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Sulfate and Sulfite Translocation via the Phosphate Translocator of the Inner Envelope Membrane of Chloroplasts

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Abstract. The permeability of the inner envelope membranes of spinach (*Spinacia oleracea*) chloroplasts to sulfite and sulfate was investigated in vitro, using the technique of silicone oil centrifugal filtration. The results show that there is a permeability towards both ions, resulting in rates of uptake of about 1.0 (SO_3^{2-}) and 0.7 (SO_4^{2-}) μmol mg chlorophyll^{-1} h^{-1} respectively (external concentration 2 mmol l^{-1}). The rates depend on the external concentration of the anions. Anion exchange experiments with ^{35}S-preloaded chloroplasts indicate that sulfite and sulfate are exchanged for inorganic phosphate, phosphoglyceric acid, and dihydroxyacetone phosphate with rates up to 14 nmol mg chlorophyll^{-1} min^{-1}. There is no exchange for glucose-6-phosphate and malate. Because of the similarities to the transport of inorganic phosphate and triose phosphates the results give evidence that the phosphate translocator of the inner envelope membrane of chloroplasts is also involved in sulfite and sulfate transport – at least in part.

Key words: Chloroplast envelope – Ion transport – Sulfate – Sulfite.

Introduction

It is generally accepted that assimilatory sulfate reduction takes place in the chloroplasts (c.f. Schiff and Hodson, 1973). There is accumulating evidence that at least the initiating steps (sulfate activation and formation of bound sulfite) occur on the thylakoids (Schwenn et al., 1976; Ziegler, 1977). This implies that the envelope membranes of chloroplasts are permeable to sulfate and sulfite.

Abbreviations: DHAP = dihydroxyacetone phosphate; PGA = 3-phosphoglycerate; P_i = inorganic phosphate; S_i = sulfite, sulfate

A similar conclusion may be drawn from experiments done by Baldry et al. (1968). They showed that in photosynthetic phosphorylation of intact chloroplasts sulfate competed with phosphate. This requires a preceding uptake of this ion into the stroma space of intact chloroplasts. However, direct demonstration of the uptake of either sulfate or sulfite has not yet been achieved, nor has the detailed mechanism of uptake been worked out.

In this study we investigated the permeability properties of the inner envelope membranes of spinach chloroplasts – that were shown to be transport limiting (see Heldt, 1976) – to sulfate and sulfite. The possible involvement of translocators, already known for other compounds (Heldt and Rapley, 1970), in SO_3^{2-}/SO_4^{2-} transport was examined.

Materials and Methods

Plastid Isolation

Chloroplasts were isolated from leaves of spinach plants, that were grown for 4–6 weeks as described previously (Libera and Ziegler, 1975). The isolation was carried out according to Jensen and Bassham (1966); for phosphate free medium, K_2HPO_4 and $Na_4P_2O_7$ respectively, were omitted.

Uptake and Exchange of Anions

Uptake of labelled anions into the intraplastidic space was measured by a silicon oil centrifugal filtration method as used by Heldt and Rapley (1970). Similarly, the amount of [^{3}H]H_2O and nonpermeating [U-^{14}C]sorbitol, taken up by the controls, was assessed in order to calculate plastid volumes and to correct for unspecific permeation into the inner membrane spaces (see Heldt and Sauer, 1971).

Studies of back exchange were carried out as described previously (Hampp and Wellburn, 1976).

Chlorophyll concentration was determined according to Arnon (1949). The chemicals used were: sodium [^{35}S]sulfite, sodium

[^{35}S]sulfate from Amersham Buchler; silicone oil (TypeAR 150) from Wacker Chemie, Burghausen (W. Germany); all other chemicals were analytical grade from Merck (Darmstadt, W. Germany).

Results

Uptake of $^{35}SO_3^{2-}$ and $^{35}SO_4^{2-}$ into the Sorbitol-Impermeable Space of Chloroplasts

In Figure 1 the increase with time of the concentrations of sulfite and sulfate within the chloroplast stroma is given, the external concentrations being 2 mmol l^{-1} for each. Shortly after application, the rates of uptake for both ions are linear up to 10 s, resulting in amounts of about 1.0 (SO_3^{2-}) and 0,7 (SO_4^{2-}) µmol mg chlorophyll^{-1} h^{-1} respectively. The rate of increase becomes lower for incubation times exceeding 10 s. The rates of uptake of sulfite as well as of sulfate depend on the extraplastidic concentrations (Fig. 2); but there was no substrate saturation in the range of molarities investigated. However, the intraplastidic concentration of the respective ion was found to be much lower than the applied external one, reaching values of about 25% maximum.

In contrast, the uptake of phosphate showed an obvious substrate saturation (Fig. 3), which is similar to that obtained by Flügge and Heldt (see Heldt, 1976).

When the uptake of $^{35}SO_4^{2-}$, into the sorbitol-impermeable space of the plastids was measured in the presence of P_i or PGA, there was no competition (Fig. 2). This was shown by the fact that the uptake of sulfate was twice the amount of that found without the addition of P_i or PGA.

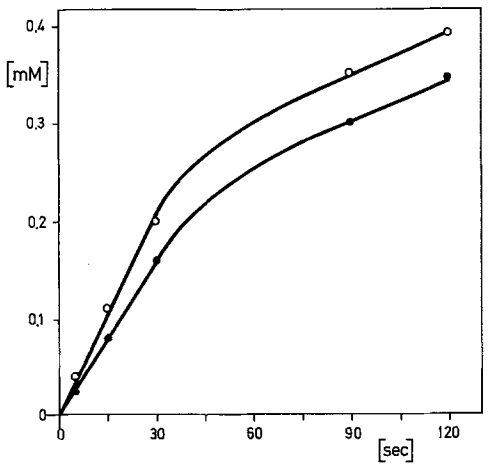

Fig. 1. Transport of sulfate (●—●) and sulfite (○–○) into the sorbitol impermeable space of spinach chloroplasts. SO_4^{2-}, SO_3^{2-} concentration in the medium: 2 mmol l^{-1}; the values are given as nmol/µl sorbitol impermeable space (≙ mmol l^{-1})

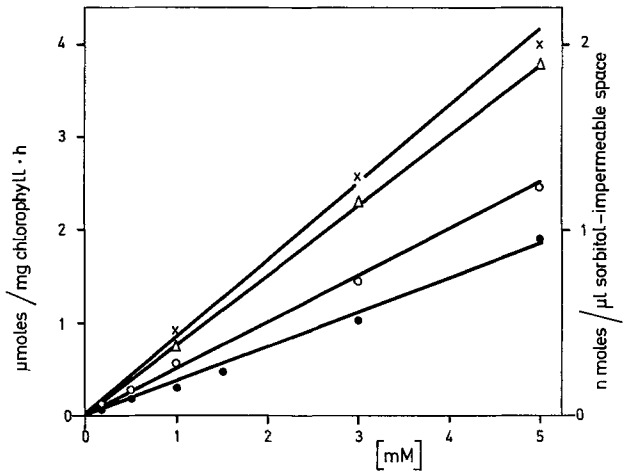

Fig. 2. Rates of uptake of sulfate (●—●) and sulfite (○—○) into the sorbitol impermeable space of spinach chloroplasts in relation to the respective concentration in the medium. In addition, rates are given for sulfate in the presence of 3-phosphoglyceric acid (5 mmol l^{-1}) (△—△) and inorganic phosphate (5 mmol l^{-1}) (×—×)

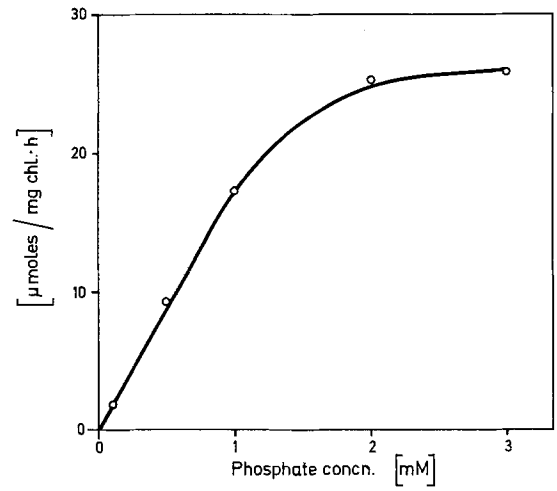

Fig. 3. Rates of uptake of inorganic phosphate into the sorbitol impermeable space of spinach chloroplasts in relation to the phosphate concentration in the medium

Rates of Exchange of $^{35}SO_3^{2-}$ and $^{35}SO_4^{2-}$ for other Metabolites

In order to determine whether transport of sulfite and sulfate occurs in exchange for other metabolites, chloroplasts were preloaded with $^{35}SO_3^{2-}$ and $^{35}SO_4^{2-}$ and subsequently washed. Chloroplasts treated in this way were incubated with various unlabelled metabolites. After an incubation period of 1 min, the activity of ^{35}S in the external medium was measured. As shown in Table 1, chloroplasts preloaded with sulfite or sulfate released the same amounts of ^{35}S after application of P_i, PGA, or DHAP as after the applica-

Table 1. Release of $[^{35}S]SO_3^{2-}$ and $[^{35}S]SO_4^{2-}$ from spinach chloroplasts on addition of various metabolites

Metabolite added (1 mmol l^{-1})	Amount of label released (nmol mg chlorophyll^{-1} min^{-1}; 0° C)[a]	
	$[^{35}S]SO_3^{2-}$	$[^{35}S]SO_4^{2-}$
Sulfate	12.8	10.4
Sulfite	13.6	10.4
Inorganic phosphate	12.0	9.6
3-Phosphoglycerate	14.4	14.4
Dihydroxyacetone phosphate	12.0	9.6
Malate	1.8	–
Glucose-6-phosphate	0.8	–

[a] Values are corrected for unspecific loss of radioactivity

tion of the respective anion. In similarity to the slightly higher rates of uptake of sulfite as compared to sulfate, there was an increased rate of exchange with sulfite.

However, when malate or glucose-6-phosphate was applied to the incubation medium, the amount of ^{35}S released was much lower. This indicates a low affinity of exchange of these metabolites for sulfite or sulfate, localized within the chloroplast stroma.

Discussion

The results presented here seem to show that the envelope membranes of spinach chloroplasts possess a certain permeability towards sulfite and sulfate ions. The calculated rates of uptake are rather low and do not show substrate saturation kinetics in the range of concentrations investigated (up to 20 mmol l^{-1}). This linear dependency on the concentration in the medium could be due to a low affinity to translocating systems or to an uptake via diffusion across the inner envelope membrane. Thus the permeabilities of these anions are quite different compared to the phosphate. In the case of P_i, there is a linear increase of uptake only with very low phosphate concentrations in the medium (below 0.2 mmol l^{-1}). The rates of uptake for P_i (48 µmol mg chlorophyll^{-1} h^{-1}; Heldt, 1976) and sulfite/sulfate (1.0/0.7) show that the envelope permeabilities for the latter ones are much lower. In addition, the maximum internal concentrations of S_i are only 25% of those in the surrounding medium, while for P_i, at low external concentrations, there is an enrichment in the intraplastidic space of up to 8 times (Heldt, 1976).

The accumulation of P_i was thought to be a consequence of a counter exchange for plastidic anions, so that by a strict coupling of influx and eflux each P_i taken up causes another P_i (PGA, DHAP) of plastidic origin to be translocated from the chloroplast stroma to the extraplastidic space.

As shown by the exchange experiments (Table 1) such a counter-exchange also seems to be involved in S_i transport. However, compared to the rates of exchange measured for P_i (Heldt and Rapley, 1970: up to 170 nmol mg chl^{-1} min^{-1}) those for S_i are much lower, reaching values of about 14 nmol mg chl.$^{-1}$ min^{-1}.

After correction for unspecific leakage, there is an enhanced release of S_i only with externally applied P_i, PGA, and DHAP, while the rates of exchange for malate or glucose-6-phosphate are quite similar to those measured as unspecific leakage. From this evidence it seems that S_i is at least partly translocated via a transport system specific to P_i, PGA, and DHAP. This translocating system is known to be a protein, located at the inner envelope membrane (Flügge and Heldt, 1976), and is named "phosphate translocator."

Assuming that P_i, PGA, DHAP, and S_i are transported into the chloroplast stroma via the same specific system, then there should be a competition for transportation similar to that shown for P_i/PGA/DHAP (Heldt and Rapley, 1970; Heldt, 1976). The values given in Figure 2 indicate that this is not true for the uptake of S_i; in fact the uptake of sulfate in the presence of P_i or PGA is even enhanced. A possible explanation could be the low affinity of the translocating system to S_i (see rates of uptake). On the other hand, the observed enhancement of uptake could also be explained by the assumption that P_i and PGA might increase the affinity of the phosphate translocator to S_i, the latter, in part, being translocated into the stroma by a kind of cotransport together with P_i or PGA.

In relation to our results, the effects of sulfate on the photosynthetic oxygen evolution (Baldry et al., 1968), mentioned earlier, are interesting. When intact spinach chloroplasts were incubated with sulfate (20 mmol l^{-1}), there was a complete inhibition of the CO_2-dependent oxygen evolution. The same concentration of sulfate, however, did not inhibit O_2-evolution from envelope-free chloroplasts, utilizing $NADP^+$ or ferricyanide as hydrogen acceptor. From the results presented in this paper, the above occurrences could be explained by a change in the stromal level of P_i. With envelope-retaining chloroplasts, using the same experimental conditions (high external sulfate concentration, low P_i concentration), an exchange transport should occur in the course of which the stromal concentration of P_i should decrease. This would result in a lower rate of photophosphorylation. However, when envelope-free chloroplasts are used,

because of the absence of compartmentalization, no shortage of P_i should occur.

Regarding sulfur metabolism, the prerequisite for optimum photosynthetic sulfate reduction seems to be met by a possible coupling of sulfate transport to that of phosphate. P_i influx is linked with the onset of photosynthetic activity in the chloroplast (Cockburn et al., 1967). According to our results, P_i enhances the influx of S_i; this process is accentuated by an exchange for photosynthetically synthesized DHAP and PGA, released from the stromal compartment. In turn, via the phosphorylating steps, the regulatory factors for optimum assimilatory sulfate reduction are provided (Ziegler and Hampp, 1977).

Finally, the markedly lower affinity of the translocator for S_i as compared to its affinity for P_i is in agreement with the drastically lower rates of sulfate reduction (3 μmol mg chlorophyll^{-1} h^{-1}; Trebst and Schmidt, 1969) compared to the phosphate requiring CO_2 reduction rates (about 150 μmol mg chlorophyll^{-1} h^{-1}).

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