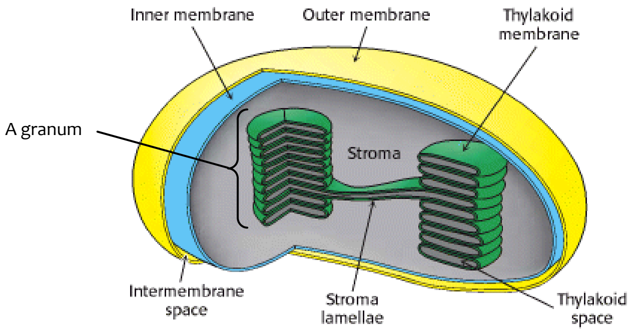
The Light Reactions of Photosynthesis

Introduction

- Essential all **free energy** utilised by biological systems arises from **solar energy** that is **trapped** by the process of **photosynthesis**, in which **water** and **carbon dioxide** *combine* to form **carbohydrates** and **molecular oxygen**.
- **Photosynthesis** occurs in **chloroplasts**:



[*Typically 5 μm long*]

Light is captured by **pigment molecules**, called **chlorophyls**, in the **thylakoid membranes**. This captured energy **excites certain electrons to higher energies**. In effect, **light** is used to create **reducing potential**. The **excited electrons** are used to produce NADPH as well as ATP in the **light reactions**, all in the **thylakoid membranes**.

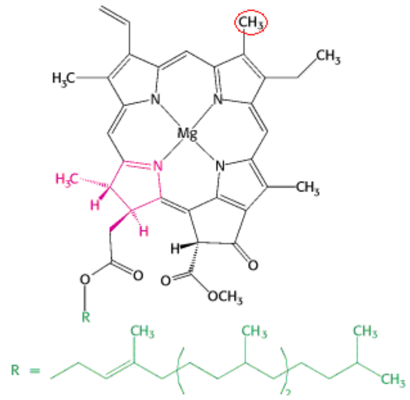
These are then used to **reduce CO_2** and covert it into **3-phophoglycerate** in a series of reactions called the **dark reactions**, or the **Calvin cycle**, which occur in the **stroma**.

As in the mitochondrion, the **outer membrane** of the chloroplast is **reasonably permeable** to small ions, whereas the **inner membranes** are **extremely impermeable**.

- The **light reactions**, as it happens, are rather similar to those of **oxidative phosphorylation**. The main difference is **where the electrons come from**.

Chlorophyl

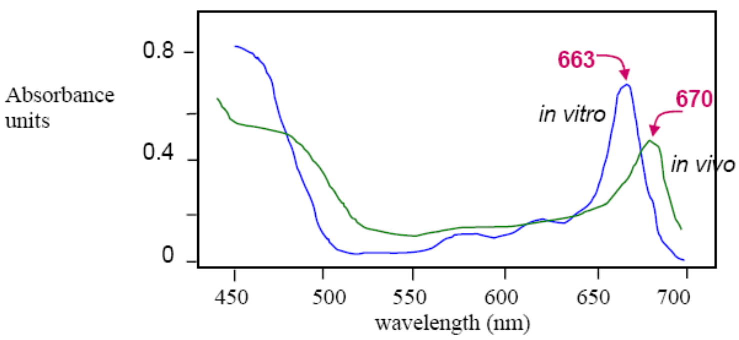
The principal **photoreceptor** in the chloroplast or **higher plants** and **organisms** is **chlorophyll**, a **substituted tetrapyrole**:



This is Chlorophyll a. In Chlorophyll b, the circled CH_3 group is replaced by a CHO group

Chlorophylls are very effective **photoreceptors** because they contain a networks of **alternating single and double bonds**, which means that a high degree of **conjugation** exists in the molecule. This gives chlorophyll its **green colour**.

Chlorophyl absorbs in the **red** and **blue** (hence why it's green). The **shape** and **position** of the **read peak** is determined by the **environment** of the molecule – **in vivo**, it looks much **flatter** and **broader**.



When a **photon** is **absorbed** by **chlorophyll**, an **electron** is **moved** to a **high energy level**. Thence, it can either **fall back** to the **ground state** and release the **absorbed energy** as **heat**, or pass to a suitable **electron acceptor** nearby. The **acceptor** then becomes **negative**, and the original molecule **positive**. This is know as **photoinduced charge separation** (the **photochemical event**). In effect, **light** moves **electrons** to **higher redox potentials**. [Note that the electron comes from the delocalised electron system, not form the magnesium, since it's not a transition metal].

The **passage** of **electrons** to the **acceptor** (rather than their falling-back to their ground state – **charge recombination**) is **favoured** by **close proximity** of the **chlorophyll** and **acceptor** in **protein complexes**, and also by the fact that charge recombination is **so thermodynamically favourable** that it takes place in the **inverted region**, where **electron transfer rates** become **slower**.

Accessory pigments (antennae)

If the **chloroplast** relied on the **light-harvesting** capabilities of the **chlorophyll *a* molecules** of the **special pair** in the **reaction centres**, the whole process would be rather inefficient, for several reasons:

- **Chlorophyll *a*** only absorbs at **specific wavelengths**, leaving a **large gap present** which falls right at the **peak** of the solar spectrum!
- The **density** of **chlorophyll *a*** molecules in the **reaction centre** is **not very great**. Thus, many photons just "**pass through**".

Thus, many other pigments are used to **absorb light** and **funnel the energy to the reaction centre**. Examples include:

- **Chlorophyll *b***, which has a spectrum similar to that of chlorophyll *a*, but where both peaks are shifted **towards the centre**.
- **Carotenoids**, which are **extended polyenes** that **absorb light** between **400** and **500 nm**. [They provide most of the yellow and red colour in fruits, are when chlorophyll molecules degrade in autumn, they are revealed].

The "**excitation**" is usually transferred from one pigment to the other and to the reaction centre by **resonance energy transfer**, which is an **electromagnetic interaction through space**, whereby the electron in *one* molecule falls back to its ground state and passes its energy to the next one along. The electron itself does not move. [The rate this happens at depends on d^{-6}, so the chlorophylls are all very close to each other (about 10Å, after which the process stops being 100% efficient)]. For reasons of **conservation of energy**, the excited state of the **acceptor** must be of **equal** or **lower** energy than the excited state of the **donor**. Thus, the **excited state of the special pair** is **lower in energy** than that of **single chlorophyll molecules**, allowing **reaction centres** to **trap** that energy. [This is because **delocalisation** occurs over the **whole** of the **chlorophyll dimmer** – this makes electrons slightly **easier** to remove from the delocalised system].

Special **protein complexes** called **photosystems** contain proteins to which the **reaction centre chlorophylls**, **light-harvesting chlorophylls** and the **acceptor molecule** are **bound**, in **precise orientations**. These are **embedded in the thylakoid membrane**.

Photosynthesis in Green Plants

In **green plants**, photosynthesis depends on the **interplay** of two kinds of membrane-bound light-sensitive complexes **photosystem I (PSI)** and **photosystem II (PSII)**:

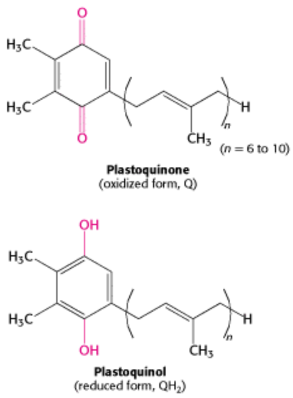
- **PSII** responds to wavelengths **shorter than 680nm**. It derives **electrons** to synthesise NADPH from **two molecules of water**.
- These electrons pass along **cytochrome *bf*** [analogous to Complex III] to **PSI**, creating a **proton gradient** at the same time.
- **PSI** responds to wavelengths **shorter than 700nm** uses these electrons to make **NADPH**.

The following scheme is known as the **Z-scheme**, and was first proposed by **Hill** and **Bendall** in **1960**.

It turns out that **light absorption** and **RET** (10^{-15} and 10^{-12}s) are ***much*** faster than the **chemical reactions** (eg: loss of electron to acceptor: 10^{-6}s).

Photosystem II

Photosystem II is an **enormous transmembrane assembly** of over 20 subunits. The **electron acceptor**, **plastoquinone**, closely resembles **ubiquinone** (a **lipid**) and cycles between an **oxidised** (PQ) and **reduced** (PQH_2) form:

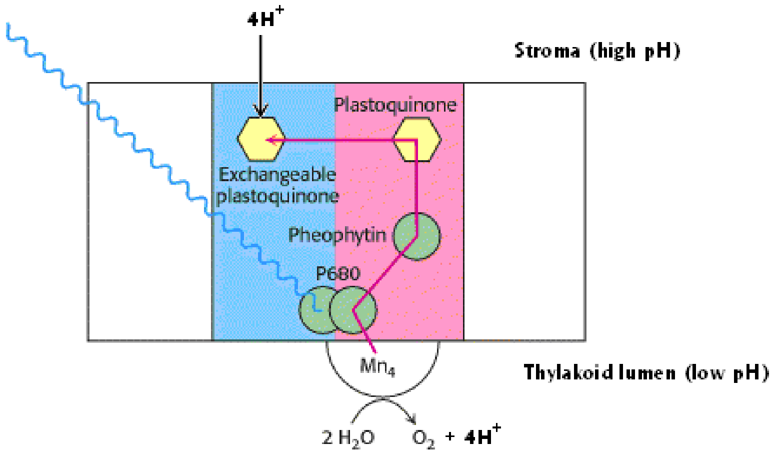


The overall reaction catalysed is:

$$2Q + 2H_2O \xrightarrow{\text{light}} O_2 + 2QH_2$$

The electrons in $\mathbf{PQH_2}$ are at a **higher redox potential** than those in $\mathbf{H_2O}$ (in oxidative phosphorylation, electrons **flowed** from PQH_2 to O_2). Hence, we are here **driving the reaction** in a **thermodynamically uphill direction** using the **free energy of light**.

The steps in the **photochemistry** of this photosystem are as follows:



1) The **special pair** of **chlorophyll *a* molecules** are **excited**. Because they absorb light at 680nm, the special pair is often called the **P680**.

2) The special pair **rapidly transfers** its electrons to a nearby **pheophytin**.
3) The electron then flows to a **fixed plastoquinone $\mathbf{Q_A}$** and then to a **mobile plastoquinone $\mathbf{Q_B}$**.
4) A **second electron excites** the P680, and $\mathbf{Q_B}$ becomes $\mathbf{PQH_2}$. In the process, it **picks up two protons**.
5) The **$P680^+$** left after **photoinduced charge separation** is a **very strong oxidant**, and it is **neutralised** by **extracting electrons** from **water molecules** bound at a **manganese** (chosen because of its ability to bind to oxygen-containing compounds and exist in a variety of oxidation states) centre [also known as the **oxygen-evolving complex**]. The electrons, however, actually come from a **tyrosine residue** (often denoted *Z*) forming a **tyrosine radical**, which then removes electrons from H_2O. This acts as a "charge store", since the photosystem can only accept electrons **one at the time**.

The **overall stoichiometry** is that **FOUR photons** extract **FOUR electrons**, and produce **$4H^+$** and **TWO PQH_2**.

Note that **PSII** is positioned such that the **manganese complex** is on the **thylakoid lumen** side whereas the site of **quinone reduction** is on the **stroma side**. Thus, the process generates a **proton gradient** across the **thylakoid membrane**.

Cytochrome *b₆f*

Electrons flow **from PSI to PSII** through the **cytochrome b_6f complex**, which catalyses the **transfer of electrons** from **PQH_2** to **plastocyanin (Pc)**, a small, soluble copper protein in the **thalakoid lumen**:

$$QH_2 + 2Pc\left(Cu^{2+}\right) \longrightarrow Q + 2Pc\left(Cu^{+}\right) + 2H^+_{\text{thyalkoid lumen}}$$

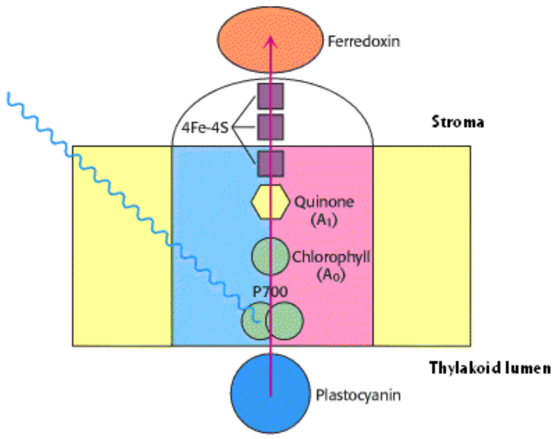
The protons released also **contribute** to the **proton gradient**.

This reaction also occurs by proceeding through the **Q-cycle**. [In reality, therefore, **2 electrons** are **removed** from the **stroma**, and **4** are **released** in the **thylakoid lumen**].

Photosystem I

This photosystem **produces NADPH**. A **special pair** of **chlorophyll *a*** molecules lie at the centre of the structure and absorb light at **700nm** (hence called **P700**). This centre initiates **photoinduced charge separation**.

The steps are as follows:



2) A **photon** of **light** is absorbed by **P700**. **Photoinduced charge separation** occurs and the **electron** travels down to **chlorophyll** at site $\mathbf{A_0}$ and **quinone** at site $\mathbf{A_1}$ to a set of **4Fe-4S clusters**.
3) The **electron** is then transferred to **ferrodoxin**, containing a **2Fe-2S cluster** coordinated to **four cysteine residues**.
4) This is then transferred to **NADP$^+$**, a much more **useful reducing agent** (because it carries ***two*** electrons). This is done by **ferrodoxin NADP$^+$ reductase**, a **flavoprotein** with an **FAD prosthetic group**. **FAD** accepts *two protons* and *two electrons* from *two reduced ferrodoxins* to form **FADH$_2$**. It then transfers a **hydride ion** to **NADP$^+$**, and releases an **H$^+$** ion. This occurs on the **stromal side** of the membrane, and so **contributes** to the **proton gradient**.

5) **$P700^+$ captures** an electron from **reduced plastocyanin** to return to **P700**.

ATP Production

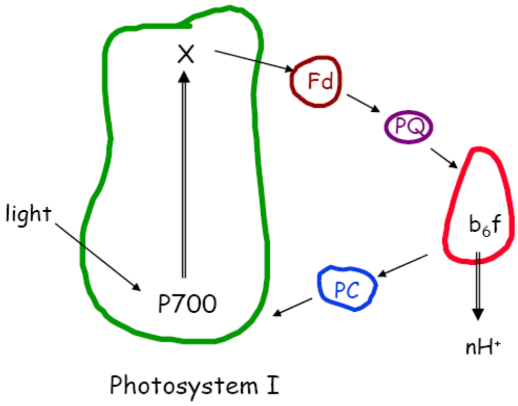
The **proton gradient** is then **used** to **generate ATP**, in a **very similar way** this is done in **mitochondria**. The **ATP synthase** of chloroplasts is also very similar, apart from the fact that it **faces the other way**, because the **proton gradient** is **reversed** in mitochondria.

In **mitochondria**, there are both **electrical** and **chemical** gradients. The **p.m.f.** is the **sum** of both these forces. In **chloroplasts**, there **electrical gradient** is **practically 0**. This is because the **thylakoid membrane** is quite permeable to **Mg^{2+}** and **Cl^-**. The **movement** of **H^+** through the membrane is therefore **coupled** with the movement of **Mg^{2+}** or **Cl^-**.

As a result of these reactions, **ATP** and **NADPH** are released in the **stromal space**. Thus, they are **appropriately positioned** for the **subsequent dark reactions**.

Cyclic Electron Flow

In some cases, when the **concentration of NADPH** is **very high**, **$NADP^+$** might not be available to accept electrons from **ferrodoxin**. In such a case, the electron in **reduced ferrodoxin** can be transferred to the **cytochrome *bf* complex** rather than to **$NADP^+$**. The **cytochrome *bf* complex** then reduces **plastocyanin**, which can then be **reoxidized by $P700^+$** to **complete the cycle**. The **net outcome** of this cycle is the setting up of a **proton gradient**, ***without*** the production of **NADPH**. Photosystem II is not involved, and O_2 is not formed from H_2O. This process is called **clyclic photophosphorylation**:



This is important, because it allows the chloroplast to produce **ATP** and **NADPH** in **varying proportions**.

Overall Stoichometry

Overall:

- **EIGHT PHOTONS** yield **ONE O_2**, **TWO NADPH** and **THREE ATP** molecules.
- In **cyclic photophosphorylation**, **TWO PHOTONS** yield **ONE ATP**.

This can be calculated by noting that there are apparently **12** rotating units in chloroplast ATP-synthase.

The Orientation of Membrane Components

Thylakoid membranes in most plants are **differentiated** into **stacked** and **unstacked** regions. **Stacking** increases the **amount** of thylakoid membrane in a given chloroplast volume. Both regions surround a **common thylakoid space**, but only **unstacked regions** make **direct contact** with the **stroma**. In general:

- **Photosystem I** and **ATP synthase** are located in the **unstacked region**.
- **Photosystem II** is present mostly in **stacked** regions.
- The **Cytochrome *bf*** complex is found in **both regions**, and moves quickly from one to the other.

PQ and **PC** act as **mobile carriers of electrons**.

Experimental Methods

The **photosystems** can be analysed by **lysing** the **chloroplasts**, **isolating the thylakoids** and **solubilising** the **membrane** with **detergent**. The **protein complexes** are **released**. It turns out that:

- **Isolated chloroplasts** will **evolve O_2** in the **absence of CO_2 fixation** – this is the **Hill reaction**.
- **Isolated thylakoids** will carry out **partial reactions**, but need **stromal extracts** for complete **electron transfer**, implying that there are **extrinsic proteins**.
- The **isolated complexes** do what they we said they did above!
- Treating **PSII** with **high salt** *removes* the **oxygen-evolving complex**, and the photosystem will now only carry out the **photochemical event**.