Short Communication

A Hexokinase-initiated Inhibition of Oxygen Uptake in Tomato Fruit Mitochondria Uncoupled by Dinitrophenol

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The stimulation of oxygen uptake in plant mitochondria uncoupled by 2,4-dinitrophenol (DNP) depends on adenine nucleotide in the absence of hexokinase (EC 2.7.1.1). Laties (9) observed this dependence with cauliflower bud mitochondria oxidizing malate. While Wiskich and Bonner (13) reported a doubling of the DNP-rate of oxygen uptake by adenine nucleotide in sweet potato mitochondria oxidizing succinate. Tomato fruit mitochondria also showed a dependence on adenine nucleotide in the presence of DNP and succinate and the absence of hexokinase (5). Heytler (7) reported a gradually suppressed oxygen uptake with increasing concentrations of the uncoupler. m-CI-carbonyl cyanide phenylhydrazone, in the presence of hexokinase, in mouse liver mitochondria oxidizing succinate. Inhibition of succinate oxidation in rat liver and potato mitochondria in the absence of hexokinase depended on the concentration of the uncoupler (12, 13).

The inhibition of succinate oxidation may be due to an inhibition of succinate dehydrogenase (EC 1.3.99.1) by oxaloacetate (13). Wiskich and Bonner (13) suggested that ATP, which could be formed by adenylyl kinase (EC 2.7.4.3) in uncoupled mitochondria, could act in 2 possible ways in reversing the inhibition caused by oxaloacetate. These are, to effect a conversion of oxaloacetate to phosphoenolpyruvate or to cause a dissociation of oxaloacetate and succinate dehydrogenase. Pyruvate and cysteine sulfonic acid prevented the inhibition of oxygen uptake in tomato fruit and mouse liver mitochondria, respectively, thus implicating oxaloacetate as the inhibitor (5, 7). Malate oxidation is also inhibited in preparations of plant mitochondria, but not in the presence of pyruvate (3, 8). In this communication we report the dependence of oxygen uptake in uncoupled mitochondria on the presence of hexokinase.

Mitochondria were isolated from the outer pericarp of mature green tomato fruit (Lycopersicon esculentum Mill.) as previously described (4). Oxygen uptake was measured by the standard Warburg method using a Gilson differential respirometer. The reaction was initiated by the addition of 0.2 ml mitochondrial suspension (the equivalent of 10 g fr wt of tissue) and equilibrated 10 min at 25°C. Oxygen uptake was determined every 3 min for the next 33 min. Aliquots for the determination of P1 by the method of Allen (1) were taken after 3 min and 33 min following equilibration. Values given are averages from duplicate flasks.

Table I. The Suppression of Oxygen Uptake in Mitochondria Oxidizing Succinate in the Presence of Hexokinase and Dinitrophenol or Oligomycin

<table>
<thead>
<tr>
<th>Expt</th>
<th>Inhibitor</th>
<th>Oxygen consumed in 30 min</th>
<th>Rate of oxygen consumption at the end of 30 min</th>
<th>P/O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µliters</td>
<td>% of control</td>
<td>µliters/30 min</td>
</tr>
<tr>
<td>a</td>
<td>DNP, mM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>84.5</td>
<td>Control</td>
<td>83.5</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>41.1</td>
<td>48.6</td>
<td>33.5</td>
</tr>
<tr>
<td>b</td>
<td>Oligomycin, µg/flask</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>80.1</td>
<td>Control</td>
<td>82.0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>42.2</td>
<td>52.7</td>
<td>44.0</td>
</tr>
</tbody>
</table>
Reaction flask contents are listed with the table and figure. Hexokinase, type C-300 (table 1) and type C-301 (fig 1), was purchased from Sigma Chemical Company, St. Louis, Missouri.

In manometric experiments with hexokinase present we noted that the average rate of O₂ uptake by tomato fruit mitochondria in the presence of 0.1 mM DNP was only 48% of the control and that the rate at the end of 30 min was 18% less than the average rate for that period (table I, a). Oligomycin also inhibited the rate of O₂ uptake, but this rate did not decrease with time (table I, b). These effects of DNP were in contrast to our results in polarographic experiments in which we had obtained a 0.1 mM DNP-rate of 90% of the state 3 rate (4) as well as a steady DNP-rate to the exhaustion of O₂ from the medium (5). In these polarographic experiments no glucose or hexokinase was present, the adenine nucleotide concentration was only 0.2 to 0.3 mM, and the DNP-rate lasted only 8 min at the most before oxygen was exhausted.

Hexokinase proved to be the factor of consequence. Figure 1 shows the pattern of oxygen uptake in a Warburg experiment with an ADP concentration of 0.8 mM in the presence and absence of both 0.10 mM DNP and 3.3 units of hexokinase. The presence of hexokinase (curve 2) resulted in a considerable inhibition compared to the DNP-uncoupled rate in the absence of hexokinase (curve 3), while this later rate was comparable to the coupled rate of oxygen uptake (curve 1).

Nitrophenols inhibit O₂-uptake at concentrations in excess of an optimum uncoupling concentration (6, 13) and this could explain the results of table 1. However, DNP did not severely inhibit at this uncoupling concentration of 0.1 mM; the observed severe inhibition was due to the presence of hexokinase (fig 1). Hexokinase could cause the inhibition by competing for the ATP with either of the enzymes involved in the 2 possible functions of ATP proposed by Wiskich and Bonner (13). The data suggest that in the control and the oligomycin-treated mitochondria, the formation of oxaloacetate through the oxidation of malate and NADH was restricted. This could be due to a reversed electron gradient through the first phosphorylation site of the coupled mitochondria in the presence of succinate. DNP would nullify such an effect of succinate and permit the formation of oxaloacetate (2).

These results show that the tacit assumption that hexokinase simply provides for the recycling of ATP to ADP and does not otherwise affect the mitochondrial reactions under study is not true with plant mitochondria oxidizing succinate. It is necessary, therefore, to state clearly whether or not reported experiments, especially with plant mitochondria, follow the customary procedure of measuring the inhibition of phosphorylation by uncouplers in the presence of hexokinase and the stimulation of oxygen uptake in the absence of hexokinase (10, 11). When succinate is the substrate, the practice of determining the extent of uncoupling as the P:O ratio in the presence of hexokinase is of questionable value.

**Literature Cited**


