Chapter 10

Sulfolipid Biosynthesis and Function in Plants

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Summary

The plant sulfolipid sulfoquinovosyldiacylglycerol accounts for a large fraction of organic sulfur in the biosphere. Aside from sulfur amino acids, sulfolipid represents a considerable sink for sulfate in plants. Plant sulfolipid is found in the photosynthetic membranes of plastids and provides negative charge in the thylakoid membrane where it is thought to stabilize photosynthetic complexes. As the plant sulfolipid is a non-phosphorous glycolipid, its synthesis does not impinge on the supply of phosphate, which is a macronutrient limiting plant growth in many natural environments. Indeed, plants evolved homeostatic mechanisms to balance the amount of sulfolipid with anionic phospholipids maintaining a proper level of anionic charge in the photosynthetic membrane. The strong anionic nature of the sugar sulfonate head group of sulfolipid also makes this lipid an interesting compound for biotechnological applications. As bacterial and plant genes encoding sulfolipid enzymes are now available, biotechnological approaches can be developed to produce the plant sulfolipid in sufficient amounts to pursue the development of practical applications.

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I. Introduction

The plant sulfolipid, sulfoquinovosyldiacylglycerol (SQDG), is a glycoglycerolipid characterized by a 6-deoxy-6-sulfoglucose head group at the *sn*-3 position of the glycerol backbone of diacylglycerol (DAG) as shown in Fig. 1. Over the past 50 years, much has been written about the presumed biosynthetic pathway of plant sulfolipid and its possible function in plants. With recent advances in molecular and biochemical analysis, data are now available for a more informed discussion of the biosynthesis and function of SQDG in bacteria, algae and plants and its biotechnological potential. Bacteria are considered here, because the first genes encoding the enzymes directly involved in SQDG biosynthesis were discovered in the purple bacterium *Rhodobacter sphaeroides* (Benning and Somerville, 1992a; Benning and Somerville, 1992b) and the cyanobacterium *Synechococcus* PCC7942 (Güler et al., 1996; Güler et al., 2000). These studies prepared the way for the final elucidation of SQDG biosynthesis in plants and algae. Aside from a brief historic overview, we will focus on the more recent literature providing the current view of sulfolipid biosynthesis and function. For additional background information the reader is referred to previous reviews on the subject (Benson, 1963; Haines, 1973; Harwood, 1980; Barber and Gounaris, 1986; Mudd and Kleppinger-Sparace, 1987; Kleppinger-Sparace et al., 1990; Heinz, 1993; Marechal et al., 1997; Benning, 1998; Okanenko, 2000; Frentzen, 2004).

A. Discovery and Historic Perspective

Sulfolipid was discovered by A.A. Benson and coworkers in alcohol extracts of [^{35}S]-sulfate-labeled algae, purple bacteria and plants (Benson et al., 1959). The structure of SQDG was subsequently elucidated as summarized by A.A. Benson (Benson, 1963) and later confirmed by mass spectrometric analysis (Budzikiewicz et al., 1973; Gage et al., 1992; Kim et al., 1997). Analyzing water soluble sulfur-labeled compounds, a sulfoquinovose nucleotide was tentatively identified in *Chlorella* extracts, as well as small sulfur-labeled compounds (Shibuya et al., 1963) interpreted as biosynthetic intermediates. As we now know, A.A. Benson correctly proposed that a sulfoquinovose nucleotide is the direct head group donor for sulfolipid biosynthesis (Benson, 1963). Moreover, G.A. Barber was the first to suggest that the sulfoquinovose nucleotide could be formed from a 4-keto-6-deoxy-α-D-glucose nucleotide or similar molecule (Barber, 1963) and Lehmann and Benson demonstrated that sulfoquinovose can be chemically generated by addition of sulfite to methyl 5,6-glucoseenide (Lehmann and Benson, 1964). A more detailed hypothesis of the nucleotide pathway was provided by Pugh and colleagues (Pugh et al., 1995b) following the genetic demonstration that a sugar nucleotide modifying enzyme was critical for sulfolipid biosynthesis in the purple bacterium *Rhodobacter sphaeroides* (Benning and Somerville, 1992a). Based on the isolation of the genes encoding the enzymes of sulfolipid biosynthesis, their biochemical analysis *in vitro*, and the structure determination of the UDP-sulfoquinovose (UDP-SQ) forming enzyme as described in detail below, it is now generally accepted that sulfolipid is synthesized in plants by the nucleotide pathway shown in Fig. 2. Sulfonated 3-carbon or 2-carbon compounds found in plants or bacteria are thought to be degradation products of SQDG rather than biosynthetic intermediates (Roy et al., 2003).

B. Occurrence in Plants and Bacteria

The plant sulfolipid SQDG represents an important component of the global sulfur cycle (Harwood and Nicholls, 1979) as it is found in most photosynthetic and a few non-photosynthetic organisms. It has been estimated that sulfolipid is approximately equal to glutathione in abundance, but presumably an order of magnitude less abundant than sulfur amino acids bound in proteins of plant tissues (Heinz, 1993). The relative sulfolipid content in plant and algae tissues has been reported to be as low as 2% and as high as 50% of total polar lipids in marine plants and algae (Harwood, 1980; Dembitsky et al., 1990; Dembitsky et al., 1991; Heinz, 1993). Because sulfolipid is

Abbreviations: DAG – diacylglycerol; ER – endoplasmic reticulum; PG – phosphatidylglycerol; SQDG – sulfoquinovosyldiacylglycerol; TLC – thin-layer chromatography; UDP-SQ – UDP-sulfoquinovose

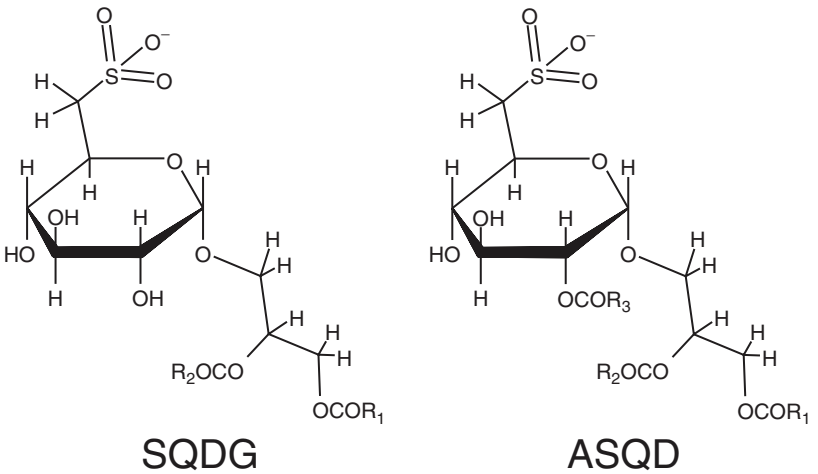

Fig. 1. Structures of sulfoquinovosyldiacylglycerol (SQDG) and 2-acyl- sulfoquinovosyldiacylglycerol (ASQD).

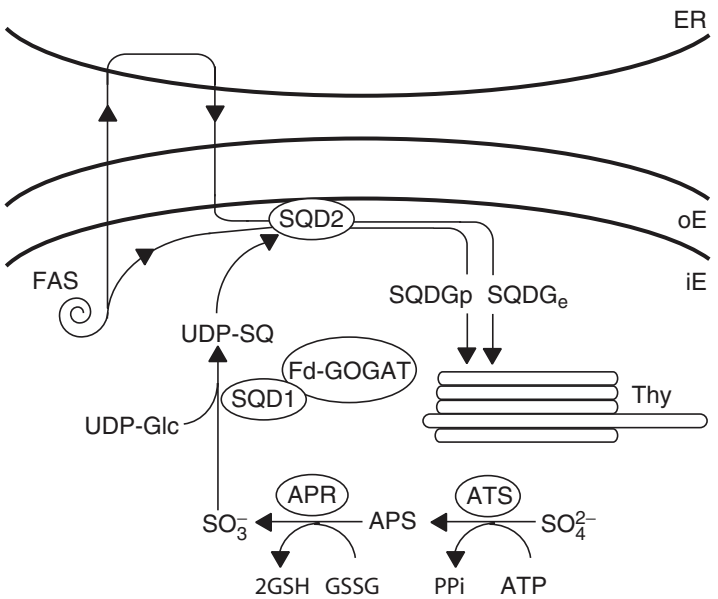

Fig. 2. Sulfolipid biosynthesis in plant chloroplasts. The inner envelope membrane (iE), the outer envelope membrane (oE), the endoplasmic reticulum (ER), and the thylakoid membranes (Thy) are shown. With the exception of the fatty acid synthase complex (FAS) depicted as a spiral, enzymes are shown as ovals. They include the UDP-SQ synthase (SQD1), the SQDG synthase (SQD2), ferredoxin-dependent glutamate synthase (Fd-GOGAT), ATP-sulfurylase (ATS) and adenosylphosphosulfate reductase (APR). Sulfolipid (SQDG) can be derived from the plastid pathway of thylakoid lipid biosynthesis ($SQDG_p$), or the ER pathway of thylakoid lipid biosynthesis ($SQDG_e$). Arrows indicate the direction of net flux in the pathways. Substrates and intermediates are: APS, adenosylphosphosulfate; ATP, adenosine triphosphate; GSSG, oxidized glutathione; GSH, reduced glutathione; PPi, orthophosphate; SO_3^-, sulfite; SO_4^{2-}, sulfate; UDP-Glc; UDP-glucose; UDP-SQ, UDP-sulfoquinovose.

found in photosynthetic membranes of bacteria and plants where it is associated with photosynthetic complexes, and because chlorophyll content seems to correlate with sulfolipid content, it was concluded that sulfolipid must be important for photosynthesis (Barber and Gounaris, 1986). However, there is no strict correlation between the competence of an organism to conduct photosynthesis and the presence of the SQDG. For example, *Sinorhizobium meliloti* (Cedergren and Hollings-

worth, 1994; Weissenmayer et al., 2000), different species of *Caulobacter* and *Brevundimonas* (Abraham et al., 1997), and even Gram-positive bacteria (Langworthy et al., 1976; Sprott et al., 2006) have been reported to contain SQDG. The sulfolipid SQDG as well as other sulfolipids are present in the non-photosynthetic diatom *Nitzschia alba* (Anderson et al., 1978). The opposite has been observed as well, for example the lack of sulfolipid genes and, therefore, SQDG in a cyanobacterium (Selstam and Campbell, 1996; Nakamura et al., 2003). It should be cautioned, though, that SQDG content of bacteria and plants can change depending on growth conditions (Gage et al., 1992; Benning et al., 1993). As full-genome sequences for many bacteria are now available, a search for genes encoding putative sulfolipid biosynthetic enzymes provides a broad picture of the distribution and evolution of sulfolipid biosynthesis in different bacteria and plants, as will be discussed below.

II. Biosynthesis of Sulfoquinovosyldiacylglycerol

During the early phase of research exploring the biosynthetic pathway for SQDG in seed plants, isolated chloroplasts served as a facile model and were employed in numerous studies (Haas et al., 1980; Kleppinger-Sparace et al., 1985; Joyard et al., 1986; Kleppinger-Sparace and Mudd, 1987; Kleppinger-Sparace and Mudd, 1990; Pugh et al., 1995a; Pugh et al., 1995b; Roy and Harwood, 1999). The general conclusion from these experiments was that chloroplasts are fully capable of synthesizing SQDG from labeled sulfate when energy requirements were met either by photosynthesis or by the supply of nucleotides. Therefore, in seed plants chloroplasts must contain the biosynthetic machinery to provide the sulfur and carbon precursors for sulfolipid biosynthesis. By synthesizing the proposed head group donor for SQDG biosynthesis, the sugar nucleotide UDP-SQ, Heinz and colleagues were able to assay the SQDG synthase in chloroplast envelopes (Heinz et al., 1989). This assay was used to characterize the SQDG synthase and determine its localization on the inside of the inner envelope membrane of chloroplasts (Seifert and Heinz, 1992; Tietje and Heinz, 1998). However, the definitive determination of the pathway of sulfolipid biosynthesis as shown in Fig. 2 was made possible by the identification of the enzymes at the molecular level and the biochemical characterization of the recombinant proteins. Sulfolipid biosynthesis in plants requires at minimum two specific enzymes: (1) the UDP-SQ synthase (SQD1), which is responsible for the biosynthesis of the head group donor, and (2) the SQDG synthase (SQD2) catalyzing the final assembly of sulfolipid.

A. Biosynthesis of UDP-Sulfoquinovose

Aside from the sulfoquinovosylated oligosaccharide side chain of a cytochrome *b* protein from an archaeon (Zähringer et al., 2000), sulfoquinovose has only been reported to be a substituent of sulfolipid or its precursor UDP-SQ. The existence of UDP-SQ in biological materials was originally suggested by A.A. Benson based on the tentative identification of a sulfur labeled sugar nucleotide in extracts of *Chlorella* (Shibuya et al., 1963). A more certain identification of UDP-SQ in an organism was accomplished in extracts of a sulfolipid-deficient *sqdD* mutant of *R. sphaeroides* (Rossak et al., 1995). This mutant lacks the presumed SQDG synthase encoded by *sqdD* and accumulated the UDP-SQ precursor to the extent that it could be readily analyzed. Subsequently, UDP-SQ was identified in extracts from different plants (Tietje and Heinz, 1998). Using genetic analysis, Benning and Somerville identified four genes, *sqdA, sqdB, sqdC*, and *sqdD* of *Rhodobacter sphaeroides* essential for SQDG biosynthesis (Benning and Somerville, 1992a; Benning and Somerville, 1992b). The first gene, *sqdA*, encodes an acyltransferase–like protein (Benning and Somerville, 1992b) and its precise role in SQDG biosynthesis remains to be shown. The *sqdB* gene forms an operon with *sqdC* and *sqdD* (Benning and Somerville, 1992a), with the *sqdD* gene encoding a predicted glycosyltransferase, the presumed SQDG synthase in this bacterium (Rossak et al., 1995). The *sqdC* gene encodes a small reductase-like protein and its targeted disruption led to the accumulation of sulfoquinovosyl-1-*O*-dihydroxyacetone in the respective mutant (Rossak et al., 1997). The original interpretation was that the SqdC and SqdD proteins form a functional SQDG synthase with SqdC

providing substrate-specificity to the enzyme (Rossak et al., 1997). However, this hypothesis needs to be revisited as it seems possible that in bacteria SQDG is assembled in a different way than shown in Fig. 2 for plants.

Neither *sqdA*, nor *sqdC* and *sqdD* of *R. sphaeroides* seem to be involved in the biosynthesis of UDP-SQ and are also not found to be conserved in cyanobacteria and plants. However, the *sqdB* encoded protein of *R. sphaeroides* resembles sugar nucleotide modifying enzymes and has its apparent orthologue in every SQDG-producing organism studied thus far (see evolution of SQDG biosynthesis below). Its discovery in *R. sphaeroides* (Benning and Somerville, 1992a) led to the identification of the respective *sqdB* orthologue in the cyanobacterium *Synechococcus* sp. PCC7942 (Güler et al., 1996), and the orthologues in plants (Essigmann et al., 1998; Shimojima and Benning, 2003) and algae (Riekhof et al., 2003; Sato et al., 2003b) designated *SQD1*, and laid the foundation for our current understanding of UDP-SQ biosynthesis. The Arabidopsis SQD1 protein was localized in the stroma of the chloroplast (Essigmann et al., 1998). The structure of SQD1 from Arabidopsis was predicted based on its similarity to other sugar-nucleotide modifying enzymes and a detailed reaction mechanism was proposed (Essigmann et al., 1999) based on a 4-keto-5,6-glucoseene intermediate as suggested by Pugh and coworkers (Pugh et al., 1995b). The SQD1 protein contains a tightly-bound NAD^+residue participating in the formation of a 4-keto hexosyl group. This 4-keto intermediate is converted to a 4-keto-5,6-glucoseene intermediate to which sulfite is added. Subsequently, the 4-keto group reverts back to the hydroxyl after accepting a hydride from the NADH bound in the active site, which thereby releases UDP-SQ and regenerates NAD^+. Detailed structural analysis by X-ray crystallography of the Arabidopsis SQD1 dimeric protein with NAD^+ and UDP-glucose (Mulichak et al., 1999) supported this reaction mechanism and identified the active site residues as shown in Fig. 3.

The recombinant SQD1 protein was subsequently shown to convert UDP-glucose and sulfite to UDP-SQ *in vitro* (Sanda et al., 2001). However, the rate at which the recombinant enzyme catalyzed this reaction was very low. This left the question whether ancillary proteins and additional cofactors might be involved *in vivo*, or whether sulfite might not be the native substrate for this enzyme. When the SQD1 protein was isolated from spinach chloroplasts, it purified as a 250 kDa complex suggesting the presence of additional proteins in the native complex (Shimojima and Benning, 2003). The K_M of this native complex for sulfite was at least fourfold reduced compared to recombinant SQD1 suggesting an increased affinity for this substrate. Further analysis of the native complex revealed that ferredoxin-dependent glutamate synthase (FdGOGAT) co-purifies and tightly associates with SQD1 (Shimojima et al., 2005). The physiological/biochemical relevance of this interaction is not yet fully understood. However, as FdGOGAT is a flavin-containing protein, it could intermittently and covalently bind sulfite and deliver it to the active site of SQD1. Modeling of the cyanobacterial FdGOGAT and Arabidopsis SQD1 into a plausible complex supports this suggestion (Shimojima et al., 2005). It was therefore proposed that FdGOGAT moonlights in the synthesis of UDP-SQ by providing sulfite to the SQD1 active site. Because sulfite is a highly cytotoxic compound that is maintained at very low level in plants, the interaction of FdGOGAT and SQD1 could overcome this impasse by channeling sulfite to SQD1. However, further experiments will be required to support this current working hypothesis.

B. Assembly of Sulfoquinovosyldiacylglycerol

For the final assembly of SQDG, *R. sphaeroides* could not serve as a universal model because plants have neither orthologues of *sqdC* nor *sqdD*. When the *sqdB* gene of the cyanobacterium *Synechococcus* sp. PCC7942 was first isolated, it was found in an operon next to a gene now called *sqdX* (Güler et al., 2000). This gene encodes a protein with similarity to other lipid glycosyltransferases (Berg et al., 2001), but different from that encoded by the *R. sphaeroides sqdD* gene. Disruption of the *sqdX* gene of *Synechococcus* sp. PCC7942 led to SQDG deficiency implicating this protein in SQDG biosynthesis in this bacterium (Güler et al., 2000). Using this cyanobacterial gene, it was subsequently possible to isolate the plant orthologue, *SQD2*, encoding the plant sulfolipid

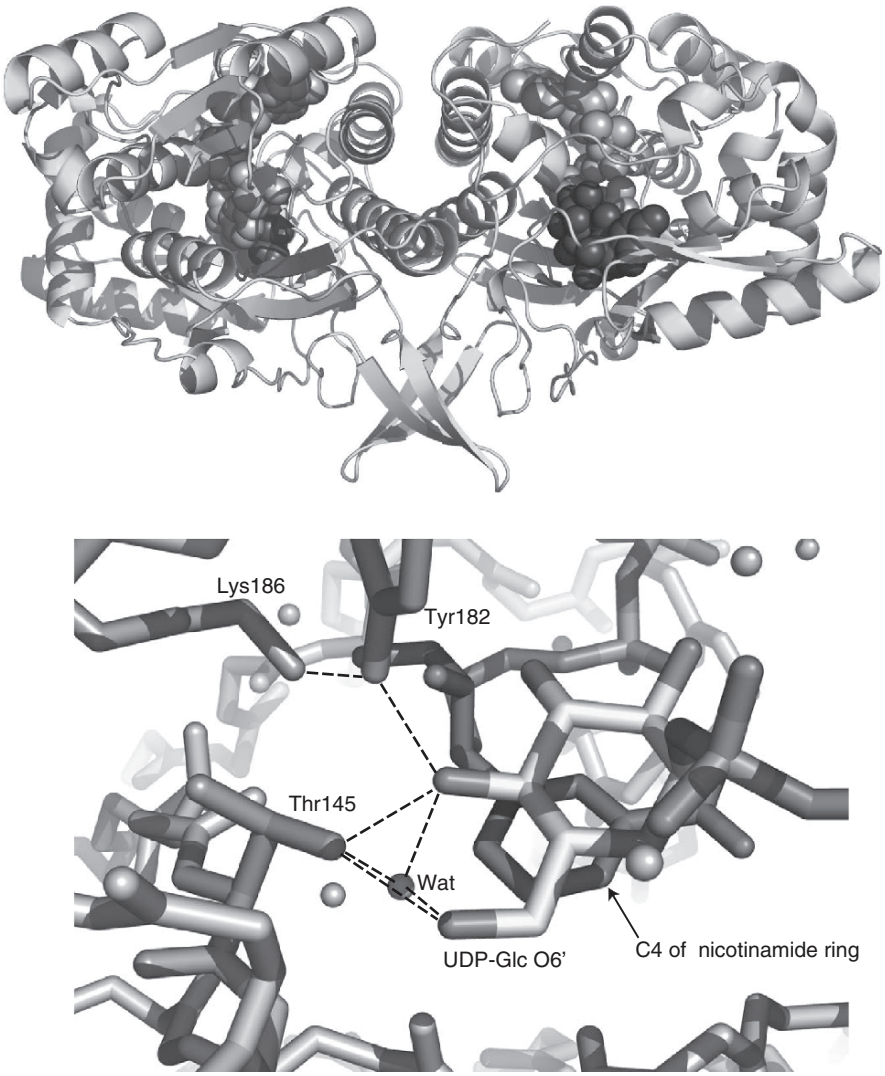

Fig. 3. Crystal structure of the Arabidopsis SQD1 protein (PDB entry 1QRR). The SQD1 dimer structure with the NAD^+ cofactors (light gray solid spheres) and the UDP-Glucose substrates (dark grey solid spheres) is shown in the top panel. The bottom panel depicts the active site of an SQD1 monomer with three critical amino acid residues that hydrogen-bond with the substrate UDP-glucose O6′ hydroxyl group, and one water molecule (Wat). The carbon 4 of the nicotine amide ring of NAD^+ cofactor is indicated.

synthase (Yu et al., 2002). Disruption of this gene by T-DNA insertion resulted in complete loss of SQDG in Arabidopsis. Moreover, co-expression of the *SQD1* and *SQD2* cDNAs from Arabidopsis in *E. coli* led to sulfolipid biosynthesis in this bacterium normally lacking this lipid (Yu et al., 2002). Thus, these two plant genes were sufficient to reconstitute SQDG biosynthesis demonstrating that the ultimate sulfur donor for SQDG biosynthesis must be a common intermediate of the sulfur assimilation pathway, such as sulfite, present in *E. coli*. The SQD2 protein was localized inside the plastid (Yu et al., 2002) consistent with biochemical data showing that the sulfolipid synthase is associated with the inside of the inner envelope membrane (Seifert and Heinz, 1992; Tietje and Heinz, 1998). *In vitro* data or crystallographic data on this membrane-bound enzyme are not yet available. Based on these data, the nucleotide pathway of sulfolipid biosynthesis in plants inside chloroplast and the proteins involved are summarized as shown in Fig. 2.

III. Evolution of Sulfolipid Biosynthesis

In the era of genomics it is now possible to scan for the presence of putative sulfolipid genes in a large number of genomes. This approach provides independent means to examine the distribution of sulfolipid biosynthetic competence in the natural world and suggests clues towards the evolution of sulfolipid biosynthesis. Taking this approach, it is immediately clear that the two plant genes *SQD1* and *SQD2* evolved at different times. While many genomes show readily identifiable *SQD1/sqdB* orthologues suggesting that the capability to produce UDP-SQ evolved early and presumably only once, SQDG synthases were recruited differently in cyanobacteria/plants versus non-cyanobacteria and archaea. The alphaproteobacteria *R. sphaeroides* and *S. meliloti*, in which the *sqd* genes have been well studied, harbor an SQDG synthase encoded by the *sqdD* gene (Rossak et al., 1995; Weissenmayer et al., 2000). This gene is not related to the cyanobacterial *sqdX* gene (Güler et al., 2000) and its plant orthologue *SQD2* (Yu et al., 2002).

Figure 4 shows the approximate relatedness of predicted SQD1/sqdB-like protein sequences in bacteria, archaea and plants. Overall UDP-SQ synthases are closely related to UDP-glucose epimerases and similar sugar nucleotide-modifying enzymes. Therefore, many of the presumed UDP-SQ synthases are not correctly annotated in the public databases. On the other hand, without biochemical analysis, one cannot be certain that the respective protein indeed represents a UDP-SQ synthase. At this time, seven UDP-SQ synthases have been experimentally verified as indicated in Fig. 4. There is a clear cutoff in sequence similarity between presumed and known UDP-SQ synthases and other enzyme classes and it is apparent that animals do not have an UDP-SQ synthase

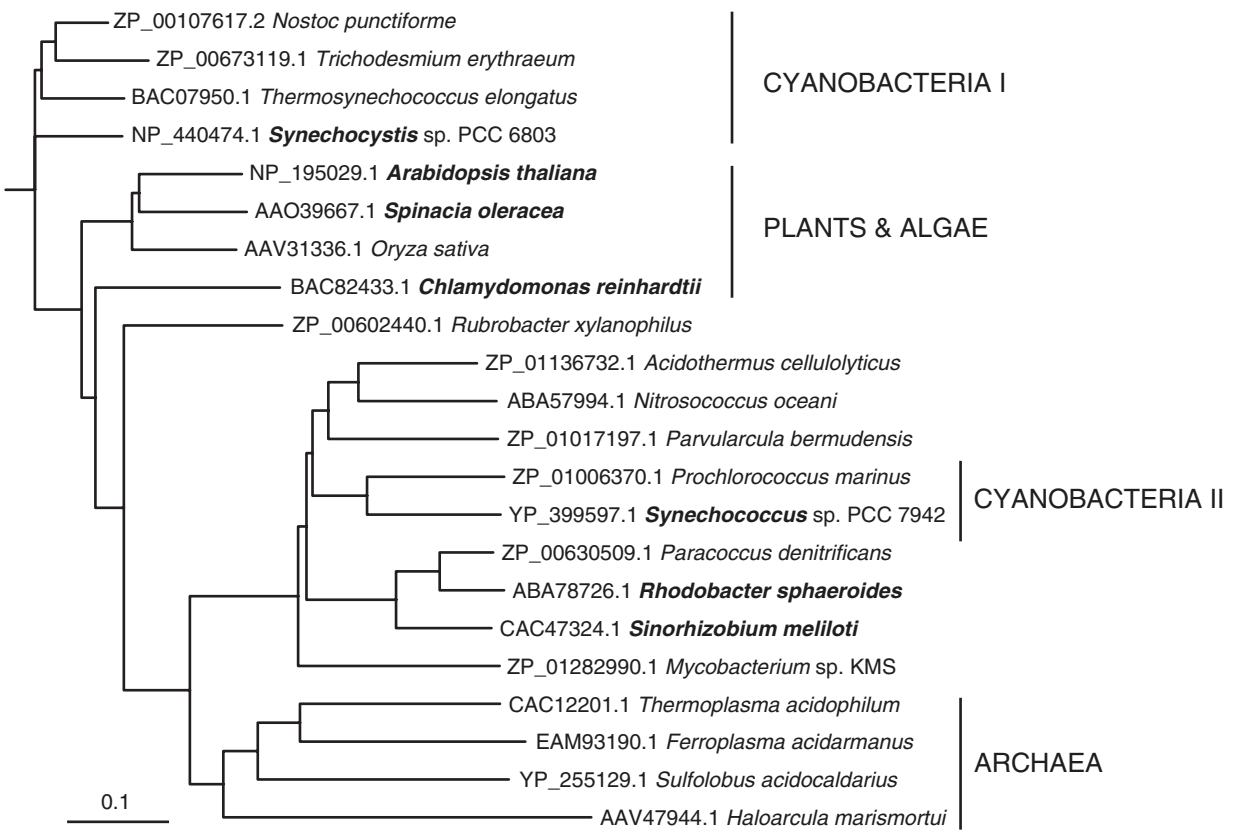

Fig. 4. Rooted tree showing the relatedness of selected SQD1/sqdB protein sequences. Sequences were aligned and the tree was built using Clustal W software (Thompson et al., 1994). Genbank accession numbers and species names are indicated. Species in which the respective UDP-SQ synthase was experimentally verified are shown in bold.

related to SQD1/sqdB consistent with their inability to produce SQDG. Only sea urchins have been reported to contain SQDG and its derivatives in their gut (Sahara et al., 1997), but it seems that these lipids are derived from their algal diet rather than being synthesized by this organism. Overall, SQD1/sqdB encoding sequences are present in a wide range of archaea, Gram + and Gram- bacteria, cyanobacteria, algae and plants. Whether all these organisms produce sulfolipid is not clear. It is also possible that UDP-SQ serves as sulfoquinovosyl donor for syntheses other than sulfolipid, exemplified by the presence of sulfoquinovose in a glycoprotein of *Sulfolobus acidocaldarius*, an archaeon (Zähringer et al., 2000). Thus, the capability to synthesize UDP-SQ evolved very early on, but SQDG biosynthesis itself arose independently at least twice, in bacteria other than cyanobacteria, and in plants and cyanobacteria.

IV. Biological Functions of Sulfolipid

A. In Vitro *and Correlative Studies*

Early inferences regarding the function of sulfolipid were mostly based on considerations of chemical and molecular properties of SQDG (Haines, 1983), the prevalence of SQDG in photosynthetic organisms, and the analysis of SQDG in typical phototrophs. For example, SQDG content increases during chloroplast development (Shibuya and Hase, 1965; Leech et al., 1973) and SQDG-specific antibodies inhibit the biochemical activity of photosynthetic membranes (Radunz and Schmid, 1992). Moreover, SQDG is associated with pigment protein complexes of photosynthetic membranes (Menke et al., 1976; Gounaris and Barber, 1985; Pick et al., 1985; Sigrist et al., 1988) and SQDG is required for the functional reconstitution of membrane-bound enzymes *in vitro* (Pick et al., 1987; Vishwanath et al., 1996). Sulfolipid specifically interacts with signal peptides of chloroplast targeted proteins suggesting a role during the import of nuclear encoded proteins into plastids (van't Hof et al., 1993; Inoue et al., 2001). Sulfolipid has also been shown to bind to an annexin in a calcium-dependent manner presumably at the cytosolic face of the outer envelope (Seigneurin-Berny et al., 2000). However, the biological significance of this interaction seen in *in vitro* experiments is not clear.

Effects of environmental factors on membrane lipid composition have led to suggestions about the conditional importance of plant sulfolipid. For example, a positive correlation has been observed between sulfolipid content and salt exposure, particularly in halophytes (Kuiper et al., 1974; Müller and Santarius, 1978; Ramani et al., 2004; Hamed et al., 2005), or drought exposure (Quartacci et al., 1995; Taran et al., 2000). Increases in sulfolipid have also been observed in cold-hardened plants (Kuiper, 1970) or during the winter season in pine needles (Oquist, 1982). However, one of the most striking environmental factors affecting sulfolipid content in bacteria and plants has been phosphate deprivation (Gage et al., 1992; Benning et al., 1993; Güler et al., 1996; Härtel et al., 1998; Essigmann et al., 1998; Sato et al., 2000; Yu et al., 2002; Yu and Benning, 2003; Sato, 2004) suggesting that sulfolipid can substitute to some extent for phosphatidylglycerol to maintain the anionic surface charge of thylakoid membranes.

B. Analysis of Sulfolipid-Deficient Mutants

The knowledge of the genes required for the biosynthesis of SQDG has provided genetic tools to study the function of the sulfolipid *in vivo*. An interesting picture has emerged from the study of SQDG-deficient mutants of bacteria, Arabidopsis and the unicellular alga *Chlamydomonas reinhardtii* as recently summarized by others (Sato, 2004; Frentzen, 2004). Bacterial mutants of the photosynthetic purple bacterium *Rhodobacter sphaeroides* (Benning et al., 1993), the cyanobacterium *Synechococcus* sp. PCC7942 (Güler et al., 1996) or the non-photosynthetic bacterium *Sinorhizobium meliloti* (Weissenmayer et al., 2000) disrupted in the *sqdB* gene essential for SQDG biosynthesis and completely lacking sulfolipid did not show any growth deficiencies under optimal growth conditions. However, it should be noted that for *Synechococcus* sp. PCC7942, subtle effects of SQDG deficiency on photosystem II chemistry were observed, but were apparently not limiting to growth (Güler et al., 1996). Lack of sulfolipid did not affect nodule formation induced by *S. meliloti* (Weissenmayer et al., 2000). However, *R. sphaeroides* and *Synechoc-*

occus sp. PCC7942 *sqdB* mutants ceased growth much earlier than the wild type under phosphate-limited growth conditions (Benning et al., 1993; Güler et al., 1996). In the respective wild-type strains, phosphatidylglycerol content decreased while SQDG content increased during phosphate deprivation suggesting that SQDG can substitute for the anionic phospholipid under phosphate-limited conditions. The respective *sqdB* mutants maintained their level of phosphatidylglycerol following the onset of phosphate limitation and therefore depleted in this essential macro nutrient sooner than did wild type. Therefore, in these bacteria sulfolipid appears to be of conditional importance.

Similar observations were made with an SQDG-deficient *sqd2* mutant of Arabidopsis which showed a growth-impairment only after severe phosphate depletion (Yu et al., 2002). Phosphatidylglycerol content remained high in the mutant following phosphate limitation as was observed for the bacteria. However, from the analysis of phosphatidylglycerol-deficient mutants it became clear that this phospholipid has roles beyond that of SQDG in Arabidopsis as *pgp1* mutants impaired in phosphatidylglycerolphosphate synthase activity showed considerable growth and photosynthesis defects depending on the severity of the allele (Xu et al., 2002; Hagio et al., 2002; Babiychuk et al., 2003). When a weak *pgp1-1* allele was combined with an insertion disruption allele of *sqd2* in Arabidopsis, total anionic lipids were reduced to one third of wild-type level leading to a more severe impairment of growth and photosynthesis than in either mutant alone (Yu and Benning, 2003). This result suggests that anionic lipids such as SQDG or phosphatidylglycerol are essential for a functional photosynthetic membrane. This result also shows that under certain circumstances, in this case when the overall anionic lipid content is low, SQDG-deficiency can cause a visible phenotype even under normal growth conditions, unlike the three bacterial examples described above. Indeed, an SQDG-deficient mutant of *Chlamydomonas reinhardtii* (Sato et al., 1995; Minoda et al., 2002; Minoda et al., 2003; Sato et al., 2003a) has shown growth defects and impairment of photosynthesis without additional phosphate-limitation. The stability of photosystem II was impaired, in particular at higher temperatures, and the mutant was more sensitive to inhibitors of photosystem II activity. However, it is difficult to conclude from these results whether the observed defects were specifically due to the lack of SQDG or due to the overall decrease in anionic lipids. Moreover, the exact molecular defect in the UV-induced *hf-2 C. reinhardtii* mutant used in these studies is not known, although the mutant has been backcrossed repeatedly to the wild-type in the more recent studies to minimize the chance of interfering background mutations. When the *SQD1* orthologue of *C. reinhardtii* was specifically disrupted by plasmid insertion, complete loss of SQDG as well as of an acylated derivative of SQDG (Fig. 2) was observed and the mutant was more sensitive to a photosystem II inhibitor confirming previous results observed for the *hf-2* mutant (Riekhof et al., 2003). However, the loss of two lipids in this *sqd1* mutant still left ambiguity with respect to a causal link between a specific SQDG-defect and the observed increased sensitivity against a photosystem II inhibitor.

A cleaner picture emerged in a direct comparison of *sqdB*-disrupted mutants of *Synechococcus* sp. PCC6803 and *Synechococcus* sp. PCC7642 (Aoki et al., 2004), where in the former strain SQDG seemed to be essential for growth under optimal conditions as was observed in the *C. reinhardtii* studies, and in the latter it was dispensable. Therefore, from the limited analysis of SQDG-deficient mutants in different organisms one must conclude that SQDG is required, either conditionally or outright, to maintain a functional photosynthetic membrane, in particularly photosystem II. One function of SQDG seems to be the maintenance of anionic lipid content in the thylakoid membrane under phosphate-limited conditions and possible other conditions yet to be discovered. It seems even possible that photosynthetic complexes have specific binding sites for anionic lipids as was observed in the crystal structure of photosystem I for phosphatidylglycerol (Jordan et al., 2001). Recently, an SQDG molecule was located close to a regulatory lysine residue of cytochrome *f* in the crystal structure of the cytochrome b_6f complex of *C. reinhardtii* (Stroebel et al., 2003), corroborating the specific interaction of this lipid with complexes of the photosynthetic membrane. As more refined and higher resolution structures become available, it is possible that more lipid-binding sites in these

complexes will be discovered which can be tested for functionality by directed mutational analysis.

V. Biotechnological Applications and Production of Sulfolipids

The discoverer of the plant sulfolipid, A.A. Benson, considers it "Nature's Finest Surfactant Molecule" (Benson, 2002). However, few detailed studies of biophysical properties of sulfolipid are available, (Shipley et al., 1973; Webb and Green, 1991; Howard and Prestegard, 1996; Matsumoto et al., 2005a). One reason might be that the plant sulfolipid is not readily available in larger quantities and with high purity to broadly study its applications as a natural detergent molecule. However, beginning with the discovery that SQDG from a marine cyanobacterium potentially has antiviral properties (Gustafson et al., 1989), attention has turned to this lipid class, and an exponentially increasing number of papers has been published documenting the biological effects of natural or synthetic SQDG or its derivatives, e.g. the monoacylated or beta-linked forms, in a number of systems. While it is outside the authors' expertise to critically evaluate the medical or physiological properties of SQDG and its derivatives in the employed model systems, the summary of the different reports in Table 1 might enable the reader to obtain an initial assessment of the biotechnological potential of this compound class.

Chemical synthesis of sulfolipid, in particular its chiral synthesis, is complex and presumably expensive (Gordon and Danishefsky, 1992; Hanashima et al., 2000a; Hanashima et al., 2000b; Hanashima et al., 2001) but has led to the limited availability of stereoisomers and different derivatives of the naturally occurring SQDG used in the studies described above. The alternative production of sulfolipids from natural resources is currently impeded by two factors: 1. While basic protocols for the isolation and purification of SQDG from plant tissues are available (O'Brien and Benson, 1964; Norman et al., 1996), they are not practical for a large scale commercial setting because they are based on halogenated solvents, which require expensive measures to contain their environmental hazards. There is a clear need for the development of an economic and environmentally friendly extraction and purification procedure for SQDG if this natural compound class should become commercially valuable. 2. The yield of SQDG from readily available agricultural materials is relatively low. Attempts at cultivation of cyanobacteria for the optimization of SQDG yield have been made (Archer et al., 1997), but what is needed is an even richer natural resource for SQDG. The availability of sulfolipid biosynthetic genes and our increasing knowledge about sulfur and sulfolipid metabolism of plants might enable us one day to engineer plants that are sufficiently rich in SQDG content to allow a commercial harvest of this valuable natural compound.

Table 1. Summary of reported biological effects of the plant sulfolipid and its derivatives.

Biological effect	Sulfolipid[a]	Citation
Anti AIDS/inhibition of reverse transcriptase	SQDG, SQMG, acylated forms of SQDG	Gustafson et al., 1989; Gordon and Danishefsky, 1992; Reshef et al., 1997; Ohta et al., 1998; Loya et al., 1998
Inhibition of mammalian polymerases	SQDG, SQMG, βSQDG	Mizushina et al., 1998; Ohta et al., 1999; Ohta et al., 2000; Murakami et al., 2002; Mizushina et al., 2003a; Murakami et al., 2003a; Murakami et al., 2003b; Mizushina et al., 2003b; Kuriyama et al., 2005; Matsumoto et al., 2005b
Anti-tumor effects	SQDG, SQMG	Shirahashi et al., 1993; Sahara et al., 1997; Ohta et al., 2001; Quasney et al., 2001; Sahara et al., 2002; Murakami et al., 2004; Hossain et al., 2005; Matsubara et al., 2005; Maeda et al., 2005
Inhibition of Telomerase	SQDG	Eitsuka et al., 2004
Immunosuppressant	βSQDG	Matsumoto et al., 2000; Matsumoto et al., 2004; Shima et al., 2005
Anti-inflammatory effects	SQDG	Vasange et al., 1997; Golik et al., 1997

[a]SQDG, sulfoquinovosyldiacylglycerol (naturally occurring α-anomeric form); βSQDG, synthetic β-anomeric form; SQMG, sulfoquinovosylmonoacylglycerol.

VI. Concluding Remarks

Research towards the biosynthesis and function of the plant sulfolipid has come a long way since the discovery of SQDG approximately 50 years ago. Based on genetic and biochemical analyses we are reasonably certain now that two proteins, SQD1 and SQD2, are crucial for SQDG biosynthesis in plants. The pathway depicted in Fig. 2 summarizes our current knowledge about SQDG biosynthesis in plants and cyanobacteria. What remains uncertain is the involvement and role of ancillary proteins that make the process sufficiently efficient *in vivo*. Although the first SQDG genes were identified in purple bacteria, their exact roles are less clear and it seems possible that sulfolipid is assembled differently in alpha-proteobacteria than in plants. The UDP-SQ synthase, on the other hand, is well conserved in many diverse organisms. This raises the question whether sulfoquinovose can be a moiety of biomolecules other than SQDG as has been reported for a bacterial glycoprotein (Zähringer et al., 2000).

The function of sulfolipid *in vivo* can be traced to its anionic non-phosphorous properties, which apparently make this lipid a highly suitable component of photosynthetic membranes. As new high-resolution structures of photosynthetic and other biosynthetic membrane complexes in the chloroplast become available, it seems likely that new specific binding sites for SQDG in these complexes might be discovered. These anticipated findings will enable more direct approaches to test specific roles of SQDG.

With the availability of the genes required for the biosynthesis of SQDG, biotechnological approaches towards the production of sulfolipid can now be devised. The number of publications reporting potentially beneficial health effects and applications for SQDG and its derivates has increased exponentially during the past three years. It seems likely that as SQDG becomes more widely available, necessary follow up studies will become more facile.

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