Activation of Soluble Succinate Dehydrogenase by Reduction

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Activation of soluble succinate dehydrogenase may be activated by reduction by NADH (in the presence of an enzyme catalysing the transfer of reducing equivalents to succinate dehydrogenase) or Na₂S₂O₄.

Since the experiments of KEARNEY 1 it has been known that both particle-bound and soluble succinate dehydrogenase can be activated by its substrate succinate and by competitive inhibitors such as fumarate, malonate and phosphate. Binding at the active centre apparently brings the enzyme in an active conformation. GUTMAN et al. 2, 3 have recently reported that NADH is as effective as succinate in activating succinate oxidation in sub-mitochondrial particles. Activation by NADH could be inhibited by rhein or piericidin, but not by thenoyltrifluoroacetone. Since activation by NADH was not possible in ubiquinone-depleted particles, they concluded that ubiquinone is necessary for the activation by NADH and that ubiquinol is the direct activator.

This paper describes the activation by NADH of solubilized succinate dehydrogenase, made by the procedure of WANG et al. 4 as modified by KEILIN and KING 5, and ZEYLEMAKER 6. Ubiquinone could not be detected in this preparation by the procedure of KRÖGER and KLINGENBERG 7; 0.01 mole ubiquinone per mole flavin would have been detected. The preparation was contaminated with a soluble NADH dehydrogenase, and 10% of the total of flavin, analysed by the method of KING et al. 8, 9, CERLETTI et al. 10 and ZEYLEMAKER 6, could be shown to be FMN. Because of this contaminating activity all of the succinate dehydrogenase flavin and iron-sulphur could be slowly reduced by NADH, as measured by spectrophotometry and EPR spectroscopy. This reduction, which was insensitive to rhein and rotenone, presumably takes place by direct reaction between the two flavoproteins.

Table I shows that the enzyme is activated, not only by succinate, malonate or phosphate, but also by the reductants NADH and Na₂S₂O₄. NAD⁺ had no effect.

Table I. Activation of succinate dehydrogenase by different compounds.

<table>
<thead>
<tr>
<th>Activator</th>
<th>µmoles succinate oxidized/min per mg protein</th>
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<tbody>
<tr>
<td>None*</td>
<td>0.68</td>
</tr>
<tr>
<td>Succinate (0.1 M)</td>
<td>1.03</td>
</tr>
<tr>
<td>NADH (1.1 MM)</td>
<td>0.99</td>
</tr>
<tr>
<td>NAD⁺ (1.0 MM)</td>
<td>0.60</td>
</tr>
<tr>
<td>Dithionite</td>
<td>1.22</td>
</tr>
<tr>
<td>Malonate (5 MM)</td>
<td>0.92</td>
</tr>
<tr>
<td>Phosphate (0.1 M)</td>
<td>0.78</td>
</tr>
</tbody>
</table>

* Not incubated at 38 °C.

These findings show that succinate dehydrogenase may be activated either by competitive inhibitors or by reduction. It may be that the active conformation promoted by binding of competitive inhibitor resembles the conformation of the reduced enzyme. Reduction of particle-bound succinate dehydrogenase by NADH via ubiquinone would seem to be an adequate explanation of the NADH-induced
Sequence around the Active Center Cystine of Lipoamide Dehydrogenase from Pig Heart, Comparison with the E. coli Enzyme *

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Sequence, active center, lipoamide dehydrogenase, enzyme

Catalysis by lipoamide dehydrogenase involves the concerted action of the flavin and a cystine residue. Peptides containing this cystine residue have been previously isolated from E. coli and now from pig heart. The sequences of amino acid residues reveal a high degree of homology indicating a strict conservation of the region around the active site cystine during the long evolutionary period between these two species. The peptide sequences suggest a likely conformation of the polypeptide chain in the region of the flavin as well as the forces involved in substrate and flavin binding.

The pyridine nucleotide-disulfide oxidoreductases, lipoamide dehydrogenase, thioredoxin reductase and glutathione reductase are catalytically similar flavoproteins characterized by the presence of a redox active cystine residue functioning together with the flavin in electron transfer \(^1\)–\(^4\). We have recently reported the structures of peptides containing the active center cystine isolated from E. coli lipoamide dehydrogenase and thioredoxin reductase and the latter structure has also been reported by Thelander \(^5\)–\(^8\). Enzymes of this group contain a single cystine and three or more cysteine residues. (There is some evidence to suggest that pig heart lipoamide dehydrogenase may contain a second disulfide \(^9\); but our data indicate that this may be artificial.) Provided certain precautions are observed in order to prevent thiol-disulfide interchange, the isolation of peptides containing the active site

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