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Short Communication

Activation of Endogenous Respiration and Anion Transport in Corn Mitochondria by Acidification of the Medium[1]

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ABSTRACT

Acidification of the suspending medium of corn mitochondria (*Zea mays* L., WF9 × Mo17) from pH 7.5 to pH 6.8 to 6.4 initiates osmotic swelling with the transportable anions citrate, sulfate, and phosphate. Swelling becomes pronounced with a combination of citrate plus sulfate or phosphate. Acidification proves to activate endogenous respiration, which is essentially zero at pH 7.5. The endogenous respiration transports citrate (in the presence of sulfate or phosphate) which then contributes to respiration and the accelerated osmotic swelling. Mersalyl will inhibit the swelling and antimycin inhibits the endogenous respiration. Magnesium appears to reduce the permeability of the membranes under the acid conditions.

It has been reported (6) that corn mitochondria show pronounced contraction or shrinkage as judged by light transmission when the medium is acidified. There were indications that certain anions, such as citrate or acetate, opposed the acid contraction, suggesting salt uptake driven by the pH gradient. However, in terms of Mitchell's chemiosmotic hypothesis (9) this should not happen. Net salt uptake requires a proton motive force ($\Delta p = \Delta\psi - 59\Delta pH$, mV at 25 C) with $\Delta\psi$ driving cation uptake as an electrophoretic uniport, and ΔpH driving anion uptake as a neutral symport or antiport. Simply acidifying the medium does not provide the requisite electrical potential.

We have investigated this problem using sulfate, phosphate, and citrate as transportable anions. Acidification of the medium by about 1 pH unit does produce osmotic swelling, but the transport is primarily due to activation of endogenous respiration.

MATERIALS AND METHODS

Mitochondria were isolated from corn shoots (*Zea mays* L. WF9 × Mo17) essentially as previously described (6), but with the isolation medium changed to 0.4 M sucrose, 50 mM TES buffer, 1 mg/ml of BSA, 3 mM EDTA, 3 mM $MgCl_2$, adjusted to pH 7.6 with KOH. Final suspension was in 0.25 M sucrose plus 1 mg/ml of BSA.

Measurements of per cent transmission, pH, and O_2 were as previously described (6) but at room temperature (23 C). Full scale transmittance was set from 20 to 40%, adjusting slit width to bring initial transmittance to 30%. Cuvettes contained 4 ml of aerated 100 mM sucrose, 10 mM TES, 1 mg/ml of BSA, adjusted to pH 7.5; however, after addition of 0.7 to 1 mg of mitochondrial protein the actual pH recorded was sometimes as low as 7.4. Additions of acids and salts to the medium were from 1 M stock solutions. Where pH recordings were not made (see Fig. 2) the amount of acid to be added, especially in the presence of phosphate and citrate, was determined separately.

RESULTS AND DISCUSSION

Figure 1A shows the effect of acidifying the medium with H_2SO_4 on the light transmission of the mitochondrial suspension. There is close correspondence between osmotic swelling as measured by per cent transmission (% T) and osmotic theory (7, 11), and assuming that this holds over the neutral pH range (pH 6-8), addition of H_2SO_4 initiates a slow osmotic swelling. Dropping the pH to 4.3 causes an abrupt "shrinkage" which slows after a few sec and takes several min to stabilize. The transition point between swelling and shrinkage was in the range of pH 5.8 to 6. Shrinkage was secured using several acids and it may represent a physical response of membrane or matrix proteins to high proton concentration rather than solute efflux driven by an acid gradient. Previous investigations, however, showed a loss of mitochondrial water at low pH (6). We limited the present investigation to the swelling phenomena which occur upon reducing pH by 1 unit or less (pH 6.8 to 6.4).

Swelling could not be obtained with HCl and HNO_3 (data not shown) but citric and phosphoric acids were effective. A result with citric acid is given in Figure 1B, which also shows that the shrinkage with deep acidification can be reversed by raising the pH. The presence of 5 mM KCl had no discernible effect on citric acid swelling, but 2 mM K_2SO_4 had a very large synergistic effect, *cf* SO_4 and citrate swelling singly (Fig. 1, A and B) and combined (Fig. 1C). Lowering resistance to K^+ transport with valinomycin greatly accelerated swelling, which mersalyl blocked (Fig. 1C). These are the responses expected from energy-linked transport. Although not shown, phosphate could be substituted for sulfate with essentially the same results. Due to its buffering capacity phosphate was not as convenient for this work as sulfate.

Acidification is potentially capable of removing surface-bound cations in exchange for H^+, and Wehrle *et al.* (14) have shown that membrane permeability in heart mitochondria is increased by removal of a small amount of superficially bound Mg^{2+}. Figure 1D shows that 1 mM Mg^{2+} transformed the swelling upon addition of H_2SO_4 (*e.g.* Fig. 1A) into a small shrinkage. Apparently, "tightening" the membranes with Mg^{2+} retards electrophoretic K^+ entry (14), and the effect of acidification is now manifest in solute efflux. We added Mg^{2+} in all subsequent experiments to reduce the possibility that acidification was primarily making membranes leaky.

Figure 2 reports the results from determining O_2 consumption upon acidification with HCl. Chloride is not actively trans-

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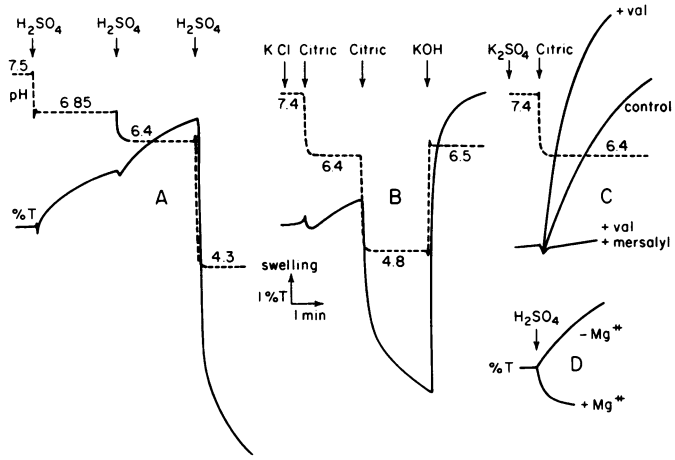

FIG. 1. Per cent transmission (% T) and pH traces for corn mitochondria suspended in the basic sucrose-TES-BSA medium. Acids and alkali were added in μl quantities to produce the pH changes shown. Upward deflection of % T indicates osmotic swelling. In B and C, the media contain initially 5 mM KCl and 2 mM K_2SO_4, respectively. In C, valinomycin was 0.25 μg/ml and mersalyl was 35 μM. In D, the H_2SO_4 addition produced pH 6.4. Mg^{2+} was 1 μM. Numbers refer to pH.

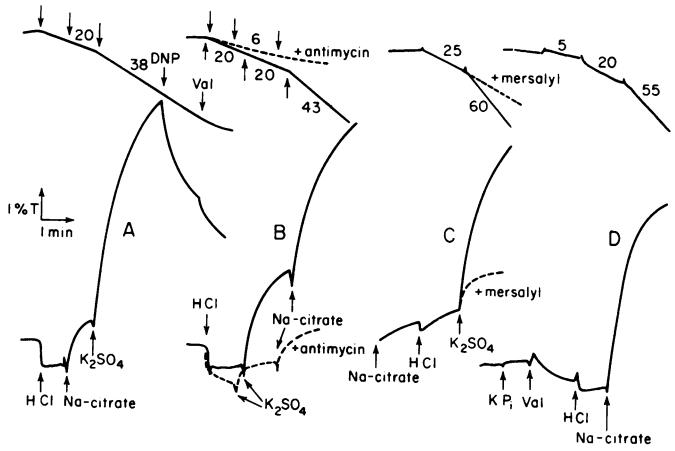

FIG. 2. O_2 concentration and % T curves for mitochondria in basic medium plus 1 mM $MgCl_2$; B, C, and D also included 2 μg/ml of oligomycin. HCl additions produced pH of 6.4 to 6.5. Additives were 1.5 mM Na-citrate, 2 mM K_2SO_4, 2 mM K-phosphate (pH 6.8), 1.25 μg/ml of antimycin A, 50 μM mersalyl, 0.25 μg/ml of valinomycin. Numbers are nmol of O_2/min/mg of protein.

ported, and HCl thus permits determination of respiration rates in the absence of active transport. Within error there was no detectable endogenous respiration at pH 7.4 in the standard medium plus Mg^{2+} (Fig. 2, A, B, and D). However, acidification to pH 6.8 to 6.4 invariably initiated a significant level of endogenous respiration (Fig. 2, A and B). Addition of citrate or sulfate alone would not stimulate this basal respiration, but the combination of citrate plus sulfate increased the respiration in association with massive swelling (Fig. 2, A, B, and C). Phosphate would substitute for sulfate (Fig. 2D and unpublished data). Clearly, the transport of citrate is limiting until sulfate or phosphate is added. The respiration and transport can be inhibited by antimycin and mersalyl, respectively (Fig. 2, B and C). Citrate cannot be transported and oxidized until the medium is acidified (Fig. 2C), which may reflect the redistribution of proton-conducting anions in favor of the matrix (12).

Uncoupling (Fig. 2A) causes loss of solute and respiration becomes transport-limited (3). Mobilization of K^+ transport with valinomycin would not substitute for acidification (Fig. 2D), although a very low respiration could sometimes be detected. The energy-linked rate of swelling is more pronounced in the presence of Mg^{2+} (Figs. 1C and 2A).

As yet we have no explanation for the increase in endogenous respiration upon acidification nor do we know the endogenous substrate, which in liver mitochondria is fatty acids (1). The oxidation of NADH, a substrate which is not transported (5), is also increased by lowering the pH to 6.5 (data not shown). Corn mitochondria oxidizing malate plus pyruvate have state 3 respiration rates independent of pH between 7.5 and 6.0 (6), but cauliflower mitochondria oxidizing malate have maximum activity between pH 6.7 and 7.0 (8) apparently due to enhanced malic enzyme activity. Succinic dehydrogenase is reported to be activated by pH 6.1 to 6.5 (10).

The activation of citrate transport by sulfate or phosphate in a mersalyl-sensitive transport reaction suggests that citrate may exchange with these inorganic anions. De Santis *et al.* (4) report that this exchange does not occur in bean mitochondria, but their experiments were with passive exchange under special conditions in KCl media without Mg^{2+}. In liver mitochondria sulfate uptake is indicated to be in exchange for phosphate (2), but this is contrary to the report of Watanabe *et al.* (13) that phosphate competitively inhibits sulfate uptake.

In conclusion, the mitochondrial swelling which occurs with transportable anions upon acidification of the medium to pH 6.8 to 6.4 is due to activation of endogenous substrate oxidation. Citrate is rapidly transported and oxidized at these low pH values provided sulfate or phosphate is present as a transport cofactor. The identity of the endogenous substrate and the mechanism of citrate transport are yet to be worked out.

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