Phosphatidylglycerol Requirement for the Function of Electron Acceptor Plastoquinone Q_B in the Photosystem II Reaction Center†

Zoltán Gombos,‡,§ Zsuzsanna Várkonyi,§ Miki Hagio,‡ Masayo Iwaki,|| László Kovács,§ Kazumori Masamoto,⊥ Shigeru Itoh,# and Hajime Wada*,‡

Department of Biology, Faculty of Sciences, Kyushu University, Ropponmatsu, Fukuoka 810-8560, Japan, Institute of Plant Biology, Biological Research Center of Hungarian Academy of Sciences, P.O. Box 521, H-6701 Szeged, Hungary, CEA/Saclay, 91191 Gif-sur-Yvette Cedex, France, Biological Laboratory, Faculty of Education, Kumamoto University, Kurokami, Kumamoto 860-0862, Japan, and Division of Material Science (Physics), Graduate School of Science, Nagoya University, Furocho, Chikusa, Nagoya 464-8601, Japan

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ABSTRACT: Phosphatidylglycerol (PG), a ubiquitous constituent of thylakoid membranes of chloroplasts and cyanobacteria, is demonstrated to be essential for the functionality of plastoquinone electron acceptor Q_B in the photosystem II reaction center of oxygenic photosynthesis. Growth of the *pgsA* mutant cells of *Synechocystis* sp. PCC6803 that are defective in phosphatidylglycerolphosphate synthase and are incapable of synthesizing PG, in a medium without PG, resulted in a 90% decrease in PG content and a 50% loss of photosynthetic oxygen-evolving activity as reported [Hagio, M., Gombos, Z., Várkonyi, Z., Masamoto, K., Sato, N., Tsuzuki, M., and Wada, H. (2000) *Plant Physiol. 124*, 795−804]. We have studied each step of the electron transport in photosystem II of the *pgsA* mutant to clarify the functional site of PG. Accumulation of Q_A^- was indicated by the fast rise of chlorophyll fluorescence yield under continuous and flash illumination. Oxidation of Q_A^- by Q_B plastoquinone was shown to become slow, and Q_A^- reoxidation required a few seconds when measured by double flash fluorescence measurements. Thermoluminescence measurements further indicated the accumulation of the $S_2Q_A^-$ state but not of the $S_2Q_B^-$ state following the PG deprivation. These results suggest that the function of Q_B plastoquinone was inactivated by the PG deprivation. We assume that PG is an indispensable component of the photosystem II reaction center complex to maintain the structural integrity of the Q_B-binding site. These findings provide the first clear identification of a specific functional site of PG in the photosynthetic reaction center.

Thylakoid membranes in chloroplasts and cyanobacterial cells provide the matrix for the primary reactions of oxygenic photosynthesis that are performed in the pigment−protein reaction center complexes which are surrounded by the bilayers of glycerolipids (*1*). The lipid composition of thylakoid membranes is highly conserved among oxygenic photosynthetic organisms (*1*−*3*). Thylakoid membranes contain uncharged monogalactosyldiacylglycerol and digalactosyldiacylglycerol, and anionic sulfoquinovosyldiacylglycerol and phosphatidylglycerol (PG).[1] In the present study, we studied the functional role of PG, a minor (at about 10% of total thylakoid lipids) but indispensable lipid in vivo.

The interaction of glycerolipids with proteins in thylakoid membranes has been assumed to modulate the electron transfer rate, and the effects of lipid modifications have been extensively studied in vitro to identify the specific function of the individual lipid classes (*1*). The decomposition of PG from thylakoid membranes by using phospholipase A_2 suppressed the photosynthetic electron transport rate (*4*, *5*). Treatment of thylakoid membranes with phospholipase C, which removes the headgroup of phospholipid molecules, also decreased the photosynthetic activity presumably in photosystem (PS) II (*6*). Kruse and Schmid (*7*) suggested a specific binding of PG to D1 protein by the use of an antibody against PG and postulated that PG anchors the D1 protein and stabilizes the PS II reaction center (RC). PG was also suggested to be required for the dimerization of PS II RC based on the effects of phospholipase A_2 digestion (*8*). These in vitro enzymatic treatments suggested the importance of PG in photosynthesis especially in the PS II function. However, none of these experiments could confirm any

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* To whom correspondence should be addressed. Tel/Fax: 81−92−726−4761. E-mail: wadarcb@mbox.nc.kyushu-u.ac.jp.

‡ Kyushu University.

§ Biological Research Center of Hungarian Academy of Sciences.

|| CEA/Saclay.

⊥ Kumamoto University.

Nagoya University.

[1] Abbreviations: CDP, cytidine 5′-diphosphate; Chl, chlorophyll; DCBQ, 2,6-dichloro-*p*-benzoquinone; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; DMBQ, 2,6-dimethyl-*p*-benzoquinone; E, Einstein; PB, phycobilin; PG, phosphatidylglycerol; PQ, plastoquinone; PS, photosystem; RC, reaction center; TL, thermoluminescence.

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specific functional site of PG in PS II on molecular level. The in vitro enzymatic treatments produce degradation products of PG at the same time, and might also have been affected by the artificial, nonspecific side effects. Direct, in vivo evaluation of specific lipid function as done here by the targeted gene manipulation has been awaited.

The core of PS II is a heterodimer of RC polypeptides D1 and D2 that show homologies with each other (*9*) and are essentially homologous to the L and M subunits, respectively, of purple bacterial RC that is also a heterodimer (*10*). The PS II heterodimer, however, does not contain a subunit corresponding to the H-subunit of the latter that covers and stabilizes the reducing side, but it attaches peripheral proteins on the oxidizing side as recently shown by X-ray crystallography with 3.8 Å resolution (*9*). The purified PS II RC complex made of D1, D2, and cytochrome b_{559} polypeptides lacks the electron acceptor plastoquinones (PQ) Q_A and Q_B (*11*) and shows weak affinities for plastoquinones as well as for artificial ones that recover Q_A function (*12*). Roles of lipids as functional factors have been suggested both on reducing and oxidizing sides of PS II that have also been a major target of many kinds of stresses such as high light stress (*13*). The sites of lipid function, however, have not been assigned clearly.

PG is synthesized from phosphatidic acid via cytidine 5′-diphosphate (CDP) diacylglycerol by CDP-diacylglycerol synthase and PG phosphate synthase. On the basis of the determination of the entire nucleotide sequence of the genome of *Synechocystis* sp. PCC6803 (*14*), we identified and disrupted the *pgsA* gene encoding a PG phosphate synthase to manipulate the content of PG in the thylakoid membranes (*15*). The deprivation of PG from the growth medium decreased the PG content of the *pgsA* mutant cells, blocked cell growth and led to a 50% decrease of oxygen evolving activity on the chlorophyll (Chl) basis (*15*), demonstrating that PG is essential for cell growth and function of electron transport system. Disruption of a gene encoding CDP-diacylglycerol synthase in *Synechocystis* sp. PCC6803 (*SNC1* mutant) (*16*) also led to the repression of PG biosynthesis and of growth indicating the essential requirement of PG. In these *SNC1* mutant cells, however, repression of the oxygen evolving activity was small on the Chl basis, although the activity per cell was decreased to about a half due to the parallel decrease of Chl content. It was suggested that the depression of the activity per cell is a consequence of the deprivation of PG that is necessary for the accumulation of Chl-proteins. PG was also assumed to play some functional roles in PS II since the oxygen evolution was suppressed by addition of *p*-benzoquinone, which served as the efficient electron acceptor for PS II in wild-type cells. The results in the two types of mutants that were defective in different steps of PG-biosynthetic pathway thus indicated the importance of PG in cell growth and photosynthesis, but did not reveal the specific action site of PG.

In the present study, we have investigated the role of PG in each step of PS II electron-transfer reaction in the *pgsA* mutant cells and demonstrate that PG deprivation in vivo specifically inactivates the function of the second electron acceptor PQ, Q_B, in the PS II RC. The results indicate that PG is an indispensable component of the PS II RC.

EXPERIMENTAL PROCEDURES

Organisms and Culture Conditions. The wild type and the *pgsA* mutant cells of *Synechocystis* sp. PCC6803 were grown photoautotrophically at 28 °C in BG-11 medium supplemented with 4 mM HEPES−NaOH (pH 7.5) and 20 μM PG as described previously (*15*). Growth of the cultures was monitored by determination of the optical density at 730 nm (OD_{730}).

Lipid Analysis. Lipids were extracted from intact cells by the method of Bligh and Dyer (*17*), and analyzed as described previously (*18*).

Measurement of Photosynthetic Activities. Photosynthetic oxygen evolving activity from H_2O to CO_2 of intact cells was measured with a Clark-type oxygen electrode (Hansatech Instruments, Kings Lynn, U.K.) as described by Gombos et al. (*19*). PS II specific oxygen evolving activity was measured from H_2O to artificial quinones exogenously added at various concentrations as indicated in the text. The cells were washed twice with BG-11 medium and were suspended in the same medium for the measurement of oxygen evolution. Light from an incandescent lamp through a red optical filter (2−61; Corning inc., New York, NY) was used for all the oxygen evolution measurements at the light intensity of 500 μEinstein (E) m^{-2} s^{-1}. The Chl concentration of the cells was adjusted to be 2 μg mL^{-1}. The concentration of Chl was determined by the method of Arnon et al. (*20*).

Fluorescence Induction Measurements. Induction of Chl *a* fluorescence under stationary illumination light was measured either with a pulse-modulated fluorometer (PAM 101, Heinz Walz GmbH, Effeltrich, Germany) or with a home-built fluorometer. In the former type of measurements, the F_m level of fluorescence associated with reduced Q_A was recorded during short (1−2 s) saturating light pulses obtained from a halogen lamp (KL-1500 Electronic, Schott Glasswerke, Wiesbaden, Germany) with filters (Calflex CK 65, Blazers; RG 630, Schott). The difference between the F_m and F_o levels was noted as variable fluorescence F_v. In the latter type of measurements, intensity of fluorescence at 685 nm was monitored by a combination of interference filter (20-nm full width at the half-maximum, Nihon Shinko Kagaku) and a photomultiplier (R-446, Hamamatsu Photonics) under the excitation with continuous blue light (400−500 nm with a maximum at 430 nm) obtained from a tungsten−iodine lamp through a band-pass glass filter (B-430, Toshiba) and a broadband interference filter. Excitation light intensity was adjusted to be enough to give the full rise of fluorescence intensities within 1 s that corresponds to the Q_A reduction in the presence of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU).

Double Flash Measurement of Microsecond Rise and Decay Kinetics of Chl a Fluorescence. The change in fluorescence yield after a strong saturating laser flash was detected with a weak probing xenon flash light of 3 μs duration at 430 nm, whose intensity was adjusted to excite only a few percentages of PS II RCs as described elsewhere (*21*). The probing xenon flash was given at varied times after the Nd:YAG laser flash (with a 10-ns duration at 532 nm, DCR-2-10, Quanta-Ray) of saturating intensity to monitor the increase of fluorescence yield that reflects the redox state of the P_{680} and Q_A after the excitation of all the PS II RC.

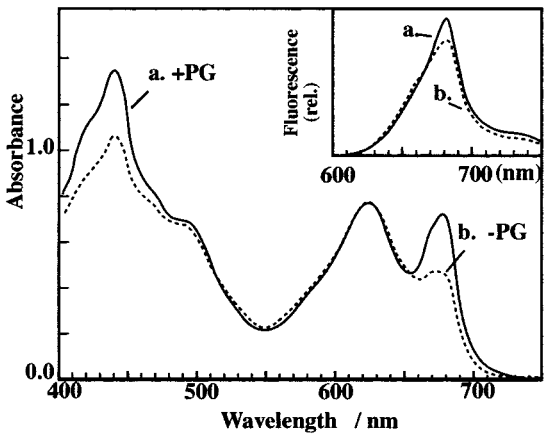

FIGURE 1: Absorption spectra of the *pgsA* mutant of *Synechocystis* sp. PCC6803. The spectra were measured using whole cells of the mutant cells grown with PG (a) and without PG (b). In the case of trace b, the cells grown in the presence of PG were transferred to the medium without PG and incubated for 6 days. An inset shows the fluorescence spectra of the intact cells grown with PG (a) and without PG (b) measured upon the excitation of the 435 nm blue peak of Chl *a*.

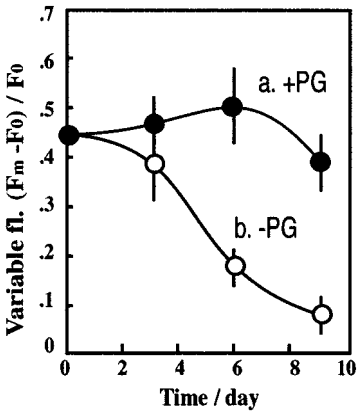

FIGURE 2: Effect of deprivation of PG on induction of room temperature Chl fluorescence in the *pgsA* mutant of *Synechocystis* sp. PCC6803. The cells grown with PG were transferred into the medium with PG (-●-) or without PG (-○-) and incubated for designated time. Fluorescence was measured in the presence of 10 μM DCMU. The values are means and standard deviations from three independent measurements. F_m, maximum fluorescence; F_0, initial fluorescence.

Measurement of Thermoluminescence (TL). The TL measurement was carried out according to Vass et al. (*22*) with minor modifications. The Chl concentration of the samples was adjusted to 10 μg mL^{-1}. Samples (0.4 mL) were preilluminated at −80 °C with continuous white light (200 μE m^{-2} s^{-1}) for 30 s, and then the glow curves were recorded at a heating rate of 20 °C min^{-1}.

RESULTS

PG Content of the pgsA Mutant Cells. When the cells of the *pgsA* mutant were grown in the presence of 20 μM PG, the cells showed high PG content [2143 nmol (mg of Chl)$^{-1}$] and normal photosynthetic activity [390 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}] as previously demonstrated (*15*). After the transfer of the cells into the medium without PG, the level of PG decreased to 215 nmol (mg of Chl)$^{-1}$ following a 6-day long PG deprivation. Simultaneously, the photosynthetic oxygen evolving activity decreased to 180 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}.

Pigment Content of the pgsA Mutant Cells. A partial decrease of pigment content was detected in the cells that lost PG. However, the loss of oxygen evolving activity was more significant than the decrease in the pigment as seen in the 50% decrease in the activity on the Chl basis. Figure 1 shows the absorption spectrum of the *pgsA* mutant cells cultured with PG (+PG cells) and without PG (−PG cells) normalized at the peak of phycobilin (PB) band. Consistent with the low Chl content, the spectrum of −PG cells showed a decreased 678 nm peak of Chl *a* with respect to the PB band. This result is in good agreement with that reported by Sato et al. (*16*), although in their case the light scattering shaded the effect of PG deprivation on pigment composition. An inset in Figure 1 shows the fluorescence spectra in these cells measured upon the excitation of the 435 nm blue peak of Chl *a*. The −PG mutant cells showed a spectrum similar to that of +PG mutant cells with a little more marked 660 nm PB fluorescence band.

Effect of Artificial Quinones on Photosynthetic Oxygen Evolving Activity. The photosynthetic oxygen evolving activity of the *pgsA* mutant cells decreased to be 50% after a 6-day deprivation of PG from the medium in parallel with the 90% deprivation of PG from the thylakoid membranes as shown earlier (*15*). The decrease in the activity suggests some changes in the electron-transfer chain from H_2O to CO_2 via PS II and PS I. To study PS II activity alone, we added quinones, which are known to function as the artificial electron acceptor for PS II, to the reaction medium. These quinones did not affect the oxygen evolution up to 1 mM in the mutant cells grown in the presence of PG (*15*). However, the addition of 2,6-dichloro-*p*-benzoquinone (DCBQ), 2,6-dimethyl-*p*-benzoquinone (DMBQ), and *p*-benzoquinone drastically decreased the rate of oxygen evolution remaining in the −PG cells and completely inhibited oxygen evolution above 20 and 400 μM, respectively (data not shown). These results implied that the quinone reaction site(s) is somewhat altered by the deprivation of PG.

Change in Fluorescence Induction. Changes in the relative extent of the variable fluorescence (F_v/F_0 ratio) were measured in the +PG and −PG mutant cells with a PAM system (Figure 2). In the +PG mutant cells, this ratio was about 0.45. The F_V/F_0 ratio gradually decreased to 0.38 after a 3-day deprivation of PG from the growth medium and became 0.08 after a 9-day deprivation. The decrease of the F_V/F_0 ratio was almost parallel with the decreases in the PG content and the oxygen evolving activity. The readdition of PG to the growth medium of PG-depleted cells restored the F_V/F_0 ratio.

Induction of Chl fluorescence was also measured under the continuous 430 nm blue excitation light. Fluorescence intensity slowly increased from the initial level F_0 during the illumination in the cells grown with PG (Figure 3) indicating the slow reduction of the electron acceptor Q_A. In the presence of DCMU that inhibits electron transfer between Q_A and Q_B, the fluorescence increased rapidly to the maximum level (F_m) indicating the faster accumulation of Q_A^- as is well-known (*23*).

In the cells cultured for 6 days without PG, similar fluorescence rise was observed in the presence of DCMU indicating the accumulation of Q_A^-, although the F_v (F_v =

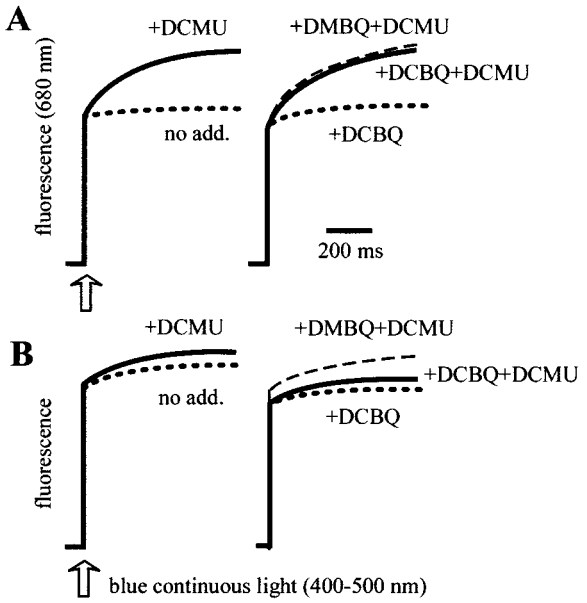

FIGURE 3: Effect of deprivation of PG on fluorescence induction of Chl *a* during the continuous illumination of blue excitation light in the *pgsA* mutant of *Synechocystis* sp. PCC6803. Kinetics of F_{680} in the mutant cells grown with PG (A) or without PG (B) were measured in the absence of DCMU or in the presence of 10 μM DCMU as indicated. In the case of panel B, the cells grown in the presence of PG were transferred to the medium without PG and incubated for 6 days. In the right figures, the cells were treated with 40 μM DCBQ or DMBQ, and the kinetics of F_{680} were measured in the absence of DCMU or in the presence of 10 μM DCMU as indicated.

$F_m - F_0$) extent was smaller. However, the rise of fluorescence intensity during the illumination was also rapid even in the absence of DCMU. This suggests that the electron transfer from Q_A^- to Q_B is slow even without DCMU in this type of cells.

The effects of quinones on fluorescence induction were also investigated. As shown in Figure 3 (right parts), both DCBQ and DMBQ at the concentration of 40 μM did not significantly affect the fluorescence induction in the +PG and −PG mutant cells. At the higher concentration of the quinones, the fluorescence was quenched dramatically in the +PG and −PG mutant cells (data not shown). These results indicate that the primary target site, which is affected by PG deprivation, is Q_B rather than Q_A.

Change in Fast Fluorescence Kinetics. We measured the yield of fluorescence excited by a weak probing xenon flash (at 430 nm with a 3 μs duration) given at varied times after the saturating 532 nm/10 ns actinic laser flash (Figure 4) to monitor the rapid change in the redox state of RC components. In this type of measurement, fluorescence is high only in the RC with a P_{680} Q_A^- state and low with a P_{680} Q_A or P_{680}^+ Q_A^- state as shown in the scheme in Figure 5. In the wild type cells (Figure 4A) and in the +PG mutant cells (Figure 4B), the fluorescence yield rose rapidly with about 2 μs half time ($t_{1/2}$), to the maximal level at 10 μs, and then decreased with a half decay time of 300 μs to one-fifth of the maximum intensity, followed by a slower 2-s decay phase to the dark-adapted F_0 level again (Figure 4A,B). The maximum extent of fluorescence yield at 10 μs was about 1.9-fold of those of F_0 levels in the +PG mutant cells and the wild-type cells grown without PG. According to the literature (*21*), the 2 μs rise ($k_2 = 2\ \mu s^{-1}$) can be assigned to

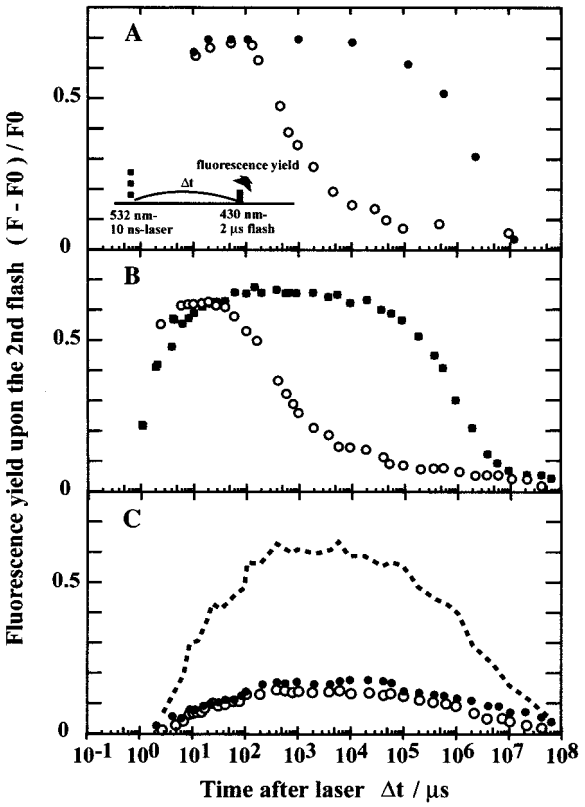

FIGURE 4: Fluorescence intensity excited by a weak flash excitation following a saturating actinic laser flash in the mutant cells of *Synechocystis* sp. PCC6803. The fluorescence was measured using whole cells of the wild-type grown without PG (A), the *pgsA* mutant grown with PG (B) or without PG (C). In the case of panel C, the mutant cells grown in the presence of PG were transferred to the medium without PG and incubated for 6 days. In the closed symbols, the fluorescence was measured in the presence of 10 μM DCMU. The broken line represents 4 times magnification of the curve obtained with the mutant cells grown without PG in the absence of DCMU to facilitate the comparison of the data.

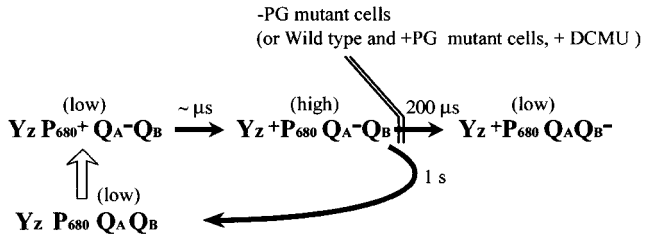

FIGURE 5: A scheme for electron transfer in PS II RC in the *pgsA* mutant of *Synechocystis* sp. PCC6803.

the reduction of light-produced P_{680}^+ by the electron from Yz, which is connected to Mn cluster of water splitting reaction at one of four Sn states. The 300 μs decay ($k_3 = 300\ \mu s^{-1}$) is assigned to the oxidation (equilibration) of Q_A^- with Q_B and the 2 s phase ($k_4 = 2\ s^{-1}$) to the oxidation of Q_B^- by PQ pool, which results in the full oxidation of Q_A^- that is in equilibrium with Q_B (see a reaction scheme in Figure 5). Upon the addition of DCMU, the decay of Q_A^- at 0.2−10 ms range disappeared indicating the decrease of k_3, and Q_A^- was stabilized more than a second. The slow decay time constant of 1 s in the presence of DCMU seems to represent the charge recombination time between Q_A^- and S_2 state in the oxidizing side (Figure 5).

In the −PG mutant cells, the excitation with the laser light induced a smaller, 30% increase of fluorescence yield at the

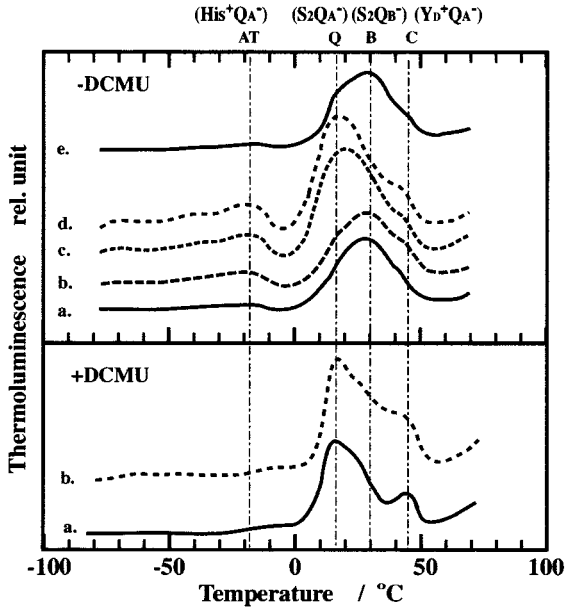

FIGURE 6: Effect of PG deprivation on the TL characteristics of the *pgsA* mutant of *Synechocystis* sp. PCC6803. (Upper) Effect of PG deprivation on TL. The cells grown in the presence of PG were transferred to the medium without PG for 0 (a), 3 (b), 6 (c), and 9 days (d). In the case of (e), the cells were grown without PG for 6 days and then with PG for 2 days. (Lower) Effect of 10 μM DCMU on the band positions of TL. The mutant cells grown with PG (a) were transferred to the medium without PG and incubated for 6 days (b).

maximum (Figure 4C). The fluorescence yield after the laser excitation increased more slowly with a 20 μs half time, i.e., with a 10 times smaller k_2 and decreased very slowly with a half time of 2 s, i.e., with a 10^4 times smaller k_3 as compared to those in the mutant cells grown with PG. Addition of DCMU did not affect the fluorescence change of the −PG cells, suggesting that PS II RCs apparently lack the function of Q_B without PG. The smaller k_2 value also suggests the slower reduction of P_{680}^+ by the component in the donor side, i.e., partial suppression of the water-splitting reaction. The very small k_3 indicates either the inhibition of electron transfer between Q_A and Q_B, or the loss of Q_B itself. The result is in consistent with the conclusion obtained from the measurement of fluorescence induction under continuous illumination in Figure 3.

Analysis of TL. The TL from Chl *a* was detected during the constant rate of warming up of the cells after illumination at −80 °C. The emission efficiency is a function of the activation energy in the reversed electron-transfer reactions in PS II (*24*). Figure 6 (upper traces) shows the TL profile of the *pgsA* mutant cells grown in the presence or absence of PG and assignment of each peak. The B band that can be ascribed to the emission in the charge recombination reaction in the PS II RC in the $S_2Q_B^-$ state was seen at 30 °C in the +PG cells. This peak shifted to 17 °C after 6-day deprivation of PG and to 16 °C after a 9-day deprivation. Two days' growth with PG after the 6-day deprivation of PG reversed the peak position to 30 °C. The 16 °C band was assigned to the $S_2Q_A^-$ recombination band (Q-band) as seen from the effect of DCMU (Figure 5, lower traces). In the presence of DCMU, the position of Q-band was observed at 16 °C both in the +PG and −PG mutant cells, indicating that the $S_2Q_A^-$ state was photoaccumulated by the illumination at −80 °C in both types of cells. In the −PG cells, the Q-band seen in the presence of DCMU was also associated with a band at 42 °C (C band), which is suggested to represent the emission in the charge recombination in the $Y_D^+Q_A^-$ state (25). These TL results indicate that the PG-depleted PS II does not accumulate Q_B^- and has more Y_Z^+, again indicating the suppression of the electron transfer between Q_A and Q_B in the acceptor side and the slower rate in the electron transfer from S_n state to Y_Z in the donor side.

DISCUSSION

The anionic lipids such as PG and cardiolipin play a determinative role as cochaperones in trafficking of proteins through or in membranes. They can affect the oxidative electron transport processes and translation of proteins involved in the mitochondrial electron transport (*26*−*28*). In thylakoid membranes, protein complexes are embedded in the lipid matrix that shows highly conserved lipid composition. In the present paper, we studied the effect of PG deprivation on each step of the PS II electron transfer. It was demonstrated previously that the *pgsA* mutant cells of a cyanobacterium *Synechocystis* sp. PCC6803 cultured without PG resulted in the loss of PG in the thylakoid membranes, and the loss was fully recovered if the cells were grown in the PG-containing medium again (*15*). The loss of PG resulted in the inhibition of cell growth, 30% decrease of Chl content in the cells and the 50% depression of the activity of the photosynthetic oxygen evolution on Chl basis. Suppression of cell growth and decrease of Chl content were also observed in the *SNC1* mutant in parallel with the loss of PG (*16*). However, the photosynthetic oxygen evolving activity on Chl basis decreased only slightly in this mutant. The difference observed in the photosynthetic activity in the two types of mutant strains might be caused by the different growth and experimental conditions since their oxygen evolving activities were highly sensitive to the light intensity of culturing condition and the growth temperature. Absorption and fluorescence emission spectra of the *pgsA* mutant cells did not significantly change upon the PG-deprivation, suggesting almost normal formation of PS II RC as assumed in the *SNC1* mutant. (*16*). The excitation of PBs in the −PG *pgsA* mutant cells led to fluorescence emission from Chl *a* indicating efficient energy transfer from the phycobilisomes to the PS II RCs (data not shown). These results suggested that the PG-deprivation affected a specific step in the electron-transfer system in the PS II RC as summarized in Figure 5.

Oxygen evolution was suppressed by artificial quinones that are known to be efficient electron acceptors for PS II (*23*) in the −PG cells of both the *pgsA* and the *SNC1* mutant cells. The result suggests an enhanced charge recombination due to some modifications of Q_A or Q_B as proposed in PS II with mutated D2 protein (*29*) by assuming replacement of PQ by artificial quinones. Although the inhibitory effect of DCBQ and DMBQ on oxygen evolving activity was more pronounced than that of *p*-benzoquinone, the fluorescence induction measurements did not indicate any specific reaction of the used artificial quinones with Q_A. The suppressive effect of artificial quinones on oxygen evolving activity in the −PG mutant cells, therefore, may reflect the change in the quinone reactivity to the Q_B site. The fluorescence induction and TL data underline the effect on the Q_B site rather than at the Q_A

site. These results are in contrast to those reported by Ermekova-Gerdes and Vermaas (*29*) that showed an enhanced charge recombination due to some modifications of Q_A site in PS II with mutated D2 protein.

The induction in the fluorescence yield of Chl *a* is known to reflect the increase of Q_A^- during illumination. In the presence of DCMU, both the +PG and −PG mutant cells showed a fast rise of fluorescence, suggesting the normal function of Q_A. The smaller F_v/F_0 ratio in the −PG cells reflect a little higher F_0 level and suggest slightly lower energy transfer efficiency among Chls. The increase in F_0 level may come from the decrease in the dimerization of D_1D_2 suggested by Kruse et al. (*8*). In the absence of DCMU the induction was very slow in the +PG cells indicating that the accumulation of Q_A^- is slow due to the oxidation by Q_B and DCMU accelerated the rise as expected. On the other hand, the induction in the −PG cells showed a fast rise and did not significantly change upon the addition of DCMU, suggesting that the PG deprivation results in the suppression of oxidation of Q_A^- by Q_B.

Direct evidence for the suppression of the Q_A^- to Q_B reaction was obtained by the measurement of flash-induced fluorescence. In the absence of DCMU, fluorescence rose with a $t_{1/2}$ of 20 μs, 10 times slower than that in the +PG cells, and decayed with a $t_{1/2}$ of 2 s, i.e., 10^4 times more slowly. The slow rise suggests the slower formation of P_{680} Q_A^- state due to the slow electron donation to P_{680}^+ from Yz. The lack of 300 μs decay phase indicates the absence of the activity of Q_B to change P_{680} Q_A^- state to P_{680} Q_A state just like the case with DCMU. Addition of DCMU only slightly changed the rise and decay kinetics confirming the inactivation of Q_B. Decreased F_v/F_0 ratio may reflect some changes in the pigment system. These effects can be also reversed again if the −PG cells were cultured in the presence of PG for a few days.

TL is emitted in the thermally activated recombination of the positive and negative charges stored on the donor and acceptor sides of PS II during illumination at −80 °C. The peak temperatures of the TL-bands reflect redox energy levels of electron transport components. In the presence of DCMU, both the +PG and −PG cells showed TL peaks at 16−17 °C corresponding to the Q-band, which relates to the recombination of charges on the Q_A^- and S_2 states. This indicates that Q_A and S_2 in the −PG cells are at the same redox potentials as those in the +PG cells. In the absence of DCMU, TL curve of the +PG cells showed a wide band at 27 °C of B-band that is known to be emitted in the recombination of charges on the Q_B^- and S_2 state (*25*). During the 6-day long PG deprivation, the peak gradually shifted to the lower temperature to the Q-band side indicating the inactivation of Q_B. The readdition of PG recovered the B band again. These results indicate the normal function of Q_A and Y_D and inactivation of Q_B in the −PG mutant cells.

It was also noticed that DCMU somewhat accelerated the fluorescence induction and modified the TL-profile even in the −PG cells. It suggests binding of DCMU to the modified Q_B site. Some modifications in the −PG cells were also suggested by the slight increase of the C band at 45 °C that is emitted in the recombination of Y_D^+ and Q_A^- (*25*). The change in the TL was somewhat different from that shown by the treatment of isolated thylakoid membranes with phospholipase C that decreased PG level (*6*). This treatment caused a rapid oxidation of Q_B^- and decreased the intensities of B and Q-bands of TL with a concomitant increase of the C band, suggesting the severe damage on the RC structure.

CONCLUSION

The decrease in the PG content of the thylakoid membranes in vivo resulted in a (i) specific inactivation of Q_B, (ii) slowdown of the electron-transfer rate from Yz to P_{680}, (iii) elevation of the F_0 level of chlorophyll fluorescence, (iv) anomalous suppressive effect of artificial benzoquinones on the oxygen evolution, (v) no apparent changes in the Q_A function, the DCMU binding to the Q_B site and the energy transfer from PB to Chl. The effect (i) suggests the PG action nearby the Q_B-binding site on D_1 subunit polypeptide, although the PG molecule has not been identified yet in the preliminary 3D structure of PS II RC complex shown at 3.8 Å resolution (*9*). The mechanism for (ii)−(iv) effects is not fully clear yet. These effects may indicate another PG target site around D_1D_2 polypeptides presumably nearby the Q_A site on D_2 subunit at a position almost symmetrical to the Q_B affecting site or nearby the PsbL/K subunits that are required for the dimer formation as well as for the efficient energy transfer between the RCs, as previously suggested by the effects of PG antibody (*7*).

The binding of lipids was actually the case in the PS I RC structure resolved at 2.5 Å (*30*) that contained two PG molecules at the periphery, and one PG molecule together with one monogalactosyldiacylglycerol molecule at the central core almost at a symmetrical position nearby the two electron acceptor phylloquinone molecules. The function of these lipids in PS I, however, is totally unknown yet. In the type II RC of purple bacteria, an anionic lipid cardiolipin and a detergent are located at 15 and 10 Å from the Q_B ubiquinone binding site, respectively (*31*, *32*). The use of lipid for the functional and structural control of quinone-binding site, thus, might be a common feature of the photosynthetic RCs or a possible heritage from a common ancestor for the types I and II RCs. We conclude that PG is an intrinsic structural component of photosynthetic membranes and is required for the proper function of Q_B plastquinone in PS II RC complex.

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