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Review

The role of lipids in photosystem II ☆

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ABSTRACT

The thylakoid membranes of photosynthetic organisms, which are the sites of oxygenic photosynthesis, are composed of monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG), and phosphatidylglycerol (PG). The identification of many genes involved in the biosynthesis of each lipid class over the past decade has allowed the generation and isolation of mutants of various photosynthetic organisms incapable of synthesizing specific lipids. Numerous studies using such mutants have revealed that deficiency of these lipids primarily affects the structure and function of photosystem II (PSII) but not of photosystem I (PSI). Recent X-ray crystallographic analyses of PSII and PSI complexes from *Thermosynechococcus elongatus* revealed the presence of 25 and 4 lipid molecules per PSII and PSI monomer, respectively, indicating the enrichment of lipids in PSII. Therefore, lipid molecules bound to PSII may play special roles in the assembly and functional regulation of the PSII complex. This review summarizes our present understanding of the biochemical and physiological roles of lipids in photosynthesis, with a special focus on PSII. This article is part of a Special Issue entitled: Photosystem II.

1. Introduction

In cyanobacteria, algae, and higher plants, the thylakoid membrane is the site of the photochemical and electron transport reactions of oxygenic photosynthesis, which are performed by two protein–cofactor complexes of photosystem II (PSII) and photosystem I (PSI), cytochrome (Cyt) b_6f complex, and ATP synthase [1,2]. In general, lipids of the thylakoid membrane provide the matrix for the photosynthetic complexes to avoid free diffusion of ions and are a prerequisite for the generation of the electrochemical potential difference across the membrane that is required to drive ATP synthase. Recent X-ray crystallographic analyses of the photosynthetic complexes have identified integral lipids that bind specifically to the complexes, suggesting that lipids are important not only for formation of the lipid bilayer but also for the structure and function of photosynthetic complexes (for a recent review, see Kern et al. [3]).

Fig. 1 shows the composition of lipids in thylakoid membranes from the cyanobacteria *Synechocystis* sp. PCC 6803 and *Thermosynechococcus vulcanus*, and the higher plant *Spinacia oleracea* (spinach). The lipid composition of thylakoid membranes is highly conserved among oxygenic photosynthetic organisms and is composed of uncharged lipids including monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), as well as anionic lipids including sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG) [4]. The majority of lipids in thylakoid membranes are glycolipids, *i.e.*, MGDG, DGDG, and SQDG [5,6], in contrast to other biological membranes in which phospholipids are the major lipid components. Fig. 2 shows the structure of lipids that constitute thylakoid membranes. Each lipid has two acyl groups esterified at the *sn*−1 and *sn*−2 positions of the glycerol moiety and a polar head group at the *sn*−3 position, which characterizes each individual class of lipid (Fig. 2). MGDG has a head group of 1 β-galactose linked to the diacylglycerol. DGDG has a head group of digalactose with the second galactose bound to the first galactose of MGDG by an α1-6 glycosidic linkage. SQDG contains 6-deoxy-6-sulfo-α1-glucose as the head group, and PG has *sn*-glycerol 1-phosphate. The latter two are categorized as acidic lipids because of their negative charge at neutral pH.

Each lipid in a different class is expected to have a specific role in photosynthesis, depending on the nature of its head group. Considerable amounts of MGDG and DGDG are thought to exist as "bulk lipids" and mainly function as structural lipids [7], although DGDG has also been recognized as important for the proper structure and function of PSII mainly on the donor side [8,9]. PG is the only major phospholipid found in thylakoid membranes [10–12]. It is likely that PG mediates indispensable interactions with the components of photosynthetic

Abbreviations: CDP, cytidine 5′-diphosphate; CDP-DG, CDP-diacylglycerol; Chl, chlorophyll; CMP, cytidine 5′-monophosphate; Cyt, cytochrome; DCMU, 3-(3, 4-dichlorophenyl)-1,1-dimethylurea; DG, diacylglycerol; DGDG, digalactosyldiacylglycerol; DM, *n*-dodecyl-β-D-maltoside; FAS, fatty acid synthase; G3P, glycerol 3-phosphate; LHC, light-harvesting complex; LPA, lysophosphatidic acid; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; MGlcDG, monoglucosyldiacylglycerol; PG, phosphatidylglycerol; PGP, phosphatidylglycerophosphate; PQ, plastoquinone; PSI, photosystem I; PSII, photosystem II; SQDG, sulfoquinovosyldiacylglycerol; TMH, transmembrane α-helices; UDP, uridine 5′-diphosphate

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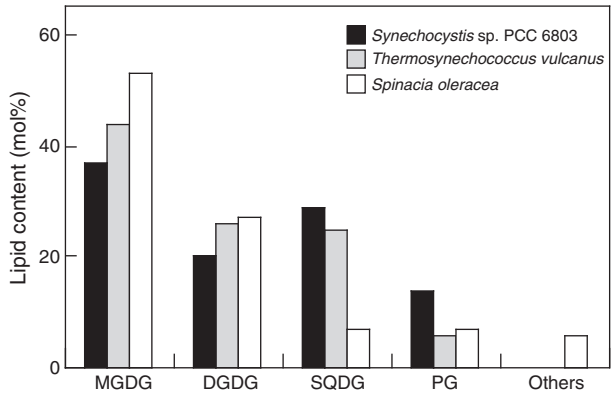

Fig. 1. Composition of lipids in thylakoid membranes from the cyanobacteria *Synechocystis* sp. PCC 6803 and *Thermosynechococcus vulcanus*, and the higher plant *Spinacia oleracea*. The original data of lipid analysis of thylakoid membranes are from Sakurai et al. [48] (*Synechocystis* and *T. vulcanus*) and Dorne et al. [11] (*Spinacia oleracea*), respectively.

complexes in thylakoid membranes, and plays a specific role in photosynthesis (for review see [13–15]). Another anionic lipid, SQDG, also appears to have an important function in photosynthesis, although the importance of this lipid differs among species (for review see [16,17]). PG and SQDG are considered to be at least partially functionally redundant, which may be related to maintenance of anionic charge on the surface of the thylakoid membrane [18].

The roles of different classes of lipid have been studied by biochemical and molecular genetic approaches. One of the most useful biochemical approaches is to analyze the effects of removal of a specific lipid class by treatment with lipases on photosynthesis. Analyses of thylakoid membranes or PSII preparations treated with phospholipases, which are specific to phospholipids (*i.e.*, PG in thylakoid membranes), have indicated that PG plays important roles in photosynthesis [13,15]. Decomposition of PG from thylakoid membranes by treatment with phospholipase A_2 [19] or phospholipase C [20] strongly inhibits the photosynthetic electron transport in PSII without any significant effect on photosynthetic electron transport in PSI. In addition to these enzymatic approaches, an immunochemical study using an antibody against PG showed that PG interacts specifically with the D1 polypeptide, one of the subunits of the PSII reaction center [21]. Leng et al. [22] compared the effects of phospholipase A_2 and lipase on the structure and function of PSII from *T. vulcanus*. Phospholipase A_2 treatment decreased PG content by 59%, leading to a decrease in oxygen evolution by 40%. On the other hand, treatment with lipase specifically decreased MGDG content by 52% and decreased oxygen evolution by only 16%. These observations indicate that PG plays a more important role in PSII than MGDG. However, based on the results of these experiments with phospholipase A_2 and lipase, it is impossible to clarify the roles of lipid molecules in PSII that are buried inside the complex because of their inaccessibility to the enzymes.

The roles of different classes of lipid in photosynthetic organisms have also been studied by molecular genetic approaches (for review, see [13–17,23]). The recent identification of genes for enzymes that are required for the biosynthesis of lipids in cyanobacteria and higher plants, as well as the subsequent generation of mutants defective in the biosynthesis of lipids, has provided powerful molecular tools for detailing the functions of lipids in photosynthetic organisms. Such studies with various mutants have revealed that in many cases reduction or depletion of specific lipids by knocking out or inactivating genes involved in lipid synthesis causes defects and/or changes in the properties of PSII but does not affect those in PSI. Based on these studies, it has become apparent that each lipid class plays an important function in photosynthesis, especially in PSII.

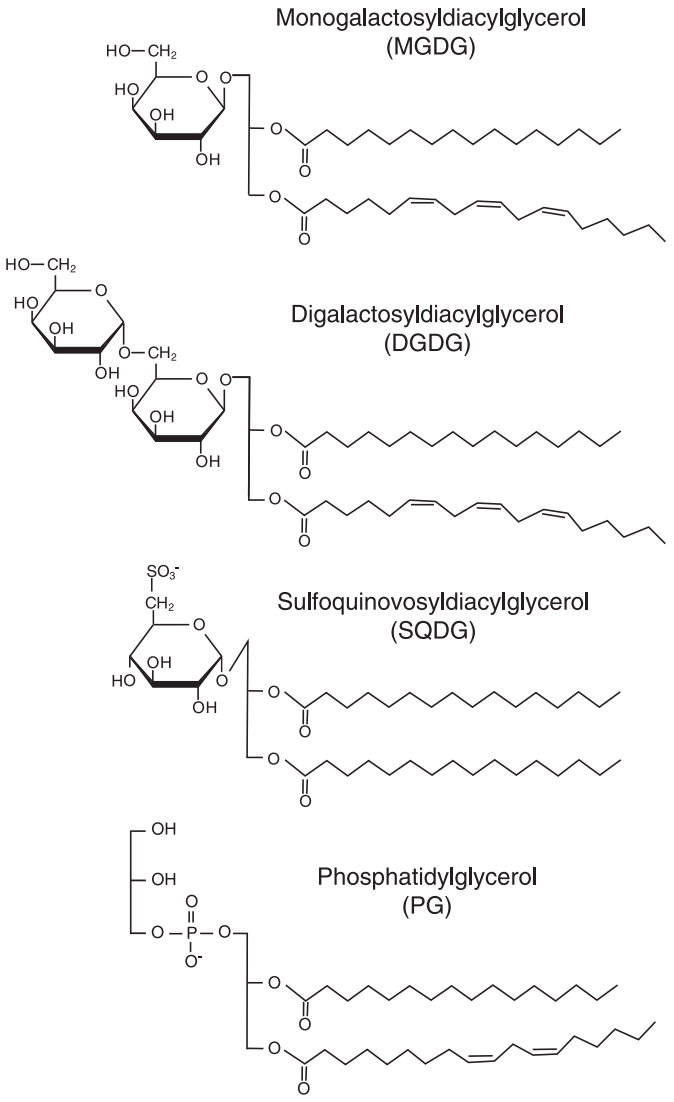

Fig. 2. Structure of lipids in thylakoid membranes, *i.e.*, monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG), and phosphatidylglycerol (PG). Fatty acids are esterified to the *sn*−1 and *sn*−2 positions of the glycerol backbone in each lipid. This figure shows the typical fatty acids bound to each class of lipid in Synechocystis sp. PCC 6803.

Recent X-ray crystallographic analysis of PSII and PSI complexes from *Thermosynechococcus elongatus* revealed the presence of 25 and 4 lipid molecules per PSII and PSI monomer, respectively. The lipid-to-transmembrane α-helix (TMH) ratios are 0.7 and 0.1 for PSII and PSI, respectively [24]. These structural data suggest the enrichment of lipids in PSII among the supra-complexes involved in photosynthesis and special functions of lipids in PSII. These findings are consistent with data obtained by biochemical and mutational analyses. Lipid molecules can also be found in the light-harvesting complex II (LHCII) attached to the PSII core complex [25].

In this review, we summarize our present understanding of the crucial role played by lipids in photosynthesis, focusing on PSII.

2. Biosynthesis of lipids

Fig. 3 shows the biosynthetic pathway of lipids in cyanobacteria. In higher plants, lipids are synthesized in three subcellular compartments, *i.e.*, the plastid, endoplasmic reticulum, and mitochondrion. The biosynthetic pathway of lipids present in the thylakoid membranes of chloroplasts in higher plants is essentially the same as that in

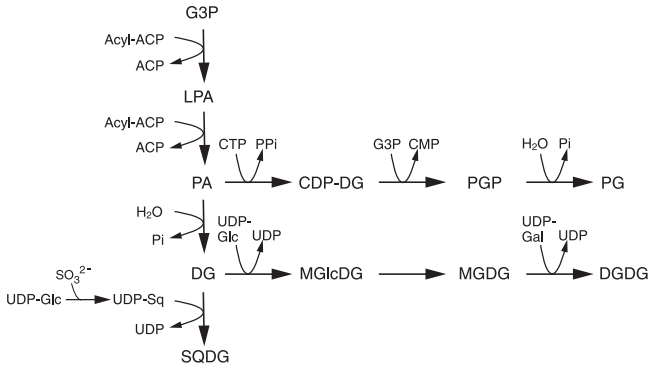

Fig. 3. Biosynthetic pathway of thylakoid lipids in cyanobacteria. ACP, acyl-carrier protein; CMP, cytidine 5′-monophosphate; CTP, cytidine 5′-triphosphate; UDP-Gal, UDP-galactose; UDP-Glc, UDP-glucose; UDP-Sq, UDP-sulfoquinovose.

cyanobacteria. Phosphatidic acid (PA) is the common intermediate in the synthesis of all classes of lipids. The glycerol backbone for the synthesis of PA is provided by glycerol 3-phosphate (G3P). G3P is acylated first at the *sn*−1 position by G3P acyltransferase (GPAT) to generate lysophosphatidic acid (LPA) and then at the *sn*−2 position by LPA acyltransferase to generate PA. In *Synechocystis* sp. PCC 6803, the genes for LPA acyltransferase (*sll1752* and *sll1848*) [26,27] have been identified. After the synthesis of PA, the biosynthetic pathway of lipids in cyanobacteria divides into branches [12,28], as is also the case in chloroplasts [6,29]. PA is dephosphorylated by PA phosphatase to yield diacylglycerol (DG) for the synthesis of glycolipids (*i.e.*, MGDG, DGDG, and SQDG) on one branch, whereas PA is converted to cytidine 5′-diphosphate-DG (CDP-DG) by CDP-DG synthase for the synthesis of PG on the other branch.

The DG produced by PA phosphatase is utilized as the substrate for the synthesis of monoglucosyldiacylglycerol (MGlcDG) and SQDG in cyanobacteria. MGlcDG is produced by the catalytic reaction of MGlcDG synthase *via* the transfer of glucose from uridine 5′-diphosphate-1α-glucose (UDP-glucose) to the *sn*−3 position of DG. Recently, *sll1377* in *Synechocystis* sp. PCC 6803 and its homolog, *all3944*, in *Anabaena* sp. PCC 7120 were identified as the genes encoding MGlcDG synthase [30]. MGDG may be synthesized by the epimerization of the glucose moiety to galactose in MGlcDG, although the gene responsible for this epimerization reaction remains to be identified. In plant chloroplasts, the biosynthetic pathway of MGDG differs in the assembly of the head group. In chloroplasts, MGDG is synthesized by the direct transfer of galactose from UDP-galactose to DG in a reaction catalyzed by MGDG synthase. There are three genes encoding MGDG synthase in *Arabidopsis thaliana* (*MGD1*, *MGD2*, and *MGD3*). MGD1 predominantly regulates MGDG synthesis in photosynthetic tissues, while MGD2 and MGD3 are mainly expressed in non-photosynthetic tissues [31].

DGDG is synthesized by DGDG synthase *via* the transfer of galactose to MGDG. The genes encoding DGDG synthase (*DGD1*, *DGD2*) were first identified in *Arabidopsis*. *DGD1* is predominantly expressed in all tissues, while the level of *DGD2* expression is extremely low under normal conditions [32,33]. As there are no homologs of DGD1 and DGD2 in cyanobacteria [34], identification of the corresponding gene in cyanobacteria is difficult. The gene encoding DGDG synthase, designated *dgdA* (*slr1508*), was recently identified in *Synechocystis* using comparative genomic analysis [9,35]. Among eukaryotic photosynthetic organisms, a homolog of the *dgdA* gene has been found only in *Cyanidioschyzon merolae*.

SQDG is synthesized from DG by a two-step reaction. First, UDP-6-sulfo-6-deoxy-1α-glucose (UDP-sulfoquinovose), which is the donor of sulfoquinovose for the synthesis of SQDG, is synthesized from UDP-glucose and sulfite by UDP-sulfoquinovose synthase. Then, SQDG is synthesized by the transfer of sulfoquinovose from UDP-sulfoquinovose to DG by SQDG synthase. The gene for UDP-sulfoquinovose synthase is named *sqdB* in cyanobacteria and *SQD1* in plants, and the gene for SQDG synthase is named *sqdX* in cyanobacteria and *SQD2* in plants [36,37]. These genes are conserved among cyanobacteria, *Arabidopsis*, *C. merolae*, and the diatom *Thalassiosira pseudonana*, which is presumed to be a secondary symbiont of red algae [38,39]. Thus, it seems that the system for the synthesis of SQDG in cyanobacteria, unlike those for the synthesis of MGDG and DGDG, has been conserved through the evolution of cyanobacteria into chloroplasts.

For synthesis of PG, the PA synthesized by the two-step acylation is converted to CDP-diacylglycerol (CDP-DG) by CDP-DG synthase, which transfers the cytidine 5′-monophosphate (CMP) moiety from CTP to PA. The resultant CDP-DG reacts with glycerol 3-phosphate to produce PG phosphate (PGP) and CMP in a reaction catalyzed by PGP synthase. The last step in this pathway is the release of the phosphate group from PGP by PGP phosphatase to generate PG. In *Synechocystis* sp. PCC 6803, genes specifically involved in the biosynthesis of PG, the *cdsA* gene (*slr1369*) for CDP-DG synthase [40], and the *pgsA* gene (*sll1522*) for PGP synthase [41] have been identified. In *Arabidopsis*, the genes for PGP synthase, *PGP1* and *PGP2*, which exhibit substantial similarity to cyanobacterial PGP synthases, have been identified. *PGP1* encodes a preprotein that is targeted to plastids and mitochondria, whereas *PGP2* encodes a microsomal enzyme [42,43].

3. Compositions and localization of lipids in photosynthetic protein–cofactor complexes

The thylakoid membranes contain several protein–cofactor complexes, *i.e.*, the PSI complex, the PSII complex, the Cyt b_6f complex, and ATP synthase [1,2]. With the exception of ATP synthase, the lipid molecules in these complexes have been analyzed by X-ray crystallography [3,24,44–47] and those extracted from purified PSI and PSII complexes have also been analyzed by thin layer chromatography and gas chromatography [48,49]. Table 1 shows the lipid molecules that have been identified by X-ray crystallography and by analysis of the extracted lipids from such complexes.

PSI is involved in the reduction of $NADP^+$ to produce NADPH, the reducing power of which is used to drive the Calvin–Benson cycle (carbon fixation), and is composed of approximately 15 protein subunits and many cofactors, such as chlorophylls (Chls) and lipids [44]. Jordan et al. [44] analyzed the structure of the PSI complex from *T. elongatus* by X-ray crystallography at 2.5 Å resolution, and they identified one molecule of MGDG and three molecules of PG per monomer of the complex. One of the three PG molecules and the MGDG molecule were located symmetrically relative to one another in the complex. These two lipid molecules may be specifically important in the formation of the photochemical reaction center of the PSI complex. A second PG molecule was located in the vicinity of the monomer–monomer interface in the trimeric structure of the PSI complex, suggesting that this PG molecule may participate in the trimerization of the complex. This was supported by the observation that monomers of the PSI complex accumulated in a *pgsA* mutant of *Synechocystis* sp. PCC 6803 after strict PG deprivation [50]. A third PG molecule was found between the PsaB and PsaX subunits, suggesting that it may contribute to the binding of PsaX to the PSI complex. Recently, we generated *Synechocystis* sp. PCC 6803 strains expressing a His-tagged PsaF or PsaJ subunit of PSI for rapid and simple purification of the PSI complex using Ni-affinity column chromatography [49]. Analysis of lipids extracted from the purified trimer complex of His-tagged *Synechocystis* PSI by thin-layer chromatography and gas chromatography identified 6 lipid molecules per PSI monomer (Table 1). Although the number of lipids was close to that found in the crystal structure of the PSI of *T. elongatus*, the lipid composition of that of *Synechocystis* was different from that of *T. elongatus*; that is, two MGDG, one DGDG, one SQDG, and two PG per monomer were identified in the PSI complex of *Synechocystis*. These findings suggest that the lipid composition of PSI depends on the cyanobacteria species, and that DGDG and SQDG molecules found in

Table 1
Lipid molecules identified in protein–cofactor complexes from thylakoid membranes by X-ray crystallographic analysis and by biochemical analysis of lipids extracted from the complexes. The number of lipid molecules per monomer is shown for each protein complex.

Protein complex	Organism	Number of lipid molecules		Reference
X-ray crystallography				
PSII	*Thermosynechococcus elongatus*	MGDG	6	Loll et al. [47]
		DGDG	4	
		SQDG	3	
		PG	1	
PSII		MGDG	11	Guskov et al. [24]
		DGDG	7	
		SQDG	5	
		PG	2	
PSI		MGDG	1	Jordan et al. [44]
		PG	3	
LHCII	*Spinacia oleracea*	DGDG	1	Liu et al. [25]
		PG	1	
Cyt b_6f	*Mastigocladus laminosus*	PC	2	Kurisu et al. [45]
Cyt b_6f	*Chlamydomonas reinhardtii*	MGDG(?)	2	Stroebel et al. [46]
		SQDG	1	
Lipid analysis				
PSII	*Synechocystis* sp. PCC 6803	MGDG	6	Sakurai et al. [48]
		DGDG	3	
		SQDG	5	
		PG	6	
PSI		MGDG	2	Kubota et al. [49]
		DGDG	1	
		SQDG	1	
		PG	2	
PSII	*Thermosynechococcus vulcanus*	MGDG	8	Sakurai et al. [48]
		DGDG	6	
		SQDG	6	
		PG	8	

Synechocystis but not in *T. elongatus* might be replaced by other lipid molecules such as PG in *T. elongatus*.

PSII is involved in the oxidation of water molecules and reduction of plastoquinone molecules, and is composed of approximately 20 protein subunits in addition to many cofactors, such as pigments, metals, and lipids [3,51–54]. The spatial arrangement of protein subunits and cofactors in PSII has been clarified by X-ray crystallographic analysis [24,47,55–57]. In the most recently determined crystal structure of PSII dimer at 2.9 Å resolution, 25 lipid molecules (11 MGDG, 7 DGDG, 5 SQDG, and 2 PG) per monomer were assigned in the PSII complex from *T. elongatus* (Table 1) [24]. Compared to the PSI complex, the PSII complex contained a relatively large number of lipid molecules. Of the 25 lipid molecules, 7 were located at the monomer–monomer interface, 3 were at the periphery of PSII, 7 formed clusters with 2 or 4 lipids in the lipid belt around D1–D2, and the remaining 8 lipids were arranged as a bilayer island forming the plastoquinone–plastoquinone exchange cavity ([24]; Fig. 4). The 15 integral lipids located in the vicinity of the photochemical reaction center may provide some structural flexibility within the reaction center. The PSII complex is inactivated by light and the inactivated PSII complex is repaired *via* a multi-step process [58–61]. The removal of the degraded D1 protein from the damaged complex and insertion of the newly synthesized D1 into the damaged PSII complex are critical to the repair process, and a flexible environment around the reaction center, created by abundant lipid molecules, may facilitate this replacement of the D1 protein.

Recently, Broser et al. [62] reported the first crystallization and structural analysis of the monomeric form of the PSII complex from *T. elongatus* at 3.6 Å that has oxygen evolution activity comparable to that of the dimeric form. Using information about lipid positions in the PSII dimer [24], they assigned 22 lipid molecules per monomer and discussed the role of lipids in dimer formation. The positions of the lipid head group surrounding the reaction center were essentially the same as those of their counterparts in the dimer: three small clusters (2–3 lipids) around the reaction center and one bilayer island forming the plastoquinone–plastoquinone exchange cavity consisting of seven lipids missing one MGDG (MGDG18) found in the dimeric PSII. The three lipids located at the periphery of PSII dimer were also found in the monomer. Significant differences were found in the monomer–monomer surface area. One molecule of MGDG (MGDG14) found in the dimer was replaced by DGDG or *n*-dodecyl-β-D-maltoside (DM) in the monomer. Two DM were additionally located at new positions in close proximity to DGDG23 [62]. It is likely that these lipid and detergent molecules specifically bound to the monomer but not in the monomer derived from the dimeric complex would interfere with dimer formation. Interestingly, one of the two expected binding niches for SQDG (SQDG12) at the monomer–monomer interface in the dimer [24] was unoccupied in the monomeric complex. These observations suggest a direct role of SQDG rather than PG in dimer formation of PSII [63].

The content of lipids bound to PSII, determined by X-ray crystallographic analysis [24], and the lipid analysis by gas chromatography [48] were similar, as shown in Table 1. However, the content of PG was considerably different. Only two PG molecules were identified in the crystal structure of PSII. However, our analysis of extracted lipids from the purified PSII complexes indicated that the PSII complexes from *T. vulcanus* and *Synechocystis* sp. PCC 6803 contain 8 and 6 PG molecules per monomer, respectively, and PG was the most abundant lipid ([48]; Table 1). These results suggest that more PG molecules are present in the complex than were identified by the crystallographic technique [48]. However, it is also possible that the difference in lipid content may be caused by the different procedures used for solubilization and purification during isolation of PSII and/or the different organisms used.

Liu et al. [25] analyzed the crystal structure of LHCII bound to the PSII core complex in higher plants and found that one PG and one DGDG molecule were bound to each monomer of the complex. They demonstrated that the complex formed a trimer, which in turn aggregated to form an icosahedral sphere consisting of 20 LHCII trimers. The DGDG molecules were located at the contact surfaces between adjacent trimers, suggesting that they may be required for maintenance of the icosahedral structure. The PG molecules were buried at the interfaces between the monomers and therefore may be required for the trimerization of monomers. The requirement for PG in the trimerization of monomers was supported by the finding that treatment of the complex with phospholipase resulted in the dissociation of trimers into monomers [64]. It has also been suggested that PG molecules may play a direct structural role in the binding of one of the antenna Chls.

Kurisu et al. [45] crystallized the Cyt b_6f complex from *Mastigocladus laminosus* and found two phosphatidylcholine molecules per monomer of the complex after exogenous phosphatidylcholine had been added during purification and crystallization of the complex. Stroebel et al. [46] crystallized the Cyt b_6f complex from *Chlamydomonas reinhardtii* and found one SQDG molecule and two other lipid molecules (probably MGDG) but no PG.

4. Roles of lipids in photosystem II

4.1. MGDG

Arabidopsis contains three MGDG synthases, and MGD1 predominantly contributes to the activity of MGDG synthase in photosynthetic tissues as described in Section 2. The role of MGDG in photosynthesis was studied using an *Arabidopsis mgd1-1* mutant in which a T-DNA tag was inserted into the promoter region of *MGD1* and the content of MGDG was reduced to 42% compared to the wild-type [65]. The mutant had a yellow-green phenotype that correlated with a 50% deficiency in total Chl per plant and the impaired development of thylakoid membranes. In the dark-grown cotyledons, however, the etioplasts in the mutant were essentially the same as those in the wild-type,

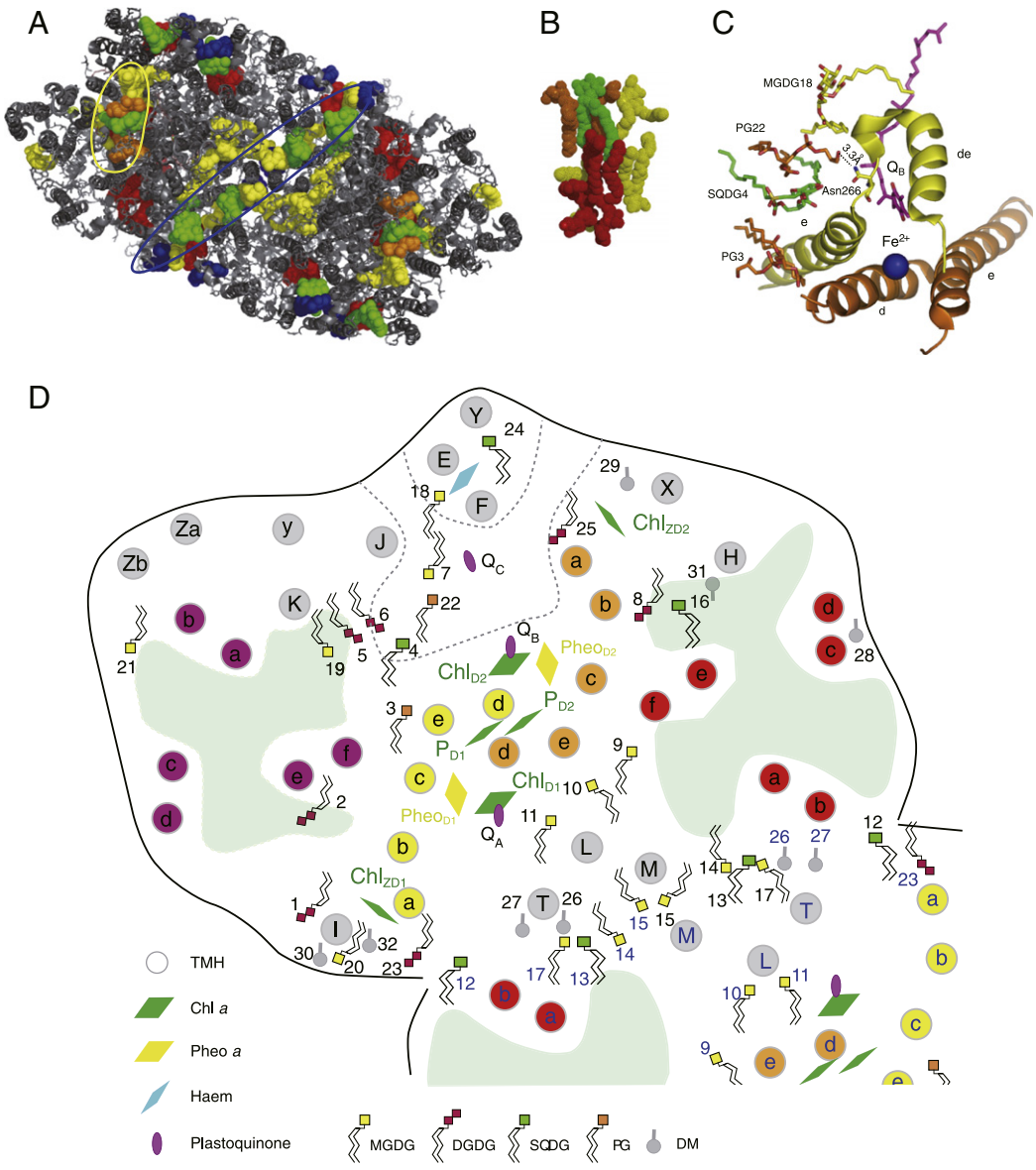

Fig. 4. Lipids in the PSII complex from *Thermosynechococcus elongatus*. (A) Location of lipids in the homodimer viewed from the cytoplasm. Membrane-embedded proteins are shown in gray, and lipids are represented in color (MGDG yellow, DGDG red, PG orange, SQDG green, DM blue). The blue and yellow ellipses indicate the monomer–monomer interface and the region forming a lipid bilayer structure enlarged in panel B, respectively. (B) View along the membrane plane of the eight lipids in the lipid cluster (indicated by the yellow ellipse in panel A) forming a bilayer structure in the PQ/PQH_2 exchange cavity. Color codes are the same as in (A). The negatively charged PG3, PG22, and SQDG4, and neutral MGDG18, are on the cytoplasmic (top) side, and the neutral MGDG19, DGDG5, DGDG6, and MGDG7 are on the luminal (bottom) side. (C) Lipids close to Q_B (violet). The negatively charged lipids PG3 (orange), PG22 (orange), and SQDG4 (green), the neutral lipid MGDG18 (yellow), non-heme Fe^{2+} (blue sphere), and part of D1 (yellow) and D2 (orange) subunits forming the Q_B binding site are shown. The conserved D1-Asn266 forms a hydrogen bond with glycerol of PG22. (D) Schematic representation of the locations of lipids and transmembrane α-helices (TMH) in PSII. One monomer and part of the other monomer of the dimeric complex viewed from the cytoplasmic side are shown as in (A). Areas indicated by light green are regions occupied with Chl *a* molecules bound to CP43 and CP47. Lipids and detergent molecules located on the luminal and cytoplasmic sides are drawn with head groups pointing "downwards" or "upwards," respectively. The PQ/PQH_2 exchange cavity is indicated by dotted lines. TMHs of the main subunits are highlighted in yellow (D1), orange (D2), red (CP47), magenta (CP43), and gray (low molecular mass subunits). The five TMHs of D1 and D2, and the six TMHs of CP43 and CP47 are labeled a–e and a–f, respectively, and the TMHs of the small subunits are labeled by capital letters in black (one monomer) and blue (the other monomer). Only Ycf12 is labeled "y." Panels A–C are drawn based on the dimer structure at 2.9 Å resolution (PDB accession codes 3BZ1 and 3BZ2) [24]. Panel D is adapted from Guskov et al. [24].

suggesting the importance of MGDG in photosynthesis. Aronsson et al. [66] studied the photosynthetic properties of the *mgd1-1* mutant. The PSII activity (F_V/F_M) was almost the same in the wild-type and *mgd1-1* mutant, indicating that a reduction in MGDG content of approximately 40% has no impact on PSII activity. It is likely that the remaining MGDG is sufficient to maintain the structure and function of PSII. Leng et al. [22] examined the effects of lipase on the structure and function of PSII using the PSII dimer complex from *T. vulcanus*. They found that lipase treatment specifically degraded MGDG from 10.0 molecules/PSII monomer to 4.8 molecules/PSII monomer (a decrease of 52%) without changing the content of other classes of lipid, but it decreased oxygen evolution by only 16%. The content of MGDG in this PSII preparation (10 molecules/PSII monomer) before lipase treatment was almost the same as that identified in the crystal structure (11 molecules/PSII monomer) of PSII from *T. elongatus* [24]. These observations indicated that five molecules of MGDG per PSII monomer digested by lipase do not participate in the important function of PSII. The remaining five molecules of MGDG, which are resistant to lipase treatment and are inherently associated with PSII, may play important roles in maintaining the structure and function of PSII.

Interestingly, the *mgd1-1* mutant contained less zeaxanthin and more violaxanthin than the wild-type and became more susceptible to photoinhibition of PSII with the impaired capacity for thermal dissipation of excess light (qE), indicating impairment of the xanthophyll cycle, *i.e.*, the conversion from violaxanthin to zeaxanthin in the mutant. In the mutant, the conductivity of thylakoid membranes was increased at high light intensities because of a change in the membrane properties by the reduction of MGDG, and the thylakoid lumen became less acidic than that in the wild-type. Thus, the reduction of qE in the mutant was ascribed to the impairment of pH-dependent activation of the violaxanthin de-epoxidase. *In vitro* studies have indicated that MGDG also supports violaxanthin de-epoxidase activity by efficiently solubilizing the xanthophyll pigments diadinoxanthin and violaxanthin [67,68]. The hexagonal structure-forming lipids MGDG and phosphatidylethanolamine are able to solubilize these xanthophyll pigments at lower concentrations than the bilayer-forming lipids DGDG and phosphatidylcholine. Kobayashi et al. [69] studied the effects of a lack of MGDG on photosynthesis and chloroplast development using a complete knockout mutant of *MGD1* (*mgd1-2*) in *Arabidopsis*. The *mgd1-2* mutant seeds germinated as small albinos only in the presence of sucrose. The seedlings lacked MGDG and DGDG, and did not develop thylakoid membranes, leading to complete impairment of photosynthetic activity and photoautotrophic growth. They lacked the major PSII proteins, such as D1, D2, and the light-harvesting complex II (LHCII). These results clearly indicate that MGDG and DGDG are essential for the development of thylakoid membranes and the stable accumulation of Chls and PSII proteins. However, it is difficult to evaluate the specific role of MGDG in PSII from studies with the *mgd1-2* mutant because, in the mutant, formation of thylakoid membranes itself is impaired due to the substantial losses of bulk lipids for the thylakoid membranes and, in addition to MGDG, DGDG is also absent. For this purpose, it would be necessary to make and analyze mutants in which the accumulation level of MGDG is varied but the level of DGDG is unaffected [66]. Disruption mutants of genes for enzymes involved in MGDG biosynthesis in cyanobacteria have not yet been isolated.

4.2. DGDG

4.2.1. Higher plants

In *Arabidopsis*, two genes (*DGD1* and *DGD2*) are specifically involved in the biosynthesis of DGDG. The role of DGDG in photosynthesis in higher plants has been investigated *in vivo* using three *Arabidopsis* mutants, *i.e.*, *dgd1*, *dgd2*, and *dgd1 dgd2*. The *dgd1* mutant, which has a point mutation in the *DGD1* gene, had markedly less DGDG content (1% of total lipids compared to 15% in the wild-type) and a dwarf phenotype, pale green leaves, and alternation of chloroplast structure [70]. Steady-state Chl fluorescence measurements revealed a decreased quantum yield of photosynthesis in the mutant [70]. Kelly et al. [33] isolated a T-DNA insertional mutant (*dgd2*) of the *DGD2* gene and generated the double mutant, *dgd1 dgd2*. The *dgd2* mutant contained similar levels of DGDG to the wild-type and showed normal growth. The double mutant contained only trace amounts of DGDG and showed more severe growth retardation than the *dgd1* mutant, with a strongly impaired capability for photoautotrophic growth. These results suggest that DGDG is important for growth, the development of chloroplasts, and optimal photosynthesis in *Arabidopsis*, and that the residual amount of DGDG in *dgd1* is functionally essential for photosynthesis. Further detailed analyses of the effects of DGDG depletion were performed using the *dgd1* and *dgd1 dgd2* mutants [8,71,72]. The results of these studies indicated that DGDG deficiency mainly affects the structure and function of PSII, primarily on the donor side. In the *dgd1* mutant, the PSII/PSI ratio was reduced to 60%, and the levels of LHCII relative to PSII core proteins (D1 and α-subunit of Cyt b_{559}) and inner antenna complexes (Lhcb4 and Lhcb5) were increased compared to those in the wild-type, but oxygen evolution activity in the mutant was almost the same as that in the wild-type [71]. Analyses of the DGDG-deficient *dgd1* and *dgd1 dgd2* mutants with a laser flash fluorometer [8,72] revealed the following results: (1) significant changes in the Chl fluorescence parameter $F(t)/F_0$ occurred due to the deficiency of DGDG; (2) in the mutants *dgd1* and *dgd1 dgd2*, the probability of the dissipative recombination reaction between $P680^+$ and Q_A^- increased by factors of about two and four, respectively; (3) the acceptor side reactions were only slightly affected; (4) excitation with saturated actinic laser flashes gave rise to elevated carotenoid triplet formation in *dgd1* and *dgd1 dgd2* mutants; and (5) the relationship between DGDG content and functional effect(s) on PSII was nonlinear. These PSII properties in the DGDG-deficient mutants probably reflect the defects on the donor side of PSII. For example, in the DGDG-deficient mutants, charge recombination reactions between $P680^+$ and Q_A^- occur more efficiently than those in the wild-type as a result of slower electron donation to PSII from water. It is also suggested that the total DGDG pool is highly heterogeneous in its functional relevance for PSII—only a small fraction of DGDG is essential for a fully active PSII complex. This small amount of DGDG is most likely specifically bound and predominantly affects the reaction properties of the PSII donor side.

4.2.2. Cyanobacteria

The recent identification of the gene encoding DGDG synthase in *Synechocystis* sp. PCC 6803 (*slr1508*, a *ycf82* homolog) has allowed the generation of disruption mutants of *dgdA* in this organism [9,35]. As the *dgdA* mutant contains no detectable amount of DGDG, it is a powerful tool for elucidating the role of DGDG in photosynthesis. We investigated the effects of lack of DGDG on photosynthetic properties in detail using the *dgdA* mutant cells, the thylakoid membranes, and PSII preparations isolated from the *dgdA* mutant [9,73,74].

In the *dgdA* mutant, concomitant with a lack of DGDG, the content of MGDG was elevated with respect to that in the wild-type cells, whereas the contents of SQDG and PG were unaffected. These results suggest that DGDG molecules are substituted with MGDG in the mutant cells. Despite a lack of DGDG, the *dgdA* mutant showed normal photoautotrophic growth under low light conditions at 30 °C and net photosynthetic activity from H_2O to CO_2 was comparable to that of the wild-type, indicating that DGDG is not essential for photosynthesis in *Synechocystis*. However, we found that growth retardation occurred in the mutant cells on exposure to environmental stresses or nutrient ion-depleted conditions. The growth of *dgdA* mutant cells was hindered under high light intensity [73] or high temperature (38 °C) [74] conditions and suppressed under $CaCl_2$-limited conditions [73]. As the growth properties were recovered by supplementation of the growth medium with DGDG, it is evident that DGDG plays an important role in growth under these conditions. Further analyses revealed markedly increased heat and NH_2OH susceptibility of oxygen-evolving activity in the mutant cells *in vivo* compared to the wild-type controls [9,74]. These photosynthetic properties were similar to those of mutants lacking the extrinsic proteins of PSII. In mutants deficient in the extrinsic proteins, such as PsbO, PsbV, and PsbU, the manganese cluster is not protected properly and oxygen-evolving activity is easily inactivated by heat [75–79] or NH_2OH treatment [80,81]. In the mutant cells lacking PsbO or PsbV, manganese ions in the manganese cluster are reduced under dark conditions and released from PSII, causing the inactivation of oxygen evolution, whereas the activity is sustained during dark incubation in mutant cells lacking PsbU [82,83]. Thus, a dark-induced decrease in oxygen-evolving activity is a useful marker for monitoring the dissociation of extrinsic proteins from PSII *in vivo*. The *dgdA* mutant retained its oxygen-evolving activity during dark incubation similar to the wild-type and PsbU-lacking mutant, suggesting that PsbO and PsbV binding are unaffected in the *dgdA* mutant cells but PsbU is dissociated from PSII *in vivo*. Analyses of the PSII complex purified from the *dgdA* mutant cells, in which a His-tag was attached to the C-terminus of CP47, indicated that the mutant PSII had extremely low O_2 evolution activity and that the extrinsic proteins PsbO, PsbU, and PsbV were substantially dissociated from the PSII complex, suggesting that PsbO and PsbV are

dissociated from the unstable PsbU-lacking PSII complex during the purification procedure [9]. This is consistent with the report that dissociation of PsbO and PsbV occurred during purification of PSII by Ni-affinity chromatography from the PsbU-lacking mutant [84]. These results demonstrate that DGDG plays important roles in PSII through the binding of extrinsic proteins required for stabilization of the oxygen-evolving complex. Awai et al. [35] also observed the dispensability of DGDG in photosynthesis using an independently generated *dgdA* mutant. Interestingly, they observed growth retardation of the mutant under phosphate-limited conditions but the mechanism was unknown.

Experiments of photoinhibition *in vivo* revealed that the *dgdA* mutant showed increased sensitivity to photoinhibition especially at an elevated growth temperature of 38 °C [73,74]. Interestingly, in the *dgdA* mutant, photodamage as well as repair processes of photosynthesis was affected and the repair process was more severely affected than the photodamage process, suggesting that DGDG plays important roles in the repair cycle of photosynthesis. A recent model for the mechanism of photoinhibition [60,85] suggested that photodamage proceeds in two steps. The first photoinhibitory event is the release of manganese ions from the manganese cluster, leading to the inactivation of oxygen evolution. The second event is the overaccumulation of positive charge on $P680^+$ due to the lack of electron donors, which causes irreversible damage to the D1 protein at the reaction center of PSII. In the case of the *dgdA* mutant, the manganese cluster exists in a highly unstable state especially at higher temperatures because of the dissociation of PsbU, and thus the release of manganese ions occurs more easily than in the wild-type leading to irreversible photodamage of P680. It is also known that the photodamage proceeds with high efficiency in the PsbU-lacking mutant [84]. The acceleration of photodamage on the donor side of PSII through this putative mechanism may lead to enhanced photoinhibition in the *dgdA* mutant under high-temperature stress. Awai et al. [35] also showed that the rate of photosynthetic oxygen evolution is reduced in the *dgdA* mutant compared to the wild-type only when measurements are performed under high light conditions. In contrast to the *dgdA* mutant in which both photodamage and repair processes were affected, only photodamage was affected in the PsbU-lacking mutant [84]. Therefore, it is likely that there are multiple sites inside PSII affected by a lack of DGDG other than the binding of PsbU protein.

Damaged PSII is repaired *via* a multistep process involving monomerization of dimers, dissociation of CP43 from monomers, degradation of the D1 protein, synthesis of the precursor to the D1 protein, reassembly including reintegration of CP43, processing of the precursor D1 to mature D1 and the formation of a manganese cluster, and dimerization of the monomers [58–61]. Nowaczyk et al. [86] isolated monomeric PSII core complexes from *T. elongatus*, either containing or lacking the Psb27 protein. Biochemical characterization of these preparations indicated that an active PSII monomer without Psb27 (referred to as PSII monomer/−Psb27) had a normal donor side composition with PsbO, PsbU, and PsbV. The other PSII monomer with Psb27 (PSII monomers/+Psb27) had no oxygen evolution activity and lacked the manganese cluster, PsbO, PsbU, and PsbV. They proposed that PSII monomer/+Psb27 is an intermediate state prior to incorporation of the manganese cluster to the oxygen-evolving complex, leading to the formation of PSII monomer/−Psb27. Interestingly, our biochemical analysis of PSII purified from the *dgdA* mutant by ultracentrifugation of the crude PSII on a glycerol density gradient showed greater accumulation of inactive PSII monomer with Psb27 and Psb28 than that of active PSII dimer [9], suggesting the inhibition of dimer construction from the inactive monomer *via* the active monomer in the *dgdA* mutant. In addition, our preliminary Blue-Native PAGE (BN-PAGE) analysis of PSII purified from *dgdA* mutant cells detected significant accumulation of the putative intermediate of PSII assembly, the CP43-lacking monomer, indicating interruption of the association of CP43 with the monomer during the repair process due to the lack of DGDG (N. Mizusawa, S. Sakata, I. Sakurai, H. Kubota, N. Sato, H. Wada, unpublished results).

Recently, the X-ray crystallographic structure of the dimeric form of PSII from *T. elongatus* has been improved compared to previous structures [47], and the authors identified seven DGDG molecules per monomer [24]. Notably, the polar head groups of all DGDG molecules face the luminal side. Four of the DGDG molecules (DGDG1, DGDG2, DGDG5, and DGDG6) are located between D1 and CP43 subunits; one molecule (DGDG23) is located close to D1, PsbI subunits, and the CP47 subunit of another monomer; another molecule (DGDG25) is located between the D2 subunit, α-subunit of Cyt b_{559}, and PsbY; and the other molecule (DGDG8) is located between D2 and CP47 subunits (Fig. 4D). However, no direct interactions among these DGDG molecules and the extrinsic proteins or the manganese cluster are apparent in the crystal structure [24]. As all DGDG molecules in the wild-type PSII may be replaced with MGDG in the *dgdA* mutant [9], it is likely that the absence of one galactose moiety from each DGDG molecule affects interactions among the PSII proteins, especially between CP43 and D1, and causes a conformational change on the luminal side of PSII that leads to dissociation of the extrinsic proteins and interruption of the smooth insertion of CP43 into the premature PSII intermediate. Hölzl et al. [87] reported that the growth defect in the *dgd1* mutant of *Arabidopsis* could be complemented by the production of glucosylgalactosyldiacylglycerol; however, this lipid did not compensate for the function of PSII. Their findings indicate an important role of the second galactose moiety of DGDG molecules in the function of PSII.

4.3. SQDG

4.3.1. Green algae

The roles of SQDG in oxygenic photosynthesis have been investigated using various SQDG-deficient mutants of cyanobacteria, green algae, and higher plants. Sato and coworkers comprehensively characterized the photosynthetic properties of SQDG-deficient mutants of the green alga *C. reinhardtii* (for review see [16]). They isolated a mutant of *Chlamydomonas*, designated *hf-2*, from a population of UV-induced mutants showing high-Chl fluorescence yields [88]. The *hf-2* cells contained extremely curled thylakoid membranes [88] and showed slightly reduced growth with a decrease in PSII activity by 30–40% but no effect on PSI activity [89]. The lowered PSII activity in the *hf-2* cells could be recovered by supplementation of the growth medium with SQDG [90], indicating the importance of SQDG in the PSII of *Chlamydomonas*. Further detailed analyses have indicated that SQDG deficiency affects both acceptor and donor sides of PSII. The PSII activity and growth in *hf-2* cells exhibit higher sensitivity to an inhibitor, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), for electron transport from Q_A to Q_B, suggesting some modification around the Q_B binding site, although the sensitivity toward another inhibitor that also inhibits electron transport from Q_A to Q_B, atrazine, remains unaffected [90,91]. Assays of photoreduction of 2,6-dichlorophenol indophenol in the presence or absence of diphenylcarbazide and flash-induced decay of Chl fluorescence in the *hf-2* thylakoid membranes revealed that the lowered activity in the mutant was derived from a decrease in the efficiency of electron donation from water to Y_Z^+, *i.e.*, redox-active Tyr_{161} of the D1 protein, not from the efficiency of the electron transport from Q_A to Q_B [92]. The PSII activity in the *hf-2* mutant was more sensitive to treatment with hydroxylamine [92] or high temperature [91], suggesting instability for binding of the manganese cluster and extrinsic proteins to PSII. These phenomena resemble those observed in the DGDG-deficient mutants (see Section 4.2.2) but the locations of DGDG and SQDG within PSII in *T. elongatus* are different ([24]; Fig. 4D). In contrast to DGDG, the polar head groups are all located on the luminal side, whereas the head groups of all SQDQ molecules (five per monomer) face the cytoplasmic side, far from the donor side of PSII. Notably, one SQDG molecule (SQDG4) is located close to the PQ-PQH_2 exchange cavity and forms a “cork” with MGDG18 and PG22 to seal the cavity (Fig. 4C). Therefore, it is likely that the change in affinity of DCMU

to the Q_B site is caused by the conformational change of the Q_B binding site induced by the lack of SQDG4. The defects in the donor side of PSII are probably indirect effects because the locations of SQDG identified are far from the water-oxidizing complex. Taking into consideration that the water-oxidizing complex containing the manganese cluster and Y_Z, and Q_B binding sites are both located on the D1 protein, it is possible that the conformational change in the D1 protein around the Q_B site due to the lack of SQDG in PSII of *hf-2* would be transmitted to the donor side to decrease the electron donation from water to Y_Z^+. However, we cannot exclude the possibilities that depletion of SQDG molecules other than SQDG4 affects the donor side of PSII, such as binding properties for extrinsic proteins in an indirect manner, and/or that SQDG directly affects the donor side if *Chlamydomonas* PSII has binding sites for SGDG close to the water-oxidizing complex. Similar but more severe dysfunctions of both donor and acceptor sides of PSII have been reported for the PG-depleted mutant (see Section 4.4.1).

4.3.2. Anoxygenic photosynthetic bacteria and cyanobacteria

The importance of SQDG in cyanobacteria is species-dependent, and the proportion of SQDG varies considerably between different species. *Prochlorococcus* cells contain SQDG at levels up to 60% of the total lipids, while *Gloeobacter* completely lacks SQDG [93]. SQDG-deficient mutants of *Synechocystis* sp. PCC 6803 [94] and *Synechococcus* sp. PCC 7942 [95] were generated through disruption of the *sqdB* gene for UDP-sulfoquinovose synthase. The *Synechocystis* mutant designated SD1 required supplementation with SQDG for photoautotrophic growth. The content of SQDG in the SD1 cells decreased when the cells grown in the presence of SQDG were transferred to medium without SQDG. The role of SQDG in photosynthesis could be investigated by observing phenomena that occur after transfer of the cells to medium without SQDG. Similar to the observations in *Chlamydomonas hf-2* mutant, SQDG deprivation in SD1 cells had little effect on the construction of PSI and PSII complexes, but brought about more severe damage to PSII. The activity was as low as 37% of the level before SQDG deprivation, and the cells showed increased sensitivity not only to DCMU but also to atrazine. In contrast, PSII activity and growth were not severely affected by disruption of the *sqdB* gene in *Synechococcus* sp. PCC 7942, which was done independently by two different groups [94,95]. There was little alternation in the PSII activity or its sensitivity to DCMU and atrazine in this mutant [94]. No requirement of SQDG for photosynthesis was also found for an anoxygenic photosynthetic bacterium, *Rhodobacter sphaeroides*. The *Rhodobacter* SQDG-deficient mutant made by disrupting the *sqdB* gene showed no detrimental phenotypes with respect to growth rate and function of the electron transport system including the reaction center complexes that are structurally related to the D1/D2 complex [96].

4.3.3. Higher plants

SQDG is not essential for photosynthesis in higher plants, as shown by studies with *Arabidopsis* mutants, *sqd1* and *sqd2*. In *sqd1*, SQDG was decreased by 30% compared to that of the wild-type by downregulation of SQD1 through antisense expression [97]. The 30% reduction of SQDG in *sqd1* had no influence on photosynthetic properties [97]. Yu et al. [37] isolated a null mutant of *SQD2* (*sqd2*) carrying a T-DNA insertion in the *SQD2* gene. As this mutant completely lacks SQDG, it is a good tool to study the role of SQDG in photosynthesis of higher plants. Under optimal growth conditions, however, the growth rate and pigment content were not altered in the mutant. Chl fluorescence measurements showed that the maximum quantum yields for PSII photochemistry (F_V/F_M) were very similar to those of the wild-type. The effective quantum yield of photochemical energy conversion in PSII (ΦPSII) was only slightly reduced in the mutant grown under optimal conditions. These results clearly indicate that SQDG is not important for the function of photosynthesis in *Arabidopsis*. However, SQDG plays an important role in higher plants under phosphate-limited conditions because the *sqd2* mutant showed reduced growth under such conditions. As a common phenomenon in photosynthetic organisms, the relative amount of total anionic thylakoid lipids is maintained by reciprocally adjusting SQDG and PG contents according to the availability of phosphate. Under phosphate-limited conditions, *SQD1* and *SQD2* expression are upregulated and SQDG content is increased [37,97]. Under conditions of phosphate starvation, PG content is decreased in the wild-type but remains constant in the *sqd2* mutant, leading to earlier phosphate starvation in the mutant compared to the wild-type [37]. Based on these observations, it was suggested that one of the functions of SQDG is to substitute for PG under phosphate-limited conditions to maintain the proper balance of anionic charge in the thylakoid membrane [37,98]. However, it remains unclear if PG buried inside PSII is actually substituted for SQDG under phosphate-limited conditions. In addition, it is difficult to evaluate the specific role of SQDG for photosynthesis under phosphate-limited conditions as phosphate limitation has pleiotropic effects on plant growth and metabolic reactions other than photosynthesis. In the SD1 mutant of *Synechocystis*, the PG content showed a complementary increase. However, the increased PG in SD1 could not recover its decreased PSII activity [94]. In contrast to the effect of SQDG deficiency, PG deficiency in *Synechocystis* caused suppression of oxygen evolution by addition of artificial *p*-benzoquinones [41,99]. Therefore, SQDG and PG play distinct roles in the maintenance of PSII properties, at least in *Synechocystis*.

4.4. PG

4.4.1. Cyanobacteria

In cyanobacteria, the role of PG in photosynthesis has been investigated in great detail using mutants of *Synechocystis* sp. PCC 6803 that are defective in the biosynthesis of PG. Among the genes for the three enzymes that are specifically involved in the biosynthesis of PG in *Synechocystis* (see Section 2), the *cdsA* gene for CDP-DG synthase [40] and the *pgsA* gene for PGP synthase [41] have been identified. The gene for PGP phosphatase has not yet been identified. Sato et al. [40] inactivated the *cdsA* gene for CDP-DG synthase and Hagio et al. [41] inactivated the *pgsA* gene for PGP synthase in *Synechocystis* sp. PCC 6803. The generated mutants were incapable of synthesizing PG and required an exogenous supply of PG for growth under photoautotrophic conditions. Other phospholipids, such as phosphatidylcholine and phosphatidylethanolamine, failed to support the growth of the mutant cells [41]. *Synechocystis* sp. PCC 6803 can grow under light-activated heterotrophic growth conditions in which cultures are incubated in the dark except that they are illuminated once a day for 5 min [100]. The growth of the cells under light-activated heterotrophic growth conditions is dependent on glucose in the growth medium, but not on photosynthesis. The *pgsA* mutant could grow under the light-activated heterotrophic growth conditions in the presence but not in the absence of PG. These findings indicate that *Synechocystis* sp. PCC 6803 cells are able to take up PG from the growth medium and PG is indispensable for their growth, even for photosynthesis-independent growth.

We used the above-mentioned mutants to investigate the role of PG in photosynthesis [40,41,50,99,101–103]. The content of PG in the mutant cells decreased when cells grown in the presence of PG were transferred to medium without PG. The role of PG in photosynthesis could be investigated by observing phenomena that occur after transfer of the cells to the medium without PG. The photosynthetic activity of the *pgsA* and *cdsA* mutants decreased markedly with a concomitant decrease of PG content in thylakoid membranes after PG deprivation within 3 days and the decreased activity was fully recovered to the original level following re-addition of PG to the growth medium. The decrease of photosynthetic activity induced by PG deprivation was attributed to the decrease of PSII activity, but not to the decrease in PSI activity, suggesting that PG plays an important role in PSII [40,41]. A decrease in PSI activity was observed only after a longer period of PG deprivation (more than 2 weeks) [50]. We further

checked each step of the electron transport in PSII of the *pgsA* mutant to clarify the functional site of PG [99]. Measurements of fluorescent yield of Chl indicated that accumulation of the reduced form of plastoquinone Q_A (Q_A^-) occurred in the *pgsA* mutant cells after PG deprivation. Thermoluminescence measurements also suggested the accumulation of $S_2Q_A^-$ following PG deprivation. These results indicate that electron transport from Q_A to Q_B was inhibited after deprivation of PG [99], and are consistent with the observation that Q_B-mediated transport of electrons was inhibited upon digestion of PG by treatment of PSII complex with phospholipase A_2 [22,48]. Recently, Laczkó-Dobos et al. [104] generated a new mutant of *Synechocystis* sp. PCC 6803 by disrupting the *cdsA* gene for CDP-DG synthase in the PAL mutant, which has no phycobilisomes. The mutant is not able to synthesize PG and has no phycobilisomes. The results obtained with this mutant were similar to those obtained with the *pgsA* mutant. In an immunological study with antibodies directed against PG, Kruse and Schmid [21] showed that PG is specifically associated with the D1 protein. These findings suggested that PG may play an indispensable role in maintaining the structure of the Q_B binding site of the D1 protein. We further characterized the *pgsA* mutant and found that PG depletion induces dysfunction not only on the electron acceptor side of PSII, *i.e.*, inhibition of electron transport from Q_A to Q_B, but also on the donor side [103]. To understand the reason why the donor side of PSII was impaired in the mutant, we purified PSII complex from the *pgsA* mutant cells. Analyses of the purified PSII complexes indicated that PSII from PG-depleted *pgsA* mutant cells sustained only ~50% of the oxygen-evolving activity compared to PSII from wild-type cells. We also observed dissociation of the extrinsic proteins PsbO, PsbV, and PsbU in PSII of the PG-depleted mutant cells. This dissociation of extrinsic proteins induced release of manganese ions from PSII, leading to a decrease in oxygen-evolving activity. The released PsbO re-bound to PSII when PG was added back to the PG-depleted mutant cells, even when *de novo* protein synthesis was inhibited. It is likely that the dissociation of extrinsic proteins observed in the purified PSII from the mutant cells occurs even *in vivo* because oxygen evolution activity of the PG-depleted *pgsA* mutant cells decreased following heat treatment as observed in Δ*psbO*, Δ*psbV*, and Δ*psbU* mutant cells. This property of the *pgsA* mutant resembles that of the *dgdA* mutant in which PsbU protein appears to be dissociated from PSII *in vivo*, but different in that the *psgA* mutant but not the *dgdA* mutant is susceptible to dark inactivation of oxygen evolution. As the inactivation of oxygen evolution under darkness is a measure of the dissociation of PsbO and/or PsbV (see Section 4.2.2), it is suggested that PSII of the PG-depleted mutant cells cannot functionally bind extrinsic proteins, possibly PsbO and/or PsbV, and that re-binding of extrinsic proteins is PG-dependent. A recent biochemical study using phospholipase A_2-treated thylakoids of *A. thaliana* also confirmed the results of our mutational analysis. They observed that, in addition to the inhibition of electron transfer from Q_A to Q_B, the phospholipase A_2 treatment affected the electron transfer from the manganese cluster to Yz^+ [105]. Interestingly, in their phospholipase A_2-treated thylakoids, charge separation of the PSII reaction center was also affected. However, it is questionable whether this phenomenon was solely caused by the decomposition of PG because the authors did not measure the changes in lipid content by phospholipase A_2 treatment.

In the PG-depleted *pgsA* mutant cells, we previously observed accumulation of the PSII monomer and suggested that PG is involved in the dimerization of PSII [101]. However, the requirement of PG for the binding of extrinsic proteins to the PSII core complex suggested that the accumulation of monomers may result from dissociation of extrinsic proteins and that PG is not directly involved in the dimerization of PSII. Our preliminary data obtained with mutant cells of extrinsic proteins showed that dimer formation was severely impaired in the Δ*psbO* mutant cells and partially impaired in the Δ*psbV* and Δ*psbU* mutant cells [103], suggesting that monomerization of the PSII complex in the *pgsA* mutant cells was indirectly induced by dissociation of extrinsic proteins. This conclusion is consistent with the X-ray crystal structure of the PSII complex from *T. elongatus*, suggesting the absence of PG in the interface between monomers ([24]; Fig. 4A and D).

Based on the findings obtained with the *pgsA* mutant, we expected PG molecules to be located close to the Q_B binding site and at the interface between the extrinsic proteins and the PSII core. Our biochemical analysis of lipids extracted from PSII complexes of the wild-type and *pgsA* mutant cells indicated that approximately 6 PG molecules per monomer are bound to the PSII of the wild-type cells, whereas 3 PG molecules per monomer are bound to the PSII of PG-depleted mutant cells [48]. The 3 PG molecules lost from the PSII of the mutant cells may be located near the Q_B binding site and at the interface between the extrinsic proteins and the PSII core. However, X-ray crystallographic analysis of the PSII complex from *T. elongatus* revealed that only 2 PG molecules per monomer are present in PSII [24]. The identified PG molecules are localized in the plastoquinone–plastoquinol cavity, their head groups face the cytoplasm, and 1 of the PG molecules (PG22) is close to the Q_B site (Fig. 4C and D) with a distance of about 8 Å between the PQ head group and the glycerol moiety of PG. These data are consistent with our observation that deprivation of PG affects the structural integrity of the Q_B site, but it seems unlikely that the identified PG molecules would be able to influence the binding of extrinsic proteins to the PSII core. Nevertheless, it is possible that several molecules of PG remain to be identified within the crystal structure and that they play roles in PSII.

In bacteria, PG is used as a substrate for lipid modification of lipoproteins, which are an abundant class of peripheral proteins anchored to membranes. Lipoproteins are synthesized as precursors and processed as follows. First, the diacylglyceryl moiety is transferred by prolipoprotein diacylglyceryl transferase from PG to the sulfhydryl group of a specific cysteine residue in the N-terminal region of precursors [106,107]. The modified precursors are processed to remove the signal sequence by signal peptidase II at the N-terminus of the cysteine residue that is modified with diacylglycerol [108]. Thus, the diacylglycerol-modified cysteine residue becomes the N-terminus of the mature protein. Finally, after cleavage of the signal peptide, the amino group of the N-terminal cysteine is acylated by apolipoprotein N-acyltransferase [109]. Since the first identification of lipoproteins in *Escherichia coli*, many lipoproteins have been identified in almost all bacteria and they have been shown to play important roles in many processes, such as uptake of nutrients and protein secretion [110,111]. It is likely that cyanobacteria also have many lipoproteins that play important roles in these organisms.

Bacterial lipoproteins possess a conserved signal sequence called the lipoprotein signal peptide [112,113]. To identify putative lipoproteins in cyanobacteria, we searched the genome database of *Synechocystis* sp. PCC 6803 using a hidden Markov model algorithm, LipoP (www.cbs.dtu.dk/services/LipoP/) [113], which is most commonly used for searching bacterial lipoproteins. We found about 40 putative lipoproteins in *Synechocystis* sp. PCC 6803 (Ujihara T, Katayama K, Sakurai I, Mizusawa N and Wada H, unpublished results). Intriguingly, half of the putative lipoproteins were substrate binding proteins of ABC transporters and three of them were PsbP, PsbQ, and Psb27, which are extrinsic proteins located in luminal side of PSII. Recently, we examined whether PsbQ and Psb27 of *Synechocystis* are real lipoproteins modified with lipid and found that the sulfhydryl and amino groups of PsbQ are modified with a diacylglycerol and a palmitic acid, respectively, whereas the sulfhydryl group of Psb27 is modified with diacylglycerol but the amino group is largely unmodified and only partially modified with palmitic acid (Ujihara T, Katayama K, Sakurai I, Mizusawa N and Wada H, unpublished results). Nowaczyk et al. [86] also analyzed Psb27 of *T. elongatus* and found that it is modified with diacylglycerol and palmitic acid. The different extent of modification of amino groups of Psb27 in *T. elongatus* and *Synechocystis* suggests that lipid modification of Psb27 is different between these species. In *Synechocystis*, PsbP, PsbQ, and Psb27 are not essential for growth, but are necessary for regulation of PSII activity [114–117].

4.4.2. Green algae and higher plants

In higher plants, the role of PG in photosynthesis was initially studied by treating thylakoid membranes with phospholipases that specifically degrade PG. Jordan et al. [19] showed that elimination of approximately 70% of the original PG from thylakoid membranes isolated from *Pisum sativum* (pea), by treatment with phospholipase A_2, almost completely blocked the photosynthetic transport of electrons in PSII without any significant effect on the transport of electrons in PSI. Similarly, Droppa et al. [20] showed that treatment of thylakoid membranes from pea with phospholipase C, which degraded approximately half of the original PG, almost completely eliminated the photosynthetic transport of electrons in PSII. These findings were consistent with those obtained with the *pgsA* mutant of *Synechocystis* sp. PCC 6803 [41,99]. Moreover, Kruse et al. [63] found that PSII complexes prepared from spinach were converted from dimers to monomers by treatment with phospholipase A_2 and monomers were reversibly converted to dimers by the addition of PG. Based on these findings, they suggested that PG is required for dimer formation of PSII. However, we have recently performed similar experiments with PSII dimers prepared from *T. vulcanus* and found that phospholipase treatment did not induce monomerization of PSII, but inhibited electron transport from Q_A to Q_B as found in the *pgsA* mutant cells [22]. The PSII dimer from *T. vulcanus* is very stable and extrinsic proteins are not dissociated from the PSII core during phospholipase treatment [22], whereas the PSII dimer from spinach is unstable and extrinsic proteins are released from the PSII core during incubation with phospholipase [63]. Thus, it is likely that PG is not directly involved in dimerization of PSII. The conversion of dimers to monomers observed in spinach PSII would be caused by dissociation of extrinsic proteins from the PSII following digestion of PG required for binding of extrinsic proteins during incubation with phospholipase. The digestion of PG could prevent the binding of extrinsic proteins that stabilize the dimer and induce monomer accumulation.

In addition to the biochemical approach, the role of PG in photosynthesis has also been studied by molecular genetic approaches. Dubertret et al. [118] isolated mutants of *C. reinhardtii* that were incapable of synthesizing molecular species of PG binding Δ3-*trans*-hexadecenoic acid because of a defect in the desaturation at Δ3 position of palmitic acid esterified to *sn*−2 position of PG. With the mutants they found that molecular species of PG binding Δ3-*trans*-hexadecenoic acid can be essential for assembly of the trimeric form of LHCII [118,119]. However, this finding is inconsistent with the observation that LHCII trimer was formed in a mutant of *A. thaliana* despite the absence of the molecular species of PG binding Δ3-*trans*-hexadecenoic acid [120]; hence, it is not clear whether the molecular species of PG are involved in trimer formation of LHCII. Nevertheless, it seems that the PG molecule itself is required for LHCII trimer formation. Hobe et al. [121,122] studied the interaction of PG with LHCII apoprotein, and reported that the negative charge on PG interacts with the N-terminal region, positions 16 to 21 from the N-terminus, of the LHCII apoprotein and this region plays a crucial role in the trimerization of the LHCII. Furthermore, Maanni et al. [123] suggested that the formation of a trimeric LHCII complex may be important for grana stacking in thylakoid membranes.

Mutants of *A. thaliana* with a defect in the biosynthesis of PG were also isolated and used to investigate the function of PG. Xu et al. [124] isolated a mutant that had a defect in the gene encoding PGP synthase (*PGP1*), which is located in plastids and mitochondria. The level of PG in leaves of mutant plants was approximately 25% lower than that of wild-type plants. Moreover, mutant plants had pale green leaves and a slightly reduced capacity for photosynthesis than wild-type plants, suggesting that PG may play an important role in the development of chloroplasts and the photosynthetic machinery. Hagio et al. [125] and Babiychuk et al. [43] isolated null *pgp1* mutants of *A. thaliana*. The growth of these mutants required the addition of sucrose to the standard growth medium, and the mutant plants had pale yellow-green leaves. In these mutant plants, the level of PG was only approximately 10% of that in wild-type controls, and the development of chloroplasts in the leaf cells of mutant plants was severely impaired. These findings suggest that PG is indispensable for the development of chloroplasts, in particular, for the development of thylakoid membranes.

5. Involvement of lipids in assembly and repair of PSII

The roles of lipids in assembly and repair processes in PSII have been studied mainly with cyanobacterial mutants of *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942, which are defective in the synthesis of lipids. The *dgdA* mutant lacking DGDG [73,74], and *pgsA* [101,103] or *cdsA* mutants depleted of PG [104,126], showed high-light-induced growth retardation due to the suppression of repair processes of PSII, which could be restored by reincorporation of exogenously added respective lipid. Therefore, it was suggested that DGDG and PG are involved in the assembly and repair cycles of PSII (also see Section 4.2.2). The effects of MGDG and SQDG depletion on PSII repair have yet to be studied.

PSII must be repaired at high rates under conditions of illumination due to the vulnerability of D1 to high light exposure levels [58–61]. PSII complexes are thought to exist in dimeric form when they are active in oxygen evolution, although recently there has been some question regarding whether PSII exists in a dimeric or monomeric form *in vivo* [127]. The repair processes of damaged PSII proceed through many complicated steps, including monomerization of the dimer, partial disassembly of the complex including detachment of CP43 and extrinsic proteins from the reaction center, degradation of D1 coupled with insertion of newly synthesized precursor form of D1 to the complex, reassociation of CP43 and extrinsic proteins, processing of the C-terminal extension of precursor form of D1, assembly of the manganese cluster, and dimerization of PSII ([128]; Fig. 5). Our studies showed that, in the PG- or DGDG-deficient mutant, the fraction containing the monomeric form of PSII, isolated by ultracentrifugation of the crude PSII fraction on glycerol density gradient, increases compared to that in wild-type [9,101,103]. Interestingly, these fractions showed low oxygen-evolving activities [9,103] and contained Psb27 and Psb28 proteins, which are thought to be involved in the assembly of PSII [61,86,128], suggesting that premature intermediates of PSII accumulate in these mutants. Our preliminary BN-PAGE analysis indicated that, in these monomer fractions, monomer as well as CP43-less monomer co-migrated (N. Mizusawa, S. Sakata, I. Sakurai, H. Kubota, N. Sato, and H. Wada, unpublished results). Laczkó-Dobos et al. [104] also showed that CP43-less monomer accumulated in the thylakoid membranes of the PG-deficient mutant, based on the results of pulse-chase experiments with [^{35}S]methionine and BN-PAGE. These results strongly suggest that the putative assembly (repair) intermediate, *i.e.*, CP43-less monomer, would accumulate due to retardation of the forward assembly steps by a lack of DGDG or PG. In the recent X-ray structure of dimeric PSII [24], four DGDG (DGDG1, DGDG2, DGDG5, DGDG6) and two PG (PG3 and PG22) molecules were located at the interface between D1 and CP43 where one SQDG (SQDG 4) molecule was also identified (Fig. 4). Therefore, it is suggested that removal of DGDG or PG and/or replacement of these lipids by others affect the reassociation of CP43 with CP43-less monomer, leading to the accumulation of CP43-less monomer. Removal of CP43 is proposed to facilitate the replacement of photodamaged D1. However, depletion of PG did not affect the rates of D1 turnover under illumination, suggesting that the removal of CP43 from the complex could occur normally in the PG-deficient mutant [101].

Recently, Nanjo et al. [129] reported that enoyl-(acyl-carrier-protein) reductase (FabI), a key component of the type-II fatty acid synthase, is loosely associated with PSII complexes. The components of type-II fatty acid synthase, including FabI, are localized in the stroma in plastids of plants [130]. However, some reports have indicated that components of type-II fatty acid synthase, such as acyl-carrier protein, are partially associated with thylakoid membranes [131]. Therefore, it is possible that the synthesis of fatty acids may also occur near PSII

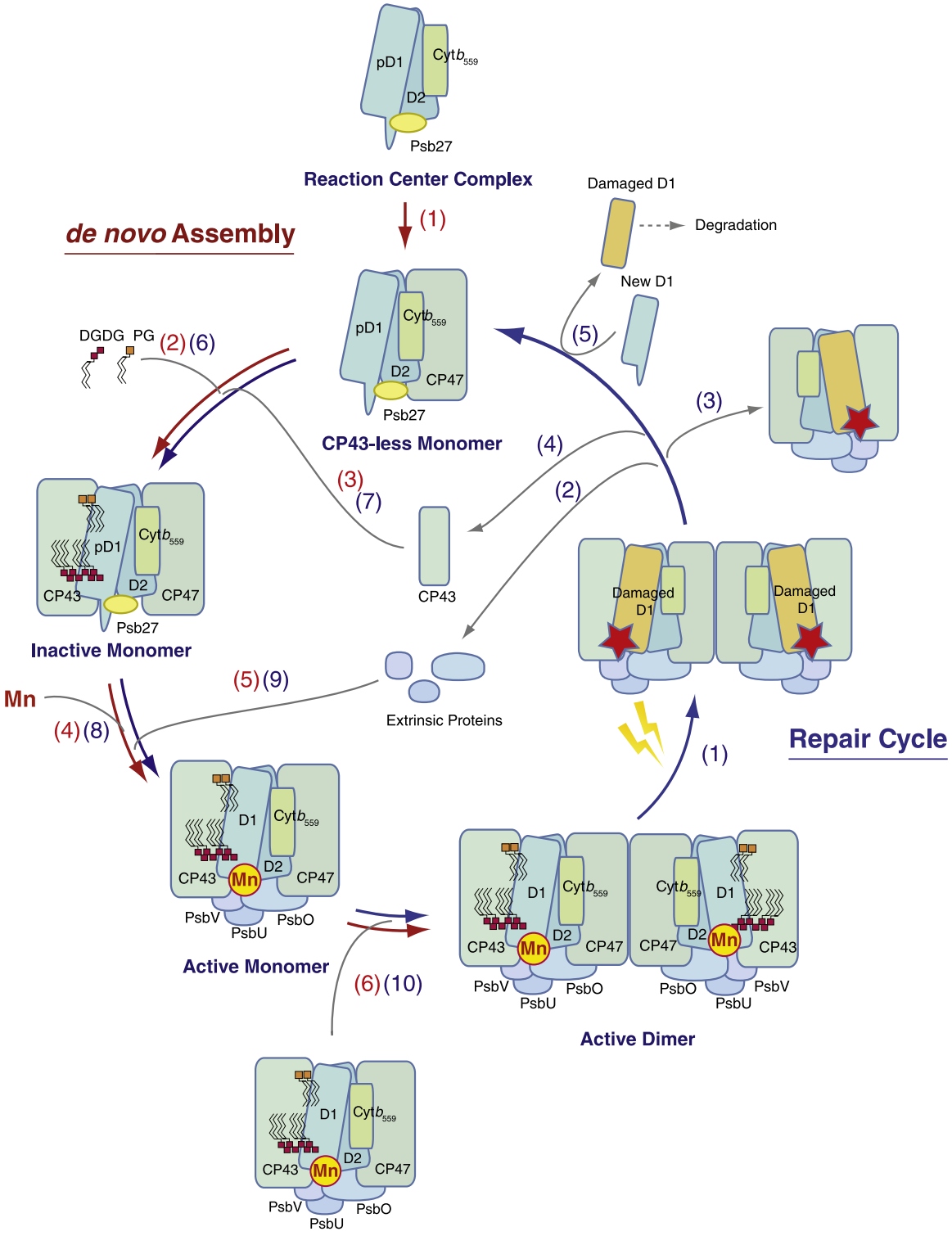

Fig. 5. A possible model for the involvement of lipids in the assembly and repair cycles of cyanobacterial PSII. Assembly (red arrows) and repair (blue arrows) processes of PSII proceed *via* multiple intermediate states as follows. In the *de novo* assembly process, the preD1/D2 reaction center complex containing Cyt b_{559} and Psb27 is formed, and then CP47 is associated with the complex to yield CP43-less monomer (assembly step 1). After its formation, DGDG and PG are presumably incorporated into the CP43-less monomer (assembly step 2), and thereby CP43 can be bound to the monomer with the aid of these lipid molecules (assembly step 3). After the binding of CP43, the C-terminal extension of precursor D1 (preD1) is processed, Psb27 is released from the complex, and the manganese cluster is formed (assembly step 4), followed by binding of extrinsic proteins such as PsbO, PsbU, and PsbV (assembly step 5). Finally, the active monomer formed at assembly step 5 is dimerized to the active dimer (assembly step 6). In the repair process, the D1 protein in the active dimer is photodamaged under strong illumination (repair step 1). In the photodamaged PSII, the extrinsic proteins and manganese ions are released from the luminal side (repair step 2), leading to monomerization of the complex (repair step 3). CP43 is then dissociated from the monomer complex (repair step 4), rendering the efficient replacement of damaged D1 with a newly synthesized preD1 (repair step 5). The damaged D1 is subjected to degradation, and Psb27 binds to the luminal side of the complex. After the formation of CP43-less monomer, the repair processes proceed according to the same pathway as the assembly processes (assembly steps 2 to 6). In this figure, only DGDG and PG molecules located between D1 and CP43 are shown for simplicity. Other lipid molecules are also incorporated into the complex at different steps in the assembly and repair processes.

complexes, although the components of type-II fatty acid synthase other than FabI have yet to be found. The authors of these studies tried to inactivate the *fabI* gene (*slr1051*) in *Synechocystis* but failed to achieve complete disruption of all the genomic copies of the *fabI* gene. The incomplete disruption was probably due to the lethal effect of the complete absence of the *fabI* gene. Despite partial inactivation of the *fabI* gene, the mutant exhibited marked impairment in the tolerance and acclimation of cells to high temperatures: photoautotrophic growth of the mutant was severely inhibited at 40 °C. Moreover, mutant cells were unable to achieve wild-type enhancement of the thermal stability of PSII when the growth temperature was raised from 25 °C to 38 °C. It was suggested that *de novo* synthesis of fatty acids as well as proteins is required for enhancement of the thermal stability of PSII during the acclimation of *Synechocystis* cells to high temperatures. Under high-temperature conditions, D1 protein turnover rate must be more rapid to maintain PSII activity than under normal-temperature conditions because the rates of damage to PSII are higher under high-temperature conditions [74]. Therefore, incorporation of lipids synthesized with fatty acids that are newly synthesized *via* FabI into PSII intermediate forms may be required to sustain the high rate of D1 turnover under high-temperature conditions.

Kanervo et al. [132] studied the role of membrane lipid unsaturation in the repair of PSII under low-temperature conditions after photoinhibition in wild-type and mutant cells of *Synechocystis* sp. PCC 6803 with genetically inactivated desaturase genes. They showed that posttranslational C-terminal processing of the precursor form of D1 is an extremely sensitive reaction in the PSII repair cycle and is readily affected by low temperature. Interestingly, the capabilities of D1 protein processing are specifically dependent on the extent of unsaturation of thylakoid membrane lipids. The mechanism underlying the perturbations due to changes in the degree of lipid unsaturation is still unclear. The C-terminal processing of the precursor form of the D1 protein is an essential reaction for manganese cluster formation and assembly of the extrinsic proteins required for stabilization of the manganese cluster [133–135]. Therefore, the inhibition of C-terminal processing of D1 results in failure of manganese cluster assembly, leading to the suppression of oxygen evolution recovery under low-temperature conditions. Effects of desaturation of PG on photoinhibition under low-temperature conditions were studied using transgenic tobacco plants containing increased amounts of disaturated PG [136]. Similar to the desaturase-inactivated cyanobacterial mutants, the transgenic plants were more chilling-sensitive than the wild-type and showed the suppression of repair process from photoinhibition [136].

It has been suggested that lipids play an important role in the dimerization of PSII because the monomer–monomer interface in the dimeric PSII is dominated by the presence of lipids and very few protein–protein interactions between the two monomers are observed (mainly contributed by the small membrane-intrinsic subunit PsbM) [24]. The monomer–monomer interface was filled with a total of 14 lipids, 7 from each monomer, as follows: 4 MGDGs (MGDG14, MGDG15, MGDG17, MGDG20), 1 DGDG (DGDG23), and 2 SQDGs (SQDG12, SQDG13) (Fig. 4D). In addition, there were eight DM molecules that may replace galactolipids during purification, suggesting the presence of additional lipids in this region when PSII is embedded in the thylakoid membrane. Depletion of PG in a *pgsA* mutant of *Synechocystis* caused monomerization of the dimeric structure of PSII. As no PG was identified in the dimeric interface, the monomerization observed in the PG mutant is likely to be caused by secondary effects, *i.e.*, dissociation of extrinsic proteins of PSII, which occur upon depletion of PG as discussed in Section 4.4.1. In DGDG-deficient *dgdA* mutant of *Synechocystis*, the increase in the monomeric form of PSII was also observed. As the dissociation of the extrinsic proteins of PSII occurred in the *dgdA* mutant, the monomerization of PSII observed in the mutant may be also a secondary effect. However, we cannot exclude the possibility that DGDG participates directly in the dimerization of PSII because one DGDG molecule (DGDG23) was found in the monomer–monomer interface. The four MGDG molecules and two SQDG molecules found in the monomer–monomer interface may play important roles in dimer formation and in the dissociation of the dimer during the repair process. Notably, the monomeric form of PSII from *T. elongatus* with high oxygen-evolving activity, which was recently analyzed by X-ray crystallography [62], lacked SQDG12, one of the two SQDG molecules located in the dimeric surface, suggesting the important function of SQDG12 in dimer formation in *T. elongatus*. Further studies using a combination of SQDG-deficient mutants and site-directed mutagenesis directed toward the putative interaction sites between the polar head group of SQDG and PSII subunits will uncover details of the roles of SQDG in dimer formation.

6. Conclusions and future perspectives

Over the past decade, many of the genes encoding enzymes that are required for the biosynthesis of lipids in cyanobacteria and higher plants have been identified using powerful approaches, such as comparative genomic analysis. This allowed us to make mutants defective in the biosynthesis of lipids to detail the functions of lipids in photosynthesis. Extensive studies with the mutants have revealed that MGDG, DGDG, SQDG, and PG depletion or reduction causes defects in PSII, and that these lipids each have important functions in PSII. PG and SQDG are required for structural integrity of the Q_B binding site of the D1 subunit. DGDG and PG are involved in the binding of extrinsic proteins, which stabilize the manganese cluster in PSII; the association of CP43 subunit with CP43-less monomer; and the formation of the dimeric structure during assembly and repair processes of PSII. Information on the role of MGDG in photosynthesis is still limited because of the difficulty in making a mutant lacking only MGDG.

Recent structural analyses of PSII from the thermophilic cyanobacteria *T. elongatus* and *T. vulcanus* have provided a great deal of information regarding the structural and functional roles of lipids in PSII. It has become evident that lipids are essential not only as the major components of the membrane matrix in which to embed the complex but also as integral components of PSII to fulfill specific important roles in assembly and repair processes. It appears that lipids are important components in PSII for providing the specific interactions between the PSII core and extrinsic proteins, but yield flexibility to avoid excessively strong binding to allow efficient dissociation of extrinsic proteins from the core complex during the assembly and repair processes of PSII. The same functions of lipids are also required in monomer–monomer interactions for dimer formation and dissociation into monomers that proceed in the assembly and repair of PSII. However, studies on the roles of lipids in PSII were performed with mutants lacking one of the thylakoid lipids, *i.e.*, whole modification of one of four lipid classes in thylakoid membranes. In future studies, it will be necessary to clarify the roles of individual lipid molecules bound to PSII by site-directed mutagenesis directed toward substitution of the specific amino acid residues involved in the binding of each lipid molecule. It will also be necessary to perform structural studies with *Synechocystis* sp. PCC 6803, in which extensive information about various mutants has already been accumulated and the methods for gene manipulation have been well established. In addition, this organism can grow even with defects in PSII in the presence of glucose; thus, it is a suitable system for mutational analysis of PSII. Elucidation of the X-ray crystal structure of PSII from various photosynthetic organisms, including *Synechocystis*, will provide valuable information for understanding the universality and diversity of roles of individual lipid molecules in the structure and function of PSII by comparing the conserved and nonconserved lipid binding sites among different photosynthetic organisms.

The latest X-ray structure of PSII with 2.9 Å resolution [24] would still not be enough to unambiguously locate all of the lipid molecules in PSII. For example, in the case of cytochrome *c* oxidase, even a 1.8 Å resolution was not enough to determine all of the lipid molecules, and

other techniques had to be used in parallel to determine all of them [137]. It might be possible therefore that in the 2.9 Å resolution structure of PSII, some of the lipid molecules are assigned ambiguously and/or incorrectly. The detailed structural analysis of PSII with higher resolution is required to determine their localization conclusively.

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