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Biosynthesis of Sulfoquinovosyldiacylglycerol in Higher Plants

THE INCORPORATION OF $^{35}SO_4$ BY INTACT CHLOROPLASTS IN DARKNESS

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ABSTRACT

Intact spinach chloroplasts incorporated $^{35}SO_4^{2-}$ into sulfoquinovosyldiacylglycerol in the dark at rates equivalent to those previously reported for illuminated chloroplasts provided that either ATP itself or an ATP-generating system was added. No additional reductant was necessary for SQDG synthesis by chloroplasts. The optimal concentration of ATP was between 2 and 3 millimolar. Rates of synthesis up to 2.6 nanomoles per milligram chlorophyll per hour were observed. UTP, GTP, and CTP could not substitute for ATP. Incubation of UTP with ATP (1:1) stimulated synthesis of sulfoquinovosyldiacylglycerol. No additional stimulation of the reaction was observed upon addition of other nucleoside triphosphates with ATP. For the generation of ATP in the chloroplast, addition of dihydroxyacetone phosphate alone did not promote synthesis of sulfoquinovosyldiacylglycerol, but in combination with inorganic phosphate and oxaloacetate, rates of synthesis up to 3.2 nanomoles per milligram chlorophyll per hour were observed. Dark synthesis was optimal in the presence of 2 millimolar dihydroxyacetone phosphate, 2 millimolar oxaloacetate, and 1 millimolar KH_2PO_4.

Previous research has shown the autonomy of the chloroplast for the synthesis of SQDG[2] from $^{35}SO_4^{2-}$ (15, 20). A light requirement for the synthesis of SQDG from sulfate has been indicated (1, 16, 20). Endogenous levels of DG in isolated spinach chloroplasts are sufficient to support nmol/mg Chl·h rates of synthesis (8, 20). Sulfate uptake into the chloroplast does not require light (22). Hence the light requirement must be restricted to incorporation of sulfate into the headgroup of SQDG. The role of light in this process has not been demonstrated.

Light may serve to generate ATP, generate reductant, either ferredoxin and/or NADPH, or to activate enzymes involved in SQDG synthesis. There could be more than one role of light in the synthesis of SQDG. Abraham and Bachhawat (1) have suggested that in *Euglena* chloroplast preparations, light provides only the ATP necessary for synthesis of an activated form of sulfate, APS or PAPS, which they postulated is then further converted to SQDG by condensation with a reduced sugar nucleotide or glycosylated lipid acceptor. Although APS is the donor for the reductive steps of sulfur metabolism in higher plants while in some cyanobacteria PAPS is the sulfate donor (25, 26), either APS or PAPS could be the donor for the condensation reaction proposed. In this case, addition of ATP alone should alleviate the light requirement for SQDG biosynthesis provided the endogenous sugar acceptor compound is present. The prerequisite for reduction of the sugar moiety of the acceptor compound at an earlier time is then implied. Speculation that a more reduced form of sulfur may be involved in synthesis of SQDG led some workers to propose the involvement of a 'sulfoglycolytic' or 'cysteic acid' pathway (6, 16, 28). Contrary to this proposal is the demonstration that cysteic acid, a key intermediate in the proposed pathway, is not directly involved in synthesis of SQDG in higher plants (23) or in isolated chloroplasts (20). Although the sulfoglycolytic pathway itself appears unlikely, the involvement of other reduced forms of sulfur cannot be excluded. If a more reduced form of sulfur is required, addition of ATP alone should not alleviate the light requirement since sulfate reduction is light-dependent, requiring a reductant such as ferredoxin or DTT *in vitro* (25, 26). Synthesis of cysteine takes place in dark incubated chloroplasts upon addition of DTT and ATP but not in the presence of ATP alone (26). The addition of ATP in conjunction with a reduced thiol such as DTT should then alleviate the light requirement for SQDG synthesis. If light activation of enzymes is involved, addition of ATP alone will not enable incorporation of sulfate into SQDG. Addition of either a reduced dithiol compound, reduced thioredoxin, or reduced ferredoxin may also be necessary to activate sulfhydryl-containing enzymes and restore the biosynthetic activity (4). Magnesium fluxes and pH changes may also be involved in light activation of the pathway.

The current work investigates the role of light in SQDG biosynthesis, distinguishing between one or more of these possibilities by examining synthesis of SQDG from $^{35}SO_4^{2-}$ in chloroplasts incubated in the dark. This study also determined which portions of the sulfur metabolic pathway are not involved in the biosynthesis of SQDG.

MATERIALS AND METHODS

Plants were grown under 11 h of light and 13 h of darkness in a growth chamber with a nighttime temperature of 19°C and a daytime temperature of 22°C. Rapidly expanding leaves from 4 to 6 week old spinach plants were harvested for chloroplast isolation. Intact chloroplasts were recovered from Percoll gradients, pelleted, then resuspended in 0.3 M sorbitol, 0.5 mM Tris at pH 7.5 as described previously (20). Chloroplasts were kept in darkness as much as possible during this procedure.

Chloroplasts were incubated in 1.0 ml of a basic reaction medium containing 0.3 M sorbitol, 2 mM $MgCl_2$, 2 mM ATP, 0.5 mM DTT, 33 mM Tricine at pH 7.9, and 100 μM $^{35}SO_4^{2-}$ (0.25 μCi/nmol) unless otherwise noted in the legends of the tables and figures. Reported data represent the average of duplicate determinations from experiments repeated two or more times. Incubation was for 30 min at 25°C in complete darkness. When

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[2] Abbreviations: SQDG, sulfoquinovosyldiacylglycerol; APS, adenosine-5′-phosphosulfate; DHAP, dihydroxyacetone phosphate; DTT, reduced dithiothreitol; OAA, oxaloacetate; PAPS, adenosine 3′-phosphate 5′-phosphosulfate; 3-PGA, 3-phosphoglyceric acid.

comparisons between light and dark incubations were made, dark incubated chloroplasts were placed in covered test tubes next to test tubes in white light (300 $\mu E/m^2 \cdot s$) in a photosynthetic reaction bath. Reactions were stopped, lipids extracted and prepared for analysis as described previously (19). After evaporation under N_2, the lipids were redissolved in chloroform, and aliquots were removed for scintillation counting. The remainder was chromatographed in the solvent chloroform:acetone: methanol:acetic acid:water(50:20:10:15:5,v/v). The distribution of radioactivity on the TLC plate was determined with a Berthold linear analyzer. In addition to SQDG, other sulfolipids were synthesized in all incubations. Since we have not identified these compounds the incorporation into them is not reported in this paper. Joyard *et al.* (19) have indicated that these other sulfolipids are forms of elemental sulfur.

RESULTS

ATP was the only nucleoside triphosphate tested which promoted dark synthesis of SQDG (Table I). Estimated rates of ATP transport, between 2 μmol/mg Chl·h (18) and 8 μmol/mg Chl·h (17), are adequate for these rates of dark synthesis. Very low amounts of SQDG were synthesized when no ATP was provided to chloroplasts incubated in the dark. This incorporation was believed due to residual levels of endogenous ATP remaining in chloroplasts kept in the dark. Other nucleoside triphosphate compounds did not substitute for ATP. Rates of transport for CTP (<30 nmol/mg Chl·h), UTP and GTP (each < 12 nmol/mg Chl·h) are known to be lower than those for ATP (18). However, rates of synthesis of SQDG were higher upon simultaneous incubation with ATP and UTP than with ATP alone. These results agree with the previously reported increased SQDG synthesis observed in light incubations upon the addition of UTP (20). CTP and GTP added alone and simultaneously with ATP had no effect on SQDG synthesis. Addition of the compounds shown in Table I did not alter the pH of the buffered solution. The optimal pH for SQDG synthesis in the dark is 7.0, identical to that previously reported (20) for SQDG synthesis in the light. Incorporation of sulfate into SQDG in the dark is linear at least for 30 min and rates are comparable to those of light incubated chloroplasts.

The addition of other adenylated compounds was also investigated, as well as the addition of Pi and PPi, to determine if compounds which might influence the state of phosphorylation in light- or dark-incubated chloroplasts affect the synthesis of SQDG (Table II). None of the compounds tested other than ATP promoted synthesis of SQDG in the dark. In the light, only ATP alone or PPi alone increased the rate of synthesis of SQDG. Simultaneous addition of ADP and Pi or of AMP and PPi failed to stimulate synthesis of SQDG in the dark and even inhibited

Table I. *Effect of Nucleotides on the Incorporation of $^{35}SO_4^{2-}$ into SQDG in the Dark*

Reaction mixtures were as described under "Materials and Methods." Each tube contained chloroplasts equivalent to 57 μg Chl.

Additions	SQDG Formation
	nmol/mg Chl·h
None	0.25
2 mM ATP	0.93
2 mM UTP	0.27
2 mM GTP	0.19
2 mM CTP	0.22
1 mM ATP	0.67
1 mM ATP + 1 mM UTP	1.10
1 mM ATP + 1 mM GTP	0.67
1 mM ATP + 1 mM CTP	0.55

Table II. *Effect of Adenosine Nucleotides on the Incorporation of $^{35}SO_4^{2-}$ into SQDG*

Reaction mixtures were as described under "Materials and Methods." Each tube contained chloroplasts equivalent to 66 μg Chl.

Additions	SQDG Formation	
	Dark	Light
	nmol/mg Chl·h	
None	0.36	1.11
2 mM ATP	1.34	1.83
2 mM ADP	0.55	0.70
2 mM Pi	0.14	0.26
2 mM ADP + 2 mM Pi	0.34	0.74
2 mM AMP	0.23	0.48
2 mM PPi	0.20	1.55
2 mM AMP + 2 mM PPi	0.15	0.64

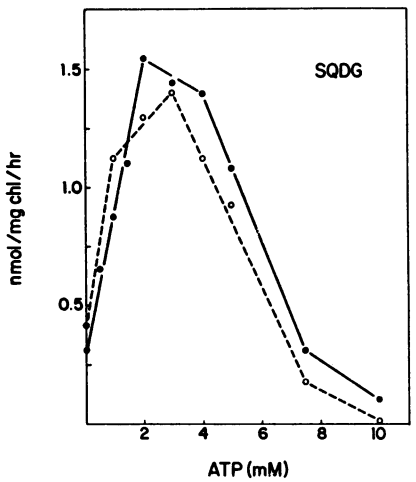

FIG. 1. Effect of increasing concentrations of ATP on the synthesis of SQDG. The reaction mixture was as indicated under "Materials and Methods" except the magnesium concentration was either 1.5 mM (●) or 4 mM (○). Chloroplasts equivalent to 77 μg Chl were added.

synthesis in the light. ADP and AMP could not substitute for ATP, as reported in a preliminary communication (21). ADP slightly increased rates of synthesis of SQDG in the dark. The presence of AMP inhibited SQDG synthesis in the light.

The incorporation of sulfate into SQDG as a function of ATP concentration was tested at two different concentrations of magnesium ion (Fig. 1), to determine the optimal ATP concentration and to establish if there was a requirement for an ATP-magnesium ion complex. Many reactions which require ATP utilize an ATP:magnesium ion complex, including the ATP sulfurylase and protein synthesis (7, 9, 10, 13, 14, 27). The optimal concentration of ATP for SQDG synthesis in the dark lies between 2 and 3 mM. Although a specific stoichiometry was not evident, magnesium ions slightly influenced the concentration of nucleotide required. At a magnesium ion concentration of 1.5 mM, 2 mM ATP is the optimal concentration for synthesis of SQDG in the dark. In the presence of 4 mM $MgCl_2$, the optimal concentration shifts to 3 mM ATP. Manganese ions could partially substitute for magnesium ions, with rates of synthesis less than half those observed in the presence of magnesium ions. The concentration of nucleotide and magnesium ions added to the reaction mixture in other experiments were each maintained at 2 mM since others have reported a loss of magnesium ions by chloroplasts when nucleotides or other chelators in excess of equimolar concentrations are added (7).

The interaction of ATP and magnesium ions in these reactions was further examined by determining sulfate incorporation into

SQDG as a function of magnesium ion concentration (Fig. 2). The incorporation of sulfate into SQDG increased with increasing concentrations of magnesium ion. A concentration of magnesium ion greater than 5 mM is necessary for optimal incorporation of sulfate into SQDG. Calcium ions could not substitute for magnesium ions. Manganese ions could substitute for the magnesium ions at the lower concentrations but at 10 mM rates of sulfate incorporation into SQDG measured in the presence of manganese ions were only 70% of those using magnesium ions. The effect of magnesium and manganese ions were additive for SQDG synthesis. In all other experiments, the concentration of magnesium ion was 2 mM to avoid possible effects of excess magnesium ion on other related chloroplastic properties including protein synthesis (7) and light-dark regulation (4).

To ensure ATP transport was not limiting, ATP was provided to chloroplasts either by the addition of ATP directly or by the addition of an ATP-generating system. As depicted in Figure 3, an ATP-generating system known as the triose phosphate shuttle, first utilized by Werdan *et al.* (31) for CO_2 fixation in the dark, can be a more efficient mechanism of providing ATP inside the chloroplast. Rates of ATP synthesis utilizing this mechanism of between 60 and 90 μmol/mg Chl·h are reported to occur (17). With this shuttle mechanism, DHAP generates ATP and NADPH via its conversion to 3-PGA inside the chloroplast. Pi is added to maintain chloroplastic internal Pi levels, the external Pi exchanging for the 3-PGA formed in the reaction and the internal Pi exchanging for DHAP via the phosphate translocator. The shuttle also requires OAA to utilize NADPH in its conversion to malate and thus regenerate NADP necessary for the conversion of DHAP to 3-PGA. For dark CO_2 fixation, the presence of all components was required (31).

Regardless of whether the ATP was supplied directly or indirectly by the shuttle mechanism, SQDG was synthesized in the dark at fairly comparable rates (Table III). In order for SQDG synthesis to proceed in the dark utilizing the shuttle mechanism, DHAP and OAA must both be present simultaneously. When Pi was present in addition to DHAP and OAA, rates of SQDG synthesis were greater than those for DHAP with OAA and for the control in which none of these compounds was present. Except for DHAP, each shuttle component added separately did not increase the incorporation of sulfate into SQDG over the control. When DHAP was present, incorporation doubled over that of the control but was still not equal to the incorporation when ATP alone was added. Addition of Pi with either OAA or DHAP alone diminished the rates of synthesis for SQDG. Addition of Pi with ATP stimulated SQDG synthesis in agreement with previously reported results (20). Simultaneous addition of DHAP or OAA with ATP caused a decrease in the incorporation of sulfate into SQDG.

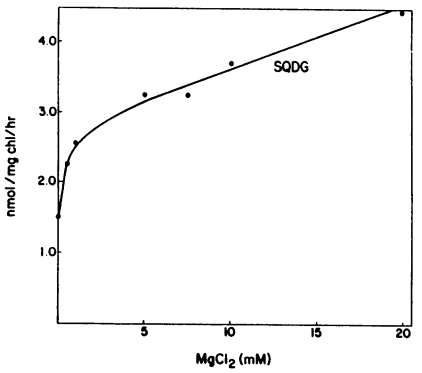

FIG. 2. Effect of increasing concentrations of magnesium ions on the synthesis of SQDG. The reaction mixture was as indicated under "Materials and Methods" with an ATP concentration of 2 mM. Chloroplasts equivalent to 74 μg Chl were added.

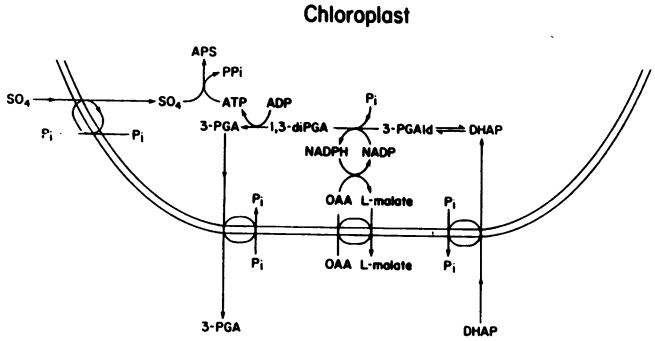

FIG. 3. Triose phosphate shuttle mechanism for generating ATP inside the chloroplasts.

DHAP and OAA together, maintained in a molar ratio of 1:1, were tested at different combined concentrations to maximize the rates of synthesis of SQDG. The data depicted in Figure 4A demonstrate a sharp optimum at 1 mM combined concentration for DHAP and OAA (0.5 mM DHAP plus 0.5 mM OAA). No Pi was added to the incubation medium, so that the transport of DHAP was dependent only on the concentrations of phosphate and triose phophate compounds in the chloroplast at the time of isolation plus any 3-PGA derived from the added DHAP during the 30 min incubation. To ensure that transport of 3-PGA out of the chloroplasts and DHAP transport into the chloroplasts was not limiting, the same concentrations of DHAP and OAA were tested in the presence of added Pi, in a ratio of 2:1 for Pi to DHAP for each concentration of DHAP. In Figure 4B, the overall rates of SQDG synthesis were greatly enhanced by the presence of Pi in addition to the DHAP and OAA. The optimum was shifted to a higher combined concentration of DHAP and OAA of around 4 mM (2 mM DHAP plus 2 mM OAA with 1 mMPi).

The necessity of reductant to generate reduced sulfur compounds or reduce enzyme sulfhydryl groups was examined by contrasting dark synthesis in the presence and absence of a thiol with that of light synthesis of SQDG (Table IV). SQDG was synthesized in the dark in the presence of DTT when ATP was supplied directly or indirectly via the shuttle mechanism at rates somewhat less than, but in the same range as synthesis in the light. Synthesis of SQDG in the light was also stimulated by providing chloroplasts with ATP indirectly via the shuttle mechanism or by the addition of ATP directly, in agreement with Table 1 and as previously reported (20, 21). A preliminary

Table III. *Effect of DHAP Shuttle on the Incorporation of $^{35}SO_4^{2-}$ into SQDG in the Dark*

Each tube contained chloroplasts equivalent to 58 μg Chl. In addition to the standard reaction mixture, 2 mM ATP, 4 mM Pi, 2 mM DHAP, and 2 mM OAA were added as indicated.

Conditions	SQDG Formation
	nmol/mg Chl·h
No additions	0.09
+ATP	0.98
+ATP, OAA	0.78
+ATP, DHAP	0.70
+ATP, Pi	1.13
+DHAP	0.23
+OAA	0.11
+Pi	0.10
+Pi, DHAP	0.30
+Pi, OAA	0.10
+DHAP, OAA	0.87
+Pi, DHAP, OAA	1.12

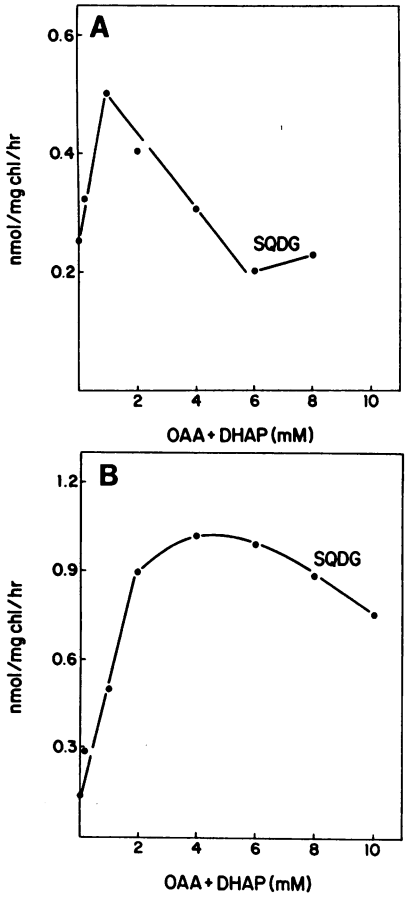

FIG. 4. Effect of increasing concentrations of DHAP and OAA on the synthesis of SQDG in the dark. A, In the absence of Pi. DHAP and OAA were added in a ratio of 1:1. The combined concentration of DHAP and OAA is indicated. Chloroplasts equivalent to 88 μg Chl were added. B, In the presence of Pi. DHAP and OAA were added in a ratio of 1:1. Pi was added in a ratio of 2:1 Pi to DHAP. The combined concentration of DHAP and OAA is indicated. Chloroplasts equivalent to 165 μg Chl were added.

Table IV. *Effects of DTT and Light on the Incorporation of $^{35}SO_4^{2-}$ into SQDG*

Each tube contained chloroplasts equivalent to 102 μg Chl. DTT, ATP, DHAP, OAA, Pi were left out of the standard reaction mixture except where indicated. DHAP and OAA were added at a concentration of 1 mM each. Pi was added at a concentration of 2 mM.

Conditions	−DTT	+DTT
	nmol/mg Chl·h	
Dark incubation		
No ATP	0.2	0.3
+2 mM ATP	1.9	2.7
+DHAP, OAA, Pi	2.0	3.3
Light incubation		
No ATP	2.1	2.3
+2 mM ATP	2.6	4.1
+DHAP, OAA, Pi	5.1	7.8

communication of these findings, showed that in the presence of DTT and when ATP was provided directly or indirectly via the shuttle mechanism, the synthesis of SQDG in the dark was in the same range as that in the light (21). In the absence of DTT, the rates were also in the same range in light and dark when the chloroplasts were provided with ATP, directly or indirectly via the shuttle mechanism. Synthesis of SQDG was greater when the shuttle mechanism provided ATP to the chloroplasts in both the light and dark incubations. These data further demonstrate that a thiol was not absolutely necessary for synthesis of SQDG since SQDG was synthesized in the dark in the absence of DTT, when ATP was added directly so as to not alter NADPH levels. Synthesis of SQDG was stimulated by the addition of DTT in both the light and dark. Stimulation by DTT was greater when the shuttle mechanism was utilized to supply ATP. The possibility of light regulation of the incorporation of sulfate into sulfolipids was further examined by comparing the effects of dithiols, DTT and DTE, and a monothiol, β-mercaptoethanol. No absolute requirements for reduction by any thiol was evident and only DTT stimulated SQDG synthesis. DTE and β-mercaptoethanol had no effect, indicating light activation of sulfhydryl-containing enzymes involved in synthesis of SQDG is unlikely. However, DTT was routinely included in the incubation medium in all other experiments since DTT did stimulate synthesis of SQDG when the shuttle mechanism was employed.

DISCUSSION

The results presented here demonstrate addition of ATP alleviates the light requirement for the incorporation of sulfate into SQDG in spinach chloroplasts. Thus, the lack of sulfate incorporation into SQDG in the dark (1, 16, 20) is attributed to low levels of ATP in chloroplasts isolated and incubated in the dark. The light requirement for sulfate incorporation into SQDG is attributed primarily to a requirement for ATP to synthesize APS since ATP alone alleviates the light requirement. No additional reductant over that which may be available in the dark is necessary, nor is light activation of enzymes or pathways essential for sulfate incorporation into SQDG to occur in the dark. This does not exclude the requirement of reductant or light activation for synthesis of the acceptor compound which is sulfonated, but merely implies a sufficient pool of precursors exists in the dark for the incorporation of sulfate into SQDG to occur when only ATP is supplied.

ATP was the only nucleotide tested which alleviated this light requirement: UTP, CTP, GTP, AMP added alone were all ineffective. The slight stimulation of SQDG synthesis observed upon the addition of ADP in the dark, as previously reported (20), can be attributed to the adenylate kinase of the chloroplast which converts 2 mol of ADP to 1 mol each of ATP and AMP at rates of up to 27 μmol/mg Chl·h (5, 30). In the light this conversion is not favored because levels of ATP produced during photosynthesis change the equilibrium of the reaction. However, AMP can deplete ATP levels in both the light and dark as it is converted to ADP via the reverse reaction of the adenylate kinase. Rates of the reverse reaction (3 μmol/mg Chl·h) are adequate to account for the observed diminished rates of synthesis of SQDG in the presence of AMP. PPi is an inhibitor of the phosphate translocator (10) and can exchange with internal adenylates to deplete the stromal adenylate pools (24, 29) thus explaining the observed inhibition of SQDG synthesis by PPi in the dark. PPi can also inhibit the loss of Pi from the chloroplast, normally required for ATP synthesis in the light (29). PPi can inhibit as well the loss of glyceraldehyde-3-phosphate, thus stimulating the rate of CO_2 fixation (2). This overall effect of PPi in preventing the loss of Pi and triose phosphates may explain the observed stimulation of SQDG synthesis in the light when PPi was present.

The stimulation of SQDG synthesis by the simultaneous addition of ATP and UTP to dark incubated chloroplasts agrees with the stimulation by UTP of SQDG synthesis in light incubated chloroplasts (20). No absolute requirement for UTP has been observed. However, UTP may have a role in the final condensation of the sugar group with a diacylglycerol moiety, for example in the formation of a nucleotide sugar such as UDP-SQ in reactions analogous to those of galactolipid synthesis (20).

This is supported by the preliminary finding by Shibuya *et al.* (28) of a sulfur-containing nucleoside sugar after incubation of *Chlorella* with $^{35}SO_4^{2-}$.

As far as the diacylglycerol moiety of the synthesized SQDG is concerned, we conclude that the small amounts of diacylglycerol present in isolated chloroplasts are sufficient to support the incorporation of sulfate we have observed. Joyard *et al.* (19) have shown that the synthesis of SQDG by isolated chloroplasts can be coupled to the *de novo* synthesis of diacylglycerol.

Optimal rates of SQDG synthesis when ATP is provided via the shuttle are in the same range as when ATP is provided directly. In some instances, the shuttle provided even higher rates of SQDG synthesis than ATP alone (Table IV). Transport of DHAP into the chloroplast occurs at rates up to 300 μmol/mg Chl·h, but conversion to 3-PGA and the production of ATP occurs at lesser rates of 60 to 90 μmol/mg Chl·h (17). Thus, the shuttle should be more efficient at providing ATP (60 μmol/mg Chl·h) than the addition of ATP exogenously when ATP must be taken up by the chloroplast (2 μmol/mg Chl·h) (17, 18). But optimal rates of synthesis using the shuttle require the presence of Pi (Fig. 3) which, at 2 mM inhibits synthesis of SQDG (Tables 2 and 3). Previously, 0.2 mM Pi was shown to have little or no effect on rates of synthesis of SQDG (20). Inhibition of synthesis at the greater concentration of Pi is expected since phosphate is reported to be a competetive inhibitor of sulfate uptake in the chloroplast (22). These effects of Pi, although lessened when added with OAA and DHAP, must be kept in mind as an explanation of why the shuttle is often no more efficient than is the direct addition of ATP for synthesis of SQDG.

The optimal ATP concentration for SQDG synthesis in the dark, between 2 and 3 mM, is only slightly influenced by the magnesium ion concentration although a 1:1 requirement for ATP and Mg^{2+} was not indicated. These results agree with others which show an interaction of magnesium ions with ATP can influence reactions within the chloroplast, such as protein synthesis (7, 9, 12–14) and protochlorophyllide formation (13). The ATP sulfurylase, the rate-limiting step in sulfur assimilation, utilizes a Mg-ATP complex (27). The concentration of nucleotide in excess of the magnesium ion causes loss of magnesium ion from the chloroplast (7). This could explain the decreased incorporation of sulfate into SQDG when the concentration of ATP exceeded that of the magnesium ion, since SQDG synthesis is optimal at magnesium ion concentrations above 5 mM (Fig. 2). Magnesium ions may not necessarily have a direct effect (12). Light-induced pH and magnesium ion concentration fluxes modulate the enzymic activity of enzymes such as fructose 1,6-bisphosphatase, ribulose bisphosphate carboxylase, and phosphoribulokinase which require magnesium ions and an alkaline pH (4).

The addition of a thiol had little effect on incorporation of sulfate into SQDG, indicating light activation of enzymes is unlikely. The slight stimulation of activity observed by DTT is attributed to enzyme stabilization and not activation. The lack of effect of other dithiols such as DTE and the monothiol, β-mercaptoethanol, on sulfate incorporation into SQDG support this conclusion. The greater effect of DTT on the stimulation of sulfate incorporation into SQDG when the shuttle mechanism was utilized instead of supplying ATP directly is attributed to the transport of triose phosphate compounds. The phosphate translocator, which transports DHAP and Pi, is known to contain sulfhydryl groups and to be affected by the pH, charge of species transported, and the reduction state (11, 18, 31).

Synthesis of SQDG in the dark upon the addition of ATP does not require a thiol for sulfate metabolism since substantial rates of synthesis are observed in the absence of DTT. When chloroplasts are incubated with ATP in the dark in the absence of reduced thiol or ferredoxin, sulfate can be converted to APS and to PAPS but only a limited amount of bound or free sulfite can be formed (27) since the APS sulfotransferase is regulated by the light-induced pH changes, AMP levels, and sulfhydryl groups (3, 4, 26). Dark rates of synthesis of APS (50 nmol/mg Chl·h), PAPS (8 nmol/mg Chl·h), and bound sulfite (40 nmol/mg Chl·h) (27) are adequate to account for the rates of synthesis of SQDG in the dark reported here. However, conversion to sulfide and to cysteine cannot occur without a reduced thiol or without light to reduce ferredoxin (26, 27). Generation of sulfite is limited to these low rates without a reduced thiol or ferredoxin to regenerate the carrier protein involved in sulfate reduction to sulfite. Thus, a more reduced form of sulfur than sulfite cannot be involved in synthesis of the headgroup of SQDG. Therefore, APS, PAPS, or bound sulfite donate the sulfur moiety directly to a carbon-compound in formation of the headgroup of SQDG. The role of light, then, is to provide ATP necessary for synthesis of APS, and possibly PAPS, for SQDG synthesis.

In summary, the requirement of chloroplasts for illumination in order to incorporate sulfate into SQDG, is consistent with the generation of ATP and the light-induced increase in the stroma concentration of magnesium ions. The light requirement for the operation of the pathway does not depend on activation of sulfhydryl-containing enzymes, light-induced alkalinization of the stroma, or generation of reductant.

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