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Roles of acidic lipids in photosynthesis

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Introduction

The main lipids of thylakoid membranes of oxygenic photosynthetic organisms comprise two galactolipids, monogalactosyl diacylglycerol and digalactosyl diacylglycerol, and two acidic lipids, sulfoquinovosyl diacylglycerol (SQDG) and phosphatidylglycerol (PG). The content of a phospholipid, PG, decreases in an anoxygenic photosynthetic bacterium, *Rhodobacter sphaeroides*, a cyanobacterium, *Synechococcus* PCC7942, a green alga, *Chlamydomonas reinhardtii*, and a higher plant, *Arabidopsis thaliana*, through a reduction in phosphorus availability during growth. The decreases in PG content are accompanied by increases in the content of sulfolipid, SQDG, as if to adjust the total content of these acidic lipids to certain levels (Benning et al. 1993, Güler et al. 1996, Essigmann et al. 1998, Sato et al. 2000b). In contrast, SQDG content decreases with an increase in PG content in *C. reinhardtii* under sulfur-limiting growth condition (Sato et al. 2000b). Such alterations imply that these anionic lipids can replace each other to some extent to maintain the functional integrity of photosynthetic membranes. The possible substitution of PG for SQDG in photosynthetic membranes can also be deduced from observation that SQDG-deficient mutants of *C. reinhardtii*, *Synechococcus* PCC7942, and *R. sphaeroides* possess higher PG contents than the respective wild type levels (Benning et al. 1993, Sato et al. 1995b, Güler et al. 1996). In this study, we produced respective mutants defective in PG- or SQDG-synthesis from *Synechocystis* sp. PCC6803 to compare physiological significances of the acidic lipids.

Materials and methods

Cells of wild type and lipid mutants of *Synechocystis* sp. PCC6803 were grown photoautotrophically in BG-11 medium under constant fluorescent lamp illumination (10 $W \cdot m^{-2}$) at 30°C, in oblong glass vessels, under the bubbling with air containing 2%CO_2. Dioleoyl-PG (Sigma) or SQDG prepared from *Synechocystis* sp. PCC6803 was sonicated in BG-11 medium for liposome production to be filtered for sterilization. The culture medium for lipid mutants, when necessary, was supplemented with the PG or SQDG liposomes. The plasmid containing the disrupted putative gene for PG or SQDG synthesis was used to transform wild type *Synechocystis* sp. PCC6803 by homologous recombination, as described in Sato et al. 2000a. The content of chlorophyll was determined after its extraction from the cells with 100% methanol. CO_2-dependent photosynthesis and PSII activity of the cells were measured in BG-11 medium containing 10 mM $NaHCO_3$ and 2mM *p*-benzoquinone, respectively, with a Clark-type electrode (Rank Brothers, London).

Results and discussion

PG and SQDG as essential lipids in Synechocystis sp. PCC6803: slr1369, an ORF on the genome of *Synechocystis* sp. PCC6803, encodes a putative CDP-diacylglycerol synthase (*cdsA* gene product) responsible for PG synthesis, whereas slr1020 encodes a putative *sqdB* gene product for SQDG synthesis. These ORFs were respectively disrupted in *Synechocystis* sp. PCC6803 by homologous recombination, which was confirmed by PCR (data not shown). The resultant disruptants as to putative genes of *cdsA* and *sqdB* were designated as SNC1 and SD1, respectively. SNC1 required PG supplementation for its growth (Fig.1). Besides, the shift of SNC1 cells from PG-supplemented to PG-free medium resulted in four-fold decrease in the cellular content of PG after one round of cell division. On the other hand, SD1 showed requirement for SQDG supplementation for the growth (Fig.1) and a decrease in the cellular content of SQDG after the shift from SQDG-supplemented to SQDG-free medium. These results indicate that SNC1 and SD1 were defective in PG and SQDG synthesis, respectively, and that both PG and SQDG are essential for *Synechocystis* sp. PCC7942. Thus, PG and SQDG proved not to substitute for each other at least as to some vital functions. It is notable that the requirement for SQDG is in contrast to the dispensability of SQDG for the growth of the other SQDG-deficient mutants so far reported for *R. sphaeroides*, *Synechococcus* sp. PCC6803, and *C. reinhardtii*(Benning et al. 1993, Sato et al. 1995b, Güler et al. 1996). This discrepancy indicates that some physiological process is specialized to depend more greatly on SQDG in *Synechocystis* sp. PCC6803.

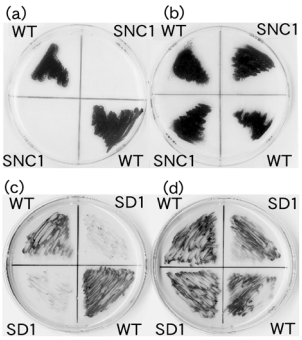

Fig. 1 Indispensability of acidic lipids in *Synechocystis* sp. PCC6803. Wild type and SNC1 cells were cultured on agar plates containing BG11 medium in the absence (a) or presence (b) of PG, whereas wild type and SD1 cells were cultured in the absence (c) or presence (d) of SQDG.

Effect of defect in PG synthesis on photosynthesis: SNC1 grown in the presence of PG, as compared with the wild type, showed almost similar values for parameters of photosynthesis such as a cellular content of Chl, CO_2-dependent photosynthesis on a Chl basis, and *p*-benzoquinone-dependent oxygen evolution, *i.e.* PSII activity on a Chl basis (Table I). However, the growth of SNC1 in the absence of PG, i.e. the decrease in a cellular content of PG, resulted in a decrease in the Chl content of a cell (Table I), indicating that PG is responsible for the accumulation of Chl-protein complexes. Dodecyl-β,D-maltoside-PAGE, a non-denaturing PAGE, of thylakoid membranes separated PSI and PSII complexes without release of Chl, showing that the decrease in Chl content was accounted for largely by the decrease in the content of PSI complex (data not shown). As to photosynthetic activities, it was notable that *p*-benzoquinone-dependent oxygen evolution was lowered by as much as 82% despite a decrease by only 21% in CO_2-dependent photosynthesis (Table I). Resultantly, *p*-benzoquinone-dependent oxygen evolution, initially higher than CO_2-dependent photosynthesis, became lower than the photosynthesis, indicating that the artifitial electron acceptor lost its ability to efficiently oxidize PSII. These results raised a possibility that the defect in PG synthesis altered the structure of the site for the action of *p*-benzoquinone, *i.e.* Q_B-binding site of D1 protein and/or lipid environment in the vicinity of the Q_B-binding site.

Table I. Photosynthetic characteristics of SNC1 and SD1

	Chl comtent[a]	O_2 evolution[a]		*p*-BQ-dep./CO_2-dep.[b]
		CO_2-dep.	p-*BQ-dep.*	
SNC1+PG[c]	0.83±0.26	1.03±0.07	0.84±0.04	1.27±0.08
-PG[d]	0.36±0.08	0.81±0.04	0.15±0.01	0.29±0.02
SD1 +SQDG[c]	1.47±0.27	0.96±0.01	0.74±0.02	1.66±0.04
-SQDG[d]	1.87±0.44	0.42±0.01	0.27±0.01	1.36±0.04

[a] Chl content in a cell and CO_2–dependent and *p*-benzoquinone-dependent O_2 evolutions were expressed as the ratio of the values of the mutants to the wild type. [b]The ratio of *p*-benzoquinone-dependent to CO_2–dependent O_2 evolutions. [c]SNC1 and SD1 cells grown in the presence of PG and SQDG, respectively. [d] SNC1 and SD1 cells grown in lipid-free medium for 3 days after the growth in PG- and SQDG-supplemented medium, respectively.

Effect of defect in SQDG synthesis on photosynthesis: SD1 grown in the presence of SQDG and the wild type were almost indistinguishable for CO_2-dependent photosynthesis (Table I). The growth of SD1 in the absence of SQDG, *i.e.* the decrease in SQDG content, brought about no decrease in cellular content of Chl (Table I), indicating that SQDG, in contrast to PG, is not required for accumulation of Chl-protein complexes. Simultaneously, CO_2-dependent photosynthesis and PSII activity were decreased by 56 and 64%, respectively, showing that SQDG is important for photosynthesis and especially for PSII activity in *Synechocystis* sp. PCC6803. The damage in PSII activity is consistent with our previous observation that SQDG-deficient mutant of *C. reinhardtii*, as compared with the wild type, showed 40% decrease in PSII activity (Sato et al. 1995a). IC_{50} of DCMU for PSII activity, the other aspect of PSII characteristics, was lowered from 115 to 45nM in SD1 by the decrease in SQDG content. The enhanced sensitivity of PSII to DCMU suggests that the structure of Q_B-binding site at which DCMU acts on and/or lipid environment surrounding the site are altered by the defect in SQDG synthesis. However, the decrease in SQDG content, distinct from that in PG content, caused no inefficiency of *p*-benzoquinone as electron acceptors of PSII (Table I). Thus, PG and SQDG appear to contribute in distinct ways to the integrity of the structure and/or lipid environment of Q_B-binding site.

Possible mechanism for acidic lipids to contribute to the functioning of PSII: On the basis of observation that localization of SQDG at PSII complex among Chl-protein complexes prepared from wild type *C. reinhardtii* corresponds to the specific defect in PSII activity for *Chlamydomonas* mutant defective in SQDG, we proposed that association of SQDG with PSII complex is prerequisite for its normal conformation of PSII and full expression of its activity. PG, similar to SQDG, showed preferential distribution to PSII complex (Sato et al. 1995a), raising a possibility that PG participates in normal functioning of PSII through the association with the complex for *C. reinhardtii*. In accordance with this, PG was previously shown to be tightly associated with the D1 subunit of PSII in a cyanobacterium, *Oscillatoria chalybea*, and the activity of PSII particles from the cyanobacterium was inhibited by treatment with phospholipase A2, which modified PG, but was restored upon PG supplementation (Kruse and Schmidt 1995). Association of SQDG and PG with PSII complex may be important also for normal functioning of PSII of *Synechocystis* sp. PCC6803. Growth of SNC1 or SD1 cells after removal of supplemented lipids would deprive PSII subunits such

as the D1 protein of PG and SQDG, respectively, to alter the conformation of the PSII complex including that of the Q_B-binding, bringing about damage to the function of the PSII.

In summary, we compared the roles of acidic lipids in the photosynthetic membranes, PG and SQDG, through analysis of PG- or SQDG-mutant of *Synechocystis* sp. PCC6803 to deduce the followings: (1)indispensability of PG or SQDG, *i.e.* existence of some vital roles of the respective acidic lipids as to which each acidic lipid can not substitute for the other one, (2)requirement for PG, but not for SQDG, in the accumulation of Chl-protein complexes, (3)contribution of PG and SQDG to normal functioning of PSII, and (4)possible responsibility of PG and SQDG for normal conformation of Q_B-binding site by distinct mechanisms. It will be the future work to specify what common and uncommon roles of PG or SQDG are among various photosynthetic organisms, and to clarify what causes differences when discrepancy was shown, *e.g.* indispensability of SQDG for *Synechocystis* sp. PCC6803, but not for the other three photosynthetic organisms as described above (Benning 1993, Sato 1995b, Güler et al. 1996).

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