Effects of Unsaturated Fatty Acids on Cyanide-resistant Respiration of Mitochondria Isolated from *Hansenula anomala*†

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Received March 3, 1992

Cyanide-resistant respiration catalyzed by an alternative oxidase, branching from the main cyanide-sensitive cytochrome pathway at the CoQ level, has been found in a variety of organisms. We reported the induction of cyanide-resistant respiration in the yeast *Hansenula anomala* and the characterization of the 36-kDa mitochondrial protein responsible for this pathway. Since a plant-like alternative oxidase was reported to serve as a sole terminal oxidase in the respiratory system of *Trypanosoma brucei brucei*, the studies on the specific inhibitors of cyanide-resistant respiration have taken on a great significance for the chemotherapy of trypanosomose infections, in addition to the characterization of the oxidase. This paper reports the inhibition by unsaturated fatty acids on cyanide-resistant respiration of the mitochondria isolated from *H. anomala*.

cis-Parinaric acid was purchased from Molecular Probes, Inc. All other fatty acids, methyl linolate, and essentially fatty acid-free BSA were obtained from Sigma Chemical Co.

The preparation of cyanide-sensitive and -resistant mitochondria and the measurement of oxygen uptake of the mitochondria using 7.5 mM malate plus 7.5 mM pyruvate as substrate were done as described previously.

Kay and Palmer reported that unsaturated C18 fatty acids selectively inhibited cyanide-resistant respiration of the intact and solubilized mitochondria isolated from the higher plant, *Arum maculatum*. Figure 1 demonstrates the effects of linoleic acid on cyanide-sensitive and -resistant O2 uptake of each mitochondria preparations. As can be seen, linoleic acid in low concentrations rather activated cyanide-sensitive respiration and then strongly inhibited it at concentrations higher than 100 μM. The inhibitory effects of unsaturated fatty acids on NADH-linked respiration, the conventional cyanide-sensitive pathway, have been reported in rat brain mitochondria. On the other hand, cyanide-resistant respiration was more sensitive to linoleic acid than cyanide-sensitive respiration. Although the data are not shown, similar results were obtained with other unsaturated fatty acids summarized in Table I except cis-parinaric acid. Stearic acid showed no significant effects on both respiration activities, while all unsaturated fatty acids with non-conjugated double bonds

![Graph](image)

**Fig. 1.** Effects of Linoleic Acid on Cyanide-sensitive and -resistant Respiration of the Mitochondria Isolated from *H. anomala*.

The cyanide-sensitive and -resistant O2 uptake activities of each mitochondria preparation using malate plus pyruvate as substrate were 0.091 and 0.125 μmol O2/min/mg, respectively. ○, cyanide-sensitive; ●, cyanide-resistant.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>CN−-sensitive</th>
<th>CN−-resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLEIC ACID</td>
<td>0.093</td>
<td>0.017</td>
</tr>
<tr>
<td>ELAIC ACID</td>
<td>0.078</td>
<td>0.028</td>
</tr>
<tr>
<td>LINOLEIC ACID</td>
<td>0.117</td>
<td>0.014</td>
</tr>
<tr>
<td>LINOLEALIC ACID</td>
<td>0.110</td>
<td>0.018</td>
</tr>
<tr>
<td>METHYL LINOLEATE</td>
<td>1.28</td>
<td>0.227</td>
</tr>
<tr>
<td>LINOLIC ACID</td>
<td>0.084</td>
<td>0.021</td>
</tr>
<tr>
<td>γ-LINOLEIC ACID</td>
<td>0.116</td>
<td>0.059</td>
</tr>
<tr>
<td>ARACHIDONIC ACID</td>
<td>0.066</td>
<td>0.031</td>
</tr>
<tr>
<td>CIS-PARINARIC ACID</td>
<td>0.400</td>
<td>0.680</td>
</tr>
</tbody>
</table>

† This research was supported in part by a Grant-in-Aid from Ciba-Geigy Foundation (Japan) for the Promotion of Science and that from the Ministry of Education, Science, and Culture of Japan.

Abbreviation: BSA, bovine serum albumin.
inhibit cyanide-resistant respiration, certain active oxygen species may be
involved in the reaction mechanism,\(^7\) and unsaturated fatty acids could
inhibit this pathway by the scavenging action. \textit{cis}-Parinaric acid, with
four conjugated double bonds, is thought to be less reactive with oxygen
radicals, which is consistent with our results that it does not exhibit the
selective, potent inhibition. Further investigation is in progress in our
laboratory to elucidate the detailed mechanism of this inhibition.

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