1. Details of Module and its structure

Module Detail	
Subject Name	Biology
Course Name	Biology 02 (Class XI, Semester - 2)
Module Name/Title	Light Reactions of Photosynthesis: Part – 2
Module Id	kebo_101302
Pre-requisites	Basic information of
Objectives	After going through this lesson, the learners will be able to understand the following: • The basic features of respiration in plants • The process of glycolysis • The process of anaerobic respiration, alcoholic and lactic acid fermentation
Keywords	Cellular Respiration, Aerobic Respiration, Aerobic Respiration, Glycolysis, Lactic Acid Fermentation, Alcoholic Fermentation, Gaseous Exchange in Plants

2. Development Team

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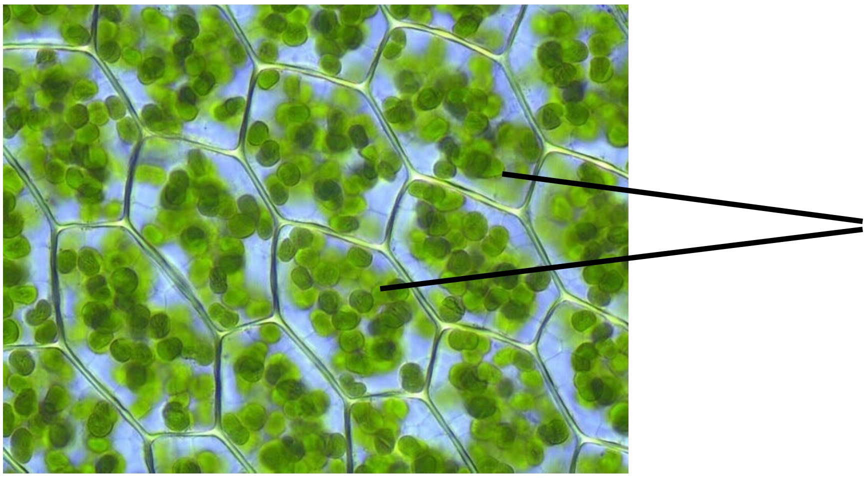
Introduction

In module 1 we learnt that photosynthesis occurs in the green parts of plants, and plant parts are green because they contain cells with green chloroplasts, the prime organelles involved in photosynthesis. About 20-50 chloroplasts are present in a mesophyll cell of an angiosperm leaf.

Chloroplasts are double-membrane bound organelles. The interior of the organelles enclosed by the two membranes, the outer and inner, is known as the **stroma**. A third distinct membrane, the thylakoid membrane, which forms disc-like sacs or the **thylakoids**, is present embedded in the stroma. At places the thylakoids stack up and form aggregates called the '**grana**'. The thylakoids are interconnected and the interior of the thylakoids is a single continuous fluid-filled region forming the thylakoid **lumen**. The thylakoids connecting the grana are called the inter-granal or stromal thylakoids.



Chloroplasts

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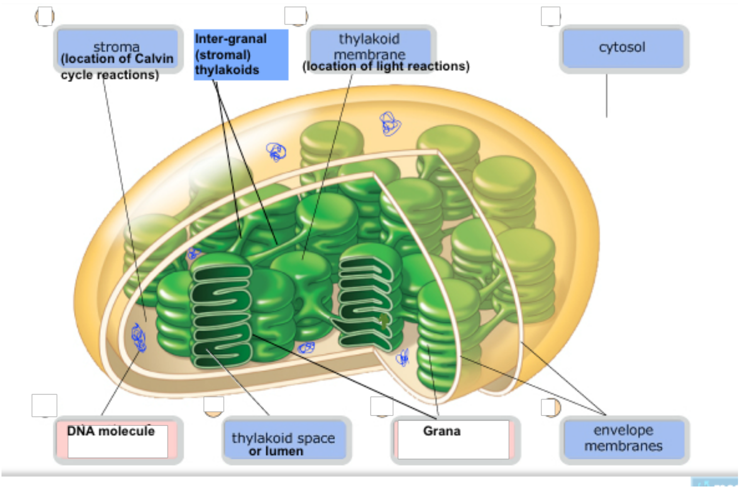

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By 1954, it was well established that photosynthesis is a two-step process consisting of the light-dependent step and the light-independent step, and in the light-dependent step, light energy is converted to chemical energy with the help of pigments and other components present in the thylakoid membranes (Module 1). Thus, the entire light-dependent process occurs in the thylakoids.

It involves a number of events:

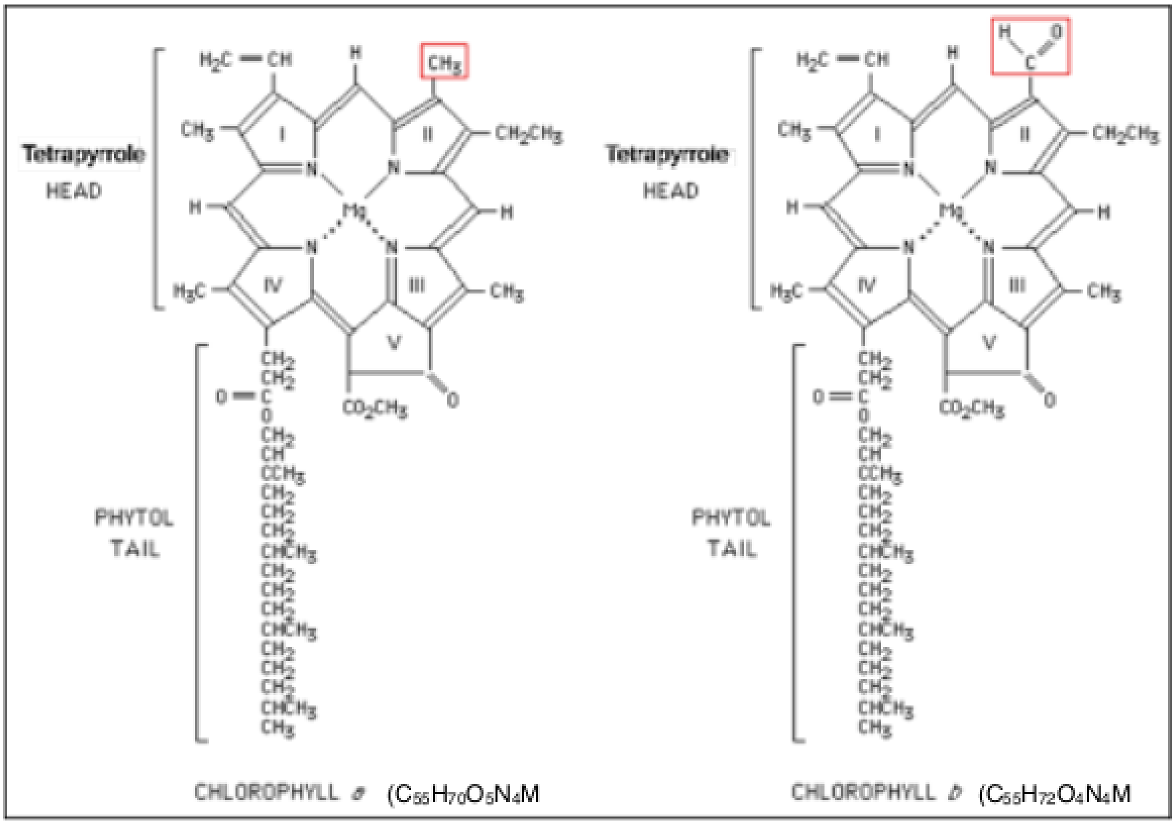
- Absorption of light energy by chloroplast pigments and generation of excited electrons
- Use of energy of excited electrons to reduce **$NADP^+$** (oxidised nicotinamide adenine dinucleotide phosphate) and phosphorylate ADP (adenosine diphosphate) to form energy-rich NADPH (reduced nicotinamide adenine dinucleotide phosphate) and ATP (adenosine triphosphate) molecules, respectively, and in the process create a strong oxidising environment.
- Splitting of water molecules because of the strong oxidising environment and release of oxygen.

Chloroplast pigments involved in light reactions

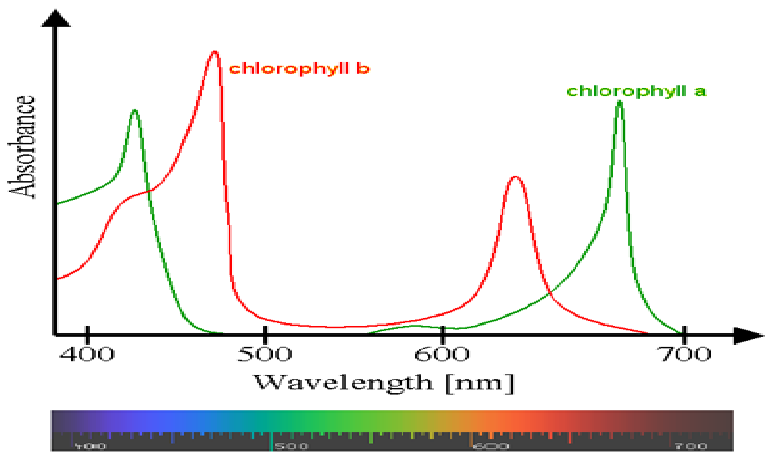
In higher plants, there are four types of pigments involved in light reactions belonging to two broad categories, the chlorophylls and carotenoids. These pigments can be easily separated from the extract of any green part of a plant by paper chromatography. The four pigments include the primary light absorbing pigments, chlorophyll a and chlorophyll b, and the accessory carotenoid pigments, carotenes and xanthophylls. Often chlorophyll b (chl b) is considered an accessory pigment as it does not directly take part in the conversion of light energy into chemical energy. Whereas chl a is the primary pigment found in all photosynthesizing organisms from bacteria to algae and higher plants, Chl b is found in some algae and all higher plants. Different types of carotenoids are found in almost all photosynthetic bacteria, algae and higher plants and apparently function in photosynthesis.

A pigment is a substance which absorbs specific wave lengths of visible light and reflects the rest of light, and therefore, appears coloured. The colour of the pigment corresponds to the colour of the reflected light. The fraction of light absorbed by a pigment at different wavelengths is generally plotted graphically and represents the **absorption spectrum** of the pigment.

Chlorophyll is the most abundant biological pigment in this world. Leaves contain upto 1 g of chlorophyll pigment /m^{2}. Chlorophyll has a planar head of four pyrrole-like rings with a magnesium atom bound in the centre. A fifth cyclopentanoic ring and a phytol chain are also attached to the molecule. The only structural difference between chlorophyll a and chlorophyll b is that chlorophyll b has an aldehyde instead of a methyl group at the C-7 position at the second pyrrole ring.

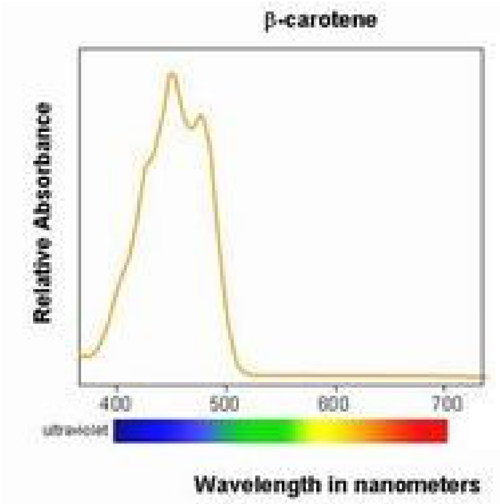


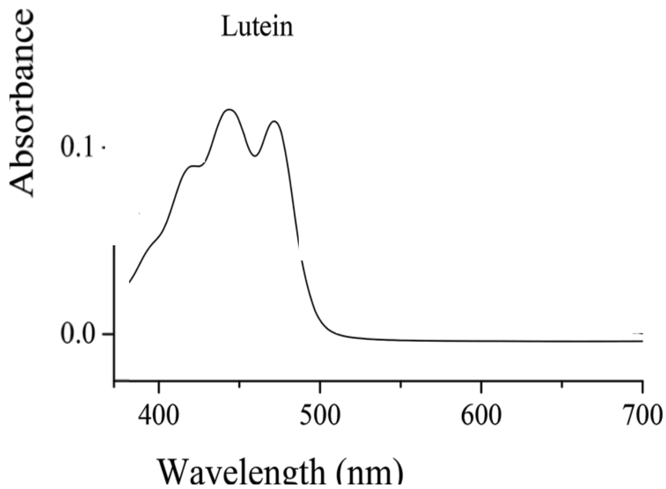
Chlorophylls (extracted from leaves in a solvent) absorb mainly violet-blue (below 480 nm) and red (550-700 nm) wave lengths of light and reflect green wave length, and therefore, are green in colour. In a paper chromatogram chlorophyll a appears bluish green while chlorophyll b appears yellowish green.

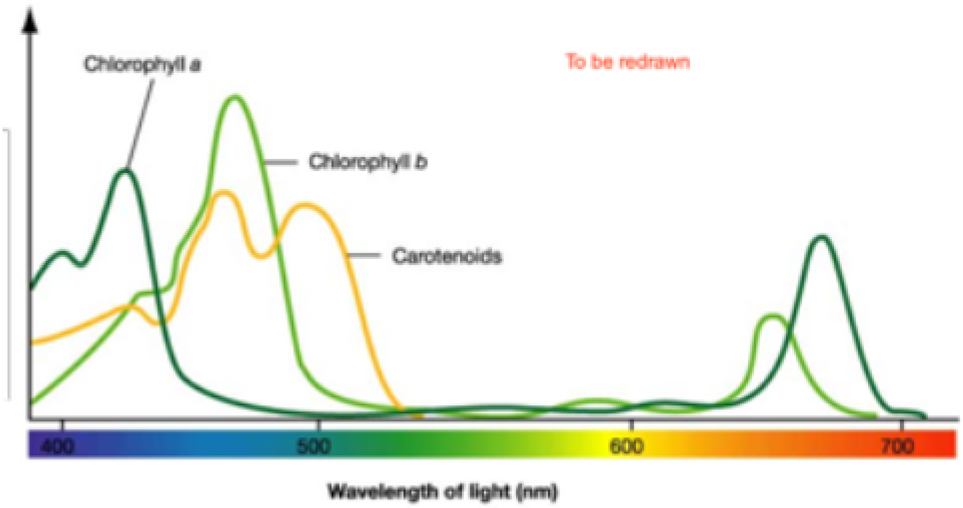


Absorption spectra of Chlorophyll pigments

Carotenoids are derivatives of tetraterpenes. There are two classes of carotenoids, carotenes (e.g. α-carotene, β-carotene, and lycopene) which are pure hydrocarbons, and xanthophylls (e.g.lutein and zeaxanthin) which contain oxygen. Extracted carotenoids generally absorb in the violet-blue range between 400 and 500nm, and hence appear yellow to orange in colour. Xanthophylls are yellow coloured while carotenes are more orangish.



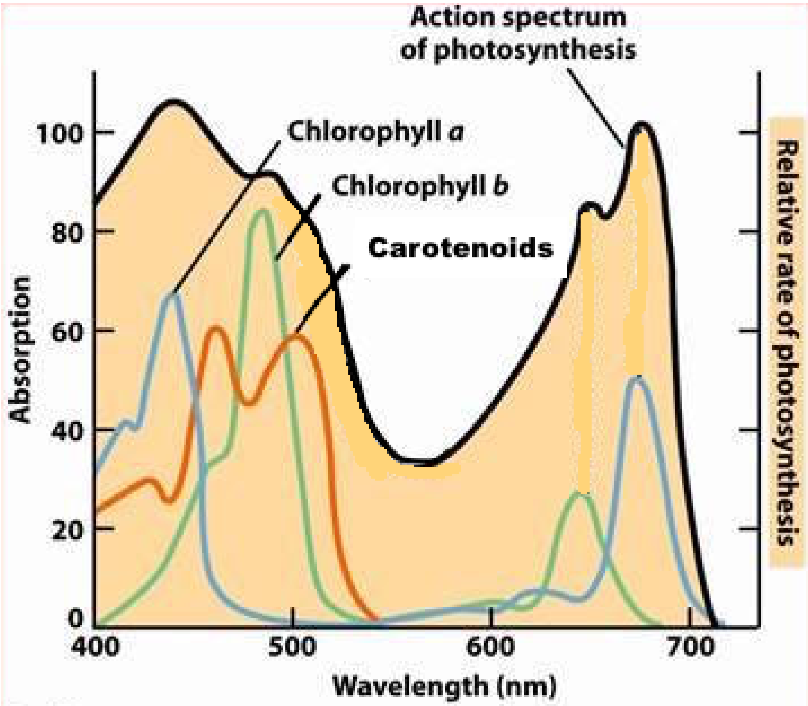




Absorption spectra of extracted chloroplast pigments

The absorption spectra of pigments in the chloroplasts indicates that maximum light is absorbed at violet-blue and orange-red wavelengths mostly by chlorophylls, less in the blue-green and yellow regions by carotenoids, and least at green wavelength light which is reflected. The chloroplasts, hence, appear green, and all chloroplast-containing parts of plant appear green.

But are these pigments, chlorophylls and carotenoids, responsible for photosynthesis? If these are the pigments absorbing and harvesting light to be used in photosynthesis, then the rate of photosynthesis will be more at the wave lengths of light at which these pigments absorb more. This becomes evident when the absorption spectrum of chloroplast pigments and the action spectrum of photosynthesis are compared. Rate of photosynthesis is maximum at violet-blue and red wavelengths, less at yellow-orange wavelengths and least at green wavelengths indicating a correlation between the absorption of light by chlorophyll and carotenoid pigments and rate of photosynthesis. It is, therefore, clear that the chlorophylls and carotenoids are the pigments involved in photosynthesis with the chlorophylls playing a major role.



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In addition, carotenoids play an important role in protecting the photosynthetic apparatus from intense sunlight. High light energy produces excess electrons which react with oxygen and produce reactive oxygen species (ROS). ROS are extremely harmful and can cause breakdown of chlorophylls, proteins and membranes. Carotenoids harmlessly eliminate the excess light energy and prevent formation of ROS.

Photosynthetic units and Photosystems

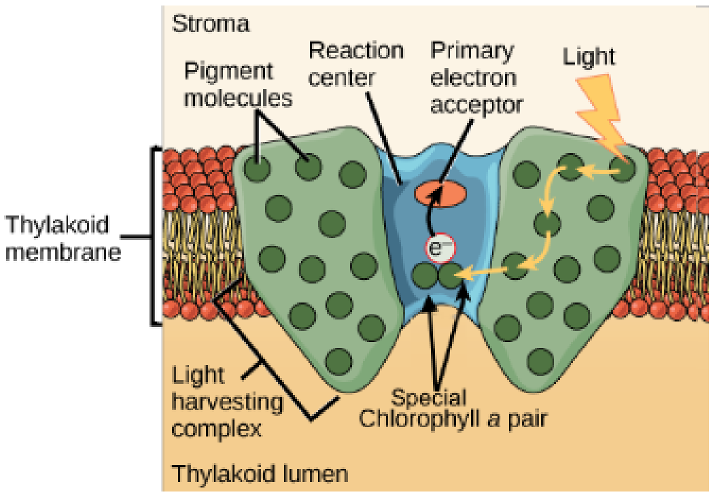
On absorption of light energy, the chloroplast pigments, especially, the chlorophyll molecules become excited, i.e. their electrons become excited and jump to a higher energy orbit. If there is a suitable electron acceptor, the excited electron is donated to the acceptor which gets reduced (Hill's experiment) resulting in a photochemical reaction. But in the absence of an acceptor, the excited electron falls to ground state releasing the energy in the form of light (red fluorescence) and heat.

The red fluorescence is readily observed when chlorophylls extracted in solvents are exposed to light.

In early 1930s, Emerson and Arnold observed that all the chlorophyll molecules individually do not bring about photochemical reaction. A group of 200-300 pigment molecules, termed the **photosynthetic unit**, act cooperatively. Only 1out of a group, known as the reaction centre molecule, performs the photochemical reaction; the rest called the antenna molecules absorb light energy and transfer it to the reaction centre molecule. Pigment molecules are bound to proteins which position them precisely to enable transfer of excitation energy. The reaction centre molecule absorbs maximum at the longest wavelength of light with lowest energy and, hence, acts as an energy trap. The photosynthetic units are pigment-protein complexes.

Later experiments by Emerson (red drop experiment in1946 and enhancement experiment in 1958) indicated the presence of two types of such complexes known as **photosystem I (PS I)** and **photosystem II (PS II)** which work in tandem. Each photosystem consists of a core region or reaction centre surrounded by light harvesting complex (LHC). The core region contains predominantly chl a molecules including a special pair of chlorophyll a molecules as the reaction centre pigment molecules. In addition, electron acceptor molecules which accept electrons from the excited reaction centre chl a molecules are located in the core region.

In PS I, the reaction centre chlorophyll a molecules absorb maximum light at 700nm wavelength and are referred to as P_{700}. Similarly, the reaction centre chlorophyll a molecules in PS II absorb 680nm light maximally, and hence, known as P_{680}. The antenna molecules of the photosystems consisting of chl a, chl b, carotenes and xanthophylls are associated with proteins forming the light harvesting complexes.



Structure of photosystem

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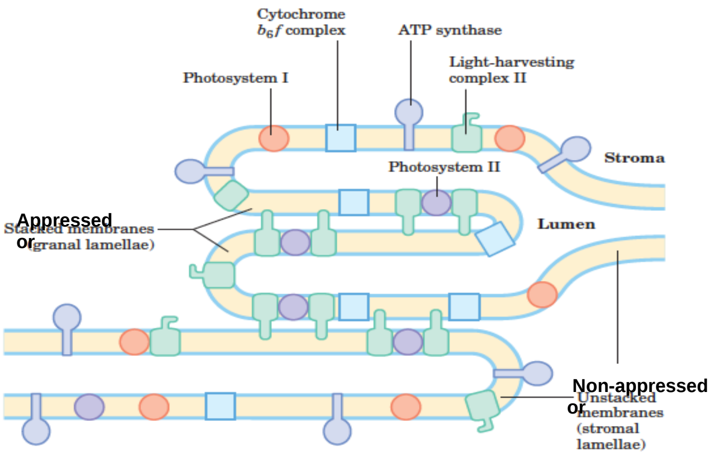
Photosystem complexes are present in the thylakoid membrane. Whereas PS I is present only in the unstacked or non-appressed regions of the thylakoid membrane, i.e., in the exposed regions of thylakoid lamellae, the PS II is mostly present in the stacked or appressed regions of the granal lamellae. In addition, an oxygen evolving complex is bound to photosystem II complex on the lumenal side of thylakoid membrane. It is a protein complex with bound manganese atoms which helps in splitting of water molecules.



Copyright protected, to be redrawn, shapes of Photosystem I, photosystem II and cytochrome b6f complex may be changed.

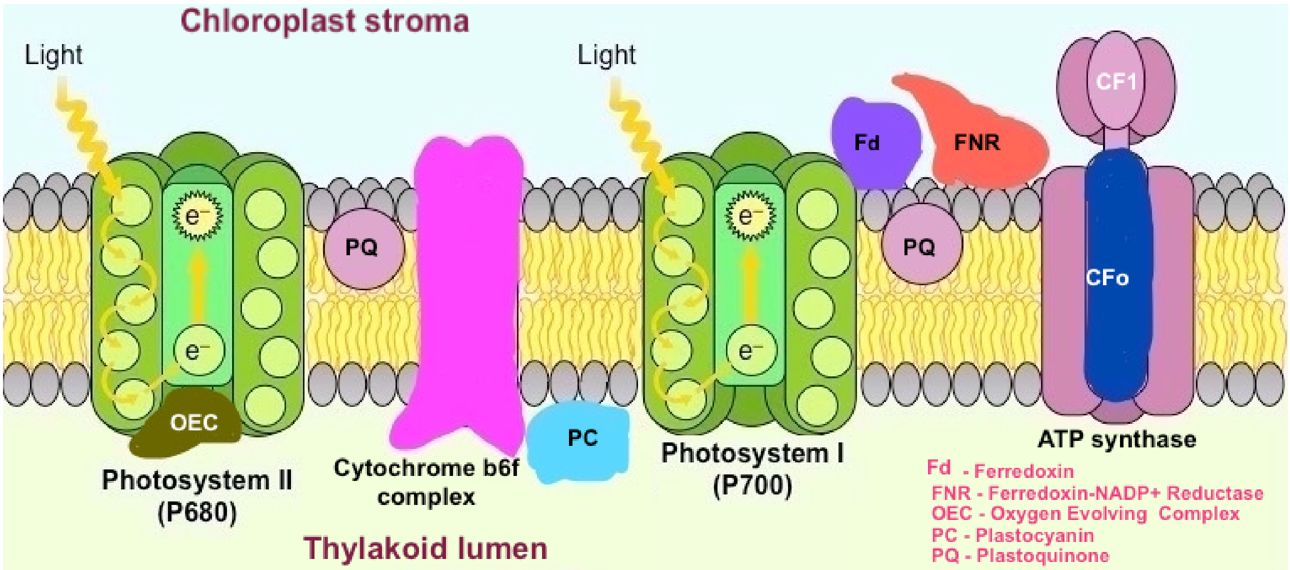
Other components of light reaction

The two photosystems are linked by an electron transport system which transfers electrons between the photosystems. The constituents of the electron transport system are plastoquinone, cytochrome b6f complex and plastocyanin. Another electron transport chain consisting of ferredoxin and ferredoxin-NADP+ reductase transfers electrons from PS I to NADP+. Cytochrome b6f complex (Cyt b6f)is protein complex uniformly distributed in the thylakoid membrane. Cytochromes are also found in inner mitochondrial membrane and function as electron transporters. Plastoquinone (PQ), a lipid soluble organic molecule, is present in the lipid bilayer of thylakoid membrane. Plastocyanin (PC) is a copper-containing protein located on the lumenal side of the thylakoid membrane. Both plastoquinone and plastocyanin are mobile electron carriers which move in and along the surface of lamellae, respectively, and deliver electrons to the acceptor molecules next in the system. A third mobile electron carrier involved in electron transfer from PS I is ferredoxin (Fd), an iron-sulfur containing protein loosely attached to the stromal side of thylakoid membrane. The last component of the electron transfer system is an enzyme, ferredoxin-NADP+ reductase (FNR) present bound to the thylakoid lamellae on the stromal side as well as free in the stroma. Both Ferredoxin and FNR are present on the non appressed regions of the thylakoid lamellae.

Another important component of light reaction is the enzyme ATP synthase involved in the synthesis of ATP. It is a multi-protein complex consisting of two parts, the CF0 , a transmembrane channel spanning the thylakoid membrane, and the knob-like CF1 attached to CF0 , and protruding out on the stromal side of thylakoid membrane. ATP synthase, like PS I, is predominantly found in the non-appressed regions of thylakoid membrane.

Generation and transport of electrons

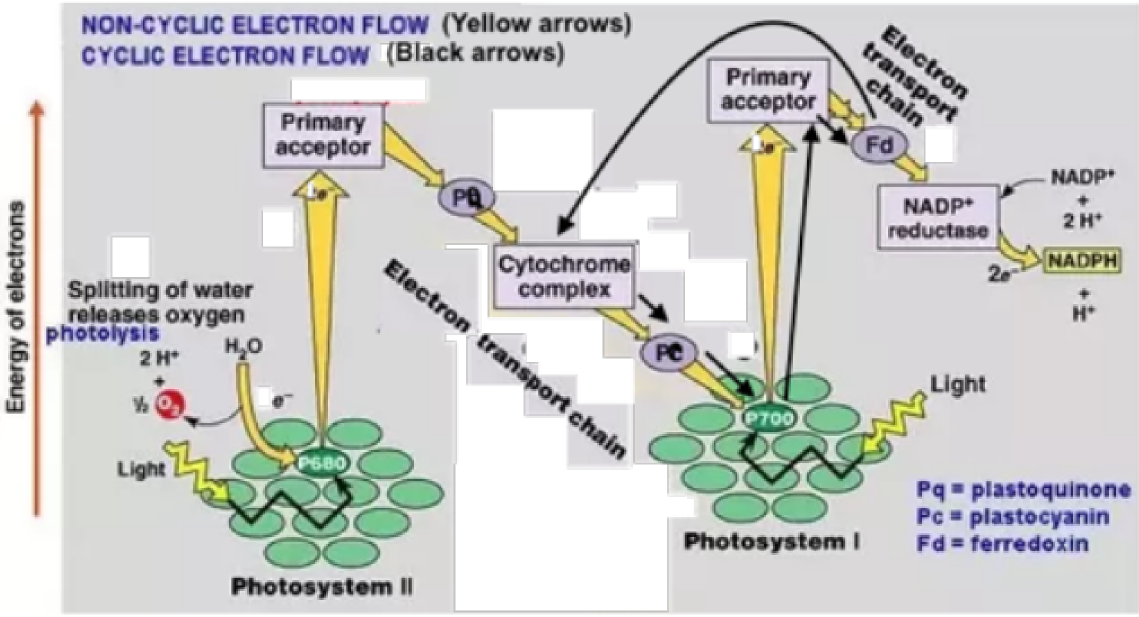
When light falls on the photosystems, the light harvesting or antenna molecules absorb light energy and transfer the energy rapidly from one molecule to other and finally, to the special pair of chl a molecules at the reaction centres. Once a specific amount of energy is absorbed by one of the two chl a molecules, it becomes excited promoting an electron to a higher energy orbital. The excited electron passes through an electron transport system (ETS), also called an electron transport chain, consisting of a series of electron acceptors. In the process, the chl a molecule at the reaction centre, which has donated the electron, acquires a positive charge or an electron hole. Hence, solar energy is used to carryout an oxidation-reduction reaction, oxidising chl a molecule in the reaction centre and generating a high energy electron.



In PS I, the excited P_{700} donates the high energy electron to an acceptor and becomes P_{700}^{+}. The ultimate electron acceptor is ferredoxin which transfers the electrons to $NADP^{+}$ reducing it to NADPH. The reduction reaction is catalysed by the enzyme, ferredoxin- $NADP^{+}$ reductase (FNR). Each $NADP^{+}$ requires two electrons and two protons (from stroma) to be reduced to NADPH.

$$NADP^+ \xrightarrow{e^-} NADP \xrightarrow{e^-} NADP^- \xrightarrow{2H^+} NADPH + H^+$$

The high energy electron of the excited P_{680} of PS II is accepted by plastoquinone (PQ) which binds with two protons from stroma and is reduced to PQH_2. Electrons of reduced plastoquinone are passed to P_{700}^+ in PS I via the cyt b_6f complex and plastocyanin. As a result, the charged P_{700}^+ gets neutralised and returns to its ground state while P_{680} acquires a positive charge (P_{680}^+).



Electrons required to neutralise P680+ and bring it to ground state are obtained from water. P680+ is a strong oxidant and with the help of oxygen evolving complex is able to oxidise water molecules and draw out electrons. This splitting of water molecules, produces not only electrons but protons and oxygen molecules as well.

$$2H_2O \xrightarrow{\text{Light}} 4e^- + 4H+ + O_2$$

Hence, it is the light energy absorbed by chloroplast pigment molecules which is responsible for splitting of water, the ultimate source of electrons for reduction of NADP+.This process of splitting of water molecules with the help of light energy is called photolysis of water.

The electrons are transferred to NADPH in the sequence —

H_2O → PS II → Plastoquinone → cytochrome b_6f → Plastocyanin →

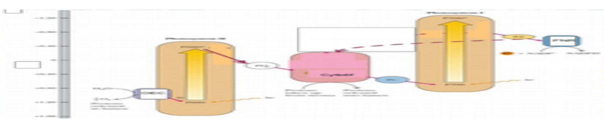
PS I → ferredoxin → NADPH

This is the **noncyclic flow of electrons** from water to NADPH and involves both PS I and PS II, and the electron acceptors of the electron transport system. Electrons generated from water are used up to reduce $NADP^{+}$.

The sequence of electron acceptors is determined by their reduction potentials. Reduction potential (also known as oxidation/reduction potential or redox potential) indicates the inclination to acquire electrons and get reduced. The excited chlorophyll molecule has a high tendency to donate its electron at higher energy orbital than retain it, and thus, has a low redox potential. It donates electron to an acceptor which has a higher or more positive redox potential which in turn transfers the electron to an acceptor with a still higher redox potential. Thus, the electron travels down a redox potential gradient, from a less to more redox potential.

The noncyclic movement of electrons from PS II to NADPH, through the sequence of acceptors arranged according to their redox potentials resembles a zigzag shape, and thus is often called the **Z scheme**.

Redox Potential (Volts)



Z scheme of Photosynthetic Electron Transfer

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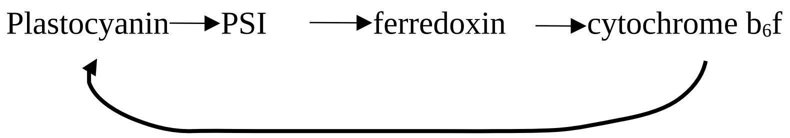
(Solid arrows indicate noncyclic and dashed arrow the cyclic flow of electrons)

An alternative light induced electron flow known as the **cyclic electron transfer** also occurs in chloroplasts. It involves only the PS I and ETS. No oxygen or NADPH are produced. The electron from the excited P_{700} is accepted by ferredoxin. Reduced ferredoxin, instead of transferring the electron to $NADP^{+}$ and reducing it, donates it to cytochrome $b_{6}f$. The electron then returns back to P_{700} via plastocyanin.



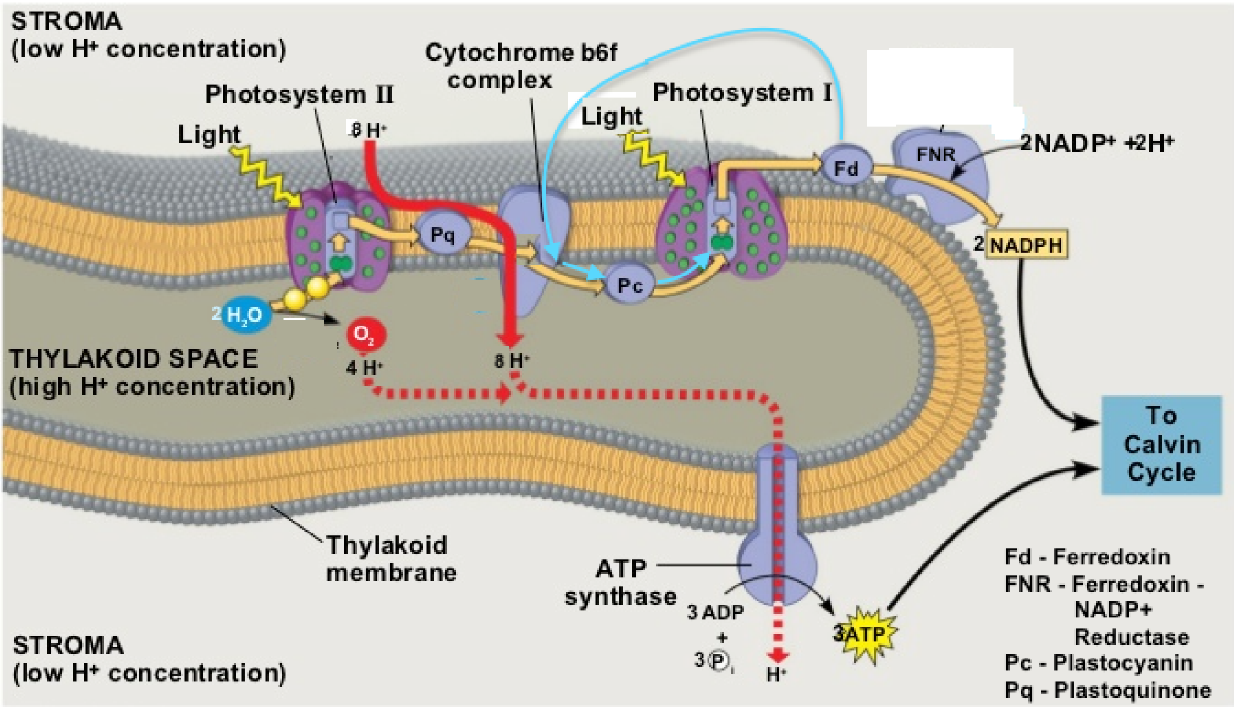
Photophosphorylation and ATP synthesis

In light reaction, apart from NADPH and oxygen, ATP is also formed. In general, ATP is synthesised by addition of a phosphate group (Pi) to ADP (adenosine diphosphate) and this process, also known as phosphorylation, requires energy. In photophosphorylation, light is the source of energy. It was in 1954 that Arnon and coworkers demonstrated the formation of ATP in isolated chloroplasts in the presence of light. Subsequently it was shown that photophosphorylation, as also oxidative phosphorylation which occurs in mitochondria, is coupled to electron transfer through an electron transport system (ETS) and requires the presence of ATP synthase. This meant that ATP synthesis will not occur in the absence of the ETS and a functional ATP synthase. This in turn indicated that the energy for ATP synthesis is generated when the electron passes down a redox potential gradient, from high energy donor molecules with more negative redox potential to lower energy acceptor molecules of the ETS. But how is the generated energy harnessed and used in the synthesis of ATP?
In 1961, Peter Mitchell postulated the chemiosmotic hypothesis for oxidative phosphorylation which was subsequently found to be true for photophosphorylation. According to the hypothesis, for electron transport coupled ATP synthesis to occur, a proton gradient is essential, and it is the proton motive force of the gradient which powers ATP synthesis. The energy generated during the electron transfer through ETS is used to pump protons across membrane and create a proton concentration gradient, expressed as a proton-motive force. When this proton gradient is broken down, the stored energy or the proton motive force is released which activates ATP synthase to phosphorylate ADP and produce ATP.
During light reaction in photosynthesis, a proton gradient is established across thylakoid membrane by three processes.

(a) When the high energy electron of the light-excited chl a molecule of the reaction centre moves along the ETS and falls to a lower energy level, energy is released. This energy is conserved by pumping protons across the thylakoid membrane from stroma into lumen by plastoquinone and cytochrome b_6f complex.

(b) Protons are released in the lumen when water is split by the oxygen evolving complex to generate electrons. Since thylakoid membrane is impermeable to protons, a high concentration of protons accumulates in the lumen.

(c) In addition, during the reduction of $NADP^+$ to NADPH, not only electrons but protons from stroma are used resulting in the reduction of protons in the stroma.



Light Reaction of Photosynthesis

Yellow arrows: path of noncyclic electron transfer, blue arrows: path of cyclic electron flow

The accumulation of protons in lumen and their reduction in stroma gives rise to a proton gradient across the thylakoid membrane. The proton gradient is dissipated by the flow of protons into stroma across thylakoid membrane through ATP synthases. Protons diffuse through the channel-like CF_0 region of ATP synthase which brings about conformational changes in the CF_1 part of the enzyme enabling the synthesis of ATP from ADP and Pi .

ATP synthesis during the noncyclic flow of electrons from water to NADPH, involving PS II, plastoquinone, cytochrome b_6f complex, plastocyanin, PSI, and ferredoxin is called **noncyclic photophosphrylation**.

Thus, noncyclic electron transfer yields all the three products of light reaction, NADPH, ATP and O_2, through splitting of water.

ATP is also formed during cyclic flow of electrons from and to P_{700}, involving PS I, cytochrome b_6f complex and plastocyanin. This process of ATP production is known as the **cyclic photophosphrylation.** The high energy electron from PS I is transferred to cytochrome b_6f complex by mobile ferredoxin. As it returns back to PS I through plastocyanin, it loses energy which is used to pump protons from stroma into lumen across the thylakoid membrane. The protom motive force of the accumulated protons drives ATP synthesis by a mechanism similar to that in noncyclic photophosphorylation. However, since fewer protons are moved into lumen by the cyclic electron flow, lesser number of ATP molecules are formed.

$$ADP + Pi \xrightarrow{\text{light}} ATP$$

In cyclic electron flow, therefore, only ATP is formed; neither NADPH nor O_2 are generated. Cyclic phosphorylation appears to operate whenever there is a need for extra ATP molecules as in C_4 metabolism.

Total Light Reaction

In general, for every molecule of oxygen released, 2 molecules of water are split producing 4 electrons. These electrons flow in a noncyclic pathway and generate 2NADPH and 3ATP molecules. The total light reaction can be represented as

$$\mathbf{2H_2O + 2NADP^+ + 3ADP + 3Pi \xrightarrow[\text{Thylakoids}]{\text{light energy}} O_2 + 2NADPH + 2H^+ + 3ATP}$$

NADPH and ATP, the products of light reaction are used in the biosynthesis of carbohydrates during the carbon reduction reactions or Calvin cycle reactions.

Summary

- During light dependent reaction of photosynthesis, light energy is converted into chemical energy; light energy is absorbed and used to form two energy-rich molecules, NADPH (reduced nicotinamide adenine dinucleotide phosphate) and ATP (adenosine triphosphate).
- Entire light reaction occurs in thylakoid membrane of chloroplast.
- Four types of pigments, chlorophyll a, chlorophyll b, carotenes and xanthophylls, are involved in light absorption, of which the chlorophylls are the main ones.
- Four types of protein complexes, photosystem I (PS I), photosystem II (PS II), cytochrome b_6f and ATP synthase, are present in the thylakoid membranes which carryout light reaction.

- PS I and PS II are pigment-protein complexes which contain groups of pigment molecules along with special chlorophyll a molecules, P_{700} and P_{680}, respectively, at the reaction centre. The pigment molecules absorb light energy and transfer it to P_{700} and P_{680} which become excited and promote an electron to a higher energy level.
- The high-energy electron of excited P_{700} is transferred to $NADP^+$ reducing it to NADPH.
- The high-energy electron of excited P_{680} is transferred to the electron deficient P_{700} via an electron transport system consisting of plastoquinone, cytochrome b_6f complex, plastocyanin with increasing redox potentials. During this movement the electron loses energy which is conserved by pumping protons across the thylakoid membrane from stroma to lumen.
- The resultant electron deficient P_{680} is a strong oxidant, which with the help of oxygen evolving complex attached to PS II, oxidises water molecules to oxygen, drawing out electrons and protons in the process. The electrons are used to fill up the electron deficiency in P_{680} , protons remain in the lumen and oxygen escapes in the form of gas.
- A high concentration of protons accumulates in lumen of thylakoids creating a proton gradient or a proton motive force.
- Diffusion of protons through ATP synthase activates it which results in phosphrylation of ADP and synthesis of ATP.
- Since the source of energy for ATP synthesis by this mechanism is light, the process is known as photophosphorylation.
- **There are two types of photophosphorylations; noncyclic phtophosphorylation occurs when the proton motive force for synthesis of ATP is generated by a noncyclic electron flow from water to NADPH involving both the photosystems, and cyclic phtophosphorylation where a cyclic transfer of electrons from and to P_{700} of PS I creates the proton motive force.**