# UNIT 12 PHOTOSYNTHESIS

## Structure



## 12.1 INTRODUCTION

In the previous units you studied some of the metabolic processes during which energy is released and ATP synthesised by the oxidation of organic fuel molecules. Oxidation of glucose is one such example. The molecules like glucose act as secondary energy sources because animals and human beings depend on plants for the supply of organic nutrients. Plants, on the other hand, can synthesise carbohydrates and other organic molecules from atmospheric carbon dioxide utilising the primary source of energy i.e., sunlight. The synthesis of carbohydrates from carbon dioxide and water under the influence of sunlight is called photosynthesis, i.e., a synthesis which depends on photo (light) energy. Photosynthesis represents a reverse of animal respiration. It may be said that the two processes are complementary to each other in the sense that they depend on each other for their starting materials. It is therefore, important to understand the process of photosynthesis too. In this unit you will study how the plants convert a primary energy source into a usable chemical form and also the utilisation of this chemical energy for carbohydrate synthesis. This will include the light and dark reactions of photosynthesis.

### Objectives

After studying this unit, you should be able to:

- describe the photosynthetic machinery of the plants,
- understand the mechanism by which the light energy is transformed into chemical energy, and
- describe how this chemical energy is utilised to drive the synthesis of carbohydrates from carbon dioxide, i.e., $CO_2$ fixation.

## 12.2 A HISTORICAL PERSPECTIVE OF PHOTOSYNTHESIS

In this section we will briefly explain the experiments which suggested the utilisation of light energy for the synthesis of carbohydrates in green plants and involvement of light and dark reactions in this process. Joseph Priestley, one of the discoverers of oxygen, was the first to demonstrate the interdependence of respiration ( or combustion ) and photosynthesis in 1780. He showed that air enclosed in a jar was depleted or injured by burning a candle in it. The depleted air could not support combustion nor the life of an animal (mouse). The same air could be "restored" by putting a sprig of mint in it. The restored air was able to support combustion. Also, a mouse placed in it did not die. Some time later, it was shown by a Dutch physician, Jan Ingenhousz, that this "restoration" of air by green plants was dependent on light. In 1842 Robert Mayer, a German physician, the discoverer of the law of conservation of energy (the first law of thermodynamics) showed that sunlight provided the required energy input for photosynthesis. He wrote "The plants take in one form of power, light, and produce another power, chemical difference".

In 1937 R. Hill at Cambridge, England, showed that leaf extract suspended in light in an aqueous medium could reduce some nonbiological electron or hydrogen acceptors with the evolution of oxygen and without the fixation of carbon dioxide. This experiment suggested that oxygen evolved during photosynthesis originated from water. This was later confirmed using $^{18}O$-enriched water when $^{18}O$ was recovered in gaseous oxygen. Secondly, it showed that evolution of oxygen and fixation of carbon dioxide were separate events. Later D.I. Arnon showed that chloroplasts irradiated in the absence of $CO_2$ developed the capacity to bring about $CO_2$ fixation in dark. These studies suggested that photosynthesis consisted of a **light reaction** during which oxygen was evolved and a **dark reaction** during which $CO_2$ was fixed. The latter is dependent on the former.

Photosynthesis is not limited to plants only but takes place in some other organisms also, e.g., blue green algae and some bacteria. These microorganisms account for more than half of all the photosynthetic activity taking place on the earth. Our discussion in this unit will be limited to plant systems only. The physicochemical principles involved in photosynthesis are the same for all organisms. However, the intracellular localisation of the photosynthetic apparatus and its organisation may vary from one species to another. You will study this in the next section.

## 12.3 SITE OF PHOTOSYNTHESIS IN PLANTS: CHLOROPLASTS

The photosynthetic bacteria and blue green algae, which are prokaryotes, lack chloroplasts. Their light absorbing molecules are localised either in the plasma membrane or in vesicular systems called chromatophores.

In plants, the photosynthetic activity is localised in chloroplasts. Their shape may vary from one plant to another. In general, they are larger than mitochondria and, like the latter, are endowed with their own genes also. You have studied the structure of chloroplasts in detail in Unit 1. Their main structural features of interest are recapitulated here with the help of a schematic drawing shown in Fig. 12.1. The chloroplasts are surrounded by an outer and an inner membrane. The latter encloses many flattened and membrane enclosed sacs or vesicles, called **thylakoids**, which are arranged in stacks or **grana** (singular **granum**). Different grana are linked by extensions of the thylakoid membranes called lamella. The medium surrounding the thylakoid and enclosed by the inner membrane is referred to as **stroma**.

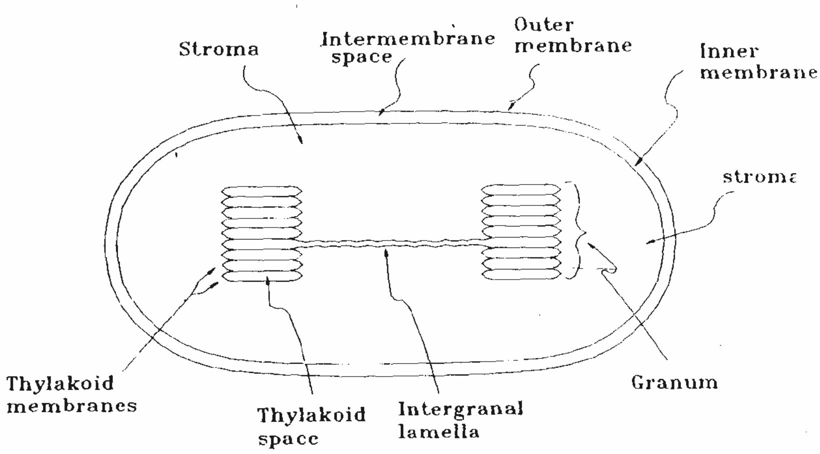


**Fig. 12.1 : Schematic diagram of a chloroplast**

The membranes of thylakoids and lamellae contain the light absorbing molecules and other substances required for transducing light energy into chemical energy. Therefore the light reactions of photosynthesis take place in the nonaqueous lipid environment of the membrane. The enzymes responsible for the actual fixation of $CO_2$ and the synthesis of carbohydrates are soluble proteins and are present in the stroma. To understand the photosynthetic reactions it is necessary to understand the process of light absorption and the factors responsible for it, which you will study in the next section. Before that try to answer the following SAQ which is based on Sec. 12.2 and 12.3.

## SAQ 1

a) On the basis of Hill's experiment and observation write the light and dark reactions of photosynthesis.

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b) Where do you think in the chloroplasts the light and dark reactions of photosynthesis take place?

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## 12.4 LIGHT ABSORPTION IN PHOTOSYNTHESIS

This section deals with mainly the factors responsible for absorbing light and converting it to chemical energy. The light reactions of photosynthesis are the energy trapping reactions where, as said before, oxygen is liberated by the splitting of water molecules, i.e.,

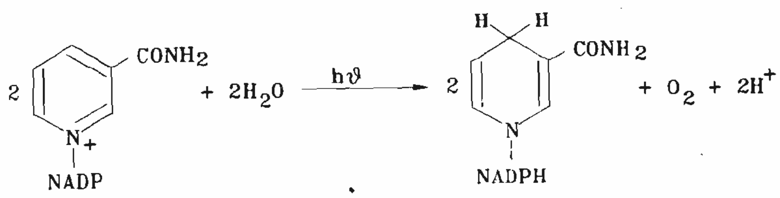
$$H_2O \longrightarrow 2e^- + 2H^+ + \frac{1}{2}O_2 \qquad \ldots(12.1)$$

As is clear from the above equation water serves as an electron donor and the light reactions must involve the transfer of an electron from water to an acceptor which then becomes a reductant, i.e., a reducing agent. Since this observation was made by Hill and coworkers it is called **Hill reaction** and can be put in a generalised form as:

$$2A + 2H_2O \xrightarrow[\text{chloroplasts}]{\text{irradiated}} 2H_2A + O_2 \qquad \ldots(12.2)$$

The acceptor A of hydrogen or electrons is called the Hill reagent.

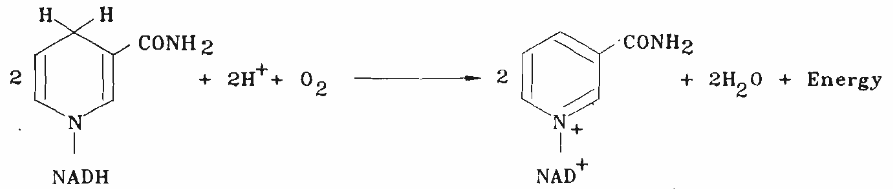
A search was made for the naturally occurring counterpart of the Hill reagent which was reduced during the light reaction in chloroplasts. In 1951, it was identified as nicotinamide adenine dinucleotide phosphate ($NADP^+$: see Unit 7 for the structure). It was reduced to NADPH. The reaction is shown in Eq. 12.3 where only the nicotinamide part of the structure of $NADP^+$ is given.




...(12.3)

As we will see la'er in this unit, another reaction which accompanies the above in the light reaction is the formation of ATP from ADP and phosphate ion.

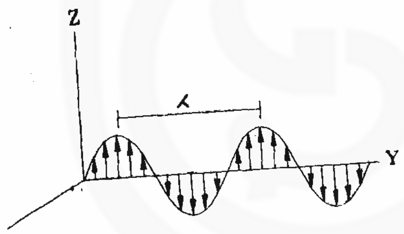
Compare the reaction given in Eq. 12.3 with the overall change in the electron transport in the mitochondria, Eq. 12.4 which was described Unit 8.



...(12.4)

The reactions of Eq. 12.3 and 12.4 are reverse of each other, except a minor difference in the structure of the coenzyme ($NAD^+$ and $NADP^+$). In the mitochondrial electron transport the electrons flow downhill from a stronger reductant ($NAD^+$/NADH pair; standard reduction potential at pH 7, $E^{o\prime} = -0.320$ V) to a stronger oxidant ($O_2/H_2O$ pair; $E^{o\prime} = +\ 0.82$ V) and the energy released in the process drives the synthesis of ATP. In contrast, the flow of electrons in photosynthesis must be uphill, i.e., from an oxidant to a reductant. This is made possible by the input of light energy. Thus Eq. 12.3 shows one of the reactions by which the light energy is transduced into chemical energy. In order to accomplish this, the incident light must first be absorbed. The nature of light absorbing substances, i.e., pigments, and the series of reactions by which the absorbed light energy is converted into chemical energy are described in the latter sections. For a better understanding of these processes, it is necessary for us to discuss the relevant aspects of the nature of light energy and fate of light energy after it is absorbed by a molecule.

### 12.4.1 Fate of Light Energy Absorbed by Molecules




You will recall that light is an electromagnetic field that oscillates sinusoidally in space and time. It interacts with matter in packets or quanta, called photons, each of which contains a definite amount of energy. The energy content of light is related to its frequency (ν) and is given by $h\nu$, where $h$ is Planck's constant $1.583 \times 10^{-34}$ cal. Since the frequency and wavelength of light are inversely related to each other, the energy is inversely proportional to the wavelength of light. The energy equivalent, $E$ in kcal of one mole or one einstein of light, i.e., of $6.023 \times 10^{23}$ photons is given by the relationship:

$$E = \frac{28{,}600}{\text{wavelength (nm)}} \text{ kcal}$$

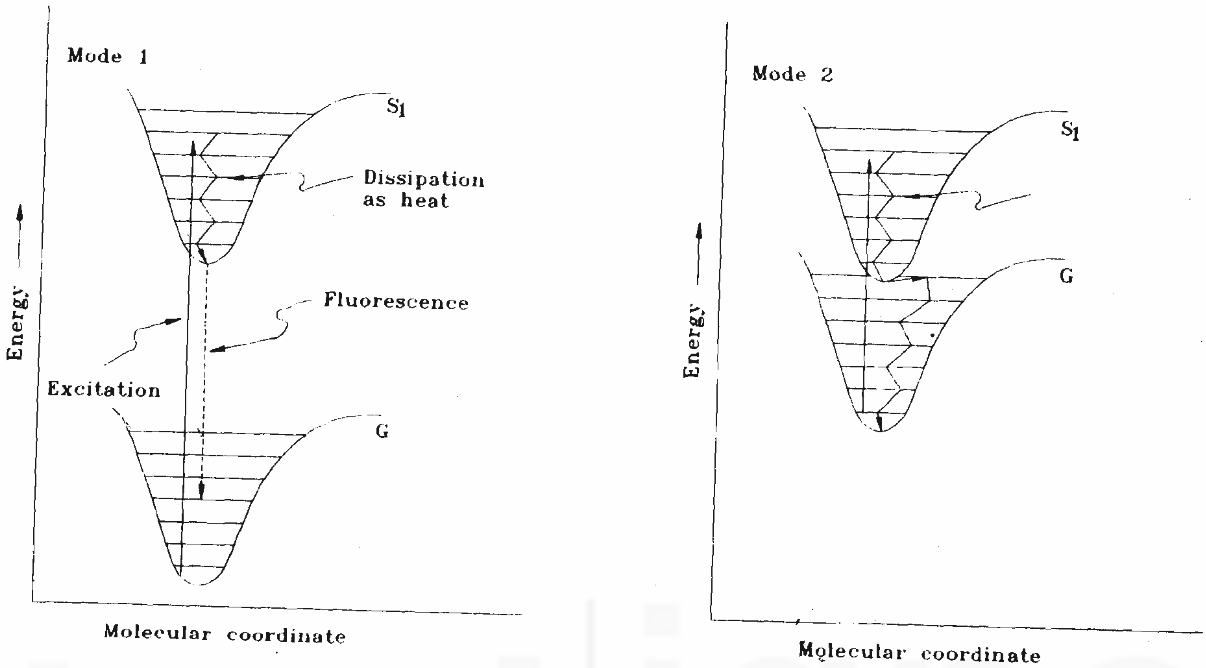
The energy equivalents of light of different wavelengths in the visible range are given in Table 12.1.

**Table 12.1 : Energy equivalent of light in the visible region**

| Wavelength (nm) | Colour of radiation | Energy/einstein (kcal) | (k J) |
|---|---|---|---|
| 400 | violet | 71.5 | 299 |
| 500 | green | 57.2 | 239 |
| 600 | orange | 47.7 | 199 |
| 700 | red | 40.9 | 171 |

Fluorescence is a phenomenon by virtue of which certain substances absorb light of one wavelength and emit light of other wavelength without undergoing a chemical reaction.

Absorption of light by a molecule takes place with a high probability if the energy of the light quantum, i.e., photon, is equal to that required to raise one of its electrons from the ground electronic state, i.e., the lowest energy state, to one of the vibrational levels of the first or higher excited electronic state, Fig. 12.2. What happens to the energy of excited molecule? The excited molecule reverts to the ground state by losing the absorbed energy. Four major modes of disposal of the absorbed energy are shown in Fig. 12.2. The absorbed energy may be dissipated as heat or emitted as light by **fluorescence**. The latter process requires a relatively longer lived zero vibrational level of the first excited state (approx. $10^{-8}$ sec). The dissipation of the absorbed energy as heat is by far the fastest and the most common mode of losing the absorbed energy. Alternatively, the excited molecule may transfer this energy on collision to another acceptor molecule in a nonradiative transfer mode, mode 3, also called **resonance energy transfer**. The excited molecule may also undergo a chemical reaction with another molecule, i.e., **electron transfer,** mode 4. The nonradiative energy transfer or electron transfer must, however, take place before the excited molecule has had a chance to fluoresce.




**Mode 3** : Nonradiative energy transfer

$D^* + A \rightarrow D + A^*$

**Mode 4** : Electron transfer

$D^* + A \longrightarrow D^+ + A^-$

**Fig. 12.2 : Different modes showing fate of light energy absorbed by a molecule**

The excited molecules, $D^*$ or $A^*$ in the figure, show markedly different chemical properties than the corresponding molecules in the ground state (D or A). For the purpose of photosynthesis we are interested in the oxidation-reduction properties, i.e., its reduction potential. It can be shown that the reduction potentials of some substances change on irradiation with light. As we will see in the following sections, the nonradiative energy transfer and changes in the reduction potential of chlorophyll on excitation are very important in photosynthesis. We have seen the fate of the light energy absorbed but what substances are responsible for its absorption. Let us study this in the next subsection.

## SAQ 2

From the equation $E = h\nu$ , calculate the energy equivalent of one mole (or one einstein) of 1000 nm photons.

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### 12.4.2 Light Absorbing Substances in Photosynthesis

As mentioned before, pigments are a class of compounds that absorb visible light. Major light absorbing pigments of thylakoid membrane are chlorophylls. These are $Mg^{2+}$ ion complexes of five-ring porphyrin derivatives (pheoporphyrins) and are responsible for the characteristic green colour of the thylakoid membrane. Structures of chlorophyll a and b are shown in Fig. 12.3.

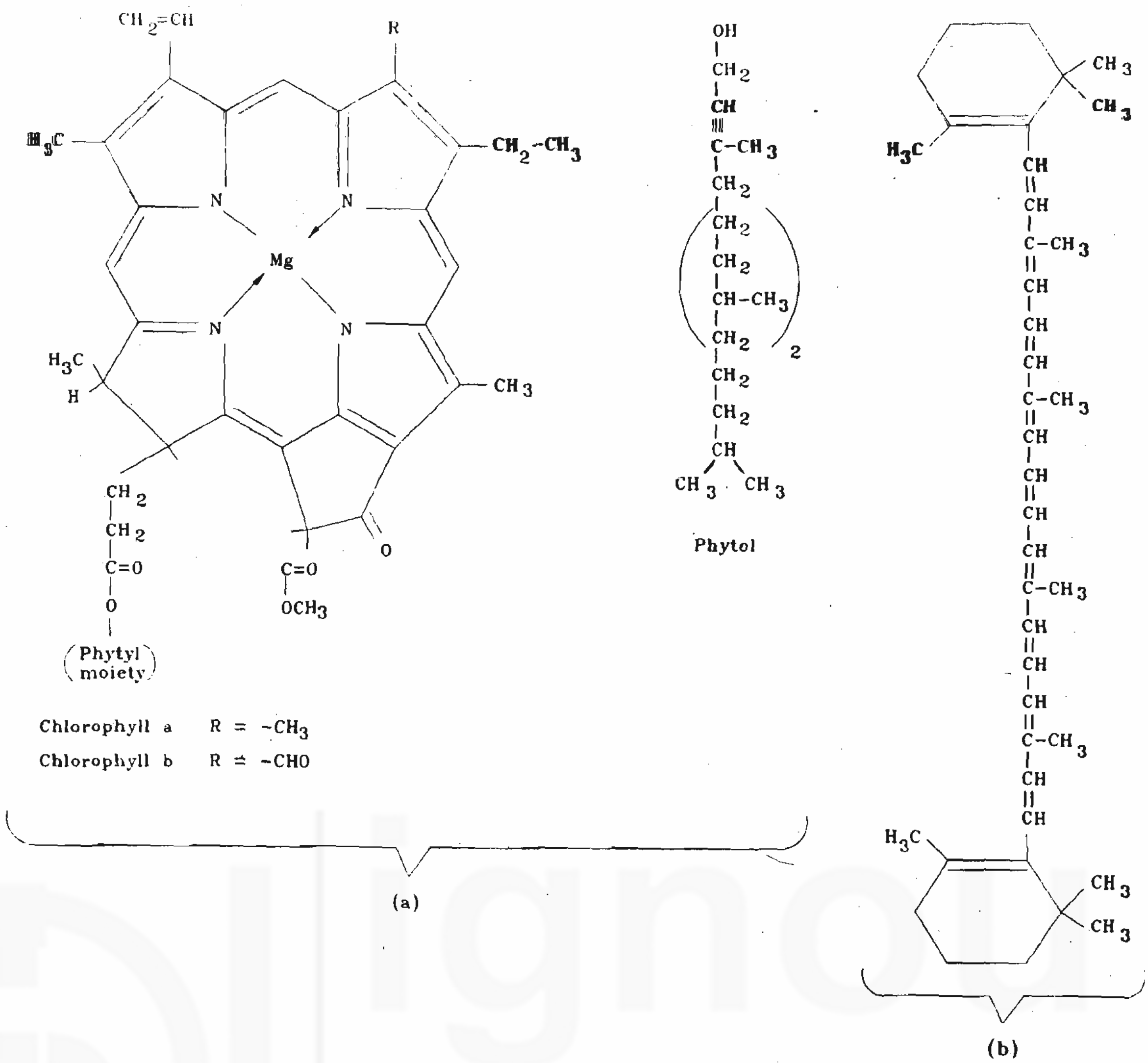


Fig. 12.3 : Structure of (a) chlorophyll a and b showing the phytol moiety separately and (b) β - carotene

When ingested by animals, each molecule of β-carotene gives rise to two of vitamin A.

Each chlorophyll has four substituted pyrrole rings, one of which is reduced. The fifth ring is not a pyrrole. A large isoprenoid alcohol, phytol, is attached by an ester linkage to a carboxylic acid side chain of chlorophyll. You can see that each chlorophyll molecule is planar and has a system of alternating single and double bonds (conjugated double bonds) which are responsible for its light absorbing, i.e., chromophoric, property. Chlorophyll molecules are bound to specific proteins which bring about small but significant changes in its spectral characteristics, e.g., the wavelength of maximum light absorbance, $\lambda_{max}$.

In addition to chlorophylls, thylakoids contain some other light absorbing substances which are collectively referred to as **accessory pigments**. These include **carotenoids** and **phycobilins**. Structure of one carotenoid, namely β-carotene, is shown in Fig. 12.3. Here also as you can see conjugated double bonds are present which together are responsible for its chromophoric property. It is red in colour.

Different accessory pigments absorb light of different wavelengths and, therefore, show a variety of colours like, yellow of xanthophyll (another carotenoid) and red of phycoerythrobilin. The accessory pigments function as supplementary light receptors which absorb light of different wavelength than chlorophylls. As we will see below, they transfer the absorbed light energy in a nonradiative transfer to specific chlorophyll molecules where transduction of light energy into chemical energy takes place. This helps enhance the efficacy of sunlight in bringing about photosynthesis, since the latter is a mixture of lights of different wavelengths.

The ratio of chlorophylls : accessory pigments and the chemical nature of the latter import characteristic colour to different photosynthetic cells, e.g., bluish green of pine needles, various shades of green in most plants and red/brown and even purple in a variety of decorative plants.

The light **absorption spectra**, i.e., relative absorption of light at different wavelengths of chlorophyll a, chlorophyll b and a leaf extract are shown in Fig. 12.4. Both the chlorophylls absorb light mostly in the wavelength ranges 400-500 nm and 600-700 nm. The accessory pigments present in the leaf extract account for the light absorption in the 500-600 nm range. The latter also helps in photosynthesis as is clear from a comparison of the absorption spectrum of the leaf extract and the photosynthetic **action spectrum.**

Action spectrum gives a measure of the efficiency of photosynthesis at different wavelengths. It is a curve obtained as a result of measurements of the quantum yield (molecules of $O_2$ evolved per quantum absorbed) with light of different wavelengths.

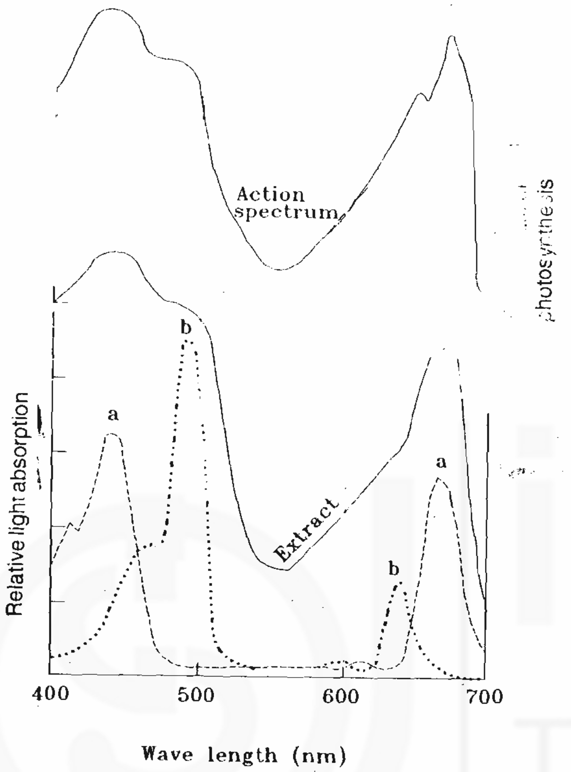


**Fig. 12.4 : Absorption spectrum of chlorophyll a (_ _ _), chlorophyll b (........) and a green leaf extract (———). The action spectrum of the leaf extract is shown in brown colour.**

You can see that the action and absorption spectra run parallel to each other except when the wavelength of light exceeds 680 nm. In this wavelength range the action spectrum shows a sudden and steep decrease, called **red drop**, as compared to the absorption spectrum. As you will study a little later, this red drop is an evidence of the existence of two photosystems.

## Photosystems and Photochemical Reaction Centres

The pigment molecules are not uniformly distributed in the thylakoid membrane but occur in clusters, called **photosystems**. For example, a typical photosystem of spinach leaf may contain about two hundred chlorophyll and about fifty carotenoid molecules. Together they account for the absorption spectrum of a leaf extract. However most of the chlorophyll present in photosystem is not photochemically active. Instead it serves as an antenna. When one of the molecules in the antenna system is excited by absorption of light it can transfer its energy to a neighbouring molecule by what is called the **resonance energy transfer**. It is the close spacing and rigid orientation of pigment molecules in the lamellae which make this kind of transfer possible. However, this transfer does not continue indefinitely and the energy is finally trapped by an electron-transfer reaction in a photochemical **reaction centre**. This reaction centre is a complex of chlorophyll bound to a protein. The energy level of the excited chlorophyll molecule at the reaction centre must be the lowest among the excited states of all the pigment molecules in a photosystem, because the energy gets trapped here in the form of its excited chlorophyll molecule and a back transfer is not possible. Thus the energy absorbed by all the chlorophyll molecules is funnelled to the reaction centre. This is called the **funnelling effect** which provides a convenient and efficient way of harvesting light energy. A schematic representation of the funnelling effect showing resonance energy transfer and an electron transfer reaction in a reaction centre is given in Fig. 12.5.

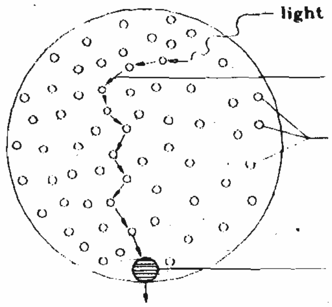


**Fig. 12.5 : Schematic representation a photosystem and photochemical reaction centre showing the funnelling effect**

The reaction centre molecule transfers the excited electron to a nonpigment acceptor in the next step. This constitutes the primary photochemical reaction.

$$\text{ChA} \xrightarrow{\text{energy transfer}} \text{Ch}^{*}\text{A} \xrightarrow{\text{electron transfer}} \text{Ch}^{+}\text{A}^{-} \quad \ldots(12.5)$$

where Ch is chlorophyll molecule at the reaction centre, A is acceptor molecule and $Ch^*$ is the excited state of Ch. Reduced A ($A^-$) may transfer electrons to other molecules and the oxidised Chlorophyll ($Ch^+$) must get electrons from a suitable donor. This donor in photosynthesis is water. The details of this reaction will be dealt with in the next section.

Coming back to photosystems, plant chloroplasts are found to contain two types of photosystems called photosystem I and II. Photosystem I absorbs light maximally at 700 nm and has a higher chlorophyll a: chlorophyll b ratio than photosystem II. The latter shows maximum absorption to 680 nm. The photosystems I and II are also referred to as P700 and P680, respectively, where P stands for pigment and the numbers refer to the wavelengths of maximum absorption of the two photosystems. The absorption of light by P680 decreases steeply at wavelengths longer than 680 nm and so does its efficiency. This results in the red drop, i.e., the fall in action spectrum when light of wavelengths longer than 680 nm is used. This shows that photosynthesis requires the participation of both the photosystems.

Excitation of photosystem I (P700) and photosystem II (P680) brings about the transfer of electrons from water to $NADP^+$ (Eq. 12.3) via a series of reactions as you will study in the next section where we discuss what actually happens in light reactions of photosynthesis. Before that, try to answer the following SAQ.

## SAQ 3

a) Why is it so that the photosynthetic pigments are arranged on chloroplast membranes and not in solution in the stroma?

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[illegible]) Match the following pairs correctly.

| | | | |
|---|---|---|---|
| 1) | absorption spectrum | i) | molecules of $O_2$ evolved per quantum absorbed |
| 2) | action spectrum | ii) | chlorophyll bound to a protein in a complex |
| 3) | reaction centre | iii) | efficiency of photosynthesis at different wavelengths |
| 4) | quantum yield | iv) | relative absorption of light at different wavelengths |

## 12.5 LIGHT REACTIONS OF PHOTOSYNTHESIS

From what was discussed in the previous section, you know that the primary photochemical reaction of photosynthesis involves the release of an electron from a molecule of chlorophyll by excitation. The chlorophyll molecules together constitute a complex acting as reaction centre and are components of the earlier mentioned two photosystems, viz., P700 and P680. To understand what actually happens in photochemical reactions of photosynthesis let us first understand the role of two photosystems.

### 12.5.1 Role of Photosystem I : Formation of NADPH

In addition to the antenna or light harvesting pigments and a chlorophyll molecule in the photochemical reaction centre, photosystem I contains acceptor chlorophyll molecules $A_o$ and $A_I$, an iron-sulphur protein, ferredoxin (Fd) and ferredoxin-NADP reductase. When P700 receives a light photon, it gets converted into its excited state $P700^*$, Eq. 12.6.

$$\underset{0.4 \text{ volt}}{P700} \xrightarrow{h\nu} \underset{-1.2 \text{ volt}}{P700^*} \qquad \text{...(12.6)}$$

standard reduction potential

You can see that the excited molecule $P700^*$ is a much stronger reductant than the same molecule in the ground state (P700). The excited molecule $P700^*$ transfers an electron to the acceptor chlorophyll $A_o$ to form $P700^+$ and $A_o^-$, Eq. 12.7.

$$P700^* + A_o \longrightarrow \underset{\text{oxidised form of P 700 ground state}}{P700^+} + \underset{\text{reduced form of } A_o}{A_o^-} \qquad \text{...(12.7)}$$

This reaction is responsible for the charge separation. The reduced form of $A_o$ ($A_o^-$) is also a very strong reductant, i.e., it has a large negative reduction potential. It transfers its electron to the acceptor chlorophyll $A_I$.

$$A_o^- + A_I \longrightarrow A_o + A_I^-$$

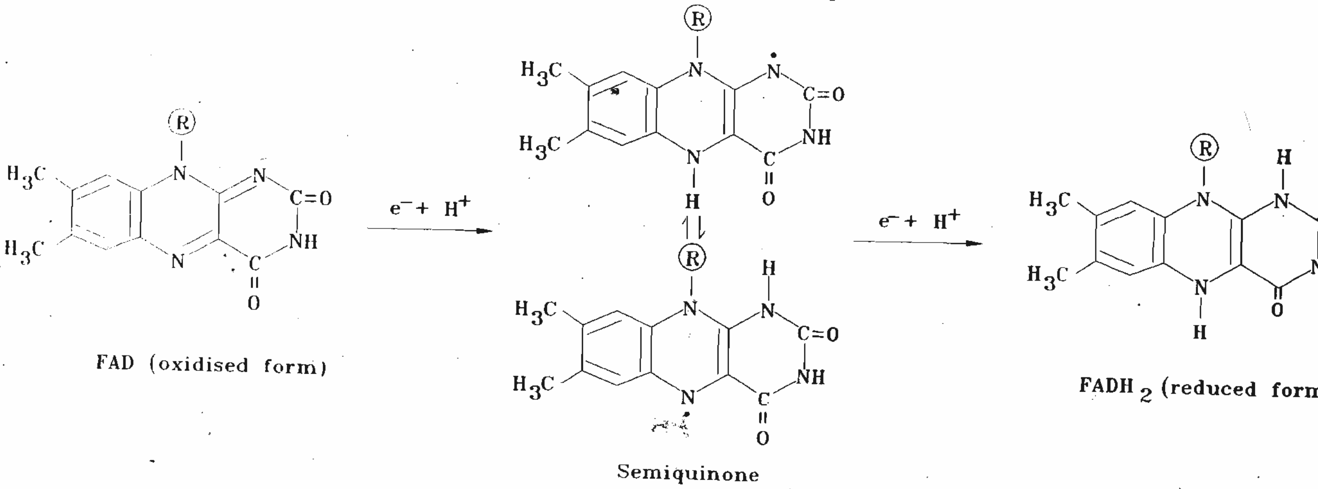
This flow of electron continues through the iron-sulphur protein, ferredoxin and ferredoxin-reductase to $NADP^+$, Scheme I. The electron flow in this scheme is downhill from a species having a more negative to one having a less negative reduction potential.

$$P700 \Rightarrow P700^* \longrightarrow A_0 \longrightarrow A_I \longrightarrow Fe\text{-}S \longrightarrow Fd \longrightarrow Fd^- \longrightarrow NADP^+$$

standard reduction potential $E^{o\prime} = 0.4$ volt; $P700^*$: $E^{o\prime} = -1.2$ volt; Fe-S: protein; Fd: ferredoxin; Fd⁻: ferredoxin reductase (a flavoprotein); $NADP^+$: $E^{o\prime} = -0.32$ volt

**Scheme - I**

Ferredoxin-reductase is a flavoprotein having FAD as the prosthetic group. FAD can undergo two successive reduction steps of one electron each with a semiquinone intermediate, Eq. 12.8. This helps to collect two electrons which are required to reduce one molecule of $NADP^+$ to NADPH, Eq. 12.9.




...(12

$$FADH_2 + NADP^+ \longrightarrow FAD + NADPH + H^+ \quad ...(12.9)$$

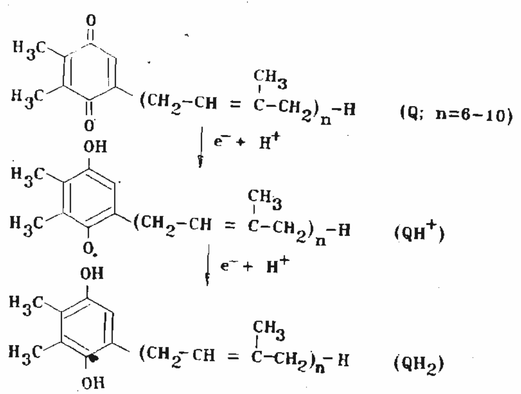
Summing up the reactions of scheme I and multiplying by two, so as to account for the transfer of two electrons, we can write the overall reaction as:

$$2\,P700^* + NADP^+ + H^+ \longrightarrow 2\,P700^+ + NADPH \quad ...(12.10)$$

The reaction takes place on the stromal side of the thylakoid membrane. Since a proton is taken up or consumed the reaction produces a proton gradient with the interior of the thylakoid becoming more acidic. Further, it leaves an electron hole in the form of $P700^+$ which must be converted back to P700 so that the reaction centre is regenerated to accept another photon. Only then can the formation of NADPH continue. Conversion of $P700^+$ to P700 is brought about by an electron transfer from photosystem II to photosystem I as described in the next subsection.

### 12.5.2 Role of Photosystem II : Splitting of $H_2O$

Photosystem II contains the usual antenna or light harvesting pigments and a chlorophyll molecule at the reaction centre (P680). In addition, it contains pheophytin, plastoquinone, cytochrome bf complex and plastocyanin. Pheophytin is a prophyrin identical to chlorophyll a without $Mg^{2+}$ ion and is bound to the membrane. Plastoquinone has a structure similar to that of ubiquinone which participates in the mitochondrial electron transport. Like all quinones, it is reduced in two steps of one electron each to plastoquinol ($QH_2$), Eq. 12.11. In actively functioning photosystem II, plastoquinone is cycled between Q and $QH_2$. Plastoquinone molecules are bound to different protein sites, referred to as $Q_A$ and $Q_B$. The cytochrome bf complex contains two cytochromes called $b_{563}$ and f (leaf cytochrome) and an iron-sulphur protein. This complex helps to transfer electrons from reduced plastoquinone ($QH_2$) to plastocyanin. The latter has a copper ion at its active site which cycles between $Cu^{2+}$ and $Cu^+$ oxidation states.




...(12.11)

On irradiation of photosystem II, an electron of chlorophyll at the reaction centre is boosted to a higher energy level giving rise to the excited state P680*. The latter is a much stronger reductant $E^{o\prime}$ (approx. 1-0.8 V) than the corresponding ground state molecule P680 ($E^{o\prime}$ = + 1.0V). This high energy electron is transferred within a few picoseconds from P680* to pheophytin (Ph) and the latter is reduced to $Ph^-$. In about one hundred picoseconds the electron is transferred to a plastoquinone molecule at the protein site A (Eq. 12.12). These two reactions ensure charge separation.

One picosecond equals $10^{-12}$ seconds.

$$\begin{bmatrix} P680 \\ Ph \\ Q_A \end{bmatrix} \xrightarrow{h\nu} \begin{bmatrix} P680^* \\ Ph \\ Q_A \end{bmatrix} \xrightarrow{<10ps} \begin{bmatrix} P680^+ \\ Ph^- \\ Q_A \end{bmatrix} \xrightarrow{100ps} \begin{bmatrix} P680^+ \\ Ph \\ Q_AH^{\bullet} \end{bmatrix} \quad ...(12.12)$$

Plastoquinone at site $Q_A$ transfers the electron to plastoquinone at site $Q_B$ giving rise to successively $QH_B$. and $QH_{2B}$. Only the fully reduced plastoquinol ($QH_2$) is released from the protein site $Q_B$ into the hydrophobic region of the thylakoid membrane. Cytochrome bf complex helps to transfer the reducing power of $QH_2$, one electron at a time, to plastocyanin (PC) which is reduced to the cuprous state, Eq. 12.13.

$$QH_2 + 2PC(Cu^{++}) \xrightarrow{\text{cytochrome bf}} Q + 2\,PC(Cu^+) + 2H^+ \quad ...(12.13)$$

Reduced plastocyanin transfers its electron to $P700^+$ of photosystem I and is thereby to the cupric state, Eq. 12.14.

$$PC(Cu^+) + P700^+ \longrightarrow PC(Cu^{++}) + P700 \quad ...(12.14)$$

Thus, the electron hole of photosystem I gets filled up. Photosystem I has been regenerated and is now ready to receive another photon. However, in doing so an electron hole has now been created in photosystem II in the form of $P680^+$. The electron poor $P680^+$ is a very strong oxidising agent and is converted back into P680 by accepting electron(s) from a water molecule which is thereby oxidised to molecular oxygen.

Water splitting enzyme, which is a constituent of photosystem II, contains a cluster of manganese ions at its active site. It can bind four or six oxygen atoms by assuming different geometries. Oxygen atoms released on transfer of electrons from water are accommodated by the enzyme active site having four bound oxygens to raise their number to six. A change in active site geometry releases two oxygen atoms in the form of an oxygen molecule and the active site gets regenerated to accept more oxygen atoms from water. The electrons released from water are transferred to $P680^+$ via an intermediary called Z. The manganese ions cluster of the water splitting enzyme helps in preventing the formation of hazardous intermediates between water and oxygen.

Combining the effect of irradiating P700 and P680, the above reactions can be summarised in the following equations.

$$2\,P700 \longrightarrow 2\,P700^*$$

$$2\,P700^* + NADP^+ + H^+ \longrightarrow 2\,P700^+ + NADPH$$

$$2\,P680 \xrightarrow{2h\nu} 2\,P680^*$$

$$2\,P700^+ + 2\,P680^* \longrightarrow 2\,P700 + 2\,P680^+$$

$$2\,P680^+ + H_2O \rightarrow 2\,P680 + 2H^+ + \frac{1}{2}O_2$$

A summation of these equations gives rise to:

$$NADP^+ + H_2O \xrightarrow{4h\nu} NADPH + H^+ + \frac{1}{2}O_2 \quad ...(12.15)$$

Multiplying the above equation by two we get Eq. 12.3. There is evidence which indicates that the two photosystems are connected in series. It will become clear when we look into how the two interact together in the next subsection.

### 12.5.3 Interaction of Photosystems I and II

A summary of the various steps described above taking place in photosystems I and II is shown in Fig. 12.6. The verticle scale in this figure gives an approximate idea of the standard reduction potentials ($E^{o\prime}$) of the reactants. A higher position in this figure represents a stronger reductant and the thermodynamically spontaneous flow of electrons is in the downward direction. An upward pumping of electrons requires an input of energy, as happens in the excitation of P700 and P680 molecules. Excitation of P700 generates a strong reductant which transfers electrons to NADP by way of several secondary electron carriers. Excitation of P680 followed by transfer an electron to $P700^+$ generates a strong oxidant, $P680^+$, which oxidises $H_2O$ to $O_2$. The reductant formed in P680 injects electrons into a chain of carriers that connect the two photosystems. This scheme is called the **Z scheme** and was first suggested by R. Hill and F. Bendall.

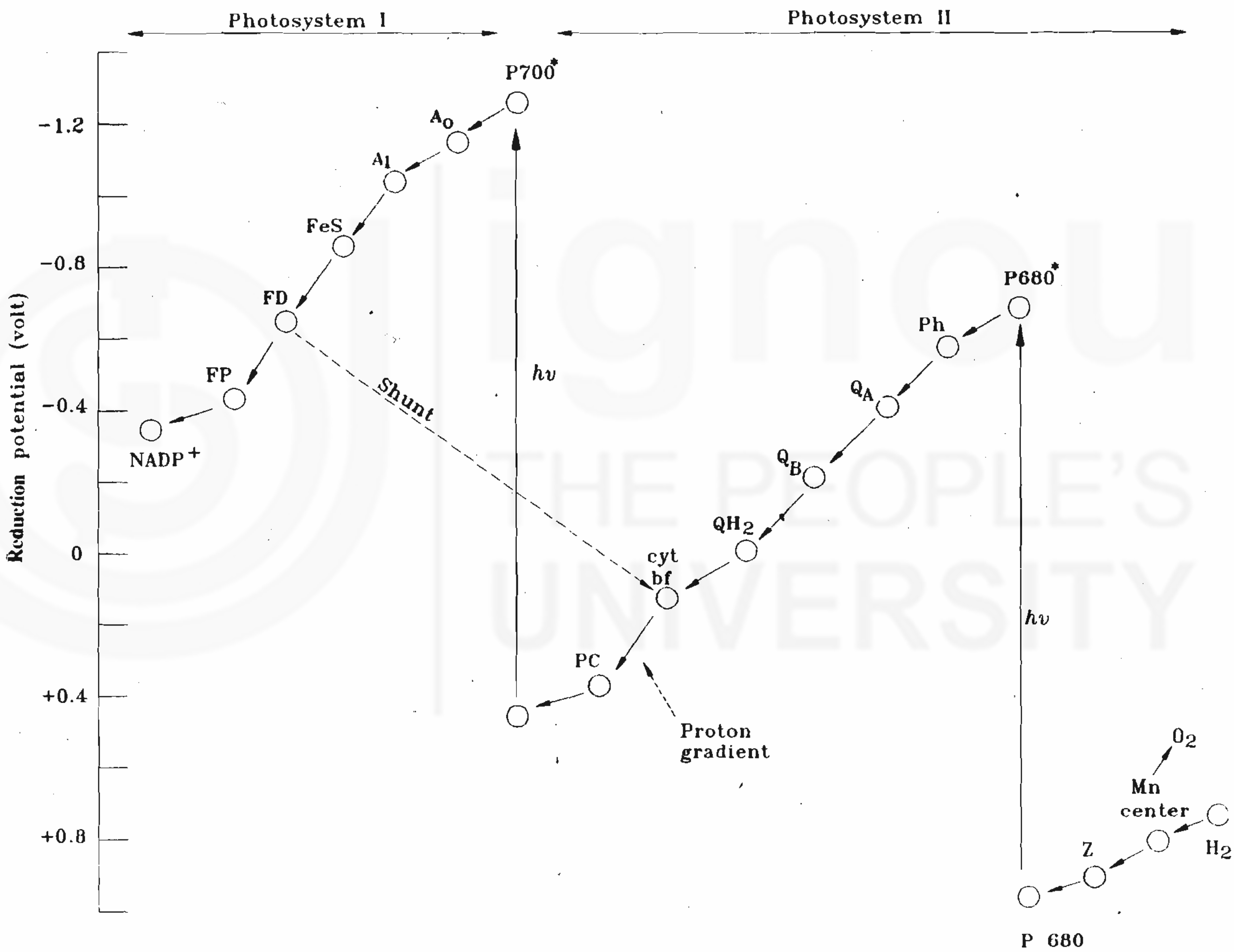



**Fig. 12.6 : Pathway of electron flow in photosystems I and II : The Z scheme FP (flavoprotein), FD (ferredoxin), Ph (pheophytin) QA and QB (plastoquinone), Cyt bf (cytochrome bf), PC (plastocyanin), Z (z protein)**

## Photophosphorylation

In addition to the electron transfer, the complete process depicted in Fig. 12.6 brings about the formation of at least one molecule of ATP for each NADPH formed as was mentioned in Sec. 8.4 also. The synthesis of ATP by photosynthetic systems is termed **photophosphorylation**. In green plants ATP can be synthesised not only during electron transport between water and NADP but also during a separate sequence of electron transfer reactions involving only photosystem I. The two processes are known as **noncyclic and cyclic photophosphorylation**, respectively. This noncyclic photophosphorylation is achieved by establishing a proton gradient as in the mitochondrial electron transport. In this case, the interior of the thylakoid is more acidic than the stromal side. The protons drive the synthesis of ATP as they flow back in a similar manner as described in Unit 8. One site where a proton gradient is known to be established is during the transfer of electron from cytochrome bf complex to plastocyanin, Fig. 12.6. The other site is not at all clear. The most commonly accepted stoichiometry of the total reaction is give below:

$$2H_2O + 2NADP^+ + 2ADP + 2P_i \xrightarrow{8h\nu} O_2 + 2NADPH + 2ATP \quad ...(12.16)$$

The relative demands of NADPH, i.e., the reducing power, and ATP, i.e., the readily utilisable free energy, may not always correspond to the ratio of their formation by the operation of photosystems I and II described above. Larger quantities of ATP are frequently required. Under such conditions ATP is produced by cyclic photophosphorylation mentioned above. For this purpose, a shunt pathway operates in which the electron is transferred from reduced ferredoxin to cytochrome bf complex from where it flows back to fill the electron hole in $P700^+$, Fig. 12.6. As indicated in the figure, this generates a proton gradient and the latter drives the synthesis of ATP. You can very well see that it is a cyclic process in which a part of the energy of a photon is utilised in the endergonic ATP synthesis.

NADPH and ATP generated in the above light reaction are then utilised to convert carbon dioxide into carbohydrates. The latter conversion, called the **dark reaction,** is accomplished via a series of reactions which require a reducing agent, NADPH, and a source of free energy, ATP. You would recall that the oxidation of glucose to carbon dioxide and water releases a large amount of energy. Correspondingly input of energy is required to convert carbon dioxide and water into glucose and oxygen. Part of this energy is provided in the form of NADPH and the rest as ATP. Since these are generated by photosystems I and II by making use of sunlight, the latter is the ultimate source of the entire amount of energy input for photosynthesis. You will study the dark reaction in the next section after solving the following SAQ.

### SAQ 4

a) The photosynthetic pigments absorbing wavelengths longer than 680 nm is:

   i) chlorophyll a

   ii) chlorophyll b

   iii) both of the above

   iv) none of the above

b) From Fig. 12.6 can you guess why has this scheme been named a Z scheme?

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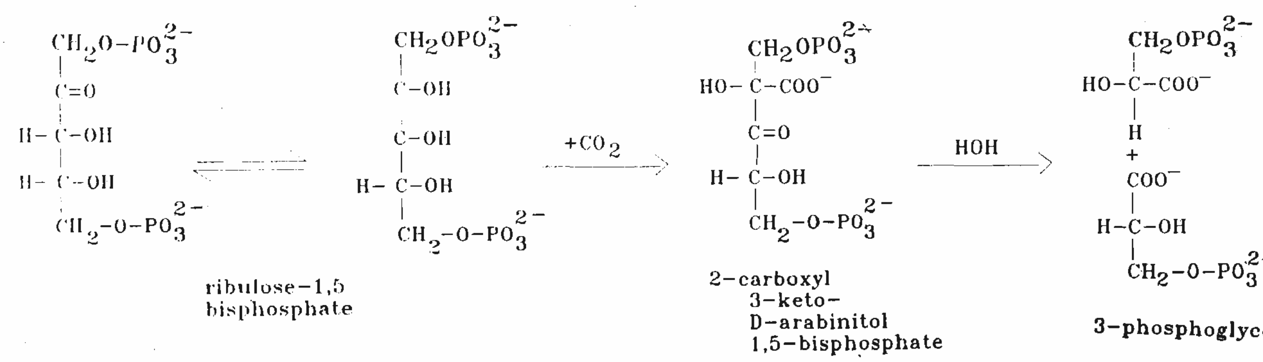
## 12.6 DARK REACTIONS OF PHOTOSYNTHESIS

The dark reactions make up the second phase of photosynthesis and result in the formation of glucose. $CO_2$ is incorporated or "fixed" into organic compounds in these reactions.

In the late 1940s, Melvin Calvin and his coworkers showed that the first detectable product formed on illuminating a suspension of green algae in the presence of labelled carbon dioxide ($^{14}CO_2$) for a few seconds was $^{14}C$-labelled 3-phosphoglycerate in which the label was localised in the carboxyl group. It was also shown that 3-phosphoglycerate could be converted into carbohydrates by plant extracts. This experiment showed that $CO_2$ is incorporated into carbohydrates. The chemistry of the conversion of $CO_2$ into 3-phosphoglycerate and the subsequent reactions has been worked out. A complicated cyclic sequence of reactions is involved, which together are referred to as the **Calvin cycle** or **reductive pentose cycle**. It is also called the **$C_3$ cycle** because the first stable products in this are the molecules with three carbon atoms.

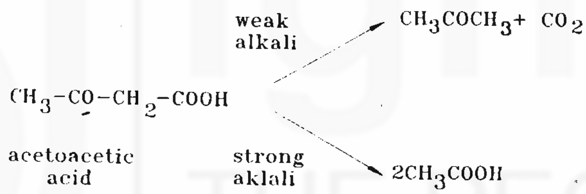
In 1961 Calvin was awarded a Nobel prize for his research.

In the first reaction, carbon dioxide reacts with ribulose-1,5-bisphosphate to give rise to two molecules of 3-phosphoglycerate, Eq. 12.17.



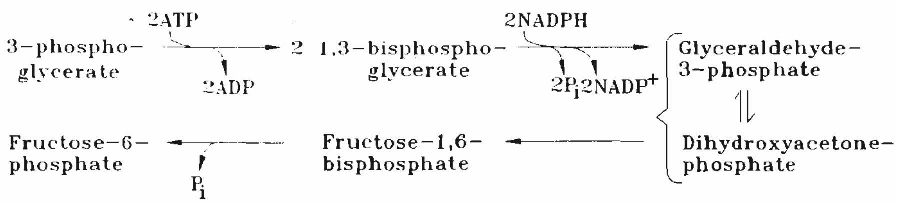

...(12.17)

You can see that the first product or intermediate formed on condensation of carbon dioxide is a β-keto acid which gets hydrolysed to form two molecules of 3-phosphoglycerate. The hydrolytic fission is similar to one of the modes of hydrolysis of the well known β-keto acid, namely acetoacetic acid.




The enzyme catalysing the reaction given in Eq. 12.17 is called **ribulose-1,5-bisphosphate carboxylase**, commonly abbreviated as **Rubisco**. It is localisd on the stromal surface of the thylakoid and constitutes approximately 15% of the total chloroplast protein. It is probably the most abundant enzyme in nature and has been well characterised.

The steps leading from 3-phosphoglycerate to fructose-6-phosphate are similar to those described for gluconeogenesis in Unit 9 and are summarised below:




... (12.18)

Two molecules of 3-phosphoglycerate formed in the reactions of Eq. 12.17 give rise to one molecule of fructose-6-phosphate, because the required dihydroxytacetone phosphate is generated by the isomerisation of one molecule of glyceraldehyde-3-phosphate. It may be noted that the reductant employed in the photosynthetic series of reactions is NADPH, instead of NADH, employed in gluconeogenesis. Summation of Eq. 12.17 and 12.18 gives rise to Eq. 12.19 and shows that these reactions bring about the reduction of one molecule of $CO_2$ to the **level** of a hexose by the expenditure of one molecule each of NADPH and ATP.

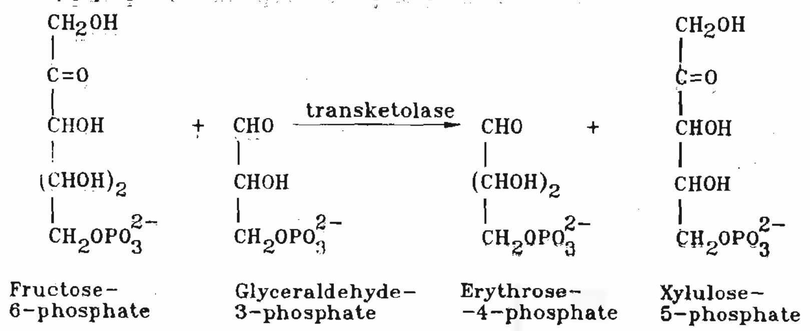
$$\text{Ribul-1,5-bis-P} + CO_2 + 2ATP + 2NADPH \longrightarrow 2G-3-P + 2NADP^+ + 2ADP + 2P_i \quad \ldots(12.19A)$$

$$2G-3-P \longrightarrow F-6-P + P_i \quad \ldots(12.19B)$$

$$\text{Ribul-1,5-bis-P} + CO_2 + 2ATP + 2NADPH \longrightarrow F-6-P + 2NADP^+ + 2ADP + 3P_i$$

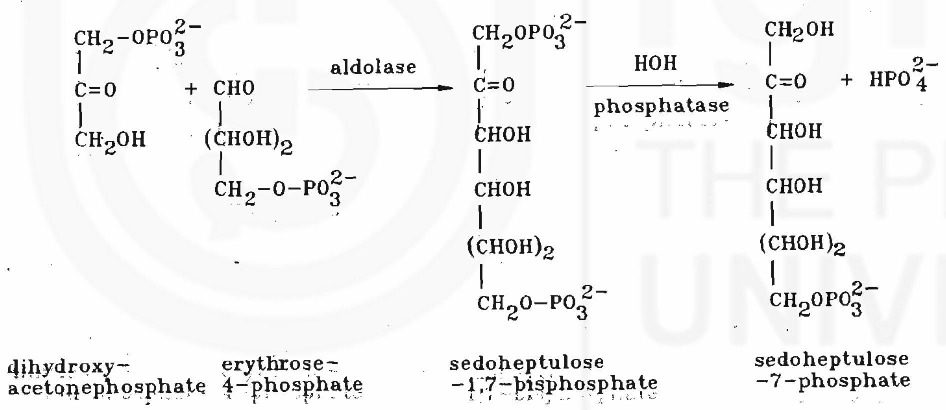
However, the starting substrate, ribulose-1, 5-bisphosphate, must be regenerated in order that this process can go on. The remaining reactions of the Calvin cycle are devoted to this purpose and involve two types of enzymes, namely, transketolases and aldolases.

Fructose-6-phosphate formed as per the reactions of Eq. 12.18 combines with another molecule of glyceraldehyde-3-phosphate in the presence of a transketolase generating erythrose-4-phosphate and xylulose-5-phosphate as shown below:



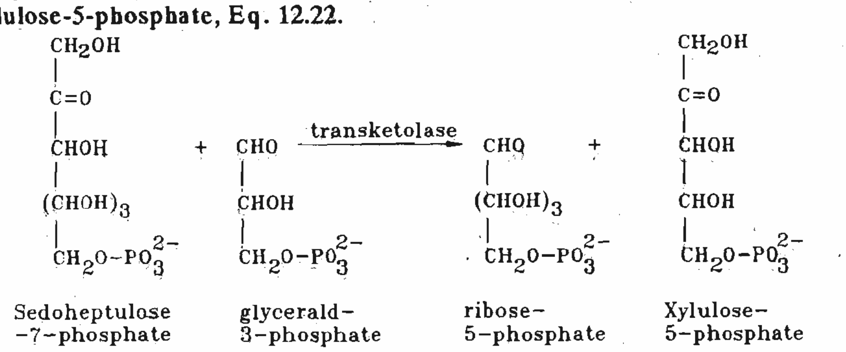

...(12.20)

Erythrose-4-phosphate goes on to combine with dihydroxyacetone phosphate to give sedoheptulose-1,7-bisphosphate, catalysed by aldolase.




...(12.21)

Seven-carbon sugar reacts with glyceraldehyde-3-phosphate in another transketolase-catalysed reaction forming ribose-5-phosphate and another molecule of xylulose-5-phosphate, Eq. 12.22.




...(12.22)

Xylulose-5-phosphate and ribose-5-phosphate both can be isomerised to ribulose-5-phosphate with the help of specific isomerases, so that the net reaction can be written as Eq. 12.23.

$$\underset{(C_6\text{-sugar})}{\text{Fructose-6-phosphate}} + 2\underset{(C_3\text{-sugar})}{\text{Glycerald-3-phosphate}} + \underset{(C_3\text{-sugar})}{\text{Dihydroxyacetone-phosphate}} \longrightarrow 3\ \underset{(C_5\text{-sugar})}{\text{Ribulose-5-phosphate}} + P_i \quad \ldots (12.23)$$

Finally, ribulose-5-phosphate is converted into ribulose-1,5-bisphosphate on reaction with ATP, which is catalysed by phosphoribulose kinase, Eq. 12.24, and completes the cycle.

$$\text{Ribulose-5-phosphate} + \text{ATP} \longrightarrow \text{Ribulose-1,5-bisphosphate} + \text{ADP} \quad \ldots (12.24)$$

In order to appreciate the cyclic nature of these set of reactions and arrive at the overall stoichiometry, try to answer SAQ 5 given at the end of this section.

Plants adapted to hot dry climates make use of another pathway for photosynthesis called the $C_4$ **pathway**. Here a $CO_2$ molecule binds in a special leaf cell to a three carbon compound rather than to the five carbon ribulose bisphosphate. This fixation results in a four carbon compound which is then transported to an adjacent cell. The advantage of this pathway is that $C_4$ plants make a more efficient use of $CO_2$ by fixing carbon upto four times as fast as $C_3$ plants do. This allows them to grow in higher temperatures and at much faster rates.

The enzyme, ribulose-1,5-bisphosphate carboxylase is absent in animals and human beings. Consequently animals and human beings cannot use carbon dioxide as carbon source for the synthesis of their biomolecules. They depend on plants for the organic compounds, which they utilise as sources of carbon as well as energy. Now you can try to answer the following SAQs.

**SAQ 5**

Find out the stoichiometry of $CO_2$ fixation, i.e., of the dark reaction of photosynthesis.

(Hint: Multiply Eq. 12. 19A by 6, 12.19B by 3, 12.23 by 2 and 12.24 by 6. Take a sum of these reactions. In the present case, the stoichiometry can be more easily arrived at by considering the number of carbon atoms in each substrate species.)

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**SAQ 6**

a) Is it necessary that the dark reactions of photosynthesis take place in dark? Explain.

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b) Tick √ on the correct answer.
Human beings cannot synthesise glucose from $CO_2$ and $H_2O$ because

i) they cannot absorb sunlight.

ii) they lack the enzyme ribulose-1,5-bisphosphate carboxylase.

iii) they depend on plants for that.

iv) they lack the pigments present in plants.

## 12.7 SUMMARY

Let us summarise what all has been discussed about photosynthesis in this unit.

Synthesis of carbohydrates from carbon dioxide under the influence of sunlight is called **photosynthesis**. It takes place in all plants, blue green algae and some bacteria. In plants, this activity is localised in chloroplasts which contain membrane-enclosed vesicles called thylakoids stacked on one another. The pigments responsible for absorption of light are present in the thylakoid membrane in clusters, called photosystems, which are of two types namely photosystem I and II. They are also called P700 and P680, respectively, indicating the wavelengths of maximum light absorption. Each photosystem has a large number of pigment molecules which absorb light and pass on the absorbed energy to specific chlorophyll molecule bound to a protein constituting the photochemical reaction centre, where the light energy is transformed into chemical energy. Both the systems must be excited by light for bringing about photosynthesis. The two photosystems work in a series and follow a Z scheme in bringing about transfer of electrons from water to $NADP^+$. In addition to the electron transfers, the two photosystems also produce a proton gradient where the interior of the thylakoid becomes more acidic than the outside medium. This proton gradient drives the synthesis of at least one mole ATP for each mole NADPH formed. This is accomplished by cyclic and noncyclic photophosphorylation. The above reactions are collectively called "light reactions" of photosynthesis, because these are light-dependent. The rest of the reactions are light independent and referred to as dark reactions. The latter utilise NADPH and ATP generated during the light reaction. A series of these reactions bring about the formation of fructose-6-phosphate from six molecules of $CO_2$ with the expenditure of 12 NADPH and 18 ATP molecules.

## 12.8 TERMINAL QUESTIONS

1) The equation for photosynthesis is frequently written as

$$6\,CO_2 + 12\,H_2O \longrightarrow C_6H_{12}O_6 + 6H_2O + 6O_2$$

Is it correct? Explain your answer.

2) The rate of photosynthesis, measured as the rate of oxygen liberation, is much higher when mixed light of 680 and 700 nm wavelengths is used than when the same intensity of either 680 or 700 nm wavelength is used. How is this explained?

3) Irradiating photosystem I (P700) with light of 700 nm wavelength changes its standard reduction potential from + 0.4 to –1.2 volts. What fraction of the light energy is transduced in the form of this "reducing power"?

4) The steady state concentrations of ATP, ADP and phosphate ions in isolated and illuminated chloroplasts are 120, 6 and 700 μM respectively.

   i) What is the $\Delta G^{\circ\prime}$ for the formation of one mole ATP in the chloroplasts?

   ii) In the cyclic photophosphorylation with light of 700 nm, what fraction of the absorbed light energy is utilised in ATP formation?

   **Note** : For questions 3 and 4 it will be necessary to apply appropriate equation from Unit 8 and the data of Table 8.1 of that unit. In each case, assume pH equal to 7.0 and temp. 298 K.

5) Define photophosphorylation. How is noncyclic photophosphorylation different from the cyclic one?

## 12.9 ANSWERS

### Self Assessment Questions

1) a) Light reaction

Dark reaction

$$CO_2 + 2NADPH \xrightarrow{ATP \quad ADP} CH_2O + 2NADH + H_2O$$

b) The light reactions take place in the membrane part while the dark reactions take place in the stroma of chloroplast.

2) Velocity of light = wavelength (λ) × frequency (ν)

$$= \frac{3 \times 10^8 ms^{-1}}{1000 \times 10^{-9} m} = 3 \times 10^{14} s^{-1}$$

$$= E = h\nu \; ; h = 6.626 \times 10^{-34} Js$$

Energy of one mole of 1000 nm photons = $h\nu \times$ Avogadro's number

$$= 6.626 \times 10^{-34} \times 3 \times 10^{14} \times 6.022 \times 10^{23}$$

$$= 119.705 \times 10^3 J$$

$$= 119.705 kJ$$

3) a) The orderly arrangement is essential for effective resonance transfer and electron transfer. Also, chlorophylls and carotenoids are lipid soluble and their high concentrations cannot remain in free solution in the aqueous stroma of the chloroplast.

b) 1 - iv, 2 - iii, 3 - ii, 4 - i

4) a) c

b) It has been named so because it looks like a letter Z on its side.

5) $6 \times (12.19A)$; $6 C_5 - bisP + 6 CO_2 + 12 ATP + 12 NADPH \rightarrow 12 C_3 - P + 12 NADP^+ \cdot 12 ADP + 12 P_i$

$3 \times (12.19B)$; $6 C_3 - P \longrightarrow 3 C_6 - P + 3 P_i$

$2 \times 12.23$ ; $2 C_6 - P + 6 C_3 - P \longrightarrow 6 C_5 - P + 2 P_i$

$6 \times 12.24$ ; $6 C_5 - P + 6 ATP \longrightarrow 6 C_5 - bis - P + 6 ADP$

sum $6CO_2 + 18ATP + 12 NADPH --> C_6 - P + 18 ADP + 12 NADP^+ + 17 P_i$

6) No, the dark reactions are light independent and can occur in dark. However, these reactions also occur in light.

b) ii)

## Terminal Questions

1) Photosynthesis consists of "light" and "dark" reactions. The light reaction produces NADPH from $NADP^+$ with a concomitant oxidation of water to molecular oxygen as per equation (A).

$$2 NADP^+ + 2H_2O \rightarrow 2 NADPH + 2H^+ + O_2 \quad \ldots (A)$$

In the dark reaction, the net overall chemical change is the "reduction" of $CO_2$ to a hexose (Eq. B).

$$6 CO_2 + 12 NADPH + 12 H^+ \longrightarrow C_6H_{12}O_6 + 6H_2O + 12 NADP^+ \quad \ldots (B)$$

Multiplying Eq. A by 6 and adding Eq. B gives the overall stoichiometry of the photosynthetic reaction. Note that 12 water molecules are required to produce the necessary amount of NADPH. Six water molecules are produced in the dark reaction. The equation given in the question explicitly states the number of water molecules required and produced in the complete process, which is not a single reaction. To that extent it is correct.

2) Photosynthesis requires the excitation and participation of two photosystems, namely photosystem I and II. These are maximally excited with light of wavelength 700 and 680 nm, respectively. Therefore, a high rate is obtained when light of both wavelengths is present. When light of only one of these wavelengths is used, one of the photosystems is optimally excited, but the other is not. Therefore, the rate of photosynthesis decreases.

3) Energy of one mole (or one einstein) of photons of 700 nm is found to be equal to 170.9 kJ. The energy required to change the standard reduction potential can be calculated with the help of the following equation which you have studied in Unit 8, Sec. 8.2.2.

$$\Delta G^{o\prime} = -n.F.\Delta E^{o\prime}$$

$$= -1 \times 96.485 \times (-1.6)$$

$$= 154.4 \text{ kJ}$$

Thus, the fraction of light energy used (or transduced)

$$= 154.4 \times 100/170.9 = 90.3\%$$

4) i) $$\Delta G' = \Delta G^{o\prime} + 2.303 \times R \times T \times \log\frac{[ATP]}{[ADP] \times [P_i]}$$

$$= (30{,}500) + 2.303 \times 8.314 \times 298 \times \log\frac{120 \times 10^{-6}}{(6 \times 10^{-6}) \cdot (700 \times 10^{-6})}$$

$$= 30{,}500 + 25{,}425 = 55{,}925 \text{ J mol}^{-1}$$

$$= 55.93 \text{ kJ mol}^{-1}$$

ii) Energy of one mole of photons of 700 nm wavelength
= 170.9 k J (See the answer to SAQ 2 for calculation).
Therefore, % of light energy utilised in ATP synthesis

$$= 55.93 \times \frac{100}{170.9} = 32.7\%$$

5) Photophosphorylation is the synthesis of ATP from ADP under anaerobic conditions in light. Noncyclic photophosphorylation involves the ATP synthesis during electron transport between water and NADP while cyclic photophosphorylation drives the ATP synthesis making use of only photosystem I during a sequence of electron transfer reactions.

## Further Readings

1) *Harper's Review of Biochemistry* (18th ed.)
D.W. Martin, P.A. Mayes and V.W. Rodwell
Lange Medical Publications, Maruzen Asia (Pte.) Ltd.
Singapore
**1981**

2) *Outlines of Biochemistry* (4th ed.)
Eric E. Conn and P.K. Stumpf
Wiley Eastern Limited, New Delhi
**1976**

3) *Principles of Biochemistry*
Albert L. Lehninger
CBS Publishers and Distributors, Delhi
**1984**

Besides the above books which will be provided in your study centres, we also suggest that you may consult the following books which may be available to you from other sources.

1) *Biochemistry - the chemistry of life*
David T. Plummer
McGraw-Hill International Editions, London
**1989**

2) *Biochemistry* (2nd ed.)
Geoffrey Zubay
Macmillan Publishing Company
**1988**

3) *Biochemistry (3rd ed.)*
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W. H. Freeman & Co., N.Y.
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