Effect of Phosphoenolpyruvate and Oxaloacetate on Ca\textsuperscript{2+} Uptake by Isolated Mung Bean Mitochondria

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ABSTRACT

Phosphoenolpyruvate partially inhibits the accumulation of Ca\textsuperscript{2+} in isolated mung bean (Phaseolus aureus Roxb.) mitochondria. Succinate-supported Ca\textsuperscript{2+} uptake is twice as sensitive to phosphoenolpyruvate inhibition as is NADH- or malate/pyruvate-supported Ca\textsuperscript{2+} uptake. Pyruvate, atractyloside, and ATP, but not ITP, reverse the phosphoenolpyruvate-induced inhibition. Oxaloacetic acid inhibits succinate-supported Ca\textsuperscript{2+} uptake completely while partially inhibiting NADH-supported Ca\textsuperscript{2+} uptake. The oxaloacetate inhibition of NADH-supported Ca\textsuperscript{2+} uptake is greater than that produced by phosphoenolpyruvate. It is suggested that inhibition of Ca\textsuperscript{2+} uptake is due to the conversion of phosphoenolpyruvate into oxaloacetate via phosphoenolpyruvate carboxykinase, with oxaloacetate responsible for the actual inhibition of Ca\textsuperscript{2+} uptake.

Both plant and animal mitochondria can accumulate a number of ionic species, including large quantities of insoluble Ca\textsuperscript{2+} and P\textsubscript{i} salt. The premise of this study was that a determination of cellular factors which affect the flow of salt across plant mitochondrial membranes can aid in interpreting the significance of the Ca\textsuperscript{2+} plus P\textsubscript{i} transport system in plants. In animal mitochondria, PEP\textsuperscript{1} has been shown to inhibit Ca\textsuperscript{2+} transport although the mechanism of inhibition remains unknown (2, 9). This study reports two organic acids, PEP and OAA, which inhibit the uptake of Ca\textsuperscript{2+} plus P\textsubscript{i} by plant mitochondria.

MATERIALS AND METHODS

Etiolated hypocotyls from 4-day-old dark mung beans (Phaseolus aureus Roxb.) were macerated with a Moulinex blender (model MP 2AV) in an ice cold isolation medium (2 ml/g of tissue) consisting of 0.3 M mannitol, 50 mM Tricine, 4 mM L-cysteine hydrochloride, 5 mM EDTA, and 1 mg BSA/ml, buffered to pH 7.4 with tris. The suspension was strained through a 45-μm nylon mesh and centrifuged at 3500g for 10 min. The mitochondria were resuspended in the average medium and layered over a discontinuous sucrose gradient consisting of 0.7 M, 1 M, and 1.6 M layers, respectively, each containing 1 mg BSA/ml and 10 mM tris-P\textsubscript{2} buffer, pH 7.5. The gradient was centrifuged at 9500g for 30 min in a swinging bucket head. Mitochondria were recovered at the interface of the 1 M and 1.6 M sucrose layers and were slowly resuspended in twice their volume of a solution containing 0.3 M sucrose, 10 mM tris-phosphate buffer, and 1 mg BSA/ml, pH 7.5. The suspension was centrifuged at 9000g for 10 min and the purified mitochondrial pellet was resuspended in 0.3 M of cold isolation medium (minus EDTA and cysteine).

Respiration was studied in a 2.4-ml glass reaction vessel with a Beckman O\textsubscript{2} electrode in a reaction medium of 0.3 M mannitol, 50 mM Tricine, 1 mg BSA/ml, 2 mM tris-phosphate, 2.67 mM MgCl\textsubscript{2}, and substrate as listed. ADP was added in 200-nmol aliquots. Mitochondrial protein was approximately 0.5 mg and determined by the Lowry method (8) with BSA as a standard.

RESULTS AND DISCUSSION

Isolated mitochondria oxidize substrates and actively accumulate Ca\textsuperscript{2+} in the presence of P\textsubscript{i} with a range of values from 400 to 700 nmol/mg protein (Table I). The respiratory values were similar to those reported for mung bean mitochondria (7). Succinate-driven Ca\textsuperscript{2+} uptake is inhibited over 50% by 1 mM PEP, while NADH- and malate/pyruvate-supported Ca\textsuperscript{2+} uptake are inhibited approximately 30% (Fig. 1). In the presence of ATP or atractylate, an adenine transport inhibitor, the PEP-induced inhibition of Ca\textsuperscript{2+} uptake is partially reversed although neither affected Ca\textsuperscript{2+} uptake by itself in the absence of PEP (Table II). ITP does not substitute for ATP in its ability to alleviate PEP-inhibited Ca\textsuperscript{2+} uptake. Pyruvate also partially reverses the PEP-inhibited Ca\textsuperscript{2+} uptake. In the presence of thiamine pyrophosphate, NAD\textsuperscript{+}, and CoA, cofactors of the pyruvate dehydrogenase reaction, pyruvate further reversed the uptake, quantitatively approaching the inhibition observed with ATP or atractylate (Table III).

OAA is a strong competitive inhibitor of succinate dehydro-
Ca²⁺ TRANSPORT IN MITOCHONDRIA

Table I. Effect of various substrates on respiration by isolated Mung bean mitochondria

<table>
<thead>
<tr>
<th>Substrate</th>
<th>State III</th>
<th>State IV</th>
<th>Respiratory ADP</th>
<th>Net Ca²⁺ Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 mM Succinate</td>
<td>185</td>
<td>73</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td>8 mM Malate +</td>
<td></td>
<td></td>
<td></td>
<td>465</td>
</tr>
<tr>
<td>8 mM pyruvate</td>
<td>122</td>
<td>30</td>
<td>3.1</td>
<td>2.2</td>
</tr>
<tr>
<td>0.2 mM NADH</td>
<td>157</td>
<td>41</td>
<td>3.8</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Total protein was between 0.4-5.0 mg.

Inhibition of NADH inactivation more endogenous than respiration driven by PEP + NAD. Pyruvate + NAD⁺ + TPP + CoA and Pyruvate + NAD⁺ + TPP + FAD also inhibit Ca²⁺ uptake by isolated mitochondria.

The inhibition of the transport of Ca²⁺ by PEP and partial reversal by ATP may involve the reversible reaction catalyzed by PEP carboxykinase. Previous workers have shown that mitochondria are a major source of this enzyme's activity (1). If externally added PEP is converted into OAA by the above reaction, the inhibition of Ca²⁺ uptake may be due to the increase in the level of OAA in the mitochondria and the inhibitory effect of OAA on citric acid cycle oxidation (4). Respiration studies show that the addition of 1 mM PEP to succinate-respiring mitochondria reduces respiration rates 20 to 30% without adversely affecting the ADP/O ratios or respiratory control values (5). However, with plant mitochondria, it is not possible to assess if this respiratory inhibition is sufficient to account for the observed effect on Ca²⁺ transport, since the component of respiration that supports Ca²⁺ transport is unknown.

The partial reversal of the PEP inhibition of Ca²⁺ uptake by ATP may reflect the reversal of the above reaction with the resulting reduction in the level of OAA. Consistent with these conclusions is the diminution of the PEP inhibition of Ca²⁺ uptake by pyruvate plus cofactors of the pyruvate dehydrogenase reaction. The formation of the product of this reaction, acetyl-CoA, would facilitate the reduction of the pool of OAA as acetyl-CoA combines with OAA to form citric acid via citrate synthase. Thus the inhibition of Ca²⁺ plus P₄ transport by PEP and OAA may reflect a general reduction in energy-linked reactions associated with the electron transport chain rather than a specific inhibition of the Ca²⁺ plus P₄ transport system as concluded from animal mitochondrial studies (2). In rat liver and heart mitochondria, the addition of PEP was reported not to reduce respiration although the incubation time may not have been sufficient to observe a reduction since maximum inhibition of Ca²⁺ transport requires a preincubation period with PEP (2). Since PEP and its conversion product OAA are normally present within the cell, these compounds may play a role in regulating Ca²⁺ plus P₄ fluxes across plant mitochondrial membranes under conditions where the transport of these salts is a major consumer of energy generated by the mitochondrial electron transport chain. One such condition has recently been suggested in slime molds where mitochondria deposit and later appear to release large amounts of Ca²⁺ plus P₄, during the formation of the outer surface of the slime mold's sporangia case (6).
LITERATURE CITED


