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PhD thesis

Structure and function of pigment-protein complexes of photosynthetic microorganisms

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Annotation

Biochemical and biophysical methods in combination with electron microscopy were used to investigate structure and function of pigment-protein complexes in thylakoid membranes of various photosynthetic organisms.

Prohlašuji, že jsem svoji disertační práci vypracovala samostatně pouze s použitím pramenů a literatury uvedené v seznamu citované literatury.

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Abbreviations

A_0	Primary electron acceptor in photosystem I, chlorophyll *a* molecule
A_1	Secondary electron acceptor in photosystem I, phylloquinone
ADP	Adenosine diphosphate
APC	Allophycocyanin
ATP	Adenosine triphosphate
BChl	Bacteriochlorophyll
b_H or b_N	High-potential heme of Cyt b_6 close to negative side of membrane
b_L or b_P	Low-potential heme of Cyt b_6 close to positive side of membrane
Cab	Chlorophyll *a*/*b* binding
Car	Carotenoid
$\mathrm{Car_{D1}}$, $\mathrm{Car_{D2}}$	β-carotene in D1 and D2 protein, respectively
CF_0	Transmembrane component of chloroplast ATP synthase
CF_0-CF_1	Chloroplast ATP synthase complex
CF_1	Catalytic component of chloroplast ATP synthase
Chl $Z_{D1,}$ Chl Z_{D2}	Peripheral chlorophyll of D1, D2 proteins, respectively
Chl(s)	Chlorophyll(s)
c_N	Covalently bound heme of Cyt b_6 close to a heme b_N
CP 43, CP 47	Core antenna proteins of photosystem II
Cys	Cysteine
Cyt b_6	Cytochrome b_6
Cyt b_6/f	Cytochrome b_6/f complex
Cyt bc_1	Cytochrome bc_1 complex
Cyt c_6	Cytochrome c_6
Cyt *f*	Cytochrome *f*
D1, D2	Photosystem II reaction center proteins of
DGDG	Digalactosyldiacylglycerol
DNA	Deoxyribonucleic acid
F_A, F_B, F_X	Iron sulphur clusters of photosystem I
FCP	Fucoxanthin chlorophyll *a*/*c* protein
Fd	Ferredoxin
FMO	Fenna-Matthews-Olson BChl *a* protein of green sulphur bacteria
FNR	Ferredoxin:$NADP^+$ oxidoreductase

GAP	Glyceraldehyde-3-phosphate
IsiA (CP 43')	Iron Stress-Induced protein A; also called CP 43'
LH1	Core light-harvesting complex of purple bacteria
LH2	Accessory light-harvesting complex of purple bacteria
LHC I	Light-harvesting complex of PS I
LHC II	Light-harvesting complex of PS II
LHC	Light-harvesting complex
Met	Methionine
MGDG	Monogalactosyldiacylglycerol
mRNA	Messenger ribonucleic acid
rRNA	Ribosomal ribonucleic acid
N	Negative
$NADP^+$	Nicotinamide adenine dinucleotide phosphate (oxidized)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
OEC	Oxygen-evolving complex
P	Positive
P_{680}	Primary electron donor in photosystem II absorbing light at 680 nm
P_{700}	Primary electron donor in photosystem I absorbing light at 700 nm
PC	Plastocyanin
Pcb	Prochlorophyte chlorophyll *a*/*b* binding protein
PCH	Phosphatidylcholine
PCP	Peridinin-chlorophyll *a* protein
PCy	Phycocyanin
PE	Phycoerythrin
PEC	Phycoerythrocyanin
PG	Phosphatidylglycerol
PGA	Phosphoglycerate
Pheo	Primary transient acceptor, pheophytin
P_i	Inorganic phosphate
pmf	Proton motive force
PQ	Plastoquinone
PQ_A	Primary stable quinone electron acceptor of photosystem II
PQ_B	Secondary stable quinone electron acceptor in photosystem II

PQ_BH_2	Plastoquinol (fully reduced)
PS I	Photosystem I
PS II core	Core of the photosystem II
PS II RC	Photosystem II reaction centre (D_1/D_2/Cyt b_{559}/PsbI complex)
PS II	Photosystem II
Q_N	Plastoquinone-binding site in the cytochrome b_6/f complex
Q_P	Plastoquinol-binding site in the cytochrome b_6/f complex
RC	Reaction centre
Rubisco	Ribulose 1,5-bisphosphate carboxylase/oxagenase
RuBP	Ribulose 1,5-bisphosphate
SDS−PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SQDG	Sulphoquinovosyldiacylglycerol
Y_D	Tyrosine in position 161 in the D2 protein
Y_Z	Tyrosine in position 161 in the D1 protein
β-Car	β-carotene
$\Delta\psi$	Transmembrane electric field

Contents

Chapter 1

1 Introduction

1.1 Overview of photosynthesis

Life on the Earth is dependent on the sun. Solar energy that reaches a surface of our planet can be directly utilized only by photosynthetic organisms in a process known as **photosynthesis** – a conversion of light energy into chemical energy. Photosynthetic prokaryotes (photosynthetic bacteria and cyanobacteria) and eukaryotes (algae and plants) are predominantly photoautotrophic organisms, which mean that they derive their energy from sunlight and all cellular carbon directly from atmospheric carbon dioxide (CO_2). They are able to synthesize energy-rich organic compounds (such as carbohydrates) from simple inorganic substances by a series of the enzymatically catalyzed redox reactions, where CO_2 is an electron acceptor and H_2A serves as a reductant and thus an electron donor (**Equation 1**). $(CH_2O)_n$ represents carbohydrates and A is a product generated by oxidation of H_2A.

$$n\mathrm{CO_2} + n\mathrm{H_2A} \xrightarrow{h\nu} (\mathrm{CH_2O})_n + n\mathrm{A} + n\mathrm{H_2O}$$

Equation 1 Photosynthesis (adapted from Malkin and Niyogi 2000 with modifications)

Regarding the electron donor, two basic types of photosynthesis are known. In the case of evolutionary younger **oxygenic photosynthesis**, the electron donor is a molecule of water. In this type, water is oxidized and released electrons are subsequently used to reduce CO_2. The final products are carbohydrates and molecular oxygen (**Equation 2**). This oxygen-evolving photosynthetic reaction is carried out by plants, algae and prokaryotic cyanobacteria.

$$n\mathrm{CO_2} + n\mathrm{H_2O} \xrightarrow{h\nu} (\mathrm{CH_2O})_n + n\mathrm{O_2} + n\mathrm{H_2O}$$

Equation 2 Oxygenic photosynthesis (adapted from Malkin and Niyogi 2000 with modifications)

Many prokaryotes perform evolutionary older **anoxygenic photosynthesis**, in which they use other electron donors than water and do not produce molecular oxygen. These anoxygenic photosynthetic organisms include purple bacteria, green sulphur bacteria, green nonsulphur bacteria and heliobacteria. For example, purple and green sulphur bacteria can use H_2S or other sulphur containing compounds as electron donors, producing elemental sulphur as a photosynthetic product (**Equation 3**).

$$n\mathrm{CO_2} + n\mathrm{H_2S} \xrightarrow{h\nu} (\mathrm{CH_2O})_n + n\mathrm{S_2} + n\mathrm{H_2O}$$

Equation 3 Photosynthetic sulphur reduction (adapted from Malkin and Niyogi 2000 with modifications)

All living organisms benefit from photosynthesis, either directly or undirectly. Photosynthetically produced saccharides and other organic products manufactured by photosynthetic organisms serve as the energy source for heterotrophs (majority of bacteria, fungi, protozoa and multicellular organisms). Aerobic heterotrophs use molecular oxygen released into the atmosphere during photosynthesis for respiration and degradation of organic substances back to carbon dioxide and water, and generating ATP. CO_2 returns to the atmosphere, to be used again by photosynthetic organisms. There exists a fundamental metabolic relationship between the photosynthetic and heterotrophic organisms that live in a balanced steady state on the Earth.

1.2 Localization of photosynthesis

In eukaryotes (algae and plants), the biophysical and biochemical processes of photosynthesis occur inside specialized subcellular structures known as **chloroplasts**.

1.2.1 Evolution of chloroplast

Currently, according to generally accepted theory chloroplasts have an endosymbiotic origin (Mereschowsky 1905, Margulis 1971). They developed from separate prokaryotic organisms which were taken inside the protoeukaryotic cell as symbionts. During evolution, they became integrated in to eukaryotic cells and ultimately lost their independence, which includes a gene transfer from the symbiont to a host (Martin *et al*. 1998 and 2002).

There is variety of evidences that these organelles arose via an ancient endosymbiosis and they are descendants of a symbiotic relationship between once free living cyanobacterium and a simple nonphotosynthetic eukaryotic cell (*for review see* McFadden 2001, Vesteg *et al.* 2009). Chloroplast has a comparable size to bacteria, generally few micrometers in diameter. Most of the genes coding chloroplast components necessary for plastid functions are located in a cell nucleus. Nucleus-encoded proteins are synthesized on cytoplasmic ribosomes and then imported into the chloroplast (Steiner and Löffelhardt 2002, Nassoury and Morse 2005), where the oligomeric complexes are assembled. Nevertheless, even today chloroplasts have retained a small prokaryote-like circular DNA. The majority of genes that remain in chloroplast code either for integral membrane proteins involved in photosynthesis or for elements required to express chloroplast genes. Many features of chloroplast internal structure and function are typically bacterial (Gray 1992 and 1999) (an organization of genes into operons, the lack of 5' cap formation and 3' polyadenylation of chloroplast mRNA, the fact that translation begins with N-formyl-methionine or 70S prokaryote-type ribosomes) or very similar to those founded in photosynthetic bacteria (the structurally comparable proteins in photosynthetic reaction centers or membrane composition).

1.2.2 Chloroplast – structure and organization

Although many different shapes and sizes can be found, a typical chloroplast has a lentil or ellipsoid shape with a size of 5–10 µm in length and 2–4 µm in width and can be easily seen in a light microscope. Like mitochondria, they are bounded by double membrane on the surface, with two complete phospholipid bilayers separated by an intermembrane space. As in mitochondria, the highly permeable outer membrane freely permits small molecules (such as water, ions, and low molecular weight metabolites) pass through pores into the aqueous compartment between membranes. An inner membrane acts as a nearly impermeable barrier that contains transport protein carriers for regulating the movement of metabolites and small neutral molecules (O_2, NH_3), into or out of the organelle. An aqueous phase enclosed by inner membrane, the chloroplast **stroma** is a site where most of chloroplast biosynthetic pathways take place and thus numerous soluble enzymes are present, in particular for CO_2 fixation and carbohydrate synthesis. It also contains one or more DNA molecules, RNA, ribosomes involved in the chloroplast protein synthesis machinery and number of other compounds required for complete chloroplast metabolism, such as enzymes needed for the synthesis of photosynthetic pigments, hemes, fatty acids, starch or for sulphur and nitrogen assimilation.

The most prominent structure inside the chloroplast is a complex internal membrane system, known as photosynthetic or thylakoid membrane, in short **thylakoids**. They are suspended in the stromal matrix, form an extensive 3D network of the flattened membrane vesicles or disc-like sacks, defining a closed inner space − **lumen**. Thylakoid membranes are highly organized structures that incorporate all pigment-protein complexes essential to photosynthesis.

In typical higher plant chloroplasts (**Fig. 1**), most striking feature of thylakoid membranes is their differentiation into two structurally characteristic regions. Thylakoids form stacks of very densely packed membranes that are pressed together, called **grana** that consist of 10–20 layers with a diameter of 300–600 nm. The cylindrical grana stacks are in turn interconnected by unstacked **stroma lamellae** of several hundred nm in length that are exposed to the surrounding fluid medium (Mustardy and Garab 2003).

Most plant chloroplasts are located in a leaf tissue named the mesophyll, in the middle part of a leaf between the upper and lower layer of epidermis.

Among algae, the chloroplast structure exhibits considerable diversity of both envelope and thylakoid membranes. Chloroplasts of three groups of algae (the green algae, the red algae and the glaucophytes) that originated from a primary endosymbiosis are enclosed only by two membranes as in the case of plants. All other groups of algae result from secondary endosymbiosis of a eukaryotic alga and contain four or in some cases three membranes surrounding the chloroplast (Palmer and Delwiche 1996, Cavalier-Smith 2000). Unlike plants, thylakoids of algae are not structured into grana but they are arranged either in separate equidistant sheets within chloroplasts or small groups of several thylakoids.

Since cyanobacteria as prokaryotic organisms do not contain membrane organelles, their thylakoids lie free in the cytoplasm; and generally they are singly and equidistant (van den Hoek *et al.* 1995).

In anoxygenic bacteria, the photosynthetic apparatus is localized in the cytoplasmic membrane. In purple bacteria, the cytoplasmic membrane can form invaginations in the form of tubes, vesicles or flat lamellae, which are called intracytoplasmic membranes (Drews and Golecki 1995).

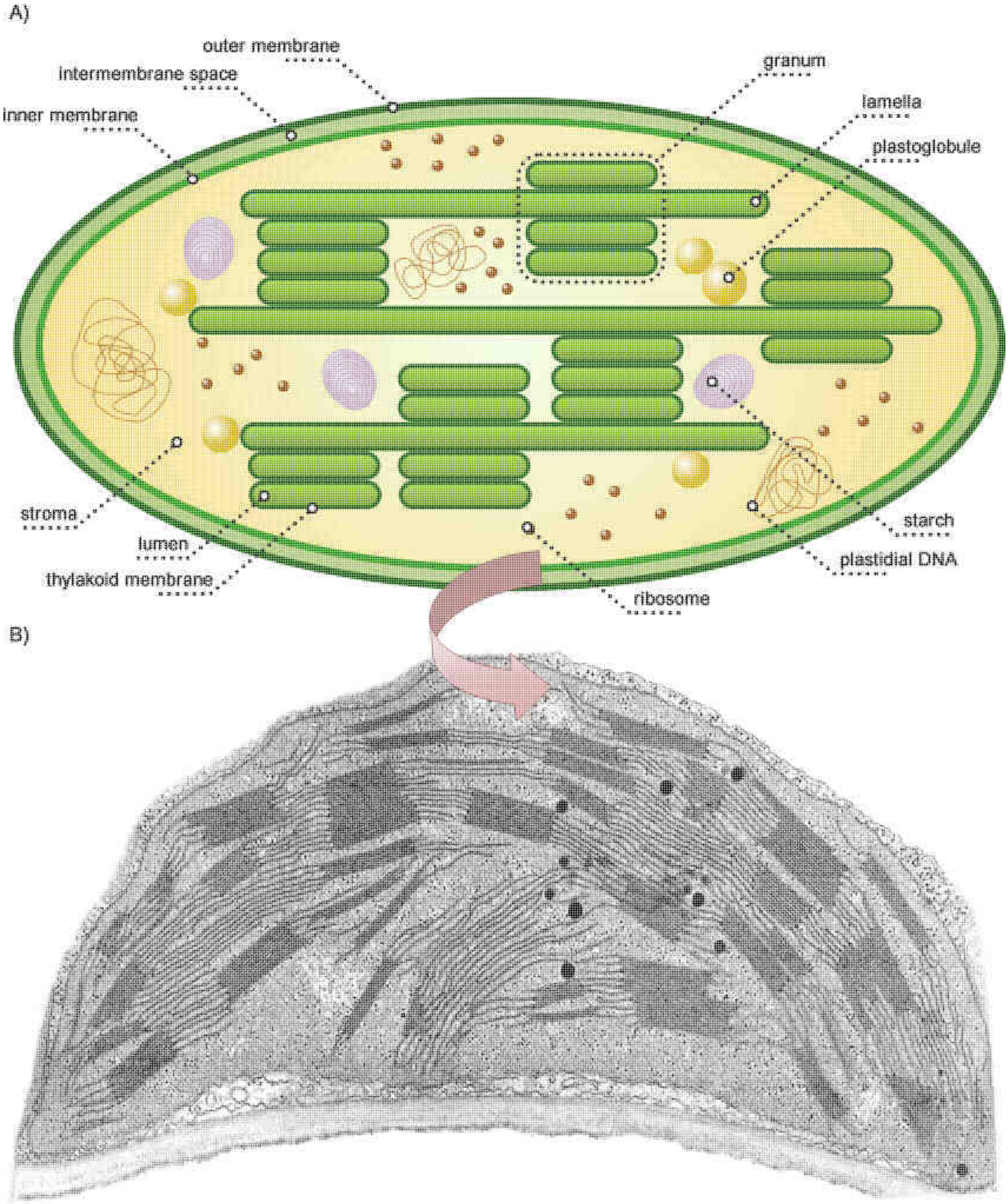


Fig. 1 A) Schematic structure of chloroplast. B) Electron micrograph of ultrathin section of chloroplast, showing stacked grana and unstacked stroma thylakoids. The dark granules are plastoglobuli, lipid droplets.

1.2.3 **Lipid composition**

Like each biological membrane, thylakoid membrane is composed mainly of proteins and lipids, forming fluid lipid bilayer. In a membrane, lipid molecules are arranged in a characteristic manner, the hydrophilic lipid headgroups are orientated toward the water phase, whereas hydrophobic tails of fatty acid chains to each other, hidden inside the membrane. Both thylakoid membrane and chloroplast envelope contain unique glycolipids containing galactose (galactolipids) – nonpolar monogalactosyldiacylglycerols (MGDG) and digalactosyldiacylglycerols (DGDG) which represent the majority of the total membrane lipids. The reminder of lipids is composed of negative charged sulpholipids – sulphoquinovosyldiacylglycerols (SQDG) and phospholipids – phosphatidylglycerols (PG) (Allen and Good 1971, Poincelot 1973). In addition to, phosphatidylcholine (PCH) is a component of the outer envelope membrane, representing the major phospholipid of chloroplast envelope (Poincelot 1976) whereas phosphatidylglycerol is a predominant phospholipid present in thylakoid membrane.

Contrary to chloroplast envelope (Bahl *et al.* 1976), lipids in thylakoid membranes have a high degree of a fatty acid unsaturation (Poincelot 1976) and due to this fact they are more fluid.

1.3 Basic principles of photosynthesis

The photosynthesis requires coordination of two different phases, traditionally referred to as **light reactions** and **dark reactions**, each localized in a specific area of chloroplast and together make up the complete process of energy conversion and storage.

In light reactions (or light-dependent reactions), chlorophyll and other pigments capture light energy and converse it in series of electron transfer reactions that lead to a reduction $NADP^+$ to NADPH and generation of a transmembrane proton gradient (or proton motive force). The proton gradient is subsequently used to synthesize ATP from ADP and P_i. Simultaneously O_2 is evolved. These chemical reactions are also known as the **thylakoid reactions** because they are catalyzed by multiprotein complexes in thylakoid membrane.

Dark reactions encompasses carbon fixation reactions (or carbon assimilation reactions), enzyme catalyzed processes driven by the end products of the light reactions – ATP and NADPH, which are consumed to reduce CO_2 and incorporate it into triose phosphates precursors of carbohydrates. These synthetic processes take place in the chloroplast stroma and therefore are also called as **stroma reactions** (Taiz and Zeiger 2006).

It is convenient to take notice that all reactions in photosynthesis are ultimately driven by light and the only strictly light dependent step is photon absorption and/or charge separation. In addition, some of the enzymes involved in the carbon fixation require activation by light and also they occur primarily during the day. Therefore, the terms light and dark reactions may be misleading.

For a detailed description, the proceeding of photosynthesis can be divided into several distinct stages:

(1) Light absorption and energy delivery by antenna systems
(2) Primary electron transfer in reaction centers (charge separation)
(3) Electron transport and generation of proton motive force (*pmf*)
(4) ATP synthesis
(5) Carbon fixation and its incorporation into carbohydrates

1.3.1 Light absorption and energy delivery by antenna systems

Molecules that absorb light, a form of electromagnetic radiation, are called pigments. Upon the absorption of a **photon,** a quantum of energy, an electron in the chromophore moves from a lower energy state to a higher energy excited state. Since the excited state is generally unstable, the excited molecules rapidly return to the ground state. There are several mechanisms of deexcitation and the energy released may take different forms. The energy can be dissipated nonradiatively as a heat; alternatively, emission of a photon can occur in a process called **luminiscence**. Since a part of the absorbed energy is lost to heat prior to emission, the energy of the emitted photon is lower than that of the photon absorbed. The third important process, called **energy transfer** mediates the movement of excited state from one excited molecule to a neighboring molecule. In photosynthesis this process occurs until the excited state reaches a reaction center (RC), in which the excitation energy is used to reduce a primary acceptor molecule through a **charge separation** event, called **photochemistry**.

The principal function of antenna systems is to make the photosynthetic process more effective by capturing solar energy and delivering the excitation energy to RCs. To increase the efficiency of light harvesting, number of pigments is systematically arranged in photosynthetic antennae, the membrane bound pigment-protein light-harvesting complexes. Light energy is funneled to reaction centers by excitation energy transfer among antenna pigments.

1.3.2 Primary electron transfer in reaction centers

The initial energy transformation of excited states to chemical energy in photosynthesis takes place in the reaction centers. These are thylakoid membrane pigment-protein complexes that bind several electron transport carriers, including specialized chlorophylls of **primary electron donor**. As a result of the photon absorption and energy transfer from the antenna complexes, the chlorophylls of primary electron donor becomes electronically excited and rapidly transfers an electron to a nearby acceptor molecule. This charge separation is the primary photochemical event in photosynthesis that takes within extremely short time (Groot *et al.* 2005, Novoderezhkin *et al.* 2005).

1.3.3 **Electron transport and generation of proton motive force**

Upon the primary electron transfer, it is absolutely essential to stabilize the separated charge by very rapid series of secondary electron transfer reactions. Electrons must be donated to the oxidized primary donor and extracted from the reduced primary transient acceptor. There are two ways to accomplish this – a **cyclic** or/and **linear electron transfer** chain (Blankenship 2002, *for a review see* Bendall and Manasse 1995, Allen 2003, Kramer *et al.* 2004).

In cyclic electron transfer pathway, the electron is transferred through a series of redox carriers back to the primary electron donor, which is thereby finally re-reduced. There is no reduction of intermediate electron acceptor ($NADP^+$) but proton translocation driven by these processes does contribute to form *pmf*. Many anoxygenic photosynthetic bacteria with one photosystem operate in this mode.

Major pathways found in oxygenic photosynthetic organisms comprise electron transfer processes (sometimes called the Z scheme), in which primary electron donor, on one side, donates the electron to electrons transport chain until they reaches the oxidized form of $NADP^+$ (to produce NADPH) and on the other side extracts electrons from water. This process also effectively translocates protons across the thylakoid membrane, establishing *pmf.*

Moreover, in oxygenic photosynthetic organisms under certain conditions both photosystems are capable of cyclic fashion.

As it was mentioned above, the electron flow in the thylakoid membrane is coupled with the movement of protons from the stroma to the thylakoid lumen, forming a difference in electrochemical potential of protons across the thylakoid membrane, so called proton motive force. The *pmf* is created by two thermodynamically equivalent components – a gradient of pH (ΔpH; $pH_{lumen} < pH_{stroma}$) and transmembrane electric field ($\Delta\psi$).

1.3.4 **ATP synthesis**

This photosynthetic energy storage reaction involves production of stable high-energy molecules of ATP that can be utilized in a variety of cellular processes. The difference in electrochemical potential across the membrane (~ 3, 5 pH unit) is the driving force for the phosphorylation of ADP by ATP synthase (Voet *et al.* 2008). The mechanism of this light-driven process known as **photophosphorylation** is equivalent to the oxidative phosphorylation in mitochondria.

1.3.5 **Carbon fixation and its incorporation into carbohydrates**

The high-energy compounds, ATP and NADPH produced by light-induced electron transport, provide energy and electrons to drive synthesis of a number of various carbohydrates. This unique reductive pentose phosphate cycle of metabolic pathways, known also as a Calvin cycle or **Calvin-Benson cycle** (Bassham *et al.* 1950), is a series of chemical reactions that can be broken down into the three phases – carboxylation, reduction and regeneration (Malkin and Niyogi 2000). In the first step, an inorganic CO_2 is built into a organic five-carbon substrate, ribulose-1,5-bisphosphate (RuBP) to form two molecules of a three-carbon 3-phosphoglycerate (PGA),the first stable intermediate. This carboxylation reaction is catalyzed by a large chloroplast enzyme complex named ribulose 1,5-bisphosphate carboxylase/oxagenyse, referred to as Rubisco. During the reduction phase, ATP and reducing equivalents NADPH are utilized to reduce PGA to triose phosphate – a carbohydrate, glyceraldehyde-3-phosphate (GAP). The regeneration phase of the Calvin-Benson cycle is a complex series of reactions in which the three-carbon triose phosphates are modified or converted into saccharides as well as regenerated into the five-carbon sugar ribulose 5-phosphate, which is phosphorylated to re-form RuBP.

Calvin-Benson cycle was found in many prokaryotes and in all photosynthetic eukaryotes, from the most primitive algae to the most advanced angiosperms (Taiz and Zeiger 2006).

1.4 **Photosynthetic pigments**

Photosynthetic pigments do not occur in a cell freely but they are bound to various proteins forming pigment-protein complexes embedded in the thylakoid membrane. They can also be associated with hydrophilic proteins linked with the membrane.

Generally, all photosynthetic organisms contain a mixture of pigments, each type serving a specific functional role.

A structure of pigments determines their chemical and spectroscopic properties and also what function they perform in photosynthetic process.

1.4.1 Chlorophylls

The key light-absorbing pigments of vast majority of photosynthetic organisms are **chlorophylls** (Chl). Plants, algae and cyanobacteria synthesize chlorophyll, whereas anoxygenic bacteria produce a variant called **bacteriochlorophyll** (BChl). Several types of chlorophylls (labelled *a–d*) and bacteriochlorophylls (*a–g*) have been identified (Scheer 2003).

Chloroplasts from higher plants as well as green algae (chlorophytes) and euglenophytes always contain chlorophyll *a* (Chl *a*) and *b* (Chl *b*). The red algae (rhodophytes) contain Chl *a* only, whereas the rest of algae possess, apart from Chl *a*, also chlorophyll *c* (Chl *c*) (van den Hoek *et al.* 1995).

Typical cyanobacteria do not contain Chl *b*, a characteristic pigment of higher plants and green algae. However, a group of atypical cyanobacteria − green oxyphotobacteria (formerly called prochlorophytes) that contains both Chl *a* and *b* has been discovered (Lewin 1976, Burger-Wiersma *et al.* 1986, Chisholm *et al.* 1988). Furthermore, *Prochlorococcus marinus* contains divinyl Chl *a*, in which substituent (ethyl) on a ring B is replaced by a vinyl. Lastly, the chlorophyll *d* (Chl *d*) was found to be a major photosynthetic pigment in cyanobacterium *Acaryochloris marina*, although this species also contains Chl *a* (Miyashita *et al.* 1996).

A structure of chlorophyll is similar to a heme, found as a prosthetic group in globins, cytochromes and some enzymes. Chemically, chlorophylls are cyclic tetrapyrroles, containing four pyrrole rings, related to porphyrins. Most of chlorophylls are classified as chlorins (dihydroporphins), by virtue of reduced ring D. Chlorophylls are produced through a common metabolic pathway as other porphyrin pigments such as heme. The branching point of biosynthetic pathway with one branch leading to heme and the other to chlorophylls is a formation of a symmetric metal-free porphyrin, protoporhyrin IX (Blankenship 2002).

In comparison with heme structure, chlorophylls has an extra fifth ring, so called isocyclic ring, and a long nonpolar hydrocarbon side chain, mostly a C_{20} phytol tail. Phytol facilitates binding of chlorophylls to the hydrophobic regions of membrane pigment-protein complexes. Four inward-oriented nitrogen atoms of tetrapyrrol ring are coordinated with a central Mg^{2+} ion, instead of Fe^{2+} found in heme.

A highly conjugated double bonds system of the cyclic tetrapyrrol strongly absorbs visible light. Two major absorption bands, one in the blue and one in the red region, give chlorophylls their characteristic green color. Various chlorophylls differ in their substituents around the ring structure. The relatively small changes in chemical structure among the chlorophylls greatly affect their absorption spectra. The central function of chlorophyll pigments is a photon capture and transfer of excited energy to the reaction center, where it also participates in the photochemistry.

1.4.2 **Carotenoids**

Second class of light absorbing pigments present in all photosynthetic organisms are **carotenoids**. Among them we can distinguish two basic types of carotenoids, the carotenes containing only carbon and hydrogen, such as α-carotene, β-carotene (β-Car) and lycopene, and their oxygenated derivates, the xanthophylls, which contain oxygen atom as a part of hydroxyl, carbonyl, carboxyl, formyl or epoxide group. Carotenoids belong to the category of tetraterpenoids (C_{40}), consisting of eight isoprene units, each with a five-carbon branched-chain (Šetlík *et al*. 1998).

In summary, during carotenoid biosynthesis the isoprene units successively condense into hydrocarbons with ten, twenty and forty carbons, ending with phytoene – precursor to all carotenoids. In the next step, phytoene is converted to lycopene in desaturation reactions. In most organisms, biosynthetic pathway continues with cyclization on both ends to generate α-carotene and β-carotene. Hydroxylation of α-carotene results in structure of lutein, the predominant carotenoid in photosynthetic plant tissue and in case of β-carotene in formation of zeaxanthin (Pavlová 2005).

Conjugated double-bond system of carotenoid polyene chain is responsible for its hydrophobic character and also for a yellow-orange coloring. Carotenoids have several essential functions in photosynthetic systems. First, they act as accessory pigments that cover the spectral region where chlorophylls do not absorb strongly, in a range between 400 and 500 nm. Second, carotenoids also play an important role in process called photoprotection, in which they quench triplet excited states of chlorophylls thereby inhibit the formation singlet state of oxygen. Therefore they protect the photosynthetic apparatus from photooxidative damage. Next, under high light intensities, they regulate the energy transfer inside antennae by xanthophyll cycle, in which the excess of energy is safely dissipated into a heat by a nonphotochemical quenching (Frank and Brudvig 2004).

1.4.3 **Phycobilins**

The last group of photosynthetic pigments found in red algae and typical cyanobacteria are **phycobilins**. Chemically, bilins are linear, open-chain tetrapyrroles, derived from the same biosynthetic pathway leading to heme and chlorophyll. These water-soluble pigments (lacking a phytol tail) are covalently linked to specific proteins via thioether bond to cysteine residues in the proteins. Four types of phycobiliproteins – allophycocyanin (APC), phycoerythrocyanin (PEC), phycocyanin (PCy) and phycoerythrin (PE) differ in their absorption maxima. Absorption is centered in a spectral range from 550 nm to 680 nm, the portion of visible spectrum that is poorly utilized by chlorophyll. Their various absorption properties are mainly affected by the interaction with proteins. The phycobiliproteins, together with a number of additional proteins known as linkers, are arranged into large peripheral membrane antenna pigment-protein complexes named **phycobilisomes** (Šetlík and Hála 1999). Molecular architecture and other characteristics of phycobilisome complex are described in a chapter 1.5.7.

1.5 Photosynthetic apparatus

The photosynthetic apparatus of modern cyanobacteria, algae and higher plants is more complex than bacterial systems with only one of the two general types of reaction center. In the thylakoid membranes of oxygenic photosynthetic organisms both photosystems cooperate. Although photosynthetic reaction centers in all organisms are structurally and functionally similar, antenna complexes are remarkably diverse.

Oxygenic photosynthesis involves four major supramolecular protein-cofactor complexes embedded in the thylakoid membrane (**Fig. 2**):

(1) photosystem II (PS II) together with oxygen-evolving complex (OEC) and light-harvesting antennae (LHC II)
(2) complex of cytochrome b_6 and f (Cyt b_6/f)
(3) photosystem I (PS I) together with light-harvesting antennae (LHC I)
(4) ATP synthase

Electron microscopy coupled with immunocytochemistry revealed a highly nonuniform characteristic distribution of protein complexes in thylakoid membrane of higher plants (Allen and Forsberg 2001). This phenomenon, called **lateral heterogeneity**, indicates that two photosystems are spatially separated from each other. PS II is localized almost exclusively in the stacked membrane regions (grana), being in contact with the thylakoid lumen. While PS I occurs mainly in the unstacked stroma lamellae or on the ends of the grana stacks, protruding into the stroma, where it has ready access to $NADP^+$. The Cyt b_6/f complex is evenly distributed throughout the membrane regions, and the ATP synthase enzyme complex is entirely situated in the stroma-exposed membranes.

Cyanobacteria and most algae do not exhibit extremes of grana stacking and lateral distribution of photosynthetic complexes observed in higher plants (Blankenship 2002).

The spatial separation of the complexes both within distinct regions of plant chloroplasts and universally in thylakoid membranes at all necessitates diffusible **electron carriers**, which shuttle electrons between the components of electron transport chain. There are hydrophobic **plastoquinol** molecules moving within the thylakoid membrane and two hydrophilic carriers – **plastocyanin**, in the lumen and **ferredoxin** present at the stroma surface of thylakoid.

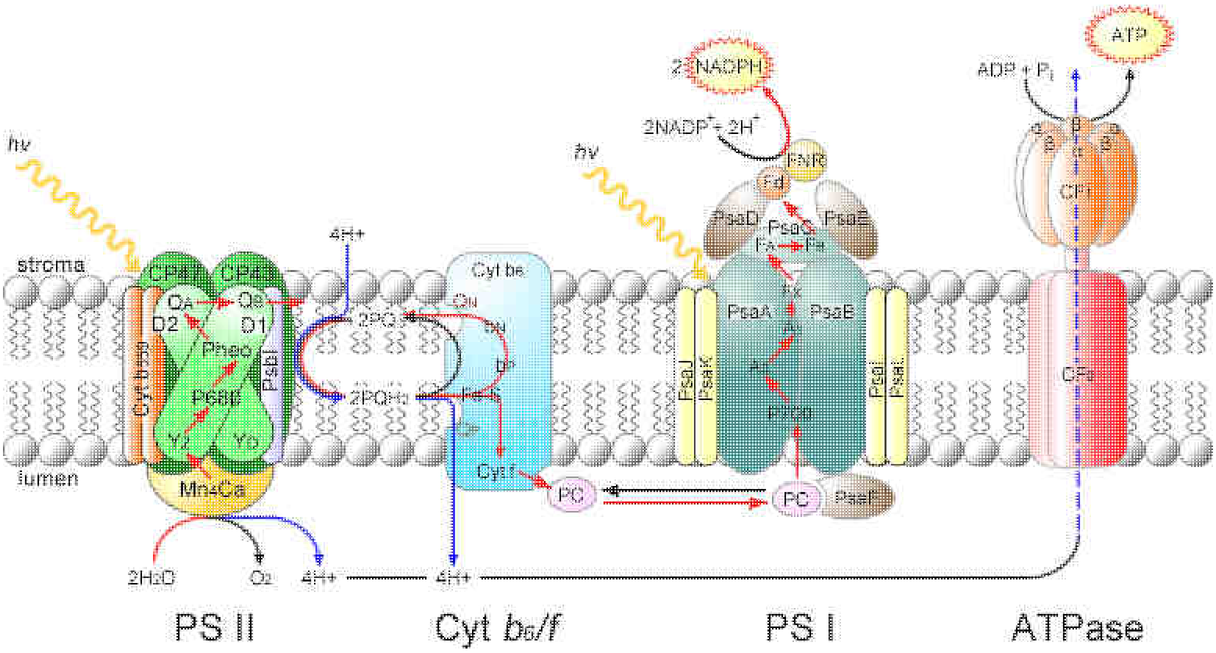


Fig. 2 Schematic diagram of the noncyclic electron transport chain, occurring in thylakoid membranes of oxygenic photosynthetic organisms. A basic stoichiometry of major reactions of photosynthesis and an arrangement of major pigment-protein complexes in membrane are also depicted. See text for details.

Symbols: PS II – photosystem II; Cyt b_6/f – cytochrome b_6/f; PS I – photosystem I; ATPase – ATP synthase; hυ – a quantum of energy; Mn_4Ca – manganese calcium cluster of the oxygen evolving complex; Y_Z, Y_D – tyrosine residues; P_{680}, P_{700} – primary electron donor of PS II and PS I, respectively; Pheo – pheophytin; Q_A, Q_B – plastoquinones of PS II; Q_P, Q_N – plastoquinone-binding sites; Fe-S – iron sulphur cluster of Rieske protein; b_P, b_N – low- and high-potential hemes, respectively; PC – plastocyanin; A_0 – chlorophyll *a*; A_1 – phylloquinone; F_A, F_B, F_X – iron sulphur clusters of PS I; Fd – ferredoxin; FNR – ferredoxin:$NADP^+$ oxidoreductase; NADPH – nicotinamide adenine dinucleotide phosphate; CF_0, CF_1 – subunits of the ATP synthase; ATP, ADP – adenosine tri- and diphosphate.

1.5.1 Photosystem II

In cyanobacteria and chloroplasts of plants and algae, the oxygenic photosynthesis is initiated in photosystem II (PS II), a large supramolecular pigment-protein complex that includes more than 25 distinct proteins (coded by genes *psbA*−*psbZ*), most of which are embedded in the thylakoid membrane. Despite the large number of components, PS II can be divided into two structurally and functionally different parts. The first is the so-called **PS II core** complex, which includes the photochemical **reaction center** (RC) and integral membrane **core antenna** complex. The second part of PS II is formed by an **oxygen-evolving complex** located on the lumenal surface of the complex.

During past few years a number of three-dimensional crystal structures, showing locations of and interactions between protein subunits and cofactors in cyanobacterial and higher plant PS II core complex, have been determined by electron (Hankamer *et al.* 2001b) or X-ray crystallography with increasingly better resolution (Zouni *et al.* 2001, Kamiya and Shen 2003, Fereirra *et al.* 2004, Biesiadka, 2004, Loll *et al.* 2005); currently the resolution is 2,9 Å (Guskov *et al.* 2009).

The **reaction center** of the PS II core complex (PS II RC), consisting of D1/D2 heterodimer coupled with a cytochrome b_{559} and PsbI peptide, was first reported by Nanba and Satoh (Nanba and Satoh 1987). The **core antenna** complex composed of two sequence-related Chl *a*-binding proteins CP43 and CP47 are intimately associated with PS II RC. There is a number of low-molecular mass proteins (PsbH−PsbZ) associated with PS II core complex (Barber *et al.,* 1997; Hankamer *et al.*, 2001a). Some of these small proteins play a role in the photoprotection, stabilization, dimerisation and assembly of PS II (Shi *et al.* 2000, Li *et al.* 2002, Aoyama 2003, Iwai *et al.* 2004, Dobáková *et al.* 2007, Bentley *et al.* 2008).

It is widely believed that the PS II complex *in vivo* exists and functions as a homo-dimer with a molecular mass of about ~650 kDa, as was shown by many crystallographic studies, cited above or using single particle analysis (Hankamer *et al.* 1999) as well as biochemical techniques (Watanabe *et al.* 2009). However, both dimeric and monomeric PS II complexes were isolated (Hankamer *et al.* 1997, Adachi *et al.* 2009) and the monomeric complex was assumed to be an intermediate form in the normal assembly pathway or in the damage repair cycle (Barbato *et al.* 1992, Hankamer *et al.* 1997). Contrary to this well-known concept, a recent study claims that deprivation of lipids during preparation caused conversion of PS II from a monomeric to a dimeric form and therefore the functional form of the PS II *in vivo* is a monomer but not a dimer (Takahashi *et al.* 2009).

Reaction center – protein composition

D1/D2 heterodimer − a major pigment-protein complex of the PS II RC which consists of D1 and D2 proteins, encoded by the chloroplast *psbA* and *psbD* gene, respectively and binds cofactors and pigments arranged on two nearly symmetric branches. Only one branch of cofactors, located on the D1 side, is involved in electron transfer pathway. These two hydrophobic polypeptides exhibit as two diffuse bands with apparent molecular weights 32 kDa (D1) and 34 kDa (D2) on SDS−PAGE gels, hence the designation D (meaning diffuse).

The D1 and D2 subunits, each with five transmembrane helices are homologous to each other (Ke 2001). In addition to that, the arrangement of helices in both D1 and D2 is remarkably similar to those of the L and M subunits of the bacterial RC (Trebst 1985). The D1/D2 heterodimer binds six Chl *a* molecules, which include a chlorophyll multimer of the primary electron donor P_{680} (Durrant *et al.* 1995) plus two peripheral chlorophyll molecules. It also binds two pheophytins (Pheo), two β-carotenes, two plastoquinones and one atom of non-heme iron.

Cytochrome b_{559} (Cyt b_{559}) − is an integral constituent of the minimal PS II RC unit still capable of primary charge separation, comprised of two small hydrophobic peptides, α (9 kDa) and β (4 kDa) subunits, encoded by chloroplast genes *psbE* and *psbF*, respectively. Each of the peptides forming Cyt b_{559} has only a single transmembrane domain. A single heme group is bound as a cofactor within structure cross-linking the two peptides into a heterodimer via a single His residue from each subunit. The possible function of the Cyt b_{559} has not been fully established and many different roles have been postulated. Cyt b_{559} is essential for PS II structure and assembly of functional PS II complex. Cyt b_{559} can be both photooxided and photoreduced by receiving electrons from PQ_B (Buser *et al.* 1990). This fact led to suggestion that it can mediate a cyclic electron transport around PS II thereby may protect PS II against photodamage by high light and photoinhibition (Stewart and Brudvig 1998, Hanley *et al.* 1999, Tracewell and Brudvig 2008).

Reaction center – electron-transport cofactors

P_{680} − is the primary electron donor of PS II. The P_{680}^{+} cation is the one of the strongest oxidizing agent exists in biological systems. Its high redox potential estimated to be about +1.3 V (Rappaport *et al.* 2002) allows oxidation of water during photosynthesis. A character "P" stands for pigment and the 680 number is its absorption maximum in the red part of the visible spectrum. Formerly, it was meant that it consists of only special pair of two Chl *a* molecules but calculations and X-ray diffraction structures of PS II core complex revealed that the primary donor is a group of four chlorophylls. P_{680} is located between D1 and D2 subunits near the center.

Pheophytin (Pheo) − is a metal-free chlorophyll-based compound, in which the central Mg^{2+} atom is replaced by two hydrogens. Pheophytin serves as a primary electron acceptor because it is easier to reduce Pheo than corresponding chlorophyll. The photochemically active Pheo is bound to D1 and the inactive Pheo to D2 protein.

Plastoquinones (PQ_A and PQ_B) − PQ_A quinone molecule, tightly bound to D2 subunit, is a one-electron acceptor from Pheo molecule that gives rise a singly reduced semiquinone. Second quinone, PQ_B, is a two-electron carrier, loosely bound to D1 subunit close to the stromal side that in doubly reduced and protonated form dissociates from PS II into the lipid bilayer of thylakoid membrane. The PQ_A quinone molecule can also be doubly reduced, but only at non-physiological conditions.

Non-heme Fe − is centrally positioned between two quinone molecules and modulates normal electron flow.

β-carotenes − two differently oriented and therefore spectroscopically distinct carotenoid molecules are bound to D1 and D2 subunits, labeled as Car_{D1} and Car_{D2}, respectively (Tomo *et al.* 1997). On the basis of their position it seems that they may have different functions. They participate in quenching of singlet oxygen produced after charge recombination to the triplet state of P_{680}^{*}(Telfer *et al.* 1994), or can acts as a secondary electron transporter if donation of electrons from water is not functional and thus in the case of danger of P_{680}^{+} accumulation they play a photoprotective role (Hanley *et al.* 1999, Telfer *et al.* 2002 and 2005 *see for reviews*, Martinez-Junza *et al.* 2008, Litvín *et al.* 2008).

Chlorophylls Z (Chl Z_{D1} and Chl Z_{D2}) − the two symmetrically located peripheral chlorophylls of PS II RC are coordinated by the His residues (D1 His118 and D2 His117). Biophysical evidence for the involvement of the redox-active Chls Z in energy transfer was obtained. The peripheral chlorophylls, Car molecules and the heme of Cyt b_{559} may functions as alternate electron donors under conditions when the primary electron donation pathway from OEC to P_{680}^{+} is inhibited, such as in Mn-depleted PS II or at low temperature (Tracewell *et al.* 2001, Tracewell and Brudvig 2003 and 2008).

Tyrosine residues (Y_D and Y_Z) – there are two specific tyrosine residues. One of them, on D1 protein in position 161 (Y_Z or Tyr_Z), functions as an intermediate electron carrier between P_{680} and the OEC. The second tyrosine is in the symmetry-related position in the D2 protein (Y_D) and apparently is not involved in the usual electron flow.

Manganese-calcium cluster (Mn_4Ca cluster) − an active site of water oxidation consists of four Mn atoms, and one atom each of Ca^{2+} and Cl^{-}. Mn_4Ca cluster is associated with OEC on the lumenal side of the PS II complex and acts as a four-electron gate in the water-oxidizing process. Calcium and chloride are necessary cofactors for the O_2 evolving reaction.

Core antenna

CP43, **CP47** − proteins encoded by the *psbC* and *psbB* genes of chloroplast genome, each possess six transmembrane α-helices (Bricker 1990). The designation CP stands for chlorophyll protein and the number refers to apparent molecular mass of 43 and 47 kDa, determined by electrophoresis. CP 43 binds thirteen Chls and three β-Car, whereas CP47 binds sixteen Chls and five β-Car molecules (Loll *et al.* 2005). CP47 being located adjacent to D2 interacts more directly and strongly with the reaction center than CP43 being adjacent to D1 (Bricker and Frankel 2002). The core antennae serve as the proximal antennae for PS II, providing a conduit for excitation energy transfer from the accessory antenna of PS II into the reaction center.

Oxygen-evolving complex

A unique part of PS II is the oxygen-evolving complex (OEC) also known as water-splitting complex (WOC). OEC has several structural components including the extrinsic proteins, the loop regions of several intrinsic membrane proteins and four oxygen-bridged manganese atoms, calcium and chloride – a redox-catalytic center, known as **Mn_4Ca cluster**. The OEC sits at the membrane-lumen interface, surrounded by four PS II core proteins (D1, D2, CP43 and CP47), but nearly all ligands for the Mn_4Ca cluster come from the D1 protein with only a single ligand contributed from CP43. According to currently published X-ray diffraction data (Fereirra *et al.* 2004, Loll *et al.* 2005 and Guskov *et al.* 2009), three Mn ions (Mn1, Mn2 and Mn3) along with one Ca ion positioned in the vertex are clustered into trigonal pyramid, with a fourth Mn4 atom asymmetrically connecting to the one of the corner of base.

Ca^{2+} is an essential structural element in the metal cluster and is also likely to be a site for ligation of the substrate H_2O molecules that undergo oxidation. Cl^- is probably utilized to regulate the Mn redox activity. These inorganic ions are both required for efficient oxidation of the Mn atoms (Yocum 2008).

A structure and catalytic function of PS II are notably conserved among a wide variety of organisms from unicellular cyanobacteria to multicellular plants (Hankamer *et al.* 2001a), but the most heterogeneity between photosynthetic organisms was found in the composition of the extrinsic OEC proteins. Depending on the type of organism, they utilize different combinations of extrinsic proteins to optimize PS II OEC activity.

The extrinsic proteins are PsbO protein (33 kDa protein), PsbP (23, 24 kDa protein), PsbQ (16–18 kDa protein in plants and 20 kDa protein in red alga), PsbR (10 kDa protein), PsbU

(12 kDa protein) and PsbV (cytochrome *c_{550}*). Many studies have shown that they are required to enhance oxygen evolution and serve important roles *in vivo* (*reviewed in* Seidler 1996).

Only the PsbO protein is ubiquitous to all oxygenic photosynthetic organisms. Higher plants and green algae have a set of four nuclear-encoded extrinsic proteins (PsbO, PsbP, PsbQ and PsbR), whereas cyanobacterial OEC contains a different set of proteins (PsbO, PsbU and PsbV) and represents the best understood OEC due to many crystal structures. In addition, the genomic and proteomic studies showed the existence of PsbP and PsbQ homologs in cyanobacteria (Kashino *et al.* 2002, De Las Rivas *et al.* 2004, Thornton *et al.* 2004) that are not present in current structural models. Therefore, cyanobacterial PS II complexes may have potentially five associated extrinsic proteins – PsbO, PsbP', PsbQ', PsbU and PsbV.

PsbO protein, a key structural component in many different types of OECs functions to stabilize Mn_4Ca cluster and modulates the Ca^{2+} and Cl^{-} requirements for oxygen evolution (Bricker and Burnap 2005). PsbP protein plays a structural role in OEC to sequester the Ca^{2+} and Cl^{-} ions and protects cluster from exogenous reductants. Genes for PsbQ protein have been identified in a number of different photosynthetic organisms (Thornton *et al.* 2004). PsbQ protein along with PsbO and PsbP functions as a structural component of OEC to protect manganese cluster. The presence of the PsbQ protein has important effects on the form and functions of the OEC, resulting in higher activity and stability (Roose *et al.* 2006). The 20 kDa extrinsic protein, originally identified in *Cyanidium caldarium* (Enami *et al.* 1995) was renamed to PsbQ' because of its significant homology to PsbQ from green algae (Ohta *et al.* 2003). PsbR protein, present in plants and green algae, is important for the optimization of electron transfer and water oxidation, but the exact function is unclear. PsbU and PsbV proteins, found in cyanobacteria and red algae serve to stabilize PS II (Roose *et al.* 2007). In addition to its typical roles as a protein component of the OEC, PsbV is an interesting water-soluble c-type monoheme cytochrome, with a very low potential (Krogmann 1991).

1.5.2 **Photosystem II function and electron transport pathway**

PS II functions as a **water:plastoquinone oxidoreductase** that utilizes the absorbed energy of photons to catalyze the reaction of water splitting and plastoquinone reduction via a complex set of energy and electron transfer events, providing electrons and protons for further photosynthetic reactions (**Fig. 2**).

After photon absorption, the excitation energy is passed along an excitonically linked network of pigments bound within a system of outer and inner light-harvesting antennae. The excitation of the PS II core induces rapid energy transfer among pigments of each of the core antennae towards the RC (de Weerd *et al.* 2002). An acceptation of excitation energy by chlorophylls of P_{680} in PS II RC produces an excited singlet state, P_{680}*, an excellent electron donor. The excitation is thought to be delocalized along all four central Chls found in the PS II RC (Raszewski *et al.* 2008). The primary donor, P_{680}*, transfers an electron to a primary transient electron acceptor, a pheophytin molecule, giving it a negative charge ($Pheo^{-}$). The resulting formation of $P_{680}^{+}Pheo^{-}$ pair is further stabilized by relocating the extra electron from reduced $Pheo^{-}$ to a primary stable acceptor, plastoquinone PQ_A, producing a plastosemiquinone PQ_A^{-}, thus forming P_{680}^{+} PQ_A^{-}. Before PQ_A^{-} can receive another electron from $Pheo^{-}$, it must transfer the electron to a second plastoquinone PQ_B, yielding plastosemiquinone PQ_B^{-}.

To regain its neutral state, the oxidized P_{680}^{+} with a high redox potential abstracts an electron via the redox-active tyrosine Y_Z from Mn_4Ca cluster, where, finally, photooxidation of water occurs.

As a result of another P_{680} excitation, the PQ_B^{-} acquires the second electron to produce doubly reduced quinol molecule, PQ_B^{2-}. Finally, the reduced PQ_B^{2-} takes up two protons from the stoma, yielding a fully reduced plastohydroquinone (PQ_BH_2). Subsequently, PQ_BH_2 leaves its binding pocket in the D1 protein and diffuses through the lipid bilayer of the membrane, where in turn it transfers its electrons to the Cyt b_6/f complex. The PQ_B site on D1 protein is filled with another plastoquinone from the pool of quinones freely floating in the membrane (*for review see* Nelson and Ben-Shem 2004, and Nelson and Yocum 2006; Pawlowicz *et al.* 2007).

When electron extraction from water is impaired, an electron might be donated to P_{680}^{+} by a photoprotective alternative electron-transfer pathway (Vasil'ev *et al.* 2003).

Photooxidation of water

Among complexes, PS II has a prominent function – a photo-induced catalysis of water oxidation leads to the production of nearly all of the oxygen in the atmosphere which is a prerequisite for aerobic life on the Earth. The whole process of water oxidation occurs in a so-called S-cycle or **Kok cycle** (Kok *et al.* 1970), which consists of a series of five redox S-states ("S" stands for storage), designated as S_0 to S_4, each representing successively more oxidized forms of Mn_4Ca cluster. The cluster cycles through these different redox states and thereby couples one-electron photochemistry occurring in the PS II RC with the four-electron chemistry of water oxidation. After each charge separation within PS II RC, oxidized P_{680}^{+} extracts one electron from OEC via a redox-active tyrosine Y_Z and thereby the system advances to the next state. Four single-electron transfers, each corresponding to the absorption of one photon, subsequently oxidize two molecules of water bound to the Mn_4Ca cluster. Only at the transition between S_4 and S_0 state the molecule of oxygen is released. The Mn_4Ca cluster is then reset to its initially reduced state, S_0. At total, two molecules of water bound to Mn_4Ca cluster are split, yielding molecular oxygen, four electrons and four protons, as shown in **equation 4**. Protons released from water remain in the thylakoid lumen (McEvoy *et al.* 2005, Brudvig 2008).

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

Equation 4 Photooxidation of water (McEvoy and Brudvig 2004)

1.5.3 Cytochrome *b₆/f* complex

The cytochrome b_6/f (Cyt b_6/f) complex is another integral membrane-bound protein complex of oxygenic photosynthesis that provides the electronic connection between PS II and PS I reaction centers. This complex resembles in structure and function the cytochrome bc_1 complex, its purple bacteria counterpart and also Complex III of the mitochondrial respiratory chain.

However, the crystal structures of the dimeric Cyt b_6/f complex from cyanobacteria (Kurisu *et al.* 2003) and green algae (Stroebel *et al.* 2003) reveal important differences compared with cytochrome bc_1 (Cyt bc_1) complex. Each monomer with a molecular weight of 110 kDa is composed of eight protein subunits and seven cofactors. In detail, there are four large subunits – that is, cytochrome *f* (Cyt *f*), cytochrome b_6 (Cyt b_6), the Rieske iron-sulphur protein (Rieske Fe-S or Fe_2S_2 protein) and subunit IV – as well as four small hydrophobic subunits (PetG, PetL, PetM, PetN) per each monomer of Cyt b_6/f complex. In total, thirteen transmembrane helices, four in Cyt b_6, three in subunit IV and one each in the remaining subunits is present per each monomer of Cyt b_6/f.

Cytochrome *f* is a c-type cytochrome with one covalently bound heme *f*. Cytochrome b_6 is a b-type cytochrome that noncovalently binds two hemes, b_H and b_L (also named b_N and b_P, respectively because of their nearness to the electrochemically negative and positive side of the lipid bilayer). In addition to these hemes, there is a further heme (heme c_N) covalently bound on the N-side of the complex, very close to intermembrane heme b_N. This structurally unique heme group with no strong field ligand might participate in cyclic electron transfer around PS I. The heme c_N as well as two cofactors with unknown function (Chl *a* and β-Car), also present in Cyt b_6/f complex, are not found in the related bc_1 complex. The Rieske protein has an unusual Fe_2S_2 cluster, in which the ligands for Fe atoms are two Cys and His residue (typical Fe-S centers have only Cys ligands), causing the Rieske centre that lies on the lumenal side of the membrane to have high redox potential.

Cyt b_6 and subunit IV are homologous to the N- and C-terminal halves, respectively, of Cyt *b* of the Cyt bc_1 complex. The Reiske Fe-S proteins of the Cyt b_6/f and Cyt bc_1 complexes are also similar (Carrell *et al.* 1997). Furthermore, PetG, PetL, PetM and PetN have no parallels in Cyt bc_1.

Cyt b_6/f complex functions as a **plastoquinol:plastocyanin/cyt c_6 oxidoreductase** that transfers electrons from reduced plastoquinone to oxidized plastocyanin (PC) and translocates protons from the stroma to the lumen and thereby generates a transmembrane electrochemical proton gradient for ATP synthesis using mechanism known as **Q cycle** (**Fig. 2**).

During this cycle, a plastoquinol PQH_2, bound at the lumenal Q_P site is oxidized by high-potential Rieske Fe-S centre and the released electron passes through Cyt *f* to plastocyanin and two protons are released from the PQH_2 to the lumen. The second electron is translocated across the membrane, through two hemes (b_H and b_L) of Cyt b_6 to reduce plastoquinone PQ that is bound at the stromal Q_N site. This cycle repeats itself to oxidize second PQH_2, with one electron being passed to PC and second being transferred to the Q_N site to produce fully reduced plastoquinol that picks up two protons from stroma and is released from Q_N site into the lipid bilayer. The net result of the Q cycle is that PQH_2 at the Q_P site is oxidized to PQ, and PQ at the Q_N site is reduced to PQH_2, two electrons are successively transferred to PC, four protons pumped from the stroma to the lumen. As a result the proton-motive force is formed across the membrane.

Plastocyanin

Electron transfer between Cyt b_6/f complex and PS I is mediated by plastocyanin (PC), a low-molecular mass, copper-containing blue protein that is resident on the lumenal surface of the thylakoid membrane, where it is freely diffusible. The X-ray diffraction crystallography showed structure of PC (Coleman *et al.* 2000) with a single Cu atom, which alternates between its Cu^{1+} and Cu^{2+} oxidation states. The Cu atom is tetrahedrally coordinated by a Cys, a Met, and two His residues.

PC first binds to Cyt *f*, where it receives an electron and is reduced ($Cu^{2+} \rightarrow Cu^{1+}$). After dissociation, PC diffuses through the lumen to PS I, recognizes and binds to a specific site, donates an electron to PS I and finally dissociates again (**Fig. 2**) (Sigfridsson 1998, Hope 2000). Many studies indicate that its acidic patch (negatively charged) has a binding and recognition function and the hydrophobic patch (positively charged) participates in electron transfer (Blankenship 2002).

In many cyanobacteria and green algae, PC is replaced by a c-type of cytochrome c_6 (Cyt c_6) (Kerfeld *et al.* 1997).

1.5.4 **Photosystem I**

A second major component involved in the photosynthetic machinery of oxygenic organisms is photosystem I (PS I), a membrane multisubunit pigment-protein complex. PS I of eukaryotes has a number of features that distinguishes it from PS I from cyanobacteria, but both have the same basic function – an electron transport from plastocyanin or cytochrome *c* in some species to ferredoxin. Eukaryotic PS I is composed of two functional parts, a **core complex** and a light-harvesting complex I (**LHC I**). LHC I belt contributes a mass of 160 kDa out of approximately 600 kDa in PS I (Amunts *et al.* 2007). Whereas the PS I core complex is relatively similar to the cyanobacterial PS I, the LHC, the membrane-embedded antenna is specific for eukaryotic PS I and completely different from the phycobilisomes serving as peripheral antenna in cyanobacteria (Scheller *et al.* 2001).

Core complex

As it was shown by the crystal structures of cyanobacterial and higher plant PS I complex (Krauss *et al.* 1996, Schubert et al. 1997, Jordan *et al.* 2001, Fromme *et al.* 2001, Ben-Shem *et al.* 2003, Amunts *et al.* 2007, *reviewed by* Allen *et al.* 2009) the heart of the PS I core complex is formed by a **heterodimer** of two large homologous subunits **PsaA** and **PsbB**, each having an approximately 80 kDa and 11 transmembrane helices; and one small subunit, the PsaC protein. These three subunits bind the major electron transfer carriers, forming two potential electron transport chains, similar to the case in PS II. Two closely associated chlorophylls near the lumenal side form a primary electron donor, the $\mathbf{P_{700}}$ special pair. Four accessory Chls, two phylloquinones and the set of three redox-active Fe-S clusters, coordinated by cysteines are also present. The electron transfer cofactors, composed of the chlorophylls and phylloquinones are arranged in two branches (A and B) as pairs of molecules. Whether one or both branches are active in electron transport remain an unsolved question. One of the Fe-S centre, termed F_X, is held between the two subunits of the core heterodimer, whereas two other Fe-S centers, called F_A and F_B, are bound to the stromal subunit PsaC (Nugent *et al.* 1996).

Like in PS II core structure, there is a number of additional proteins in PS I core complex, with no cofactors bound. In addition to PsaA/PsaB heterodimer, the cyanobacterial PS I core complex contains at least ten protein subunits that are denoted PsaC–PsaF and PsaI–PsaM and PsaX. Green plants do not have PsaM and PsaX subunits, but do contain three further larger membrane proteins (PsaG, PsaH and PsaO) and one more extrinsic protein PsaN exposed to the thylakoid lumen (Scheller *et al.* 2001, Knoetzel *et al.* 2002, Jensen *et al.* 2003).

Many of these proteins have yet unknown function. The extrinsic PsaD and PsaE subunits located on the stromal surface of membrane participate in the docking of ferredoxin (Fromme *et al.* 2001). On the lumenal side, the most noticeable distinction between plant and cyanobacterial RC is the helix-loop-helix domain contributed by the longer N-terminal domain of plant PsaF, which enables more efficient plastocyanin binding in plant (Amunts *et al.* 2007). Several subunits are probably involved in association with LHC I and in binding of LHC II. For example, PsaH subunit, which is not present in cyanobacteria was proposed to enable the binding of LHC II, and therefore to be essential for state transitions in plant photosynthesis (Lunde *et al.* 2000). The subunits also participate in the maintenance of complex integrity and probably have other functions.

In cyanobacteria, the PS I complex can exists as a monomer and trimer depending on growth condition (Boekema *et al.* 1987, Ford and Holyenburg 1988, Rogner *et al.* 1990), the trimerisation of PS I was observed in Chl *b* containing cyanobacteria (van der Staay *et al.* 1993, Bibby *et al.* 2001b, Bumba 2005) or in iron-stressed cyanobacteria (Bibby *et al.* 2001a, Boekema *et al.* 2001b), while in eukaryotic organisms only monomeric PS I was found (Boekema *et al.* 2001a, Germano *et al.* 2002, Kargul *et al.* 2003, Gardian *et al.* 2007).

1.5.5 Photosystem I function and electron transport pathway

PS I functions as a light-driven **plastocyanin:ferredoxin oxidoreductase** that catalyses the electron transport from PC on the lumenal side of the membrane to ferredoxin (Fd) at the stromal side by a chain of electron carriers (**Fig. 2**) (Chitnis 2001, Jensen *et al.* 2007).

The light-induced charge separation starts in PS I with excitation of P_{700}, a chlorophyll *a* heterodimer (Watanabe *et al.* 1985), by excitation transfer from the antenna pigments. The excited P_{700}^* loses an electron and a primary electron acceptor, called A_0 (Chl *a* monomer) is reduced, creating A_0^- and P_{700}^+. The strong oxidizing agent P_{700}^+ quickly extracts an electron from PC and the strong reducing agent A_0^- donates its electron to one of the phylloquinones (A_1). Subsequently, an electron from A_1 acceptor passes through three redox-active Fe_4S_4 centers in PS I, the F_X, and finally to F_A and F_B. From here, the electron moves to ferredoxin, a soluble protein loosely associated with the membrane that contains a Fe_2S_2 center. This electron carrier does not transfer electrons directly to $NADP^+$, but via a **ferredoxin:$NADP^+$ oxidoreductase** (**FNR**). It is a flavoprotein with the fourth Fe-S electron carrier in the chain, that transfers electrons from reduced Fd to $NADP^+$, leading to a formation of NADPH and H^+.

In addition to the major function of reducing $NADP^+$ via FNR, ferredoxin also serves as an electron donor in a number of biosynthetic and regulatory processes. Fd provides electrons for the soluble enzymes involved in nitrogen and sulphur metabolism, synthesis of the glutamate, and through thioredoxin it regulates the ATP synthase activity and carbon metabolism (Knaff *et al.* 1996).

Under some conditions, the reduced Fd can function in a cyclic electron flow around PS I, in which electrons are finally returned back to P_{700} via Cyt b_6/f complex and PC, with generating *pmf* and ATP but no NADPH (Fork and Herbert 1993, Bendall and Manasse 1995).

1.5.6 **ATP synthase**

The last supramolecular protein complex embedded in thylakoid membrane with no pigments bound is ATP synthase (ATPase), also often called the coupling factor (**Fig. 2**).

Chloroplast ATP synthase is about 400 kDa enzyme consisting of two major functional segments: a transmembrane protein portion, called CF_0 and a hydrophilic, extrinsic portion located on the stromal surface, called CF_1 (C denoting its location in chloroplast). The complex is often referred to as the **CF_0−CF_1 complex**. The main structural features of the thylakoid ATPase are highly conserved and its structure and function is, in most respects, similar to those of bacterial and mitochondrial ATPases. CF_0 functions as a channel for protons passing through the membrane which provides the driving force to the CF_1 subunit where the actual conversion of ADP and P_i into ATP takes place.

In total, ATPase of chloroplast contains nine different polypeptide subunits that are both chloroplast and nuclear-encoded gene products (Henning and Hermann 1986). The hydrophobic CF_0 part has four subunit types, labeled I, II, III (c) and IV in a probable stoichiometry 1:1:14:1. Fourteen III subunits form a ring-like structure that is equivalent to the c-ring of other ATPases (McCarty *et al.* 2000). CF_1 is composed of three copies of each of two relatively large subunits, α and β (both with molecular mass of about 50 kDa), forming $\alpha_3\beta_3$ hexamer and one copy of each of three smaller subunits, labeled γ, δ and ε in the order of decreasing molecular weight. The overall subunit stoichiometry is $I_1II_1III_{14}IV_1\alpha_3\beta_3\gamma\delta\varepsilon$ (Richter 2004).

The regulatory α and catalytic β subunits, alternating in hexameric ring, bind ADP and P_i and phosphorylate ADP into ATP. The γ and ε subunits together form a central stalk connecting the CF_0 and CF_1 segments. The ε subunit links the γ subunit to the III subunit ring that is involved in proton transport (Capaldi and Schulenberg 2000). A second, side stalk comprised of δ and subunits I and II attaches CF_0 to CF_1 and is considered to acts as stator. The whole CF_0-CF_1 complex functions as a tiny rotary proton-driven motor, in which the stationary subunits are I, II, IV, δ, α and β while the γ, ε and III subunits rotate (Nelson and Ben-Shem 2004).

The ATP synthesis occurs by way of the so-called binding-change mechanism (Boyer 1997) that involves alternating conformational changes in the three nucleotide-binding sites of the catalytic β subunits. The binding of proton to the c-subunit drives the rotation of the c-ring and the central stalk which causes an interconversion of the binding sites and also changes their affinity for the nucleotides. According to this mechanism the changes in conformation are driven by *pmf* whereas the own ATP formation does not require additional energy.

The active form of ATP synthase can catalyze both the formation and hydrolysis of ATP. The regulation of chloroplast ATP synthase activity is light-dependent and is carried out by a process called thiol modulation (also called redox regulation), which is unique to algae and higher plants (Ort and Oxborough 1992, Ritcher 2004). In the presence of light, the enzyme maintains high synthetic activity through the reduction of a specific intra-peptide disulphide bond that is located in the γ subunit of CF_1. *In vivo*, γ subunit reduction is mediated by a thioredoxin, which is present in the chloroplast stroma and photoreduced by PS I via ferredoxin in a reaction catalyzed by **ferredoxin:thioredoxin oxidoreductase** (Ketcham 1984). The reduction of the regulatory disulphide bridge is thought to cause the conformational changes in the enzyme complex thereby decreasing the *pmf* required to maintain catalytic activity (Nalin and McCarty 1984).

1.5.7 Light-harvesting antenna complexes

Despite a highly conserved molecular structure of photosynthetic reaction center complexes found among various classes of photosynthetic organisms, a remarkable variety of antenna complexes have been identified. Antenna complexes differ widely both in overall structural arrangement and in the nature, number and organization of the absorbing pigments without apparent correlation to each other (Collini 2009). This suggests that major classes of antennae appear to be independent evolutionary innovations and that they were originated several times during the evolution of photosynthesis in response to environmental selection pressures (Blankenship 2001).

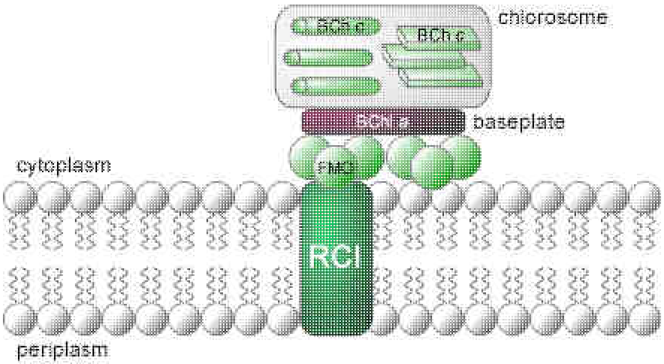
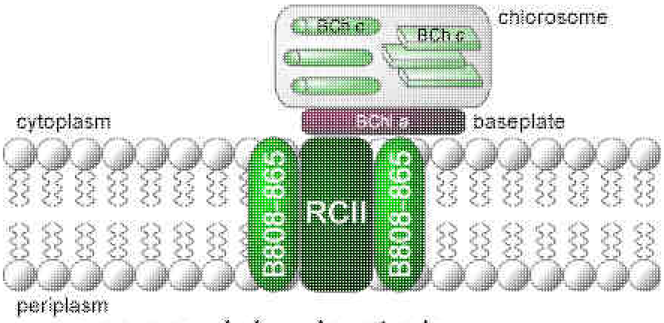
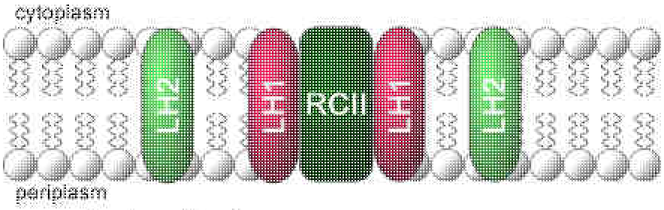
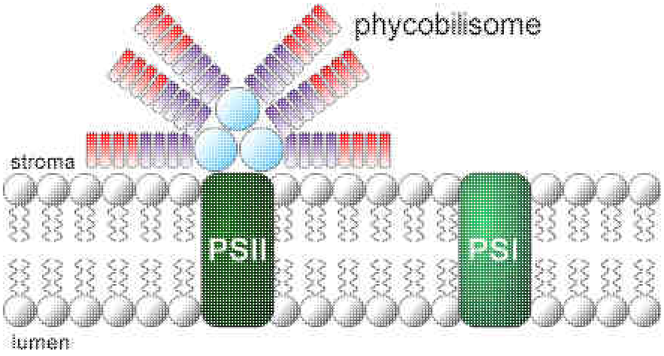
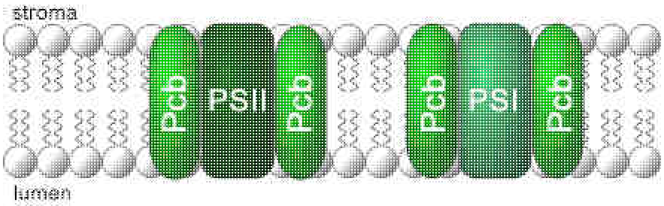
In general, the sessile plants utilize a limited repertoire of pigment types compared to photosynthetic organisms living in aquatic environment where penetrating wavelengths of light may be variedly weakened by the water column. It is also known that in many systems the size of the photosynthetic antenna is flexible depending on the light quality in their different habitats.

Antenna complexes can be broadly classified into two categories according to their position with respect to the thylakoid membrane:

Peripheral membrane antennae, which are constantly linked to one side of the membrane, but does not itself cross the membrane. This class includes phycobilisomes of red algae and cyanobacteria, chlorosomes and Fenna-Matthews-Olson (FMO) proteins of green bacteria and peridinin-chlorophyll proteins of dinophytes.

Integral membrane antennae contain proteins that traverse the lipid bilayer. Within this second class additional types of antennae can be distinguished: **core** antenna, tightly bound to RC (CP43 and CP47 of PS II or LH1 complex of purple bacteria) and **accessory** antenna, always found in addition to core antenna (LHC I, LHC II and LH2 complex of purple bacteria) (Blankenship 2002).

In **Fig. 3** schematic models of major light-harvesting antenna complexes in different photosynthetic organisms are depicted. In the following subheads, the antenna complexes involved in oxygenic photosynthesis will be described in more detail.

Prokaryotes with no chloroplasts	 green sulphur bacteria
	 green nonsulphur bacteria
	 purple bacteria
	 cyanobacteria
	 prochlorphytes

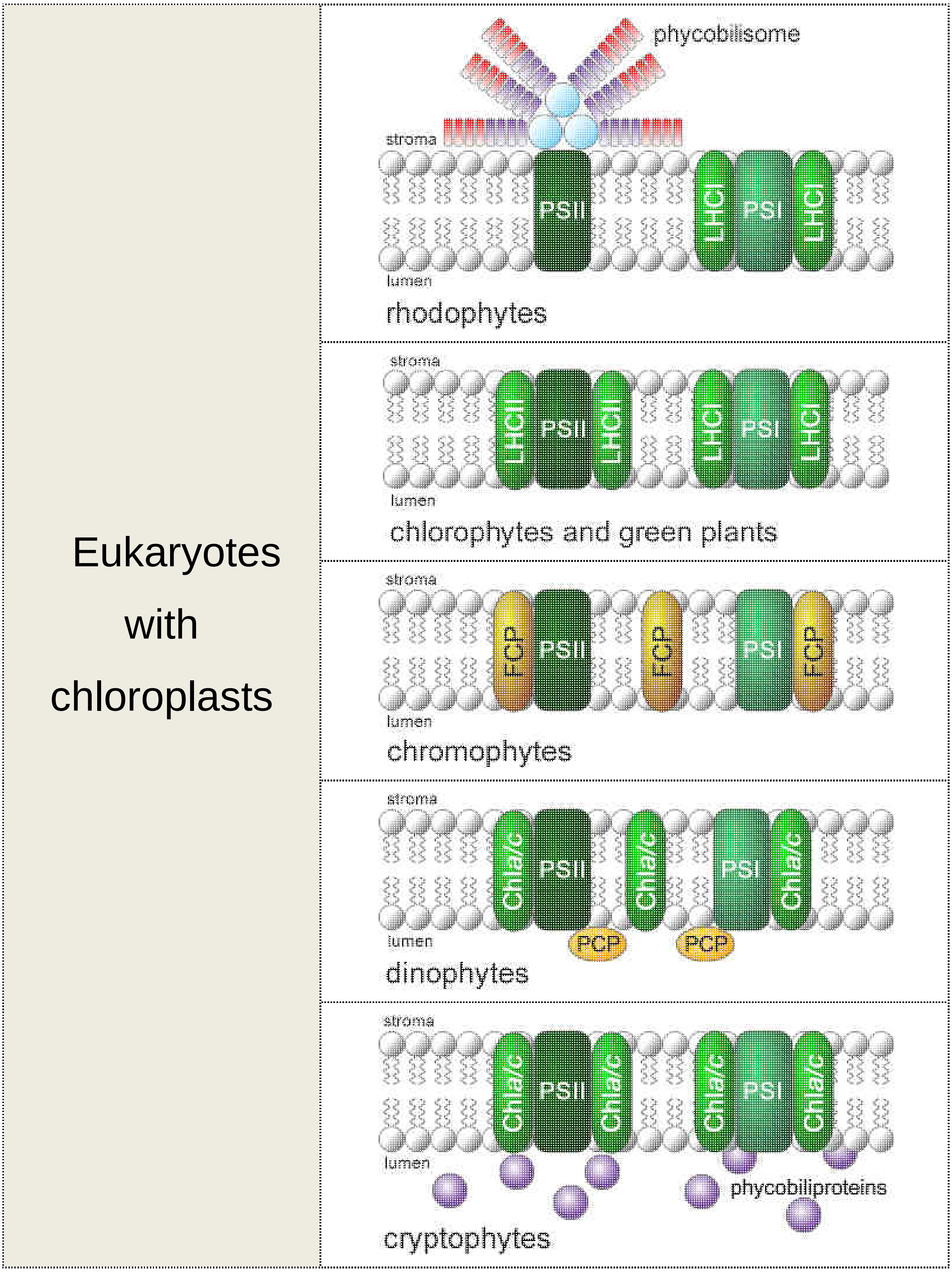


Fig. 3 Schematic models of light-harvesting antenna complexes in different photosynthetic organisms and their position with respect to the membrane and reaction centers. See the subhead 1.5.7 for a detailed description.

Phycobilisomes

Several different types of phycobilisomes in a fanlike, cylindric or hemidiscoidal arrangement, most commonly containing three major types of phycobiliproteins (APC, PC and PE), were found among cyanobacteria and red algae (Glazer 1985, Sidler 1994, Grossman *et al.* 1995, MacColl 1998). They are constructed from plenty of disc-shape subunits. A basic structural unit of all phycobiliproteins, a dimer of α and β peptides (also named as $\alpha\beta$ monomer) further assembles to form trimers $(\alpha\beta)_3$ or hexamers $(\alpha\beta)_6$. The basic structure of the most widely studied hemidiscoidal phycobilisome consists of a core made up of APC and several rods, the stacked disks of PCy and PE that are radiating from the central core (Mimuro and Kikuchi 2003). In this respect, phycobilisomes represent an ingenious funnel system, in which the light energy they absorb is transferred efficiently towards RC, i.e. from phycoerythrin, present on the ends of rods, absorbing at the shortest wavelengths through intermediate phycocyanin to allophycocyanin that absorbs at the longest wavelengths (Blankenship 2002). These supramolecular complexes are attached to the core of the photosystem from the stromal side of the thylakoid membrane with the help of an anchor peptide, usually in close association with PS II.

Apart from the three most frequently occurring phycobiliproteins mentioned above, many different phycobiliproteins are found in various organisms. For example, in the case of cryptophytes the phycobiliproteins are not organized into a phycobilisome (*see* **Fig. 3**), but they are present as $(\alpha_1\beta)(\alpha_2\beta)$ heterodimers that are located in the thylakoid lumen contrary to phycobilisomes of cyanobacteria and red algae (Doust *et al.* 2004, van der Weij-De Wit *et al.* 2006).

LHC superfamily

The light-harvesting complexes (LHCs) usually referred as **LHC I** and **LHC II**, depending on their association with PS I or PS II, are members of a large and abundant protein family of intrinsic membrane chlorophyll *a*/*b*-binding proteins present in all photosynthetic eukaryotes. Due to chlorophyll *a* and *b* presence, they are also often called as Cab proteins.

Proteins that make up these complexes are encoded by *Lhc* nuclear gene superfamily (Jansson 1999) and they have three transmembrane α-helix regions sharing high similarity. Despite their sequence and structural homology they considerably vary in oligomeric state.

Light-harvesting complex I

Light-harvesting complex I (LHC I) consists of four different membrane proteins, designated Lhca1 to Lhca4, each with molecular mass of about 25 kDa (Jansson 1994). The crystal structure of plant PS I−LHC I (Ben-Shem *et al.* 2003, Amunts *et al.* 2007) revealed the assembly of Lhca proteins around PS I as dimers Lhca1/Lhca4 and Lhca2/Lhca3 in contrast to the trimeric formation of Lhcb in LHC II. In agreement with previous model determined by electron microscopy (Boekema *et al.* 2001a) it was shown that the binding of LHC I to the RC is asymmetric and Lhc heterodimers form a half-moon shaped belt on one side of the RC. Each of the Lhca protein binds about 8 Chl *a* and 2–3 Chl *b* molecules and 2–3 carotenoids (Schmid *et al.* 2002; Croce *et al.* 2002, Mozzo *et al.* 2006, Corbet *et al.* 2007).

Light-harvesting complex II

Most of the chlorophylls that are associated with PS II are harboured in the LHC II found in plants and many algae, ranging from 130 to 250 antenna Chls per PS II core dimer (Jansson *et al.* 1997). The X-ray crystal structures of spinach and pea LHC II were reported at 2.7 Å (Liu *et al.* 2004) and 2.5 Å (Standfuss *et al.* 2005) resolution, respectively. In the membrane, LHC II assembles spontaneously from monomers into trimers consisting of various combinations of three very similar subunit proteins, Lhcb1, Lhcb2 and Lhcb3. The three subtypes of the major LHC II are very similar in polypeptide sequence and have the same pigment content and spectroscopic properties (Standfuss and Kühlbrandt 2004, Palacois *et al.* 2006). Fourteen Chls in each monomer can be distinguished as eight Chl *a* and six Chl *b* molecules and four carotenoids. The number of trimers attached to the PS II varies with irradiance level; usually there are two to four copies of LHC II bound in dimeric PS II supercomplexes (Dekker and Boekema 2005). Previously, it was estimated up to eight LHC II trimers per PS II core dimer (Peter and Thornber 1991), implicating the presence of a pool of non-bound or very loosely bound LHC II.

Besides its main light-harvesting function LHC II also plays an important role in the dissipation of excess light energy under high-light conditions (Ruban *et al*, 1994). In these cases, the main photoprotective mechanisms are the **xanthophyll cycle** (*reviewed by* Jahns *et al.* 2009) and **photophosphorylation** of LHC subunits (discussed below).

In addition, more distantly related member of LHC superfamily, a very hydrophobic PsbS protein, which is unique to eukaryotic PS II, also acts in photoprotection and is required for a nonphotochemical quenching (Li *et al.* 2002, Bergantino *et al.* 2003).

LHC II is not connected to PS II core directly, but the energy transfer from LHC II to CP43/CP47 and to D1/D2 is mediated by the so-called minor antenna complexes, referred to as CP24, CP26 and CP29 (Bassi and Dainesse 1992, Camm *et al.* 2004). They are also encoded by nuclear *Lhc* genes and originate from the gene products Lhcb6, Lhcb5 and Lhcb4, respectively. These proteins show a high homology with LHC II and one copy of each protein is found per PS II RC (Bassi *et al.* 1997). Similarly as LHC II proteins, they bind approximately 8–10 Chls, besides several xanthophylls.

State transition

Oxygenic photosynthetic organisms adapted to changes in light quality and quantity by redistribution of light excitation energy between the two photosystems through an elegant mechanism, known as **state transition** (Allen and Forsberg 2001, Wollman 2001, Haldrup *et al.* 2001 Allen 2003).

The association of LHC II with PS II is regulated by light intensity. In bright sunlight, an excess of energy is being delivered to PS II that produces electrons faster than PS I can utilize them. The accumulation of PQH_2 results in activation of a specific protein kinase that phosphorylates amino acid residues on LHC II, thereby changing the surface charge on the protein. Activation of the stromal kinase is regulated specifically by binding of PQH_2 in the Q_P pocket of the Cyt b_6/f complex (Zito *et al.* 1999). After phosphorylation, the interaction between negatively charged LHC II and PS II becomes weakened and a portion of LHC II dissociates and moves from the hydrophobic core of the grana region to the less hydrophobic stromal lamellae that are enriched in PS I. The state, in which LHC II is phosphorylated and becomes connected to PS I, is known as **state 2**. This connection requires the PsbH subunit (Lunde *et al.* 2000). As PQH_2 becomes more oxidized by PS I, the imbalance between the fluxes of excitations in PS I and PS II is reversing. The kinase becomes less active and a phosphatase acts to dephosphorylate LHC II, allowing it to migrate back to the grana stacks. The resulting state, in which dephosphorylated LHC II is attached to PS II, is called **state 1**.

Lateral displacement of LHC II from the PS II-rich grana to the PS I-rich stroma lamellae leads to decrease in light absorption by PS II and to increase in energy delivery to PS I. This reorganization of antenna systems results in enhanced photosynthetic quantum yield.

Peridin-chlorophyll proteins

In many dinoflagellates, a unique peridinin-chlorophyll *a* protein (**PCP**) complexes were found, containing an atypical water soluble carotenoid – peridinin that serves as a main light-harvesting pigment compare with chlorophylls in other classes of antennae. PCPs form trimers, including 32 peridinine and 6 Chl pigments (Hofmann *et al.* 1996), delivering energy to an integral membrane Chl *a*/*c* binding antenna complex, which, in turn, transfers it to the PS II RC.

Fucoxanthin-chlorophyll proteins

LHC antenna proteins of chromophytic algae, i.e. diatoms (*Bacillariophyceae*) and brown algae mainly bind fucoxanthin and Chl *a* and Chl *c*, and are known as fucoxanthin-chlorophyll *a*/*c* binding proteins (**FCP**s). These antenna systems contain high amount of xanthophylls, with fucoxanthin, being the most prominent carotenoid in these organisms (hence the name FCP complexes) and diadinoxanthin alternating with diatoxanthin in a xanthophyll cycle in several algal groups (Stransky *et al*. 1970). Each FCP possesses three transmembrane α-helices that are highly homologous to other proteins of LHC superfamily (Wolfe *et al.* 1994, Durnford *et al.* 1996) with small differences in molecular weight (Gugliemelli 1984, Caron *et al.* 1988). FCP antenna complexes in trimeric or higher oligomeric states were described (Büchel *et al.* 2003, Guglielmi *et al.* 2005, Beer *et al.* 2006, Brakemann *et al.* 2006, Lepetit *et al.* 2007).

Prochlorophyte-chlorophyll *b* binding proteins

Although phycobilisome-containing cyanobacteria constitute the vast majority of oxygenic photosynthetic bacteria, there are several unique kinds of prokaryotic oxygenic phototrophs that utilize intrinsic membrane antenna complexes, **Pcb** proteins, binding Chl *b* or in some cases Chl *d* in addition to Chl *a*. Based on phylogenetic studies of 16S rRNA (Turner *et al.* 1997), these significantly different species are classified as cyanobacteria, including Chl *b* containing group, inappropriately called prochlorophytes, namely genera *Prochloron, Prochlorococcus* and *Prochlorothrix* and also Chl *d* containing organisms, such as the best known *Acaryochloris marina*.

The name "prochlorophytes" expresses an original assumption that they could represent the prokaryotic ancestors of the chloroplasts of green algae and plants, because Chl *b* was found in these lineages of photosynthetic prokaryotes in contrast to typical cyanobacteria. However, as was demonstrated later by analyses of genetic markers (Bergey's Manual of systematic Bacteriology 2001), this three genera are highly diverged polyphyletic group, not affiliated with chloroplasts, thus the possible evolutionary relationship was not proved. It is therefore more accurate to use expression “green oxyphotobacteria” rather than “prochlorophytes” in spite of the fact that this term is rooted.

The Pcb (prochlorophyte chlorophyll *b* binding) proteins, encoded by *pcb* genes, having three transmembrane α-helices are completely unrelated to the Chl *a*/*b* proteins of LHC superfamily (Durnford *et al.* 1999), instead they are closely related to the CP43 core antenna protein and to the IsiA (often called CP43’), an iron-stress-induced Chl *a* binding antenna protein of cyanobacteria (LaRoche *et al.* 1996).

These Pcb-containing atypical cyanobacteria differ from each other in a number of copies of *pcb* genes in genome depending on species, strain and ecotype. In detail, the *Prochlorothrix hollandica* has a cluster of three *pcb* genes (*pcbA*, *pcbB* and *pcbC*) (van der Staay *et al.* 1998). In the case of *Prochlorococcus marinus*, the *pcb* genes are present in multiple copies in low-light adapted strains but as a single copy in high-light strains (Garczarek *et al.* 2001). Both *Prochloron didemni* and prochlorophyte-like cyanobacterium *Acaryochloris marina* contain two *pcb* tandem genes, *pcbA* and *pcbC,* which encode Chl *b* and Chl *d* binding antenna proteins PcbA and PcbC, respectively (Chen *et al.* 2005a and 2005b).

Electron microscopy revealed formation of PS I–IsiA as well as PS I–Pcb supercomplexes composed of 18 subunits forming a ring around the trimeric PS I reaction centre (Bibbi *et al.* 2001a and 2001b, Boekema *et al.* 2001b, Bumba *et al.* 2005, Chen *et al.* 2005b). The production of multiple copies of IsiA and Pcb proteins greatly enhances the light-harvesting capacity (Yeremenko *et al.* 2004, Boichenko *et al.* 2007) in response either to a poor supply of iron in the case of IsiA or to light-limiting conditions in natural habitats in the latter case.

1.6 **Outline of the thesis**

This work was focused on a study of structural organization and function of photosynthetic pigment-protein complexes. To realize this purpose, a number of biochemical methods in combination with spectroscopy and electron microscopy were used. The biochemical methods were used as a main tool for structural and functional characterization of antenna complexes, as well as to examine a light-harvesting strategy.

Chapter 2 presents results of a biochemical study conducted on Pcb antennae isolated from an atypical cyanobacterium *Prochlorothrix hollandica*. This study, based on identification and localization of the Pcb proteins, provides information about light-harvesting strategy and function of the single types of Pcb antenna proteins.

In **chapter 3**, role of carotenoids and chlorophylls in the energy transfer and quenching processes in isolated PcbC antennae and PcbC–PS I supercomplexes from *Prochlorothrix hollandica* is reported.

Chapter 4 describes an organization of photosystem I and photosystem II in red alga *Cyanidium caldarium*. This organization of photosynthetic pigment-protein complexes was investigated with a consideration of endosymbiotic origin of red algae.

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Chapter 2

2 Localization of Pcb antenna complexes in the photosynthetic prokaryote *Prochlorothrix hollandica*

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2.1 Abstract

The freshwater filamentous green oxyphotobacterium *Prochlorothrix hollandica* is an unusual oxygenic photoautotrophic cyanobacterium differing from most of the others by the presence of light-harvesting Pcb antenna binding both chlorophylls *a* and *b* and by the absence of phycobilins.

The pigment-protein complexes of *P. hollandica* SAG 10.89 (CCAP 1490/1) were isolated from dodecylmaltoside solubilized thylakoid membranes on sucrose density gradient and characterized by biochemical, spectroscopic and immunoblotting methods. The Pcb antennae production is suppressed by high light conditions (> 200 µmol photons.m^{-2}.s^{-1}) in *P. hollandica*. PcbC protein was found either in higher oligomeric states or coupled to PS I (forming antenna rings around PS I). PcbA and PcbB are most probably only very loosely bound to photosystems; we assume that these pigment-protein complexes function as low light-induced mobile antennae. Further, we have detected α-carotene in substantial quantities in *P. hollandica* thylakoid membranes, indicating the presence of chloroplast-like carotenoid synthetic pathway which is not present in common cyanobacteria.

Překlad abstraktu

Prochlorothrix hollandica je neobvyklá fotoautotrofní sinice s oxygením typem fotosyntézy žijící ve sladkých vodách. Její fotosyntetický aparát se liší od ostatních sinic tvorbou specifických světlosběrných antén, tzv. Pcb proteinů. Tyto antény neobsahují žádné fykobiliny, ale je v nich vázán chlorofyl *a* i chlorofyl *b*.

Tylakoidní membrány *P. hollandica* SAG 10.89 (CCAP 1490/1) byly solubilizovány dodecylmaltosidem a pigment-proteinové komplexy izolovány centrifugací v hustotním gradientu sacharózy a dále charakterizovány pomocí biochemických, spektroskopických a imunodetekčních metod. V podmínkách vysoké ozářenosti světlem (> 200 µmol fotonů

m^{-2} s^{-1}) je tvorba Pcb antén potlačena. Protein PcbC se vyskytuje buď ve vyšších oligomerních stavech, nebo je vázán k fotosystému I a vytváří prstenec okolo něj. Proteiny PcbA a PcbB jsou s největší pravděpodobností jen velmi volně vázány k fotosystémům a slouží jako světlem indukované mobilní antény. Stanovení α–karotenu v tylakoidních membránách *P. hollandica* ukazuje na existenci eukaryontního typu syntézy karotenoidů, který není přítomen u běžných sinic.

Autorský podíl

Miroslava Herbstová je prvním autorem této publikace, její podíl tvoří 75 %.

Chapter 3

3 Carotenoids in energy transfer and quenching processes in Pcb and Pcb–PS I complexes from *Prochlorothrix hollandica*

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3.1 Abstract

Chlorophyll (Chl) *a*/*b*-binding proteins from *Prochlorothrix hollandica* known as Pcb antennae were studied by femtosecond transient absorption technique to identify energy transfer rates and pathways in Pcb and Pcb–PS I complexes. Carotenoids transfer energy to Chl with low efficiency of ~25% in Pcb complexes. Interestingly, analysis of transient absorption spectra identified a pathway from the hot S_1 state of zeaxanthin and/or β-carotene as the major energy transfer channel between carotenoids and chlorophylls whereas the S_2 state contributes only marginally to energy transfer. Due to energetic reasons, no energy transfer is possible via the relaxed S_1 state of carotenoids. The low overall energy transfer efficiency of carotenoids recognizes chlorophylls as the main light-harvesting pigments. Besides Chl *a*, presence of Chl *b*, which transfers energy to Chl *a* with nearly 100% efficiency, significantly broadens the spectral range accessible for light-harvesting and improves cross-section of Pcb complexes. The major role of carotenoids in Pcb is photoprotection.

Překlad abstraktu

Světlosběrné komplexy vázající jak chlorofyl *a*, tak chlorofyl *b*, známé jako Pcb antény byly izolovány ze sinice *Prochlorothrix hollandica*. Pomocí femtosekundové absorpční spektroskopie byl v těchto anténách studován přenos excitační energie. Bylo zjištěno, že energie je přenášena z karotenoidů na chlorofyly s nízkou účinností (~25%). K přenosu energie dochází především z vyšších vibračních hladin prvního singletního stavu (S_1) zeaxantinu, zatímco druhý singletní stav (S_2) přispívá k přenosu energie jen minimálně. Přenos energie z vibrační hladiny S_1 stavu, která je při pokojové teplotě nejpravděpodobnější, není z energetických důvodů možný. Chlorofyly slouží tedy jako hlavní světlosběrné pigmenty, právě s ohledem na málo efektivní karotenoidy. Chlorofyl *b* přenáší energii na chlorofyl *a* s téměř 100% účinností, podstatně rozšiřuje spektrální oblast využitelného záření a zlepšuje i prostorové pokrytí anténního systému pro zachycení fotonu např. při nízkých intenzitách světla. Hlavní rolí karotenoidů v Pcb anténách je fotoprotekce.

Autorský podíl

Miroslava Herbstová je druhým autorem článku, její podíl tvoří 20 %.

Chapter 4

4 Organisation of photosystem I and photosystem II in red alga *Cyanidium Caldarium*: encounter of cyanobacterial and higher plant concepts

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4.1 Abstract

Structure and organisation of Photosystem I and Photosystem II isolated from red alga *Cyanidium caldarium* was determined by electron microscopy and single particle image analysis. The overall structure of Photosystem II was found to be similar to that known from cyanobacteria. The location of additional 20 kDa (PsbQ') extrinsic protein that forms part of the oxygen evolving complex was suggested to be in the vicinity of cytochrome c-550 (PsbV) and the 12 kDa (PsbU) protein. Photosystem I was determined as a monomeric unit consisting of PsaA/B core complex with varying amounts of antenna subunits attached. The number of these subunits was seen to be dependent on the light conditions used during cell cultivation. The role of PsaH and PsaG proteins of Photosystem I in trimerisation and antennae complexes binding is discussed.

Překlad abstraktu

Pomocí elektronové mikroskopie byla studována struktura a organizace fotosystém I a II červené řasy *Cyanidium caldarium*. Celková struktura fotosystému II této řasy se podobá dimerní struktuře fotosystému II známé u sinic. Bylo zjištěno, že protein PsbQ' o molekulové hmotnosti 20 kDa, jež je součástí komplexu vyvíjejícího kyslík se nachází v blízkosti cytochromu c-550 (PsbV) a 12 kDa proteinu PsbU. Fotosystém I se však na rozdíl of sinic vyskytuje v monomerním stavu s proměnlivým počtem vázaných antén v závislosti na světelných podmínkách použitých během kultivace. V práci je také diskutována role proteinů PsaH a PsaG fotosystému I při trimerizaci a vazbě anténních komplexů.

Autorský podíl

Miroslava Herbstová má na publikaci podíl 10 %.

Chapter 5

5 Summary

Life on the Earth ultimately depends on energy derived from the sun. In this regard, photosynthesis is the only process of biological importance whereby the pure energy of light is collected and stored by a series of enzyme-catalyzed reactions. Thylakoid membrane is the site where a conversion of solar energy into the biochemical energy takes place. Oxygenic photosynthesis performed by plants, algae and cyanobacteria is responsible for production of both molecular oxygen and organic matter on the Earth. Therefore, photosynthesis establishes the composition of the biosphere and supplies all life forms with essential food and fuels.

The process of photosynthesis cannot be understood without a detailed knowledge of the structure and function of its single components. Oxygenic photosynthesis relies on cooperative interaction between two types of separate photosystems, photosystem I (PS I) and photosystem II (PS II), both consisting of two functional parts – core complex and light-harvesting antenna complex. Photosynthetic energy storage process begins with a photon absorption and energy delivery to the reaction center complex by antenna systems. Although general structural organization of PS I and PS II seem to be similar in even distantly related organisms, a remarkable diversity of antenna systems exists among photosynthetic organisms, reflecting their different evolutionary patterns. Antenna systems represent a powerful tool to optimize the light-harvesting process and regulate energy input, in adaptation to the various habitat conditions in which different organisms live. Hence, light-harvesting antennae are a subject of considerable importance and practical interest.

In this thesis, biochemical methods were used as a main tool for structural and functional characterization of antenna complexes, as well as to investigate a light-harvesting strategy.

Chapter 1 introduces into the general concepts that provide a foundation for understanding of photosynthesis. These concepts include the basic principles underlying photosynthetic energy storage, properties of pigments, and the functional roles of various pigments. This chapter also describes the architecture of photosynthetic apparatus, the structure of its components, as well as mechanisms of electron transport and ATP synthesis, occurring in thylakoid membranes of oxygenic photosynthetic organisms. A closing part of the chapter gives an overview of various light-harvesting antenna complexes in different photosynthetic organisms.

Chapter 2 describes the results of a biochemical study of Pcb antenna complexes isolated from the photosynthetic prokaryote *Prochlorothrix hollandica*. The production of Pcb proteins is strongly regulated by light intensity. In response to unfavorable light-limiting conditions PcbC antenna complexes are expressed first, followed by PcbA and PcbB expression. Under high light conditions production of Pcb antennae is suppressed. PcbC protein, identified as a major antenna of light-harvesting system of *P. hollandica* was found either in higher oligomeric states or coupled to PS I, forming antenna ring around PS I. In contrast to PcbC antennae, we suppose that PcbA and PcbB antenna proteins, only very loosely bound to photosystems serve as low light-induced mobile light-harvesting complexes. Further, the substantial quantities of α-carotene in *P. hollandica* thylakoid membranes were detected, indicating the presence of chloroplast-like carotenoid biosynthetic pathway, absent in other cyanobacteria.

Chapter 3 presents results of femtosecond transient absorption spectroscopy. This method was used to reveal the role of carotenoids and chlorophylls in energy transfer and quenching processes in PcbC and PcbC–PS I complexes isolated from an unusual cyanobacterium *Prochlorothrix hollandica*. The light-harvesting process in these complexes is primarily provided by Chl *a* and Chl *b*, whereas carotenoids play rather minor role in antenna function. On the other hand, the presence of carotenoids as photoprotective pigments regulating energy flow within the PcbC–PS I supercomplexes was proposed.

In **chapter 4**, the supramolecular organization of the photosystem I and photosystem II of red alga *Cyanidium caldarium*, determined by electron microscopy and single particle image analysis is reported. As a result of a fact that red algae are derived from primary endosymbiosis of a cyanobacterium in a eukaryotic host, we found cyanobacterial type of photosystem II in red alga *C. caldarium*. On the contrary, typical monomeric structure of photosystem I known from higher plants with a belt of LHC I bound along one side of the PS I complex, was revealed. The amount of attached antenna subunits varied depending on the light conditions used during cell cultivation. Since PsaH subunit is missing in both cyanobacteria and red algae (Scheller *et al.* 2001), and as well only PS I monomers were observed in *C. caldarium*, we suggest that the trimerization, which is unique to cyanobacteria is not caused by a simple absence of PsaH subunit, as it was suggested previously (Ben-Shem *et al.* 2003). We also assume that presence of PsaG subunit is not essential for binding of LHC I subunits, because we were able to see one or two rows of LHC I antenna complexes attached to the PS I of red alga *C. caldarium*, where PsaG subunit is absent.