A Null Mutant of Synechococcus sp. PCC7942 Deficient in the Sulfolipid Sulfoquinovosyl Diacylglycerol*

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The sulfolipid 6-sulfo-o-quinovosyldiacylglycerol is associated with the thylakoid membranes of many photosynthetic organisms. Previously, genes involved in sulfolipid biosynthesis have been characterized only in the purple bacterium Rhodobacter sphaeroides. Unlike plants and cyanobacteria, photosynthesis in this bacterium is anoxygenic due to the lack of a water splitting photosystem II. To test the function of sulfolipid in an organism with oxygenic photosynthesis, we isolated and inactivated a sulfolipid gene of the cyanobacterium Synechococcus sp. PCC7942. An extensive analysis of the sulfolipid-deficient null mutant revealed subtle changes in photosynthesis related biochemistry of O₂. In addition, a slight increase in the variable room temperature chlorophyll fluorescence yield was observed. Regardless of these changes, it seems unlikely that sulfolipid is an essential constituent of a functional competent water oxidase or the core antenna complex of photosystem II. However, reduced growth of the mutant under phosphate-limiting conditions supports the hypothesis that sulfolipid acts as a surrogate for anionic phospholipids under phosphate-limiting growth conditions.

Photosynthesis is a function of highly organized pigment protein complexes that are embedded in the polar lipid matrix of thylakoid membranes. Some of the lipids found in this membrane are generally absent from nonphotosynthetic membranes. A typical example is the sulfolipid sulfoquinovosyl diacylglycerol, which occurs in almost all photosynthetic organisms (1) with the exception of a few photosynthetic bacteria (2) and Rhizobium meliloti, a nonphotosynthetic but plant-associated bacterium (3) with sulfolipid in its membranes. Although in higher plants and cyanobacteria two photoactive pigment-containing complexes exist, photosystems I and II, only one is present in purple bacteria. The photosynthetic reaction center of purple bacteria shows high structural and functional homology to that of photosystem II of cyanobacteria and plants (for a review see Ref. 4), but only photosystem II catalyzes the light-induced reduction of plastoquinone with electrons from water, thereby releasing oxygen (for a review see Ref. 5). The water splitting system is absent from purple bacteria, which use hydrogen donors with relatively low redox potential and evolve no oxygen. Because of the similar organization of the photosynthetic apparatus in cyanobacteria and higher plants, cyanobacteria have been used as model systems to genetically dissect the protein complexes of the thylakoid membrane (for reviews see Refs. 6 and 7). Because some cyanobacterial strains can grow heterotrophically, genes encoding individual protein components of the two photosystems can be inactivated by gene replacement, and the analysis of the resulting null mutants can reveal the function of the affected proteins. This genetic approach can also be applied to investigate the role of polar lipids for the formation and maintenance of protein lipid complexes required for oxygenic photosynthesis. However, no cyanobacterial mutants have been available that completely lack a class of polar lipids of the thylakoid membrane. But it should be noted that heterocyst mutants deficient in the biosynthesis of glycolipids specifically associated with this specialized, nitrogen-fixing cell type are known (8). Furthermore, the fatty acid composition of cyanobacterial polar lipids has been altered by genetic engineering in order to study the influence of fatty acid composition on thermal tolerance of the organism (9).

The almost exclusive occurrence of sulfolipid in photosynthetic membranes and its unusual sulfoquinovosyldiacylglycerol head group (10) has stimulated debate over a specific role for this lipid in photosynthesis (11). Sulfolipid has been identified as integral component of photosystem II protein complexes (12, 13). Furthermore, in reconstitution experiments with chloroplast ATP synthase, sulfolipid was found to be required in stoichiometric amounts with other lipids for successful restoration of enzymatic activity (14). These results led to the conclusion that sulfolipid functions as essential boundary lipid. Based on a more recent analysis of a sulfolipid-null mutant of the purple bacterium Rhodobacter sphaeroides, it can be assumed that sulfolipid plays no specific role in anoxygenic photosynthesis (15), because photosynthetic electron transport rates were not altered and growth under optimal conditions was not reduced. However, upon transfer to phosphate-limiting conditions, growth of the mutant ceased earlier than that of wild type cells. In addition, a strong reduction in phospholipid content and a concomitant increase in novel lipids as well as sulfolipid was observed in the phosphate-stressed cells of R. sphaeroides (16). Taken together, these observations led to the conclusion that sulfolipid may play a role as substitute for anionic phospholipids under phosphate-limiting growth conditions in purple bacteria and possibly other photosynthetic organisms.

Recently, a sulfolipid-deficient mutant of Chlamydomonas reinhardtii, which was induced by exposure to UV light, has been isolated based on its abnormal chlorophyll fluorescence (17). The chlorophyll fluorescence phenotype is an indication that photosynthesis is affected in this mutant. However, it has not clearly been demonstrated that the fluorescence phenotype...
and the sulfolipid deficiency are due to the same genetic defect, leaving the causal relation between the two phenotypes open.

To further address the question of whether sulfolipid plays an essential role for oxygenic photosynthesis, we isolated and inactivated a gene involved in sulfolipid biosynthesis from the cyanobacterium Synechococcus sp. PCC7942. The only previously known genes encoding sulfolipid biosynthetic enzymes were the sqd genes from R. sphaeroides (18, 19), of which one served as a molecular probe to isolate the homologous gene from the cyanobacterium. The resulting sulfolipid null mutant was analyzed with regard to its photosynthetic characteristics and growth under different conditions. Possible modifications of the photosystem II reaction kinetics due to the lack of sulfoquinovosyl diacylglycerol were investigated by measuring the oxygen yield in response to a regime of short flashes.

**MATERIALS AND METHODS**

**Bacterial Strains, Plasmids, Media, and Growth Conditions—**Synechococcus sp. PCC7942 wild type (kindly provided by P. Wolke) and mutant lines were grown photoautotrophically at 28°C in liquid or agar-solidified (1.5%) BG-11 medium (20). Light was provided by fluorescent lamps at 100 mmol m⁻² s⁻¹. If required, the growth medium was supplemented with kanamycin (15 μg ml⁻¹) or ampicillin (1 μg ml⁻¹). The phosphate content of the medium was reduced as indicated in the text by replacing KH₂PO₄ with KCl.

**Lipid Analysis**—In whole cells as described by Collier and Grossman (29). The preparation of radiolabeled lipids—Cultures (50 ml) of sulfolipid mutant and wild type strains were grown to midlog phase in BG-11 medium. Cells were washed in sulfate-free BG-11 medium (MgSO₄ was replaced by MgCl₂) and resuspended in 1 ml of the same medium. Following the addition of 100 μCi of sodium [³²P]sulfate (specific activity, 100 mCi/mmol), the cells were incubated for 16 h and harvested by centrifugation. Lipid extracts were prepared and separated as described above.

**Oxygen Evolution Measurements—**Oxygen evolution from whole cells under continuous white light was determined polarographically with 10 mM NaHCO₃ as acceptor in a buffer solution of 25 mM Hepes-NaOH, pH 7.0 (26). The concentration of cells was equivalent to 5 μg ml⁻¹ chlorophyll. The oscillation pattern of flash-induced oxygen yield produced by a series of short flashes from a Xenon lamp (FWHM ~ 10 μs) was monitored with a photometric-type electrode as described (27).

**Absorption, 77 K, and Room Temperature Fluorescence Emission Measurements—**In vivo room temperature absorption and 77 K fluorescence measurements were recorded as described previously (26). Room temperature chlorophyll a fluorescence was determined with a pulse amplitude modulation fluorometer (PAM101, Walz, Effeltrich, Germany) according to the method of Clarke et al. (28) essentially as described (26). Phycocyanin and chlorophyll content were determined in whole cells as described by Collier and Grossman (29).

**Nucleotide Sequence—**The nucleotide sequence reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number U45308.

**RESULTS**

**Isolation and Characterization of a Cyanobacterial Genes with Sequence Similarity to sqdB of R. sphaeroides—**A prerequisite for the construction of a completely sulfolipid-deficient cyanobacterial strain by gene replacement is the availability of wild type genes coding for sulfolipid biosynthetic enzymes. With the goal to isolate the first cyanobacterial gene involved in sulfolipid biosynthesis, cross-hybridization between the different sqd genes from R. sphaeroides and genomic DNA from Synechococcus sp. PCC7942 was tested. This cyanobacterium was chosen, because it is naturally competent to take up DNA. In addition, the DNA of Synechococcus sp. PCC7942 has a GC content intermediate to that of DNA from R. sphaeroides and higher plants (30). Of three sqd genes of R. sphaeroides, only sqdB gave a strong positive hybridization signal with genomic DNA of Synechococcus sp. PCC7942. Probing a genomic library prepared in λ-ZAP-Express, several clones hybridizing to sqdB of R. sphaeroides were isolated. Following the excision of plasmids from the phage and restriction analysis, it became apparent that the inserts of all clones overlapped. The two clones containing the longest DNA fragments, pSY2 and pSY3, were recombined by ligation of a 1600-base pair KpnI/Sall fragment from pSY3, a 1500 Sall/BamHI fragment from pSY2, and pBluescript II-SK⁺ cut with KpnI and BamHI giving rise to plasmid pSY8 (Fig. 1). The overlapping DNA region of pSY2 and pSY3 was sequenced on both strands, as indicated in Fig. 1. Sequence analysis revealed an open reading frame predicted to encode a protein of the molecular mass of 44.6 kDa consisting of 402 amino acids (Fig. 2). A GTG triplet is proposed to serve as the initiation codon because it is preceded by a perfect ribosome binding site, no suitable in-frame ATG codon was present, and the predicted N-terminal amino acid sequence corresponded well with the N-terminal amino acid sequence of sqdB of R. sphaeroides. Comparing the amino acid sequence over its total length with that of sqdB of R. sphaeroides, a sequence identity of 63% and a sequence similarity of 77.3%, taking into account conservative substitutions, was determined.
Construction of a Sulfolipid-deficient Null Mutant—As part of a strategy to demonstrate that the isolated open reading frame represents a gene coding for a protein involved in sulfolipid biosynthesis in *Synechococcus* sp. PCC7942, we inactivated the wild type copy of the gene by replacing an internal fragment with a kanamycin resistance cassette in opposite orientation. The kanamycin-resistant lines were tested by Southern hybridization (Fig. 3). This experiment revealed the complete disappearance of wild type genome copies and the expected replacement of the targeted open reading frame in all putative mutant lines tested. There was only one hybridizing band of approximately 6 kilobases pairs present in wild type samples, a result that is in agreement with the presence of a single copy DNA sequence. Analysis of the composition of lipid extracts prepared from wild type and mutant cells by thin layer chromatography and quantification of individual lipids by gas chromatography of fatty acid methyl esters derived from the lipids (Table I) revealed no detectable amount of sulfolipid in extracts from the mutant lines. Furthermore, using the most sensitive method available, the analysis of lipid extracts from cells labeled with [35S]sulfate, no traces of residual sulfolipid were detected in mutant samples (Fig. 4). Apparently, the inactivation of the open reading frame abolished sulfolipid biosynthesis in the affected cells and gave rise to a sulfolipid-deficient null mutant of *Synechococcus* sp. PCC7942.

The Effects of Phosphate-limiting Conditions on Growth and Lipid Composition of Mutant and Wild Type—The isolation of genetically pure sulfolipid-deficient mutants of *Synechococcus* sp. PCC7942 suggests that sulfolipid is not essential for photoautotrophic growth of this cyanobacterium. Accordingly, no difference in growth rates was observed under optimal growth conditions (Fig. 5A). However, 10-fold reduction of the phosphate concentration in the medium caused the mutant to cease growth after 6 days, whereas the wild type continued to grow (Fig. 5B). Comparable results were obtained using optical density (Fig. 5) or live cell counts (data not shown) for measuring growth of the cultures.

Under phosphate-limiting growth conditions the relative amount of the major phospholipid phosphatidyl glycerol was reduced in wild type cells to 7.2 mol % compared with cells grown under optimal conditions (16.6 mol %, Table I). Concomitantly, an increase in the relative amount of sulfolipid and digalactosyl diacylglycerol was observed in the mutant, whereas the relative amount of monogalactosyl diacylglycerol was slightly decreased. In the mutant, the relative amount of

![Fig. 1. Plasmids used for the characterization and inactivation of the sqdB gene from *Synechococcus* sp. PCC7942. Plasmids were constructed as described in the text. Small solid arrows, directions of sequence reactions; gray arrow, sqdB open reading frame; open arrow, neomycin phosphotransferase gene; cross-hatched box, fragment used for Southern hybridization. Restriction sites: A, *Bam*HI; E, *Sph*I; H, *Hind*III; O, *Xho*I; P, *Pst*I; S, *Sal*I. The asterisks indicate a Sau3A/*Bam*HI ligation site.](http://www.jbc.org/)

![Fig. 2. DNA and deduced amino acid sequence of the *Synechococcus* sp. PCC7942 sqdB gene. The nucleotide sequence is shown from the *Kpn*I site to the *Xho*I site of pSYB. The protein sequence is given below the DNA sequence. The underline indicates a putative ribosome binding site.](http://www.jbc.org/)
photosynthesis, first the rate of oxygen evolution was deter-
dant—to elucidate the effect of sulfolipid deficiency on oxygenic
found in wild type cells under both growth regimes. The approximate length of DNA frag-
ments is indicated (kilobase pairs).

Effect of phosphate nutrition on the polar lipid composition of wild type and mutant.
The values are the means ± standard errors of three independent cell cultures grown in BG-11 medium supplemented with Pi as indicated. MGD, monogalactosyldiacylglycerol; PG, phosphatidylglycerol; DGD, digalactosyldiacylglycerol; SQD, sulfoquinovosyldiacylglycerol; nd, not detected (<0.5 mol%).

| Lipid       | Wild type 0.18 mM Pi, mol % | SY-SQDB 0.18 mM Pi, mol % |
|-------------|-----------------------------|---------------------------|
| MGD         | 60.6 ± 1.1                  | 42.5 ± 0.9                |
| PG          | 16.6 ± 0.3                  | 7.2 ± 0.5                 |
| DGD         | 12.5 ± 0.8                  | 28.0 ± 0.6                |
| SQD         | 10.3 ± 0.2                  | 22.3 ± 1.0                |
|             | nd                          | nd                        |

Fig. 3. Southern hybridization of wild type (WT) and SY-SQDB mutant. Genomic DNA was cut with HindIII and probed with a 1470-base pair Spd/Khol fragment from sqdB containing the open reading frame and adjacent sequences. The approximate length of DNA fragments is indicated (kilobase pairs).

Fig. 4. Separation of 32P-labeled lipids of wild type (WT) and SY-SQDB mutant by thin layer chromatography. Approximately equal amounts of total lipids were spotted in case of undiluted extracts (undil.). In addition, 10-, 100-, and 1000-fold dilutions of the wild type extracts were loaded for estimation of the reduction of sulfolipid in the mutant extract. Radioabeled lipids were visualized by autoradiography. F, solvent front; O, origin; SQD, sulfoquinovosyldiacylglycerol; U, unidentified compound.

phosphatidylglycerol (28.4 mol %) was increased under optimal growth conditions and did not decrease as dramatically under phosphate-limiting conditions (23.2 mol %, Table I). The relative amounts of the galactolipids were comparable with those found in wild type cells under both growth regimes.

Photosynthetic Characteristics of the Sulfolipid-deficient Mutant—To elucidate the effect of sulfolipid deficiency on oxygenic photosynthesis, first the rate of oxygen evolution was deter-
mined as function of photon flux density. Essentially, identical curves were obtained for the wild type and the mutant (Fig. 6).

To examine the possibility of subtle changes in the reaction kinetics of photosystem II, the characteristic period four oscillation pattern of flash-induced oxygen evolution was compared in dark adapted wild type and mutant cells. The maximum oxygen yield is generated by the fourth flash (Fig. 7). This feature is typical for thoroughly dark-adapted cyanobacteria. With regard to the active site tyrosine (YD) of the D2 protein, this pattern is indicative of an apparent population of redox states below S1YD (31). Within the frame work of an extended Kok model, in which a cyclic sequence of redox states adopted by the water oxidase during catalysis is postulated (33), the data can be satisfac-
torily described by the probability of misses (α = 0.23) and double hits (β = 0.01) and apparent S1-state dark populations of [S1] = 0.47, [S0] = 0.39, and [S−1] = 0.12. Preillumination with a short saturating flash and subsequent dark incubation for 3 min leads to a shift of oxygen yield maximum to the third flash. This observation shows that the apparent high population of S0 is mainly due to the presence of YD in its reduced form (32). Both oscillatory patterns exhibited virtually the same features (Fig. 7, A and B) except for the pronounced oxygen uptake in the mutant sample after the first two flashes of the sequence (Fig. 7B). In an attempt to test whether this phenomenon was restricted to the first two flashes, the measurements were repeated in the presence of hydrazine. Under these conditions the redox state S−1 is highly populated, and the maximum of oxygen yield is shifted toward the sixth flash (32). Likewise, virtually no oxygen is evolved during the first four flashes. Contrary to the wild type, the oxygen yield pattern of hy-
drazine-treated mutant cells revealed a marked oxygen uptake during the first four flashes in the SY-SQDB mutant (data not shown).

Comparing room temperature chlorophyll fluorescence in the wild type and the mutant (Table II), a similar dark level fluorescence yield (F0) was observed for both strains. Because state 2-state 1 transitions can be important for the determination of the maximum fluorescence yield (Fm), cells were first illuminated with low intensity white light to induce state 1 prior to the addition of the electron transfer inhibitor 3,4-
dichlorophenyl)-1,1-dimethyleurea to close photosystem II re-
action centers. Under these conditions, the mutant showed a higher Fm value and hence a higher variable fluorescence yield (FV). Consequently, the ratio of FV/FM, which is a measure of the photochemical efficiency of photosystem II, was slightly in-
creased in the mutant (Table II). Based on statistical analysis, this increase was significant. In search for further alterations
than 6% of each value. The mean of three independent measurements. The standard error was less than 6% of each value. 

A Joliot-type electrode. Positive peaks indicate oxygen evolution, and wild type and mutant strains obtained after chlorophyll fluorescence spectra were recorded. The 77 K fluorescence spectra of emanate from photosystem II. The latter two can be mainly where the two peaks at 685 nm and approximately 695 nm peak at 717 nm is predominantly derived from photosystem I, whereas the two peaks at 645 and 665 nm, respectively. Because no difference in the phycocyanin/chlorophyll ratio was observed (data not shown), this result can be taken as an indication for a higher efficiency of excitation energy transfer from phycobilins to the photosystem II reaction center chlorophyll a in the mutant.

Fig. 6. Rate of oxygen evolution as function of photon flux density by Synechococcus sp. PCC7942 wild type (closed circles) and SY-SQDB mutant (open circles). Each value represents the mean of three independent measurements. The standard error was less than 6% of each value.

Fig. 7. Flash-induced changes of oxygen evolution or uptake by Synechococcus sp. PCC7942 wild type (A) and SY-SQDB mutant (B). The polarographic signals (arbitrary units) were detected by a Joliot-type electrode. Positive peaks indicate oxygen evolution, and negative peaks indicate uptake.

| Parameters | Wild type | SY-SQDB |
|------------|-----------|----------|
| Fm | 64.5 ± 7.0 | 65.3 ± 6.6 |
| Fv | 131.5 ± 13.8 | 153.9 ± 13.9 |
| Fv/Fm | 0.51 ± 0.04 | 0.58 ± 0.02 |

in the antenna system of the mutant, low temperature fluorescence spectra were recorded. The 77 K fluorescence spectra of wild type and mutant strains obtained after chlorophyll a excitation at 440 nm are shown in Fig. 8A. The large emission peak at 717 nm is predominantly derived from photosystem I, whereas the two peaks at 685 nm and approximately 695 nm emanate from photosystem II. The latter two can be mainly attributed to the core antenna proteins CP43 (34) and CP47 (35), respectively. No difference in the relative amplitudes of the emission maxima were observed between both strains. When excited at a wavelength of 590 nm, which corresponds to the maximum for the excitation of phycobilins, the intensity of the emission at approximately 655 nm was considerably reduced in the mutant (Fig. 8B). This peak presumably represents overlapping emissions for phycocyanin and allophycocyanin with maxima at 645 and 665 nm, respectively. Because no difference in the phycocyanin/chlorophyll ratio was observed (data not shown), this result can be taken as an indication for a higher efficiency of excitation energy transfer from phycobilins to the photosystem II reaction center chlorophyll a in the mutant.

DISCUSSION

To study the possible role of sulfolipid in oxygenic photosynthesis in a definitive way, we created a sulfolipid-deficient null mutant of Synechococcus sp. PCC7942. During the course of this work, we isolated for the first time and disrupted a gene involved in sulfolipid biosynthesis in an organism with oxygenic photosynthesis. This gene of Synechococcus sp. PCC7942 shares considerable sequence identity with the sqdB gene of R. sphaeroides and is therefore also designated sqdB. However, further experiments will be required to demonstrate functional homology between the two genes in R. sphaeroides and Synechococcus sp. PCC7942. Our current inability to detect cross-hybridization between other sqd genes of the two bacterial strains suggests that these are less conserved. Unfortunately, we still do not know the function of the sqdB gene product, and further experiments to elucidate its biochemical role may also allow us to solve the long standing mystery of sulfolipid biosynthesis.

Inactivation of the putative sqdB gene of Synechococcus sp. PCC7942 wild type gives rise to an otherwise isogenic null mutant, which was designated SY-SQDB and completely lacks sulfolipid, one of the four polar lipids found in this bacterium. This deficiency has no lethal consequences. It does not even lead to reduced growth under optimal conditions for photoautotrophic growth (Fig. 5), suggesting that sulfolipid is not essential for oxygenic photosynthesis. Apparently, the loss of the anionic sulfolipid is mainly compensated by an increased relative amount of phosphatidylglycerol (Table I), which is the second anionic lipid found in the membranes of Synechococcus sp. PCC7942. Maintaining a certain level of anionic lipids in the membranes seems to be crucial for the organism, because the reduction in phosphatidylglycerol under phosphate limitation in the wild type is compensated by an increased level of sulfolipid. The sulfolipid-deficient mutant SY-SQDB cannot respond in the same way to phosphate limitation and has to maintain a higher level of phosphatidylglycerol. Because it cannot replace lipid-bound phosphor by sulfur under conditions of phosphate limitation, it becomes phosphate-depleted and enters the stationary growth phase at an earlier time point than the wild type (Fig. 5). The same phenomenon has been previously observed for R. sphaeroides (15). In addition, both bacteria accumulate dihexosyl lipids under phosphate limitation. Although Synechococcus sp. PCC7942 does not accumulate glucosylgalactosyl diacylglycerol, as was observed for phosphate-limited R. sphaeroides (16), the relative amount of digalactosyl diacylglycerol is increased (Table I).

Normal growth of the SY-SQDB mutant under optimal laboratory conditions does not exclude the possibility of a more subtle role of sulfolipid in oxygenic photosynthesis relevant under natural conditions, e.g. high photon flux densities. However, the light response curves for oxygen evolution by wild type and mutant cells were nearly identical (Fig. 6).
This finding indicates that the lack of sulfolipid neither affects the overall electron transport rate nor the optical cross-section of oxygen evolution. More subtle changes in the reaction kinetics of photosystem II were expected to become apparent by monitoring the characteristic period four oscillation pattern of flash-induced oxygen evolution in dark-adapted wild type and mutant cells. A comparison of oscillatory patterns revealed that both strains exhibit virtually the same features except for the pronounced oxygen uptake in the mutant sample after the first two flashes (Fig. 7B). Because hydrazine-treated mutant cells showed also a marked increase in oxygen uptake during the first four flashes, it seems most likely that the enhancement of oxygen uptake in the SY-SQDB mutant is not necessarily directly related to the water splitting activity of photosystem II. Instead, increased oxygen uptake could be either due to the reduction of O$_2$ by components of the electron transport chain or increased respiratory activity. Nevertheless, the data presented in this study clearly show that sulfolipid is not an essential constituent of a functionally competent water oxidase.

Low temperature fluorescence measurements suggest that the lack of sulfolipid in the null mutant most likely has no effect on the structural organization of the reaction center/core antenna complex of photosystem II. The similarity of the emission spectra following chlorophyll a excitation at 440 nm (Fig. 8A) indicates that neither the binding environment of the chlorophyll a emitting from the core antenna proteins CP43 and CP47, nor the excitation energy transfer to the reaction center is affected in the mutant. Moreover, based on the 77 K fluorescence emission spectra following the excitation at 590 nm (Fig. 8B), it appears that excitation energy transfer from phycobilins to chlorophyll a of photosystem II reaction centers is increased. This finding can be explained in terms of structural modifications within the phycobilisome complex or an altered coupling between phycobilisomes and thylakoids. An increase in energy transfer from phycobilins to chlorophyll a may explain the increased variable chlorophyll fluorescence yield in the mutant observed during measurements of room temperature fluorescence (Table II). Generally, the increase in the variable chlorophyll fluorescence yield in the mutant could arise from either an increased activity of photosystem II reaction centers or an increased number of photosystem II (26) as well as a decreased number of photosystem I reaction centers (36). An altered number of reaction centers seems rather unlikely because neither differences in the pigment content nor in the low temperature chlorophyll fluorescence ratio of photosystem II to photosystem I were observed (Fig. 8A). Thus, photosystem II activity should be increased, and an elevated oxygen evolution rate under saturating light in the mutant would be expected. However, the maximal rate of oxygen evolution seems to be not increased in the mutant (Fig. 6). The enhanced light-induced oxygen uptake in the mutant observed during polarographic measurements with the J libre-type electrode (Fig. 7) may be a reasonable explanation for this apparent discrepancy.

The subtle alterations in photosynthesis observed for the SY-SQDB mutant would not have been sufficient to isolate this mutant from a randomly mutagenized population. On the contrary, a leaky sulfolipid-deficient mutant of *C. reinhardtii* has been isolated based on its high fluorescence phenotype following random mutagenesis (17). However, a detailed analysis of the photosynthetic characteristics of this mutant is not available for comparison. Furthermore, it has not clearly been demonstrated that the fluorescence phenotype and the lipid phenotype are indeed caused by the same genetic defect. Therefore further experiments will be required to test whether sulfolipid may play a different role in chloroplasts as compared with cyanobacterial cells.

In summary, the extensive examination of a sulfolipid-deficient null mutant of *Synechococcus* sp. PCC7942 suggests that sulfolipid does not play a specific role for oxygenic photosynthesis. A similar conclusion was drawn for nonoxygenic photosynthesis of *R.* *sphaeroides* (15). However, subtle changes in the biochemistry of O$_2$ and an increased variable room temperature chlorophyll fluorescence yield were observed for the cyanobacterial mutant. As was concluded for *R.* *sphaeroides*, the biosynthesis of sulfolipid may have evolved and been maintained during evolution, primarily not to provide an essential component for photosynthetic processes but to provide a surrogate anionic lipid for conditions of phosphate limitation. Further experiments with higher plants and algae will be required to answer the question of whether this concept is ubiquitous.

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