J. Biochem. **140**, 201–209 (2006)
doi:10.1093/jb/mvj141

Lipids in Oxygen-Evolving Photosystem II Complexes of Cyanobacteria and Higher Plants

Isamu Sakurai[1], Jian-Ren Shen[2], Jing Leng[2], Shunsuke Ohashi[3], Masami Kobayashi[3] and Hajime Wada[1,*]

[1]Department of Life Sciences, Graduate School of Arts and Sciences, University of Tokyo, Komaba 3-8-1, Meguro-ku, Tokyo 153-8902; [2]Department of Biology, Faculty of Science, Okayama University, Tsushima-naka, Okayama 700-8530/PRESTO, JST; and [3]Institute of Materials Science, University of Tsukuba, Ibaraki 305-8573

Received April 13, 2006; accepted May 24, 2006

Lipids in dimeric photosystem II complexes prepared from two species of cyanobacteria, *Thermosynechococcus vulcanus* and *Synechocystis* sp. PCC6803, and two higher plants, spinach and rice, were analyzed to determine how many lipid molecules and what class of lipids are present in the photosystem II complexes. It was estimated that 27, 20, 8, and 7 lipid molecules per monomer are bound to the dimeric photosystem II complexes of *T. vulcanus*, *Synechocystis*, spinach, and rice, respectively. In each of the organisms, the lipid composition of the photosystem II complexes was quite different from that of the thylakoid membranes used for preparation of the complexes. The content of phosphatidylglycerol in the photosystem II complexes of each organism was much higher than that in the thylakoid membranes. Phospholipase A_2 treatment of the photosystem II complexes of *Synechocystis* that degraded phosphatidylglycerol resulted in impairment of Q_B-mediated but not Q_A-mediated electron transport. These findings suggest that phosphatidylglycerol plays important roles in the electron transport at the Q_B-binding site in photosystem II complexes.

Key words: lipid, phosphatidylglycerol, photosynthesis, photosystem II complex, thylakoid membrane.

Abbreviations: Chl, chlorophyll; DCBQ, 2,6-dichloro-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1, 1-dimethylurea; DGDG, digalactosyldiacylglycerol; DM, *n*-dodecyl β-D-maltoside; DMBQ, 2,6-dimethyl-*p*-benzoquinone; Fecy, potassium ferricyanide; MGDG, monogalactosyldiacylglycerol; MALDI-TOF MS, matrix-assisted laser desorption ionization time of flight mass spectrometry; PG, phosphatidylglycerol; Pheo, pheophytin; PLA_2, phospholipase A_2; PSI, photosystem I; PSII, photosystem II; SQDG, sulfoquinovosyldiacylglycerol; X:Y(Z), fatty acid containing X carbon atoms with Y double bonds in the *cis* configuration at position Z counted from the carboxy terminus.

In thylakoid membranes, the sites of primary photosynthetic reactions, electrons are transferred through a series of photosynthetic machineries to convert light energy into biologically useful chemical energy (*1*). The lipids that constitute thylakoid membranes show unique compositions compared with other biological membranes (*2*, *3*). Specifically, thylakoid membranes have high proportions of glycolipids monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), and sulfoquinovosyldiacylglycerol (SQDG). They also have low proportions of phospholipid phosphatidylglycerol (PG). Biochemical and molecular biological analyses have indicated the importance of these lipids in the photosynthesis and heterogeneous distribution of lipids in various protein complexes in thylakoid membranes. The depletion of PG from thylakoid membranes by treatment with a phospholipase caused suppression of the electron transport activity in photosystem II (PSII) (*4*, *5*). The oxygen-evolving activity of a PSII preparation derived through solubilization of spinach thylakoid membranes with Triton X-100 was stimulated by exogenously added DGDG and phosphatidylcholine (*6*). The treatment of a PSII complex prepared from thylakoid membranes of spinach with phospholipase A_2 (PLA_2), which cuts the ester bond at the *sn*-2 position of phospholipids, resulted in dissociation of the complex from dimer to monomer, suggesting that PG is involved in dimerization of the PSII complex (*7*). The activity of the cytochrome b_6/f complex was reconstituted with DGDG, PG, and phosphatidylcholine, but not with MGDG or SQDG (*8*). DGDG and PG are bound to the trimeric form of light-harvesting chlorophyll *a*/*b* binding protein complexes (LHCII) and play important roles in the construction of these protein complexes (*9*). The roles of lipids in photosynthesis were also investigated with mutants defective in the biosynthesis of thylakoid lipids. An *mgd1* mutant of *Arabidopsis thaliana* that is deficient in the biosynthesis of MGDG in chloroplasts was isolated and used to demonstrate that MGDG is required for the development of chloroplast membranes (*10*). Involvement of the molecular species of PG which contains a Δ3-*trans*-hexadecenoic acid [16:1(3t)] in the trimerization of LHCII was suggested by a series of studies involving a mutant of *Chlamydomonas reinhardtii* defective in the biosynthesis of 16:1(3t) (*11*). The role of SQDG in PSII complexes was studied with the *hf-2* mutant of *C. reinhardtii*, and the results demonstrated that it has an important role in the electron transfer from water to

*To whom correspondence should be addressed. Tel/Fax: +81-3-5454-6656, E-mail: hwada@bio.c.u-tokyo.ac.jp

tyrosine Z on the donor side of PSII (*12*, *13*). We recently constructed mutants of *Synechocystis* sp. PCC6803 defective in the biosynthesis of PG and investigated the function of PG in photosynthesis (*14–16*). In a series of reports, we demonstrated that PG deficiency induces conformational changes in the binding site for plastoquinone Q_B in the D1 protein and thereby leads to impairment of electron transport from Q_A to Q_B in the PSII reaction center. These findings suggested that PG has an indispensable role in maintenance of the structure of the Q_B-binding site of D1 protein (*16*). PG was found to be necessary also for the assembly and stabilization of the photosynthetic machineries, because the presence of PG enhanced the formation of dimeric PSII complexes and trimeric photosystem I (PSI) complexes (*17–19*).

In addition to the findings in the biochemical and molecular biological studies mentioned above, from a structural viewpoint the importance of thylakoid lipids in the protein complexes involved in photosynthesis has become apparent. One molecule of MGDG and three molecules of PG were identified in the crystal structure of the PSI complex, suggesting that these lipids contribute to the structure of this protein complex (*20*). It has also been reported that phosphatidylcholine is needed to obtain high-quality crystals of the cytochrome b_6/f complex because it stabilizes this protein complex, although it is not a lipid present in thylakoid membranes (*21*). The crystal structure of the PSII complex at different resolutions has been reported (*22–24*), and 14 lipids, namely, four DGDG, six MGDG, three SQDG, and one PG, were assigned in the most recently determined crystal structure of the PSII complex at 3.0 Å resolution (*25*). In the structure, some other lipid-like components, which were not assigned, were also found, especially in the cavity harboring the Q_B-binding site. These findings suggest that the lipids have structural and functional important roles in the PSII complex, but the actual numbers of lipid molecules bound to the PSII complex remain obscure. Further biochemical analyses are required to determine the numbers of lipids, and to understand their structural and functional roles in the PSII complex.

In this study, we analyzed the lipid compositions in dimeric PSII complexes to determine how many lipid molecules and what classes of lipids are present in PSII complexes. The PSII complexes used for lipid analysis were purified from two species of cyanobacteria, *Thermosynechococcus vulcanus* and *Synechocystis* sp. PCC6803, and from two higher plants, spinach and rice. In addition to the lipid analysis, we also examined the effects of PLA_2 treatment on the activity of the PSII complex prepared from *Synechocystis* to study functional roles of PG in the PSII complex.

MATERIALS AND METHODS

Organisms and Growth Conditions—For the purification of PSII complexes from *Synechocystis* sp. PCC6803, a transformant with six histidine residues as a tag at the C-terminus of the CP47 subunit of a PSII complex was constructed as described by Bricker *et al*. (*26*). The obtained transformant of *Synechocystis* sp. PCC6803 carrying the six-histidine tag is referred to as *Synechocystis*. Wild-type cells of *T. vulcanus* and *Synechocystis* cells were grown photoautotrophically at 55°C and 30°C, respectively, as described previously (*27*). Spinach was purchased from a local market and rice was grown in a local field for 2 months.

Preparation of Thylakoid Membranes and PSII Complexes—Thylakoid membranes and PSII dimer complexes from *T. vulcanus* were purified as described by Shen and Inoue (*28*). Thylakoid membranes and PSII complexes of *Synechocystis* were prepared according to Kashino *et al*. (*29*) with minor modifications as follows. Protein complexes in thylakoid membranes were solubilized with 1% *n*-dodecyl β-D-maltoside (DM) (D4641; Sigma) and the solubilized protein complexes were subjected to Ni-affinity column chromatography (Ni-NTA column; Qiagen) to purify the PSII complexes. The resultant PSII complexes were further purified by ultracentrifugation through a linear glycerol density gradient containing 5–30% glycerol (w/v) in a medium comprising 10 mM $MgCl_2$, 5 mM $CaCl_2$, 1 M glycine betaine, 0.03% DM, and 50 mM MES-NaOH (pH 6.5), made with a Gradient Master (Model 107ip; Biocomp Inc., New Brunswick, Canada). The gradient was centrifuged at 180,000 × *g* for 16 h at 4°C (P40ST rotor; Hitachi). Fractions containing the dimeric form of PSII complexes were collected and concentrated by ultrafiltration (Amicon Ultra-4; Millipore).

PSII dimer complexes of spinach and rice were prepared from BBY-type (*30*) PSII membranes (*31*). The PSII membranes were treated with 2.3% *n*-heptyl β-D-thioglucoside to remove major LHCII proteins (*32*). The resultant PSII particles were treated with 1.2% DM at a chlorophyll (Chl) concentration of 1 mg/ml at 4°C for 30 min and then purified on a MonoQ column (Amersham Pharmacia Biotech). The obtained PSII was concentrated by ultrafiltration through a membrane filter (Amicon YM30; Millipore) and then treated again with 1.0% DM at 4°C for 30 min, followed by size-exclusion chromatography on two columns of HiLoad 26/60 Superdex 200pg (Amersham Pharmacia Biotech) linked serially. The obtained PSII dimer complexes described above were used for lipid analysis. The concentration of Chl was determined by the method of Arnon *et al*. (*33*), and the molar ratio of Chl to pheophytin (Pheo) was determined by HPLC as described previously (*34*)

SDS-PAGE—The polypeptide compositions of PSII dimer complexes prepared from *T. vulcanus* and *Synechocystis* were analyzed by SDS-PAGE according to the method described by Kashino *et al*. (*35*), with a gradient gel of 18–24% polyacrylamide containing 6 M urea. While those of PSII dimer complexes prepared from spinach and rice were analyzed by SDS-PAGE as described by Ikeuchi and Inoue (*36*), with a gradient gel of 16–22% polyacrylamide containing 7.5 M urea. Polypeptides separated on the gels were visualized with Coomassie Brilliant Blue R250 (Fulka).

Mass Spectrometry—Subunits of PSII dimer complexes prepared from *Synechocystis* were identified by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS). Individual polypeptides separated by SDS-PAGE were excised and subjected to in-gel digestion with trypsin (V5280; Promega). The digested peptide fragments were extracted from the gel and analyzed by MALDI-TOF MS after removal of salts

on a reversed-phase micro-column (Zip-Tip; Millipore). MALDI-TOF MS was performed on AXIMA-CFR (Shimadzu) using α-cyano 4-hydoroxycinnamic acid (C2020; Sigma) as the matrix. Proteins were identified as the highest ranking result by searching in the National Center for Biotechnology Information database using Mascot (http://www.matrix-scinence.com).

Measurement of Photosynthetic Activities—Photosynthetic activities were measured by means of oxygen exchange with a Clark-type oxygen electrode as described by Gombos *et al*. (*37*). In the case of *T. vulcanus*, the activities were measured at 30°C in a medium comprising 30 mM Mes (pH 6.0), 20 mM NaCl, and 3 mM $CaCl_2$. The concentrations of 2,6-dichloro-*p*-benzoquinone (DCBQ) and potassium ferricyanide (Fecy), which were used as electron acceptors, were 0.5 mM and 1 mM, respectively. The activities of the PSII dimer complex prepared from *Synechocystis* were measured at 30°C in a medium comprising 50 mM Mes (pH 6.0), 10 mM NaCl, 20 mM $CaCl_2$, and 1 M sucrose. The concentrations of DCBQ and Fecy were 0.5 mM and 6 mM, respectively. In the case of spinach and rice, the activities were measured at 25°C in a medium comprising 30 mM Mes (pH 6.5), 20 mM NaCl, 5 mM $CaCl_2$, and 0.5 mM each of electron acceptors.

Lipid Analysis—Lipids were extracted from thylakoid membranes and PSII dimer complexes by the method of Bligh and Dyer (*38*). Lipids from thylakoid membranes and PSII complexes, which were not treated with PLA_2, were separated into lipid classes on TLC plates as described previously (*39*, *40*). Lipids extracted from the PLA_2-treated PSII dimer complex of *Synechocystis* were separated into lipid classes by two-dimensional TLC. For the first dimension development was performed with $CHCl_3/CH_3OH/28\%$ NH_4OH (65:35:5, by volume) and for the second dimension with $CH_3COCH_3/C_6H_6/H_2O$ (91:30:8, by volume). Quantification of lipid classes by gas chromatography was performed as described previously (*39*). The mobility of the DM that was extracted together with lipids in the PSII complexes was similar to that of DGDG on the TLC plates. However, DM did not interfere with the quantification of DGDG, because fatty acid methylesters derived from DM were detected on gas chromatography at retention times different from those of fatty acid methylesters derived from DGDG.

Phospholipase A_2 Treatment—The PSII dimer complex of *Synechocystis* was diluted in a medium comprising 10 mM $MgCl_2$, 5 mM $CaCl_2$, 25% glycerol (w/v), and 50 mM MES-NaOH (pH 6.0) to the concentration of 0.5 mg Chl/ml. The PSII complex was incubated with or without PLA_2 (P9279; Sigma) at a concentration of 200 units/mg Chl in the dark at 20°C. To monitor the changes in oxygen-evolving activity on PLA_2 treatment, 2, 6-dimethyl-*p*-benzoquinone (DMBQ) was used as an electron acceptor in addition to DCBQ and Fecy. 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was used at 1 μM to inhibit the electron transport at the Q_B-binding site.

RESULTS

The photosynthetic oxygen-evolving activities of the dimeric PSII complexes are shown in Table 1. The activities of the PSII complex prepared from *T. vulcanus* were very high and similar to those of the PSII complex that was used for crystal structure analysis (*41*). In the case of *Synechocystis*, the activities of the PSII complex before separation into the monomer and dimer by ultracentrifugation through a glycerol density gradient were similar to those of the PSII complex purified previously by Kashino *et al*. (*29*) (data not shown). Although the activities slightly decreased after separation of the dimer on ultracentrifugation, they were still very high. The PSII complexes prepared from spinach and rice exhibited reasonably high levels of oxygen-evolving activities that were similar to those reported previously (*31*, *42*). The polypeptide compositions of the dimeric PSII complexes are shown in Fig. 1. Major PSII subunits CP47, CP43, PsbO (manganese-stabilizing protein), D2, D1, and PsbE were found in all PSII complexes. In addition to these subunits, PsbU and PsbV, which are known as extrinsic polypeptides in cyanobacterial PSII complexes, were also found in the PSII complex of *T. vulcanus*. These results demonstrate that the PSII complexes prepared from each organism are active and highly purified, and thus are suitable for lipid analysis.

The lipid compositions of thylakoid membranes and PSII dimer complexes are shown in Table 2. The lipid compositions of thylakoid membranes prepared from all organisms were similar to those reported previously (*14*, *43–45*). MGDG accounted for about 40 to 50% of the total lipids in all organisms. DGDG and SQDG together occupied a large part of the remaining total lipids. PG was a minor

Table 1. **Photosynthetic oxygen-evolving activities of photosystem II complexes.**

Acceptor	*T. vulcanus*	*Synechocystis*	Spinach	Rice
		(μmol O_2/mg Chl/h)		
2,6-DCBQ	2,920 ± 190	1,040 ± 110	460 ± 60	420 ± 50
Fecy	1,210 ± 100	2,080 ± 200	840 ± 100	710 ± 80

The values are averages and SD of independent measurements.

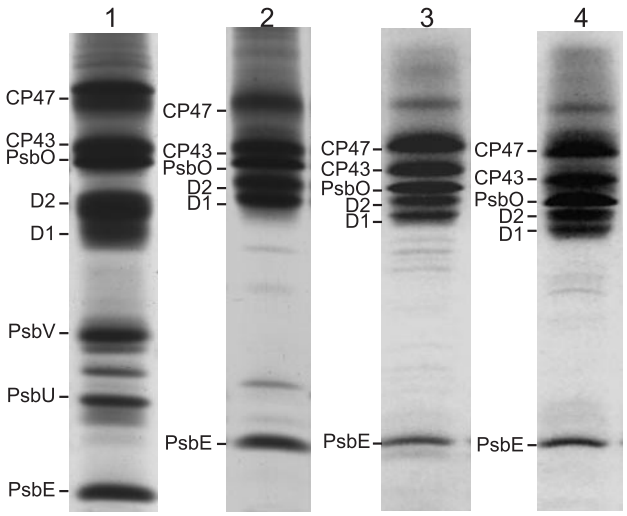

Fig. 1. **Polypeptide compositions of the dimeric forms of PSII complexes.** PSII complexes of *T. vulcanus* (lane 1), *Synechocystis* (lane 2), spinach (lane 3), and rice (lane 4) corresponding to 5 μg Chl were loaded on each lane.

Table 2. **Lipid compositions of thylakoid membranes and photosystem II complexes.**

Organism	Sample	Total	Lipid class			
			MGDG	DGDG	SQDG	PG
		(nmol/μg Chl)	(mol%)			
T. vulcanus	TM	2.63 ± 0.03	43.5 ± 1.5	25.6 ± 0.2	24.8 ± 1.1	6.1 ± 0.6
	PSII	0.79 ± 0.04	29.8 ± 1.0	21.1 ± 2.1	20.4 ± 2.3	28.7 ± 2.3
Synechocystis	TM	2.39 ± 0.08	37.4 ± 1.8	20.0 ± 1.6	28.9 ± 0.6	13.7 ± 0.8
	PSII	0.60 ± 0.03	28.7 ± 2.1	16.4 ± 0.8	25.5 ± 0.8	29.4 ± 1.6
Spinach	TM	1.39 ± 0.06	40.1 ± 0.7	32.1 ± 0.8	15.2 ± 0.0	12.6 ± 0.1
	PSII	0.25 ± 0.02	39.9 ± 1.9	25.1 ± 0.3	9.7 ± 0.6	25.3 ± 3.7
Rice	TM	2.65 ± 0.07	48.4 ± 0.1	32.9 ± 0.1	13.9 ± 0.3	4.8 ± 0.3
	PSII	0.23 ± 0.02	40.2 ± 0.3	26.2 ± 1.3	11.3 ± 0.1	22.3 ± 1.0

The values are averages and SD of independent measurements. TM, thylakoid membranes.

Table 3. **Numbers of Chl and lipid molecules in photosystem II complexes.**

Organism	Chl	Lipid				
		Total	MGDG	DGDG	SQDG	PG
		(numbers/2 Pheo)				
T. vulcanus	38.8 ± 3.0	27.4 ± 0.9	8.2 ± 0.5	5.8 ± 0.6	5.6 ± 0.5	7.8 ± 0.9
Synechocystis	37.4 ± 2.8	20.1 ± 1.1	5.8 ± 0.1	3.3 ± 0.3	5.1 ± 0.3	5.9 ± 0.6
Spinach	36.2 ± 2.8	7.9 ± 0.2	3.1 ± 0.2	2.0 ± 0.1	0.8 ± 0.1	2.0 ± 0.3
Rice	32.0 ± 2.4	6.6 ± 0.5	2.7 ± 0.2	1.7 ± 0.2	0.7 ± 0.1	1.5 ± 0.2

Table 4. **Fatty acid compositions of thylakoid membranes and photosystem II complexes prepared from cyanobacteria *T. vulcanus* and *Synechocystis*.**

Organism	Fatty acid	Thylakoid membrane				PSII complex			
		MGDG	DGDG	SQDG	PG	MGDG	DGDG	SQDG	PG
		(mol%)							
T. vulcanus	16:0	45.1	43.6	62.0	47.9	51.1	57.0	58.3	52.2
	16:1(9)	15.5	15.1	3.9	10.7	16.4	14.3	2.1	4.9
	18:0	3.9	2.5	8.0	8.9	3.6	2.4	19.8	12.6
	18:1(9)	27.9	28.1	20.9	26.3	25.1	22.8	14.6	26.9
	18:1(11)	7.6	10.7	5.2	6.2	3.8	3.5	5.2	3.4
Synechocystis	16:0	53.1	51.4	69.1	51.3	51.7	50.6	62.9	51.6
	16:1(9)	5.4	7.8	7.8	3.4	5.9	7.2	9.5	5.4
	18:0	0.2	1.5	nd	3.9	1.9	2.6	2.5	6.9
	18:1(9)	9.7	4.6	12.3	16.1	9.4	6.9	14.0	18.6
	18:2(9,12)	11.7	10.6	10.2	21.2	12.3	13.8	9.3	15.4
	18:3(6,9,12)	19.9	24.1	nd	1.9	18.8	18.9	nd	nd
	18:3(9,12,15)	nd	nd	0.6	2.2	nd	nd	2.0	2.1

The values are averages of independent measurements. Deviation of values was within 3%. nd, not detected.

lipid, comprising 6%, 14%, 13%, and 5% of the total lipids in the thylakoid membranes of *T. vulcanus*, *Synechocystis*, spinach, and rice, respectively. There was a remarkable difference between the lipid composition of the thylakoid membranes and that of the PSII complexes. The contents of MGDG, DGDG, and SQDG were lower in the PSII complexes than in the thylakoid membranes. By contrast, the contents of PG in the PSII complexes of *T. vulcanus*, *Synechocystis*, spinach, and rice were 5, 2, 2, and 5 times higher than those in the thylakoid membranes prepared from the respective organisms. These results demonstrate that PG is enriched in PSII complexes. The numbers of lipid molecules bound to the PSII dimer complexes were estimated based on the contents of Chl and Pheo in the PSII complexes. As shown in Table 3, it was estimated that 27, 20, 8, and 7 lipid molecules per monomer are bound to the PSII dimer complexes of *T. vulcanus*, *Synechocystis*, spinach and rice, respectively.

Tables 4 and 5 show the fatty acid compositions of lipid classes in thylakoid membranes and PSII complexes prepared from the cyanobacteria, *T. vulcanus* and *Synechocystis*, as well as those from the higher plants, spinach and rice. The fatty acid compositions of lipid classes in the thylakoid membranes were similar to those reported previously (*39*, *43–45*). In each organism, the fatty acid compositions of the lipid classes in the thylakoid membranes were slightly different from those of the lipid classes in the PSII complexes. In the PSII complexes of *T. vulcanus*, the contents of 16:0 in MGDG, DGDG, and PG, and of 18:0 in SQDG and PG were significantly higher than those of the lipid classes in the thylakoid membranes. By contrast, the contents of 16:0 in

Table 5. **Fatty acid compositions of thylakoid membranes and photosystem II complexes prepared from spinach and rice.**

Organism	Fatty acid	Thylakoid membrane				PSII complex			
		MGDG	DGDG	SQDG	PG	MGDG	DGDG	SQDG	PG
		(mol%)							
Spinach	16:0	3.1	6.7	49.2	15.6	1.4	10.1	46.6	13.5
	16:1(7)	0.3	0.2	1.4	nd	nd	1.6	0.8	nd
	16:1(3t)	nd	nd	nd	46.8	nd	nd	nd	44.6
	16:3(7,10,13)	13.6	4.1	1.0	nd	18.5	3.5	nd	nd
	18:0	0.6	0.6	2.3	0.4	1.2	2.5	5.9	2.4
	18:1(9)	1.1	1.7	2.9	nd	2.1	4.3	6.9	3.5
	18:3(9,12)	3.1	2.4	6.3	2.2	1.7	3.3	7.0	3.4
	18:1(9,12,15)	78.2	84.3	36.9	35.0	75.1	74.7	32.8	32.6
Rice	16:0	2.1	14.0	50.9	28.2	2.9	10.9	47.4	30.4
	16:1(3t)	nd	nd	nd	40.6	nd	nd	nd	43.1
	18:0	0.6	3.2	3.5	4.7	0.9	3.6	7.6	2.6
	18:1(9)	1.0	1.3	1.8	9.2	1.1	2.1	6.2	3.8
	18:2(9,12)	4.8	5.6	4.5	8.5	4.2	4.2	3.5	7.1
	18:3(9,12,15)	91.5	75.9	39.3	8.8	90.9	79.2	35.3	13.0

The values are averages of independent measurements. Deviation of values was within 3%. nd, not detected.

Table 6. **Effect of PLA_2 treatment on photosynthetic oxygen-evolving activity of the PS II complex of *Synechocystis*.**

Treatment	Acceptor					
	DCBQ		Fecy		DMBQ	
	–DCMU	+DCMU	–DCMU	+DCMU	–DCMU	+DCMU
	($\mu molO_2$/mg Chl/h)					
0 min	1,040 ± 110	420 ± 40	2,080 ± 200	1,020 ± 140	370 ± 50	40 ± 10
120 min –PLA_2 (Control)	970 ± 90	480 ± 60	1,900 ± 170	1,080 ± 100	410 ± 70	40 ± 10
120 min +PLA_2	860 ± 90	670 ± 90	1,600 ± 90	1,280 ± 80	120 ± 20	90 ± 20

The values are averages and SD of independent measurements.

SQDG, 18:1(11) in MGDG and DGDG, and 18:1(9) in DGDG in the PSII complexes were lower than those of the lipid classes in the thylakoid membranes. In *Synechocystis*, the contents of 18:0 and 18:1(9) in PG in the PSII complexes were higher than those of PG in the thylakoid membranes, whereas the contents of 18:2(9,12) were lower than those of PG in the thylakoid membranes. The contents of 16:0 in SQDG and 18:3(6,9,12) in DGDG in the PSII complexes were lower than those of the lipid classes in the thylakoid membranes, whereas the contents of 18:0 in SQDG and 18:2(9,12) in DGDG in the PSII complexes were higher than those of the lipid classes in the thylakoid membranes. In the higher plants, significant differences between the PSII complexes and thylakoid membranes were observed mainly in the contents of 18:0 and 18:1(9) in SQDG and PG, and 16:0 and 18:3(9,12,15) in DGDG. Although many differences between the fatty acid compositions of lipid classes in the PSII complexes and thylakoid membranes were found in each of the organisms, as described above, it was clarified that the specific molecular species of each lipid class are not enriched in PSII complexes compared to those of each lipid class in thylakoid membranes.

As mentioned above, it was demonstrated that PG is enriched in the PSII complex. This finding suggests that PG may play important roles in the PSII complex. To examine the functional importance of PG in the PSII complex, the PSII dimer complex of *Synechocystis* was treated with PLA_2, which degrades PG in the complex, and the change in oxygen-evolving activity was monitored. In addition to DCBQ and Fecy, DMBQ was used as an electron acceptor. As shown in Table 6, the oxygen-evolving activity of the PSII complex with DCBQ or Fecy was partially inhibited by DCMU to 40% and 49%, respectively. However, DCMU almost completely inhibited the oxygen-evolving activity of the PSII complex when DMBQ was used. These results indicate that DCBQ and Fecy can accept electrons not only from Q_B but also from Q_A, while DMBQ preferentially accepts electrons from Q_B, consistent with the previous findings reported by Satoh *et al*. (*46*). After PLA_2 treatment for 120 min, the oxygen-evolving activity with DCBQ or Fecy decreased to 83% and 77%, respectively, of the initial level (Table 6 and Fig. 2, A and B). By contrast, the oxygen-evolving activity monitored with DMBQ decreased to about 30% of the initial level (Table 6 and Fig. 2C). It was also found that the PLA_2 treatment slightly decreased the extent of inhibition of the activity by DCMU (Table 6), indicating that the decrease in the PG content in the PSII complex increased the affinity of the electron acceptors to the Q_A site. This is also consistent with the finding previously reported that the extent of inhibition of activity by DCMU decreased in the PSII complex, which does not sustain the native structure of the Q_B-binding site (*47*). In addition to these results, it was also found that PLA_2 digested only PG, *i.e*., not other lipids, in the PSII complex (Table 7). The content of PG in the PSII complex decreased to more than half of the initial level after the PLA_2 treatment. The subunit composition of the PSII complex was also analyzed before and after the treatment with PLA_2 to confirm that inactivation of the PSII complex was not

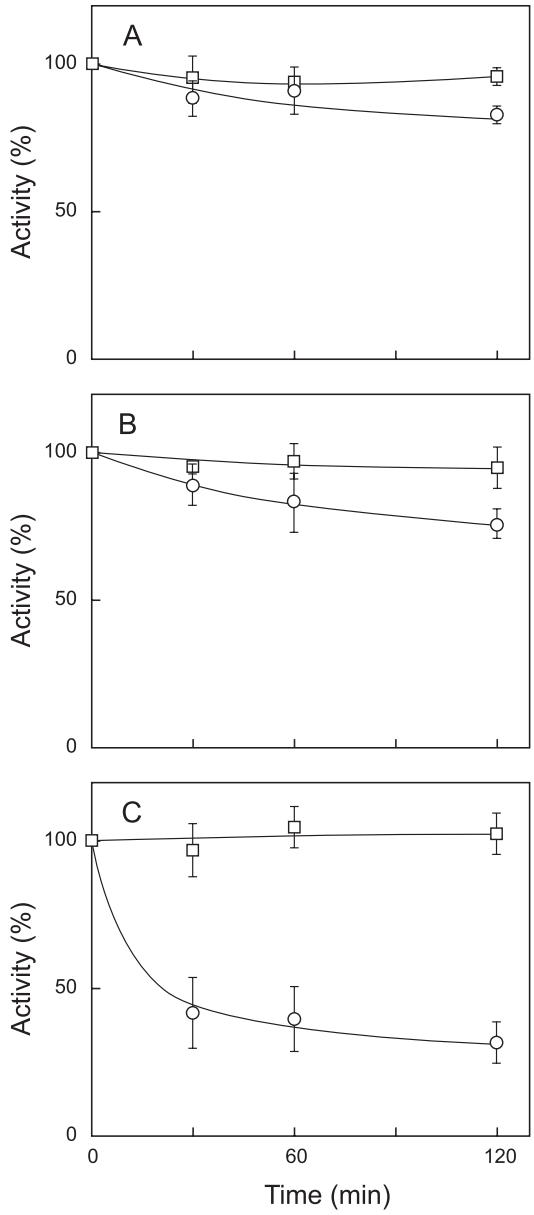

Fig. 2. **Changes in photosynthetic activity of the PSII dimer complex of *Synechocystis* on PLA_2 treatment.** The oxygen-evolving activity of the PSII complex, which was incubated without (square) or with (circle) PLA_2, was monitored using DCBQ (A), Fecy (B), or DMBQ (C) as the electron acceptor. The initial activities are shown in Table 6. Bars represent the SD ($n > 3$).

induced by degradation of some subunits. As shown in Fig. 3, no change in the subunit composition was observed with the exception of an extra band for the PLA_2-treated PSII complex that corresponds to PLA_2. These results suggest that some of PG molecules degraded on treatment with PLA_2 play important roles in the electron transport at the Q_B-binding site in the PSII complex.

DISCUSSION

Lipid molecules bound to PSII complexes were analyzed previously using PSII preparations, which were solubilized from thylakoid membranes with *n*-octyl β-D-glucopyranoside or Triton X-100 (*44*, *48*). Compared with these PSII preparations, the PSII dimer complexes used in this study were highly purified and retained higher oxygen-evolving activities, mainly because of solubilization of the thylakoid membranes with DM (Table 1 and Fig. 1). This suggested that the DM-solubilized PSII complexes had kept the native structure of the PSII complex in the thylakoid membranes. With these purified PSII complexes, we were able to analyze lipid molecules bound to PSII complexes more precisely (Table 2). The PSII complexes of higher plants contain much lower amounts of SQDG and higher amounts of MGDG compared with the PSII complexes of cyanobacteria. Interestingly, the lipid compositions of the PSII complexes were quite different from those of the thylakoid membranes in each of the organisms. The numbers of lipid molecules per monomer were 27, 20, 8, and 7 in the dimeric PSII complexes of *T. vulcanus*, *Synechocystis*, spinach, and rice, respectively (Table 3). The lipid contents in the PSII complexes of the higher plants were lower than those in the PSII complexes of the cyanobacteria. This difference might be caused by the structural difference between the PSII complexes of cyanobacteria and those of higher plants. The PSII complexes of higher plants are surrounded by LHCII in their thylakoid membranes (*1*) and the binding of lipid molecules might be limited compared to that in PSII complexes of cyanobacteria, in which phycobillisomes are attached to the surface of PSII complexes in thylakoid membranes as antennae (*49*). On the other hand, a remarkable and common characteristic was found in the lipid composition of the PSII complexes prepared from each of the organisms. The PG content was 2 to 5 times higher in the PSII complexes than in the thylakoid membranes, occupying about 20–30% of the total lipids present in the PSII complexes (Table 2). The enrichment of PG in PSII membranes prepared from cucumber was also reported previously by Yang *et al*. (*50*), and it was observed even under phosphate-deficient conditions, under which the PG content in thylakoid membranes decreased. These findings are consistent with our previous findings that PG plays important roles in the electron transport and dimerization of PSII complexes in *Synechocystis* sp. PCC6803 (*16*, *17*).

It was estimated that 8 and 6 molecules of PG per monomer are bound to the PSII dimer complexes of *T. vulcanus* and *Synechocystis*, respectively (Table 3). These numbers of PG molecules in the PSII complexes are much higher than that found in the most recent crystal structure of the PSII complex of *T. elongatus* at 3.0 Å resolution, in which only one PG molecule was identified in the complex (*25*). This difference suggests that many PG molecules still remain to be assigned in the crystal structure. As described in that paper (*25*), two or three disordered lipophylic molecules were found in the cavity harboring the Q_B-binding site. In addition, our previous findings suggested that PG plays important roles in electron transport at the Q_B-binding site of the PSII complex (*14*, *16*). This information indicates the possibility that unassigned lipid-like structures in the Q_B-binding site can be attributed to PG. Supporting our hypothesis, PLA_2 treatment of the PSII complex purified from *Synechocystis* resulted in a significant decrease in the oxygen-evolving activity with DMBQ as the electron acceptor, but there was only a small effect when DCBQ or Fecy was used instead of DMBQ (Fig. 2). These results

Table 7. **Effect of PLA_2 treatment on the lipid composition of the PSII complex.**

Treatment	Lipid class			
	MGDG	DGDG	SQDG	PG
	(nmol/μg Chl)			
0 min	0.18 ± 0.01	0.10 ± 0.01	0.15 ± 0.02	0.18 ± 0.03
120 min –PLA_2(Control)	0.21 ± 0.02	0.13 ± 0.02	0.17 ± 0.03	0.19 ± 0.02
120 min +PLA_2	0.22 ± 0.01	0.13 ± 0.02	0.17 ± 0.03	0.07 ± 0.01

The values are averages and SD of independent measurements.

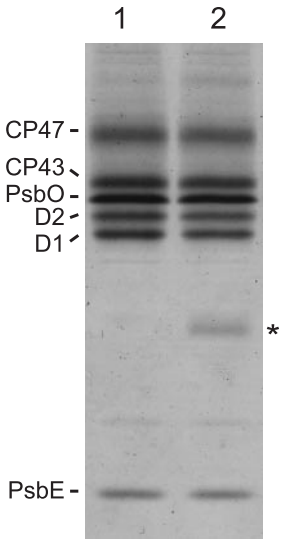

Fig. 3. **Effects of PLA_2 treatment on polypeptide compositions of the PSII dimer complex prepared from *Synechocystis*.** The polypeptide composition of the PSII complex before (lane 1) and after (lane 2) PLA_2 treatment for 120 min. The star in lane 2 indicates the polypeptide band corresponding to PLA_2.

indicate that the decrease in the PG content in the PSII complex induces a severe defect in electron transport mediated by Q_B, suggesting that some PG molecules are required to support the Q_B-mediated electron transport in the PSII complex. After the PLA_2 treatment, about 3 molecules of PG still remained in the PSII complex. These PG molecules would be embedded in the PSII complex, thus they were not degraded on the PLA_2 treatment. One of these PG molecules can be attributed to the PG molecule that has been identified in the crystal structure (*25*). The other PG molecules might be located at the monomer–monomer interface in PSII complexes since it was previously found that PG is involved in the dimerization of the PSII complex (*7*, *17*).

Kruse *et al*. (*7*) reported that the monomeric and dimeric PSII complexes prepared from spinach contained about 110 and 74 lipid molecules per monomer, respectively. The content of lipids in the dimeric PSII complex is 9 times higher than that of lipids in the dimeric PSII complex of spinach analyzed in this work (Table 3). This contradictory result might be caused by the differences in the procedures used for purification of the PSII complex. In their case, PSII membranes isolated from spinach were solubilized with *n*-octyl β-D-glucopyranoside and DM, and then subjected to size-exclusion chromatography to purify the PSII complex. Although we used similar procedures for the solubilization of PSII membranes, PSII complexes were purified by ion-exchange chromatography on a MonoQ column and by size-exclusion chromatography on two columns linked serially. It might be possible that the purification of the PSII complexes by ion-exchange chromatography and size-exclusion chromatography caused the removal of lipid molecules. However, in spite of the much lower content of lipids in our PSII complex compared with that of Kruse *et al*. (*7*), the activities of both the PSII complexes were almost the same. Thus it is likely that lipids lost from our PSII complex during the purification steps are non-specifically and/or loosely bound lipids that are not required for the activities of the PSII complex.

When lipid molecules bound to PSII complexes were identified, we expected that specific molecular species of lipids would be bound to PSII complexes. It was reported that 16:1(3t) bound to PG was enriched in PSII complexes prepared from spinach compared with thylakoid membranes, and that the dimeric PSII complex contains a higher level of PG containing 16:1(3t) than the monomeric PSII complex (*7*). This finding suggested that the PG molecular species containing 16:1(3t) is involved in dimerization of the PSII complex. However, concerning the fatty acid composition of lipid classes, we found no remarkable differences between the thylakoid membranes and PSII complexes in any of the organisms (Tables 4 and 5).

As demonstrated by the previous studies involving mutants of cyanobacteria and higher plants defective in fatty acid desaturases, the fatty acid composition of lipids in thylakoid membranes affects the process of recovery of PSII activity from photoinhibition of photosynthesis (*51*, *52*). It has also been demonstrated, with a *pgsA* mutant of *Synechocystis* sp. PCC6803 defective in the biosynthesis of PG, that the effect of PG on the recovery from photoinhibition of photosynthesis depends on the molecular species of PG (*17*). Although the fatty acid composition of lipids in PSII complexes was similar to that of lipids in thylakoid membranes, it should be noted that the acyl groups of lipid molecules also contribute to the function of PSII complexes.

In summary, 27 and 20 lipid molecules per monomer were identified in the dimeric forms of PSII complexes of two cyanobacteria, *T. vulcanus* and *Synechocystis*, as were 8 and 7 lipid molecules in those of the higher plants, spinach and rice, respectively. The lipid composition of PSII complexes was remarkably different from that of thylakoid membranes. In particular, the content of PG was significantly higher in PSII complexes than in thylakoid membranes. Digestion of PG in the PSII complex of *Synechocystis* by PLA_2 treatment resulted in a decrease in the oxygen-evolving activity with DMBQ as the electron acceptor, suggesting that PG plays important roles in the

electron transport at the Q_B-binding site in the PSII complex.

This work was supported by Grants-in-Aid for Scientific Research (No. 16570029 to HW, and No. 14340257 to JRS) from the Ministry of Education, Science, Sports, and Culture of Japan.

REFERENCES

1. Malkin, R. and Niyogi, K. (2000) Photosynthesis in *Biochemistry and Molecular Biology of Plants* (Buchanan, B.B., Gruissem, W., and Jones, R.L., eds.) pp. 568–628, American Society of Plant Physiologists, Rockville, Maryland
2. Joyard, J., Maréchal, E., Miège, C., Block, M.A., Dorne, A.-J., and Douce, R. (1998) Structure, distribution and biosynthesis of glycerolipids from higher plant chloroplasts in *Lipids in Photosynthesis: Structure, Function and Genetics* (Siegenthaler, P.-A. and Murata, N., eds.) pp. 21–52, Kluwer Academic Publishers, Dordrecht, Germany
3. Wada, H. and Murata, N. (1998) Membrane lipids in cyanobacteria in *Lipids in Photosynthesis: Structure, Function and Genetics* (Siegenthaler, P.-A. and Murata, N., eds.) pp. 65–81, Kluwer Academic Publishers, Dordrecht, Germany
4. Jordan, B.R., Chow, W.-S., and Baker, A.J. (1983) The role of phospholipids in the molecular organization of pea chloroplast membranes: Effect of phospholipid depletion on photosynthetic activities. *Biochim. Biophys. Acta* **725**, 77–86
5. Droppa, M., Horváth, G., Hideg, É., and Farkas, T. (1995) The role of phospholipids in regulating photosynthetic electron transport activities: Treatment of thylakoids with phospholipase C. *Photosynth. Res.* **46**, 287–293
6. Gounaris, K., Whitford, D., and Barber, J. (1983) The effect of thylakoid lipids on an oxygen-evolving photosystem II preparation. *FEBS Lett.* **163**, 230–234
7. Kruse, O., Hankamer, B., Konczak, C., Gerle, C., Morris, E., Radunz, A., Schmid, R.H., and Barber, J. (2000) Phosphatidylglycerol is involved in the dimerization of photosystem II. *J. Biol. Chem.* **275**, 6509–6514
8. Chain, R.K. (1985) Involvement of plastoquinone and lipids in electron transport reactions mediated by the cytochrome *b6-f* complex isolated from spinach. *FEBS Lett.* **180**, 321–325
9. Nußberger, S., Dörr, K., Wang, D.N., and Kühlbrandt, W. (1993) Lipid-protein interactions in crystals of plant light-harvesting complex. *J. Mol. Biol.* **234**, 347–356
10. Jarvis, P., Dörmann, P., Peto, C.A., Lutes, J., Benning, C., and Chory, J. (2000) Galactolipid deficiency and abnormal chloroplast development in the *Arabidopsis MGD synthase 1* mutant. *Proc. Natl. Acad. Sci. USA* **97**, 8175–8179
11. Trémolières, A. and Siegenthaler, P.-A. (1998) Reconstitution with lipids in *Lipids in Photosynthesis: Structure, Function and Genetics* (Siegenthaler, P.-A. and Murata, N., eds.) pp. 175–189, Kluwer Academic Publishers, Dordrecht, Germany
12. Sato, N., Tsuzuki, M., Matsuda, Y., Ehara, T., Osafune, T., and Kawaguchi, A. (1995) Isolation and characterization of mutants affected in lipid metabolism of *Chlamydomonas reinhardtii*. *FEBS J.* **230**, 987–993
13. Minoda, A., Sonoike, K., Okada, K., Sato, N., and Tsuzuki, M. (2003) Decrease in the efficiency of the electron donation to tyrosine Z of photosystem II in an SQDG-deficient mutant of *Chlamydomonas*. *FEBS Lett.* **553**, 109–112
14. Hagio, M., Gombos, Z., Várkonyi, Z., Masamoto, K., Sato, N., Tsuzuki, M., and Wada, H. (2000) Direct evidence for requirement of phosphatidylglycerol in photosystem II of photosynthesis. *Plant Physiol.* **124**, 795–804
15. Sato, N., Hagio, M., Wada, H., and Tsuzuki, M. (2000) Requirement of phosphatidylglycerol for photosynthetic function in thylakoid membranes. *Proc. Natl. Acad. Sci. USA* **97**, 10655–10660
16. Gombos, Z., Várkonyi, Z., Hagio, M., Iwaki, M., Kovács, L., Masamoto, K., Itoh, S., and Wada, H. (2002) Phosphatidylglycerol requirement for the function of electron acceptor plastoquinone Q_B in the photosystem II reaction center. *Biochemistry* **41**, 3796–3802
17. Sakurai, I., Hagio, M., Gombos, Z., Tyystjärvi, T., Paakkarinen, V., Aro, E.-M., and Wada, H. (2003) Requirement of phosphatidylglycerol for maintenance of photosynthetic machinery. *Plant Physiol.* **133**, 1376–1384
18. Domonkos, I., Malec, P., Sallai, A., Kovács, L., Itoh, K., Shen, G., Ughy, B., Bogos, B., Sakurai, I., Kis, M., Strzalka, K., Wada, H., Itoh, S., Farkas, T., and Gombos, Z. (2004) Phosphatidylglycerol is essential for oligomerization of photosystem I reaction center. *Plant Physiol.* **134**, 1471–1478
19. Sato, N., Suda, K., and Tsuzuki, M. (2004) Responsibility of phosphatidylglycerol for biogenesis of the PSI complex. *Biochim. Biophys. Acta* **1658**, 235–243
20. Jordan, P., Formme, P., Witt, H.T., Klukas, O., Saenger, W., and Krauß, N. (2001) Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution. *Nature* **411**, 909–917
21. Kurisu, G., Zhang, H., Smith, J.L., and Cramer, W.A. (2003) Structure of the cytochrome b_6f complex of oxygenic photosynthesis: Tuning the cavity. *Science* **302**, 1009–1014
22. Zouni, A., Witt, H.-T., Kern, J., Fromme, P., Krauß, N., Saenger, W., and Orth, P. (2001) Crystal structure of photosystem II from *Synechococcus elongatus* at 3.8 Å resolution. *Nature* **409**, 739–743
23. Kamiya, N. and Shen, J.-R. (2003) Crystal structure of oxygen-evolving photosystem II from *Thermosynechococcus vulcanus* at 3.7-Å resolution. *Proc. Natl. Acad. Sci. USA* **100**, 98–103
24. Ferreira, K.N., Iverson, T.M., Maghlaoui, K., Barber, J., and Iwata, S. (2004) Architecture of the photosynthetic oxygen-evolving center. *Science* **303**, 1831–1838
25. Loll, B., Kern, J., Seanger, W., Zouni, A., and Biesiadka, J. (2005) Toward complete cofactor arrangement in the 3.0 Å resolution structure of photosystem II. *Nature* **438**, 1040–1044
26. Bricker, T., M., Morvant, J., Masri, N., Sutton H., M., and Frankel, L., K. (1998) Isolation of a highly active Photosystem II preparation from *Synechocystis* 6803 using a histidine-tagged mutant of CP47. *Biochim. Biophys. Acta* **1409**, 50–57
27. Shen, J.-R., Ikeuchi, M., and Inoue, Y. (1992) Stoichiometric association of extrinsic cytochrome c_{550} and 12 kDa protein with a highly purified oxygen-evolving photosystem II core complex from *Synechococcus vulcanus*. *FEBS Lett.* **301**, 145–149
28. Shen, J.-R. and Inoue, Y. (1993) Binding and functional properties of two new extrinsic components, cytochrome *c-550* and a 12-kDa protein, in cyanobacterial photosystem II. *Biochemistry* **32**, 1825–1832
29. Kashino, Y., Lauber, W.M., Carroll, J.A., Wang, Q., Whitmarsh, W.J., Satoh, K., and Pakrasi, H.B. (2002) Proteomic analysis of a highly active photosystem II preparation from the cyanobacterium *Synechocystis* sp. PCC 6803 reveals the presence of novel polypeptides. *Biochemistry* **41**, 8004–8012
30. Berthold, D.A., Babcock, G.T., and Yocum, C.F. (1981) A highly resolved, oxygen-evolving photosystem II preparation from spinach thylakoid membranes. EPR and electron-transport properties. *FEBS Lett.* **134**, 231–234
31. Shen, J.-R. (1998) Possible functional differences between dimer and monomer of photosystem II complex in *Photosynthesis: Mechanisms and Effects* (Garab, G, eds), Vol. II, pp. 941–944, Kluwer Academic Publishers, Dordrecht, Germany
32. Enami, I., Kamino, K., Shen, J.-R., Satoh, K., and Katoh, S. (1989) Isolation and characterization of photosystem II complexes which lack light-harvesting chlorophyll *a/b* proteins but

retain three extrinsic proteins related to oxygen evolution from spinach. *Biochim. Biophys. Acta* **977**, 33–39
33. Arnon, D.I., McSwain, B.D., Tsujimoto, H.Y., and Wada, K. (1974) Photochemical activity and components of membrane preparations from blue-green algae. I. Coexistence of two photosystems in relation to chlorophyll *a* and removal of phycocyanin. *Biochim. Biophys. Acta* **357**, 231–245
34. Kobayashi, M., Maeda, H., Watanabe, T., Nakane, H., and Satoh, K. (1990) Chlorophyll *a* and β-carotene content in the D1/D2/cytochrome *b*-559 reaction center complex from spinach. *FEBS Lett.* **260**, 138–140
35. Kashino, Y., Koike, H., and Satoh, K. (2001) An improved sodium dodecyl sulfate-polyacrylamide gel electrophoresis system for the analysis of membrane protein complexes. *Electrophoresis* **22**, 1004–1007
36. Ikeuchi, M. and Inoue, Y. (1988) A new 4.8-kDa polypeptide intrinsic to the PS II reaction center, as reveled by modified SDS-PAGE with improved resolution of low-molecular-weight proteins. *Plant Cell Physiol.* **29**, 1233–1239
37. Gombos, Z., Wada, H., and Murata, N. (1991) Direct evaluation of effects of fatty-acid unsaturation on the thermal properties of photosynthetic activities, as studied by mutation and transformation of *Synechocystis* PCC6803. *Plant Cell Physiol.* **32**, 205–211
38. Bligh, E.G. and Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, 911–917
39. Wada, H. and Murata, N. (1989) *Synechocystis* PCC6803 mutants defective in desaturation of fatty acids. *Plant Cell Physiol.* **30**, 971–978
40. Hagio, M., Sakurai, I., Sato, S., Kato, T., Tabata, S., and Wada, H. (2002) Phosphatidylglycerol is essential for the development of thylakoid membranes in *Arabidopsis thaliana*. *Plant Cell Physiol.* **43**, 1456–1464
41. Shen, J.-R. and Kamiya, N. (2000) Crystallization and the crystal properties of the oxygen-evolving photosystem II from *Synechococcus vulcanus*. *Biochemistry* **39**, 14739–14744
42. Hankamer, B., Nield, J., Zheleva, D., Boekema, E., Jansson, S., and Barber, J. (1997) Isolation and biochemical characterization of monomeric and dimeric photosystem II complexes from spinach and their relevance to the organization of photosystem II *in vivo*. *FEBS J.* **243**, 422–429
43. Toriyama, S., Hinata, K., Nishida, I., and Murata, N. (1988) Prominent difference of glycerolipids among anther walls, pollen grains and leaves of rice and maize. *Plant Cell Physiol.* **29**, 615–621
44. Murata, N., Higashi, S., and Fujimura, Y. (1990) Glycerolipids in various preparations of photosystem II from spinach chloroplasts. *Biochim. Biophys. Acta* **1019**, 261–268
45. Kiseleva, L.L., Horvàth, I., Vigh, L., and Los, D.A. (1999) Temperature-induced specific lipid desaturation in the thermophilic cyanobacterium *Synechococcus vulcanus*. *FEMS Microbiol. Lett.* **175**, 179–183
46. Satoh, K., Koike, H., Ichimura, T., and Katoh, S. (1992) Binding affinities of benzoquinones to the Q_B site of Photosystem II in *Synechococcus* oxygen-evolving preparation. *Biochim. Biophys. Acta* **1102**, 45–52
47. Ikeuchi, M. and Inoue, Y. (1986) Characterization of O_2 evolution by a wheat photosystem II reaction center complex isolated by a simplified method: Disjunction of secondary acceptor quinone and enhanced Ca^{2+} demand. *Arch. Biochem. Biophys.* **247**, 97–107
48. Gouanaris, K. and Barber, J. (1985) Isolation and characterisation of a photosystem II reaction centre lipoprotein complex. *FEBS Lett.* **188**, 68–72
49. Sidler, W.A. (1994) Phycobilisome and phycobiliprotein structures in *The Molecular Biology of Cyanobacteria* (Bryant, D., A., eds), pp 139–216, Kluwer Academic Publishers, Dordrecht, Germany
50. Yang, W., Liu, S., Feng, F., Hou, H., Jiang, G., Xu, Y., and Kuang, T. (2004) Effects of phosphate deficiency on the lipid composition in cucumber thylakoid membranes and PSII particles. *Plant Sci.* **166**, 1575–1579
51. Gombos, Z., Wada, H., and Murata, N. (1994) The recovery of photosynthesis from low-temperature photoinhibition is accelerated by the unsaturation of membrane lipids: A mechanism of chilling tolerance. *Proc. Natl. Acad. Sci. USA* **91**, 8787–8791
52. Moon, B.Y., Higashi, S., Gombos, Z., and Murata, N. (1995) Unsaturation of the membrane lipids of chloroplasts stabilizes the photosynthetic machinery against low-temperature photoinhibition in transgenic tobacco plants. *Proc. Natl. Acad. Sci. USA* **92**, 6219–6223