Photosynthesis

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Photosynthesis is a process in which the plants use light energy to make carbohydrate from carbon dioxide and water. The overall reaction of photosynthesis can be given in a simple form as follows:

$$6CO_2 + 6H_2O \xrightarrow{sunlight+chlorophyll} C_6H_{12}O_6 + 6O_2$$

In green plants, water is the hydrogen donor and it undergoes oxidation to produce oxygen.

Photosynthesis occurs in two stages, viz. light reaction and dark reaction. The light reaction is light-dependent. Light energy is captured in this stage and is utilised to make the energy-storage molecules ATP and NADPH. Dark reaction is light-independent reaction. Dark reaction is utilised to capture and reduce carbon dioxide. Dark reaction doesn't mean that it happens in the absence of light.

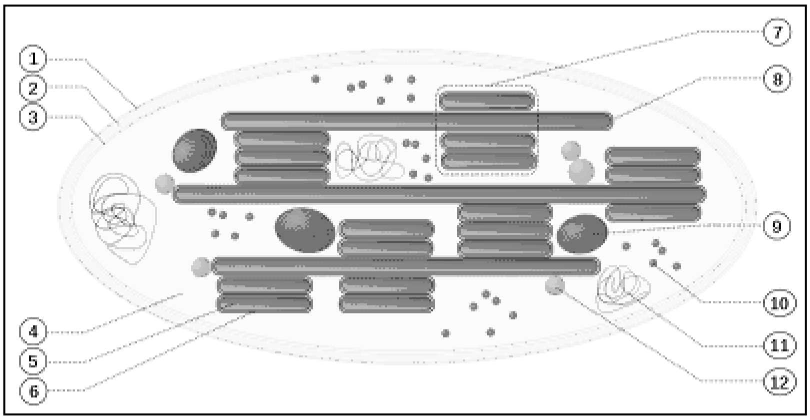
Importance of photosynthesis:

Primary source of food

Release O2 to atmosphere

Early Discoveries

- Joseph Priestly: Candle with bell jar and mouse experiment – He concluded that air is necessary for the growth of a plant. He discovered the fact that plants restore oxygen in the air.
- Jan Ingenhousz: Experiment with aquatic plant in light and dark – He concluded that sunlight is essential for plant processes that purify the air.
- Julius Von Sachs: Green parts of plant make glucose and store as starch.
- T.W. Engelmann: Spilt light using prism into 7 colours (VIBGYOR) - Green algae Cladophora placed in a suspension of aerobic bacteria - Bacteria were used to detect the sites of O2 evolutions.
- Cornelius van Niel: He did experiment with purple and green bacteria and demonstrated photosynthesis is a light dependent process with hydrogen from H2O reduces CO2 to carbohydrates. He concluded that oxygen comes from H2O, and not from CO2. Finally, the correct equation for photosynthesis was discovered.
  - $6CO_2 + 12H_2O$ $C_6H_{12}O_6 + 6H_2O + 6O_2$



Site of Photosynthesis

- o Green leaves, green stems and floral parts (sepal)
- o Chloroplast - found in mesophyll cells of leaves
  - In chloroplast – the membrane system is responsible for trapping the light energy and also for the synthesis of ATP and NADPH. Where stroma has enzymes for the reduction of CO2 in to carbohydrates (sugars)

Pigments Involved in Photosynthesis: 4 types of pigments may be present in leaves:

Chlorophyll a Chlorophyll b Xanthophylls Carotenoids

- **An absorption spectrum** is the graph plotted against the fraction of light absorbed by the pigment.
- **An action spectrum** is the rate of a physiological activity plotted against the wavelength of light.
- Photosystems are pigments that are organized in the thyllakoid membrane in to two different photosystems (PS 1 & PS 11)
- Each PS has one specific chlorophyll – a, and many other accessory pigments bound by proteins.
- Chlorophyll – a forms the reaction centre (actual reaction takes place) other pigments form the light harvesting complex (LHC) called antennae.
- PS 1 reaction centre is p700 (chlorophyll –a absorbs light at 700 nm)
- PS 11 reaction centre is p680 (chlorophyll –a absorbs light at 680 nm)

CHLOROPLAST

Chloroplast is the cell organelle where photosynthesis takes place in plants and algae. A typical plant cell may contain about 10 to 100 chloroplasts. Chloroplast is enclosed by a membrane. This membrane is composed of an inner, outer and an intermediate membrane. An aqueous fluid; called stroma is present within the membrane.

Stacks of thylakoids are present in the stroma. A stack is called a granum. Thylakoids are the sites of photosynthesis.

A thylakoid is a flattened disc. It is bound by a membrane. The lumen or thylakoid space is present within the membrane. The tyhlakoid membrane is the site of photosynthesis. It contains integral and peripheral membrane protein complexes. Pigments which absorb light energy are also present on the membrane. The protein complexes and the pigments form the photosystems.

Chlorophyll is the main pigment to absorb light. Additionally, carotenes and xanthophylls are also used by plants to absorb light energy. Algae also use chlorophyll for absorbing light.

These pigments are embedded in plants and algae in special antenna-proteins. The pigments are ordered in these proteins so that they can work in perfect coordination. Such a protein is also called a light-harvesting complex.

All the cells in the green parts of a plant have chloroplasts but most of the energy is captured in the leaves. The mesophyll of the leaf can contain between 450,000 and 800,000 chloroplasts for every square millimeter of leaf. The surface of the leaf is uniformly coated with a water-resistant waxy cuticle which protects the leaf from excess evaporation of water. It also decreases the absorption of ultraviolet or blue light to reduce heating. The epidermal layer of the leaf is transparent. It allows light to pass through to the palisade mesophyll cells where most of the photosynthesis takes place.

LIGHT REACTION

The light reaction is also called the Photochemical phase. It includes light absorption, water splitting, oxygen release and the formation of high-energy chemical intermediates (ATP and NADPH). Many complexes are involved in the process.

The pigments are organized into two discrete photochemical light harvesting complexes (LHC) within the Photosystem I (PS I) and Photosystem II (PS II). The photosystems are named in the sequence of their discovery and it has nothing to do with their function during the light reaction.

Each photosystem has all the pigments; except one molecule of chlorophyll a. The single chlorophyll a molecule forms the reaction centre. In PS I the reaction centre chlorophyll a has an absorption peak at 700 nm, hence it is called PS700. In PS II the absorption maxima is at 680 nm and hence it is called PS680.

Transfer of light energy from accessory pigment to chlorophyll:
Light energy absorbed by the pigment other than chlorophyll a transferred to chlorophyll -a by resonance of photons. In the chlorophyll-a primary photochemical reaction takes place.
There are two pigment systems in the chlorophyll with p-690 and p-700.

Activation of chlorophyll-a molecule and photo excitation of the pigment:
When pigment molecule in a photo system receives a photon of light, become excited and expels the extra energy level called excited second singlet state; then it comes to Meta stable state is called triplet state. From which it receive electron from outer source and return back to ground state.
Light-harvesting antennas and photochemical reaction centers: The absorption of the light energy is a cooperation between many chlorophylls and carotenoid molecules (**Figure 2.5**). The majority of the pigments serve as an **antenna complex**, collecting light and transferring the energy to the **reaction center complex**, where the chemical oxidation and reduction reactions leading to long-term energy storage take place. Even in bright sunlight, a single chlorophyll molecule absorbs only a few photons each second. If there were a reaction center associated with each chlorophyll molecule, the reaction center enzymes would be idle most of the time, only occasionally being activated by photon absorption. However, if a reaction center receives energy from many pigments at once, the system is kept active a large fraction of time. Several hundred pigments are associated with each reaction center, and each reaction center must operate four times to produce one molecule of oxygen – hence the value of 2500 chlorophylls per O2. The reaction centers and most of the antenna complexes are integral components of the photosynthetic membrane. In eukaryotic photosynthetic organisms, these membranes are found within the chloroplast; in photosynthetic prokaryotes, the site of photosynthesis is the plasma membrane or membranes derived from it.

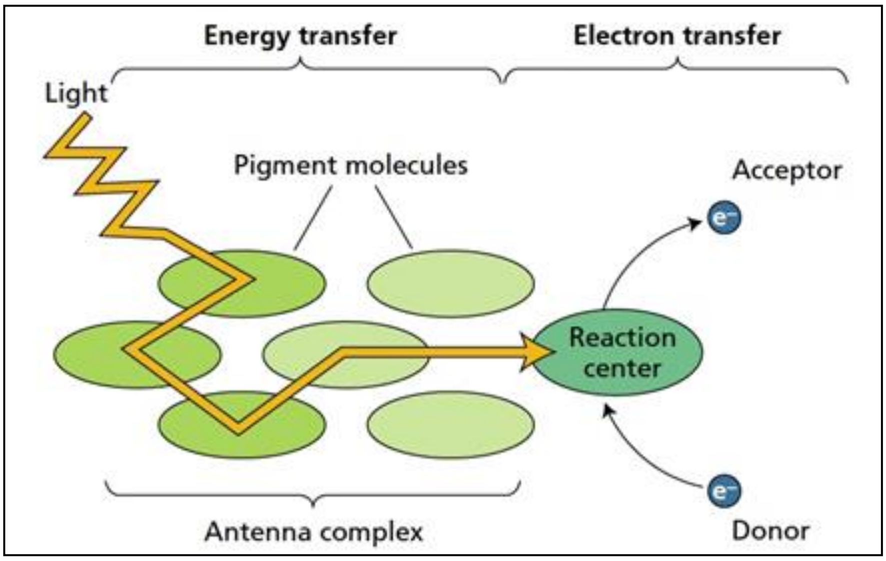


Figure 2.5 Basic concept of energy transfer during photosynthesis (*source: Taiz L., Zeiger E., 2010*)

Oxygen-evolving organisms have two photosystems
The quantum yield of photochemistry is nearly 1.0, the actions of about ten photons are required to produce each molecule of O2, so the overall maximum quantum yield of O2 production is about 0.1. Any photon absorbed by chlorophyll or other pigments is as effective as any other photon in driving photosynthesis.

However, the yield drops dramatically in the far-red region of chlorophyll absorption (greater than 680 nm). Emerson discovered the **enhancement effect**. He measured the rate of photosynthesis separately with light of two different wavelength and then used the two beams simultaneously (**Figure 2.6**). When red and far-red light were given together, the rate of photosynthesis was greater than the sum of the individual rates. These and others observations were eventually explained by experiments performed in 1960 that led to the discovery that two photochemical complexes, now known as **photosystem I and II** (**PSI and PSII**), operate in series to carry out the early energy storage reactions of photosynthesis. Photosystem I absorbs far-red light, photosystem II absorbs red light. Another difference between the photosystems is that:

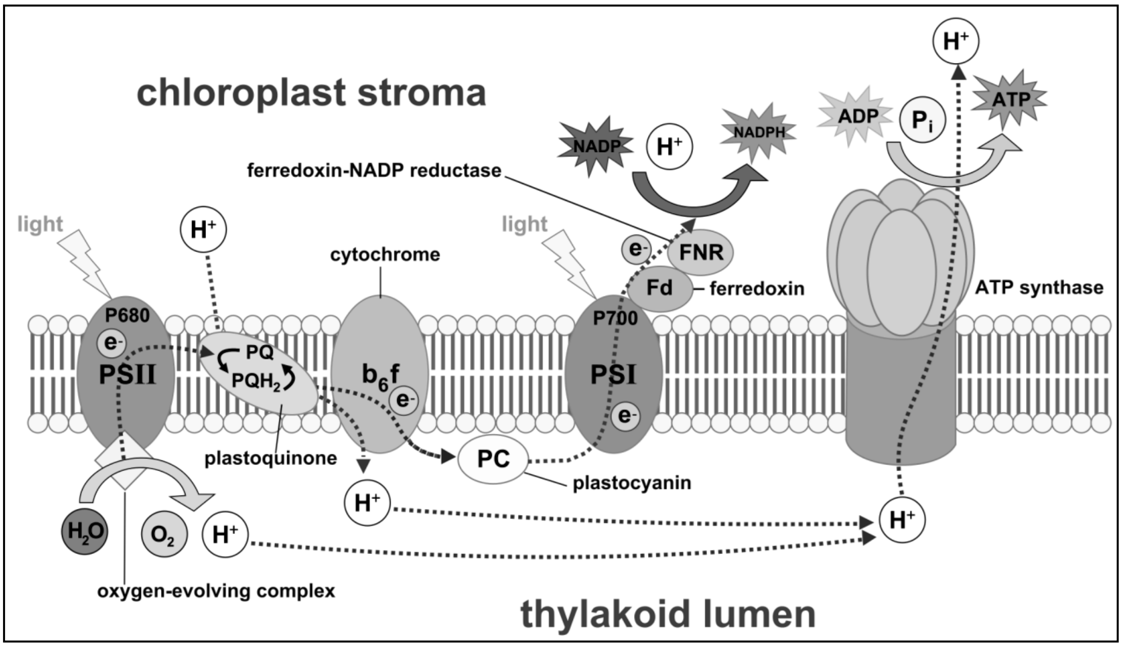
THE ELECTRON TRANSPORT

The reaction centre in PS II absorbs 680 nm wavelength of red light. This causes electrons to become excited and jump into an orbit farther from the atomic nucleus. These electrons are picked up by an electron acceptor and sent to an electrons transport system consisting of cytochromes.

In terms of an oxidation-reduction potential scale, this movement of electrons is downhill. The electrons are not used up as they pass through the electron transport chain. They are passed on to the pigments of PS I.

At the same time, electrons in the reaction centre of PS I are also excited when they receive red light of wavelength 700 nm. They are then transferred to another acceptor molecule which has a greater redox potential.

Z Scheme: These electrons are then moved downhill again, to a molecule of energy-rich NADP+. The addition of these electrons reduces NADP+ to NADPH+H+. This whole transfer of electrons from PS II to the acceptor, to PS I, to another acceptor and finally to NADP+ is called the Z scheme, because of its characteristic shape.



Splitting of Water

Water is split into H^+, [O] and electrons. The splitting of water is associated with PS II. This creates oxygen. Photosystem II provides replacement for electrons removed from PS I.

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

- Water splitting complex is associated with PSII.
- Manganese, chlorine, etc., play an important role.
- The light-dependent splitting of water is called photolysis
- Electrons formed are used for replacing the electrons lost from P680.
- P680 absorbs light and becomes as a strong oxidizing agent and splits a molecule of water to release oxygen. Oxygen is liberated as a by-product of photosynthesis.
- Protons are used for the formation of reducing power NADP to NADPH+.

<u>Photo-Phosphorylation</u>

Synthesis of ATP from ADP and inorganic phosphate in the presence of light is called photophosphorylaton.

Photo-phosphorylation is of two types:

- Non-cyclic photo-phosphorylation
- Cyclic photo-phosphorylation

Cyclic and Non-cyclic Photo-phosphorylation

When the two photosystems work in a series; first PS II and then the PS I; a process called non-cyclic photophosphorylation occurs.

- PSII absorbs 680 nm wavelength of red light, causing electrons to become excited and these electrons are then accepted by an electron acceptor, which sends them to an electron transport system.
- Electron transport system transfers the electrons to PSI.
- Electrons in PSI are simultaneously excited on receiving a wavelength of 700 nm.
- From the electron acceptor, electrons are transferred to the molecule of NADP+.
- Addition of these electrons reduces the NADP+ to NADPH+ H+.
- Since the electrons lost by PSII do not come back to it, this process of formation of ATP is called non-cyclic photo-phosphorylation.

When only PS I is functional, the electron is circulated within the photosystem and the cyclic flow of electrons leads to phosphorylation. The stroma lamellae are the possible location of phosphorylation. The stroma lamellae lack PS II and NADP reductase enzyme. The excited electron does not pass on to NADP+ but is cycled back to the PS I complex. Hence, the cyclic flow results only in the synthesis of ATP but not of NADPH+H+. Cyclic photophsophorylation also occurs when only light of wavelengths beyond 680 nm are available for excitation.

- In this scheme, only PSI is functional. Hence, the electrons are circulated within the photosystem.
- This results in a cyclic flow of electrons.
- This scheme could possibly be occurring in stroma lamellae because it lacks both PSII and NADP reductase enzyme.
- This cyclic flow results only in the synthesis of ATP, and not of NADPH + H+.

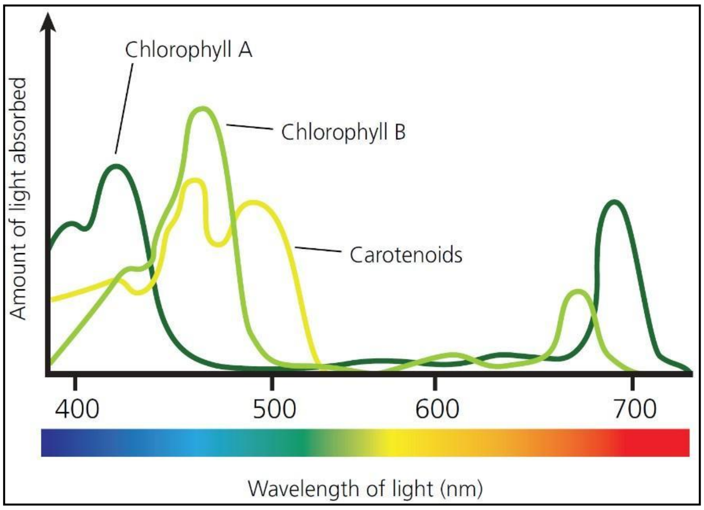
	Non- cyclic Photophosphorylation	Cyclic Photophosphorylation
1.	Photolysis of water takes place.	No photolysis of water occurs.
2.	Both PS I and PS II are involved.	Only PS 1 is involved.
3.	Electrons are not cycled.	The electrons released by PS I come back to PS I itself.
4.	Both ATP and NADPH are produced.	Only ATP is formed.
5.	Oxygen is liberated	Oxygen is not liberated.

Chemiosmotic Hypothesis

Synthesis of ATP in chloroplast can be explained by chemiosmotic hypothesis. The way it happens in respiration, ATP synthesis during photosynthesis happens because of development of a proton gradient across a membrane, i.e. membrane of the thylakoid. The following steps are involved in development of proton gradient across the thylakoid membrane.

- When the electrons move through the photosystems, protons are transported across the membrane. The primary acceptor of electron is located towards the outer side of the membrane. It transfers its electrons no to an electron carrier but to an H carrier. Due to this, it removes a proton from the stroma while transporting an electron. When electron is passed to the electron carrier on the inner side of the membrane, the proton is released into the inner side or the lumen side of the membrane.
- The NADP reductase enzyme is located on the stroma side of the membrane. Protons are also necessary for the reduction of NADP+ to NADPH+H+. These protons are also removed from the stroma.
- Thus, protons in the stroma decrease in number and accumulate in the lumen. This results in development of a proton gradient across the thylakoid membrane. Additionally, there is a measurable decrease in pH in the lumen.
- The breakdown of this gradient leads to release of energy. The movement of protons across the membrane to the stroma results in breakdown of this gradient. The movement of protons takes place through the transmembrane channel of the F0 of the ATPase.

- The ATPase enzyme consists of two parts. One part is called the F0 and is embedded in the membrane. This forms a transmembrane channel which carries out facilitated diffusion of protons across the membrane. The other portion is called F1. It protrudes on the outer surface of thylakoid membrane on the lumen side.
- The breakdown of the gradient provides enough energy to cause a change in the F1 particle of the ATPase which results in synthesis of several molecules of energy-packed ATP.



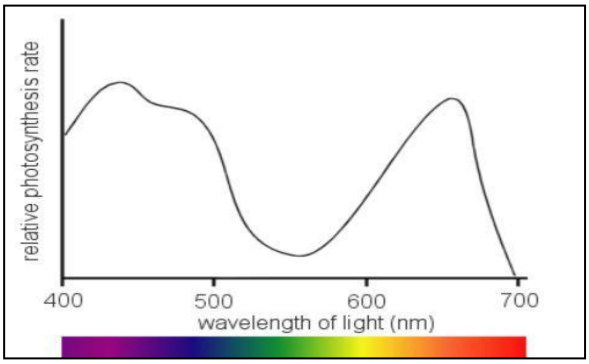
To summarise, it can be said that chemiosmosis requires a membrane, a proton pump, a proton gradient and ATPase. Energy is utilised to pump protons across a membrane, to create a gradient or protons within the thylakoid membrane. ATPase has a channel. This channel allows diffusion of protons back across the membrane. The diffusion of protons releases enough energy to activate ATPase enzyme. The ATPase enzyme catalyses the formation of ATP. NADPH and the ATP are used in the biosynthetic reaction which takes place in the stroma. This reaction is responsible for fixing CO2 and for synthesis of sugars.

(a) ABSORPTION SPECTRA

A plot (graph) showing absorption of light of different wavelength a pigment is called absorption spectrum. A solution of a pigmy placed in a **spectrometer.** It gives the wavelength absorbed by pigment. The light absorbed by the pigment is plot against wavelength gives absorption spectrum.

• Both chlorophyll a and b show maximum absorption in the **violet** and **orange-red** region of visible spectrum. Green and light is absorbed very little. The blue and red light are used by the green plants as energy source for photosynthesis. The absorption of chlorophyll a Shows t 0 peaks at about 680 and 700 tun.

- Carotenoids absorb radiant energy between 449 and 490 mu. Carotenes show absorption peaks at 449 and 478 nm.
- The xanthophylls shows peak at 440 and 490 niii.

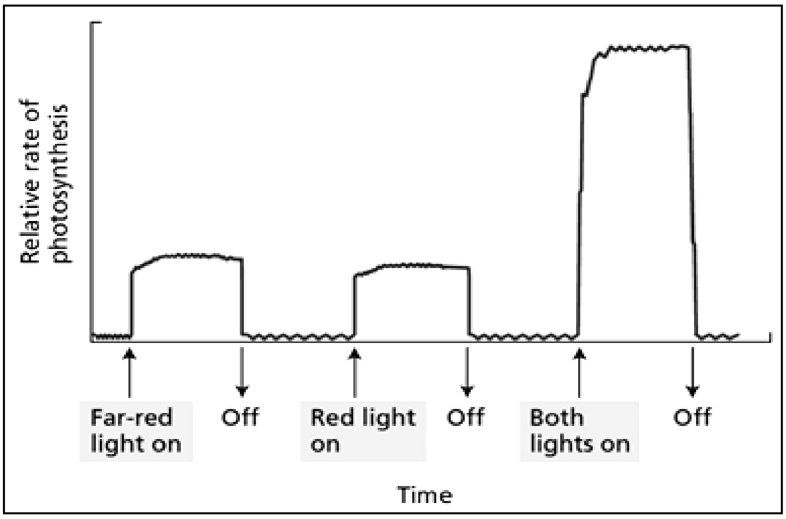


b) Action Spectra

The plot showing relative effectiveness of different wavelengths of ht in photosynthesis is called action spectrum. Action spectrum ratifies the pigments actually involved in the photosynthesis. The two photo system was also discovered by action spectrum. The action ctra are measured by spectrographs. A sample is placed in larger area tram. Monochromatic light is passed through it. Then its action is measured by spectrograph.

Comparison of absorption spectrum and action spectrum

Absorption spectrum is given by all the pigments (Chl a. b, carotene and xanthophylls). But the action spectrum is given by only chlorophyll a. It means chlorophyll a forms the reaction centre. There is a great difference between the absorption spectrum and action spectrum of chlorophyll a. The peaks of its action spectrum have more height and it valleys are much narrow than the absorption spectrum. It indicates that all other pigments are accessory. They transfer their energy to chlorophyll a. So it performs more photosynthesis than it actually absorbs light. Therefore, its action spectrum looks more efficient than the absorption spectrum.



quantum requirement: the number of quanta of light absorbed required for the transformation of one molecule; the inverse of the quantum yield.

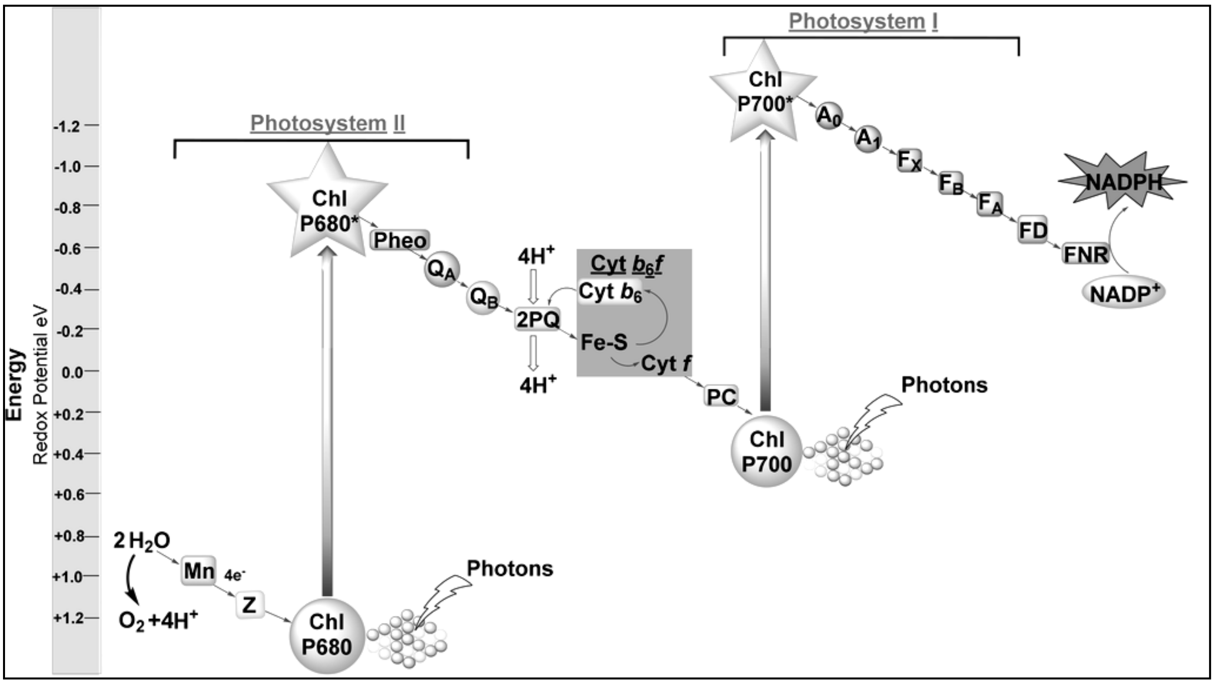
quantum yield (φ):
the number of molecules transformed (for example, via a reaction) per quantum of light absorbed; the inverse of the quantum requirement.
Synonym(s): **quantum efficiency**

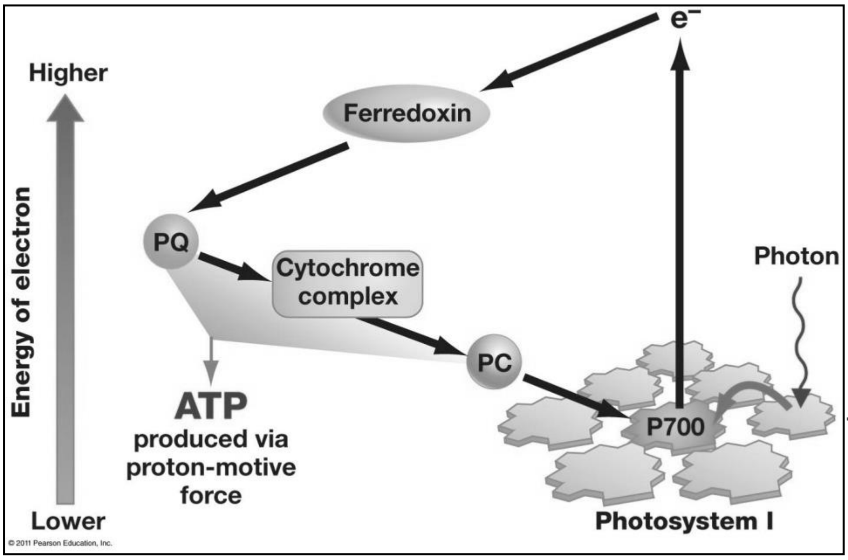
Red Drop and Enhancement Effect

The phenomenon of red drop shown by Emerson and Lewis was quite puzzling. This is not due to decrease in light absorption because the quantum yield measures only light that has actually been absorbed.

This indicates that light of wavelengths greater than 680 nm is much less efficient than light of shorter wavelengths. In subsequent experiments, Emerson and his colleagues measured photosynthesis using red and far-red light after adjusting their fluorescence rates to give equal rates of photosynthesis. They observed that the quantum yield obtained using both red and far-red light simultaneously was much higher than the sum of the yields obtained with red and far-red light separately. This phenomenon is known as Emerson enhancement effect or often as Emerson effect.

These puzzling phenomena of red drop and enhancement effect led to the conclusion that two different reaction centers or photochemical events are involved in photosynthesis. One event is driven by red light (= 680 nm) and the other driven by far-red light (>680 nm). Optimal photosynthesis occurs when both events are driven simultaneously or in rapid succession. These two photochemical events are now known as Photosystem II and Photosystem I and they operate in series to carry out photosynthesis optimally. Photosystem II absorbs red light of 680 nm well and is driven very poorly by far-red light. On the other hand Photosystem I absorb preferentially far-red light of wavelengths greater than 680 nm.




Higher
Energy of electron
Lower
Ferredoxin
e⁻
PQ
Cytochrome complex
PC
ATP
produced via proton-motive force
Photon
P700
Photosystem I
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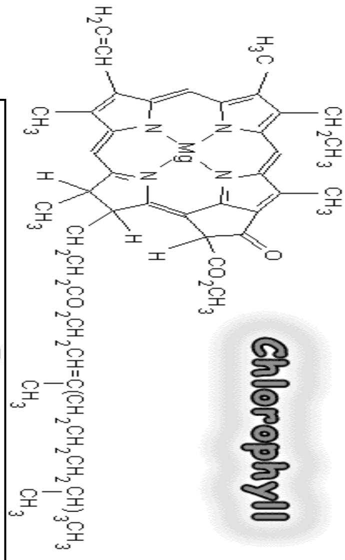
Chlorophyll