Energy-linked Sulfate Uptake by Corn Mitochondria via the Phosphate Transporter

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ABSTRACT

Corn shoot mitochondria possess an energy-linked transport system for sulfate uptake as demonstrated by osmotic swelling and $^{[35S]}$SO$_4^{2-}$ accumulation. Maximum uptake is secured in the presence of Mg$^{2+}$ and oligomycin with sucrose for osmotic support. Neither phosphate nor dicarboxylate anions are required. When added simultaneously, millimolar concentrations of phosphate block $^{[35S]}$SO$_4^{2-}$ uptake after the initial minute. Mersalyl, N-ethylmaleimide, and 2,4-dinitrophenol are strong inhibitors of sulfate uptake; n-butylnalonate is a weak inhibitor. These inhibitors act in the same fashion on phosphate uptake. It is concluded that sulfate uptake in the absence of phosphate is by the phosphate transporter.

Studies on mitochondrial swelling driven by ammonium gradients show either no sulfate swelling in the absence of phosphate (2, 3) or a slow swelling attributed to a H$^+$-sulfate symport (13). Sulfate swelling in the presence of phosphate (3) and passive phosphate/sulfate exchange studies with animal and plant mitochondria have led to the conclusion that sulfate enters by sulfate-phosphate exchange on the dicarboxylate carrier (3, 6). Earlier investigations with respiration-driven sulfate transport show points of difference. There is no requirement for added phosphate in labeled sulfate uptake (15, 19, 20, 22) and phosphate is a competitive inhibitor of sulfate uptake (15, 19).

Recently, our laboratory has observed respiration-linked, mersalyl-sensitive sulfate swelling by corn mitochondria which is not dependent upon added phosphate (10). Here, we report on the characteristic features of this sulfate uptake. In the absence of phosphate, respiration-linked sulfate transport demonstrates properties which parallel those of phosphate transport: strong inhibition by mersalyl, NEM, and DNP; weak inhibition by n-BM; promotion by Mg$^{2+}$, oligomycin and osmotic support from sucrose. Catalytic concentrations of phosphate do not increase sulfate swelling, and mM concentrations block $^{[35S]}$SO$_4^{2-}$ uptake after an initial rapid influx. The data suggest that respiration in the absence of phosphate creates a protonotive force sufficiently large to drive sulfate uptake via the phosphate transporter.

MATERIALS AND METHODS

Corn shoot mitochondria (Zea mays L.) were isolated from 3.5-day-old etiolated shoots as previously described (1). Two reaction media were used: SBTK buffer consisting of 0.2 m sucrose, 1 mg/ml BSA, 20 mM Tes, and 20 mM KCl adjusted to pH 7.5 with KOH; and KT buffer consisting of 125 mM KCl and 20 mM Tris-HCl (pH 7.5). All reactions were carried out at 22 C.

The uptake of $^{[35S]}$SO$_4^{2-}$ from 2 mM labeled K$_2$SO$_4$ (4 µCi/ml) was measured by Millipore filtration procedure as described (1). The filters were prewetted for about 5 min in 2 mM unlabeled K$_2$SO$_4$ to reduce the radioactivity absorbed by the filter from the labeled reaction medium. Reaction medium blanks (no mitochondria) were used to determine the radioactivity retained by the filter, which was measured by scintillation counting (1). Radioactive sulfate was purchased from New England Nuclear, n-BM from Aldrich, mersalyl from SerVa, and other reagents from Sigma.

Percentage transmission (swelling) at 520 nm was measured as previously reported (1), with full scale transmittance set from 20 to 40%. Protein was determined by the Biuret method (7); BSA (fraction V) was used as a standard.

RESULTS

Energy-linked Sulfate Uptake. Table I shows the dependency of $^{[35S]}$SO$_4^{2-}$ uptake upon respiratory energy (NADH was used to avoid dicarboxylate uptake and exchange), and the promotion of uptake produced by Mg$^{2+}$. Mg has been observed to promote phosphate uptake (1), and since the stimulation of NADH oxidation by Mg$^{2+}$ is quite small (12) and effect appears to be exerted on transport. The inhibitory effect of the KCl medium may be related to the ready permeability of corn mitochondria to high concentrations of salts (5, 17). It seems probable that experiments in salt media in the absence of Mg$^{2+}$ might fail to show sulfate uptake by mechanisms other than exchange.

Figure 1 shows the energy dependence of sulfate swelling in the absence of phosphate. Uncoupling causes rapid efflux of accumulated salt. The swelling and shrinkage establish that this is net K$_2$SO$_4$ accumulation, not just anion exchange. If sulfate transport were by means of phosphate/sulfate exchange, the endogenous phosphate (about 20 nmol/mg protein or about 5 µM if diffused into the medium) would have to recycle very rapidly from very low concentrations. Phosphate swelling shows biphasic kinetics with half-saturation of the first phase at about 300 µM phosphate (8). Adding 120 µM phosphate does not alter the rate or extent of sulfate swelling as would be expected if phosphate were a required transport catalyst (Fig. 2A). Increasing phosphate to 5 mM produces rapid and pronounced swelling, but the response appears to be additive with sulfate (Fig. 2, B–D). Phosphate uptake is more rapid and efficient than sulfate uptake, meeting less resistance (10), and when both anions are present the combined rate is like that of phosphate (Fig. 2D). After preloading with phosphate, the initial rate of sulfate swelling is more rapid (Fig. 2C). Thus it seems that with mM phosphate concentrations there is rapid phosphate-catalyzed sulfate uptake. Total uptake at steady-state reflects the combined anion concentration.

Inhibition of Sulfate Uptake by Inhibitors of Phosphate Trans-
Table 1. Energy-linked sulfate uptake by corn mitochondria.

Mitochondria (2 mg of protein) were added to 1.35 ml of either SBTK medium of 0.2 M sucrose, 1 mg/ml of BSA, 20 mM TES, and 20 mM KCl (pH 7.5), or KT medium of 125 mM KCl and 20 mM Tris-HCl (pH 7.5). When present, NADH (2.1 μmol/mg of protein) and MgCl₂ (2 mM) were added before the mitochondria. After 1 min of preincubation the addition of 2 mM [³⁵S]K₂SO₄ (4 μCi/ml) started the reaction. (') = number of assays.

<table>
<thead>
<tr>
<th>Minutes of incubation</th>
<th>SBTK medium</th>
<th>KT medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Addition:</td>
<td>nmoles [³⁵S]SO₄²⁻/mg protein</td>
<td>nmoles [³⁵S]SO₄²⁻/mg protein</td>
</tr>
<tr>
<td>None</td>
<td>1.27 ± 0.24</td>
<td>1.74 ± 0.19</td>
</tr>
<tr>
<td>NADH</td>
<td>3.80 ± 0.22</td>
<td>4.17 ± 0.27</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1.47 ± 0.14</td>
<td>1.48 ± 0.14</td>
</tr>
<tr>
<td>NADH + MgCl₂</td>
<td>4.35 ± 0.35</td>
<td>5.81 ± 0.11</td>
</tr>
</tbody>
</table>

From labeled sulfate and phosphate uptake studies confirming the swelling data of Figure 3; in corn mitochondria n-BM has an inhibitory effect on phosphate and sulfate transport under conditions where the dicarboxylate transporter would not be expected to be operating (no substrate anions). The lesser per cent inhibition with phosphate is probably due to the higher concentration of phosphate used.

Additional results on the inhibition of [³⁵S]SO₄²⁻ uptake are given in Figure 5. Again, mersalyl, NEM, and 2,4-DNP are very inhibitory. Oligomycin is promotive, a result noted by Watanabe et al. (20), and probably due to blockage of a proton leak in the

Figure 1. Energy-linked swelling of corn mitochondria in the presence of sulfate. Mitochondria (2.25 mg protein) were preincubated for 1 min in 4 ml of SBTK medium in the absence (B) or in the presence (A) of 4 mM MgCl₂ + 5 μmol NADH. Addition of 12.5 mM K₂SO₄ started the reaction. 2,4-DNP represents addition of 0.18 mM 2,4-dinitrophenol.

The inhibitors of phosphate transport, mersalyl and NEM (11, 18), effectively inhibit sulfate transport in the absence of phosphate (Fig. 3). Since NEM is known to be ineffective with the dicarboxylate carrier (9), a fact used to establish that phosphate-sulfate exchange is by this carrier (3, 6), the inhibition of sulfate uptake by NEM (Figs. 3 and 5) is indicated to be on the phosphate transporter. The inhibitor of the dicarboxylate carrier, n-BM (16), inhibits malate and succinate transport in corn mitochondria with some indication that it interferes partially with phosphate transport (4). Figures 3 and 4 show that the weak inhibition of phosphate transport by n-BM in the absence of dicarboxylates is shared by sulfate uptake. Table II gives data...
FIG. 4. Effect of n-BM on energy-linked swelling of mitochondria by sulfate or phosphate. Experimental conditions were as in Figure 3. Addition of sulfate or phosphate started the reaction.

![Diagram](image)

FIG. 5. Inhibition of energy-linked \[^{35}\text{S}]\text{SO}_4^{2-}\) uptake by mitochondria. As in Table I, the mitochondria were preincubated 1 min in SBTK medium in the presence of NADH + MgCl\(_2\) in the presence of one of the indicated inhibitors: 1 mM NEM, 50 \(\mu\)M mersalyl, 0.18 mM 2,4-DNP, or 1.25 \(\mu\)g/mg protein of oligomycin. When present, \(\text{Pi}\) was added at the same time with 2 mM labeled sulfate.

![Diagram](image)

coupling ATPase (14). When phosphate is added at the same time as labeled sulfate, there is very rapid \[^{35}\text{S}]\text{SO}_4^{2-}\) uptake within the first min, followed by complete inhibition (Fig. 5). As with Figure 2, C and D, there is strong indication for phosphate/sulfate exchange following the very rapid phosphate uptake from mM concentrations.

**DISCUSSION**

These results on sulfate uptake by corn mitochondria in the absence of phosphate are reminiscent of those obtained some years ago with respiration-driven sulfate uptake in animal mitochondria (15, 20, 22) prior to the evidence for passive phosphate and sulfate exchange on the dicarboxylate carrier (3, 6). They are also in accord with the suggestion of Mitchell and Moyle (13) for a sulfate/proton symport (or sulfate/hydroxyl antiport).

We do not question that phosphate/sulfate exchange occurs and that it could provide a mechanism for sulfate uptake; present data on swelling and \[^{35}\text{S}]\text{SO}_4^{2-}\) uptake are suggestive of this (Figs. 2 and 5). The point made here is that energy-linked sulfate uptake in the absence of phosphate is by a mechanism subject to the same type of inhibition (Figs. 3–5) and promotion (\(\text{Mg}^{2+}\) and oligomycin, Table I, Fig. 5, and ref. 1) as phosphate uptake. The uptake of sulfate is not affected by catalytic amounts of phosphate (Fig. 2A). It is clear that sulfate can be accumulated via the phosphate transporter, or by a mechanism which is so similar that it cannot be distinguished at this time. The phosphate transporter may not be as selective as is sometimes thought.

The principal distinction is in the rate of net transport (Fig. 2 and ref. 10); steady-state osmotic swelling is reached in less than 1 min in phosphate while about 3 min are required with sulfate. There is thus higher “resistance” to sulfate transport, which is clearly illustrated by the opposed responses to valinomycin addition at steady-state (additional swelling with phosphate, but shrinkage with sulfate [10]). Sulfate transport by this mechanism requires the high protonmotive force derived from respiration, plus osmotic support from sucrose and \(\text{Mg}^{2+}\) to reduce membrane permeability (ref. 21 and Table I). It is perhaps for this reason that sulfate swelling in \((\text{NH}_4)_2\text{SO}_4\) is not seen without addition of phosphate or sulfite (3).

**LITERATURE CITED**


