result in a change in either molecular weight or sedimentation coefficient in the concentration range studied. However, since the enzymatic activity of the dehydrogenase is studied at concentrations of 20 to 2 μg per ml, it is of great interest to know the physical state of the enzyme in this concentration range, as opposed to the range from 10 to 1 mg per ml, where the physical studies have been carried out. Studies now in progress in this laboratory have as their goal the determination of the physical properties of both the normal and the activated enzyme at very low concentrations. Preliminary results of the work strongly suggest that the enzyme does undergo dissociation at low concentrations and furthermore that methylmercuric bromide has an effect on this dissociation phenomenon. Evidence reported by Frieden (10) for the normal enzyme also is suggestive of a dissociation phenomenon.

REFERENCES

Energy-requiring Reduction of Pyridine Nucleotide by Ascorbate in the Presence of Coenzyme Q or Menadione

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Mitochondrial fragments from beef heart catalyze an adenosine triphosphate-requiring reduction of nicotinamide adenine dinucleotide by succinate (Reaction 1) (1-3). This enzyme system has been partially resolved into a particulate component

\[
\begin{align*}
\text{Succinate} + \text{NAD} & \xrightarrow{\text{ATP}} \text{fumarate} + \text{NADH} + \text{H}^+ \\
\text{Ascorbate} + \text{NAD} & \xrightarrow{\text{ATP}} \text{quinone} \\
\text{dehydroascorbate} + \text{NADH} + \text{H}^+ & \quad \text{dehydroascorbate} + \text{NADH} + \text{H}^+
\end{align*}
\]

and a soluble, heat-labile fraction (2). The electron transport chains originating from both NADH and succinate are implicated in this reversal of oxidative phosphorylation. This communication reports a simplification of the system in which the respiratory chain concerned with the oxidation of NADH alone may be involved.

Fig. 1 shows the reduction of NAD by ascorbate in the presence of CoQ or menadione. The reaction medium contained 50 mM Tris at pH 7.5, 3.3 mM magnesium chloride, 1 mM potassium cyanide, 2 mM ATP, 2.0 mg of bovine serum albumin, and 0.75 mg of mitochondrial particles in a total volume of 3.0 ml. In addition, O—O contained 6.7 mM succinate, △—△ contained 20 mM ascorbate and 33 μM menadione, and □—□ contained 20 mM ascorbate and 27 μM CoQ. The solid symbols indicate the rates with no added ATP. The reaction was carried out at 30°C.

**TABLE I**

<table>
<thead>
<tr>
<th>Washed particles</th>
<th>Soluble protein</th>
<th>ΔA260 μg/3 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction 1</td>
<td>Reaction 2</td>
<td></td>
</tr>
<tr>
<td>0.75</td>
<td>2.4</td>
<td>0.050 0.068</td>
</tr>
<tr>
<td>0.75</td>
<td>2.4</td>
<td>0.178 0.189</td>
</tr>
</tbody>
</table>
Approximately 12 to 15 μg of the antibiotic per mg of protein were necessary for 50% inhibition. The effects of the inhibitors are similar to those obtained in Reaction 1 (2).

We had previously reported that repeated washing of the mitochondrial fragments resulted in loss of activity in Reaction 1 (2). The activity could be restored by the addition of a soluble protein fraction derived from the sonic disruption of mitochondria. Reaction 2 also behaves in a similar manner. Table I shows the marked stimulation of NAD reduction caused by the addition of the soluble protein fraction to washed particles. The extent of stimulation is nearly the same as in Reaction 1.

The data suggest that ascorbate reduces menadione or CoQ4 nonenzymatically, and the resulting hydroquinone then reduces NAD enzymatically at the expense of the energy from ATP. The electron transfer sequence may be the reverse of the NAD-CoQ reductase reaction (4). The low sensitivity of Reaction 2 to antimycin suggests that the classical antimycin binding site is not involved. This interpretation is consistent with the observation that the purified NADH-CoQ reductase contains little or no cytochromes b and c1. The results also indicate that the primary phosphorylation step in NADH oxidation may operate in the segment of the respiratory chain between the pyridine nucleotide and CoQ.

REFERENCES


Enzymic Synthesis of Niacin Nucleotides from 3-Hydroxyanthranilic Acid in Mammalian Liver*

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In 1945 Krehl et al. (1) reported that niacin could be replaced by tryptophan in the rat for the growth and maintenance of the animal. Since then, a number of nutritional, isotopic, and genetic studies have shown that tryptophan is the precursor of niacin in animals and Neurospora. However, attempts to elucidate the mechanism of the conversion in vitro have been uniformly unsuccessful, since quinolinate (2) and picolinate (3) are the only pyridine carboxylic acids obtained thus far from 3-hydroxyanthranilic acid.

In this communication, we wish to report that 3-hydroxyanthranilic acid is converted to nicotinic acid, which is the precursor of 5-phosphoribosyl-1-pyrophosphate by an enzyme preparation obtained from rat liver, and to propose that 2-acrolyl-3-aminoacrylic acid and quinolinol are intermediates in this conversion (Scheme 1).

Mature albino rats were decapitated, and the livers were immediately removed, chilled, and homogenized for 2 minutes with 2 volumes of 0.14 M KCl in a glass homogenizer. The homogenate was centrifuged for 20 minutes at 12,000 x g, and the supernatant solution was treated with charcoal and centrifuged in a Spino centrifuge, model L, at 105,000 x g for 2 hours. The clear supernatant fraction was dialyzed for 3 hours against several liters of 0.02 M phosphate buffer, pH 7.0.

A reaction mixture 8.0 ml in volume and containing 417 μmoles of 3-hydroxykynurenine-C14 uniformly labeled in the benzene ring (300,000 c.p.m. per μmole), 40 μmoles of ATP, 10 μmoles of PP-ribose-P, 20 μmoles of MgCl2, 500 μmoles of potassium phosphate buffer, pH 7.0, and 30 μg of the supernatant fraction was incubated for 6 hours at 25°. When the reaction was stopped by the addition of 0.5 ml of 5 N H2SO4, 108 μmoles of C14O2 (5400 c.p.m.) were recovered. After the addition of 2 to 10 μmoles each of picolinic acid, quinolinol, nicotinic acid, NAD, deamido-NAD, and nicotinamide as carriers, the reaction was allowed to proceed for 3 hours. The radioactivity of each of these carriers was determined, and the results are given in Table I. The tryptophane → 3-hydroxyanthranilate → 2-acrolyl-3-aminoacrylic acid pathway was confirmed by the isolation of the intermediates 2-acrolyl-3-aminoacrylic acid and quinolinol.

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