Biochimica et Biophysica Acta 1817 (2012) 287–297

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Two functional sites of phosphatidylglycerol for regulation of reaction of plastoquinone Q_B in photosystem II

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ARTICLE INFO

Article history:
Received 11 April 2011
Received in revised form 7 October 2011
Accepted 11 October 2011
Available online 18 October 2011

Keywords:
Chlorophyll fluorescence
Lipid
Oxygen evolution
Phosphatidylglycerol
Photosystem II assembly
Q_B plastoquinone

ABSTRACT

Functional roles of an anionic lipid phosphatidylglycerol (PG) were studied in *pgsA*-gene-inactivated and *cdsA*-gene-inactivated/phycobilisome-less mutant cells of a cyanobacterium *Synechocystis* sp. PCC 6803, which can grow only in PG-supplemented media. 1) A few days of PG depletion suppressed oxygen evolution of mutant cells supported by *p*-benzoquinone (BQ). The suppression was recovered slowly in a week after PG re-addition. Measurements of fluorescence yield indicated the enhanced sensitivity of Q_B to the inactivation by BQ. It is assumed that the loss of low-affinity PG (PG_L) enhances the affinity for BQ that inactivates Q_B. 2) Oxygen evolution without BQ, supported by the endogenous electron acceptors, was slowly suppressed due to the direct inactivation of Q_B during 10 days of PG depletion, and was recovered rapidly within 10 h upon the PG re-addition. It is concluded that the loss of high-affinity PG (PG_H) displaces Q_B directly. 3) Electron microscopy images of PG-depleted cells showed the specific suppression of division of mutant cells, which had developed thylakoid membranes attaching phycobilisomes (PBS). 4) Although the PG-depletion for 14 days decreased the chlorophyll/PBS ratio to about 1/4, florescence spectra/lifetimes were not modified indicating the flexible energy transfer from PBS to different numbers of PSII. Longer PG-depletion enhanced allophycocyanin fluorescence at 683 nm with a long 1.2 ns lifetime indicating the suppression of energy transfer from PBS to PSII. 5) Action sites of PG_H, PG_L and other PG molecules on PSII structure are discussed.

1. Introduction

The negatively charged lipid phosphatidylglycerol (PG) is a ubiquitous component of thylakoid membranes of cyanobacterial and plant chloroplasts and constitutes 5–10% of total lipids [1]. The loss of PG affects the structure of thylakoid membranes [2,3], and damages the function of both PS I and PS II reaction centers (RC) [4–7]. In the structure of photosystem I reaction center complex (PS I RC) of *Thermosynechococcus elongatus*, the X-ray crystallography study [8] revealed four lipid molecules on the reducing side of the PsaA and PsaB polypeptides. Two PG molecules are at the peripheral moiety and one PG and one monogalactosyldiacylglycerol (MGDG) are located near the electron acceptor phylloquinone molecules (A_1 and A_1') [8]. The amount of PS I was decreased in the PG-depleted mutant cells of *Synechocystis* sp. PCC6803 [4]. Especially PSI trimers were decreased significantly in parallel with the decrease of PsaL subunit suggesting that PG is located at the center of PS I trimers associated with the PsaL subunits too [4].

X-ray crystallography studies of PS II RC of *Thermosynechococcus elongatus* [9,10] have indicated multiple lipid molecules. Two PG molecules are identified in a 3.0 Å PSII structure [10]. One PG molecule (denoted as PG_2 in Fig. 1) was located between the D1 and CP43 proteins at the postulated pathway for the exchange of Q_B quinone. Biochemical assays, on the other hand, indicated associations of 6–7 molecules of PG in the purified PS II RC of *Thermosynechococcus elongatus* and plant PS II RCs [11] indicating that some PSII-bound PG molecules were not identified yet in the structure. A recent 1.9 Å structure of PSII of this organism [12] identified these two PG too and revealed another 3 PG (altogether 5 PG) with other 6 monogalactosyldiacylglycerol (MGDG), 5 digalactosyldiacylglycerol (DGDG) and 4 sulfoquinovosyldiacylglycerol (SQDG) molecules as

Abbreviations: *APC*, allophycocyanin; *BQ*, *p*-benzoquinone; Chl, chlorophyll; $D_{(BQ)}$ and $D_{2(int)}$, decay of fluorescence yield measured with and without BQ; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; $O_{2(BQ)}$ and $O_{2(int)}$, oxygen evolution activity with and without BQ, respectively; PAM, pulse amplidue modulation; PC, phycocyanin; PG, phosphatidylglycerol; PG_H and PG_L, PG bound to the high- and low-affinity sites that regulate the Q_B function, respectively; PG1-5, PG molecules identified in the structure of PS II; PS, photosystem; Q_A and Q_B, first and second electron acceptor plastoquinoneof PSII; RC, reaction center; SQDG, sulfoquinovosyldiacyglycerol; WT, wild type

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doi:10.1016/j.bbabio.2011.10.002

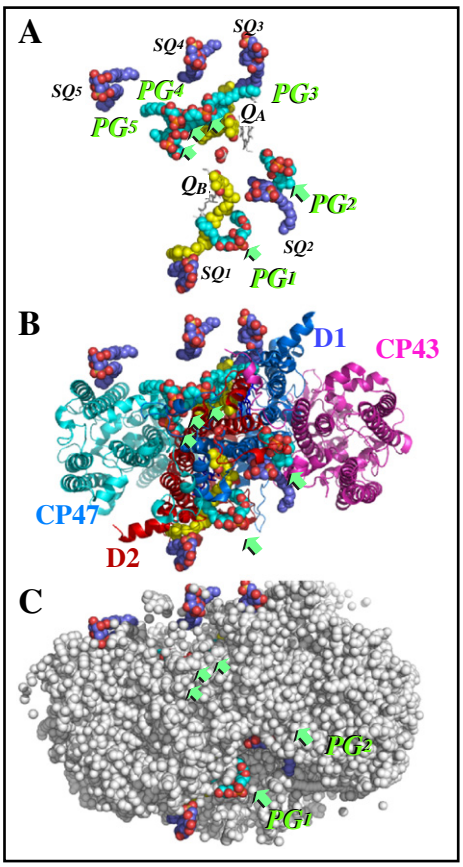

Fig. 1. Locations of negatively charged lipids, PG and SQDG (SQ), at the reducing-side surface of PS II RC monomer structure at 1.9 Å-resolution. A. Locations of cofactors. PG, SQDG and plastoquinone (Q_A and Q_B) are shown in space-filling models, and pheophytin *a* is shown as a stick model. Green arrows indicate the positions of head groups of PG. B. Locations of cofactors and protein subunits. Protein subunits, D1, D2, CP43 and CP47 are added to cofactors in A. C. A view of PSII surface on the reducing side. Cofactors and all the protein subunits including those in B are drawn in space filling models to show the exposure of lipids to the outer medium. Images were drawn with a MacPymol software and 3ARC.pdb data [12]. PG_{1-5} correspond to LHG:E772, D714, D702, L694 and D664, respectively. SQ_{1-5} correspond to SQDG:D768, A659, A667, L668, and B668, respectively, of 3ARC.pdb file.

shown in Fig. 1. Interestingly, the distributions of lipids are highly asymmetric. All the negatively charged lipids (SQDG and PG) are located on the reducing side surface of PSII as shown in Fig. 1 except for one MGDG at the periphery, and only the uncharged lipids are bound to the oxidizing side surface. It is, therefore, suggested that each PG molecule is specifically bound to a unique site on PSII and bears a specific functional/structural role together with the associated protein subunits. We attempted to identify the role of each PG identified on the structure in this study.

The role of lipids in photosynthesis has been studied either in *in vitro* extraction or after *in vivo* lipid degradation [1,13,14]. The treatment of thylakoid membranes with phospholipase C led to the suppression of PS II activity [13]. A specific role of PG in PS I was also suggested by Ikegami [15,16] who demonstrated that the addition of PG, but not that of MGDG, contributed to the reconstitution of chlorophylls (Chls) into ether-extracted PS I particles that are depleted of 95% of antenna Chls, two phylloquinones and most of lipids. The role of PG has been also studied *in vivo* in a mutant strain of *Synechocystis* sp. PCC6803 that has an inactivated gene encoding PG-phosphate synthase (pgsA) [3]. The *pgsA*-inactivated mutant strain produced by Hagio et al. [6] has been widely used to explore the role of PG. The mutant cells grew normally in media supplemented with PG, but showed suppressed oxygen-evolving activity with a concomitant decrease in Chl *a* and PG content [6]. Gombos et al. [5] have shown that the PG depletion induces the inactivation of the PS II secondary electron acceptor, plastoquinone Q_B. Sakurai et al. [7] pointed out that PG depletion leads to the destabilization of PS II dimers. Similar results were also reported by the inactivation of another gene *cdsA* that encodes cytidine 5′-diphosphate (CDP)-diacylglycerol synthase that is also required for PG synthesis [3,17]. PG was assumed to be important also for the formation of PS II dimer structure [7]. However, the 1.9 Å structure of PSII dimer crystal [12] does not contain PG at the binding surface of PSII monomers.

We studied oxygen evolution, Q_A to Q_B electron transfer reaction and fluorescence at 77 K to analyze the effects of depletion and re-addition of PG in the *ΔpgsA*- and *PAL/ΔcdsA*-mutant strains of *Synechocystis* sp. PCC6803. The results indicated two distinctly different PG functions in the regulation of Q_B together with the candidates of other PG action sites.

2. Materials and methods

2.1. Organisms and culture conditions

Cells of a Δ*pgsA* mutant strain [17] and a *PAL/ΔcdsA* strain [6] of *Synechocystis* sp. PCC6803 were grown photoautotrophically at 30 °C at a light intensity of 40 μmol m^{-2} s^{-1} in a BG11 medium supplemented with 5 mM HEPES-NaOH buffer (pH 7.5), 20 μg mL^{-1} kanamycin and 20 μmol dioleoyl-PG as described previously [4]. For the depletion of PG, cells grown in the PG-supplemented (+PG) medium were sedimented by centrifugation, washed twice with PG-free (−PG) culture medium, and then inoculated into the fresh PG-free medium. Wild-type cells were grown in the PG-free medium.

2.2. Measurement of absorption spectra and pigment concentration

Absorption spectra of cell suspensions were recorded with a wavelength resolution of 1 nm by a Shimadzu UV-1601 spectrophotometer in the cell chamber closer to a detector. Cell density was monitored by absorption at 720 nm that is mainly contributed by the light scattering of cells. Chl *a* concentration was calculated by the absorbance at 665 nm after the extraction of cells with 90% methanol with an extinction coefficient of 78.95 mM^{-1} cm^{-1} [18,19].

2.3. Electron microscopy

The harvested cells were fixed in 1% paraformaldehyde and 1% glutaraldehyde for 4 h at 4 °C and post-fixed in 1% osmium tetroxide. The samples were dehydrated in aqueous solutions of increasing ethanol concentrations, and then embedded in Spurr resin. Following polymerization, 85–90 nm ultra thin sections were cut out by a Reichert Ultracut E ultramicrotome. The sections were treated with uranyl acetate and lead citrate and subjected to electron microscopy in a Zeiss EM 902 electron microscope.

2.4. Measurements of oxygen evolution and fluorescence yield

Oxygen evolution was measured with a Clark-type electrode (Hanza Tec. Co.). The cells suspended in the growth medium inside a 1 cm-diameter tube were illuminated with white light from a halogen tungsten lamp through heat-cut filters at a nearly saturating intensity of 500 μmol photons m^{-2} s^{-1} as described previously [17].

Changes in the yield of chlorophyll fluorescence of cell suspension in a 10 mm light-path cuvette were monitored using a PAM fluorometer with a probing LED blue excitation flash, which was given at varied delay times with respect to the strong actinic excitation at a nearly saturating intensity with a red LED (Fl3000 Photon Systems Instruments, Kolackova, and Czech). Fluorescence yields