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Proteins, glycerolipids and carotenoids in the functional photosystem II architecture

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1. ABSTRACT

Photosystem II (PSII), the first supercomplex of the electron transport chain, governs the energy transfer using harvested light energy, which is transformed into biochemical energy. Phosphatidylglycerol and sulfoquinovosyl diacylglycerol, the anionic lipids of photosynthetic organisms, together with a neutral lipid, digalactosyldiacylglycerol, assist in the assembly of photosynthetic complexes. These lipids and carotenoids serve as mortar for the proteins which act as bricks in the construction of the active photosynthetic machinery, and they have determinative roles in the oligomerization of protein subunits. X-ray crystallographic localization of glycerolipids and carotenoids revealed that they are present at functionally and structurally important sites of both the PSI and PSII reaction centers. Phosphatidylglycerol is involved in the formation of the reaction-center oligomers and controls electron transport at the acceptor site of PSII. Digalactosyldiacylglycerol, together with phosphatidylglycerol, is involved in the electron transport at the donor site. Phosphatidylglycerol and carotenoids are needed to glue CP43 to the reaction center core. Carotenoids are protective agents, which prevent photosynthetic complexes from degradation caused by reactive oxygen species.

2. INTRODUCTION

Photosynthesis is a process by which the energy of sunlight is converted to chemical energy. In cyanobacteria, algae and higher plants, photosynthesis is an oxygen-evolving process based on water-oxidation, while in purple bacteria, green filamentous bacteria, green sulfur bacteria and heliobacteria, hydrogen sulfide is utilized as electron donor in an oxidative electron transport. The photophysical and photochemical reactions of oxygenic photosynthesis are localized in the thylakoid membranes. Photosystem II (PSII), cytochrome b_6f, photosystem I (PSI) and ATP-synthase, the major photosynthetic complexes, are imbedded in the thylakoid membranes. Both the PSI and the PSII reaction centers are surrounded by light-harvesting antenna systems, which consist of protein-pigment complexes. Cyanobacteria contain phycobilisomes

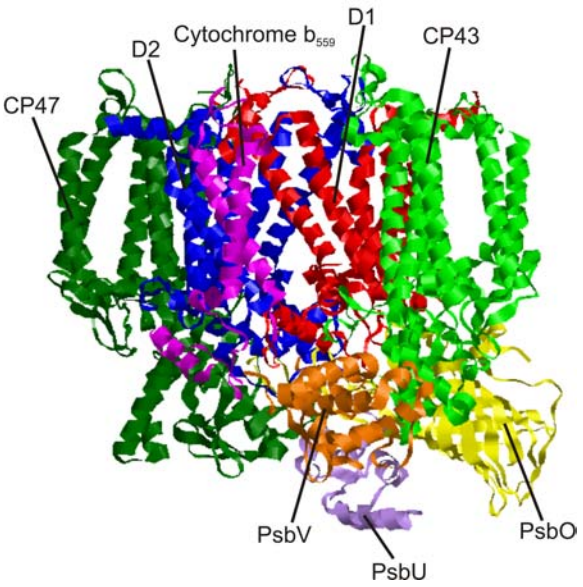

Figure 1. Structural arrangement of the main PSII and the OEC proteins. The figure was generated by using the program RasTop 2.2 (http://www.geneinfinity.org/rastop/) and the PDB file 3BZ1 (7). Side view (stromal surface above). Red, D1; blue, D2; bright-green, CP43; green, CP47; pink, Cytochrome b_{559}; yellow, PsbO; lavender, PsbU; orange, PsbV.

(PBS), distinct light-harvesting antenna complexes, which are attached to the cytoplasmic side of the PSII complex.

The oxygen-evolving complex (OEC) of the PSII reaction center (RC) is the most light- and temperature-sensitive component of the photosynthetic machinery. PSI is a complex fairly resistant to temperature stresses, although its sensitivity to low-temperature photoinhibition has been demonstrated earlier (1,2). The stress resistance of the cytochrome b_6f complex has not been investigated thoroughly, yet. Disruption of the manganese (Mn) cluster in the OEC is considered to be the first event in the heat-induced inactivation of PSII (3-5).

The PSII core complex is composed of protein subunits, pigments and lipids, most of which are evolutionarily conserved among cyanobacteria and plants. The main membrane intrinsic protein subunits of the PSII complexes are the D1 and the D2 proteins, the CP47 and the CP43 inner antenna proteins, the heterodimeric PsbE and PsbF of cytochrome b_{559} and a number of low-molecular-weight proteins of still unknown functions. In cyanobacterial PSII, three membrane extrinsic proteins related to the OEC - PsbO, PsbU and PsbV (cytochrome c_{550}) - are associated at the lumenal side of the core assembly (Figure 1). A wide variety of PSII complexes are found in monomeric and/or dimeric forms (6). A crystal structure is suggested for the dimeric PSII core complex in thermophilic cyanobacteria (7,8). In *Synechocystis* sp. PCC6803 (hereafter referred to as *Synechocystis*), the dimeric structure of PSII is supported by specific lipids and carotenoids (Cars) present in the PSII core complex (9,10).

The roles of lipids and Cars in the photosynthetic apparatus were investigated using various eukaryotic and prokaryotic mutants. *Arabidopsis thaliana* (hereafter referred to as *Arabidopsis*) and *Chlamydomonas reinhardtii* (hereafter referred to as *Chlamydomonas*) mutants were the most frequently used eukaryotic mutants, while *Synechocystis* and *Synechococcus* sp. PCC7942 (hereafter referred to as *Synechococcus*) were the cyanobacterial favorites.

In the cyanobacterium *Synechocystis de novo* synthesis of fatty acids is required for the stabilization of PSII against high temperature and for thermotolerance (11). The biochemical reaction of lipid desaturation has been demonstrated to be a key mechanism in providing resistance to both thermal and light stresses. In cyanobacteria, the unsaturation of lipids is determined directly by the growth temperature. The unsaturation level of lipids was manipulated in higher plants and cyanobacteria by inactivating acyl-lipid desaturases and, as a result, the sensitivity of photosynthesis to low-temperature photoinhibition was severely affected (12-15). A decrease in the lipid desaturation of photosynthetic organisms resulted in an elevated high-temperature destabilization of PSII activity (16).

Besides lipid-desaturation, the head group of membrane glycerolipids is also considered to be a determinative factor involved in the protection of PSII against thermal stresses. Negatively charged glycolipids, sulfoquinovosyl diacylglycerol (SQDG) and phosphatidylglycerol (PG), act in the stabilization of photosynthetic complexes (17-20). They are involved in forming functionally active complexes. The photosynthetic characteristics of wild-type and SQDG-deficient mutant cells were compared to determine the structural and functional roles of SQDG in the PSII complex. The determinative role of SQDG in the tolerance of PSII to high temperature and high irradiation has already been suggested (21). PG is a ubiquitous phospholipid in almost all organisms. The phosphate group in the PG molecule is negatively charged at neutral pH, thus, PG can be classified as an anionic phospholipid. In cyanobacteria and higher plants, which are able to carry out oxygenic photosynthesis, the majority of PGs is found in the thylakoid membranes, where the light reactions of photosynthesis are performed. Thus, it has been suggested that PG, the only phospholipid in photosynthetic membranes, is an indispensable component, and it plays a crucial role in several physiological processes such as photosynthesis and cell division (22).

The neutral lipids, the main components of photosynthetic membranes, are also important stabilizing factors of PSII. Monoglucosyldiacylglycerol, a precursor of monogalactosyldiacylglycerol (MGDG), is a heat shock lipid (23) which accumulates when the cyanobacterial cells are exposed to high temperatures. An unknown epimerase, which regulates the final step of MGDG formation, may be heat sensitive explaining the accumulation of the intermedier. Digalactosyldiacylglycerol (DGDG) is present in the thylakoid membranes of oxygenic photosynthetic

organisms, such as higher plants, algae and cyanobacteria. Earlier, the *dgdA* gene of *Synechocystis* has been identified, which encodes a DGDG synthase involved in the biosynthesis of DGDG (24). The *dgdA* mutant of *Synechocystis* displayed an increased sensitivity to high light exposure (25). The oxygen-evolving activity of PSII was significantly decreased in the mutant.

Cars are one of the most abundant pigments in nature, and more than 700 molecular species belong to this pigment family (26). Cars consist of isoprenoid blocks and can be defined as nonsaturated terpenoids containing 40 carbon atoms per molecule. In all the photosynthetic organisms, the first step in carotenogenesis is the condensation of two geranylgeranyl pyrophosphate molecules resulting in phytoene (27). Phytoene is then converted to various carotenes, which serve as substrates for the synthesis of a variety of xanthophylls. The important spectroscopic and structural properties of Cars are determined by the longer than 9 conjugated double bond system (C=C), which constructs a rigid, rod-like skeleton. This feature seems to play a key role in the stabilization of the lipid membrane structure with proteins (28). Cars can be divided into two classes: carotenes, which are purely hydrocarbons, and xanthophylls, which are oxygenated derivatives of carotenes. The delocalized electrons in the backbone of Cars make them capable of serving as chromophores and quenchers, and they can act as anti-oxidants.

The Car composition of higher plants is highly conserved. The PSII core complex binds beta-carotene, whereas the outer antennae, composed of light-harvesting complexes, contain lutein, neoxanthin, and violaxanthin under normal light conditions. Zeaxanthin is produced through de-epoxidation of violaxanthin under light stress conditions. Cars are bound to the light-harvesting complex of PSII (LHCII) (29,30).

Xanthophylls are involved in harvesting light energy and they can determine the structure of the protein architecture of PSII (31). They are also essential for quenching triplet chlorophyll (Chl) and reactive singlet oxygen. Zeaxanthin has a primary role in scavenging reactive singlet oxygen species produced by photosynthetic processes in higher plants (32) and in cyanobacteria (33). The phenomenon known as non photochemical quenching (NPQ) shields higher plants as well as cyanobacteria from light stress. In LHCII, lutein and zeaxanthin are responsible for NPQ. In contrast, 3`hydroxyechinenone and echinenone can activate the orange carotenoid protein (OCP), which initiates NPQ in cyanobacteria. In contrast to xanthophylls, beta-carotene is essentially needed for the PSII assembly (34). The localization of beta-carotene in the PSII core complex is optimized for quenching triplet-state Chls and reactive singlet oxygen. Beta-carotene may have a role as a "molecular wire" in a putative secondary electron transfer when the Mn_4Ca cluster is not functional or is absent (35).

In the present review, we summarize the newest results concerning the importance of lipids, especially that of PG and Cars, in constructing the PSII complex and their effect on functionality**.**

3. LIPID DRIVEN CONSTRUCTION OF THE PSII COMPLEX

3.1. *In vitro* studies

The first main supercomplex of the photosynthetic electron transport chain is PSII, which is a large multiprotein complex, and it catalyses water splitting and plastoquinone reduction necessary to transform sunlight into chemical energy. Oligomer structures that result from specific lipid-protein interactions in the thylakoid membranes are required for efficient energy transfer and for the stabilization of a charge-separated state of the RCs. The protein subunits, their binding and their relationship with each other were studied using *in vitro* systems in various photosynthetic organisms. *In vitro* studies of PG indicated that PG is crucial for both the trimerization (36,37) and the reconstitution (38) of LHCII complexes. Phospholipase A2 treatment of PSII complexes from spinach blocked the dimer formation of the PSII complex suggesting the involvement of PG in dimer formation (39). In addition, it has been demonstrated that the interaction of PG, as an anionic lipid, with the transit peptide of chloroplast precursor proteins is needed for the import of proteins into chloroplasts (40).

3.2. The structural role of the anionic lipids PG and SQDG in eukaryotes, higher plants and green algae.

In eukaryotic systems, PG molecules are essential components of the functional photosynthetic apparatus. Siegenthaler and coworkers (41,42) reported the localization and positional distribution of PG molecules in thylakoid membranes. An analysis of the transmembrane distribution of PG showed that the molar outside/inside distribution of PG was 58/42 in thylakoid membranes. *In vitro* experiments with isolated PSII complexes of spinach revealed an important role of PG in the dimer formation of PSII complexes (39). Using a PG mutant of *Chlamydomonas,* it has been demonstrated that green algal cells need PG for the dimerization of PSII complexes, similarly to higher plants (43). The results obtained with the *Chlamydomonas* mutant also highlighted the importance of PG molecular species containing delta-3-trans-hexadecenoic acid [16:1(3t)] in the formation of trimeric species of LHCII. Thus, it became possible to detect PG in trimers (29). In the absence of this PG species, the formation of monomeric rather than the dimeric PSII complexes was detected (44,45). This finding provides further evidence for the role of PG in the oligomerization of protein complexes.

A compensatory mechanism, which regulates the balance between the relative amounts of two anionic lipids, PG and SQDG, operates in the thylakoid membranes of both higher plants and *Chlamydomonas*. Structural and functional properties of PSII were compared between a mutant of *Chlamydomonas* defective in SQDG (*hf-2*) and the wild type to examine the role of SQDG in thylakoid membranes. The SQDG-deficient *Chlamydomonas* mutant strain showed a structural fragility of the PSII core

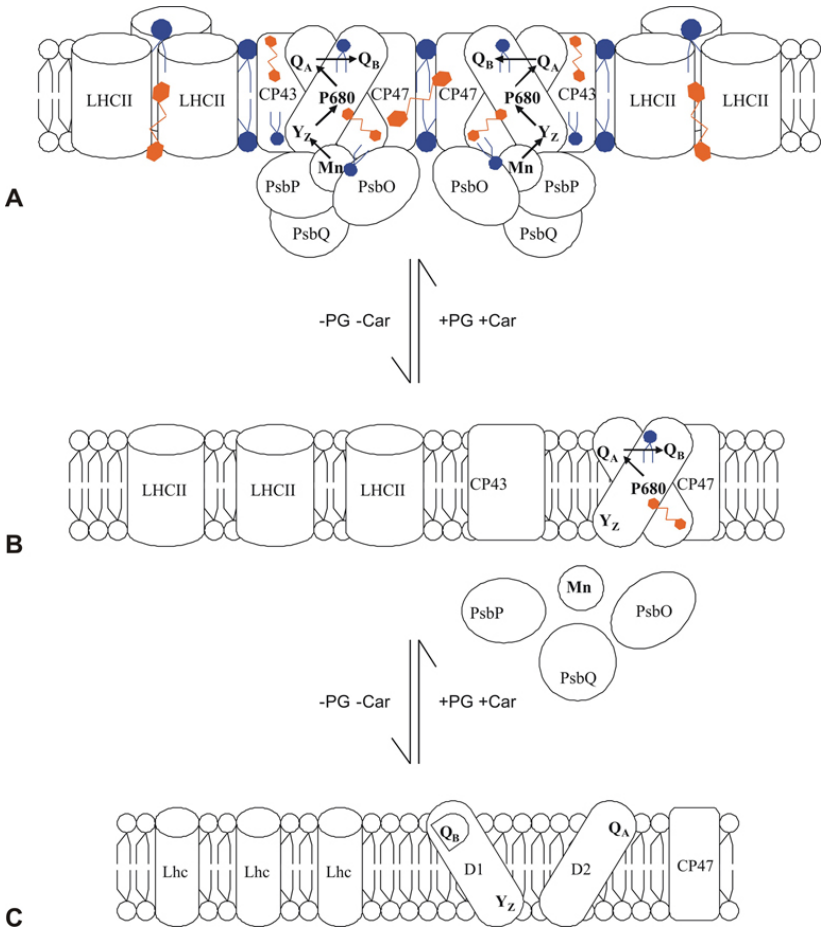

Figure 2. The involvement of PG and carotenoids in formation of dimeric PSII RC in higher plants and algae. A: Completely assembled dimeric PSII RC together with LHCII trimers. B: Disassembled PSII RC and LHCII in PG and Car depleted membranes. **C:** Complete disassembly of PSII RC and disassembeled protein subunits of LHCII in the absence of PG and carotenoid. PG and carotenoids are represented in blue and orange, respectively.

complex, which suggests that lipid depletion results in an altered stability of the PSII complex. Heat treatment significantly decreased PSII activity (21).

Under phosphate-restricted growth conditions, the reduction of the level of PG was counterbalanced by a relative increase in the level of SQDG. To eliminate this compensation process, Benning and co-workers (46) generated a transgenic *Arabidopsis* mutant (*sqd2 pgp1-1*) in which the *sqd2* gene encoding the SQDG synthase was disrupted by T-DNA insertion in the genome of the *pgp1-1* mutant. This transgenic plant contained a reduced amount of PG and no SQDG. Reduction of the overall anionic lipid content in the mutant resulted in a reduction of the level of Chl. The structure of the chloroplasts was severely affected and the number of mesophyll cells was remarkably reduced, as found in the *pgp1* knockout mutant (47,48). The chloroplasts of the mutant plants almost lacked granum stacks and the amount of thylakoid membranes was significantly reduced. On the basis of these findings, we conclude that anionic lipids, especially PG, play a determining role in the development of thylakoid membranes. PG and DGDG are glues for the protein machinery. The presence of PG in a trimer of the LHCII was detected by an X-ray study that allowed a resolution of 2.7 angstrom (29). A small substantial decrease in the maximum photochemical efficiency of PSII (*Fv/Fm*) was observed in the PG mutant of *Arabidopsis*, when compared to that of wild type (49). The partial depletion of lipids that are localized in the vicinity of PSII RCs could result in both the monomerization of PSII dimers and the detachment of peripheric protein subunits from the membranes. It can lead to complete inhibition of the oxygen-evolving activity (Figure 2A.B.). Further PG elimination could lead to the degradation of peripheric protein subunits (Figure 2C.).

3.3. Structural importance of glycerolipids in the architecture of PSII in cyanobacteria

X-ray crystallography of PSII complexes from the cyanobacteria *Thermosynechococcus elongatus* and *Thermosynechococcus vulcanus* revealed the presence of five core transmembrane alfa-helices in the D1 and D2 proteins which together comprise the heterodimer in the PSII RC (50,51). The antenna proteins, CP43 and CP47,

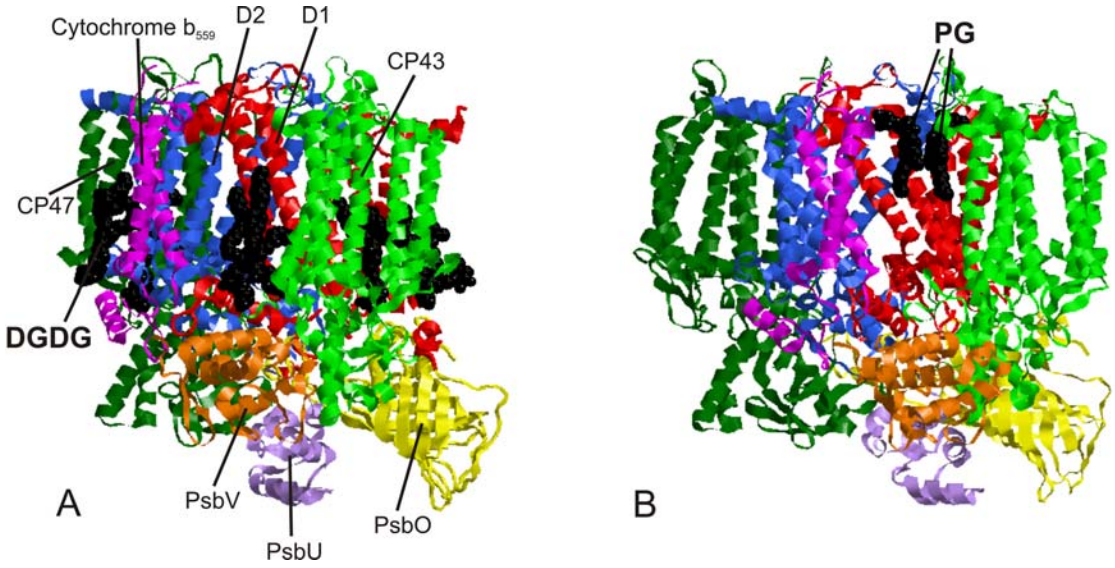

Figure 3. The localization of structurally important lipids in the PSII complex.The figure showing the positions of DGDG (A) and PG (B) were generated with the same method and use the same color coding as Figure 1. Lipids are shown in black. Side view (stromal surface above).

contain six alfa-helices. Earlier crystallographic studies of PSII complexes went as high as 3.5 angstrom, but this was still not high enough to identify lipid molecules in the PSII complex (52). The resolving power of recent crystallographic methods enabled a closer look into the detailed structure of PSII RCs (7,8). The crystal structure of PSII in *Thermosynechococcus elongatus* (*T. elongatus*) at 3.0 angstrom resolution allowed the assignment of 14 integral lipids within the protein scaffold, all of them located at the interfaces of various protein subunits. This result points to the structural and functional importance of these molecules in both the flexibility and the assembly of PSII. The X-ray crystallographic structure provides information about the Mn4Ca cluster, where oxidation of water takes place. One molecule of PG was found in the vicinity of the D1 protein, close to CP43. This PG molecule may play an important role in the energy transfer from Q_A to Q_B as has been demonstrated earlier (53). The RC subunits, D1 and D2, are encircled by a belt of 11 lipids providing a flexible environment for the exchange of D1 and protein trafficking. Three lipids are located in the dimerization interface; they mediate interactions between the PSII monomers. Several lipids are located close to the binding pocket of the mobile plastoquinone, forming a part of a postulated diffusion pathway for plastoquinone. Further two lipids were found, each ligating an antenna Chl *a*. A detailed analysis of lipid-protein and lipid-cofactor interactions allows us to postulate some specific roles of lipid-protein interactions, which may be involved in photosynthetic processes. The crystal structure of the *T. elongatus* PSII dimer suggests that lipids are a new class of cofactors in photosynthetic complexes (7). Lipid molecules not only seal PSII RC in the membrane and couple it with motions of the bulk lipid environment, but the lipid-protein and lipid-cofactor interactions in PSII RC may influence the stability of the entire complex and modulate specifically the functions of protein subunits and cofactors. The prime function of lipids is related to the flexible nature of their acyl chains, which provide deformable interfaces for the adaptation of PSII RC to high-light treatment by the replacement of the photodamaged components of the RC or transition between monomeric and dimeric states. The flexible, lipophilic environment provided by fatty acid moieties allows for the diffusion of the plastoquinone between its binding site in PSII and the thylakoid membrane. The lipid head groups are more rigid and tightly bound to the protein matrix. As an integral part of PSII, they influence the redox potential of electron transport components. Recent studies demonstrated that lipids bound to the RCs assist in the binding of proteins involved in photosynthetic reactions. These protein assemblies provide the functional centers of photosynthesis.

3.3.1. The involvement of DGDG in the construction of cyanobacterial PSII

Recent X-ray crystallographic analyses of protein-cofactor supercomplexes in thylakoid membranes revealed that DGDG molecules are present in the PSII complex (seven molecules per monomer), suggesting that DGDG molecules play an important role in the folding and assembly of the subunits in PSII complexes (7) (Figure 3A). However, the specific role of DGDG in PSII has not been fully clarified yet. To study the functional and structural role of DGDG, a DGDG-less mutant of *Synechocystis* was constructed. The *dgdA* gene, which presumably encodes a DGDG synthase involved in the biosynthesis of DGDG, was inactivated. Despite the lack of DGDG, the growth rate of the mutant cells was similar to that of wild-type cells, indicating that DGDG is not essential for growth in *Synechocystis*. However, the oxygen-evolving activity of PSII was significantly decreased in the mutant. Analyses of the PSII complex

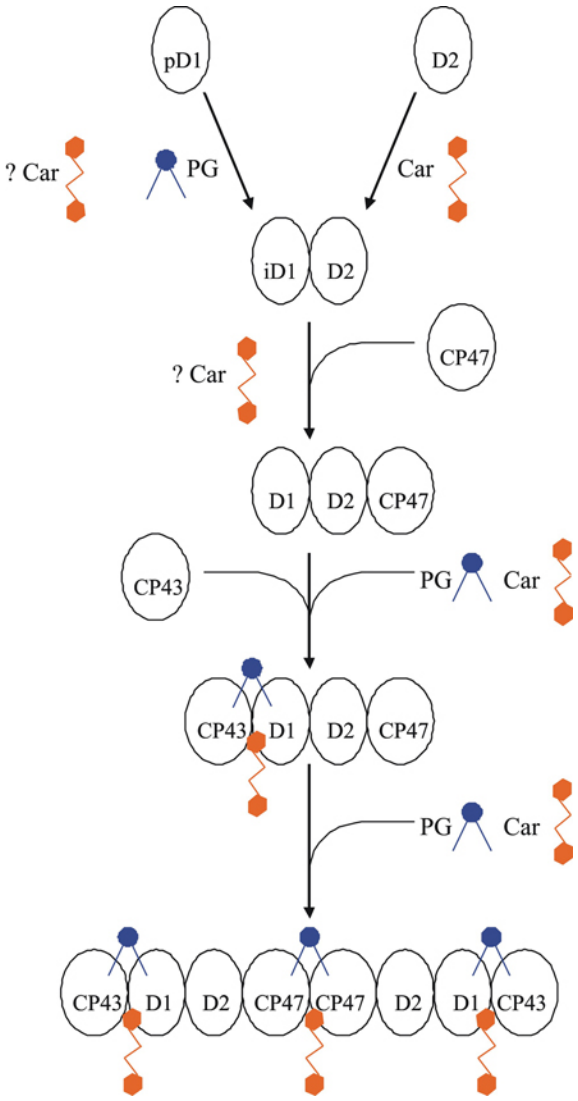

Figure 4. PG and carotenoid assisted assembly of PSII. pD1, D1 precursor: iD1, unprocessed, incomplete D1; D1 and D2, two core proteins; CP43 and CP47, intrinsic chlorophyll-protein complexes; PG, phosphatidylglycerol; Car, carotenoid; ?Car, potential involvement of carotenoid molecules. The PG and carotenoids are shown in blue and orange, respectively.

purified from the mutant cells indicated that the extrinsic proteins PsbU, PsbV, and PsbO, which stabilize the OEC, were dissociated from the PSII complex. This finding suggests that DGDG plays an important role in PSII: it binds extrinsic proteins required for the stabilization of the OEC (54). Nevertheless, a loss of DGDG did not lead to the appearance of lethal phenotypes, while a loss of PG proved to be lethal.

3.3.2. The role of PG in the assembly of photosynthetic complexes

Lipids in dimeric PSII complexes prepared from two species of cyanobacteria and two higher plants were analyzed. The content of PG in the PSII complexes of each organism was much higher than that in the thylakoid membranes. A specific binding of PG to the PSII complex was suggested based on the results obtained using antibodies against PG. It has been postulated that PG anchors the D1 protein to the PSII RCs (55). The determining role of PG in the dimer formation of PSII complexes was supported by results obtained with a mutant of *Synechocystis* defective in PG biosynthesis (20). In this mutant, a *pgsA* gene, encoding a PGP synthase involved in the biosynthesis of PG, was disrupted to manipulate the content of PG in the mutant cells (56). The mutant could only divide in a medium containing PG, and the content of PG in the thylakoid membranes decreased when the cells grown in the presence of PG were transferred to a medium without PG (22). According to Sakurai *et al.* (57), PG plays an important role in the electron transport at the donor side of PSII, i.e., the oxygen-evolving system. Analyses of purified PSII complexes indicated that PSII from PG-depleted *Synechocystis pgsA* mutant cells sustained only approximately 50% of the oxygen-evolving activity of wild-type cells. Dissociation of the extrinsic proteins PsbO, PsbV, and PsbU, which are required for the stabilization of Mn clusters after the release of a Mn atom, was observed in the PSIIs of PG-depleted mutant cells. The released PsbO reassociated with PSII when PG was re-added to the PG-depleted mutant cells, even when *de novo* protein synthesis was inhibited.

The PBS-less mutant was mutated by inactivating the *cdsA* gene, which encodes an enzyme involved in PG biosynthesis. This mutation resulted in the decomposition of PSII dimers into monomers. One of the intrinsic Chl complexes, protein CP43, was detached from a major part of the PSII core complex. The detachment resulted in the formation of RC47 which is an RC possessing only CP47. This result indicates that protein CP43 is glued to the RC core complex by PG, further emphasizing its role in the assembly of PSII (Figure 4). The above results suggest that PG plays an important role in binding extrinsic proteins required to sustain a functional Mn cluster on the donor side of PSII. The binding of PG to proteins is not strong enough to resist detergent treatment. This may also be the reason why the presence of PG in PSII could hardly be detected by X-ray crystallographic methods. Cooperative binding could explain the character of PG in protein interactions. PG cements together the protein subunits that provide the structural backbone of the photosynthetic apparatus and this intimate PG-protein interaction may explain the effect of PG on the photosynthetic electron transport. Functionally, both the electron transport in PSII around the primary and secondary quinones and PSI activity were affected by PG depletion (17,53). PG depletion of the cells resulted in elevated light susceptibility, as signaled by the bleaching of cellular pigments (20). The results obtained with the *Synechocystis pgsA* mutant confirmed that PG depletion has a deleterious effect on the structure of PSII complexes. Owing to the *in vivo* loss of PG molecules from the PSII complex, the dimeric structure of the complex was gradually transformed into a monomeric one. The cells that developed following PG depletion contained an elevated level of the monomer/dimer ratio of the PSII complex as compared to

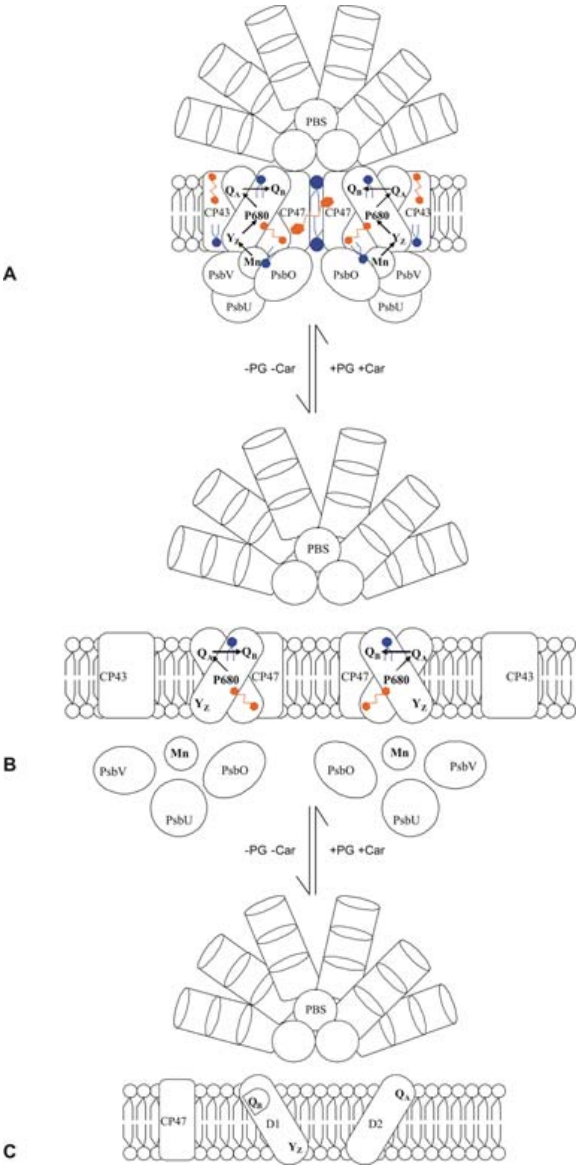

Figure 5. PG- and carotenoid-dependent formation of dimeric PSII RC of cyanobacteria. A: Completely assembled dimeric PSII RC together with phycobilisome (PBS). B: Disassembled dimeric PSII RC with detached PBS and detached manganese (Mn) complex in PG and carotenoid depleted cells. C: The complete disassembly of PSII RC with detached PBS and without manganese (Mn) complex in the absence of PG and Car. PG and carotenoids are represented in blue and orange, respectively.

that of the cells grown in the presence of PG. This *in vivo* result underlines the importance of PG in the oligomerization of PSII complexes. In addition, the unbalanced monomer to dimer ratio resulted in an enhanced light susceptibility of the cells. PG depletion led to a complete bleaching of cell pigments after exposure to high light. Monomerization of the PSII complex may be associated with an increased susceptibility to high light and with the inactivation of PSII in the cells. Since the photoinhibitory processes can be initiated by the monomerization of the PSII complex (58,59), we suggest that the cells need PG-dependent dimer formation of the PSII complex for the recovery of PSII activity. The PG-supported dimerization is presented in Figure 5A. The partial depletion of PG resulted in the monomerization of PSII, together with a partial detachment of peripheric protein subunits that anchor the Mn complex to the thylakoid membranes (Figure 5B). The complete elimination of PG from the photosynthetic membranes initiated the degradation of OEC-related protein subunits, together with the Mn complex. PG depletion induced the disappearance of CP43 from the membrane and a significant decrease in CP47 content (Figure 5C). Although PG depletion resulted in the detachment of PBS from the thylakoid membranes, it did not cause the degradation of phycobiliproteins, which became non-functional following the inactivation of PSII but remained intact and localized in the cytosol.

A recent publication described the assignment of eleven new integral lipids emphasizing the importance of lipids in the perfect functioning of PSII. Moreover, a third plastoquinone molecule (Q_C) and a second quinone transfer channel have been revealed, making it possible to consider different mechanisms for the exchange of plastoquinone/plastoquinol molecules. In addition, possible transport channels for water, dioxygen and protons have been identified (60).

We have to raise the important question of how PG participates in the assembly of the functional units of the electron transport chain. Recent studies on the structural and functional role of PG revealed that this lipid species plays a crucial role in the assembly of both PSI and PSII RCs (17,20,57). Using X-ray crystallography, PG molecules were found to be localized within the RCs (8,61-63). PG molecules were found in the vicinity of functionally important sites of the PSII complex which suggests that they have determinative functions in the photosynthetic processes rather than in constructing the architecture (Figure 3B.).

4. HOW DO GLYCEROLIPIDS AFFECT PSII PROCESSES

4.1. Functioning proteins and glycerolipids are the bricks and mortar in constructing the PSII architecture

The protein content of the cyanobacterial PSII is similar to that of the eukaryotic PSII supercomplex, although there are some substantial differences. Besides D1 and D2, the PSII reaction complex contains the alfa and beta subunits of a membrane bound cytochrome b_{559}, small peptides, which are involved in the OEC formation and two core antenna complexes known as CP43 and CP47 (64-67). There are also some other small protein subunits which support the construction of the PSII architecture. Light-harvesting-complex trimers of LHCIIs bind to the PSII in higher plants. Instead of LHCII, cyanobacteria contain PBSs, which are peripheral antenna complexes. The proteins that form the OECs are significantly different in different species. The PsbO protein plays an important role

in maintaining the stability and activity of the Mn cluster of the OEC in oxygenic photosynthetic organisms (68). Besides PsbO, PsbV and PsbU also help to construct the functional OEC in cyanobacteria, while in higher plants, PsbP and PsbQ have the above function. In the last few years, high resolution X-ray crystallography of PSII provided detailed information about its structure (7,8,51,60).

Based on its function, the PSII complex is also called "water-plastoquinone oxidoreductase". Excitation of the reaction center Chl initiates the primary charge separation with the formation of oxidized Chl ($P680^+$) and the reduction of pheophytin ($Pheo^-$). $P680^+$ successively extracts four electrons from two water molecules through the manganese cluster and tyrosine Y_Z, and, during this process, one molecule of oxygen and four protons are released into the lumen side (69,70). The electron on $Pheo^-$ is rapidly transferred to a tightly bound quinone (Q_A), which, in turn, passes this electron to a loosely bound plastoquinone (Q_B). For every pair of water molecule two fully reduced and protonated plastoquinols are formed. When Q_B has acquired two electrons from Q_A in two successive photoacts and extracted two protons from the stroma (or the cytoplasm), it is replaced by a plastoquinone at the binding site. Plastoquinol transfers electrons to the cytochrome b_6f complex (69,71,72).

Since PSII is embedded in the thylakoid membrane, it is in interaction with the neighboring lipid molecules. Lipids have been identified in the PSII crystal structure indicating that lipid molecules may influence and may even have a role in the structural organization and function of PSII. Thylakoid membranes contain three types of glycolipids: MGDG, DGDG and the negatively charged SQDG, as well as PG as the only phospholipid constituent. The fatty-acid composition of these membrane lipids may affect membrane associated processes. PSII is the main target of various environmental stresses like high light and low temperature. To maintain the photosynthetic processes under such stress conditions, the exchange of the D1 protein of the PSII is required. Fatty acid desaturation of the membrane lipids is a tool to regulate overall membrane fluidity, and it can also influence specific lipid-protein interactions, causing structural and functional alteration in the proteins. Studies on genetically modified *Arabidopsis* and desaturation mutants of cyanobacteria demonstrated the crucial role of membrane lipid unsaturation in the D1 protein exchange mechanism to maintain the function of PSII (11,73-76). Besides their general influence on membrane fluidity and desaturation level, the various lipid molecules may also play distinct roles in the photosynthetic processes. The identification of lipids at specific sites within photosynthetic complexes by X-ray crystallography implies their involvement in photosynthetic processes. Specific lipid-protein interactions have been described using biochemical methods and by genetic manipulation in cyanobacteria, green algae and higher plants.

4.2. The functional role of lipid molecules in the PSII of plants and algae

4.2.1. The involvement of MGDG in photosynthesis-related processes

A number of *in vitro* and also *in vivo* studies have been performed to reveal the role of lipid molecules in photosynthetic processes. The role of MGDG molecules was investigated in reconstituted liposomes containing thylakoid lipids, with or without MGDG, and photosynthetic membrane proteins, LHCII and PSII core complex (77). An increase in the MGDG content increased the oxygen evolution, which was more pronounced in proteoliposomes containing LHCII and PSII than in those containing only the PSII core complex. MGDG seems to act through the stimulation of PSII activity by energy transfer from LHCII. We have to take into account that these results refer to the loosely attached MGDG molecules since MGDG molecules were not removed from the PSII RCs during their preparation. However, they demonstrate that MGDG molecules have a role in the energy transfer between LHCIIs and PSII RCs. Lipid molecules may also be involved in the function of LHCII. MGDG has been suggested to support the ability of LHCII macro-aggregates to undergo light-induced structural changes (78). The conformational changes of the PSII light-harvesting system, among others, are controlled by the conversion of the light-harvesting pigment, violaxanthin into antheraxanthin and zeaxanthin in the so-called xantophyll or violaxanthin cycle (79). Recent *in vitro* enzyme assays on isolated LHCII complexes revealed that MGDG, which is located in the vicinity of the LHCII, strongly enhances the de-epoxidation of LHCII-associated violaxanthin (80). Characterization of an *Arabidopsis* mutant with a T-DNA insertion in the MGDG synthase-encoding *mgd1* gene demonstrated that the decrease of the MGDG content causes severe defects in the chloroplast structure (81). The abundance of the MGDG lipid in *mgd1* mutant leaves was reduced by 42% compared to the wild type. The *mgd1* mutant contained a decreased amount of Chl and was less green than the wild type. The inefficient operation of the xanthophyll cycle resulted in an impaired capacity for thermal dissipation of excess light in the *mgd1* mutant leaves (82).

4.2.2. The role of DGDG in photosynthetic processes

The role of DGDG in photosynthetic processes was studied in the galactolipid deficient *dgd1* mutant of *Arabidopsis.* This mutant contains only 10% of the DGDG content of wild-type (83,84). It is not surprising that the mutant is not completely deficient of DGDG since it has been reported that *Arabidopsis*, as well as other plant species, contain another gene besides *dgd1*, *dgd2,* which encodes an enzyme with DGDG synthase activity (85). However, the expression level of *dgd2* is very low in plants grown under normal conditions and the mutation in *dgd1* leads to a 90% reduction of DGDG in the chloroplast, making the *dgd1* mutant a good subject for photosynthetic studies. The mutant displays a stunted growth, a pale green leaf color and the overall chloroplast ultrastructure in the leaves shows dramatic alterations. The abundance of specific pigment-protein complexes, which are in the vicinity of the PSII core, is reduced by the decreased

DGDG content. This observation suggests that DGDG is required for the stabilization of these complexes (84). The *dgd1* mutant exhibits a slight enhancement in sensitivity to high-temperature stress, supposedly because of the general effect of DGDG deficiency on the structure of the PSII complex (86). The oxygen-evolving activity of the *dgd1* mutant is not altered significantly (84) compared to that of the wild type. Further characterization of mutant plants suggested that the decrease in DGDG content mainly affects the donor side of the PSII through the modification of the properties of the water-oxidizing complex, resulting in the modification of $P680^+$ reduction by Y_Z (86-88). Glycosyl-galactosyldiacylglycerol (GlcGDG) was produced in the *dgd1* mutant by introducing a bacterial glucosetransferase from *Chlorofelexus auranticus* (89). GlcGDG complemented the growth defect of the *dgd1* mutant; however, the degree of LHCII trimerization and the photosynthetic efficiency were only partially restored. A double mutant *dgd1dgd2* contained only a trace amount of DGDG (90). The photosynthetic efficiency and the growth of the double mutant plants were affected more severely than those of the *dgd1* mutant. GlcGDG accumulation complemented plant growth in the double mutant as well, but the photosynthetic efficiency was decreased (91), implying that the presence of galactose is crucial for maintaining maximal photosynthetic efficiency.

Characterization of MGDG-deficient and DGDG-deficient mutants indicated that MGDG and DGDG do not simply provide a membrane environment for the physical support of the photosynthetic complexes, but they also take part in various photosynthesis-related processes with an individual contribution (87,92).

4.2.3. The role of SQDG in the photosynthetic function of PSII

The *Chlamydomonas* mutant *hf-2,* defective in the synthesis of SQDG, exhibits a reduced PSII activity (93). Incubation of the thylakoid membrane of *hf-2* with SQDG significantly raised the PSII activity (94) suggesting that SQDG contributes to the activity of the PSII RC by associating with PSII core and LHCII complexes. SQDG contributes to maintaining the conformation of PSII complexes, mainly by stabilizing D1 polypeptides and influencing the electron donation Mn cluster to the tyrosine Y_Z radical (95). In the absence of SQDG in *hf-2* cells, repression of recovery of PSII from heat-induced damage occurred, probably as a result of a conformational disorder of the PSII complex or a dysfunction of the repair mechanism (21). The *sqd2* gene, which encodes an enzyme that catalyzes the transfer of sulfoquinovose moiety from UDP-sulfoquinovose to diacylglycerol, was inactivated by T-DNA insertion in *Arabidopsis* (96). The mutation led to a complete lack of SQDG. Interestingly, the *sqd2* mutant did not show any obvious signs of altered morphology, growth or photosynthetic parameters under normal conditions. However, the mutant displayed reduced growth under phosphate-limited growth conditions. It was proposed that SQDG and PG, the two anionic lipids of the thylakoid membrane, can complement each other under certain conditions to keep a constant anionic lipid content, since the SQDG and PG contents showed inverse correlation during nutrient limitation (96-98). Studies on an SQDG and PG-deficient *sqd2 pgp1-1* double mutant of *Arabidopsis* also support this hypothesis (46). Besides a T-DNA insertion in the structural gene of SQDG synthase, this mutant carries a point mutation in the structural gene of PG-phosphate synthase, thus, it does not contain SQDG and the PG content is reduced by 30%. In the mutant, the fraction of total anionic lipids is reduced by approximately one-third. The double mutant was not able to assemble thylakoid membranes at a rate required for normal growth. The electron transport rate through PSII, especially when a herbicide, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was added, was reduced as compared to the single mutant or the wild type. DCMU fits into the Q_B-binding site of the D1 protein and blocks the electron transfer between Q_A and Q_B,, since it has a higher affinity to the Q_B-site than plastoquinone itself. It was suggested that the increased sensitivity to DCMU is a consequence of the reduction of anionic lipids in general. At the same time, characterization of the single mutants indicates that SQDG and PG may have specific and distinct functions in photosynthesis.

4.2.4. PG depletion affects photosynthetic activities

The *Chlamydomonas mf*1 and *mf*2 mutants have a reduced PG content with a total loss of its delta-3-trans hexadecenoic acid-containing form (45). It has been demonstrated that delta-3-trans hexadecenoic acid-containing PG has a role in the formation of the trimeric LHCII (99). The mutants lose their PSII activity as a result of a severely decreased synthesis of two subunits, D1 and apoCP47, which is not due to a decrease in translation initiation (100). Only trace amounts of PSII core monomers were detected, which were unable to oligomerize in the PSII-LHCII supercomplex.

Phospholipase C treatment of pea thylakoids, which removes the head group of phospholipids and eliminates around 50% of the phospholipids, drastically decreased photosynthetic electron transport (101). To study the role of PG in the electron transfer and the structural organization of PSII, *Arabidopsis* thylakoids were also treated with phospholipase A2 (PLA2) to decrease the PG content (102). The PLA2 treatment exerted its effect around the Q_B site; it inhibited electron transfer from the primary quinone acceptor Q_A to the secondary quinone acceptor Q_B of PSII. The electron flow was also retarded in the donation of electron from the Mn cluster to oxidized tyrosine Y_Z. The removal of PG by the PLA2 treatment of thylakoids led to the partial dissociation of PSII supercomplexes into PSII dimers that do not possess the complete LHCII antenna and even to the dissociation of PSII dimers and LHCII trimers to monomers. The importance of PG in the resistance to photoinhibition, mainly at lower temperatures, was also demonstrated on isolated functionally active PSII monomers and dimers (103).

Since in many cases a significant amount of the investigated lipid molecules remained in the thylakoid membrane following the enzymatic treatment or genetic modification of the plant, the role of these molecules in the

photosynthetic processes could not be totally revealed. In plants, we also have to take into account the traffic between organelles. Cyanobacteria, the ancestor of chloroplasts could be the ideal model organisms to study photosynthetic processes.

4.3. The functional role of lipid molecules in the PSII of cyanobacteria

4.3.1. The role of DGDG in photosynthetic processes

The role of lipid molecules in cyanobacteria has been studied using either biochemical or molecular genetic approaches. Mutants of lipid metabolism pathways are useful tools to investigate *in vivo* the specific roles of lipids in photosynthesis. The DGDG synthase-encoding *dgsA* gene was identified in *Synechocystis*. A mutant defective in DGDG synthesis was constructed by the disruption of the *dgsA* gene (54). DGDG is not essential for *Synechocystis* since the growth of the mutant cells was almost the same as that of the wild type. However, the efficiency of energy transfer from the PBSs to the RC of PSII and in PSII decreased, and the OEC was found to be instable in the mutant cells. Heat susceptibility, but not dark-induced inactivation of the oxygen-evolving activity, was remarkably increased in the mutant cells (54). The impairment and instability of PSII was caused by the dissociation of extrinsic proteins required for the stabilization of the Mn cluster of the OEC in mutant cells. DGDG is required for the PsbU subunit binding and, thus, the stabilization of the PsbO and PsbV subunits. DGDG may play a role in growth under high-temperature stress by the stabilizing the Mn cluster (24). DGDG might also play an important role on the acceptor side of PSII, since it seems that the physical properties in the quinone-exchange cavity of PSII are changed by the lack of DGDG. The *dgdA* mutant displayed an increased sensitivity to photoinhibition (25). The repair and photodamage processes of the photosynthetic machinery were affected, suggesting that DGDG plays an important role in the photosynthetic repair cycle.

4.3.2. The involvement of SQDG in photosynthesis

Mutant strains of *Synechocystis* and *Synechococcus* defective in SQDG synthesis were constructed by the inactivation of putative *sqdB* genes (98,104). SqdB catalyzes the formation of UDP-sulfoquinovose. It has been demonstrated that *Synechocystis* cells require SQDG for growth. SQDG-deficient mutant cells can be maintained by providing external SQDG supply, since cyanobacterial cells are able to take up SQDG from the culture medium. With the reduction of the SQDG content, the *Synechocystis sqdB* SD1 mutant cells exhibited a decreased PSII activity (104). In contrast, the *Synechococcus* SQDG-null mutant could grow without SQDG and it displayed a normal level of PSII activity with little effect on its sensitivity to PSII herbicides, atrazine and DCMU (104). Atrazine, like DCMU, associates with the Q_B-binding site of the D1 subunits of PSII and, therefore, blocks the electron flow from Q_A to Q_B. Since the sensitivity of PSII activity to DCMU and atrazine increased with the decrease of the SQDG content in *Synechocystis* SQDG-deficient mutant cells, SQDG may have a role in maintaining the normal conformation of the Q_B binding site of the D1 protein and the normal state of its lipid environment. The SQDG content was decreased with a simultaneous increase in the PG content in SD1, although the increased PG content could not recover the normal PSII activity. The PSII complex of *Synechocystis* was proposed to be more evolved than that of *Synechococcus*, because SQDG is not required for the functioning of the *Rhodobacter sphaeroides* photosystem, the prototype of the PSII complex, but it is responsible for the functioning of the PSII complex of the green alga, *Chlamydomonas* (104). Supposing it is true, the importance of SQDG in PSII activity might vary according to the organism and it might have changed during the evolution. This hypothesis and the molecular mechanism of the interaction between SQDG and PSII require further elucidation.

4.3.3. PG: an indispensable lipid component of photosynthetic complexes

Two enzymes required for PG biosynthesis and their encoding genes have been identified in *Synechocystis*. Cytidine 5'-diphosphate (CDP)-diacylglycerol synthase, which catalyzes the conversion of phosphatidic acid to CDP-diacylglycerol and PG phosphate synthase, which catalyzes the conversion of CDP-diacylglycerol to PG phosphate are encoded by the *cdsA* and *pgsA* genes, respectively. PG-deficient mutant *Synechocistys* strains can be constructed by inactivating one of these genes. Though PG was found to be an essential lipid, cyanobacterial cells are able to take up PG from the culture medium through the cell wall, and PG-deficient mutant cells can be maintained by supplying external PG. The effect of PG depletion and the role of PG in photosynthesis can be studied by PG free culturing of the mutant cells. The oxygen evolution of *cdsA* mutant cells, in the absence of PG, is slightly suppressed and the oxygen-evolution measurements in the presence of p-benzoquinone (*p*BQ) indicated a conformation change of the Q_B-binding site of the D1 protein (105). *p*BQ is used to measure the oxygen evolution rate of PSII (106,107). PG deficiency decreased the ability of PSII to use *p*BQ as an electron acceptor (105). In *pgsA* mutant cells, the PSII activity dramatically decreased with a concomitant decrease of the PG content (56). In the absence of PG, the light-susceptibility of the *pgsA* mutant cells increased and resulted in a severe photoinhibition of photosynthesis upon high-light treatment, mainly caused by the impairment of the restoration process (20). PG is needed for the dimerization of the PSII monomer complexes in the recovery process after photoinhibition (Figure 4). Further investigations demonstrated that inactivation of the PSII by PG depletion in the *pgsA* mutant cells was due to the suppression of the secondary electron acceptor plastoquinone Q_B reduction (53). Besides the specific inactivation of Q_B, the decrease in the PG content slowed down the electron transfer rate from Y_Z to photooxidized P680. Results suggested that PG plays a role in the stabilization of the donor side of PSII as well (57). Dissociation of PsbO, PsbV, and PsbU, the extrinsic proteins of the OEC, was observed in the PSII of the PG-depleted cells, as in the case of the DGDG-deficient mutant strain. When PG was added to the PG-depleted cells, the released PsbO rebound to PSII indicating that PG may play

an important role in the binding of extrinsic proteins, which is required to sustain a functional Mn cluster on the donor side of PSII.

For a more detailed investigation of the effect of PG on PSII function, the *cdsA* gene was inactivated in the PAL mutant of *Synechocytis* (108), in which the photosynthetic function of the cells with missing PBS is compensated by a high cellular content of PSII (9). The results obtained on PAL/delta-*cdsA* confirmed the specific effect of PG molecules on the acceptor side in the PSII electron transfer and on the dimerization of PSII. Additionally, it was shown that PG depletion affects the binding of the antenna CP43 to the peripheral region within the PSII RC (Figure 4). Since the donor side damage is not obvious in the PAL/delta-*cdsA* mutant, PG may affect the donor side of the PSII in a later stage of PG depletion, and this effect seems to be small and might be associated with the release of CP43, as this antenna Chl protein is directly involved in liganding the Mn atom.

As SQDG, the other anionic lipid, displayed differed importance in *Synechocystis* and *Synechococcus,* it was intriguing to investigate the role of PG in *Synechococcus* as well. The *cdsA* gene was inactivated in *Synechococcus* (109). Mutant cells required a continuous supply of PG for growth, indicating that PG is essential for the cells. Suppression of photosynthetic activity was even more extensive in *Synechococcus* delta-*cdsA* than in the PG-deficient *pgsA Synechocystis* mutant cells during PG depletion. Since not even the increase in SQDG content on the expense of PG could restore photosynthetic activities in *Synechococcus* mutant cells, it was concluded that PG is indispensable for this organism as well. These results also indicated that maintaining a constant sum of negatively charged lipids is not enough to maintain the active electron transport processes. In *Synechococcus* delta-*cdsA* cells, the PSII dimers decomposed to monomers and ^{35}S methionine-labeling showed that PG depletion did not block the *de novo* synthesis of PSII proteins, but it slowed down the assembly of the newly synthesized D1 protein into the PSII core complex. The relatively rapid decrease in PSII oxygen-evolving activity during PG depletion was related to perturbation on the acceptor side of PSII, while its donor side was not primarily inactivated. In contrast to *Synechocystis,* perturbation of the PSII acceptor side in *Synechococcus* is not caused by the PG-deficiency-induced inhibition of the electron transport between Q_A and Q_B, but seems to be due to a decreased equilibrium constant for sharing the electron between the $Q_A^-Q_B$ and $Q_AQ_B^-$ states.

In order to study the role of MGDG and PG, the PSII core complex dimer from *Thermosynechococcus vulcanus* was treated with lipase and PLA2, and their effect on PSII structure and function were examined (110). PLA2 treatment, which decreases the PG content, decreased the oxygen-evolving activity more severely than the lipase treatment, which specifically decreases the MGDG content. This indicates that PG plays a more important role than MGDG in PSII. The degradation of PG resulted in the inhibition of the electron transfer from Q_A to Q_B. However, neither of the two enzymatic treatments induced PSII dimer dissociation or any loss of polypeptides. The degradation of PG specifically damaged the Q_B-binding pocket, leading to a decreased affinity of Q_B and, therefore, to an impaired electron transfer from Q_A to Q_B. The specific degradation of MGDG caused a slight decrease in oxygen evolution which could not be ascribed to damage either in the oxygen-evolving site or in the acceptor side of the PSII. However, we have to take into consideration that a few molecules of PG and MGDG molecules do remain in the PSII following the enzymatic treatments, thus, the role of these molecules still awaits to be explored.

The lack of lipid molecules drastically affected the photosynthetic processes in several cases indicating their crucial role in these reactions. Alteration of the photosynthesis may have marginal consequences on other cell functions. Like in the case of PG depletion in *Synechocystis pgsA* mutant cells, the repression of the photosynthetic processes seems to lead to the generation of reactive oxygen species. As a consequence, to protect PG-depleted cells from light-induced damage, the echinenone and myxoxanthophyll content of the cells increase suggesting that PG depletion regulates the biosynthetic pathway of specific Cars (111). Cars mostly play a photoprotective role in photosynthetic organism but at the same time, like lipid molecules, they can also be part of the structure and function of photosynthetic complexes.

5. THE STRUCTURAL INVOLVEMENT OF CAROTENOIDS IN THE PSII COMPLEX

Cars are integral and essential components of the photosynthetic membranes, and their partial or complete elimination by molecular biological methods provides a tool for the modification of the structure and function of the PSII. Cars participate in light harvesting and serve as protective pigments against high-light stress. Several investigations have been performed to reveal how these molecules carry out their diverse functions. Detailed X-ray analyses of the structure of photosynthetic complexes indicated that Cars are involved in constructing the architecture.

Early studies of the structure of the PSII core complex isolated from spinach utilized electron microscopy and revealed the relative positions of the main Chl binding protein subunits (65,67,112-116). The accurate localization of Cars in cyanobacterial PSII was carried out by X-ray crystallographic measurements (7,8,52). Chl *a* and beta-carotene are the only pigments in the PSII core complex. Preferentially, these are bound to CP43 and CP47, the inner antenna Chl-protein complexes. The latest PSII structural model provides a complete modeling of 35 Chl *a* and 12 beta-carotene molecules (7). On the basis of X-ray studies, the localization of Cars was demonstrated in the cyanobacterial PSII architecture (Figure 6). LHCII, the PSII outer antenna in plants, is a large, strictly organized pigment-protein complex in the chloroplast membrane. LHCII is organized in heterotrimers and is composed of the Lhcb1, Lhcb2 and Lhcb3 polypeptides. The minor complexes, CP24, CP26, and CP29 are present as monomers. In the high-resolution crystal structure of

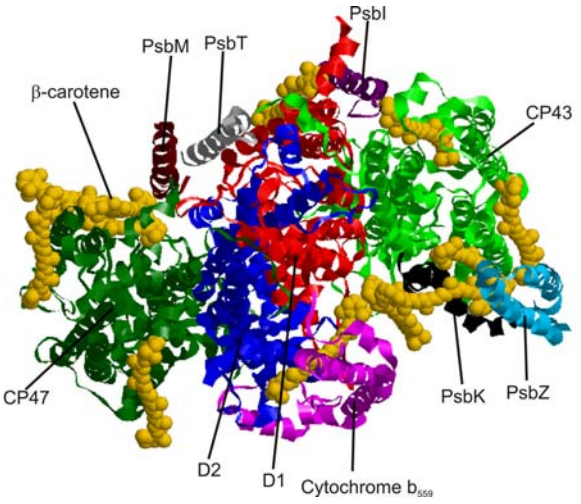

Figure 6. Carotenoid positions in the PSII complex. The figure was generated with the same method and use the same color coding as Figure 1. Violet, PsbI; dark red, PsbM; light gray, PsbT; black, PsbK; sky blue, PsbZ; Stromal side view.

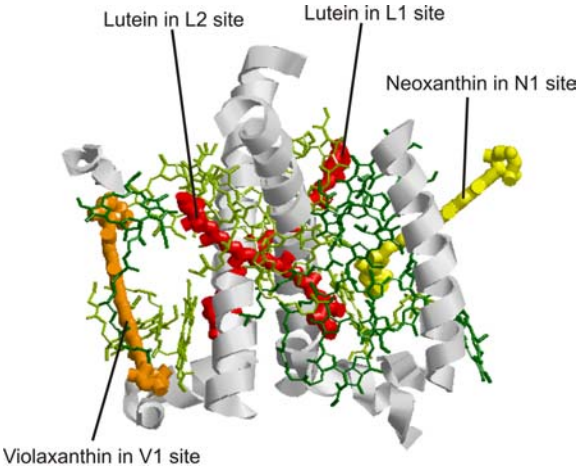

Figure 7. Xanthophyll binding sites in the monomeric LHCII apoprotein.Light gray, helices; red, lutein; yellow, neoxanthin; light orange, violaxanthin; green, chlorophyll *b*; lime, chlorophyll *a*. The figure was created with the software RasTop 2.2 (http://www.geneinfinity.org/rastop/) and the PDB file 2bhw (30).

spinach and pea LHCII complexes, 42 Chl *a* and *b* and 12 Car molecules were identified. Two luteins with all-*trans* configuration, one 9'-*cis* neoxanthin and one xanthophyll-cycle Car were found in each LHCII monomer. The luteins form a "cross-brace" with two central transmembrane alfa-helices. Their specific binding sites are termed L1 and L2. Site N1 is located near the transmembrane c-helix and is highly specific for the more polar neoxanthin. V1, the less stable and peripherally located site contains the xanthophyll-cycle Cars (29,30,117) (Figure 7).

5.1. The structural importance of carotenoids in the PSII of higher plants and algae

Earlier experiments demonstrated that Cars are essential for the assembly of a functional PSII in eukaryotic algae and higher plants. Norflurazon, a herbicide, can inhibit the synthesis of colored Cars (118). Using this herbicide to induce Car-deficiency, high-light treatment resulted in a block of the turnover of the D1 protein and of the reassembly of functional PSII in *Chlamydomonas* (119). Markgraf and Oelmüller also used norflurazon to reduce the content of Cars in mustard seedlings, and, as a consequence, no PSII complex was assembled (120). It has been shown in a Car-less *Chlamydomonas* mutant that Car deficiency decreases the accumulation of PSII, but Chl and protein biosynthesis is essentially unaffected. However, the Chl-protein complexes become unstable, which increases the degradation of the Chl and its apoproteins (121).

The simultaneous lack of beta-carotene and xanthophylls prevents the formation of LHCII and PSII core complexes. *Scenedesmus obliquus C-6E*, a Car-deficient mutant strain, is characterized by a complete lack of Chl *b* and by the presence of Chl *a* exclusively in the PSI. In this mutant, PSI activity is fully retained, however, PSII core and LHCII complexes can not be detected and there is no active PSII complex (122). Although PSII supercomplexes and LHCII trimers were not formed in several xanthophyll mutants of green algae and *Arabidopsis*, the PSI-LHCI and the PSII RC were not altered significantly (123-128), which is in agreement with the observation that there was no change in the amount of beta-carotene. Therefore, normal xanthophyll composition is essential for maintaining LHCII trimers and for the macroorganization of the PSII, while the integrity of the PSII RC requires the presence of beta-carotene.

Reconstitution experiments demonstrated that lutein is needed for stable LHCII formation (129-131). Green alga and higher plant mutants lacking lutein showed that LHCII trimer formation *in vivo* also requires lutein (123,124,126,127). Furthermore, although lutein was essential, and alone was sufficient for trimerization, the stability of the individual proteins did not decrease if lutein was replaced with violaxanthin. Even though lutein, violaxanthin and zeaxanthin can all bind to sites L1 and L2, it seems that trimers can only be formed if luteins are present in sites L1 and/or L2 (125,132). The occupancy of L1 is crucial for protein folding (31). The lutein-dependent formation of LHCII trimers is demonstrated in Figure 8 depicting that Car-less conditions initiate monomerization, which can be reversed by the readdition of Cars.

Among the xanthophylls of the LHCII, neoxanthin appears to be the most strongly bound to the Lhc proteins (133). Reconstitution experiments revealed that neoxanthin was not essential in the presence of lutein, violaxanthin or zeaxanthin (31). None of the antenna complexes could be folded in the presence of neoxanthin alone (129,134). In the absence of neoxanthin, refolded recombinant proteins can bind a corresponding amount of violaxanthin. The *Aba4-1 Arabidopsis* mutant completely lacks neoxanthin but retains all the other xanthophylls. In this mutant, neoxanthin was replaced by 9-*cis*-violaxanthin in the N1 binding site. The relative proportions of the minor LHCII proteins changed, but trimerization was not affected (135).

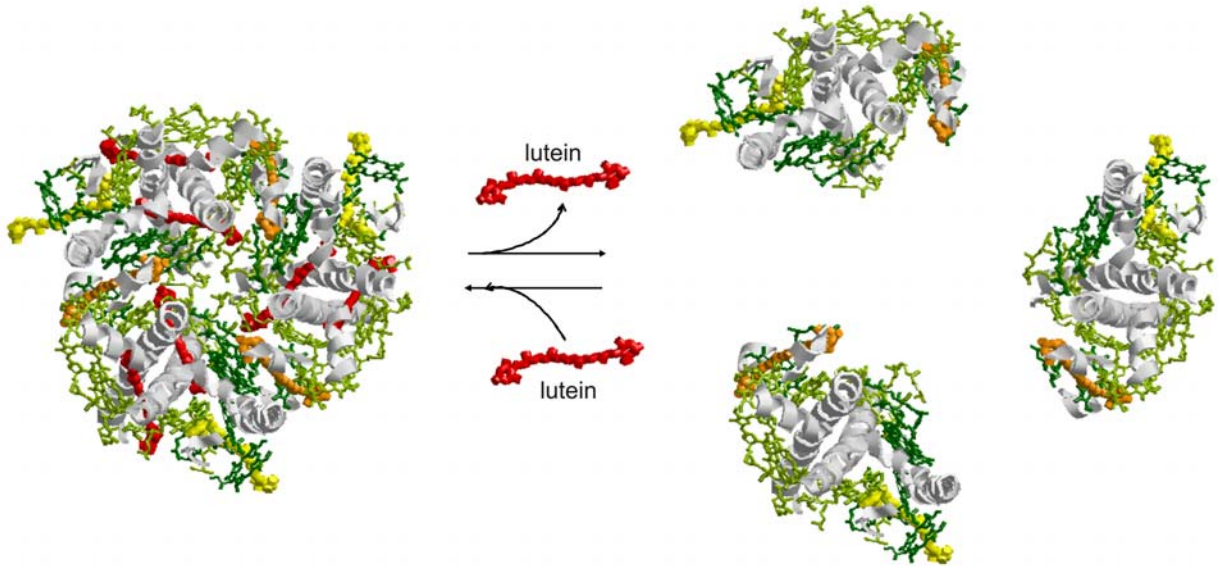

Figure 8. Disassemble of LHCII trimer upon lutein depletion. Color codes are the same as in Figure 7.

Among LHCII bound xanthophylls, violaxathin has the lowest binding affinity (117,129). During the xanthophyll cycle, violaxanthin is converted to zeaxanthin, which is needed for NPQ. Due to its low binding affinity, violaxanthin can be easily removed and can interact with the de-epoxidase enzyme. The resulting zeaxanthin binds to the violaxanthin site of the LHCII trimers, and then trimers can dissipate excess light as thermal energy (30). A tempting hypothesis suggests that LHCII quenching may be associated with a conformation change which converts the active antenna into a quenching state (136-138). However, this model has been challenged (139), and further investigations are required to solve this controversy.

The proposed localization of Cars in the PSII structure is shown in Figure 2A. A partial depletion of Cars may induce the disassembly of PSII dimers and the detachment of peripheric small protein subunits and the Mn_4Ca complex, resulting in the inactivation of the OEC of higher plants and algae (Figure 2B.). The complete loss of Cars leads to the degradation of peripheric protein subunits, as well as to the disappearance of CP43 and to a partial loss of CP47 (Figure 2C.). The Car-less condition may induce a modification of D1 or D2; however, this process is only suggested, it has not been proven yet.

5.2. The contribution of carotenoids to the formation of cyanobacterial PSII complexes

The major Cars in cyanobacteria are beta-carotene; its hydroxyl derivatives, zeaxanthin and nostaxanthin; its keto derivatives, echinenone and canthaxanthin; and the Car glycosides, myxoxanthophyll and oscillaxanthin (26). However, Car composition depends on the growth stage, light intensity, nitrogen source, and the strain type within a given species (140). Beta-carotene is found in all cyanobacteria. In *Synechococcus*, beta-cryptoxanthin, zeaxanthin, caloxanthin and nostoxanthin are the main Car species besides beta-carotene. In *Synechocystis,* the major Car components are as follows: beta-carotene, myxoxanthophyll, zeaxanthin, and echinenone (33,141,142).

Based on X-ray crystallography analyses, 12 beta-carotene molecules were identified in the PSII RC of *T. elongatus* (7). Two beta-carotene molecules are bound to the D1 and D2 proteins (8). The Car close to the D1 protein participates in transferring and/or quenching Chl triplet states and quenching singlet oxygen produced by P680 triplets. The Car close to D2 has a key role as a "molecular wire" in a putative secondary electron transfer when the Mn cluster is not functional or is absent (35). Additionally, three beta-carotenes are bound to CP43 and five to CP47. Most of the Cars located in the antenna subunits are in van der Waals contact with the Chl *a* molecules (8). Myxol glycosides are located in the cytoplasmic and the outer membranes without contact with Chl *a* (143). Under high light conditions, accumulation of zeaxanthin could be detected mainly in the cytoplasmic membranes of *Synechococcus* (144). An increase in the xanthophyll content was observed in both the thylakoid and the cytoplasmic membranes, and the xanthophyll ratio between the two membranes depended on the stress conditions (111,144).

The *Synechocystis crtR* mutant does not contain zeaxanthin and myxoxanthophyll, while its oxygen-evolving activity is similar to that of the wild-type cells (34,145). These findings suggest that zeaxanthin and myxoxanthophyll are not required for the assembly of RCs (34). The lack of xanthophylls does not prevent the accumulation of functional PSII complexes, although the mutant cells show an enhanced light sensitivity (33).

During *Synechocystis* carotenogenesis, all Cars are synthesized from 15-*cis* phytoene. This reaction is catalyzed by the *crtB* gene-encoded phytoene synthase,

involving two stepwise desaturations to form 7, 9, 7', 9'-tetra-*cis*-lycopene. The conversion to all-*trans* lycopene is governed by photoisomeration or by the *cis* to *trans* carotene isomerase encoded by *crtH* (140,146). In light, the Car composition of the delta-*crtH*-mutant cells is indistinguishable from that of the wild type (146). However, under light-activated heterotrophic growth (LAHG) conditions (147), no xanthophylls and only a negligible amount of beta-carotene were produced in delta-*crtH* cells (146). The primary products found were *cis*-lycopenes. The mutant cells grown under LAHG conditions contained a highly decreased level of D1 protein, and no oxygen-evolving activity of the PSII was detected. Beta-carotene and the D1 protein reappeared, and a high level of PSII activity was detected following the transfer of cells to continuous light conditions. These results suggest that beta-carotene is required for the assembly of PSII (34). Bautista and coworkers genetically modified the carotene composition of *Synechocystis* (148). When beta-carotene was replaced with linear Cars, active PSII could not be detected, however, PSI activity was observed. The Car composition of *Synechocystis* was altered by the inactivation of genes involved in beta-carotene synthesis, and the phytoene desaturase gene of *Rhodobacter capsulatus* was expressed. In the mutant, beta-zeacarotene, a compound with one beta-ionylidene ring, which has a chemical structure to that of beta-carotene, was sufficient to support PSII assembly. These results suggest that beta-carotene plays an indispensable structural role in the PSII assembly, and that the presence of a Car containing at least one beta-ionylidene ring is necessary and sufficient for PSII assembly (148).

The first completely Car deficient oxygenic photosynthetic prokaryotic mutant was generated by the inactivation of the *crtB* gene in *Synechocystis* (10). Among the photosynthetic membrane complexes in the Car-less mutant, the PSII complex was the one most severely affected by the absence of Cars. The mutant cells exhibited no oxygen-evolving activity suggesting the absence of photochemically active PSII complexes. Only a very small amount of monomeric PSII core complex, detectable only by radioactive labeling, was formed. This PSII core was unstable, and CP43 quickly dissociated resulting in an RC47 subcomplex, which lacks CP43. This result indicates that Cars are needed for the insertion of CP43 into PSII.

Recent structural models of the cyanobacterial PSII (7,8,52) demonstrated the presence of beta-carotene at the interface between the initial transmembrane helices of the large and small subunits of PSII (Figure 6.). It has been shown previously that unassembled large PSII subunits bind some of these small PSII subunits. They stabilize the unassembled large Chl-binding proteins (149-151). 2D gel electrophoresis showed bands with faster electrophoretic mobility of unassembled CP47, CP43 and D2 proteins in Car-deficient mutant of *Synechocystis* compared to that of wild type, which suggests the absence of the small subunits from these bands related to the absence of Cars. Interestingly, the expression of all four Chl-binding subunits of the PSII was severely suppressed, especially that of the antenna proteins CP47 and CP43, however, the accumulation of phycobiliproteins was not affected. Most of the beta-carotene molecules are located in the vicinity of the transmembrane alfa-helices of CP47 and CP43. Chls that are not in complexes with the protective Car molecules can be in triplet states in light and can produce highly damaging singlet oxygen. The structure of cyanobacterial PSII is presented in Figure 5. In the presence of Cars, the PSII dimer is assembled, while partial depletion of Cars may result in the monomerization and loss of OEC, which is related to the detachment of peripheric small protein subunits and the Mn complex (Figure 5A.B.). Thus, missing Cars seem to affect not only the synthesis of PSII subunits but also the stability of binding between them (10). A complete loss of Cars can induce the complete degradation of OEC, together with the elimination of CP43 and a significant decrease in CP47 content (Figure 5C.).

6. CAROTENOIDS – REGULATORY COMPONENTS IN OXYGENIC PHOTOSYNTHESIS

It is known that Cars are involved in the light regulation of photosynthesis via the processes of light harvesting and photoprotection. Under light-limiting conditions, Chl-protein complexes may not be sufficient to harvest all the light needed. Chl is only able to absorb light within a narrow range in the whole absorption spectrum. Therefore, under light-limiting conditions, all photosynthetic organisms solve the problem of light limitation by utilizing additional pigments in antenna complexes, which can extend the wavelength range of light absorbance and can provide more efficient light absorption. Higher plants use the large protein complex LHCII, which contains Chl *a*, Chl *b* and various xanthophylls (30). Some marine organisms, such as diatoms, utilize xanthophylls, Chl *a*, and Chl *c* within a light-harvesting complex (152). Red algae and cyanobacteria contain phycobilins and Cars (153,154). Cars, especially xanthophylls, are important components of the light-harvesting complexes of many oxygenic photosynthetic organisms. The major functions of Cars are photoprotection and light harvesting both in the RCs and in the antenna complexes (155). The specific light-harvesting function of Cars consist of two steps: the capturing of light in the blue-green parts of the solar spectrum at approximately 400-570 nm, followed by singlet-energy transfer to nearby Chls via resonance transfer (156,157). The Chls subsequently transfer the captured energy to the RCs, where the excited state energy is fixed by a series of electron transfer reactions.

However, overexcitation causes incomplete photochemical quenching, which leads to a longer life time of singlet state Chl, with an increased possibility of triplet excited-state-Chl formation through intersystem crossing. In contrast to the singlet-excited state of Chl, triplet-excited-state Chl is relatively long-lived (158). Triplet-excited-state Chl can interact with molecular oxygen and form singlet-excited-state oxygen, which is a highly damaging reactive oxygen species (ROS). ROS can attack pigments, proteins and lipids in the membrane (159). Car molecules can efficiently accept the excess energy by quenching the triplet state of Chls and singlet state of reactive oxygen, forming triplet state of Cars. This

photoprotective function of Cars is utilized by all oxygenic photosynthetic organisms (32). This process is followed by another function of Car pigments: thermal energy dissipation. During the relaxation of the Car triplet to its ground state, it releases the excess absorbed light energy as heat (160). Thermal dissipation is thought to protect photosynthesis by decreasing the life time of the singlet state of Chl to minimize the generation of excited singlet oxygen in the PSII LHC and RC.

6.1. The functional importance of carotenoids in higher plants and algae

In algae and higher plants, Cars are normally synthesized only in the chloroplast (161). The Car composition undergoes modifications during short- and long-term photoprotection. The xanthophyll cycle, the main protective mechanism, protects eukaryotic organisms from short term fluctuation of light intensities (162). The xanthophyll cycle involves zeaxanthin, antheraxanthin and violaxanthin. Zeaxanthin is absent in the thylakoid membrane in normal light conditions. Under excess light illumination, lumenal pH decreases and the violaxanthin de-epoxidase converts violaxanthin to zeaxanthin. During the xanthophyll cycle, the rapidly reversible pH changes across the membrane trigger energy quenching induction (qE) (163), the predominant component of NPQ. During NPQ, the excess absorbed light energy in PSII is thermally dissipated, which prevents the photosynthetic apparatus, especially PSII, from photooxidative damage (164) and can be measured as a light-dependent quenching of Chl fluorescence originated by PSII (165). Single and double mutations of the Car biosynthesis pathway in *Arabidopsis* and *Chlamydomonas* demonstrated that Cars and especially their oxygenated derivatives, the xanthophylls, play a key role in qE. *Chlamydomonas* and *Arabidopsis npq1* mutants are violaxanthin de-epoxidase activity deficient, lack zeaxanthin and have greatly reduced NPQ (166,167). On the other hand, *npq2* mutants of *Chlamydomonas* and *Arabidopsis*, which lack zeaxanthin epoxidase activity, accumulate zeaxanthin and contain only trace amounts of antheraxanthin, violaxanthin and neoxanthin, and display a faster induction of NPQ (166,167). These results revealed the primary role of zeaxanthin in qE induction. Lutein is the most abundant alfa-branch xanthophyll in plant and algae chloroplasts, where it plays an important role in LHCII assembly and function (168). To reveal the additive effect of lutein on NPQ, a *lut2npq1* double mutant lacking both zeaxanthin and lutein was created, in which no NPQ could be detected (169). The *Arabidopsis lut2* mutant, lacking only lutein, displays low NPQ, which is believed to originate from zeaxanthin (170). Violaxanthin and neoxanthin are not determinant components of qE. The *Arabidopsis szl1 npq1* mutant lacks zeaxanthin, has lower levels of violaxanthin, antheraxanthin, and neoxanthin and a higher level of lutein and alpha-carotene accumulation. The increase in the level of lutein strongly suggests that lutein can replace zeaxanthin, indicating the direct role of lutein in qE (171). The above results, together with NPQ kinetics of wild-type *Arabidopsis* and different xanthophyll pathway mutants studied by Silvia de Bianchi and coworkers, suggest that both zeaxanthin and lutein are important for qE-related photoprotection. Moreover, they exert their specific effects on qE separately from each other (172). Although neoxanthin and violaxanthin are not involved in NPQ, neoxanthin preserves PSII and protects membrane lipids from photooxidation by ROS (135). *Arabidopsis* mutants lacking alfa-branch or beta-branch xanthophylls are extremely photosensitive. Characterization of these mutants demonstrated that alfa-branch- and beta-branch-xanthophylls specialize in triplet quenching and ROS scavenging, respectively. Furthermore, beta-xanthophylls are also indispensable Cars for the activation of qE (173).

6.2. The functional roles of carotenoids in Cyanobacteria

6.2.1. Carotenoids: protective agents

Cyanobacteria were the first oxygenic photosynthetic organisms to face the deleterious effects of photooxidation. The formation of ROS can be lethal for photosynthetic organisms, mainly due to their deleterious effect on PSII. Cyanobacteria utilize various Cars as scavengers of ROS. The beta-carotene derivative xanthophylls determine the antioxidative properties. In general, their hydroxyl derivatives, zeaxanthin and nostoxanthin, are good in inactivating peroxy radicals (174), whereas their keto derivatives, echinenone and canthaxanthin, are more efficient in quenching ROS (175). In *Synechocystis*, which is one of the most extensively studied cyanobacteria due to its adaptability to environmental stresses, the major Car components are beta-carotene, myxoxanthophyll, zeaxanthin, and echinenone (33,146). Wild-type *Synechocystis* is very well protected against photooxidation. It contains a well-balanced antioxidative system to handle photooxidative stress. In the echinenone-deficient *crtO* mutant, the photosynthetic activity of the cells does not differ significantly from that of wild-type cells (176). However, complete zeaxanthin and myxoxanthophyll deficiency results in a strong photoinhibition of PSII as well as of the overall photosynthetic electron transport under high-light conditions in *crtR* mutant cells (145). In this mutant, myxoxanthophyll is modified to desoxy myxoxanthophyll. Due to the spectral properties and the rather high polarity attributed to the sugar moiety, it can be assumed that myxoxanthophyll and desoxy myxoxanthophyll may be functionally equivalent (142). The *crtRO* double mutant is deficient in echinenone, zeaxanthin and myxoxanthophyll (33). Under high-light treatment, the oxygen-evolving activity and maximum quantum yield of PSII dramatically dropped. Moreover, the remaining beta-carotene and desoxy myxoxanthophyll are unable to protect photosynthesis. These results suggest that zeaxanthin is an essential Car for PSII protection against photoinhibition caused by reactive singlet oxygen in *Synechocystis*. Results obtained with *Synechococcus* also support the efficient antioxidant activity of zeaxanthin, since UV-B stress increased the level of zeaxanthin (177). Investigations on the photoprotective function of myxoxanthophylls in cyanobacterium *Plectonema boryanum* cells exposed to UV radiation revealed a reduced efficiency of energy transfer to PSII RCs and an elevated level of myxoxanthophyll (178). Under high-light treatment of *T. elongatus,* the level of specific Cars increased, while there were only minor

alterations in the level of beta-carotene. The level of zeaxanthin was the highest in the cells grown in high light. Interestingly, the level of myxol glycoside, in which the dimethyl fucoside moiety of myxoxanthophyll is probably replaced by an anhydrohexose (142), was greatly elevated in cultures exposed to high light (179). In *crtH*, a cis-trans-isomerase mutant, beta-carotene can not be synthesized in darkness, only in light (34,146). The PSII activity of the mutant cells was activated by the presence of beta-carotene in light. Under continuous light illumination, PSII activity was detected at a level similar to that of wild type. The Car-less mutant cells were light sensitive and could grow only under LAHG conditions in the presence of glucose (10). Car deficiency did not significantly affect the cellular content of phycobiliproteins, while the Chl content of the mutant cells was decreased. The mutant cells exhibited no oxygen-evolving activity suggesting the absence of photochemically active PSII complexes. Thus, Cars are indispensable constituents of the photosynthetic apparatus; they participate not only in antioxidative protection, but also in the efficient synthesis and accumulation of photosynthetic proteins, especially that of PSII antenna subunits.

6.2.2. The Orange Carotenoid Protein and non photochemical quenching of PSII in cyanobacteria

Cyanobacteria were not considered capable of NPQ for many years until the OCP was found. The OCP is a water-soluble protein containing a single Car (180,181). Most cyanobacterial species contain OCP and perform OCP-related NPQ associated with PBS (182,183). The OCP is a new member of the family of blue light photoactive proteins. It is a unique example of a photoactive protein containing a Car as the photoresponsive chromophore. Upon illumination with blue-green light, the OCP undergoes a reversible transformation from its stable dark orange form to a red “active” form. The red form is essential for the induction of the photoprotective mechanism. The illumination-induced structural changes affect both the Cars and the proteins (184). 3’-hydroxyechinenone, which is the key inducer of the photoprotection mechanism, is the principle Car detected in the OCP of *Synechocystis*. In a *Synechocystis* mutant, the overexpressed OCP contains mainly echinenone. Echinenone can replace hydroxyechinenone in the photoprotective mechanism, and it can efficiently generate the red OCP form. This indicates that the hydroxyl group in 3’-hydroxyechinenone, which presumably makes a hydrogen bond to the N terminal domain of OCP, is not crucial in photoprotection. In the *crtO* mutant, 3’hydroxyechinenone and echinenone are replaced by zeaxanthin in the OCP, and, as a result, the binding stability of the Car to the protein is decreased, and light is unable to photoconvert the dark form into the red active form. These results suggest that the presence of the Car carbonyl group in echinenone and 3’hydroxyechinenone is essential for the photoactivation of the OCP and the induction of the photoprotection mechanism. Zeaxanthin lacks the oxygen atom needed for hydrogen bonding, thus, the OCP binding with zeaxanthin is not photoactive and is unable to induce fluorescence quenching under blue-green light (185). Recent structural analyses based on protein-Car interactions, which determine the optical properties of the OCP, suggest the role of several key amino acids in the function of the OCP and demonstrate that photoconversion and the photoprotective responses of the OCP to blue-green light can be decoupled (186).

7. PERSPECTIVES

Several *in vivo* studies using biochemical and genetic approaches suggested that lipid molecules determine the assembly and the functions of the PSII complex. Interestingly, there are some signs indicating that besides the lipid-head group fatty acid chains may also have specific roles in photosynthetic structures and functions. It has been demonstrated that exogenously added dioleoyl-PG molecules are retailored in PG-deficient *Synechocystis* cells to generate natural PG molecules (187). These findings indicate that specific lipid molecular species may play distinct roles in specific photosynthetic processes, necessitating further studies. It is also essential to reveal the enzymatic processes involved in lipid remodeling and the corresponding genes. Having identified the genes, mutants can be generated to be used as tools to study the roles of lipids in photosynthesis.

Cars, especially xanthophylls, are indispensable constituents of photoprotective mechanisms. In LHCII, the molecular mechanism of NPQ has not been elucidated yet. The dissipation of excess energy has been investigated in various model organisms by several research groups, and the result gained so far are rather contradictory.

The crystal structure of the whole PSII has been determined in prokaryotic cyanobacteria but not in eukaryotic organisms. In order to reveal the structural differences between higher plants and cyanobacteria, a high-resolution structure of the eukaryotic PSII is required. Adachi and coworkers have successfully purified PSII dimers from the red alga *Cyanidium caldarium* and crystallized them (188). This was a very important step towards resolving the structure of a eukaryotic PSII.

The first oxygenic prokaryotic photosynthetic organism that is completely Car deficient has been generated recently. In this mutant, neither assembled PSII complexes nor functional PSIIs were detected, and the overall membrane protein synthesis rate was considerably decreased. The regulation mechanism behind this suppression has not been elucidated yet. Since Car-assisted PSII construction can not occur, there is no oxygen-evolving activity in the mutant cells. Turnover of the D1 protein is needed to maintain functional PSIIs under stress conditions. The repair process of D1 includes the degradation of the damaged protein and the de novo synthesis of the D1 precursor (pD1), together with a posttranslational processing of the incomplete D1 (iD1) following its partial processing and binding to D2. D1 protein replacement in the mutant occurred, however, pD1 and iD1 accumulated in PSII subcomplexes. It has been demonstrated that lipid molecules play an important role in the D1 protein turnover, which raises the question whether

Cars, the other mortar of PSII, also affect repair processes and at which steps (Figure 4).

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Abbreviations: *Arabidopsis*: *Arabidopsis thaliana*, Car: carotenoid, *Chlamydomonas*: *Chlamydomonas reinhardtii*, Chl: chlorophyll, CP43: 43 kDa chlorophyll-protein complex, CP47: 47 kDa chlorophyll-protein complex, DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethylurea, DGDG: digalactosyldiacylglycerol, GlcGDG: glycosyl-galactosyldiacylglycerol, LAHG: light-activated heterotrophic growth, LHCII: light-harvesting complex of photosystem II, MGDG: monogalactosyl-diacylglycerol, NPQ: non-photochemical quenching, OCP: orange carotenoid protein, OEC: oxygen-evolving complex, PBS: phycobilisome, *p*BQ: p-benzoquinone, PG: phosphatidylglycerol, PLA2: phospholipase A2, PSI: photosystem I, PSII: photosystem II, Q_A: primary quinone acceptor of photosystem II, Q_B: secondary quinone acceptor of photosystem II, qE: energy quenching induction, RC: reaction center, ROS: reactive oxygen species, SQDG: sulfoquinovosyl diacylglycerol, *Synechocystis*: *Synechocystis* sp. PCC6803, *Synechococcus*: *Synechococcus* sp. PCC7942, *T. elongatus*: *Thermosynechococcus elongates,* Y_Z: tyrosine Z of photosystem II,

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