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Effect of phosphatidylglycerol on molecular organization of photosystem I

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Abstract

Phosphatidylglycerol (PG) is the only anionic phospholipid in photosynthetic membrane. In this study, photosystem I (PSI) particles obtained from plant spinach were reconstituted into PG liposomes at a relatively high concentration. The results from visible absorption, fluorescence emission, and circular dichroism (CD) spectra reveal an existence of the interactions of PSI with PG. PG effect causes blue-shift and intensity decrease of Chl *a* peak bands in the absorption and 77 K fluorescence emission. The visible CD spectra indicate that the excitonic interactions for Chl *a* and Chl *b* molecules were enhanced upon reconstitution. Furthermore, more or less blue- or red-shift of the peaks characterized by Chl *a*, Chl *b*, and carotenoid molecules are also occurred. Simultaneously, an increase in α-helix and a decrease particularly in the disordered conformations of protein secondary structures are observed. In addition, the same effect also leads to somewhat more tryptophan (Trp) residues exposed to the polar environment. These results demonstrate that some alteration of molecular organization occurs within both the external antenna LHCI and PSI core complex after PSI reconstitution.
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Keywords: Molecular organization; Phosphatidylglycerol; Photosystem I; Pigment; Protein secondary structure; Tryptophan residues

1. Introduction

Photosystem I (PSI) is a supermolecular pigment–protein complex and acts as the light-driven plastocyanin–ferredoxin oxidoreductase in the thylakoid membranes of cyanobacteria and chloroplasts. In algae and higher plants, PSI is composed of a core complex and a light-harvesting complex (LHCI) [1,2]. The LHCI serves as an antenna to trap light and funnel its energy to the core complex. LHCI binds 80–120 antenna Chls, both Chl *a* and total Chl *b*. LHCI consists of four different polypeptides denoted Lhca1 to Lhca4 which belong to the group of Chl *a*/*b* binding proteins of the super-gene family Lhc [3–5]. It was previously assumed that there are six to eight Lhca proteins per PSI [6,7]. However, the recently crystal structure of PSI from pea [8] and biochemical analysis of PSI from *Arabidopsis thaliana* [9] revealed that only four Lhca proteins surround the core of PSI. In *Arabidopsis thaliana*, two new genes (Lhca5 and Lhca6) that encode additional putative LHCI proteins have been identified [10,11].

The PSI core complex is consisted of at least 13 protein subunits and contains approximately 90~100 Chl *a* and ~22 carotenoid molecules in total [1,2]. In eukaryotic PSI core complex, all the carotenoids and almost all Chl *a* are bound with the core reaction center PsaA/PsaB. It was known that both PsaA and PsaB subunits contain 11 transmembrane α-helices, in which five was located in C-terminal and the remaining six was in N-terminal [12,13]. In addition, most of the loop regions also contain the secondary structure elements as α-helices and β-sheet. The C-terminal domains of PsaA and PsaB bind 25 Chls of the antenna system (12 in PsaA and 13 in PsaB). The N-terminal α-helices of PsaA and PsaB coordinate 54 Chls (28 in PsaA and 26 in PsaB).

The core complex of PSI also contains the primary electron donor *P*700 (a dimer of Chl a molecules) located in PsaA/B proteins, and five different electron carriers, i.e.,

Abbreviations: Chl, chlorophyll; CD, circular dichroism; LHC, light-harvesting complex; MGDG, monogalactosyldiglycerol; PG, phosphatidylglycerol; PSI, photosystem I; PSII, photosystem II; Trp, tryptophan.

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primary electron acceptor A_0 (a Chl *a* molecule), secondary electron acceptor A_1 (a phylloquinone molecule), and F_X (iron–sulfur center X) in PsaA/B protein, and F_A and F_B (iron–sulfur centers A, B, respectively) in peripheral protein PsaC on the stromal side of the PSI complexes [14]. Some tryptophan (Trp) residues in PsaA/PsaB are also suggested to be involved in the electron transfer process of PSI [8,12,13,15–19], in which there are about 62 Trp residues per *P*700 in PSI [20].

Oxygenic photosynthesis is carried out in thylakoid membranes in which highly organized pigment–protein complexes are embedded in the lipid matrix. Thylakoid membranes are characterized by a unique lipid composition. Compared to the animal, bacterial and nonchloroplastic cellar membranes dominated by phospholipids, thylakoids contain a small quantity of phospholipids. In contrast, the major lipids of thylakoid membranes are the uncharged galactolipids, namely, monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG). These galactolipids account for at least 70 mol% of the total thylakoid lipids. In addition, photosynthetic membranes are specific in that there is a negatively charged sulfolipid, sulfoquinovosyl diacylglycerol (SQDG), which seems to be defective in nonphotosynthetic organism. The only significant phospholipid is anionic phosphatidylglycerol (PG), rather than phosphatidylcholine (PC) [21, 22].

Although only a small amount of PG (about 5~15 mol% of total acyl lipids) is contained in thylakoid membrane, there is increasing evidence indicating that PG plays an important role in photosynthesis, in particular for photosystem II (PSII). It was shown that either charge or electron transfer in PS II depends on the presence of PG [23–25]. PG with the unusual fatty acid *trans*-Δ^3-hexadecenoic acid (16:1- $\Delta^3 tr$) was found to be involved in the in vitro dimerization of PS II [26] and trimerization of light-harvesting complex of PSII (LHCII) [27–29]. Furthermore, PG has been shown to be bound to the D1 protein, where PG might sustain the optimal conformation of D1 polypeptide [30]. More recently, several studies on the mutant defective in PG have provided the direct evidence that PG is an indispensable component of PSII for photosynthesis [31–35]. PG was also shown to be required for the biogenesis of PSII core complex in *Chlamydomonas reinhardtii* mutants *mf1* and *mf2* with the decreased rate of translation of the D1 and CP47 owing to PG deficiency [36].

In contrast, the structural and functional aspects of lipids in PSI complex are not yet clear. Recently, the electron crystallographic PSI structure at resolution 2.5 Å from thermophilic cyanobacterium *Synechococcus elongatus* revealed the presence of four lipid molecules, three of which are negatively charged PG, the other is an uncharged MGDG [12]. They are located in the central core of PsaA and PsaB. For the three PG molecules, two of them are respectively bound to PsaA and PsaB, while the third one is coordinated by the phosphodiester group with an antenna Chl *a*. These facts clearly indicate that these three PG molecules, together with MGDG, are integral components and may stabilize the structure of cyanobacterial PSI complex. Indeed, PG was recently confirmed to be essential for the oligomerization of cyanobacterial PSI [37,38]. Thus, these facts obviously demonstrate that the PG molecules are necessary for the maintenance of the structural organization of PSI complex in cyanobacteria.

The previous results by Makewicz et al. using antilipid antibodies of PSI preparations from tobacco species and spinach have revealed the presence of only MGDG and PG with the molar ratio at about 2:1 [39]. They found that PG molecules are only associated with PSI core complexes of the analyzed higher plants. Nevertheless, the existence of PG in plant PSI complexes needs to be further verified, which is mostly dependent on, for example, the analysis of PSI crystallographic structure with a high resolution. Although the crystal structure of PSI from higher plant (*Pisum sativum* var. *alaska*) has been determined recently, a resolution at 4.4 Å did not allow the detection of lipid molecules [8]. However, one can expect the existence of PG molecules in PSI core complex of higher plant chloroplasts as in cyanobacterium due to the conservation of structure and components of PSI core complex.

In this regard, however, little information has yet been available on the interaction between PSI and PG, and the structure–function relations in the interactions between this lipid and PSI complexes are not completely understood. A reconstitution system is very useful for analyzing specific lipid action without interference of other components such as pigments and proteins. By incubating pigment–protein with lipid liposomes, additional lipid can be introduced to produce an average lipid enrichment in the reconstituted system. Such a manipulation offers the chance to investigate the role of lipids in terms of their chemical and physical properties as the support matrix for the functional proteins [40]. Although there are a few studies about PS I reconstitution focusing on the functional or structural properties of PS I [38,41–43], little attention was paid to the regulative effect of PG on the PSI. This prompted us to explore the involvement of PG in functional and structural roles in PSI. In the present work, the characteristics of molecular organization within PSI reconstituted into the PG liposomes have been investigated by absorption, fluorescence, and circular dichroism (CD) spectroscopy.

2. Materials and methods

2.1. Materials

Phosphatidylglycerol (PG) was purchased from Sigma Chemical and used without further purification. The fatty acid chains composition determined by gas chromatography experiment of PG (mol% in parentheses) is: 16:0 (15.1);

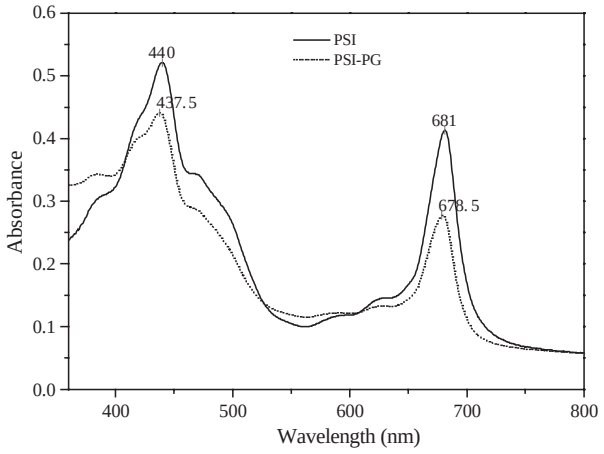

Fig. 1. Room temperature absorption spectra of PSI and proteoliposomes with PSI. Chl concentration is 10 μg/ml.

16:1 (0.4); l8:0 (20.3); 18:1 (33.5); l8:2 (21.6); and 18:3 (9.7). The average molecular weight of PG was determined to be 771.

Spinach (*Spinacia oleraceal* L.) was from local market in spring season.

2.2. Preparation of the PSI particles

PSI particles were prepared from the fresh spinach leaves, using a modified procedure of Mullet et al. [44] and Bassi et al. [45], with discontinuous sucrose gradient centrifugation (100,000×*g*) for only 6 h. The PSI preparation was dialyzed against 5 mmol/L Tricine buffer (pH 7.8) for 3 h and then pelleted by centrifugation at 40,000×*g* for 30 min to remove excess Triton X-100. The pellet was resuspended in a buffer containing 10 mmol/L NaCl and 20 mM Tricine-NaOH (pH 7.8), and then stored at −80 °C until use. In this work, the ratios of Chl/*P*700 and Chl *a*/*b* in PSI particles were determined to be about 200 and 6.0, respectively.

Chlorophyll (Chl) concentrations were determined in 80% (v/v) acetone solutions using the method of Arnon [46].

2.3. Preparation of proteoliposomes reconstituted with PSI particles

The PG liposomes were prepared according to the procedures reported in literature [47], in which lipid was firstly dissolved in chloroform and dried under a stream of N_2, then dispersed in a buffer containing 10 mmol/L NaCl, 20 mM Tricine-NaOH (pH 7.8), followed by sonication for over 20 min with a microtip-equipped ultrasonic crasher equipped with microtips Model JY92-II (Ningbo Scientz Biotechnology, China) at an output of 60 W in 30-s bursts with 1-min cooling intervals. After that, aliquots of PSI solutions in the same buffer were added to the liposome solution to make a required ratio of lipid to Chl. The concentration of PG used in this study is 50 mg per mg Chl. These preparations, namely PSI complexed with PG, are denoted hereunder as the PSI–PG complex.

2.4. Absorption spectra measurements

Absorption spectra were recorded at room temperature with a Shimadzu UV-Vis 2550 spectrophotometer using 1-cm path length cuvettes. The incubation media contained 10 mmol/L NaCl and 20 mmol/L Tricine-NaOH (pH 7.8).

2.5. Fluorescence measurements

The low-temperature (77 K) fluorescence spectra of the samples were obtained using a Hitachi F-4500 fluorescence spectrophotometer with 440 nm as the excitation wavelength. The fluorescence emission spectra were measured between 650 nm and 780 nm. The Trp fluorescence spectra were recorded in the 320–400 nm range at room temperature and were excited at 295 nm in order to avoid interference from PG.

2.6. Circular dichroism measurements

The circular dichroism (CD) spectra were recorded with a Jasco J-715 spectropolarimeter at a scanning speed of 200 nm/min, with a bandwidth of 2.0 nm and an accumulation of four times. CD spectra in the far-ultraviolet region (190–250 nm) and red region (360–800 nm) were measured in quartz cuvettes with path length of 1 mm. All spectra were measured at room temperature. The calculation of the contents of protein secondary structure was performed with the software program package Dicroprot 2000 (release 1.0.4) from the Internet (http://www.dicroprot-pbil.ibcp.fr/). The evaluations were carried out by using the Selcon3 program developed by Sreerama and Woody [48].

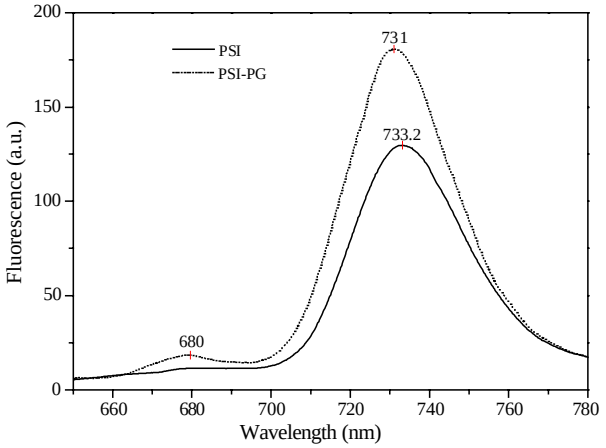

Fig. 2. Fluorescence emission spectra at 77 K of PSI and PSI–PG complexes on excitation at 440 nm. Chl concentration is 10 μg/ml.

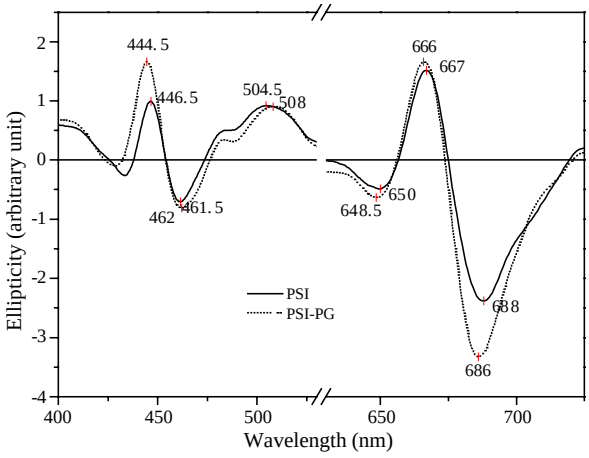

Fig. 3. Visible CD spectra of PSI and the proteoliposomes reconstituted with PSI. Chl concentration is 100 μg/ml. Other experimental conditions are given in Materials and methods.

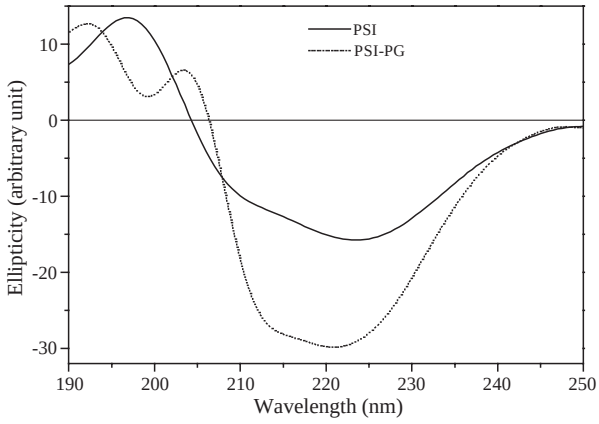

Fig. 4. Far ultraviolet CD spectra of PSI and the proteoliposomes reconstituted with PSI. Chl concentration is 100 μg/ml. Other experimental conditions are given in Materials and methods.

In this study, the concentration of Chl used for absorption and fluorescence measurements is 10 μg/ml, whereas for CD experiments is 100 μg/ml.

3. Results

Fig. 1 presents the absorption spectra of PSI complexes in the absence of PG and in its presence. The spectra of control PSI preparations show typical absorption peaks at 681 and 440 nm contributed by Chl *a*, the shoulders at 650 and 470 nm originated from Chl *b* in LHCI. As shown in Fig. 1, incorporation of PSI into PG liposomes caused a decrease in the amplitude of the adsorption peak of Chl *a*, in which the ratio of A_{681}/A_{440} decreased by 20%. Meanwhile, the peak position of Chl *a* was shifted toward the blue in both the blue and red region, demonstrating that the microenvironment around Chl *a* molecules of PSI has been altered.

Fig. 2 displays the fluorescence emission spectra at 77 K of the PSI. An emission peak at 733.2 nm is observed while no clear emission band at 680–685 nm appears. This emission peak at 733.2 nm is typical characteristic of PSI and is believed to be dominated by the emission from LHCI-730 [4]. Fig. 2 also shows 77 K fluorescence emission spectra of reconstituted PSI complexes. It was found that the effect of PG induced the blue-shift of the maximum emission peak of PSI from 733.2 nm to 731 nm, indicating the modification of the microenvironment around Chl *a* molecules within LHCI. Furthermore, a small increase in its intensity at 680 nm was also observed after reconstitution. The ratio of F730–735/F680 in the proteoliposomes was about 9.3, compared with a value of 11.3 for the control PSI. The intensity at 680 nm was 22% higher than that of control PSI. It means that some dissociation of LHCI-680 [45,49] occurred due to the presence of PG in the reconstituted PSI complex.

The visible CD spectroscopy is very useful to probe the pigment–pigment and/or pigment–protein interactions [50–53]. The visible CD spectra of PSI and reconstituted PSI complexes are displayed in Fig. 3. Three bands in the Q_y region are observed with peaks at (−) 650 nm, (+) 667 nm, and (−) 688 nm in control PSI particles. According to Refs. [45,54,55], the two major bands at (+) 667 nm and (−) 688 nm are due to Chl dimers caused by the excitonic interaction of Chl *a* in PSI, while the negative peak at 650 nm is the characteristic of Chl *b*. In the Soret region, the positive bands peaking at 446.5 nm and 504.5 nm are originated from Chl *a* and carotenoids, respectively, while the negative peak at 461.5 nm is characterized by Chl *b*. It was found that when the LHCI was removed from PSI complexes, the negative peaks at 650 nm and 461.5 nm would disappear [45]. This implied that the peaks of 650 nm and 461.5 nm are of the characteristics of LHCI.

As shown in Fig. 3 and Table 1, when PSI was reconstituted into liposomes, prominent changes in the CD spectra both in the Q_y- and the Soret-band regions were occurred. In the Q_y-band region, the signal intensities of the positive peak at 667 nm and the negative peaks at 688 nm and 650 nm became higher; meanwhile, these three peaks shifted to the blue after reconstitution. In the Soret-band region, PG effect led to an increase in the intensities of both bands at 446.5 and 461.5 nm. Interestingly, the band at

Table 1
The CD peaks and corresponding intensities of PSI in the absence of PG and in its presence

PG/PSI (mg PG/mg Chl)	Chl *a* (nm)			Chl *b* (nm)		Carotenoid (nm)
0	667 (100%)	688 (100%)	446.5 (100%)	650 (100%)	461.5 (100%)	504.5 (100%)
50	666 (110%)	686 (140%)	444.5 (168%)	648.5 (130%)	462 (115%)	508 (99%)

The number in parentheses indicates the relative intensity of CD peak. 100% is the intensity of control PSI.

Table 2
The contents of the protein secondary structures in PSI and the proteoliposomes reconstituted with PSI

PG /PSI (mg PG/mg Chl)	α-helix (%)	β-sheet (%)	Turn (%)	Disorder (%)
0	40	12	13	35
50	49	12	10	29

504.5 nm was red-shifted to the 508 nm with no change in its intensity upon reconstitution into PG liposomes. These results indicate that the pigment–pigment and/or pigment–protein interactions in PSI complexes were affected by PG addition, which came from the changes in microenvironment of pigments or/and protein secondary structure of PSI complexes.

To look for conformational changes in the secondary structure of the protein backbone of PSI produced by the reconstitution with PG, the CD spectroscopy was also carried out in the far-ultraviolet region (190–250 nm) of peptidic bond absorption. Fig. 4 shows the CD spectra of PSI and PSI–PG complexes in the 190–250 nm range.

The UV-CD spectral analysis indicates that PSI contains about 40% α-helix, 12% β-sheet, 13% turn, and 35% disordered structure (Table 2), similar to the results determined by Fourier transform infrared (FT-IR) spectroscopy [56]. Incorporation of PSI into PG liposomes increased the helical content with a corresponding decrease in disordered and turn structures. The UV-CD spectra data clearly demonstrate the alterations of the protein secondary structures in PSI upon incorporation.

The intrinsic fluorescence of aromatic amino acid residues (tryptophan, tyrosine, and phenylalanine) of proteins is an excellent built-in indicator [57]. It is very useful in monitoring the changes in emission induced by conformational changes and anything that affects the local environment surrounding these residues. Fig. 5 shows the emission spectra of fluorescent Trp residues in PSI particles

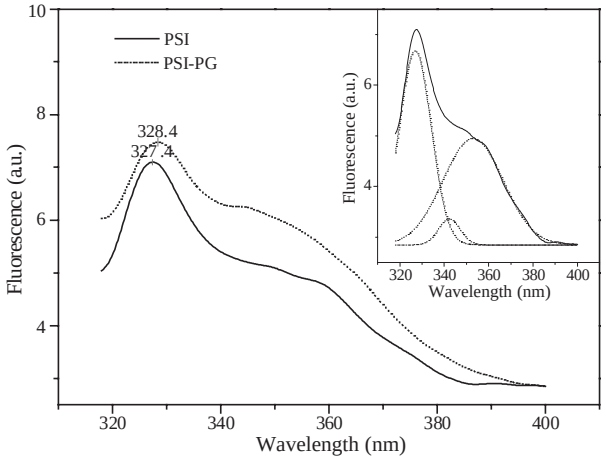

Fig. 5. Ultraviolet fluorescence emission spectra at room temperature of PSI and PSI–PG complexes on excitation at 295 nm. The inset shows the deconvolution of spectrum for control PSI. Chl concentration is 10 μg/ml.

Table 3
The relative contents of the tryptophan residues in PSI and the proteoliposomes reconstituted with PSI

PG/PSI (mg PG/mg Chl)	Trp peak (nm)	Relative content (%)
0	327	45
	342	5
	353	50
50	328	40
	346	3
	353	58

upon the incubation with and without PG. The maxima of the spectra are at 327.4 and 328.4 nm for control PSI and proteoliposome, respectively.

The gaussian curve-fitting analysis of Trp fluorescence spectrum of control PSI discloses three spectral classes as plotted in the inset of Fig. 5, indicating heterogeneity in the emission of three Trp residues for PSI. Three similar subbands are also found to exist for proteoliposomes reconstituted with PSI (not shown here). An increase in band intensity was also observed in the proteoliposomes. The detailed subband peaks and their corresponding contents are indicated in Table 3.

Three discrete spectral classes may exist in Trp residues of proteins [58], depending on whether they are buried in nonpolar regions of proteins with max ~330 nm, or completely exposed to water with max ~350 nm, or located in an environment of intermediate polarity with max ~340 nm. As indicated in Table 3, Trp residues in PSI proteins are almost equally situated at polar and nonpolar environment.

An obvious increase in the content of Trp component at ~353 nm together with the decrease in the level of Trp components at ~330 and ~340 nm thus suggests a somewhat more hydrophilic environment for Trp in the proteoliposome. This is in agreement with a small red-shift of fluorescence emission maxima for proteoliposome compared to the control PSI particles. The modification of the polarity in the environment of the Trp residues seems to occur with the conformational change since the observed increase in the fluorescence emission intensity of PSI produced by interaction of PSI with PG could arise from a decreased static quenching caused by the conformational change [57].

4. Discussion

In the present study, we have employed the absorption, fluorescence and CD spectroscopy to characterize the molecular reorganization of reconstituted PSI within PG environment.

As displayed in Fig. 1, PG effect resulted in blue-shift for the adsorption peak in both the blue and red region, concomitantly with a decrease in the absorption intensity of A_{681}/A_{440}. The absorption band at 440 and 681 nm reflects Chl *a* electronic absorption properties or the

electronic state of Chl *a*. A small blue-shift of the absorption band in both the blue and red region caused by PG effect indicated that the Chl *a* binding state to the protein is somewhat affected even if it is still bound to the protein [59]. The Chl *a* molecules with maximum absorption at 681 nm are mainly attributed to LHCI [44,45]. Therefore, the changes in absorption spectra in this study demonstrated that the microenvironment around Chl *a* molecules within LHCI of PSI was altered upon reconstitution.

The modification of microenvironment around Chl *a* molecules of LHCI was also indicated by the 77 K fluorescence characteristic of reconstituted PSI particles in the present work. It was reported that the long-wavelength Chl of a maximum fluorescence at 730~735 nm is associated primarily with LHCI-730, while the long-wavelength Chl with a maximum at about 680 nm is related to LHCI-680 [45,54]. It was clearly seen from Fig. 2 that the effect of PG gave rise to a blue-shift of emission peak of PSI, reflecting the alteration of the microenvironment around the Chl *a* molecules of LHCI-730. In addition, an appearance of characteristic fluorescence of dissociated LHCI due to the PG addition was also observed. As shown in Fig. 2, PSI incorporation into PG liposomes experienced the blue-shift of the emission peak at 733.2 nm and the decrease in the ratio of F730–735 to F680. This is accompanied by the increase of fluorescence intensity at 680 nm. The occurrence of emission fluorescence at 680 nm is believed to be the dissociation of LHCI-680 from the core complex, in which LHCI-680 stays as a non-aggregated monomeric state [45,60]. It was reported that LHCI-680 is weakly attached to the PSI core complexes, compared to the strong binding for LHCI-730 [8]. That may mean the easier segregative property of LHCI-680. The blue-shift of fluorescence peak at 733.2 nm as well as the decrease of the ratio of F730–735 to F680 suggests that the reconstitution of PSI into PG liposomes not only alters the microenvironment around the Chl molecules, but also induces partial Chl reorganization in LHCI within PSI due to the presence of PG.

Molecular reorganization of Chls within PSI was also reflected in the visible CD spectra. Intensities of both the Chl *a* and Chl *b* bands increased in the CD spectrum of Fig. 3 when PSI was reconstituted into liposomes, indicating that the excitonic interactions for Chl *a* and Chl *b* molecules were enhanced. Furthermore, PG effect resulted in more or less blue- or red-shifts of the peaks characterized by Chl *a*, Chl *b*, and carotenoid molecules. In PSI, Chl *b* molecules are only contained in LHCI. In addition, the reported CD spectra of LHCI show no positive bands of carotenoids in the region of 500~510 nm [60,61]. Indeed, PSI core complex contains most of carotenoids while LHCI proteins coordinate a small amount of carotenoids [60,62]. In this context, we believe that the positive bands peaking at about 504.5 nm are most probably originated from the carotenoids in PSI core complex, specifically in PsaA/PsaB [62]. The blue- or red-shift of CD spectra may indicate an alteration in the internal environment or the orientation of the pigment molecules [63], so the CD data in this study indicate that the reconstitution of the PSI particles induces some alteration of pigment organization within both the external antenna LHCI and PSI core complex, and new interactions between pigments or pigments and proteins seem to appear in PSI in the lipid environment. It therefore means that PG effect does affect pigment–pigment/pigment–protein interactions of PSI.

On the other hand, pigment–protein interactions may also be responsible for molecular CD, which originates from a protein-induced chirality of the chromophore [64]. In this respect, the changes in pigment–protein or pigment–pigment interactions are related to the modification of protein secondary structure of PSI complexes. Indeed, alterations in the protein secondary structures of PSI complexes were observed after reconstitution into PG liposomes. Detailed analysis of the ultraviolet CD spectra reveal that PG effect primarily caused a decrease in disordered structures coupled with an increase in α-helical conformations, showing a more ordered structural organization of proteins in PSI.

The conformational rearrangement is also probably responsible for some changes in the surrounding of the Trp residues in PSI proteins. In this study, a small red-shift of the maximum with obvious fluorescence quenching was observed upon the interaction with PG. The exposure of Trp residues to a more polar environment or the interaction with polar ligands results in the Trp fluorescence quenching and a red-shift of the maximum wavelength [57]. It is possible that following an increase in α-helical content of PSI coupled with a decrease in the disordered structures, the new structure of the protein allows Trp residues become exposed to the solvent, and the new polar tryptophan environment is created by the protein structure.

In summary, the results in the present work clearly disclosed that in vitro PG effect can induce molecular reorganization in both the core complex and peripheral antenna of PSI complex, although the changes were not too much. The observations reported here are comparable with the studies on PG deficiency-induced structural organization of PSI. It was earlier reported that *Synechocystis* sp. PCC6803 mutants with the disruption of the *pgsA* or the *cdsA* gene, encoding a PGP synthase and a CDP-diacylglycerol synthase, respectively, have severe defects in growth and photosynthesis [32,33]. The growth and concomitant photosynthetic activity of these mutants have an absolute requirement for PG supplementation, and vary with the different levels of PG that can be manipulated in the mutant cells by exogenous addition of PG, indicating the specific dependence of the function of photosynthesis on PG concentration. More recently, it was found that a 21-d PG depletion from *pgsA* mutant resulted in the degradation of PSI trimers and the concomitant accumulation of monomer PSI simultaneously with the decrease in PSI activity. Nevertheless, the trimeric structure of PSI reaction center could be restored by readdition of PG. It was quite

interesting since it presented strong evidence in vivo of the essential role of PG for structural organization of cyanobacterial PSI [37], compatible to the observation that in vitro oligomerization of cyanobacterial PSI can be induced by liposome-based reconstitution [38].

In the present work, the large amount of exogenous PG would bind non-covalently to the surface of PSI particles. In this context, it appears that PG affected the molecular organization of PSI in this way through interaction with LHCI or the three extrinsic proteins of PsaC, PsaD, and PsaE. The alterations in the spectroscopic properties of Chl visible absorption, low-temperature fluorescence, and CD of LHCI in this study may reflect this case.

Another way should be considered for PG action is that PG molecules may incorporate into the PSI complex. In this way, PG insertion into the gap between components of PSI would perturb the binding state of the pigments or native lipids, for example, MGDG and PG, to the polypeptides of PSI. In this study, a small detachment of LHCI-680 from the PSI core complex was observed upon reconstitution. In this respect, some dissociation of LHCI-680 is most likely related to the effect of excess lipids that introduce additional phospholipids into the somewhat large space among LHCI and the PSI core complex [8]. Siegel et al. [65] have also shown that the thylakoid particles became more widely spaced upon incorporation into liposomes of PC at high concentration, reflecting that the insertion of more lipid molecules into the natural lipid matrix results in a spatial separation of membrane components.

The third way for PG effect on the molecular organization of PSI is the replacement of zwitterion detergent Triton X-100 by PG substitution from the PSI samples isolated by using Triton X-100. In the present work, the preparation of PSI particles described in Material and methods was inevitably suffered from more or less an amount of Triton X-100 being remained due to incomplete removal by the common dialysis method. According to Ref. [66], the reconstitution of PSI into lipid matrix can cause the effect that almost all of the detergent interacts with the lipids. Therefore, the replacement of Triton X-100 from the PSI can modify the binding site for Triton X-100 that associates with pigments and/or polypeptides of PSI. In fact, it was found that the Triton X-100 treatment could cause the changes in Chl binding state to proteins in PSII and the protein secondary structures of PSII and PSI [56,67].

Based on the considerations mentioned above, one can expect that either some or all of three ways for PG action could cause more or less changes in molecular organization of PSI.

As a conclusion, a straightforward consequence from the above results and consideration is the existence of the interaction between PSI and PG molecules as disclosed by the fact that PG effect induces the changes in the molecular organization of pigments and proteins in both the external antenna LHCI and PSI core complex. In other words, PG perturbation can affect the molecular organization of PSI, or in general, PG has an ability to regulate the integrity of assembly of PSI particles.

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References

[1] P.R. Chitinis, Photosystem I: function and physiology, Annu. Rev. Plant Physiol. Plant Mol. Biol. 52 (2001) 593–626.
[2] H.V. Scheller, P.E. Jensen, A. Haldrup, C. Lunde, J. Knoetzel, Role of subunits in eukaryotic photosystem I, Biochim. Biophys. Acta 1507 (2001) 41–60.
[3] J. Kargul, J. Nield, J. Barber, Three-dimensional reconstruction of a light-harvesting complex I–photosystem I (LHCI–PSI) supercomplex from the green alga *Chlamydomonas reinhardtii*. Insights into light harvesting for PSI, J. Biol. Chem. 278 (2003) 16135–16141.
[4] D.G. Durnford, J.A. Deane, S. Tan, G.I. Mcfadden, E. Gantt, B.R. Green, A phylogenetic assessment of the eukaryotic light-harvesting antenna proteins, with implications for plastid evolution, J. Mol. Evol. 48 (1999) 59–68.
[5] A. Ben-Shem, F. Frolow, N. Nelson, Light-harvesting features revealed by the structure of plant photosystem I, Photosynth. Res. 81 (2004) 239–250.
[6] E.J. Boekema, R.M. Wynn, R. Malkin, The structure of spinach photosystem I studied by electron microscopy, Biochim. Biophys. Acta 1017 (1990) 49–56.
[7] E.J. Boekema, P.E. Jensen, E. Schlodder, J.F.L. van Breemen, H. van Roon, H.V. Scheller, J.P. Dekker, Green plant photosystem I binds light-harvesting complex I on one side of the complex, Biochemistry 40 (2001) 1029–1036.
[8] A. Ben-Shem, F. Frolow, N. Nelson, Crystal structure of plant photosystem I, Nature 426 (2003) 630–635.
[9] M. Ballottari, C. Govoni, S. Caffarri, T. Morosinotto, Stoichiometry of LHCI antenna polypeptides and characterization of gap and linker pigments in higher plants photosystem I, Eur. J. Biochem. 271 (2004) 4659–4665.
[10] S. Jansson, A guide to the Lhc genes and their relatives in *Arabidopsis*, Trends Plant Sci. 4 (1999) 236–240.
[11] S. Storf, E.J. Stauber, M. Hippler, V.H.R. Schmid, Proteomic analysis of the photosystem I light-harvesting antenna in tomato (*Lycopersicon esculentum*), Biochemistry 43 (2004) 9214–9224.
[12] P. Jordan, P. Fromme, H.T. Witt, O. Klukas, W. Saenger, N. Krauss, Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution, Nature 411 (2001) 909–917.
[13] P. Fromme, P. Jordan, N. Krausß, Structure of photosystem I, Biochim. Biophys. Acta 1507 (2001) 5–31.
[14] K. Brettel, W. Leibl, Electron transfer in photosystem I, Biochim. Biophys. Acta 1507 (2001) 100–114.
[15] M. Guergova-Kuras, B. Boudreaux, A. Joliot, P. Joliot, K. Redding, Evidence for two active branches for electron transfer in photosystem I, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 4437–4442.
[16] I.P. Muhiuddin, P. Heathcote, S. Carter, S. Purton, S.E.J. Rigby, M.C.W. Evans, Evidence from time resolved studies of the $P700^{+}/A_1^{-}$ radical pair for photosynthetic electron transfer on both the PsaA and

PsaB branches of the photosystem I reaction centre, FEBS Lett. 503 (2001) 56–60.
[17] S. Purton, D.R. Stevens, I.P. Muhiuddin, M.C.W. Evans, S. Carter, S.E.J. Rigby, P. Heathcote, Site-directed mutagenesis of PsaA residue W693 affects phylloquinone binding and function in the photosystem I reaction center of *Chlamydomonas reinhardtii*, Biochemistry 40 (2001) 2167–2175.
[18] R. Agalarov, K. Brettel, Temperature dependence of biphasic forward electron transfer from the phylloquinone(s) A1 in photosystem I: only the slower phase is activated, Biochim. Biophys. Acta 1604 (2003) 7–12.
[19] H. Ishikita, E.-W. Knapp, Redox potential of quinones in both electron transfer branches of photosystem I, J. Biol. Chem. 278 (2003) 52002–52011.
[20] H.V. Scheller, I. Svendsen, B.L. Msller, Subunit composition of photosystem I and identification of center X as a [^{4}Fe–^{4}S] iron–sulfur cluster, J. Biol. Chem. 264 (1989) 6929–6934.
[21] P.A. Siegenthaler, N. Murata, Lipids in Photosynthesis: Structure, Function and Genetics, Kluwer Academic Publishers, The Netherlands, 1998.
[22] M.S. Webb, B.R. Green, Biochemical and biophysical properties of thylakoid acyl lipids, Biochim. Biophys. Acta 1060 (1991) 133–158.
[23] P.A. Siegenthaler, A. Rawyler, J. Smutny, The phospholipid population which sustains the uncoupled non-cyclic electron flow activity is localized in the inner monolayer of the thylakoid membrane, Biochim. Biophys. Acta 975 (1989) 104–111.
[24] S. Duchene, J.P. Smutny, A. Siegenthaler, The topology of phosphatidylglycerol populations is essential for sustaining photosynthetic electron flow activities in thylakoid membranes, Biochim. Biophys. Acta 1463 (2000) 115–120.
[25] Z. Gombos, Z. Varkonyi, M. Hagio, M. Iwaki, L. Kovacs, K. Masamoto, S. Itoh, H. Wada, Phosphatidylglycerol requirement for the function of electron acceptor plastoquinone Q(B) in the photosystem II reaction center, Biochemistry 41 (2002) 3796–3802.
[26] O. Kruse, B. Hankamer, C. Konczak, C. Gerle, E. Morris, A. Radunz, G.H. Schmid, J. Barber, Phosphatidylglycerol is involved in the dimerization of photosystem II, J. Biol. Chem. 275 (2000) 6509–6514.
[27] S. Nuβberger, K. Dörr, D.N. Wang, W. Kuhlbrandt, Lipid–protein interaction in crystals of plant light-harvesting complex, J. Mol. Biol. 224 (1993) 347–356.
[28] S. Hobe, S. Prytulla, W. Kuhlbrandt, H. Paulsen, Trimerization and crystallization of reconstituted light-harvesting chlorophyll *a*/*b* complex, EMBO J. 13 (1994) 3423–3429.
[29] Z. Liu, H. Yan, K. Wang, T. Kuang, J. Zhang, L. Gui, X. An, W. Chang, Crystal structure of spinach major light-harvesting complex at 2.72 Å resolution, Nature 428 (2004) 287–292.
[30] O. Kruse, G.H. Schmid, The role of phosphatidylglycerol as a functional effector and membrane anchor of the D1-core peptide from photosystem II particles of the cyanobacterium *Oscillatoria chalybea*, Z. Naturforsch. 50c (1995) 380–390.
[31] E. Babiychuk, F. Muller, H. Eubel, H.P. Braun, M. Frentzen, S. Kushnir, *Arabidopsis* phosphatidylglycerophosphate synthase 1 is essential for chloroplast differentiation, but is dispensable for mitochondrial function, Plant J. 33 (2003) 899–909.
[32] M. Hagio, Z. Gombos, Z. Varkonyi, K. Masamoto, N. Sato, M. Tsuzuki, H. Wada, Direct evidence for requirement of phosphatidylglycerol in photosystem II of photosynthesis, Plant Physiol. 124 (2000) 795–804.
[33] I. Sakurai, M. Hagio, Z. Gombos, T. Tyystjarvi, V. Paakkarinen, E.M. Aro, H. Wada, Requirement of phosphatidylglycerol for maintenance of photosynthetic machinery, Plant Physiol. 133 (2003) 1376–1384.
[34] N. Sato, M. Hagio, H. Wada, M. Tsuzuki, Requirement of phosphatidylglycerol for photosynthetic function in thylakoid membranes, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 10655–10660.
[35] C.C. Xu, H. Hartel, H. Wada, M. Hagio, B. Yu, C. Eakin, C. Benning, The *pgp1* mutant locus of *Arabidopsis* encodes a phosphatidylglycerolphosphate synthase with impaired activity, Plant Physiol. 129 (2002) 594–604.
[36] B. Pineau, J. Girard-Bascou, S. Eberhard, Y. Choquet, A. Tremolieres, C. Gerard-Hirne, A. Bennardo-Connan, P. Decottignies, S. Gillet, F.A. Wollman, A single mutation that causes phosphatidylglycerol deficiency impairs synthesis of photosystem II cores in *Chlamydomonas reinhardtii*, Eur. J. Biochem. 271 (2004) 329–338.
[37] I. Domonkos, P. Malec, A. Sallai, L. Kovacs, K. Itoh, G. Shen, B. Ughy, B. Bogos, I. Sakurai, M. Kis, K. Strzalka, H. Wada, S. Itoh, T. Farkas, Z. Gombos, Phosphatidylglycerol is essential for oligomerization of photosystem I reaction center, Plant Physiol. 134 (2004) 1471–1478.
[38] J. Kruip, N.V. Karapetyan, I.V. Terekhova, M. Rogner, In vitro oligomerization of a membrane protein complex. Liposome-based reconstitution of trimeric photosystem I from isolated monomers, J. Biol. Chem. 274 (1999) 18181–18188.
[39] A. Makewicz, A. Radunzm, G.H. Schmid, Comparative immunological detection of lipids and carotenoids on peptides of photosystem I from higher plants and cyanobacteria, Z. Naturforsch., C 51 (1996) 319–328.
[40] P.A. Millner, J.P. Grouzis, D.J. Chapman, J. Barber, Lipid enrichment of thylakoid membranes: I. Using soybean phospholipids, Biochim. Biophys. Acta 722 (1983) 331–340.
[41] J. Cladera, J.L. Rigaud, H. Bottin, M. Dunach, Functional reconstitution of photosystem I reaction center from cyanobacterium *Synechocystis* sp PCC6803 into liposomes using a new reconstitution procedure, J. Bioenerg. Biomembranes 28 (1996) 503–515.
[42] S. Hoshina, S. Itoh, Characterization of photosystem I chlorophyll–protein complexes reconstituted into phosphatidylcholine liposomes, Plant Cell Physiol. 28 (1987) 599–609.
[43] E. Nabedryk, P. Biaudet, S. Darr, C.J. Arntzen, J. Breton, Conformation and orientation of chlorophyll–proteins in photosystem I by circular dichroism and polarized infrared spectroscopies, Biochim. Biophys. Acta 767 (1984) 640–647.
[44] J.E. Mullet, J.J. Burke, C.G. Arnezen, Chlorophyll proteins of photosystem I, Plant Physiol. 65 (1980) 814–822.
[45] R. Bassi, D. Simpson, Chlorophyll–protein complexes of barley photosystem I, Eur. J. Biochem. 163 (1987) 221–230.
[46] D.I. Arnon, Copper enzymes in isolated chloroplasts: polyphenyloxidase in *Beta vulgaris*, Plant Physiol. 14 (1949) 1–15.
[47] E.K. Nenonene, M. Fragata, Interaction of photosystem II proteins with non-aggregated membranes constituted of phosphatidylglycerol and the electrically neutral phosphatidylcholine enhances the oxygen-evolving activity, Chem. Phys. Lipids 91 (1998) 97–107.
[48] N. Sreerama, R.W. Woody, A self-consistent method for the analysis of protein secondary structure from circular dichroism, Anal. Biochem. 209 (1993) 32–44.
[49] T.Y. Kuang, J.H. Argyroudi-Akoyunoglou, H.Y. Nakatani, J. Watson, C.J. Arntzen, The origin of the long-wavelength fluorescence emission band (77 K) from photosystem I, Arch. Biochem. Biophys. 235 (1984) 618–627.
[50] K.D. Philipson, V.L. Sato, K. Sauer, Exciton interaction in the photosystem I reaction center from spinach chloroplasts. Absorption and circular dichroism difference spectra, Biochemistry 11 (1972) 4591–4595.
[51] R. Bassi, M. Silvestri, P. Dainese, I. Moya, G.M. Giacometti, Effects of a non-ionic detergent on the spectral properties and aggregation state of the light-harvesting chlorophyll *a*/*b* protein complex (LHCII), J. Photochem. Photobiol., B Biol. 9 (1991) 335–354.
[52] A.V. Ruban, F. Calkoen, S.L.S. Kwa, R.V. Grondelle, P. Horton, J.P. Dekker, Characterization of LHCII in the aggregated state by linear and circular dichroism spectroscopy, Biochim. Biophys. Acta 1321 (1997) 61–70.

[53] O.J. Somsen, R. van Grondelle, H. van Amerongen, Spectral broadening of interacting pigments: polarized absorption by photosynthetic proteins, Biophys. J. 71 (1996) 1934–1951.
[54] E. Lam, W. Ortiz, R. Malkin, Chlorophyll *a*/*b* proteins of photosystem I, FEBS Lett. 168 (1984) 10–14.
[55] P. Haworth, J.L. Waeson, C.J. Arntzen, The detection, isolation and characterization of a light-harvesting complex which is specifically associated with photosystem I, Biochim. Biophys. Acta 724 (1983) 151–158.
[56] X. Ruan, J. Wei, Q. Xu, J. Wang, Y. Gong, X. Zhang, T. Kuang, N. Zhao, Comparison of the effects of Triton X-100 treatment on the protein secondary structure of photosystem I and photosystem II studied by FT-IR spectroscopy, J. Mol. Struct. 525 (2000) 97–106.
[57] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, 2nd edition, Kluwer Academic/Plenum, New York, 1999.
[58] E.A. Brustein, N.S. Vedenkina, M.N. Irkova, Fluorescence and the location of tryptophan residues in protein molecules, Photochem. Photobiol. 18 (1973) 263–279.
[59] J. Wang, J. Shan, Q. Xu, X. Ruan, Y. Gong, T. Kuang, N. Zhao, Spectroscopic study of trypsin, heat and Triton X-100-induced denaturation of the chlorophyll-binding protein CP43, J. Photochem. Photobiol., B Biol. 58 (2000) 136–142.
[60] R. Croce, T. Morosinotto, S. Castelletti, J. Breton, R. Bassi, The Lhca antenna complexes of higher plants photosystem I, Biochim. Biophys. Acta 1556 (2002) 29–40.
[61] S. Castelletti, T. Morosinotto, B. Robert, S. Caffarri, R. Bassi, R. Croce, Recombinant Lhca2 and Lhca3 Subunits of the photosystem I antenna system, Biochemistry 42 (2003) 4226–4234.
[62] H.V. Scheller, P.E. Jensen, A. Haldrup, C. Lunde, J. Knoetzel, Role of subunits in eukaryotic photosystem I, Biochim. Biophys. Acta 1507 (2001) 41–60.
[63] P. Mohanty, B. Vani, J.S.S. Prakash, Elevated temperature treatment induced alteration in thylakoid membrane organization and energy distribution between the two photosystems in *Pisum sativum*, Z. Naturforsch. 57c (2002) 836–842.
[64] M. Germano, A.Y. Shkuropatov, H. Permentier, R. de Wijn, A.J. Hoff, V.A. Shuvalov, H.J. van Gorkom, Pigment organization and their interactions in reaction centers of photosystem II: optical spectroscopy at 6 K of reaction centers with modified pheophytin composition, Biochemistry 40 (2001) 11472–11482.
[65] C.O. Siegel, A.E. Jordan, K.R. Miller, Addition of lipid to the photosynthetic membrane: effects on membrane structure and energy transfer, J. Cell Biol. 91 (1981) 113–125.
[66] M. Ollivon, S. Lesieur, C. Grabielle-Madelmont, M. Paternostre, Vesicle reconstitution from lipid–detergent mixed micelles, Biochim. Biophys. Acta 1508 (2000) 34–50.
[67] X. Ruan, D. Li, Q. Xu, H. Mao, G. Li, Y. Gong, T. Kuang, N. Zhao, Phosphatidylcholine-induced reactivation of photosystem II membranes pretreated with Triton X-100, J. Photochem. Photobiol., B Biol. 67 (2002) 109–115.