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

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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 (\( \Delta p = \Delta \phi - 59 \Delta \text{pH} \), mV at 25 C) with \( \Delta \phi \) driving cation uptake as an electrophoretic unipot, and \( \Delta \text{pH} \) driving anion uptake as a neutral sympot 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 mM sucrose, 50 mM TES buffer, 1 mg/ml of BSA, 3 mM EDTA, 3 mM MgCl₂ adjusted to pH 7.6 with KOH. Final suspension was in 0.25 mM sucrose plus 1 mg/ml of BSA.

Measurements of per cent transmission, pH, and O₂ 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 mM 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 H₂SO₄ 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 H₂SO₄ 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 HNO₃ (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 K₂SO₄ had a very large synergistic effect, cf. SO₄ and citrate swelling singly (Fig. 1A, 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 Mg²⁺. Figure 1D shows that 1 mM Mg²⁺ transformed the swelling upon addition of H₂SO₄ (e.g. Fig. 1A) into a small shrinkage. Apparently, “tightening” the membranes with Mg²⁺ retards electrophoretic K⁺ entry (14), and the effect of acidification is now manifest in solute efflux. We added Mg²⁺ in all subsequent experiments to reduce the possibility that acidification was primarily making membranes leaky.

Figure 2 reports the results from determining O₂ consumption upon acidification with HCl. Chloride is not actively trans-
Numbers are oligomycin. Media plus 1.5 mM 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$_2$SO$_4$, 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.

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.

LITERATURE CITED

CORRECTIONS

Jones, Michael G. K., William H. Outlaw, Jr., and Oliver H. Lowry. Enzymic Assay of $10^{-7}$ to $10^{-14}$ Moles of Sucrose in Plant Tissues.
Page 381, column 1, paragraph 9, lines 2–3 should be corrected to read: The reagent is the same as for method B except that the buffer is 20 mM imidazole base.

Vol. 60: 518–520, 1977
Schneider, Monika M., Rüdiger Hampp, and Hubert Ziegler. Envelope Permeability to Possible Precursors of Carotenoid Biosynthesis during Chloroplast-Chromoplast Transformation.
Page 518, column 2, paragraph 3, line 14 should be corrected to read:

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\text{Citrate} + \text{CoA} \rightarrow \text{H}_2\text{C} \rightarrow \text{SCoA} + \text{ADP} + \text{P}_i
\]

Vol. 60: 933–934, 1977
Kimpel, Janice A., and John B. Hanson. Activation of Endogenous Respiration and Anion Transport in Corn Mitochondria by Acidification of the Medium.
Page 934, column 1, Figure 1 legend, last line should be corrected to read: Mg$^{2+}$ was 1 mM.