DNA Research **10**, 229–237 (2003)

Identification of a Gene for UDP-sulfoquinovose Synthase of a Green Alga, *Chlamydomonas reinhardtii*, and Its Phylogeny

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(Received 8 August 2003; revised 15 October 2003)

Abstract

Sulfoquinovosyl diacylglycerol is responsible for the structural and functional integrity of the photosystem II complex of a green alga, *Chlamydomonas reinhardtii*. We cloned a cDNA of *C. reinhardtii* containing an open reading frame for a protein 36–64% identical in the primary structure to known UDP-sulfoquinovose synthases, which are required for SQDG synthesis, in other organisms. Through the introduction of the cDNA, a cyanobacterial disruptant as to the UDP-sulfoquinovose synthase gene recovered the ability to synthesize sulfoquinovosyl diacylglycerol, thus confirming that the cDNA encodes the UDP-sulfoquinovose synthase. On the genome, the cDNA was divided into 14 exons, and the gene designated as *SQD1* was present as one copy. The molecular phylogenetic tree for the UDP-sulfoquinovose synthase showed grouping of *C. reinhardtii* together with species that require sulfoquinovosyl diacylglycerol for the functioning of the PSII complex, but not with those that do not utilize the lipid for photosynthesis. The role of sulfoquinovosyl diacylglycerol in the functioning of the photosynthetic membranes might evolve in harmony with the system of the membrane lipid synthesis such as UDP-sulfoquinovose synthase gene.

Key words: *Chlamydomonas reinhardtii*; photosystem II; *SQD1*; sulfoquinovosyl diacylglycerol; UDP-sulfoquinovose synthase

1. Introduction

An acidic glycolipid, sulfoquinovosyl diacylglycerol (SQDG) of photosynthetic membranes, distinct from the other non-charged glycolipids, monogalactosyldiacylglycerol and digalactosyl diacylglycerol, is distributed extensively among not only oxygenic photosynthetic organisms, but also anoxygenic ones,[1] which has tempted researchers to envisage some fundamental role of SQDG in the photosynthetic apparatus. However, the contribution of SQDG to photosynthesis has been shown not to be universal, but instead to depend on the photosynthetic organisms, through characterization of the photosynthetic apparatus in mutants of several photosynthetic organisms deficient in SQDG synthesis: SQDG is indispensable for the maintenance of PSII activity in the green alga *Chlamydomonas reinhardtii*[2–5] and the cyanobacterium *Synechocystis* sp. PCC6803,[6] while it contributes little to photosynthesis in another cyanobacterium *Synechococcus* sp. PCC7942[7] or the anoxygenic photosynthetic bacterium *Rhodobacter sphaeroides*.[8] We have shown that, in *C. reinhardtii* and *Synechocystis* sp. PCC6803, SQDG contributes to the structural integrity of the PSII complex through associating with the complex, thereby enabling the complex to exert its normal activity.[3–5] On the other hand, the dispensability of SQDG in *R. shaeroides* and *Synechococcus* sp. PCC7942 may be interpreted as an indication that SQDG had little involvement in the functioning of the photosynthetic apparatus at the early phase of evolution of photosynthetic organisms, but that it began to participate in photosynthesis during the evolution of photosystem II (PSII), since the reaction center of photosynthesis of *R. spharoides* is postulated to be the prototype of PSII.[9]

It is thus of interest to examine how the role of SQDG synthesis in the functioning of the photosynthetic membranes has evolved. SQDG is synthesized in two steps, the assembly of UDP-glucose and sulfite into UDP-sulfoquinovose by UDP-sulfoquinovose synthase, and the subsequent transfer of sulfoquinovose from UDP-sulfoquinovose to diacylglycerol to produce

Communicated by Masayuki Ohmori

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‡ Abbreviations: DCMU, 3-(3,4-chlorophenyl)-1,1-dimethylurea; PSII, Photosystem II; SQDG, sulfoquinovosyl diacylglycerol.

SQDG by UDP-sulfoquinovosyl: diacylglycerol sulfoquinovosyl transferase.[1] *R. sphaeroides*, about which the most is known concerning the genes for SQDG synthesis, was shown through genetic analysis to contain at least four genes for the synthesis: *sqdA*, *sqdB*, *sqdC*, and *sqdD*.[10–12] On the other hand, only two genes have so far been found to participate in SQDG synthesis in cyanobacteria and a higher plant, e.g., the *sqdB* and *sqdX* genes for *Synechococcus* sp. PCC7942 and *Synechocystis* sp. PCC6803,[6,7,13] or the *SQD1* and *SQD2* genes for a higher plant, *Arabidopsis thaliana*.[14,15] The UDP-sulfoquinovose synthases encoded by the *sqdB* and *SQD1* genes, irrespective of the species, were shown to have significant identity in the primary structure, while UDP-sulfoquinovosyl: diacylglycerol sulfoquinovosyl transferase were structurally separated into two groups. One group involves the transferase of the anoxygenic photosynthetic organism encoded by the *sqdD* gene and the other contains those of the oxygenic photosynthetic organisms encoded by the *sqdX* or *SQD2* gene. It is also intriguing that homologs of *sqdA* and *sqdC* of *R. sphaeroides*, the functions of which remain to be identified, are absent from the genome of cyanobacteria and *A. thaliana*.

As described above, information on the system of SQDG synthesis is currently available only for prokaryotes and higher plant, whereas little is known about organisms such as green algae that are midway in the evolutionary process between prokaryotic photosynthetic organisms and higher plants. In this study, we cloned a cDNA for UDP-sulfoquinovose synthase from a green alga, *C. reinhardtii*, to give a clue to elucidate the evolution of the system of SQDG synthesis, and discussed its evolution in relation to changes of the roles of SQDG in the functioning of photosynthetic membranes.

2. Materials and Methods

2.1. Cell culture

Cells of *C. reinhardtii* CC125, or *Synechococcus* sp. PCC7942 and its transformant, were grown in oblong glass vessels under constant fluorescent illumination as previously described.[3,16]

2.2. DNA and RNA isolation

The cells of *C. reinhardtii* and *Synechococcus* sp. PCC7942 grown as described above were harvested by centrifugation (8000 × g, 10 min, 4°C). The collected cells were frozen in liquid N_2 and preserved at −80°C until use. DNA or RNA was isolated from these cells as previously described.[17]

2.3. Search of cDNA and genes for UDP-sulfoquinovose synthase in the EST and genome databases

A search of the Kazusa DNA Research Institute *C. reihardtii* EST dataset (http://www.kazusa.or.jp/en/plant/chlamy/EST/) revealed that the deduced amino acid sequence of a reading frame in a cDNA clone (CMO23h12r) is highly homologous to the amino acid sequence of known UDP-sulfoquinovose synthases (data not shown). Additionally, open reading frames (ORFs) for the putative UDP-sulfoquinovose synthase were found on the cyanobacterial genome databases of the Kazusa DNA Research Institute (http://www.kazusa.or.jp/cyano/) and the DOE Joint Genome Institute (http://www.jgi.doe.gov/JGI_microbial/html/index.html).

2.4. Amplification of a part of the UDP-sulfoquinovose synthase-related cDNA of C. reinhardtii

Poly(A)$^+$ RNA was prepared from total RNA with Oligotex-dT30 (Takara), according to the manufacturer's protocol. This RNA was then used for the synthesis of cDNA by reverse transcriptase with a commercially available kit (Time Saver cDNA synthesis kit, Amersham). The cDNA was then used as a template for amplification of a fragment corresponding to a part of CMO23h12r by PCR with the sense and antisense oligonucleotide primers, 5′-GCAGTGCTCAACAATGCAGC-3′ and 5′-CCTTCTGCGTCAATCATGAG-3′, respectively. PCR was performed with a DNA Thermal Cycler (Perkin-Elmer) with the following thermocycle regimen: 2 min at 95°C, followed by 25 cycles of 30 sec at 95°C, 60 sec at 55°C, and 90 sec at 72°C. The product of 0.38 kbp was purified from the agarose gel after electrophoresis.

2.5. Screening of cDNA library of C. reinhardtii

The cDNA fragment of 0.38 kbp was used as a probe for screening of cDNA library of *C. reinhardtii* CC125 constructed in bacteriophage λDASHII (Stratagene) through plaque hybridization, and the DNA sequence of the isolated clone was determined as previously described.[17]

2.6. Construction of a mutant of Synechococcus *sp. PCC7942 with disruption of the* sqdB *gene*

A DNA fragment covering the coding region of *sqdB* gene was amplified from genomic DNA of *Synechococcus* sp. PCC7942 through PCR reaction with the oligonucleotide primers 5′-TTGGGTGGCGATGGTTTCTG-3′ and 5′-GAGAGAGTGCGACTTTAGCG-3′. A product of 1.2 kbp was purified from the agarose gel after electrophoresis, then ligated to pGEM-T Easy Vector (Promega) for production of a plasmid designated as pSBC. We cut pSBC with *Bal* I, the site of which resides in the middle of the *sqdB* gene, and inserted a fragment of 1.4 kbp carrying the kanamycin-resistance cassette of pHSG298 to yield a plasmid, pSBBK. Disruption of the

sqdB gene in *Synechococcus* sp. PCC7942 was conducted with pSBBK through homologous recombination as previously described.[16]

2.7. *Transformation of* sqdB*-disruptant of* Synechococcus *sp. PCC7942 with* SQD1 *of* C. reinhardtii

A DNA fragment corresponding to the N-teminal truncated SQD1 protein was amplified through PCR reaction using a cloned cDNA of *C. reinhardtii* as a template. The primers used were 5′-CCTCC*GAATTC*ATGGCT GCGAGCCGTGCTA-3′ and 5′-CCGGG*GAATTC*TTA ACGGGCCACCGCAGCG-3′ with attachment of *Eco*RI sites (italic sequences), where the underlined codons are the start and stop codons, respectively. The fragment was cut with *Eco*RI, then inserted just after the promoter of the *cpc* operon of pANY1e (a kind gift from Dr. I. Kobayashi at National Institute of Agrobiological Sciences) that was derived from pANY1 by deletion of the luxAB operon.[18]

2.8. *Lipid analysis*

The total lipids were extracted from the cells of *Synechococcus* sp. PCC7942 for determination of individual lipid compositions through two-dimensional TLC and subsequent analysis by capillary GLC.[2]

3. Results

3.1. *Isolation of cDNA encoding a protein homologous to known UDP-sulfoquinovose synthases*

A cDNA clone, the deduced amino acid sequence of which shows significant similarity to those of known UDP-sulfoquinovose synthases (the product of *SQD1* or *sqdB* gene), was found in the *C. reinhardtii* EST dataset of the Kazusa DNA Research Institute. A fragment of the cDNA was amplified by PCR reaction from cDNA synthesized from *C. reinhardtii* mRNA to be used as a probe for screening of its cDNA library. We thus obtained five independent cDNA clones with a similar size (ca. 2.2 kbp) and the same restriction patterns (data not shown), and then arbitrarily selected one clone designated as pSCR1 for determination of the DNA sequence. The sequence was 2151 bp in the length (DDBJ accession no. AB116936), containing two possible ORFs with the same stop codon, but with distinct start codons: one is 1440 bp long and encodes a putative protein of 479 amino acid residues with a molecular mass of 52.9 kDa, while the other is 1425 bp long and encodes a protein shorter by 5 residues at the N-terminus with a molecular mass of 52.3 kDa (Fig. 1). The longer ORF was preceded by an upstream region of 28 bp, the first 10 bp of which appeared to be attached by some artifact (see below), being followed by a 3′-untranslated region of 683 bp (data not shown). We consider that the upstream region, although not possessing any in-frame stop codons, does not encode polypeptide, in view of probable transit peptide present at the N-terminus of the putative protein (see below). On the other hand, the 3′-untranslated region contained a trace 16-bp poly(A)-tail at its 3′-terminus with the sequence TGTAA (the putative polyadenylation signal of *C. reinhardtii*[17]) 15 bp upstream of the trace. These results showed that the isolated cDNA is of full length.

The amino acid sequence deduced from the longer ORF was 62% and 64% identical to those of SQD1 of higher plants, *A. thaliana* and *Spinacia olearacea*, respectively, and 55% to that of SqdB of a cyanobacterium, *Synechocystis* sp. PCC6803 (Fig. 1).[6,14,19] Relatively limited but significant homology was also observed with SqdB of the other cyanobacterial strain, *Synechocystis* sp. PCC7942 (37%) and an anoxygenic photosynthetic bacterium, *R. sphaeroides* (36%).[7,11] A hydropathy plot of the putative protein of *C. reinhardtii*, made according to the method of Kyte and Doolittle, is similar to those of SQD1 and SqdB of the other organisms and shows a relatively hydrophilic property (mean hydrophobicity of -0.24 in *C. reinhardtii*, cf. -0.27 in *A. thaliana*, Fig. 2a). The protein was also characteristic in the prediction of no transmembrane helices with the TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/, data not shown). Furthermore, the putative protein as well as SQD1, when aligned with SqdB of the prokaryotes, exhibited an N-terminal extension of more than 80 amino acid residues from the first Met residue of the prokaryotic sequences (Fig. 1). The extensions of higher plant SQD1, owing to their characteristic amino acid compositions, have been postulated to correspond to transit peptide for chloroplasts,[14,19] being in agreement with actual localization of SQD1 at chloroplast stroma.[19] The extension of *C. reinhardtii* also showed features of chloroplast transit peptide in *C. reinhardtii* such as the high contents of Ala (24%) and Arg (9%) with few acidic residues (only two Glu).[20] This finding, together with the prediction of no transmembrane helices in the whole protein, raised the possibility that the putative protein, like SQD1 of higher plants, is located at chloroplast stroma. Using three criteria based on the sequence analysis, e.g., high similarity with known SQD1 and SqdB in the amino acid sequence and hydropathy profile, and its probable localization at chloroplasts, it is probable that the cDNA isolated from *C. reinhardtii* encodes the UDP-sulfoquinovose synthase. Since the other protein deduced from the shorter ORF also possessed these features (data not shown), it would be interesting to find where the translation starts for the putative UDP-sulfoquinovose synthase mRNA.

We then surveyed the draft DNA sequence of the *C. reinhardtii* genome, released recently by Joint Genome Institute (http://genome.jgi-psf.org/chlre1/chlre1.home.html), to find a sequence that corresponds to the isolated cDNA. However, the first 10 bp of the cDNA did not match the genomic sequence of *C. reinhardtii CC125*.

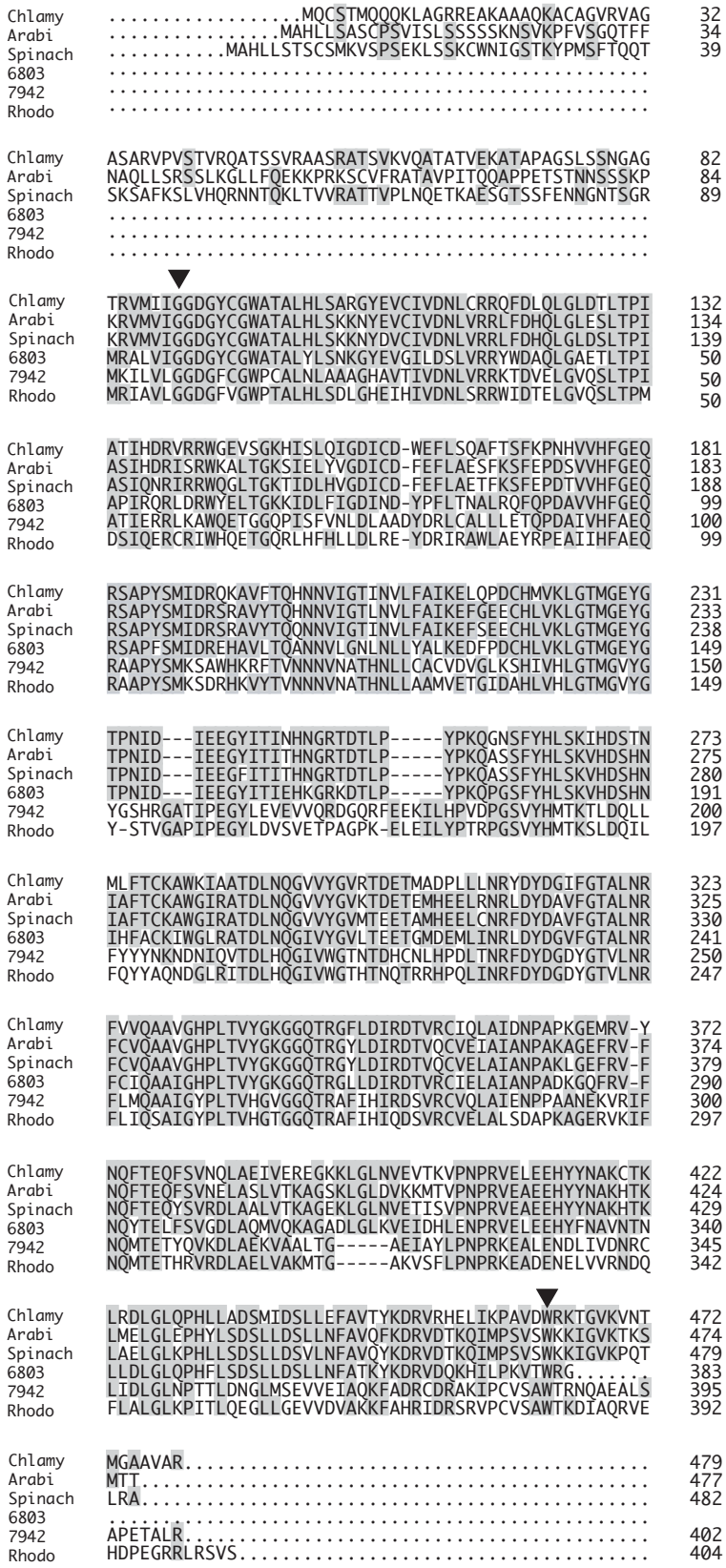

Figure 1. Alignment of the amino acid sequences of the putative UDP-sulfoquinovose synthases of *C. reinhardtii* and its orthologs of higher plants and prokaryotic photosynthetic organisms. Chlamy, *C. reinhardtii* (this study); Arabi, *A. thaliana*;[14] Spinach, *Spinacia oleracea*;[19] 6803, *Synechocystis* sp. PCC6803;[6] 7942, *Synechococcus* sp. PCC7942;[7] Rhodo, *R. sphaeroides*.[11] Arrowheads indicate the region for molecular phylogenic analysis in Fig. 4.

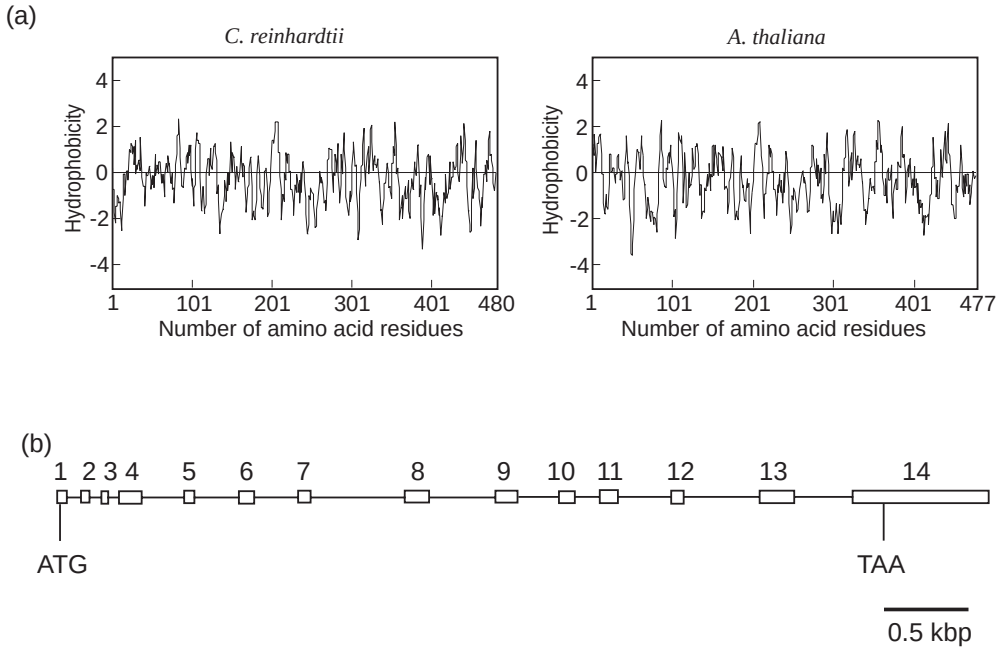

Figure 2. Structural features of the putative UDP-sulfoquinovose synthase of *C. reinhardtii* and its gene. (a) Hydropathy plot of the putative SQD1 of *C. reinhardtii* and its ortholog of *A. thaliana*. (b) *SQD1* gene of *C. reinhardtii*. Boxes, exons; lines, intron.

The origin of the cDNA (data not shown), as well as that of the database, indicate that the additive 5′-terminal sequence was due not to differences in the strains but to some artifact. As shown in Fig. 2b, it was confirmed that the cDNA was composed of 14 exons on the genome. A protein model constructed on the basis of the genome sequence alone, similar to our results, identified 14 exons on the genome, but may be wrong to contain an extra pentapeptide to the sequence we deduced, owing to the failure to identify the range of the 8th exon (data not shown).

3.2. Functional identification of SQD1 *homolog of* C. reinhardtii

We then tried to identify the function of the *SQD1* homolog of *C. reinhardtii* through investigating whether or not its expression in a disruptant as to the *sqdB* gene of *Synechococcus* sp. PCC7942 complements the SQDG-defective phenotype (Table 1). The putative SQD1 of *C. reinhardtii* was expressed in the disruptant under the control of a strong promoter of the *cpc* operon, with deletion of 49 amino acid residues from the 2nd to 50th residues. We consider that part of the N-terminal extension is absent from prokaryotic SqdB (Fig. 1), and therefore is dispensable for the functioning of *Synechococcus* sp. PCC7942. The SQDG content of the wild-type was 18% of total lipids, while that of the *sqdB*-disruptant was below the detectable level. Interestingly, the transformant into which the cDNA for the truncated SQD1 had been introduced showed accumulation of SQDG up to as much as 2.9% of total lipids together with a significant level of the transcript of the SQD1 cDNA (data not shown), confirming that the cDNA we obtained indeed encodes UDP-sulfoquinovose synthase. We thus designated the gene for the cDNA as *SQD1* after its orthologs of higher plants.

Table 1. Expression of the cDNA of *C. reinhardtii* for *SQD1* in a disruptant of *Synechococcus* sp. PCC7942 as to the *sqdB* gene.

Lipid	Wild type	Disruptant	Transformant
		mol(%)	
MGDG	52.9±2.3	51.5±1.0	48.3±1.5
DGDG	11.6±1.4	18.2±1.7	18.2±1.5
SQDG	18.0±2.2	-[a]	2.9±0.5
PG	17.4±1.7	30.2±2.6	30.6±0.3

The values are the mean ±SD for three independent experiments.
[a] The SQDG content was undetectable in the disruptant.

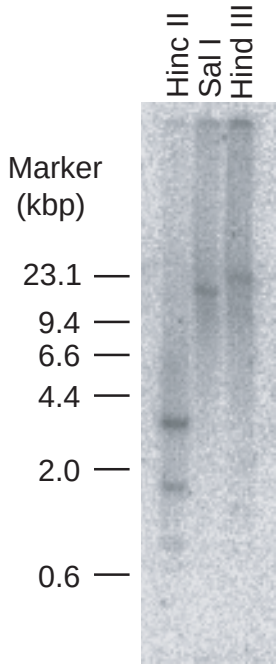

Figure 3. Genomic Southern analysis of the *SQD1* gene. Genomic DNA (5 μg) digested with the indicated enzymes was electrophoresed, blotted onto a membrane, and then probed with a ^{32}P-labeled cDNA fragment.

3.3. Genomic Southern and Northern hybridization

In the genomic Southern hybridization under stringent conditions, only one signal was observed with the genomic DNA digested with *Hin*dIII and *Sal* I, respectively, and three signals with *Hin*cII digestion (Fig. 3). The large size of the single signal with *Hin*dIII or *Sal* I digestion was consistent with the absence of a restriction site for either restriction enzyme within the *SQD1* gene, while the sizes of three signals with *Hin*cII digestion were in agreement with those postulated from the genomic sequence (data not shown). We thus conclude that the *SQD1* gene is present as one copy on the genome, and that the ambiguous part of the genome, the sequences of which are yet to be clarified, contains no candidates for the isogene with significant identity. On the other hand, Northern analysis exhibited a signal at 2.5 kbp (data not shown), which supported our consideration that the ca. 2.2-kbp cDNA which we cloned without a large part of the poly(A) tail is of full length.

3.4. Molecular phylogenetic tree of the products of SQD1 *and* sqdB *genes*

The genes and/or cDNAs for UDP-sulfoquinovose synthase have been isolated from prokaryotic photosynthetic organisms[6,7,10–13] and higher plants,[14,15] but not from green algae. Identification of the *SQD1* gene of *C. reinhardtii* thus gave us an opportunity to examine in more detail the evolution of the system of SQDG synthesis through construction of a molecular phylogenetic tree for UDP-sulfoquinovose synthases and the putative synthases (Fig. 4). The tree separated two phylogenetic groups based on the number of amino acid substitutions: one comprises *R. sphaeroides* and four cyanobacterial strains, while the other comprises the remaining three cyanobacterial strains, *C. reinhardtii*, and the higher plants *A. thaliana* and *S. olearace* (Fig. 4). It is noted that the cyanobacteria does not construct one phylogenetic group, but is divided into two groups. *C. reinhardtii* was positioned between the cyanobacteria and the higher plants in the latter group, which is compatible with the notion that the green algae and higher plants originated from the same cyanobacterial ancestor.

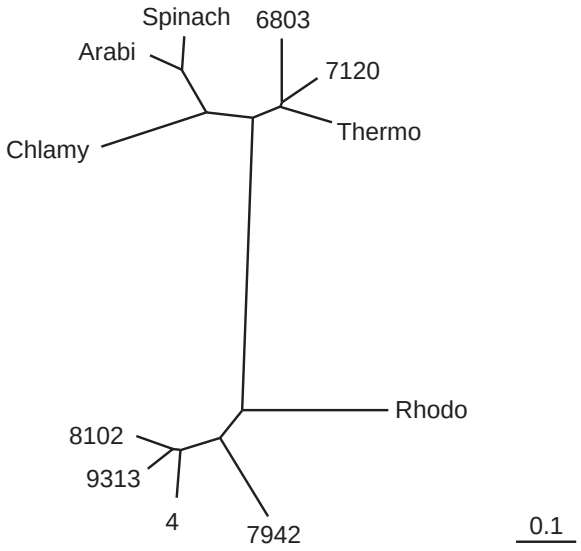

Figure 4. The phylogenetic tree of UDP-sulfoquinovose synthases and the putative trees of photosynthetic organisms. The region between the two amino acid residues indicated by arrowheads in Fig. 1 was used for construction of the tree by the neighbor-joining method.[22] The abbreviations used are the same as in Fig. 1, except that 7120, Thermo, 8102, 9313, and 4 represent *Anabaena* sp. PCC7120, *Thermosynechococcus elongatus* BP-1, *Synechococcus* sp. WH8102, *Prochlorococcus marinus* MIT9313, and *Prochlorococcus marinus* MED4, respectively. As to these cyanobacterial strains, ORFs for putative UDP-sulfoquinovose synthase, which were found on the genome database, were used for construction of the tree (*Anabaena* sp. PCC7120, alr1744; *T. elongatus* BP-1, tll0398; *Synechococcus* sp. WH8102, SYNW0052; *P. marinus* MIT9313, PMT0050; *P. marinus* MED4, PMM1665).

4. Discussion

SQDG, the sole glycolipid that distributes among photosynthetic membranes of almost all of oxygenic and anoxygenic photosynthetic organisms, has been one of the targets for the study of the physiological significances of membrane lipids in the functioning of membrane systems. We have shown that the decrease in

the specific activity of PSII by the mutational lack of SQDG in *C. reinhardtii* or *Synechocystis* sp. PCC6803 is accompanied by enhanced sensitivity of PSII activity to a PSII herbicide, DCMU, and/or greater fragility of the PSII complex upon treatment with a detergent, dodecyl-β, D-maltoside, which are indicative of conformational changes of the PSII complex.[3–6, 21] We therefore concluded that SQDG, through interaction with the PSII complex, adjusts the complex to the normal conformation for its optimal functioning, in view of that in *C. reinhardtii*, SQDG is bound specifically to the PSII complex but not to the PSI complex.[3] In contrast, we recently observed that the deficiency in SQDG in a disruptant of *Synechococcus* sp. PCC7942 as to the *sqdB* gene little affected its PSII property including its activity and sensitivity to DCMU,[21] which is reminiscent of no deleterious effect following the mutational loss of SQDG on the photosynthetic parameters in *R. sphaeroides*.[8]

In this study, we clarified a gene for SQDG synthesis of *C. reinhardtii* that to date has only been reported in an algal species. There are no reports in the literature on the genes for SQDG synthesis that examine the evolution of the photosynthetic membrane system with regards to SQDG synthesis. A cDNA cloned in this study was identified as encoding the UDP-sulfoquinovose synthase through sequence and functional analyses of the putative protein. This putative protein showed high similarity to known UDP-sulfoquinovose synthases of other organisms regarding the amino acid sequence and hydropathy profile, the presence of a N-terminus corresponding to the chloroplast transit peptide (Figs. 1 and 2), and the complementation of the SQDG-lacking phenotype of a cyanobacterial disruptant as to the UDP-sulfoquinovose synthase gene (Table 1). The gene corresponding to the cDNA, designated as *SQD1* after its orthologs in higher plants, was present as one copy in the genome of *C. reinhardtii* (Fig. 3), as is the case of the other species so far investigated.[6,7,10]

Interestingly, the molecular phylogenetic tree for the UDP-sulfoquinovose synthase comprises two phylogenic groups, and the cyanobactrial SqdB proteins are incorporated into either group depending on the strain (Fig. 4). These results suggest that SqdB proteins may have followed two evolutionary pathways from anoxygenic to oxygenic photosynthetic prokaryotes: one could result from evolutionary change not so drastic, as is represented by SqdB of *Synechococcus* sp. PCC7942 with relatively high similarity to that of *R. sphaeroides* (Figs. 1 and 4), while the other would be caused by significant changes, as observed with SqdB of *Synechocystis* sp. PCC6803. Grouping of SQD1 of *C. reinhardtii* and higher plants with SqdB of *Synechocystis* sp. PCC6803 indicates that the SQD1 proteins originated from the common ancestral SqdB protein of a cyanobacterium that had followed the significant evolutionary modification pathway.

It is of interest also that the classification of the two groups by the phylogenetic tree coincides well with the presence or absence of SQDG-dependency of the PSII complex and its prototype. The evolution of the system of SQDG synthesis, such as UDP-sulfoquinovose synthase genes, might parallel the appearance of role of SQDG in the functioning of the photosynthetic membranes. It is possible that the less striking change of SqdB of anoxygenic photosynthetic organisms into those of the cyanobacterial group including *Synechococcus* sp. PCC7942 would be accompanied by inheritance of the dispensability for SQDG from the photosynthetic reaction center complex to the PSII complex. In contrast, the remarkable change into the other type of cyanobacterial SqdB, such as that of *Synechocystis* sp. PCC6803, might occur concomitantly with modification of the photosynthetic complex for the SQDG requirement to appear. We consider that the latter type of cyanobacterial SqdB further evolved into SQD1 of green algae such as *C. reinhardtii*, together with inheritance of the SQDG requirement of the PSII complex.

Important for verification of the proposal described above is the analysis of the effects of SQDG-deficiency on PSII in cyanobacterial disruptants as to the putative *sqdB* genes (Fig. 4). On the other hand, chlorophyll fluorescence analysis did not show a prominent effect on photosynthetic parameters in an SQDG-deficient mutant of *A. thaliana* recently isolated.[15] However, the results by themselves did not prove that SQDG has nothing to do with the functioning of PSII, since the SQDG-deficient mutant of *C. reinhardtii*, with a reduced PSII oxygen-evolving rate (60% of the wild-type), also shows little change in chlorophyll fluorescence parameters such as F_v/F_m ratio, owing probably to the main defect in the oxygen-evolving system of PSII but not in its reaction center.[23] Examination of PSII in the *Arabidopsis* mutant in more detail would indicate the direction in which the PSII complex is evolving with regards to the requirement of SQDG. Such a study based on the evolution of the system of the lipid synthesis, together with the molecular phylogenetic strategy to date for the membrane proteins, should provide a new aspect for discussion of the issue not only regarding the evolution of the photosynthetic membrane systems but also regarding the evolution among cyanobacteria, e.g., from monocellular to multicellular strains, and the cyanobacterial ancestor of chloroplasts.

The *C. reinhardtii* SQD1 expressed heterologously in *Synechococcus* sp. PCC7942 under the control of the strong promoter of the *cpc* operon contributed significantly to SQDG synthesis, but at much lesser level in comparison to the innate SqdB (Table 1). Transformation of *Escherichia coli* with two cDNAs of *A. thaliana* for SQD1 and SQD2 resulted in the appearance of SQDG, although at barely detectable levels, providing support for the idea that combined expression of these two genes are fundamentally enough for SQDG synthesis.[15] However,

a native form of the *S. oleracea* SQD1 was recently reported to be ca. 250 kDa in molecular mass, much larger than the ca. 90-kDa homodimer that is formed by the recombinant SQD1, and to show fourfold higher affinity to sulfite than the recombinant SQD1, thus raising the possibility that SQD1 association with some unidentified components in vivo is needed for its proper functioning.[19] The lower level of SQDG synthesis in *Synechococcus* sp. PCC7942 by the *C. reinhardtii* SQD1 than by the native SqdB can be accounted for by the inappropriate combination of the *C. reinhardtii* SQD1 with the other components for SQDG synthesis originally present in the cells of *Synechococcus* sp. PCC7942, in view of the relatively low structural homology of the SQD1 to the SqdB (Fig. 1). Alternatively, it is also probable that the expression level of SQD1, owing to the highly biased codon usage of *C. reinhardtii* nuclear genes, was too low to completely cover the loss of SqdB.

The transformation of *Synechococcus* sp. PCC7942 with SQD1 cDNA is the first report, to our knowledge, on the successful expression of *C. reinhardtii* nuclear genes in cyanobacteria, the postulated ancestor of chloroplasts, thus opening a way to verify the functions of unidentified genes of *C. reinhardtii* in the cyanobacterium. The system will allow us to identify genes that are related particularly to construction of the photosynthetic apparatus, including the genes for SQDG synthesis other than the *SQD1* gene.

Acknowledgements: This work was supported by grants from the Ministry of Education, Science, Sports and Culture (13740463, 13874112, 12206002), the Promotion and Mutual Aid Corporation for Private Schools of Japan, and CREST of JST (Japan).

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