

1 Introduction

1.1 Photosynthesis

Photosynthesis is one of the most fundamental processes on our planet, converting sunlight to chemical energy. Early organisms lived on small organic molecules (e.g. CH₄, H₂, H₂S, malonate) occurring in a reductive atmosphere. Later, micro-organisms using the sunlight, created O₂-rich air. These organisms were living under water, which protected them from the damaging UV-radiation. The released oxygen created an oxidative atmosphere and an oxygen-derived ozone layer protected our planet from aggressive UV-B radiation (Kasting and Siefert, 2002). This made higher life possible. Outside the water these dramatic changes were one of the key events in the development of life on earth, and the present oxygen level is determined by the balance between photosynthesis and respiration. The widespread occurrence of photosynthetic bacteria as early as 3.5 billion years ago (Awramik, 1992) is indicated by fossil evidence (stromatolites). The molecular machinery of oxygenic photosynthesis is the most powerful biologically converter of energy.

In principle there are two independent ways how to use solar energy: first, classical photosynthesis characterised by the use of cyclic or acyclic tetrahydropyrrol-pigments, where the transformation of light to chemical energy involves several oxidation and reduction steps. Second, photoreception takes place in archaea (Trissl, 1990) establishing an ion gradient across the membrane. The membrane-intrinsic protein bacteriorhodopsin collects light with a covalently bound retinal (vitamin A-aldehyde) and undergoes a thermo-reversible, photochemical 13-*cis-trans*-isomerisation, which pumps protons inside the cell. The resulting electrochemical gradient of protons across the membrane is used to power ATP-synthetase. The synthesised ATP is used to drive a wide range of energy-requiring reactions. This allowed photoautotrophic forms like halophilic bacteria to fill this niche. This kind of photosynthesis is of minor importance and symbolises an alone standing way.

1.2 Historical

J.B. van Helmont (1577-1644) observed early that a willow that had been cultivated in a container for five years with enough watering gained more than 25 kg weight although only two ounces of the container's soil were lost. He concluded that plants received their nutrition

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from water and/or soil. S. Hales (1677-1761) understood that air, before the composition of air became known, and light are necessary for the nutrition of green plants. In 1771, J. Priestley (1733-1804) observed that green plants release oxygen and thus improve the air. J. Senebier (1742-1809) discovered that the regeneration of air is based on the use of carbon dioxide. These observations were confirmed and broadened by studies of J. Ingenhousz (1730-1799) who recognised both the meaning of light and the fact that the whole carbon contained in plants is of atmospheric origin. In 1804, T. des Saussure (1767-1845) discovered that the plant's increase in weight cannot solely be caused by the uptake of carbon and minerals, but is also based on the binding of the water components. J. v. Sachs (1832-1897) could finally prove that chlorophyll is involved in photosynthesis. In addition he showed that starch is produced in chloroplasts as a result of photosynthetic activities. These results are in accord with the first law of thermodynamics, whose discoverer J. R. Mayer postulated already in 1842 that plants take up energy in the form of light and that they transform it into biochemical energy. In 1894, T. W. Engelmann (1843-1909) constructed a gadget out of a modified microscope condenser that allowed him to expose parts of photosynthetically active cells to a thin ray of light. Whenever parts of the chloroplast were illuminated, the bacteria concentrated in this area (where oxygen and light were available). He observed that the bacteria accumulated mainly in blue and red light rays, suggesting that photosynthesis depends on the spectrum of light that resembles roughly the absorption spectra of chlorophyll *a* (Chl*a*) and Chl*b*.

In 1864, Boussingault could show that the volume of reacting CO₂ is approximately the same as that of the produced oxygen. In 1925 O. Warburg (Kaiser-Wilhelm-Institut, later Max-Planck-Institut für Zellphysiologie in Berlin-Dahlem) manifested the existence of two classes of photosynthetic reactions: the light and the dark reactions. R. Willstätter (1872-1942) was working on chlorophyll molecules and in cooperation with A. Stoll he discovered that the quotient between CO₂/O₂ is exactly one. A first experimental verification that the oxygen developed during photosynthesis stems from water was provided by R. Hill. He detected that isolated chloroplasts release oxygen in the presence of unnatural reducing agents like iron oxalate, ferricyanide or oxidising agents like benzoquinone after exposure to light.

After light exposure to plants, S.M. Ruben and A.P. Vinogradow could demonstrate that ¹⁸O₂ is the product of H₂¹⁸O. The discovery of molecules involved in electron transfer falls in the middle of the 20th century: 1947 plastoquinone by M. Koffler, 1951 cytochrome (cyt) *b* and *f*

by R. Hill, 1960 plastocyanin by Kato and 1961 ferredoxin by I.K. Tagawaga and D.I. Arnon. In 1960, the Z-scheme (Fig. 1.5) was suggested by R. Hill and F. Bendall, based on the existence of two parallel light reactions acting in series across the membrane. In the same year, the groups of B. Kok, L.N.M. Duysens and H.T. Witt could prove the existence of two PS. Eight years later G. Döring, H.H. Stiel and H.T. Witt identified P680, formed by a pair of Chla, as reaction centre (RC) of PSII (Witt *et al.*, 1961), and another three years later the same group discovered the charge separation across the thylakoid membrane.

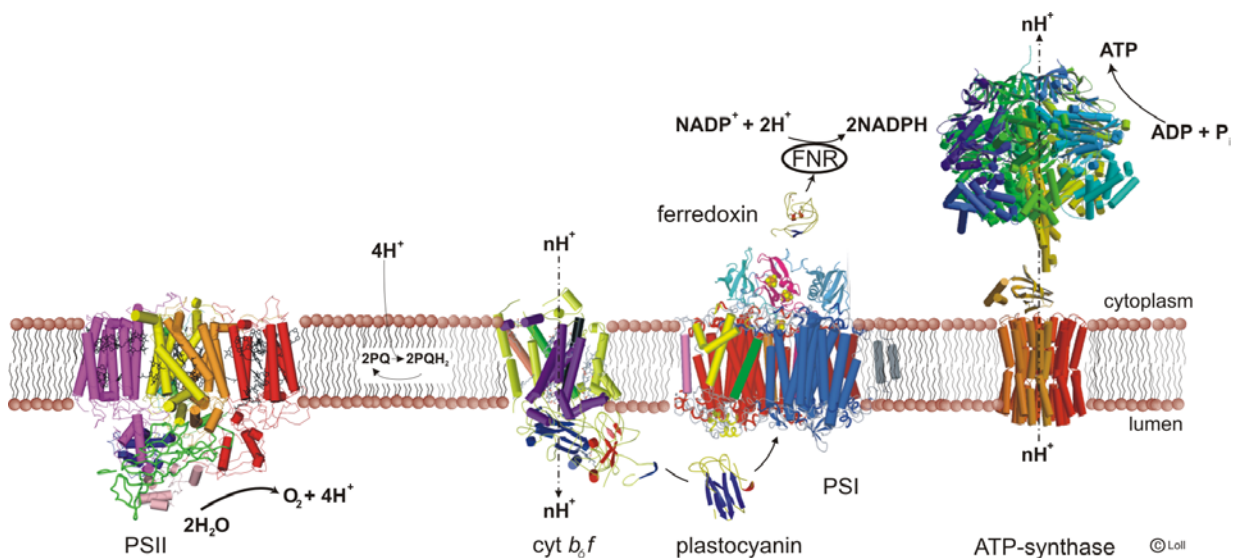


Fig. 1.1: Overview of the protein complexes involved in oxygenic photosynthesis and small electron transferring proteins that are located in the thylakoid membrane. For clarity all proteins are shown as monomers. From the left to the right: PSIIcc (PDB entry: 1W5C), PQ (plastoquinone), PQH₂ (plastoquinone), cyt *b₆f* (PDB entry: 1Q9O (Stroebel *et al.*, 2003)), plastocyanin (PDB entry: 1BXV (Inoue *et al.*, 1999)), PSI (PDB entry: 1JB0 (Jordan *et al.*, 2001)), ferredoxin (PDB entry: 1CJN (Hatanaka *et al.*, 1997)), FNR (ferredoxin-NADP⁺-reductase) and the mitochondrial ATP-synthase (PDB entry: 1QO1 (Stock *et al.*, 1999)). The indicated stoichiometries are related to the release of one mol O₂.

In the mid of 1980ies, the interest in structural information was awaking. A milestone was the structure of the purple bacterial reaction centre (PbRC) (Deisenhofer *et al.*, 1985). The structure solved by J. Deisenhofer, H. Michel and R. Huber gave new insights in protein-cofactor interaction and inspired the field of photosynthesis and the field of membrane protein crystallisation. In the meantime several photosynthetic complexes could be structurally characterised. Structures were obtained for the light-harvesting complex II (LHCII) by W. Kühlbrandt (Kühlbrandt *et al.*, 1994; Liu *et al.*, 2004). The first high resolution X-ray structure of bacteriorhodopsin became available in 1997 (Pebay-Peyroula *et al.*, 1997). The

molecular picture of oxygenic photosynthesis was completed by the structures of PSI by Jordan *et al.* (Jordan, 2001; Jordan *et al.*, 2001), the cyt *b₆f* complex (Kurusu *et al.*, 2003; Stroebel *et al.*, 2003) and a first models of PSIIcc at low resolution (Vasil'ev *et al.*, 2001; Zouni *et al.*, 2001; Kamiya and Shen, 2003; Ferreira *et al.*, 2004).

1.3 Absorption of light

The main photoreceptor of photosynthetic organisms is chlorophyll, a tetrapyrrol derivate. The cyanobacterium *T. elongatus* contains exclusively Chl*a*. The main differences to the haem group are (1) the central metal ion is Mg²⁺ (2) pyrrol-ring III has an additional cyclopentanone-ring (pyrrol-ring IV) (3) pyrrol-ring IV is partially reduced (4) the propionate group of pyrrol-ring IV is condensed with a phytol tail (Fig. 1.2). The conjugated π -system efficiently absorbs sunlight. Small differences in the protein-surrounding can modulate the spectral properties of Chl*a*.

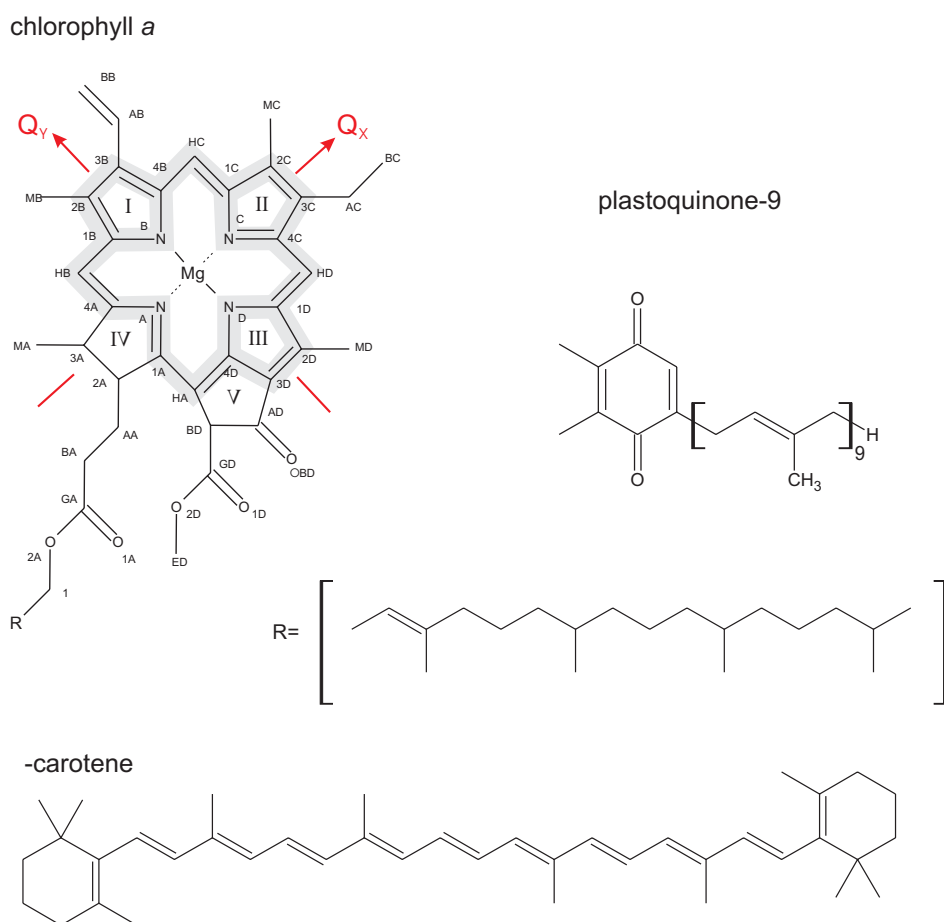


Fig. 1.2: Structure formula of cofactors. R indicates the phytol chain. The conjugated π -system of Chl*a* is shown in grey background. The transition dipole vectors Q_x and Q_y are shown by red arrows labelled on their tips.

Cyanobacteria make use of carotenoids to fill the absorption gap, covering the spectral region where Chl a are less absorbing. Carotenoids are linear polyenes like e.g. β -Car (Fig. 1.2). Moreover PSII contains pheophytin (Pheo a). Pheo a takes part in the electron transfer reactions. The only difference to Chl a is the absence of the central metal ion (Mg $^{2+}$). They are mainly stabilised by hydrophobic interactions with the protein matrix. Finally, plastoquinones are the terminal electron acceptors of PSII (Fig. 1.2).

1.4 The photosynthetic reaction centre

The central unit of photosynthesis is the photosynthetic RC. Its purpose is the conversion of light energy to biochemically usable energy. Under biochemical aspects, the RC is the smallest biophysical unit that can drive charge separation. Of special interest is the primary electron donor and its functional relationship to other cofactors of the ETC, whereas the importance of the protein scaffold is often underestimated. The definition of RC is sometimes not clearly stated in the literature.

In this thesis, the RC describes all protein subunits which are directly involved in binding of cofactors belonging to the ETC. All other protein subunits or cofactors are not considered to belong to the RC. This definition is based on the structure, as the biochemical preparation of such RC might be not possible. In terms of the bacterial RC this definition comprises the protein subunits L and M as well as the embedded cofactors. In the case of PSI the RC comprises the C-terminal domains of PsaA, PsaB and subunit PsaC, whereas in PSII, the RC comprises the protein subunits D1 (PsbA) and D2 (PsbD).

The term PS comprises an RC which is surrounded by an inner central antenna system, as in PSII or PSI of oxygenic photosynthesis and the RC complex of green sulphur bacteria or heliobacteria.

The RC has a number of characteristics: with its peripheral antenna system it is able to collect light energy. Its unique primary electron donor can be excited to conduct a directed charge separation and to convert light energy into a stable form which can be subsequently used for chemical reactions.

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RC are embedded in the photosynthetic membrane. In most of oxygenic organisms, this is a closed system built up by the membrane system of the thylakoids. The surface, where the RC is donating electrons is called the "negative" side (n-side), whereas the opposite side where electrons are accepted is named in analogy "positive" side (p-side). Near the p-side of all RC the primary electron donor (P) is located. The following number indicates the wavelength (in nm) of its absorption maximum (e.g. P680).

After excitation, the primary electron donor is converted to an excited state P^* with a short life time, which leads within a few pico-seconds to charge separation. The electron is very rapidly donated to the first electron acceptor (A). The resulting radical pair $P^{*\bullet}A^{\bullet}$ is labile and stabilised by stepwise reduction of the following electron-acceptors ($A_1...A_t$) of the ETC. The fast transfer guarantees the uni-directionality avoiding possible charge recombination. Finally, the electron reaches the terminal acceptor A (A_t), close to the p-side. The oxidised primary electron donor P^{+} is reduced by an external electron donor on the luminal side and returns to its ground state.

1.5 Classification of reaction centres

Generally, two classes (Fig. 1.3) of reactions are employed in distinguishing RC complexes: organisms of oxygenic photosynthesis (often incorrectly referred to as plant-type photosynthesis) contain two RC-complexes, PSI and PSII, while those of anoxygenic photosynthesis (incorrectly referred to as bacterial photosynthesis) possess only one.

The RC can also be divided according to their terminal electron acceptor. In type-II RC (purple non-sulphur bacterial, green filamentous bacterial and oxygenic PSII) quinones function as the terminal electron acceptors (quinone-type RC), whereas this role is performed by iron-sulphur clusters in type-I RC (green sulphur bacterial PSC, heliobacterial PSH and oxygenic PSI). No species capable of tetrapyrrole-pigment-based photosynthesis is known amongst the Archaea, while algae and higher plants amongst the eucaria have acquired their photosynthetic ability by endosymbiotically incorporating oxygenic bacteria (Woese *et al.*, 1990; Pace, 1997).

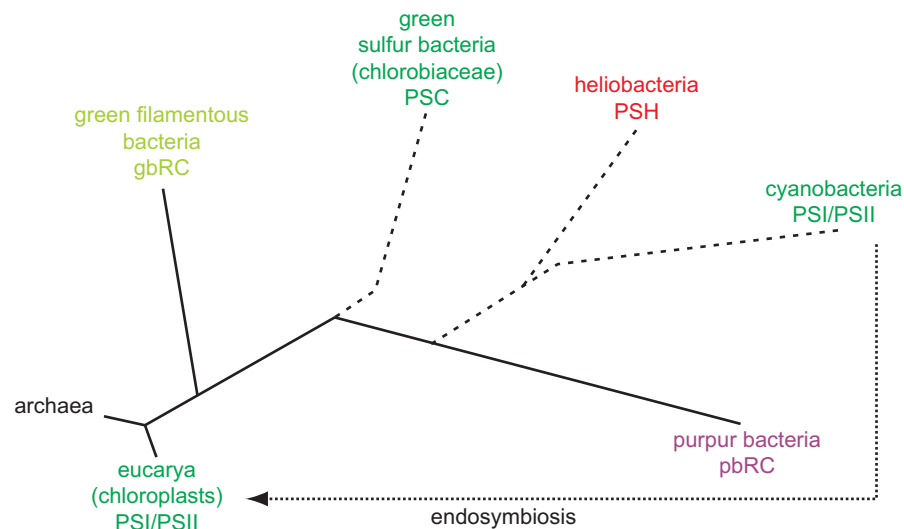


Fig. 1.3: Evolutionary relations between the different photosynthetic organisms (Nitschke and Rutherford, 1991). Two classes of RC are indicated by dashed lines (type-I) and solid lines (type-II). Remarkably, cyanobacteria and eucarya contain both classes of RC. Under the organism name, the abbreviation for the RC is indicated.

1.6 Endosymbiotic theory - thylakoid system

According to the endosymbiotic theory, cyanobacteria-like cells were incorporated into the eukaryotic systems and developed into the plastids of algae and higher plants (Margulis, 1981; Stiller and Hall, 1997), thereby extending oxygenic photosynthesis to the eukaryotes (Fig. 1.3).

There is evidence that chloroplasts were once free living bacteria that invaded a non-photosynthetic cell long ago. They have retained some of the DNA necessary for their assembly, but much of the DNA necessary for their biosynthesis is located in the cell nucleus. This enables a cell to control the biosynthesis of chloroplasts.

In plants, the photosynthetic process occurs inside chloroplasts, which are organelles found in certain cells. Each chloroplast is defined by an inner and an outer envelope membrane and is shaped like a convex lens that is 5-10 μm in diameter (Fig. 1.4). The inner envelope membrane acts as a barrier, controlling the flux of organic and charged molecules in and out of the chloroplast. Water passes freely through the envelope membranes, as do other small neutral molecules like CO_2 and O_2 other than positively charged protons.

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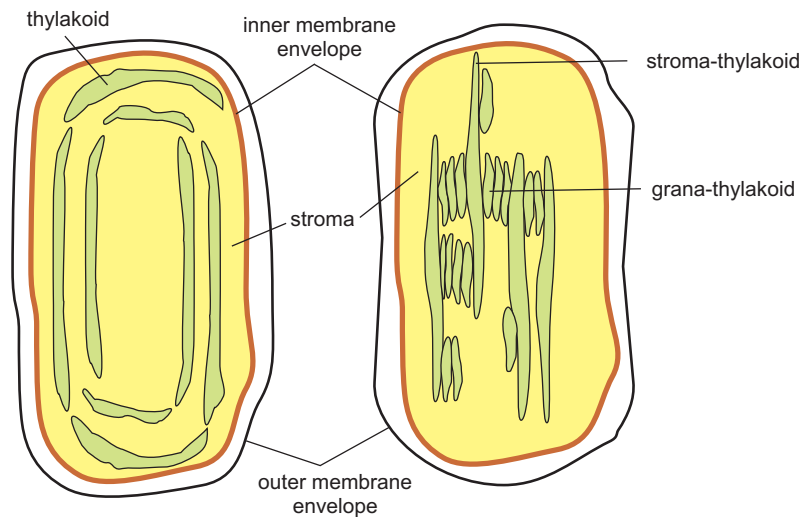


Fig. 1.4: Photosynthetic membrane: outer membrane envelope (black), inner membrane envelope (orange), stroma/cytoplasm (yellow) and thylakoid (green). On the left side, membrane system of cyanobacteria and on the right side chloroplast of higher plants developed from photosynthetic prokaryotes according to the endosymbiotic theory.

Inside the chloroplast there is a third membrane system, known as the photosynthetic membrane (or thylakoid membrane), that contains most of the proteins required for the light reactions. The proteins required for the fixation and reduction of CO_2 are located outside the photosynthetic membrane in the surrounding aqueous phase.

The photosynthetic membrane is vesicular, defining a closed space with an outer water space (stromal or cytoplasmic phase) and an inner water space (lumen). The organisation of the photosynthetic membrane can be described as groups of stacked membranes (grana-thylakoids), interconnected by non-stacked membranes (stroma-thylakoids) that protrude from the edges of the stacks. Experiments indicate that the inner aqueous space of the photosynthetic membrane is likely to be continuous inside the chloroplast. It is not known why the photosynthetic membrane forms such a convoluted structure. PSII occurs monomeric in the stroma-thylakoids, whereas the grana-thylakoids contain mainly dimeric PSII.

The overall architecture of the cyanobacterial thylakoid system differs from that of higher plants (Fig. 1.4). As the phycobilisomes are membrane-extrinsic and attached to the surface of PSII, stacking of the thylakoids is not possible. Therefore, no differentiation for the thylakoid system is observed (Fig. 1.4). Cyanobacterial PSII dimers and the associated phycobilisomes

are arranged in the row, with inserted PSI and *cyt b₆f* complexes as well as ATP-synthetase. The occurrence of monomeric PSII in the thylakoid membrane has not been observed so far.

As a result of endosymbiosis, most of the genes required for photosynthesis are chloroplast encoded. Consequently all these proteins have to be imported across the thylakoid membrane from the cytosol, where they were synthesised. All proteins destined for interior locations within plastids are synthesised as precursor proteins carrying cleavable transit peptides. Transit peptides direct proteins into plastids *via* the general protein import pathway.

1.7 Oxygenic photosynthesis

Two categories of phototrophic organisms have to be distinguished: if water is oxidised to molecular oxygen as the product of photosynthesis, we are describing oxygenic photosynthesis occurring in prokaryotic cyanobacteria as well as in eukaryotes such as algae and higher plants. In all phototrophic prokaryotes except cyanobacteria, we do not observe the release of oxygen as for example green sulphur bacteria convert small organic substances like H₂S to sulphur, and purple bacteria convert succinate to fumerate. In such case we discuss anoxygenic photosynthesis.

The primary processes of oxygenic photosynthesis are light-induced charge separation reactions across the thylakoid membrane. These reactions are carried out by two large multi-subunit complexes, PSI and PSII, embedded in the photosynthetic membrane (Fig. 1.1). This is in contrast to anoxygenic photosynthesis, where only one membrane-intrinsic protein complex is involved. The numbering of the PS reflects their discovery and is not functionally related. Both systems are able to absorb light and to transfer the excitation energy to their primary electron donors P700 in PSI and P680 in PSII that are composed of pairs of Chl_a and are called "special pair". Further electron transfer generates an electrochemical gradient of protons across the membrane that powers ATP-synthetase. This reaction is known as the light-reaction. The two main events are the charge separation steps at the special pairs of PSII and PSI. Fig. 1.5 shows the energy diagram (Z-scheme) of the electron transport by embedded cofactors across the thylakoid membrane, first suggested by Hill and Bendall (1960).

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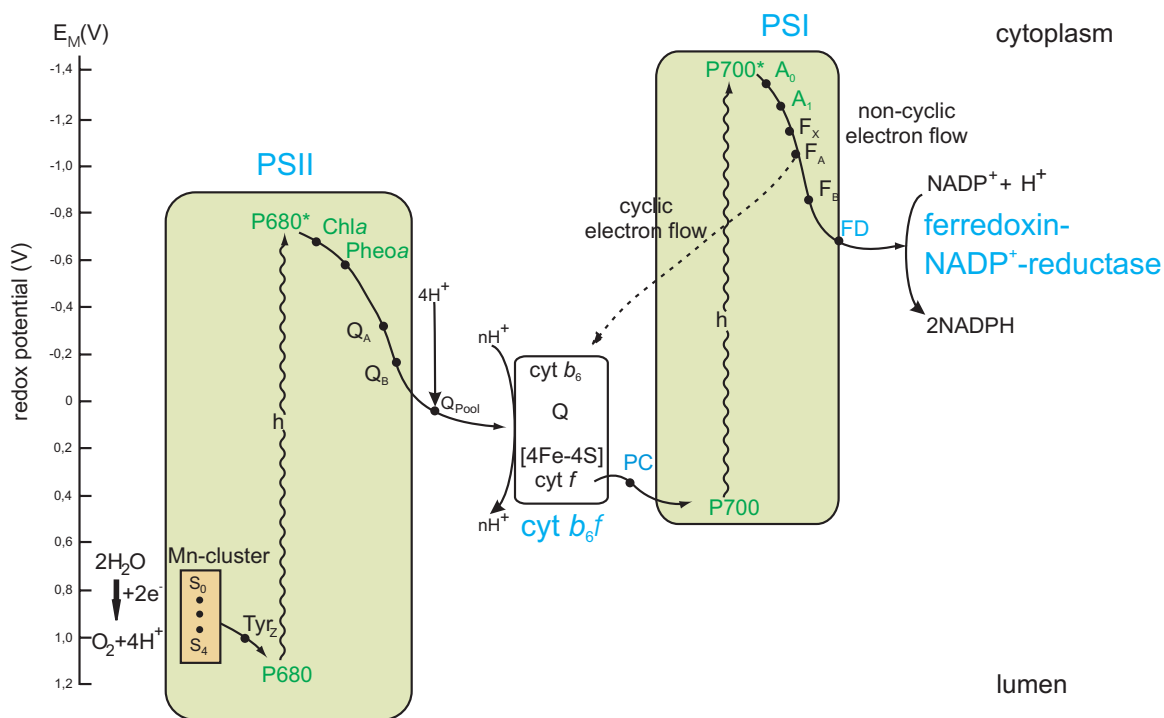
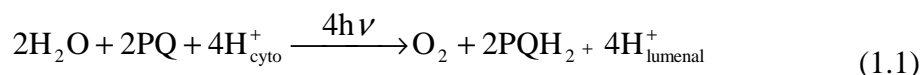


Fig. 1.5: Z-scheme of cyanobacterial photosynthesis: Energy diagram of light-reactions of oxygenic photosynthesis. The axis on the left side gives the electrochemical potential in [V]. All cofactors involved in electron transfer reactions are indicated according to their redox-potentials. The reduction potentials increase downward so that electrons flow spontaneously in this direction. Protein-pigment complexes involved are, from the left to the right side: PSII, *cyt b₆f* and PSI. Absorption of one photon by P680 ejects an electron along the ETC to the final electron acceptor Q_B. At P680 the electron-gap (after release of an electron) is filled by an electron derived *via* Tyr_Z from the Mn-Ca-cluster catalysing the oxidation of two water molecules to oxygen and four protons. The resulting plastoquinol diffuses through the thylakoid membrane and in turn reduces the *cyt b₆f* complex, with the concomitant translocation of protons into the thylakoid lumen. *Cyt b₆f* then transfers the electrons to plastocyanin (PC) and the PC regenerates photooxidised P700 in PSI. The electron ejected from P700 travels along the ETC to the electron-acceptors (A_x) and then to the iron-sulphur clusters (F_x). The electron carrier ferredoxin (FD) transfers the electrons to the ferredoxin:NADP⁺-reductase, which catalyses the reduction of NADP⁺ to NADPH in non-cyclic electron transport. Alternatively, the electron may return to the *cyt b₆f* complex in a cyclic process that translocates additional protons into the thylakoid lumen. The dashed arrow indicates probable cyclic electron flow.

After light absorption, the primary electron donor (P680) of PSII transfers one electron from its excited state (P680^{*}) along the ETC that is composed of several bound cofactors, to a bound plastoquinone (Q_B) and becomes a strong oxidant having one of the most positive midpoint potentials (~ 1.12 V) known for biological complexes. After two cycles, doubly reduced and protonated plastoquinone (PQ) is released as plastoquinol PQH₂ into the plastoquinone pool located in the thylakoid membrane (eq. 1.1).



On the electron donor side, the chlorophyll cation radical P680⁺ oxidises a redox-active tyrosine residue (Tyr_Z), which in turn oxidises a Mn-Ca-cluster composed of four Mn and one Ca cations at the catalytically active centre of PSII.

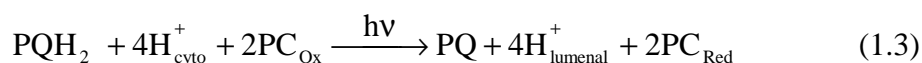
Four oxidising equivalents must accumulate at this single catalytic centre before molecular oxygen is produced and released. Eq. 1.2 summarises the reaction.



There must be four light-induced redox turnovers of P680 and Tyr_Z to produce the four oxidising equivalents accumulated at the Mn-Ca-cluster. The S-state cycle for the oxygen evolution reaction, as first suggested by Kok *et al.* (1970), was later modified to accommodate the model proposed by Babcock. The scheme is based on the observation, that the evolution of oxygen follows a period of four, when a photosynthetic organism is exposed to a series of saturating flashes of light. Oxygen is produced on the S₄ to S₀ transition. According to the hypothesis of Babcock (Tommos and Babcock, 2000; Tommos, 2002), each photo-induced step of the S-state cycle (S₀ to S₄) involves the concerted removal of an electron and a proton from two bound water molecules. During the cycle the four Mn atoms change their oxidation state. The knowledge of geometry and redox-states of all involved Mn atoms is essential to understand the mechanism of water oxidation.

In addition to the above, some side reactions can occur under certain conditions, including the oxidation of a β-carotene molecule, a Chl_a molecule and a high potential cyt *b*-559 bound within the PSII_{cc}.

After the translocation of two electrons, reduced PQ²⁻ is protonated to PQH₂ which is released from PSII and diffuses through the membrane to the membrane-intrinsic cyt *b*_{6f} complex and is re-oxidised. One plastoquinol molecule (PQH₂), gives rise to the reduction of two plastocyanin (PC) molecules *via* the cyt *b*_{6f} complex (eq. 1.3).



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The cyt *b₆f* complex provides the connection between PSII and PSI reaction centres of oxygenic photosynthesis and generates a vectorial electrochemical proton gradient across the membrane. According to the modified Q-cycle (Trumpower, 1990), a total of four protons are released to the lumen during the oxidation of one water molecule. Finally, the soluble electron carrier PC transfers electrons to PSI (Fig. 1.5). In these reactions the cyt *b₆f* complex converts the redox-potential energy of plastoquinol into a transmembrane electrochemical charge gradient of protons. The cyt *b₆f* complex is also essential for cyclic electron flow around PSI (Fig. 1.5).

PSI catalyses the light-driven transmembrane electron transfer from PC or cyt *c₆* located in the lumen to the mobile electron carrier proteins ferredoxin or flavodoxin at the cytoplasmic side of the membrane and is therefore also named cytochrome *c₆*-ferredoxin:oxidoreductase. Like PSII, PSI establishes an electron gradient across the thylakoid membrane. The main difference is the much lower redox potential of the primary electron donor P700. As a result, PSI is able to reduce ferredoxin (FD) on the cytoplasmic side. PSI retrieves the lost electrons from PC or cyt *c₆* on the luminal side. Macroscopically, the formula could be written:



The main difference between PSI and PSII are the redox potentials (Fig. 1.5) of the bound cofactors. After electron transfer from P680* *via* the ETC the redox potential of the first electron acceptor (pheophytin) is so much lowered that it could barely reduce plastoquinone, whereas the redox potential of PSI is low enough to reduce the [Fe₄S₄]-cluster. In contrast, the redox potentials of the PSI bound cofactors are not positive enough to take part in water oxidation.

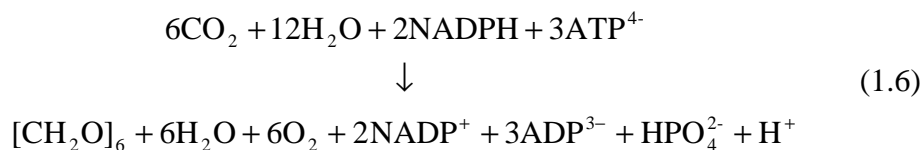
PSI reduces FD which in turn reduces NADP⁺ in a reaction driven by the ferredoxin-NADP⁺-reductase (FNR) to NADPH (eq. 1.5) which is the final product of the light reaction.



Another functional importance of FD is the partaking in the cyclic electron flow around PSI (Fig. 1.5). The processes of cyclic electron transfer between PSI and the *cyt b₆f* complex are not understood.

The electron transfer reactions in both PS are coupled in series, finally leading to the production of NADPH. As the thylakoid is a closed compartment, the lumen is acidified by the protons released from PSII and the *cyt b₆f* complex. The proton gradient across the membrane is used by ATP-synthetase to convert ADP under consumption of inorganic phosphate (P_i) to ATP. Afterwards, NADPH and ATP deliver the chemical energy needed for the CO₂-assimilation (Calvin cycle), also named the dark reaction.

The free energy of hydrolysis of phosphate bonds of ATP, and reducing power of NADPH, are used to fix and reduce CO₂ to drive the endergonic reaction to carbohydrates. For the sugar glucose (one of the most abundant products of photosynthesis) the equation is:



Enzymes and intermediates of the Calvin cycle are located in the chloroplast stroma, a compartment analogous to the matrix of the mitochondrion (see chapter 1.6). Ribulose-bisphosphate:carboxylase-oxidase (EC 4.1.1.39) catalyzes CO₂ fixation. Because it can alternatively catalyze an oxygenase reaction, the enzyme is also called Rubisco and is the most abundant enzyme on earth.

Under stress conditions, PSI is able to maintain a minimal electron transport independent from PSII and is therefore able to satisfy its demand on energy (Scheller, 1996). Under iron starvation cyanobacteria developed mechanisms to compensate for this. Hence, they express a protein named CP43' (according to its sequence homology to CP43 of PSII) that forms a well-structured light-harvesting antenna ring, composed of 18 copies, around the trimeric PSI (Bibby *et al.*, 2001a; Bibby *et al.*, 2001b; Bibby *et al.*, 2001c; Boekema *et al.*, 2001). This increases the antenna size of PSI probably as a response to the lowering of the phycobiliprotein level and decreases the PSI:PSII ratio.

1.8 Photosystem II

A broad community of scientists is interested to understand the underlying mechanistic processes of photosynthesis. As a result, a multitude of spectroscopic and biochemical articles have been published during the last decade. The function of PSII is of special interest, as it catalyses the unique oxidation of water to atmospheric oxygen. Most scientific publications are based on plant PSII from spinach and cyanobacterial PSII from *Synechocystis* sp. PCC 6803 as well as *T. elongatus*. Based on these publications, characteristics of PSII will be briefly described. In terms of function, PSII is a light-driven water-quinone:oxidoreductase located in the thylakoid membrane. In higher plants, monomeric as well as dimeric PSII-complexes are observed, whereas in cyanobacteria the PSII complex exists exclusively as dimer.

One source of structural information on PSII is microscopy, especially electron microscopy of single particles, as well as cryo-electron crystallography on 2D crystals, yielding 2D and 3D information. This improved our understanding of the organisation of the super-complexes of PSII in plants, including several different light-harvesting complexes together with the PSII core complex (PSIIcc; PSII without the peripheral antenna system) in the thylakoid membrane. Furthermore, single particle work on PSII from different species, plants, algae and cyanobacteria gave insights in differences in organisation, size and subunit composition (Boekema *et al.*, 2000; Nield *et al.*, 2000; Yakushevskaya *et al.*, 2001). A further important step towards a detailed structural understanding of PSIIcc was the elucidation of a 2D and later 3D structure of a PSIIcc complex at a resolution of 6-8 Å by cryo-electron-microscopy by Rhee and coworkers (Rhee *et al.*, 1998; Rhee, 2001). For the first time the arrangement of the central subunits as well as cofactors and the membrane-intrinsic antenna system could be identified, but as this complex was not active in water oxidation, no information about the catalytic site of water splitting could be gained.

So far several groups reported 3D crystals of PSIIcc from different sources: spinach, rice, cyanobacteria (Adir *et al.*, 1992; Tsiotis *et al.*, 1996; Adir, 1999; Kuhl *et al.*, 2000), but the crystals were not suitable for X-ray structure analysis.

The crystal structures of fully active PSIIcc from two related cyanobacteria, *T. elongatus* (Zouni *et al.*, 2001; Ferreira *et al.*, 2004) and *T. vulcanus* (Kamiya and Shen, 2003) have been

described at medium resolution of 3.8 Å to 3.5 Å, respectively and provide for the first time a picture of the three-dimensional structure of the PSIIcc.

A different source of structural information on PSII was obtained by spectroscopy, for example distance measurements by electron paramagnetic resonance (EPR), measurements of electrochromic effects. Many insights could be gained especially about the structure of the Mn-Ca-cluster by comparative EPR and/or X-ray spectroscopic measurements (EXAFS) on PSII and metal-organic model compounds.

Cyanobacterial PSIIcc is composed of at least 17 protein subunits (Fig. 1.6). According to their genes, they are named (PsbA to PsbF, PsbH to PsbO, PsbU, PsbV and PsbX). The RC is formed by the subunits PsbA (D1) and PsbD (D2) that contain five transmembrane α -helices (TMH) each and harbour all cofactors of the ETC. D1 and D2 show homology to the RC subunits L and M (Deisenhofer *et al.*, 1995) in PbRC as well as the antenna domains of PsaA and PsaB in PSI. PsbA and PsbD are surrounded by the inner antenna subunits PsbB (CP47) and PsbC (CP43). The primary sequences of both subunits show similarity to the N-terminal antenna domain of PsaA and PsaB of PSI. All antenna Chl a are embedded in the two antenna proteins. The α - and β -chains of cyt *b*-559 are membrane-intrinsic as well. The remaining 12 TMH belong to smaller subunits, with largely unknown function.

PsbO, PsbU and PsbV (cyt *c*-550) are membrane-extrinsic and attached at the luminal side of the PSIIcc, shielding the redox-active Mn-Ca-cluster. Whereas PsbO is present in all oxygenic photoautotrophic organisms, the cyanobacterial PsbU and PsbV are functionally replaced by PsbQ (17 kDa) and PsbP (23 kDa) in green algae and in higher plants.

Of special interest is the catalytic centre, where water oxidation takes place. It is formed by a Mn-Ca-cluster composed of 4 Mn and 1 Ca²⁺ cation and probably by an additional Cl⁻ anion.

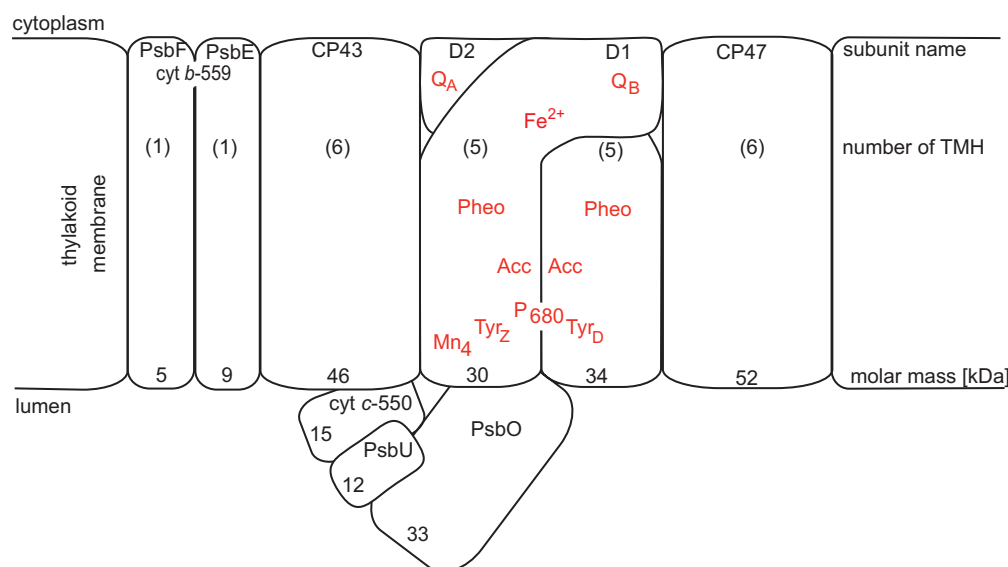


Fig. 1.6: Schematic drawing of PSIIcc monomer. For clarity small membrane-intrinsic subunits are omitted. Subunit name (top), the number of transmembrane α -helices (TMH) in parentheses and molar mass [kDa] (bottom) are indicated. In red the cofactors of the ETC from the luminal to the cytoplasmic side: Mn-Ca-cluster (Mn_4), Tyr_Z , Tyr_D , P680, accessory Chla (Acc), Pheoa, non-haem Fe^{2+} and plastoquinones (Q_A and Q_B). All other cofactors embedded in the antenna proteins (CP47 and CP43) and in *cyt b-449*, *cyt c-550* are not shown.

1.9 Photosystem I and cytochrome *b₆f* complex

Recently, two structures of algae and cyanobacterial *cyt b₆f* complexes (Fig. 1.1) were published by two independent groups (Kurisu *et al.*, 2003; Stroebel *et al.*, 2003).

The *cyt b₆f* complex contains four large subunits including *cyt f*, *cyt b₆*, the Rieske iron-sulphur protein and subunit IV as well as four small subunits (Fig. 1.1). Four haem, one [2Fe-2S] cluster, one Chla, one carotenoid, one plastoquinone and one additional quinone-analog are embedded in the protein-complex. The core of the *cyt b₆f* complex is similar to the analogous respiratory *cyt bc₁* complex, but the domain arrangement outside the core and the complement of prosthetic groups are strikingly different. Surprisingly one additional haem was identified, probably playing a role in cyclic electron flow.

The crystal structure of PSI from the thermophilic cyanobacterium *T. elongatus* (Fig. 1.1), published at 2.5 Å resolution, identifies 12 proteins which coordinate 128 cofactors (96 Chla, 2 phylloquinones, 3 [Fe₄S₄]-clusters, 22 carotenoids, 4 lipids and a putative Ca²⁺ ion). A possible docking site for *cyt c₆* and PC and for FD or flavodoxin were suggested (Jordan,

2001; Jordan *et al.*, 2001). This structural model of PSI at atomic resolution provides new insights into the function of this complex photosynthetic apparatus.

1.10 Aim of this work

At the beginning of this PhD thesis, only low resolution models of PSII_{cc} (Fig. 1.7) were available (Vasil'ev *et al.*, 2001; Zouni *et al.*, 2001), and a number of computer-based models for D1 and D2 subunits derived from sequence homology with the PbRC subunits L and M (Svensson *et al.*, 1990; Xiong *et al.*, 1996; Xiong *et al.*, 1998). Of great interest was a 3-dimensional X-ray structure at medium or even atomic resolution, as the present model was mainly a C α -model without sequence assignment (Fig. 1.7). The loop regions of D1, D2, CP47 and CP43 were not built and the membrane-extrinsic subunit PsbU was not localised. The empty space within the membrane-extrinsic subunits and the membrane-intrinsic subunits illustrate the incomplete model (Fig. 1.7).

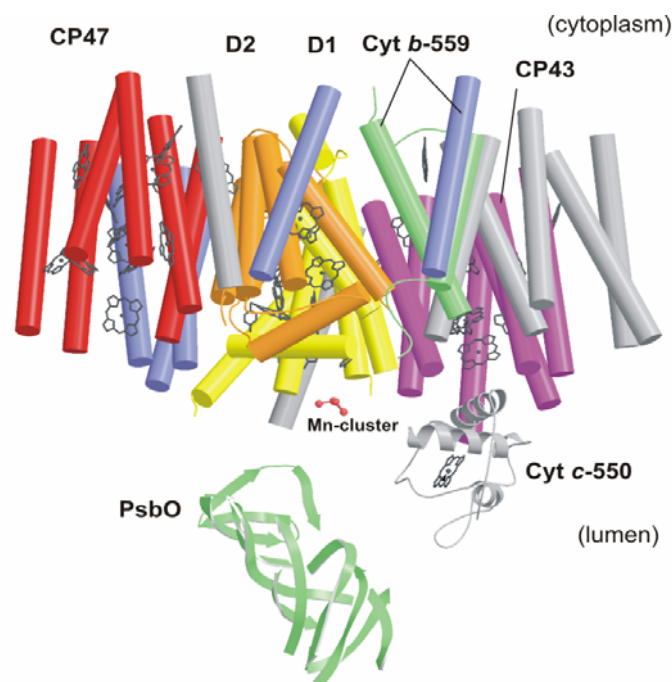


Fig. 1.7: Side view of PSII_{cc} monomer at 3.8 Å resolution (Zouni *et al.*, 2001), slightly tilted against the membrane plane. Assigned subunits in magenta (CP43), yellow (D1), orange (D2), red (CP47), α - (green) and β -chain (cyan) of cyt *b*-559, membrane-extrinsic subunits in green (PsbO) and blue (cyt *c*-550). Unassigned TMH are shown in grey.

Introduction

Since the 90ties, the group of Prof H.T. Witt (Max-Volmer Laboratorium/Institut für Biophysikalische und Biochemie der TU Berlin) and Prof W. Saenger (Institut für Kristallographie an der FU Berlin) were working to build the first structural model of PSIIcc. This was a huge step forward to obtain much new functional information based on the structure.

In cooperation with Drs A. Zouni and K.-D. Irrgang, PSII was purified and characterised. The crystallisation of PSIIcc had to be optimised to obtain higher resolution data. In cooperation with Dr J. Biesiadka the X-ray diffraction data should be evaluated, finally leading to a more complete structural model of PSIIcc. Based on such a model, spectroscopic as well as biochemical data should be discussed to understand the molecular basis of protein-cofactor interaction. The purpose was the explanation of electron transfer along the ETC and secondary electron transfer. Special attention should be addressed to the redox-active Mn-Ca-cluster to elucidate its ligands as well as its internal structure to gain sound knowledge of the light-induced water splitting mechanism, which is of special importance for the design of a new generation of solar cells.

Besides the RC, the internal antenna system was of great interest to reveal the functional connection of the antenna system to the ETC. Based on this knowledge, mutagenesis experiments can be reliably designed to elucidate spectroscopic properties of PSIIcc and to complete the molecular picture of oxygenic photosynthesis.