. Carboxylation of RuBP is followed by cleavage of the unstable six-carbon intermediate leading to formation of two molecules of 3-PG, one of which bears the new carbon introduced as CO_2 in its carboxyl group. Driving force for the highly exergonic reaction ($\Delta G^{\circ\prime} = -35.1$ kJ.mol^{-1}) is provided by the cleavage of the β-keto acid intermediate to yield an additional resonance-stabilized carboxylate group. Rubisco is located in the chloroplast stroma. It is a very slow enzyme catalyzing the fixation of 1–12 CO_2 molecules on each active site per second, unlike many other enzymes which catalyze at the reaction rate to the order of 10^3 to 10^5 s^{-1}. Slow activity of Rubisco is compensated by the large amount of the enzyme being present, so much so that it can constitute up to 50% of the leaf protein. It is the most abundant protein in the biosphere. Enormous amount of nitrogen is invested for synthesis of Rubisco. Another shortcoming of the enzyme is failing to discriminate between CO_2 and O_2. It wastes one carbon every time enzyme reacts with O_2. During oxygenation reaction, a molecule of 3-PGA and a two-carbon compound 2-phosphoglycolate are produced (Fig. 5.22).

Besides being the slowest, Rubisco is also one of the largest enzymes. Four distinct forms of Rubisco have been identified in the organisms which fix CO_2. In all the eukaryotic photoautotrophs, form I Rubisco is present which consists of 16 subunits of two types, i.e., L and S (a hexadecamer, L_8S_8, molecular mass 560 kDa). Each larger subunit (L) has a molecular weight of 55 kDa, while molecular weight of each small subunit (S) is 15 kDa. Large subunits are present as four dimers $(L_2)_4$. There are two of tetrameric small subunits which are present on top and bottom of aggregate of large subunits. Rubisco holoenzyme is expressed as $(L_2)_4(S_4)_2$. Larger subunit has got the catalytic side and is encoded in plastid genome (rbcL), whereas a family of nuclear rbcS genes encodes nearly identical smaller subunits in all photoautotrophic land plants and green algae, which are synthesized by cytosolic ribosomes and are transported into the plastids. Following post-translational processing, molecular chaperones help in the assembly of subunits in plastids. Form I Rubisco is the only type of Rubisco which consists of small subunits also along with the large subunits. Homodimers of catalytic large subunits are common in all forms. Form II consists of dimer of only large subunits and is present in dinoflagellates which is encoded in nuclear genome (unlike in algae and land plants). In archaebacterial, Rubisco is neither form I nor II but a decamer of five large subunits dimers.

5.4.3 Reduction Phase

Reduction of 3-PGA to glyceraldehyde 3-phosphate (GAP) occurs in two steps. In the first step transfer of phosphate from ATP to 3-PGA occurs, yielding 1,3-bisphosphoglycerate (BPG). Reaction is catalyzed by phosphoglycerate kinase. In the second step, NADPH-specific glyceraldehyde 3-phosphate dehydrogenase catalyzes the reduction of BPG to GAP. Reduction of BPG is the major energy-requiring step of the cycle. Triose phosphate isomerase catalyzes the conversion of GAP to dihydroxyacetone phosphate (DHAP). Equilibrium of this reversible reaction favors synthesis of ketone. Both GAP and DHAP are together called triose phosphates. In addition to being used for RuBP generation, GAP has several other fates in the metabolism. It may be metabolized via glycolysis in the plastid itself or is used for starch biosynthesis (in chloroplasts) or is transported out of chloroplast to cytosol for biosynthesis of sucrose. Export of triose phosphates occurs in exchange of cytosolic P_i. This is facilitated by the antiporters located in the inner membrane of plastid (Fig. 5.17).

5.4.4 RuBP Regeneration Phase

As RuBP is consumed during initial carboxylation phase, it must be constantly regenerated. This phase is significant for continuation of CO_2 assimilation. Out of every six molecules of triose phosphates, three molecules of RuBP (15 C-atoms) are regenerated by reshuffling of carbon atoms. Reactions involved are the following:

Aldol Condensation Reaction Condensation of two triose phosphates, i.e., dihydroxyacetone-3-phosphate (DHAP) with GAP, is catalyzed by aldolase yielding fructose-1,6-bisphosphate (FBP).

Dephosphorylation Reaction Fructose 1,6-bisphosphatase catalyzes the irreversible dephosphorylation of FBP to yield fructose 6-phosphate and inorganic phosphate. This is followed by transfer of two-carbon unit (C-1 and C-2) of fructose 6-phosphate to the third molecule of GAP to form xylulose 5-phosphate**.** The reaction is catalyzed by **transketolase**. The remaining carbons (C-3 to C-6) of fructose-6-phosphate form erythrose 4-phosphate.

Second Aldol Condensation Reaction Another condensation reaction, catalyzed by aldolase, involves erythrose 4-phosphate and DHAP (fourth molecule of triose phosphate), which yields sedoheptulose 1,7-bisphosphate (seven-carbon sugar). This is followed by the **dephosphorylation** of sedoheptulose 1,7-bisphosphate to yield sedoheptulose 7-phosphate. This is another irreversible reaction of the pathway which is catalyzed by a specific phosphatase. Further reaction involves transfer of two-carbon unit (C-1 and C-2) of sedoheptulose 7-phosphate to a fifth molecule of GAP to form xylulose 5-phosphate, while remaining carbon (C-3 to C-7) of sedoheptulose 7-phosphate generate ribose 5-phosphate. Reaction is catalyzed by

transketolase. Phosphopentose epimerase catalyzes **epimerization** of the two molecules of xylulose 5-phosphate to produce two molecules of ribulose 5-phosphate, while ribose 5-phosphate isomerase catalyze isomerization of ribose 5-phosphate into ribulose 5-phosphate. The last step in the Calvin cycle involves phosphorylation of three molecules of ribulose 5-phosphate consuming three ATP molecules to generate ribulose 1,5-bisphosphate (RuBP). The reaction is catalyzed by ribulose 5-phosphate kinase also known as Phosphoribulo kinase (PRK). With this regeneration stage is completed (Fig. 5.18). In this way three RuBP molecules, consumed during carboxylation using 3CO_2, are regenerated. Reactions occurring during interconversion of various intermediates during regeneration phase of Calvin

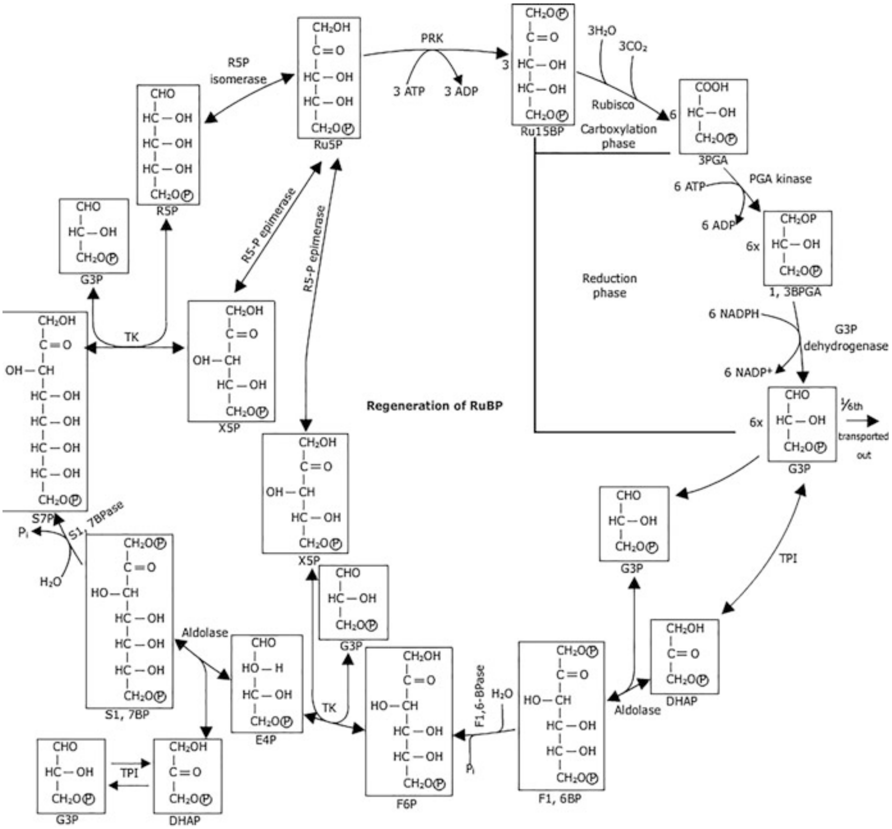


Fig. 5.18 Calvin cycle includes carboxylation phase, reduction phase, and phase involving regeneration of RuBP. 1/6 of the trioses produced are transported out of plastids, while 5/6 are utilized for regeneration of RuBP. Reversible and irreversible reactions have been demonstrated by using appropriate arrows. *PRK* phosphoribulokinase, *PGA* phosphoglyceric acid, *BPGA* biphosphoglyceric acid, *G3P* glyceraldehyde 3-Phosphate, *TPI* triose phosphate isomerase, *TK* transketolase, *E4P* erythrose 4-Phosphate, *S1,7BP* sedoheptulose 1,7-biphosphate, *DHAP* Dihydroxyacetone 3-phosphate, *S1,7BPase* sedoheptulose 1,7 biphosphatase, *F1,6-BPase* fructose 1,6 biphosphatase

cycle (also known as reductive pentose phosphate pathway) are quite similar to those which occur during oxidative pentose phosphate pathway. However, the two unique reactions in photosynthesis are dephosphorylation of sedoheptulose 1,7-biphosphate to sedoheptulose 7-phosphate and ribulose 5-phosphate to ribulose 1, 5-bisphosphate which are catalyzed by a phosphatase and phosphoribulose kinase, respectively. Reason for production of so many intermediates during the regeneration of RuBP is not obvious, but possibly besides being utilized for RuBP generation, these are to be used for other metabolic processes also. Erythrose 4-phosphate is utilized in shikimic pathway for generation of aromatic amino acids in plastids, while ribose 5-phosphates are used as precursor for nucleotide biosynthesis.

5.4.5 ATP and NADPH (Energy Sources in CO_2 Fixation)

The carboxylation reaction is exothermic ($\Delta G^{\circ\prime} = -35$ kJ.mol^{-1}) and is, thus, energetically favorable. But formation of GAP from 3-PGA and regeneration of RuBP require energy. This is provided by ATP and NADPH which are generated during light reaction. Three molecules of RuBP condense with three CO_2 molecules to form six molecules of 3-PGA. These are reduced to six molecules of GAP, using six ATP and six NADPH molecules. One out of every six molecules of GAP is either used for synthesis of temporary starch (transient starch) in plastid or is transported out of the plastids in exchange of P_i through antiporters located in the inner envelope of chloroplast which is utilized for synthesis of sucrose to be transported out of mesophyll cells. Carbon atoms of the remaining five GAP molecules ($(3\times5) = 15$ carbon atoms) are rearranged to regenerate 3 R5P molecules ($(5\times3) = 15$ carbon atoms) which are further required for CO_2 fixation and continuation of the cycle. Phosphorylation of 3 R5P molecules requires three ATPs to produce three molecules of RuBP. Thus, for every molecule of triose phosphate transported out, six NADPH and nine ATP are required.

$$\begin{gathered}3CO_2 + 3RuBP + 3H_2O + 6NADPH + 6H^+ + 6ATP \\ \rightarrow 6\text{Triose phosphate} + 6NADP^+ + 6ADP + 6P_i\end{gathered}$$

Out of six triose phosphates, five are used for regeneration of RuBP (Fig. 5.22).

$$5\text{Triose phosphate} + 3ATP + 2H_2O \rightarrow 3\ RuBP + 3ADP + 3P_i$$

Since it is only one triose phosphate out of six that is used for storage or further metabolism, net reaction can be written as:

$$3CO_2 + 5H_2O + 6NADPH + 9ATP \rightarrow GAP + 6NADP^+ + 9ADP + 9P_i$$

Efficiency for energy use in photosynthesis can be calculated easily. For each mole CO_2 reduction $\Delta G^{\circ\prime}$ is +478 kJ. Energy of light of 600 nm wavelength is

1593 kJ/mole. Energy of 680 nm and 700 nm wavelength will be lesser than this. Thus efficiency of photosynthesis is at least 30% (478/1593 × 100).

5.4.6 Autocatalytic Regulation of Regeneration of RuBP for Continuous CO_2 Assimilation

Autocatalytic regulation of Calvin cycle is responsible for maintaining balance in between RuBP regenerated and RuBP consumed for CO_2 fixation. If all ten molecules of triose phosphates, produced for every five molecules of CO_2 fixed in Calvin cycle, are utilized for regeneration of RuBP, there will be net gain of one RuBP, since six molecules of RuBP will be produced while five are consumed. Increase in RuBP will result in increased enzyme activity. In that case none of the triose phosphates will be diverted for further utilization. Shutting down the photosynthetic process during night results in reduction of intermediates of the Calvin cycle, because these are consumed in various metabolic reactions. Consequently, at the start of photosynthesis the following day, CO_2 fixation is severely affected because of availability of RuBP being low. As a result, all of ten triose phosphates generated during fixation of five CO_2 molecules are consumed for regeneration of RuBP, and none of the triose phosphates is transported out of chloroplast. This will result in net gain of one RuBP, resulting in lag phase for triose phosphate being transported out. This lag phase is known as **photosynthetic induction period**. Once photosynthesis reaches steady state of CO_2 assimilation, one-sixth of triose phosphates generated during Calvin cycle start getting diverted for carbohydrate biosynthesis maintain the balance between RuBP produced and RuBP being consumed. As a result, sucrose is synthesized in cytosol.

$$\begin{aligned}&6CO_2 + 12H_2O + 18ATP + 12NADPH + 12H^+\\&\quad \rightarrow C_6H_{12}O_6 + 18ADP + 18P_i + 12NADP^+ + 6O_2 + 6H_2O\end{aligned}$$

5.4.7 Regulation of Calvin-Benson Cycle

During day, green plants, being autotrophs, carry out photosynthesis to fulfill their energy requirements by using light as an energy source, whereas at night, like other heterotrophic organisms, they use their nutritional reserves to generate NADH and NADPH through glycolysis and oxidative pentose phosphate pathway, respectively. CO_2 assimilation occurs during Calvin cycle at the expense of NADPH and ATP. On the contrary, release of CO_2 occurs coupled with reduction of $NADP^+$ to NADPH during oxidative-reductive pentose phosphate (ORPP). ORPP also occurs in stroma of chloroplasts. If both cycles occur simultaneously, it will result in **futile cycle** since NADPH generated in ORPP will be consumed during Calvin cycle. This is checked by the regulation of enzymes of both pathways. Plants have a light-sensitive control mechanism to prevent Calvin cycle operating in the dark (Fig. 5.19). The activity of Rubisco is light-dependent. Rubisco has very low catalytic activity and must be

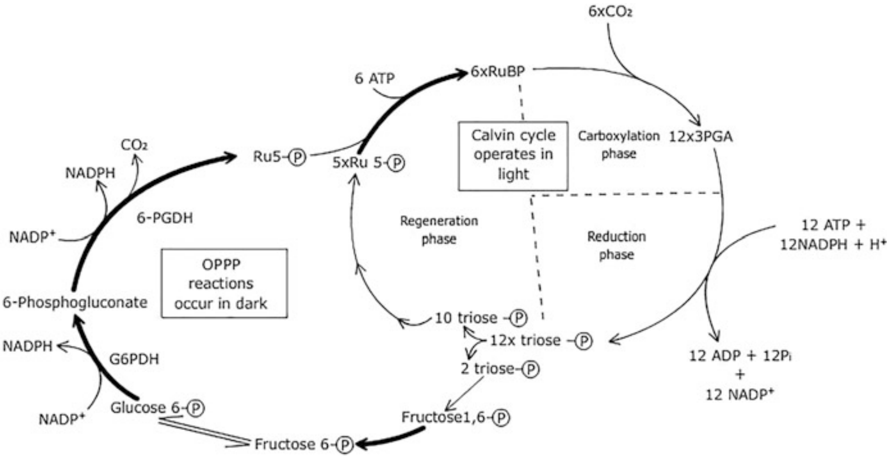


Fig. 5.19 Potential for futile cycle in chloroplast is avoided by difference in timings of the two cycles, i.e., OPPP (oxidative pentose phosphate pathway) and Calvin cycle. OPPP occurs during the night, while Calvin cycle (reductive pentose phosphate pathway) occurs during the day. Bold lines indicate irreversible regulatory steps of Calvin cycle and OPPP

activated before acting as a catalyst. Since Rubisco is the first enzyme in CO_2 fixation by Calvin cycle, it is the prime target for regulation. The enzyme is inactive until carbamylated, which occurs due to its complex interaction with CO_2, high pH of stroma, and Mg^{2+}. pH of stroma increases from 7 (in dark) to 8, since during daytime protons move into the thylakoid lumen in the light reaction. Consequently, Mg^{2+} moves out of the lumen to compensate for the proton flux in the opposite direction, increasing Mg^{2+} concentration from 3 to 6 mM in the stroma. The allosteric site is present in larger subunit (LSU) of the enzyme, which is separate and distinct from the substrate binding site of the enzyme. The model proposed for in vitro activation of Rubisco takes into account all three factors: CO_2, Mg^{2+}, and pH. According to the proposed model, the CO_2 molecules first binds with ε-amino group of lysine residue present in position 201 of the 470 amino acid long protein of large subunit (LSU) at the allosteric site to form carbamate. This is different from the one in which CO_2 is required as the substrate by the enzyme. Carbamate formation requires release of two protons from the ε-amino group of lysine residue, which is favored by increase in pH of stroma. Mg^{2+} which has moved out of lumen to stroma coordinates with the carbamate to form carbamate-Mg^{2+} complex, which is the active form of Rubisco (Fig. 5.20). However, binding of sugar phosphates to the enzyme, such as ribulose 1,5-bisphosphate, prevents carbamylation of Rubisco. In vitro experiments have indicated that the Mg^{2+}, CO_2, and pH differences alone are not sufficient to account for more than half of the expected activation level of Rubisco. An *Arabidopsis* mutant, *rca*, has been isolated, in which Rubisco fails to be activated in light, even though the enzyme which had been isolated from the mutant is apparently identical to the one isolated from the wild type. Electrophoretic analysis of chloroplast proteins has revealed that the mutant lacked a soluble chloroplast protein, and full activation of Rubisco could be

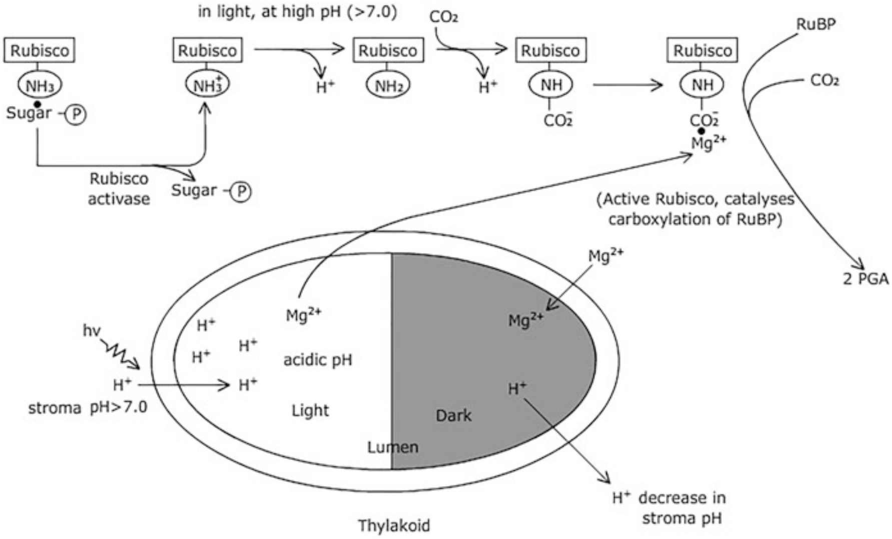


Fig. 5.20 In dark activity of Rubisco is inhibited because of binding of sugar phosphates such as RuBP or CA1P (2-carboxyarabinitol 1-phosphate) to the active site of the enzyme. CA1P and RuBP bind to the uncarbamylated and carbamylated Rubisco, respectively. Inhibition is relieved in light by removal of sugar phosphates in an ATP-dependent catalysis by Rubisco activase. Increase in stromal pH, because of protons being transferred from stroma to lumen in light, results in loss of H^+ from ε-amino group of lys^{210} of the enzyme protein followed by carbamate formation by CO_2. The carbamylated form of the enzyme forms a complex with Mg^{2+} resulting in the activation of enzyme

restored in vitro simply by adding that missing protein to a reaction mixture containing Rubisco, RuBP, and physiological levels of CO_2. This protein has been named **Rubisco activase**, which signifies its role in regulation of Rubisco activity. Rubisco activase brings about a conformational change in the Rubisco protein, causing release of bound sugar phosphates and allowing carbamylation to occur. This is an ATP-dependent process. In dark, a proton gradient created during light reaction is dissipated which causes Mg^{2+} to flow back to the lumen from the stroma. The decrease in Mg^{2+} concentration as well as decrease in pH in stroma results in the inactivation of Rubisco. 2-carboxyarabinitol-1-phosphate (CA1P) also acts as an inhibitor of Rubisco**.** CA1P is the structural analog of six-carbon intermediate of the carboxylation reaction, which is generally synthesized in high concentrations in dark in the leaves of legumes. It alters the activity of Rubisco by binding to its activation site and thus keeping a control on Calvin cycle. But in light, specific phosphatase releases the phosphate group from CA1P, thereby making it incompetent to bind to the activation site of the enzyme. The activity of Rubisco activase is also regulated by light making it active in the light and inactive in the dark. Interconversion of active and inactive forms of Rubisco activase is regulated by ferredoxin-thioredoxin system discussed below.

Ferredoxin-thioredoxin system regulates Calvin-Benson enzymes. Calvin cycle enzymes, which includes fructose-1,6-bisphosphatase, sedoheptulose-1,7-

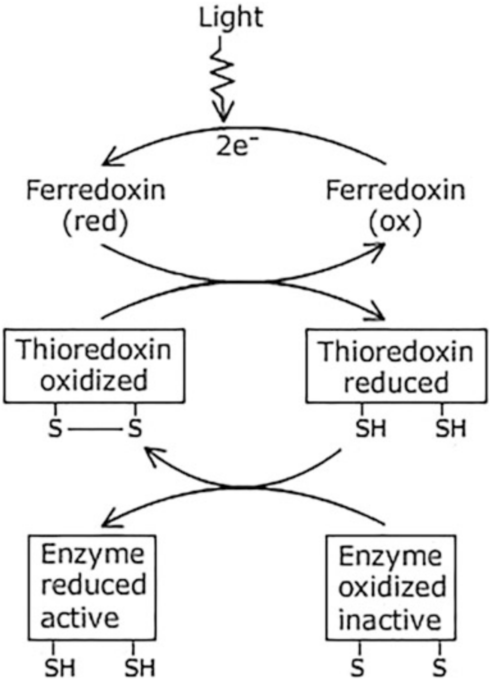


Fig. 5.21 Regulation of Calvin cycle enzymes by thioredoxin system

bisphosphatase, phosphoribulokinase, and NADP-glyceraldehyde 3-phosphate dehydrogenase, are regulated due to interconversion of thiol (reduced) and sulfhydryl (oxidized) forms of cysteine residues of the enzyme proteins. This is mediated by ferredoxin-thioredoxin system, which requires proteins ferredoxin (Fdx), ferredoxin-thioredoxin reductase (FTR), and thioredoxin (Trx), a small protein-disulfide reductase. The activation of photosystem I (P700) results in the reduction of ferredoxin in the light. Reduced ferredoxin transforms the disulfide bond (-S-S-) of the ubiquitous regulatory protein **thioredoxin** to its reduced state (-SH-HS-). The reaction is catalyzed by the enzyme ferredoxin-thioredoxin reductase. Reduced thioredoxin subsequently reduces the appropriate disulfide bonds of the target Calvin cycle enzymes, resulting in their activation (Fig. 5.21). This process is reversed in the dark, and inactivation of the target enzymes is observed due to oxidation of cysteine residues involved. Oxidation converts thioredoxin and target enzyme from their reduced active state (-SH HS-) to the oxidized inactive state (-S-S-) leading to their inactivation.

Another means for regulation of activity of Calvin cycle enzymes is due to their non-covalent interactions with proteins to form supramolecular complex. This mechanism is important especially when plants are exposed to quick-changing light conditions either in cloudy conditions or due to change in plant canopy. This includes enzymes viz., phosphoribulose kinase and glyceraldehyde 3-phosphate dehydrogenase whose activity is also regulated by NADPH directly. In the dark, these enzymes associate with a nuclear-encoded small chloroplast protein CP12

(~8.5 kDa protein) to form a large complex, which results in their inactivation. NADPH, generated in light reactions, on binding to this complex, leads to the release of the enzymes. Thus, activity of these enzymes depends both on reduction by thioredoxin and NADPH-mediated release from CP12.

5.5 Photorespiration

CO_2 is also released in a pathway which is distinct from mitochondrial respiration. The pathway is known as **photorespiration** or **C2 oxidative photosynthetic carbon cycle** or **photosynthetic carbon oxidation cycle (PCO)**. Rubisco possess carboxylase as well as oxygenase activities, since both CO_2 and O_2 compete for same catalytic site of the enzyme. Rubisco reacts with its second substrate, RuBP, to generate an unstable intermediate that splits into 2-phosphoglycolate and 3-phosphoglycerate in the presence of light and O_2 (Fig. 5.22). In 1920, Otto Warburg, a German biochemist, observed in *Chlorella* that photosynthesis was inhibited by O_2. Similar observations were made in many other plants. In 1955, Decker observed that photosynthesizing plants when transferred from light to dark,

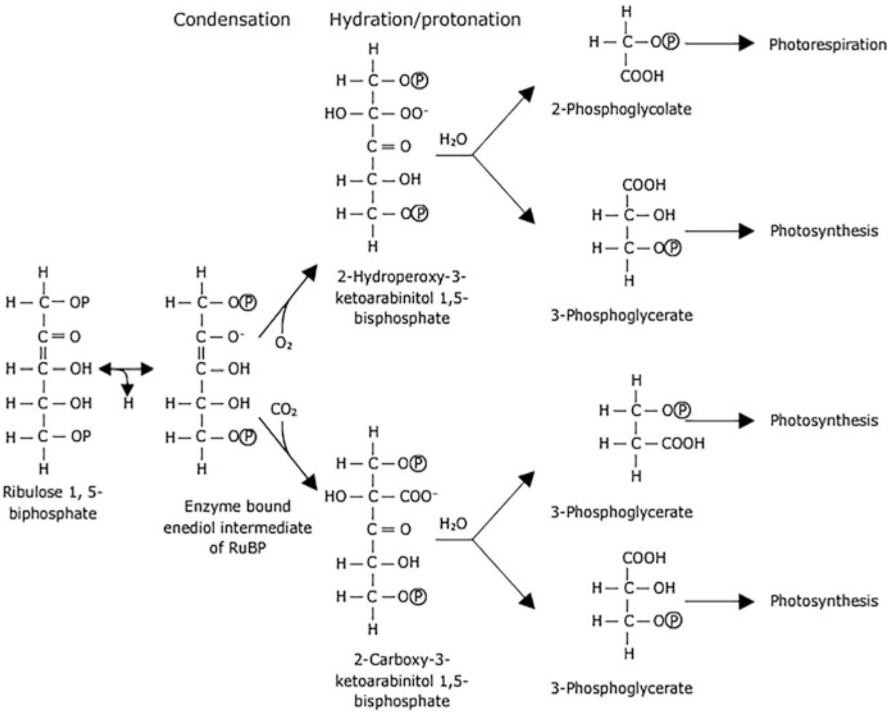


Fig. 5.22 Carboxylation and oxygenation of RuBP catalyzed by Rubisco. Binding of RuBP with Rubisco results in formation of an enzyme bound enediol intermediate which can react either with CO_2 or O_2 depending upon their availability

CO_2 production during initial 1–4 min was much more than CO_2 produced later on, a phenomenon which he called as "post-illumination burst of CO_2." Factors which influenced rate of photosynthesis during the light period also affected post-illumination burst of CO_2. This light-stimulated CO_2 production was called photorespiration. Several scientists including Zelitsch, Tolbert, and their associates related glycolate metabolism to photorespiration. Though glycolate pathway was different from mitochondrial respiration (also called dark respiration), this was called as photorespiration, since, similar to respiration, O_2 was consumed and CO_2 was released. However, unlike mitochondrial respiration there is no ATP production, but rather ATP is consumed, making it an energy-wasteful process. Since Rubisco possess both carboxylase and oxygenase activity, it is named RuBP carboxylase/oxygenase, which is abbreviated as Rubisco. Affinity of the enzyme for oxygen is much lower than for carbon dioxide. K_m value of Rubisco for oxygen is 535 $\mu mol.L^{-1}$, while for CO_2 it is 5 $\mu mol.L^{-1}$. Both gases, O_2 and CO_2, are present in low concentrations in chloroplasts. O_2 concentrations in chloroplasts are 250 $\mu mol.L^{-1}$, while concentrations of CO_2 are about 8 $\mu mol.L^{-1}$. Though affinity of the enzyme for O_2 is much lower, oxygenase activity of the enzyme is quite significant, because of comparatively high oxygen concentration in chloroplast. Oxygenase activity of the enzyme usually proceeds at the rate which is 25% of the carboxylation rate at normal O_2 and CO_2 concentration. It means that one oxygenation reaction occurs for every three carboxylation reactions. At high levels of O_2, oxygenation reaction of the enzyme results in formation of 3-phosphoglycerate and 2-phosphoglycolate. Photorespiratory glycolate pathway was discovered in 1972 by American scientist Edward Tolbert. 2-phosphoglycolate, produced during oxygenation of RuBP, is recycled to generate RuBP through this pathway. Phosphate group of 2-phosphoglycolate is hydrolyzed to form glycolate by chloroplast-specific enzyme, glycolate phosphatase. Subsequent metabolism of the glycolate involves the participation of other two organelles: peroxisomes and mitochondria (Fig. 5.23). Glycolate is exported out of chloroplasts through specific transporters present in the inner envelope of the plastid. It diffuses into peroxisomes through porins present in their membranes. In peroxisomes, oxidation of glycolate to glyoxylate is catalyzed by glycolate oxidase which contains flavin mononucleotide (FMN) as the cofactor. H_2O_2, which is produced during the reaction, is highly toxic and is degraded by peroxisome-localized catalase to yield O_2 and water.

$$H_2O_2 \rightarrow H_2O + \tfrac{1}{2}O_2$$

Glycolate oxidation does not occur in chloroplasts, since both products of the reaction are toxic for chloroplast-localized enzymes. Glyoxylate is toxic for Rubisco activity, and H_2O_2 being highly oxidizing agent may cause damage to thylakoids, besides inactivating Calvin cycle enzymes. Glyoxylate undergoes transamination to form glycine. Transamination of glycine occurs by two reactions which occur in 1:1 ratio. One of them is catalyzed by glutamate:glyoxylate aminotransferase, requiring glutamate as the amino group donor. The enzyme may also use alanine as the amino

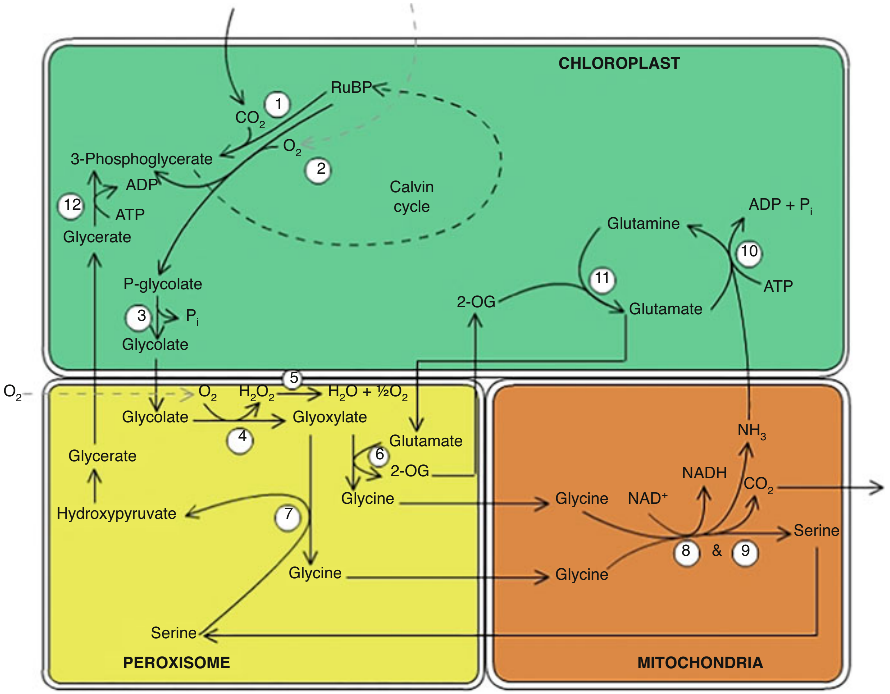


Fig. 5.23 Photorespiratory cycle involving three organelles chloroplast, peroxisomes, and mitochondria. Enzymes catalyzing reactions include (1) RuBP carboxylase, (2) RuBP oxygenase, (3) P-glycolate phosphatase, (4) glycolate oxidase, (5) catalase, (6) glutamate/glyoxylate aminotransferase, (7) serine/glyoxylate aminotransferase, (8) glycine decarboxylase complex, (9) serine hydroxymethyltransferase, (10) glutamine synthetase, (11) GOGAT (12) glycerate kinase

group donor. Another transaminase catalyzing the reaction is serine:glyoxylate aminotransferase, which use serine as the amino group donor. Glycine is released from peroxisomes via porins and is transported to mitochondria through specific transporters located in the inner mitochondrial membrane. In mitochondria, glycine decarboxylase, a multienzyme complex, catalyzes the condensation of two molecules of glycine (4 carbon), which are oxidized to yield one molecule of three-carbon amino acid serine, CO_2 and NH_3. This oxidative reaction is coupled with reduction of NAD^+ to NADH and is catalyzed by the enzyme glycine decarboxylase-serine hydroxymethyl transferase complex, which is present in large concentrations in the matrix of plant mitochondria. It consists of four proteins, namely, H-protein (a lipoamide-containing polypeptide), P-protein (a 200 kDa, homodimer, pyridoxal phosphate-containing protein), T-protein (containing tetrahydrofoliate as the prosthetic group), and L-protein (a dihydrolipoate dehydrogenase). Glycine decarboxylase constitutes as much as 30–50% of the total soluble protein in mitochondria. The enzyme serine hydroxymethyltransferase is in close proximity which facilitates transfer of the formyl group to another molecule of glycine resulting in synthesis of serine. Ammonia, which is released during the reaction in mitochondria, diffuses rapidly to chloroplasts, where it is fixed through GS/GOGAT pathway involving Fd-GOGAT (Chap. 11). One of the glutamates, produced during the reaction, is transported to peroxisomes. When photorespiration rate is high, the rate of NH_3 release in mitochondria is much higher. It has been found to be ten times higher than produced during nitrate reduction. NADH is either oxidized through electron transport chain localized in mitochondria resulting in ATP generation or is transported out of mitochondria to cytosol through malate-oxaloacetate NADH shuttle system to be used for reduction reactions such as nitrate reduction. Serine is transported from mitochondria to peroxisomes through the transporters localized in the inner mitochondrial membrane, where it is converted to hydroxypyruvate by transamination. In peroxisomes hydroxypyruvate is reduced to glycerate utilizing NADH. As there is no endogenous source for NADH in peroxisomes, it is produced during malate dehydrogenase catalyzed oxidation of malate to OAA. Malate is imported from cytosol through malate-oxaloacetate shuttle. Glycerate is transported to chloroplast via glycolate-glycerate transporter located in inner membrane of the plastid where it is phosphorylated to 3-phosphoglycerate by glycerate kinase and is metabolized through the Calvin cycle (Table 5.3). C2 oxidative photosynthetic carbon cycle is dependent on Calvin cycle for RuBP formation, and the balance between the two cycles is determined by three factors: Rubisco kinetics, substrate concentration (CO_2 and O_2), and temperature. With increase in temperature, affinity of oxygenase activity of Rubisco for O_2 increases. On a hot bright day, when photosynthesis has depleted CO_2 in the chloroplasts and raised the level of O_2, the rate of photorespiration may approach that of photosynthesis (Fig. 5.24). This phenomenon is, in fact, a major limiting factor in growth of many plants. Indeed, plants possessing a Rubisco with significantly less oxygenase activity would not only have increased photosynthetic efficiency but would need less water because they could spend less time with their stomata (the pores leading to their internal leaf spaces) open acquiring CO_2 and would have a reduced need for fertilizers because they would require less Rubisco. The control of photorespiration is, therefore, an important unsolved agricultural problem that is presently being attacked through genetic engineering research.

5.5.1 Significance of Photorespiration

Overall stoichiometry of the PCO cycle is:

$$2\text{RuBP} + 3\text{O}_2 + 2\text{Fd}_{\text{red}} + 2\text{ATP} \rightarrow 3,3\ \text{PGA} + \text{CO}_2 + 2\text{Fd}_{\text{ox}} + 2\text{ADP} + 2\text{P}_{\text{i}}$$

It indicates that one in ten carbon atoms from carbon acceptor molecule (RuBP) is lost during photorespiration besides NADPH and ATP which also are consumed during assimilation of NH_3. Thus photorespiration increases the energy cost of CO_2 assimilation. A number of photons which are required for CO_2 assimilation increase from 8 to nearly 14. Thus photorespiration reduces the efficiency with which plants fix CO_2, which is usually measured as the quantum efficiency (i.e., moles of CO_2

Table 5.3 Reactions of oxidative photosynthetic carbon cycle

S. No.	Reaction	Enzyme	Site
1.	$2\text{RuBP} \rightarrow 2\text{P} - \text{Glycolate} + 23 - \text{PGA}$	RuBP oxygenase	Chloroplast
2.	$2\text{P} - \text{Glycolate} + 2\text{H}_2\text{O} \rightarrow 2\text{Glycolate} + 2\text{P}_\text{i}$	Phosphoglycolate phosphatase	Chloroplast
3.	$2\text{Glycolate} + 2\text{O}_2 \rightarrow 2\text{Glyoxylate} + 2\text{H}_2\text{O}_2$	Glycolate oxidase	Glyoxysome
4.	$2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$	Catalase	Glyoxysome
5.	$2\text{Glyoxylate} + 2\text{Glutamate} \rightarrow 2\text{Glycine} + 2\text{x}2 - \text{oxoglutarate}$	Glutamate: glyoxylate aminotransferase	Glyoxysome
6.	$2\text{Glycine} + \text{NAD}^+ + \text{H}_2\text{O} \rightarrow \text{Serine} + \text{NADH} + \text{NH}_4^+ + \text{CO}_2$	(i) Glycine decarboxylase complex	Mitochondria
		(ii) Serine hydroxymethyltransferase	
7.	$\text{Serine} + 2 - \text{oxoglutarate} \rightarrow \text{Hydroxypyruvate} + \text{Glutamate}$	Serine:2-oxoglutarate aminotransferase	Glyoxysome
8.	$\text{Hydroxypyruvate} + \text{NADH} + \text{H}^+ \rightarrow \text{Glycerate} + \text{NAD}^+$	Hydroxypyruvate reductase	Glyoxysome
9.	$\text{Glycerate} + \text{ATP} \rightarrow 3 - \text{PGA} + \text{ADP}$	Glycerate kinase	Chloroplast
10.	$\text{Glutamate} + \text{NH}_3 + \text{ATP} \rightarrow \text{Glutamine} + \text{ADP} + \text{P}_\text{i}$	Glutamine synthetase	Chloroplast
11.	$2 - \text{oxoglutarate} + \text{Glutamine} + 2\text{Fd}_\text{red} + 2\text{H}^+ \rightarrow 2\text{Glutamate} + 2\text{Fd}_\text{ox}$	GOGAT	Chloroplast

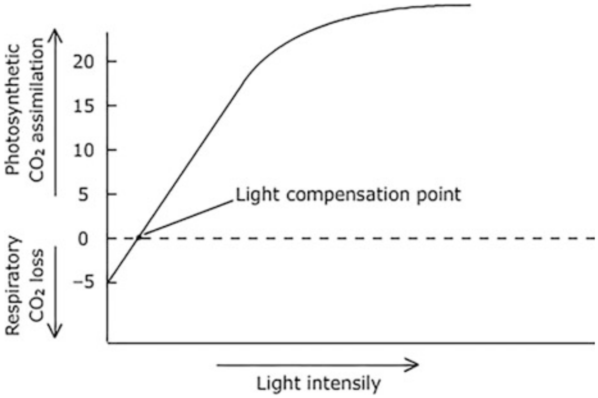


Fig. 5.24 Graph showing effect of light intensity on respiratory CO_2 loss and photosynthetic CO_2 assimilation. Light intensity, at which respiratory CO_2 loss equals to CO_2 assimilated during photosynthesis resulting in no apparent change in CO_2 concentration, is known as light compensation point

fixed per quanta absorbed). With increase in temperature, quantum efficiency decreases further, because photorespiration increases more in comparison with photosynthesis. Relative rates of carboxylase and oxygenase activity depends upon the affinity of Rubisco for CO_2 and O_2 and also on the ratio of CO_2 and O_2 present in the cell solution. With rise in temperature not only solubility of CO_2 decreases more in comparison with that of O_2, but affinity of enzyme for CO_2 also decreases in comparison with O_2. As a result, glycolate is produced more in comparison with 3PGA especially in C3 plants. Though being a wasteful process, photorespiration is significant for plants.

- One of the significance associated with photorespiration is believed to its scavenging role. Since glycolate production could not be avoided, because of inherent oxygenase activity of Rubisco, photorespiratory cycle must have evolved to remove glycolate causing minimum loss of carbon. Seventy-five percent of carbon lost from Calvin cycle as 2-phosphoglycolate is recovered in the C2 oxidative photosynthetic carbon cycle. Out of ten carbons of RuBP (two molecules), only one carbon is lost as CO_2, while nine carbons are recycled as 3-PGA.
- Involvement of three organelles for removal of glycolate possibly is because glyoxylate and H_2O_2 are toxic for Rubisco and Calvin cycle enzymes. Glycolate is transported out of chloroplasts and is oxidized to produce glyoxylate in peroxisomes and not in chloroplast. Otherwise photosynthesis would have been inhibited in chloroplasts. Peroxisomal matrix has got a very efficient system for removal of H_2O_2 as well for conversion of glyoxylate to glycine. Glyoxylate, H_2O_2, and hydroxypyruvate, the intermediates of glycolate metabolism, are not released because of substrate channeling in a multienzyme complex. It is observed that even if peroxisomal membrane is broken, the enzyme complex remains intact, indicating its protective function. However, in case of any leakage, there are enzymes present in cytosol required for conversion of glyoxylate to glycolate.
- Another significance associated with photorespiration is believed to be its role in preventing **photoinhibition.** In high light intensity and high temperature, stomata

of the plants close in order to prevent water loss. Because of restricted supply and constant utilization in photosynthesis, there is a decrease in concentration of CO_2, in comparison with that of O_2. As a result of this, CO_2 concentration decreases to much below the **compensation point**. Because of the decrease in availability of CO_2, intermediates reduced during light reaction accumulate. In absence of CO_2, ATP is not consumed leading to reduction in proton gradient created across thylakoids. As a result of this photosynthetic electron transport is also not dissipated leading to ROS production, which causes damage to photosynthetic apparatus. Consumption of NADPH and ATP through photorespiration may be a safety measure strategy adopted by plants, which can utilize NADPH as well as ATP thus protecting the photosynthetic apparatus and thylakoids from damaging effects of light. ATP is also required in photorespiration for conversion of glycerate to phosphoglycerate and for assimilation of NH_3 in chloroplasts. Net result of this complex photorespiration cycle is useless dissipation of some of the ATP and NADPH generated by the light reaction. Thus, under insufficient CO_2 concentration, Rubisco may undergo oxygenase activity to protect the photosynthetic apparatus from photooxidative damage but resulting in decrease in photosynthetic efficiency also.

- NADH, generated during conversion of glycine to serine, is oxidized through ETC in mitochondria.
- In addition to role of photorespiration in carbon economy, it plays significant role in nitrogen economy. When two molecules of glycine are converted to serine in mitochondria, one nitrogen atom, which is lost as NH_3, is re-assimilated in chloroplasts by GS/GOGAT reactions, thus conserving nitrogen. During NH_3 assimilation, reducing equivalents are utilized as reduced ferredoxin and NADPH.
- Photorespiratory cycle also plays an important role in synthesis of two essential amino acids, glycine and serine. Alternate pathways for synthesis of these amino acids function when photorespiration is suppressed.

Photorespiration is generally believed to be primarily an evolutionary remnant. It is speculated that Rubisco in bacteria which might have existed in anoxic conditions almost 3.5 billion (3.5×10^9) years ago has the sequence similarity with that of plants. Later around 1.5 billion years ago when concentration of free O_2 in air increased due to oxygenic photosynthesis, RuBP oxygenase activity increased possibly because of inability of the enzyme to discriminate in between CO_2 and O_2. Ratio of oxygenase to carboxylase activity decreased possibly due to selection of modification of the active site of the enzyme. Rubisco in anaerobic bacteria has higher oxygenase activity than carboxylase activity, when exposed to aerobic conditions which suggest possible alteration in the enzyme activity having lower oxygenase activity in plants. One of the possible reasons for retaining oxygenase activity of Rubisco activity might have been because oxygenase activity could not be modified without affecting the carboxylase activity.

5.6 C4 Pathway and Types of C4 Plants

Plants have developed strategies to reduce carbon loss by photorespiration. This is achieved by increasing CO_2 concentration at the Rubisco site, especially by plants growing at high light intensities, high temperatures, and under arid conditions. In these plants, the first stable product of CO_2 fixation is a four-carbon compound, viz., oxaloacetic acid, instead of 3-PGA. Earliest observations in this context were made in 1950s by Kortschak and coworkers at the Hawaiian Sugar Planters Association Experimental Station. In order to identify photosynthetic intermediates, leaves of sugarcane were exposed to $^{14}CO_2$, and surprising results were obtained. Unlike in Calvin cycle, the first stable compounds formed as a result of $^{14}CO_2$ fixation in sugarcane were four-carbon compounds—malate and aspartate. Since labeled carbon appeared first in four-carbon dicarboxylic acid and not in 3-PGA, it was known as C4 dicarboxylic acid pathway. The pathway is also called as **Hatch and Slack pathway** after the name of the scientists who elucidated the pathway. This pathway is operative in many tropical grasses, such as sugarcane, maize, sorghum, and *Amaranthus*. In contrast to C3 plants, C4 plants exhibit a different type of leaf anatomy. Vascular bundles in leaves of these plants are surrounded by large parenchyma cells which form a sheath. Chloroplasts present in bundle sheath cells are larger, lack grana and contain starch grains, and are morphologically and functionally distinct from the chloroplasts of mesophyll cells which are smaller and contain grana. This peculiar anatomy in leaves of C4 plants is called **Kranz anatomy**. The bundle sheath cells are bigger and look like a ring or wreath. Kranz in German means wreath, and hence this anatomical feature of the leaves of C4 plants is also referred to as Kranz anatomy. Mesophyll and bundle sheath cells have plasmodesmata connections, which provide symplasmic connections in between them. Unlike mesophyll cells in many C4 plants, there is deposition of suberin in cell walls of the bundle sheath cells. However, in some aquatic plants dimorphic chloroplasts occur within the same cell. Initial fixation of CO_2 occurs in cytosol of mesophyll cells which are exposed to both O_2 and CO_2 in stomatal chamber. Phosphoenolpyruvate, a three-carbon compound, is the acceptor of CO_2, and the enzyme which catalyzes the carboxylation reaction is PEP carboxylase (PEPC) (Fig. 5.25). Unlike Rubisco substrate for PEPC is HCO_3^-, and oxaloacetate is formed as a result of carboxylation reaction. Carboxydismutase catalyzes the formation of H_2CO_3, which gets ionized to produce HCO_3^-.

$$H_2O + CO_2 \rightarrow H_2CO_3$$

$$H_2CO_3 \rightarrow H^+ + HCO_3^-$$

$$PEP + HCO_3^- \rightarrow OAA + P_i$$

In C4 plants, both C4 and C3 pathways are spatially separated; C4 pathway occurs in mesophyll cells, while C3 in bundle sheath cells. Basic scheme for C4 pathway involves four steps which include: (i) carboxylation of PEP in cytosol of mesophyll cells is catalyzed by PEPC, resulting in synthesis of C4-dicarboxylic acid OAA; (ii) conversion of OAA to malate occurs in cytosol of mesophyll cells, which is transported to bundle sheath cells through plasmodesmatal connections; (iii) in bundle sheath cells malate is decarboxylated to generate CO_2, and a three-carbon compound, pyruvate, is produced. The released CO_2 is fixed by Rubisco through Calvin cycle in the chloroplasts of bundle sheath cells; (iv) the three-carbon compound, pyruvate, is transported back to the chloroplasts of mesophyll cells, where it is phosphorylated to produce 2-phosphoenolpyruvate in an enzyme-catalyzed reaction, which then is transported back to cytosol and is ready for another carboxylation reaction (Fig. 5.25). The last step is catalyzed by the enzyme pyruvate orthophosphate dikinase (PPDK) consuming two ATP equivalents.

$$\text{Pyruvate} + \text{ATP} + \text{P}_\text{i} \xrightarrow{\text{PPDK}} \text{PEP} + \text{AMP} + \text{PP}_\text{i}$$

The characteristics of this pathway are (1) high CO_2 concentration at the site of Rubisco (bundle sheath cells), which is produced as a result of decarboxylation of four-carbon compound. As a result, oxygenase activity of Rubisco is reduced to great extent because of high CO_2/O_2 ratio since the enzyme is localized deep inside the tissue and is not exposed to O_2 directly. (2) In some C4 plants deposition of suberin in cell wall of bundle sheath cell prevents any escape of CO_2. Besides having different pathways operating in bundle sheath and mesophyll cells, the chloroplasts of the two type of cells differ in their structure and function. (3) Bundle sheath cell chloroplasts lack grana and PSII. PSI operates in the bundle sheath chloroplasts which is responsible for cyclic photophosphorylation and ATP generation.

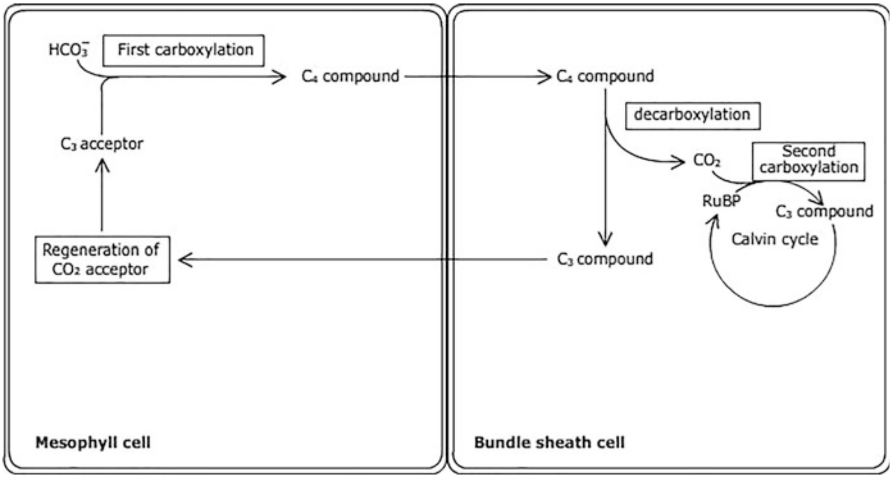


Fig. 5.25 Overview of C4 pathway. Mesophyll cells are interconnected with bundle sheath cells through plasmodesmata

3-phosphoglycerate, the product of RuBP carboxylation in bundle sheath cells, is transported to mesophyll cell chloroplasts where it is reduced to triose phosphate using NADPH and ATP produced during photosynthetic non-cyclic electron transport since chloroplasts of mesophyll cells have grana and PSII. Triose phosphates are transported back to bundle sheath chloroplasts. (4) Unlike in maize and sorghum, there are some aquatic and land plants which do not have Kranz anatomy. There are single-cell C4 plants in which dimorphic chloroplasts may be present in the same cell; e.g., in *Bienertia sinuspersici* chloroplasts of peripheral and central domain of the cell differ in their structure and function. Peripheral chloroplasts are similar to mesophyll cell chloroplasts, while chloroplasts of the central domain are similar to those of bundle sheath cells. Carboxylation reaction catalyzed by PEPC occur toward outside, while Rubisco has been pushed inside to reduce oxygenase activity (not exposed to O_2) so that photorespiratory loss of carbon skeleton is reduced.

Variations in the Basic Pathway However, variation of the scheme of C4 pathway has been observed. On the basis of the decarboxylation of four-carbon compound, C4 plants belong to three categories.

NADP-ME Types (NADP-Malate Enzyme Types) These include *Sorghum bicolor* and *Zea mays*. In these types of C4 plants, four-carbon exported out of mesophyll chloroplasts to bundle sheath cell is malate, while three-carbon which comes back to mesophyll cells is pyruvate. Oxidative decarboxylation of malate, in chloroplasts of bundle sheath cells, results in release of CO_2. The decarboxylation reaction is coupled with reduction of $NADP^+$ to NADPH. NADPH produced during oxidative decarboxylation of malate is used for reduction phase in Calvin cycle as no PSII is present in the bundle sheath chloroplasts, since they lack grana. However, ATP requirement is met through cyclic photophosphorylation which occurs in the agranal thylakoids, as PSI is present. Around 50% of the requirement of NADPH is met through oxidative decarboxylation of malate. 3-PGA is transported back to mesophyll cell chloroplasts where PSII operates, and NADPH generated due to non-cyclic photosynthetic electron transport is used for reduction of 3-PGA to G3P, which is the transported back to chloroplasts of bundle sheath cells (Fig. 5.26).

NAD-ME Types (NAD-Malate Enzyme Types) *Panicum miliaceum* and *P. virgatum* are included in this category. In these type of C4 plants exchange of carbon compounds between mesophyll cells and bundle sheath cells occurs as amino acids. Four-carbon compound exported out of mesophyll cells is aspartate, and the three-carbon compound which is returned is alanine. Transamination is carried out by cytosolic aspartate aminotransferase and alanine aminotransferase which are localized in cytosol of mesophyll and bundle sheath cells, respectively. Once in bundle sheath cells, aspartate is first converted back to OAA by aminotransferase in mitochondria, followed by its reduction to malate. The reduction is catalyzed by

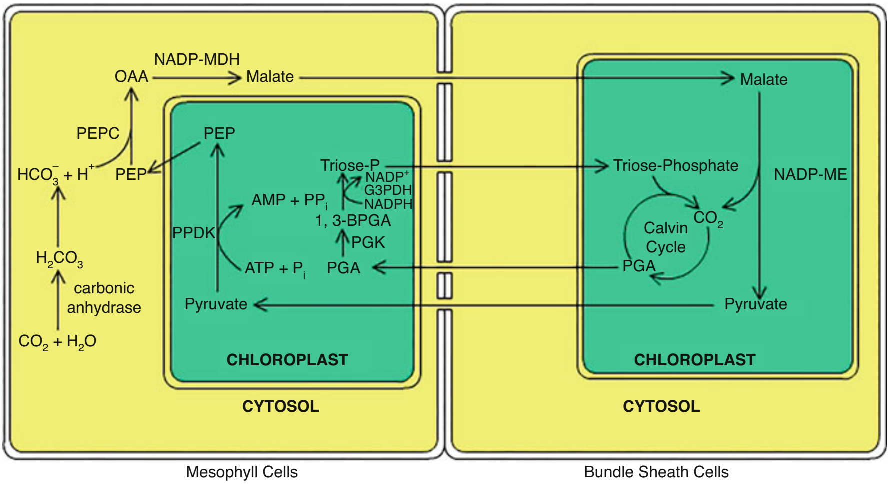


Fig. 5.26 NADP-ME type of C4 plants, e.g., *Sorghum bicolor* and *Zea mays*. *PEPC* phosphoenolpyruvate carboxylase, *G3PDH* glyceraldehyde 3-phosphate dehydrogenase, *NADP-ME* NADP-malate enzyme

mitochondrial NADH-dependent malate dehydrogenase. Decarboxylation of malate occurs in mitochondria, which is catalyzed by the NAD^+-dependent malate enzyme. CO_2 produced during decarboxylation diffuses into the chloroplasts and is fixed through Calvin cycle. Pyruvate is transported out of the mitochondria to cytosol where it's conversion to alanine is catalyzed by the aminotransferase. Alanine returns to mesophyll cells through plasmodesmatal connections and is converted to pyruvate by another cytosolic alanine aminotransferase of the cell. In mesophyll cells pyruvate is transported to chloroplasts, where it is converted to PEP catalyzed by the enzyme pyruvate dikinase. PEP is transported out of the chloroplasts to cytosol, where carboxylation reaction catalyzed by PEPC takes place (Fig. 5.27).

PEPCK (PEP Carboxykinase) These types of C4 plants include *Megathyrsus maximus*. There are two types of pathway operate in these category of plants. In one type of pathway, 4C compound and 3C compound which are exchanged in between mesophyll cells and bundle sheath cells are aspartate and PEP. Aspartate is converted back to OAA by the cytosolic aminotransferase in bundle sheath cells, which is decarboxylated, resulting in the formation of PEP and release of CO_2. Reaction, catalyzed by cytosolic PEPCK, is ATP requiring. Released CO_2 is fixed through Calvin cycle in chloroplasts, and PEP is returned to mesophyll cells. The other pathway includes exchange of C4 and C3 compound as malate and alanine, respectively. In mesophyll cells some OAA is reduced to malate in the chloroplasts and is transported to mitochondria of bundle sheath cells. Malate is decarboxylated and oxidized by NAD^+-dependent malate enzyme. The released CO_2 is fixed by Calvin cycle in the chloroplasts of bundle sheath cells. Pyruvate is converted to alanine by aminotransferases in cytosol of bundle sheath cells. Alanine returns to the

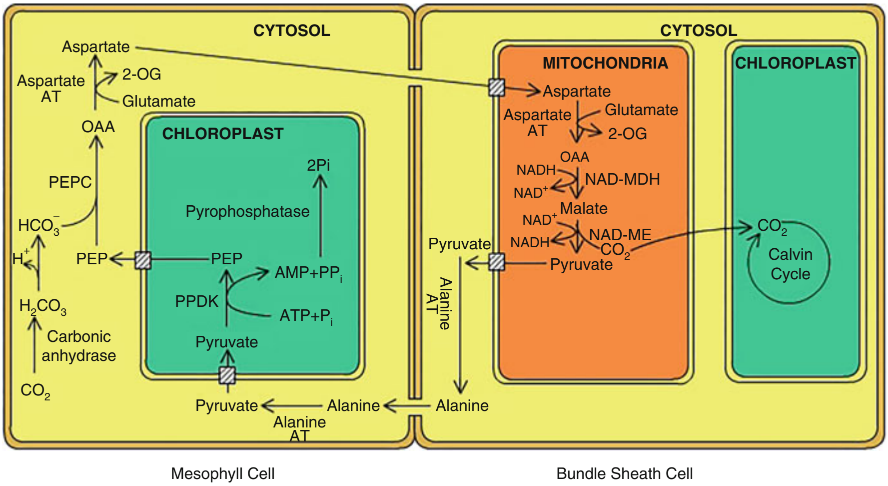


Fig. 5.27 NAD-ME type of C4 plants, e.g., *Panicum miliaceum* and *P. virgatum*. *PEPC* phosphoenolpyruvate carboxylase, *2-OG* 2-oxoglutarate, *NAD-MDH* NAD-malate dehydrogenase, *NAD-ME* NAD-malate enzyme, *PPDK* pyruvate-phosphate dikinase

mesophyll cells, where it is converted to pyruvate by aminotransferases in the cytosol. Pyruvate is converted to PEP after being transported to the chloroplasts of mesophyll cells (Fig. 5.28).

5.6.1 Regulation of C4 Pathway

Similar to regulation of Calvin cycle in C3 plants, C4 pathway is also regulated by light, so that there is coordination in between the two pathways. Three enzymes of C4 pathway which are activated in light, include PEPC, PPDK, and NADP-ME. NADP-ME is regulated by ferredoxin-thioredoxin system. The regulation of PEPC (PEP carboxylase) and PPDK (pyruvate-phosphate dikinase) occurs through phosphorylation and dephosphorylation of the enzyme proteins. Phosphorylated PEPC is active while dephosphorylated form is inactive. In the presence of light, activation of PEPC occurs due to phosphorylation of the enzyme protein at a specific serine residue, which is catalyzed by PEPC kinase. PEPC kinase is active in light, and inactive in dark possibly due to ferredoxin-thioredoxin system. PEPC has more affinity for PEP in light, and less affinity for malate, which acts as inhibitor in dark. Unlike PEPC, phosphorylated PPDK is inactive and dephosphorylated form is active (Fig. 5.29). Phosphorylation of PPDK at threonine residue occurs in dark. Dephosphorylation of the enzyme protein in light restores its activity. Phosphorylation and dephosphorylation of PPDK is carried on by a regulatory protein (RP). ADP is the phosphate group donor in case of PPDK whose availability is influenced by light. In dark ADP concentration is high and ATP concentration is low, reverse is true in light.

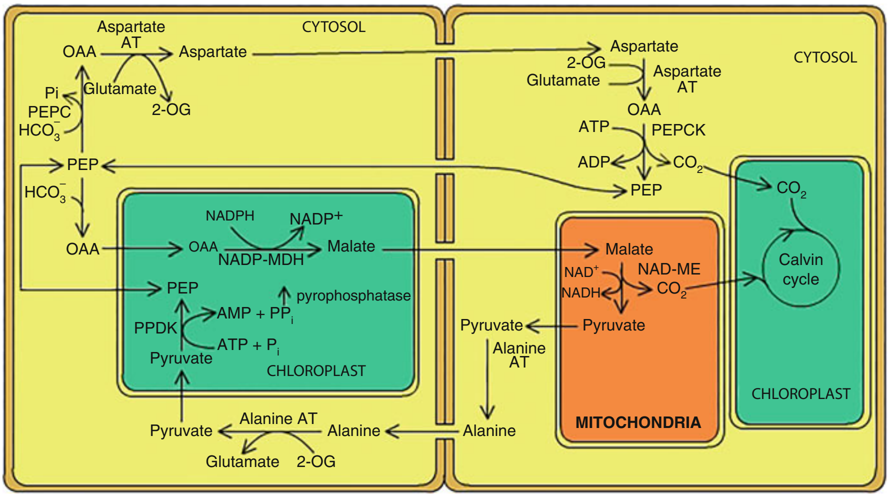


Fig. 5.28 PEPCK type of C4 plants, e.g., *Megathyrsus maximus. PEPCK* PEP carboxykinase, *AT* aminotransferase, *PPDK* pyruvate-phosphate dikinase, *NADP-MDH* NADP-malate dehydrogenase, *NAD-ME* NAD-malate enzyme

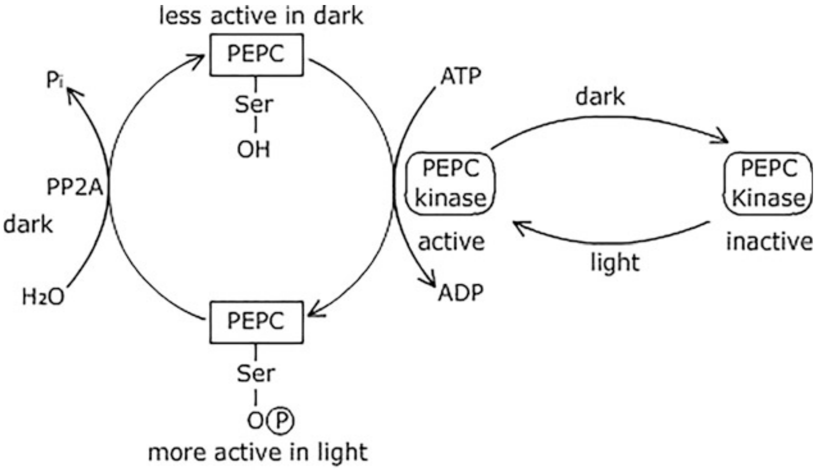


Fig. 5.29 Regulation of PEP carboxylase (PEPC) by light in C4 plants. *PP2A* type 2 protein phosphatase, *PEPC* PEP carboxylase

5.6.2 Energy Requirement for CO_2 Fixation by C4 Pathway

Since in all C4 plants, both C4 and C3 pathways operate, it indicates that additional energy must be required. Two ATPs and three NADPH are required for fixation of one CO_2 through C3 pathway, while two additional ATPs are required for regeneration of PEP from pyruvate in C4 pathway. One additional ATP is used by the enzyme PPDK and another by adenylate kinase. The reaction catalyzed by phosphoenolpyruvate dikinase (PPDK) is:

$$\text{Pyruvate} + \text{ATP} + \text{P}_\text{i} \xrightarrow{\text{PPDK}} \text{PEP} + \text{AMP} + \text{PP}_\text{i}$$

$$\text{PP}_\text{i} \xrightarrow{\text{Pyrophosphatase}} 2\text{P}_\text{i}$$

One molecule of ATP is used for generation of ADP from AMP, in a reaction catalyzed by adenylate kinase.

$$\text{AMP} + \text{ATP} \rightarrow 2\text{ADP}$$

AMP is produced in the reaction catalyzed by PPDK. Thus, for assimilation of one molecule of CO_2, at least five ATPs are consumed. Overall equation for synthesis of glucose by C4 plants is as follows:

$$6CO_2 + 24H_2O + 12\text{NADPH} + 12H^+ + 30\text{ATP}$$
$$\rightarrow C_6H_{12}O_6 + 12\text{NADP}^+ + 30\text{ADP} + 30\text{P}_\text{i}$$

5.6.3 Evolutionary Significance of C4 Pathway

Though C4 plants consume additional energy for CO_2 fixation, they are photosynthetically more efficient than C3 plants. Maize, sorghum, and sugarcane have been found to be world's most productive crops. Most of the C4 plants are tropical plants. When exposed to higher temperature and reduced water supply, stomata of a plant are closed so as to reduce water loss. As a result CO_2 uptake is reduced causing decrease in the ratio of CO_2/O_2 in stomatal chamber. At high temperature, affinity of Rubisco for O_2 also increases due to change in kinetic property of the enzyme. Temperature also influences solubility of the gases. At high temperature O_2 is less affected, while there is decrease in solubility of CO_2. As a result Rubisco is exposed to relatively higher O_2 concentration resulting in higher oxygenase activity of the enzyme. There will be increase in carbon loss because of photorespiration. In C4 plants, Rubisco is localized deep inside the tissue, i.e., in bundle sheath cells where it is not exposed to atmospheric O_2. There is increased CO_2 concentration at the Rubisco site because of CO_2 concentration mechanism. Initial fixation of CO_2 by PEPC occurs at mesophyll cells resulting in synthesis of four-carbon compound which is transferred to the site of Rubisco where it gets decarboxylated, thus acting like a CO_2 pump. CO_2 is concentrated as much as 20-folds in mesophyll cells which is the site of functioning of Rubisco, thus reducing oxygenase activity of the enzyme. Another structural feature of C4 plants is the presence of suberin in the cell walls of bundle sheath cells, which prevents any leakage of CO_2 adding to increased CO_2

concentration at the site of Rubisco action. Unlike Rubisco substrate for PEPC is HCO_3^-. This has two advantages, (i) it is distinct from CO_2 in its structure thus there is no competition from O_2 for binding with active site of the enzyme, and (ii) HCO_3^- concentration in the medium is five times higher than CO_2. Some scientists believe that it is not hot and dry climate rather it is the decrease in CO_2 concentration which might have led to development of C4 pathway. Until the end of crustacean period almost 100 million years ago, CO_2 would have been higher which fell dramatically during past 100 million years to around 200 ppm. Decrease in CO_2 concentration might have led to development of C4 pathway. Increase in CO_2 probably would result in reduced photorespiratory loss and increase in photosynthetic efficiency, a phenomenon known as "CO_2 fertilization effect."

5.7 Crassulacean Acid Metabolism (CAM): CO_2 Fixation in Dark

CAM plants are usually succulents which are adapted to grow under extremely adverse xeric conditions. Mesophyll cells of the plants have larger number of chloroplasts, and, unlike C_4 plants, vascular bundles are not surrounded by the well-defined bundle sheath cells. In these plants, stomata remain open during night when temperature is low and there is a humid condition but are closed during daytime so as to reduce water loss. They are not as photosynthetically efficient as C4 plants but are better suited to conditions of extreme desiccation. Only 5% of the vascular plants use CAM as their photosynthetic pathway, while some of these may exhibit CAM activity when needed. Earliest observations were made by Romans that leaves of some of the plants tasted bitter early in the morning than the leaves which were harvested later in the day. Benjamin Heyne had made similar observations in 1815 with the leaves of *Bryophyllum calycinum*, a member of family Crassulaceae. In 1804 *Theodore de Saussure* had published his observations that gas exchange in plants such as cactus differed from the thin-leaved plants. Ranson and Thomas possibly had given the term Crassulacean acid metabolism in 1940, but they were not able to discover the cycle. The discovery of CAM can be attributed to the cyclical acidification and deacidification. It was after the discovery of C4 pathway, almost 150 years after the observation were made by Benjamin Heyne, that an understanding about the CAM pathway was made. C_4 and CAM pathways are not alternate pathway to C3 cycle, rather they occur in addition to Calvin cycle (C_3 pathway). In C_4 plants there is spatial separation, while in CAM, there is temporal separation of C_3 and C_4 pathways (Fig. 5.30).

Acidification Diurnal fluctuations of acidity in leaves of succulents were observed due to the accumulation of malic acid during night and decrease in malic acid during daytime. The amount of stored carbohydrates (starch) also fluctuated. Starch accumulated during daytime, while it disappeared during night. Stomata of succulents are open in night so as to reduce water loss, when temperature is low

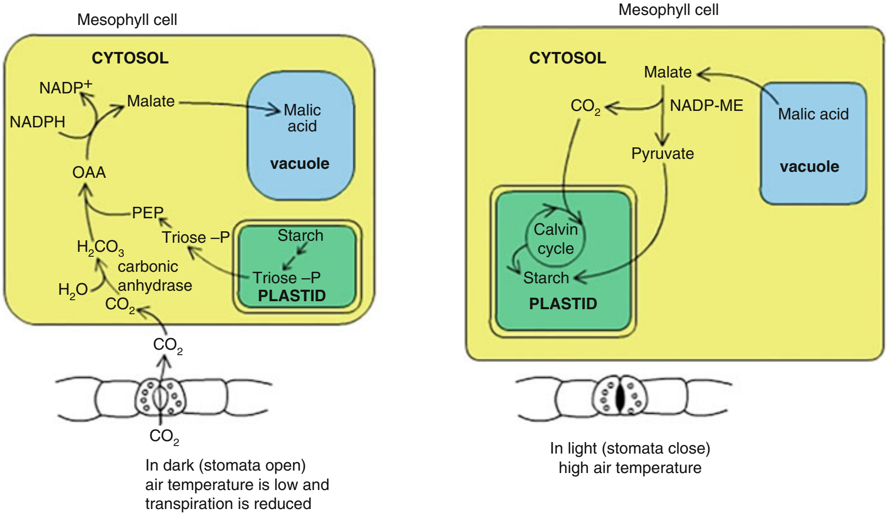


Fig. 5.30 Comparison of C4, C3, and CAM plants

and air is humid and are open during the daytime. During night CO_2 diffuses through the open stomata and is converted to HCO_3^- in cytosol of mesophyll cells. PEPC catalyzes carboxylation of phosphoenolpyruvate (PEP) using HCO_3^- as the substrate.

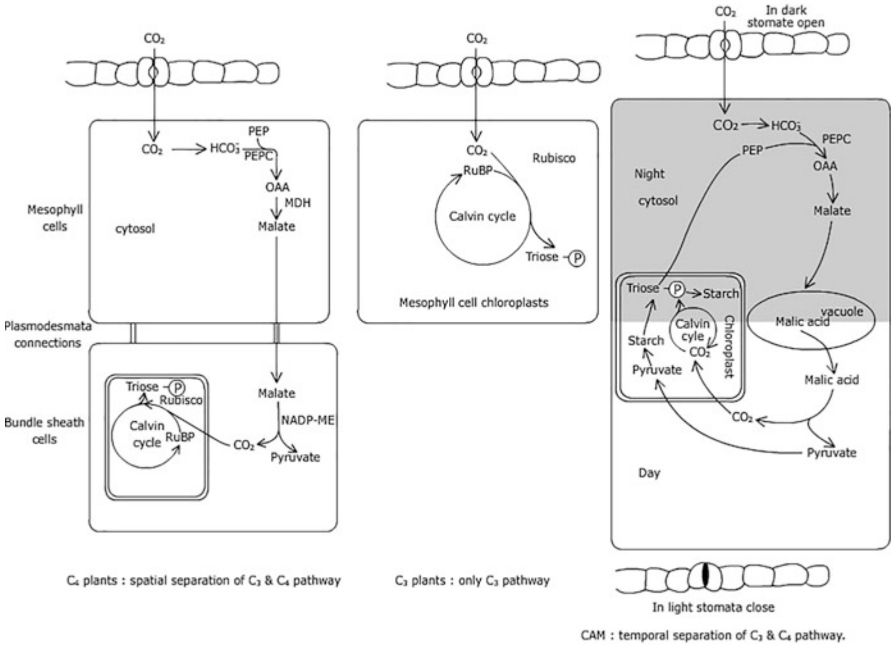
$$\text{Phosphoenol pyruvate} + HCO_3^- \xrightarrow{\text{PEP Carboxylase}} \text{Oxaloacetate} + P_i$$

Oxaloacetate is reduced to malate by the enzyme malic acid dehydrogenase. The reaction requires NADPH.

$$\text{Oxaloacetate} + \text{NADPH} + H^+ \xrightarrow{\text{Malic acid dehydrogenase}} \text{Malate} + \text{NADP}^+$$

Malate which is produced is transported to vacuole through a translocator located in tonoplast and is stored there as malic acid which is responsible for the increase in acidity of the tissues during night.

Deacidification Following morning, when the stomata are closed, malic acid moves out of the vacuole and is decarboxylated in cytosol to produce CO_2 and pyruvate. The reaction is catalyzed by $NADP^+$-specific malate enzyme. $NADP^+$ is reduced during the reaction. Since stomata are closed CO_2 can't escape and is refixed in chloroplasts through Calvin cycle. Calvin cycle enzyme becomes active in light. pH increases due to decrease in malic acid. The process is called deacidification.



C₄ plants : spatial separation of C₃ & C₄ pathway

C₃ plants : only C₃ pathway

CAM : temporal separation of C₃ & C₄ pathway.

Fig. 5.31 Crassulacean acid metabolism (CAM). In these plants the occurrence of two pathways of CO_2 fixation, i.e., C4 and C3 pathways, is separated by the time. C4 occurs during night when stomata are open, while C3 occurs during daytime when stomata are closed

$$\text{Malate} + \text{NADP}^+ \rightarrow \text{Pyruvate} + CO_2 + \text{NADPH} + H^+$$

Pyruvate is converted to triose phosphate during Calvin cycle which accumulates in plastids as **transitory starch.** Transitory starch is the source of PEP during dark, which is required as an acceptor of CO_2 by PEPC (Fig. 5.31).

Carboxylation of PEP by PEPC during nighttime and decarboxylation of malate during daytime are called phase I and phase III. However, phase II and phase IV are also included in CAM. Phase II refers to CO_2 fixation in the morning both by PEPC and Rubisco when stomata open briefly. When plants are subjected to extreme xeric conditions, both light signal and low internal CO_2 concentration act as signal for opening of stomata. As malate is decarboxylated CO_2 concentration increases and stomata close and phase III begins. At times during later part of the day when malate is depleted and CO_2 is exhausted stomata open and CO_2 diffuse into the stomatal chamber which is fixed both by PEPC and Rubisco activity resulting in phase IV of CAM. Phase IV occurs in response to environmental signal especially when the plants are exposed to adequate water supply. However, under extreme xeric conditions, stomata remain closed both during day and night, and CO_2 is produced because of respiration that is fixed through PEPC activity. There are some CAM which are facultative CAM, e.g., *Mesembryanthemum crystallum*, which change from C3 to C4 when there is decrease in rainfall. In *Kalanchoe blossfeldiana* also CAM is expressed in warmer long days and reduced water supply. Phytochrome might be involved in perception of signal. Unlike C4 plants in which PEPC is active in light, in CAM it is active in dark and is inactive in light. Similar to C4 plants phosphorylated PEPC is active. PEPC kinase is responsible for phosphorylation of PEPC. In CAM, the source of ATP during dark is respiration, while in C4 plants, it is photophosphorylation. Another regulatory factor for the activity of PEPC is malate. When malate accumulates at the end of dark period, PEPC activity is inhibited, while decrease in malate at the end of day PEPC is activated. A comparison of C3, C4, and CAM plants is given in Table 5.4.

5.7.1 Ecological Significance of CAM Plants

CAM plants open their stomata during night when exposed to xeric conditions so that water loss due to transpiration is reduced. These have high water-use efficiency (WUE) which can be calculated by the following formula:

$$\text{Water use efficiency} = \frac{\text{Moles of } CO_2 \text{ assimilated}}{\text{Moles of } H_2O \text{ transpired}}$$

WUE of CAM plants is high (4–20 mmol mol^{-1}) when compared to C4 and C3 plants which have value of WUE 4–12 mmol mol^{-1} and 2–5 mmol mol^{-1}, respectively. Though CAM plants are superior to other types in WUE but their photosynthetic rates and growth rates are much lower. Another way to relate transpiration rates to uptake of CO_2 is measurement of transpiration ratio (TR), which is reciprocal of WUE.

$$\text{Transpiration ratio} = \frac{\text{Moles of } H_2O \text{ transpired}}{\text{Moles of } CO_2 \text{ assimilated}}$$

CAM plants have low transpiration ratio (TR) in the range of 50–100, which is substantially lower than that of C3 and C4 plants, which are typically in the range of 200–350 and 500–1000, respectively. Low TR of CAM and C4 plants indicate their capacity to maintain high rates of photosynthesis while effectively conserving water.

Table 5.4 Comparison among C3, C4 and CAM plants

Characteristics	C3 plants	C4 plants	CAM plants
Optimal conditions for their growth	Temperate	Tropical	Arid
Examples	Wheat, barley, rice	Maize, sugarcane, millets (*Panicum* sp.)	Cacti, *Agave*, pineapple, succulents
Pathways for CO_2 fixation	C3	C4 and C3	C4 and C3
Anatomy of leaves	Mesophyll cells, bundle sheath cells are absent	Kranz anatomy, presence of large chloroplast containing bundle sheath cells present around vascular bundle which are distinct from other mesophyll cells	Mesophyll cells, bundle sheath cells are absent
Chloroplasts	Only one type of chloroplasts is present in all mesophyll cells	Chloroplasts are morphologically and functionally dimorphic	Only one type of chloroplasts is present
Location of the pathway	Only C3 in all mesophyll cells	C4 in bundle sheath chloroplasts while C3 in mesophyll cell chloroplasts (spatial separation of the two pathways)	In mesophyll cells only, C4 in night and C3 during daytime (temporal separation of the two pathways)
First fixation product of CO_2 site of its production	3-PGA in all mesophyll cells	Oxaloacetate in mesophyll cells in daytime	Oxaloacetate in mesophyll cells in night
Carboxylating enzyme carrying out initial fixation of CO_2	Rubisco	PEPC	PEPC
Energy requirement for fixation one CO_2	3 ATP + 2 NADPH	5 ATP + 2 NADPH	6.5 ATP + 2 NADPH
Photorespiration	High	If present very less	If present very less
Net assimilation rate, i.e., g dry matter produced per unit leaf area (m^2) per day	10–25	40–80	6–10
Transpiration ratio	450–950 g g^{-1}	250–350 g g^{-1}	18–125 g g^{-1}
Optimum temperature for photosynthesis (°C)	15–25	30–47	35
Water use efficiency (moles of CO_2 fixed $mole^{-1}$ of water loss)	1.05–2.22	2.85–4.00	8.0–55.0

5.8 Summary

- Green plants and certain bacteria are photoautotrophs since these are able to convert light energy into chemical energy by photosynthesis and store it in the form of organic compounds. Green plants carry out oxygenic photosynthesis thus releasing free O_2 as the by the product of the process.
- Light is transmitted in the form of waves, while it is absorbed and emitted in the form of particles known as photons. Energy of a photon is expressed as quanta. Energy of light is generally expressed as mole of quanta, which is known as Einstein. Electromagnetic spectrum of light includes those frequencies which are utilized by the biological systems. Less than 1% of the solar light reaching earth is utilized for photosynthesis.
- Photosynthetic pigments absorb light to be used for photosynthesis. These include chlorophylls, carotenoids, and phycobiliproteins. In nature multiple chlorophylls are present, Chl a, Chl b, Chl c, and Chl d. All chlorophylls consist of porphyrin ring which is esterified to phytol (except in Chl c in which phytol tail is not present). Chl a is present in all photosynthesizing organisms. Role of carotenoids is in harvesting the sunlight and also in protecting photosynthetic apparatus from photooxidative damage. Phycobiliproteins help in harvesting light energy in red and blue-green algae. It is the characteristic for each pigment to absorb specific wavelengths of light which when plotted that gives absorption spectrum. Similarly, each photobiological process requires specific light frequency, which when plotted give action spectrum. By comparing absorption spectrum of a pigment and action spectrum of a photobiological process, the role of a pigment can be understood.
- A pigment is excited after absorbing specific photon since its electron is elevated to higher energy level (singlet state). The excited electron can lose its energy through various pathways including through fluorescence. The electron may be lost to another molecule. As a result the electron donor is oxidized, while the molecule on accepting the electron is reduced. Photochemical reaction is very fast; thus the excited state of the molecule having the lowest lifetime will be responsible for carrying out photochemical reactions.
- It was the work of Blackman, Emerson, and Arnold which led to understanding that photosynthesis includes one light-dependent phase and a light-independent enzymatic phase. Experiment of Robert Hill is also a landmark in photosynthetic research since it led to understanding that light reaction is responsible for photolysis of water and generation of reduced intermediates before CO_2 is reduced. These intermediates were later identified to be $NADP^+$.
- While studying action spectrum of photosynthesis in *Chlorella*, Emerson et al. observed sudden decline in quantum yield of photosynthesis in red region of spectrum, while light was still absorbed by chlorophylls. This led to the discovery of two light reactions in photosynthesis, one requiring shorter wavelengths while another requiring longer wavelengths. Duysens conceptualized existence of two pigment systems, which are called photosystem I and photosystem II.

- Each photosystem consists of light-harvesting complex, LHC, and a reaction center. LHC is responsible for absorbing light and funneling it to the reaction center where photochemical reaction occurs. Photochemical reaction includes charge separation event which occurs because of losing of an electron of the primary chlorophyll molecule (P_{680} and P_{700} in PSII and PSI, respectively) and acceptance of the electron by another variants of chlorophyll a. Various electron carriers are involved in transfer of electrons from water to $NADP^+$ including cyt_{b6f} complex.
- Mobile electron carriers, plastoquinone and plastcyanine, mediate electron transport in between the three complexes, PSII, Cyt_{b6f}, and PSI, which are localized in thylakoid membrane. Energy of electron transport downhill is coupled with proton transport across thylakoids resulting in accumulation of H^+ in thylakoid lumen, as a result of which proton motive force is created, and ATP synthesis coupled with protons movement is known as photophosphorylation. Thus the net outcome of light reaction is photolysis of water resulting in release of O_2, NADP reduction to NADPH, and ATP generation.
- NADPH and ATP generated during light reaction are utilized in CO_2 assimilation, which occurs in stroma. Path of CO_2 assimilation has been traced by Melvin Calvin using radioactive carbon, ^{14}C, and autoradiography. First compound was identified to be a three-carbon compound, 3-phosphoglycerate, and the CO_2 acceptor was identified to be ribulose 1, 5 bisphosphate, which is regenerated during CO_2 fixation. The pathway was called as Calvin cycle. Calvin cycle includes three phase, carboxylation phase, reduction phase, and regeneration phase. Rubisco is the enzyme catalyzing the carboxylation phase. During reduction phase, reducing power NADPH and ATP, which is generated during light reaction, is utilized. 1/5 of the triose phosphate produced is diverted out of the chloroplast to cytosol for product biosynthesis, while the remaining 5/6 are used for regeneration of RuBP. Calvin cycle occurs only during light because many of Calvin cycle enzymes are active in light. As a result of this occurrence of futile cycle is avoided.
- In some tropical grasses, initial CO_2 fixation product is not a three-carbon compound but is a four-carbon compound. The pathway was elucidated by Hatch and Slack. The plants in which this pathway occurs are called C4 plants. C4 plants have characteristic leaf anatomy known as Kranz anatomy. C4 pathway is not alternative to C3 but rather occurs in addition to C3 pathway. In C4 plants both C4 and C3 pathways are spatially separated. Initial fixation of CO_2 in C4 plants involves activity of PEP carboxylase in mesophyll cell cytosol. C4 compound is transported to bundle sheath chloroplasts and is decarboxylated. Released CO_2 is refixed through Calvin cycle. Depending upon decarboxylation reaction of C4 compound, three types of C4 plants have been identified.
- Plants belonging to family Crassulaceae have developed a mechanism to conserve water while still carrying out photosynthesis. CO_2 is fixed in dark when stomata are open, and the 4C compound is stored in vacuoles in the form of malic acid, while during daytime when stomata are closed, malic acid comes out of vacuole and is decarboxylated. Released CO_2 is refixed through C3 pathway.

Thus both C4 and C3 pathways operate in C4 and CAM plants, but these are spatially separated in C4 plants, while there is temporal separation of these two pathways in CAM plants.

- Because of RuBP oxygenase activity in C3 plants, glycolate is also produced in addition to 3PGA which is the substrate for a process known as photorespiration. The process results in loss of almost 25% of the carbon fixed through photosynthesis. Plants have developed a metabolism to get rid of glycolate, which is known as glycolate metabolism. This involves many reactions and various organelles which include chloroplast, glyoxysome, and mitochondria. Photorespiration may act as energy spill-over mechanism.

Multiple-Choice Questions

1. Excited state of chlorophyll molecule responsible for the photochemical reaction of photosynthesis is:
   (a) First singlet state
   (b) Second singlet state
   (c) Triplet state
   (d) None of the above
2. Photochemical reaction requires:
   (a) 10^{-3} seconds
   (b) 10^{-12} seconds
   (c) 10^{-9} seconds
   (d) 10^{-2} seconds
3. Phenomenon of loss of energy of excited pigment molecule as light wavelength of longer wavelength than the wavelength of absorbed light is known as:
   (a) Homogenous energy transfer
   (b) Resonance
   (c) Fluorescence
   (d) Phosphorescence
4. Primary electron acceptor from excited P_{680} in PSII is:
   (a) Q_A
   (b) Phylloquinone
   (c) Tyr_z
   (d) Pheophytin
5. Primary electron donor to P_{680}^+ in PSII is:
   (a) Tyr_z
   (b) H_2O
   (c) Oxygen-evolving complex
   (d) Q_B

6. Quantum yield in photosynthesis is defined as:
   (a) Number of quanta required for release of one O_2
   (b) Number of O_2 molecules produced per quanta absorbed
   (c) Number of chlorophyll molecules required to absorb one quanta
   (d) Number of chlorophyll molecules responsible for release of one O_2
7. (Tick which is not right) Activation of Rubisco by light is due to:
   (a) Decrease in pH of lumen
   (b) Increase in pH of stroma
   (c) Due to release of Rubisco from thylakoids into the stroma
   (d) Due to Mg^{+2} moving out of thylakoid lumen to stroma
8. Which of the following reactions in Calvin cycle is reversible?
   (a) Conversion of sedoheptulose 1,7-bisphosphate to sedoheptulose 7-phosphate
   (b) Synthesis of ribulose 1,5-bisphosphate from ribose 5-phosphate
   (c) Synthesis of 3-phosphoglyceraldehyde from 3PGA
   (d) Conversion of xylulose 5-phosphate to ribulose 5-phosphate
9. The three types of C4 plants differ from each other on the basis of:
   (a) Chemical nature of C4 compound transported out of mesophyll cells
   (b) Decarboxylation reaction of C4 compound in bundle sheath cells
   (c) Chemical nature of C3 compound which is returned to mesophyll cells
   (d) The enzyme which is responsible for initial carboxylation of CO_2 acceptor
10. Tick incorrect statement:
    (a) Oxidation of glycolate to glyoxylate occurs in peroxisomes
    (b) P-glycolate is dephosphorylated to glycolate in peroxisomes
    (c) NH_3 is produced in mitochondria
    (d) α-hydroxypyruvate is reduced to glycerate in glyoxysomes

Answers

1. a 2. b 3. c 4. d 5. a 6. b 7. c
8. d 9. b 10. b

Suggested Further Readings

Jones R, Ougham H, Thomas H, Waaland S (2013) The molecular life of plants. Wiley-Blackwell, Chichester, pp 284–326

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