Biochemical Properties of Rat Liver Mitochondrial Aldehyde Dehydrogenase with Respect to Oxidation of Formaldehyde

(Received for publication, October 9, 1975)

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The oxidation of formaldehyde by rat liver mitochondria in the presence of 50 mM phosphate was enhanced 2-fold by exogenous NAD+.* Absolute requirement of NAD+ for formaldehyde oxidation was demonstrated by depleting the mitochondria of their NAD+ content (4.6 nmol/mg of protein), followed by reincorporation of the NAD+ into the depleted mitochondria. Aldehyde (formaldehyde) dehydrogenase activity was completely abolished in the depleted mitochondria, but the enzyme activity was restored to control levels following reincorporation of the pyridine nucleotide. Phosphate stimulation of formaldehyde oxidation could not be explained fully by the phosphate-induced swelling which enhances membrane permeability to NAD+, since stimulation of the enzyme activity by increased phosphate concentrations was still observed in the absence of exogenous NAD+

The \( K_m \) for formaldehyde oxidation by the mitochondria was found to be 0.38 mM, a value similar to that obtained with varying concentrations of NAD+. Both \( V_{max} \) values were very similar, giving a value of 70 to 80 nmol/min/mg of protein. The pH optimum for the mitochondrial enzyme was 8.0. Inhibition of the enzyme activity by anaerobiosis was apparently due to the inability of the respiratory chain to oxidize the generated NADH.

The inhibition of mitochondrial formaldehyde oxidation by succinate was found to be due to a lowering of the NAD+/NADH ratio, thereby blocking aldehyde oxidation, causing an accumulation of formaldehyde during mixed function demethylation reactions.

A variety of structurally unrelated drugs, such as codeine, ethylmorphine, imipramine, aminopyrine, SKF 525-A, undergo N-dealkylations catalyzed by the microsomal terminal oxidase, cytochrome P-450, to yield aliphatic aldehydes. Those and other aldehydes, like glutaraldehyde, a protein cross-linking agent, were found to be rapidly degraded by rat liver mitochondria (1). Malonaldehyde, a product of peroxidation of unsaturated fatty acids (2), was also shown to be metabolized by rat liver mitochondria (3).

In the early 1950s, Walkenstein and Weinhouse (4) reported the presence of a nonspecific aldehyde-oxidizing enzyme in rat liver mitochondria which catalyzed the consumption of a variety of aliphatic aldehydes, as measured by oxygen uptake; the enzyme was shown to require NAD+ (9). Aldehyde dehydrogenase activity has also been found in other subcellular fractions (5-9), and isolated and partial purifications have suggested the coexistence of more than one NAD+-linked aldehyde dehydrogenase (10-14). Studies have indicated that the cytosol contains more than one aldehyde dehydrogenase which can oxidize formaldehyde, yet show different specificities for...
larger aldehydes (10, 15, 16). One cytosolic enzyme was found to require reduced glutathione and to be specific for formaldehyde (17, 18), and has been highly purified from human liver (19). The latter enzyme was reported to have nonselective cofactor requirements, accepting either NAD+ or NADP+. At present, the relationship between the cytosolic and mitochondrial enzymes is unknown.

The apparently nonspecific mitochondrial aldehyde dehydrogenase has been reported to be localized on the outer mitochondrial membrane (20), however, more recent reports have indicated it to be in the inner membrane fraction (21, 22). Based upon kinetic parameters, it appears that more than one NAD+-dependent aldehyde dehydrogenase is also present in rat liver mitochondria (14).

Our interest in the mitochondrial aldehyde dehydrogenase stems from our observation that interactions occurred between the hepatic endoplasmic reticulum and the mitochondria during oxidative N-dealkylation (23, 24). The interaction was later found to be due to inhibition of formaldehyde consumption in the mitochondria by succinate. In this report, the location and properties of the rat liver mitochondrial aldehyde dehydrogenase are described.

**MATERIALS AND METHODS**

**Materials**—Tricarboxylic acid cycle substrates obtained from Sigma Chemical Co., St. Louis, were buffered to the pH of the assay medium (7.5) and added to the reaction flasks in final concentrations of 10 mM, unless otherwise indicated. NAD+, NADH, ATP, and antimycin A (type III) were also purchased from Sigma. Rotenone was obtained from Aldrich Chemical Co. All other chemicals and biochemicals were of the highest purity available commercially and were not purified further.

**Preparation of Mitochondria**—Male Sprague-Dawley rats (225 to 275 g) from Charles River were used in this study. The animals had free access to Purina Laboratory Chow and tap water at all times. Following decapitation, livers were removed and treated as described previously (23). Liver mitochondria were generally prepared by differential centrifugation of a 10 to 20% liver homogenate in 0.25 M sucrose, and resedimented as before; this washing procedure was repeated twice. During each wash, care was taken not to include the loose fluffy layer above the mitochondrial pellet. The final pellet was resuspended in 0.25 M sucrose containing 50 mM phosphate buffer (pH 7.5) and added to the reaction flasks in final concentrations of 10 mM, unless otherwise indicated. NAD+, NADH, ATP, and antimycin A (type III) were also purchased from Sigma. Rotenone was obtained from Aldrich Chemical Co. All other chemicals and biochemicals were of the highest purity available commercially and were not purified further.

**Enzyme Assay**—Unless indicated otherwise, all assays were performed at 37°. Aldehyde dehydrogenase activity was measured by two different methods: (a) determination of the rate of disappearance of formaldehyde by the Hantzsch reaction as described by Nash (30), and (b) spectrophotometric measurement of the rate of reduction of NAD+ at 340 nm with formaldehyde as the substrate. The assay medium used to measure formaldehyde disappearance generally consisted of 0.25 M sucrose containing 50 mM phosphate buffer (pH 7.5), 1 mM NAD+, and 1 mM formaldehyde in a volume of 3 ml. As indicated in the figure legends, phosphate, NAD+, formaldehyde, and pH were varied depending on the experiment. The spectrophotometric assay contained, in addition to the above, 2 μM rotenone (added in 10 μl of acetone) to block mitochondrial NADH oxidase activity. Pyrazole (0.2 mM), often added to inhibit contaminating alcohol dehydrogenase activity, had no effect on the reduction of NAD+, and hence, was omitted from the cuvette in later experiments. Swelling occurred when the mitochondria were added to the assay media; hence, it was necessary to run simultaneously a blank which contained the same medium minus the substrate. Monoamine oxidase activity was measured in the various mitochondrial fractions by the method of Schnaitman et al. (28), a modification of the method of Tabor et al. (31). Malate dehydrogenase activity was determined by the method of Ochoa (32) in a medium containing 2 μM rotenone.

Oxidation of acetaldehyde was determined by the procedure of Gupta and Robinson (33) as described by Lieber and DeCarli (34).

**RESULTS AND DISCUSSION**

**Comparison of Methods Employed to Measure Aldehyde (Formaldehyde) Dehydrogenase Activity**—A number of reports (11, 13, 14, 16, 22) concerned with aldehyde dehydrogenase activity have used the spectrophotometric assay procedure which measures the formation of the 340 nm absorption peak following reduction of NAD+ by aldehydes. We have tested this procedure and found it to give erroneous values because of the presence of rotenone used to inhibit NADH oxidase. When rotenone was not present, NADH accumulation did not occur, despite the fact that appreciable amounts of formaldehyde were consumed, as measured by the Nash procedure (30). The addition of rotenone prevents NADH oxidation, and the rate of NAD+ reduction can then be measured at 340 nm. However, this rate did not agree with the rate of formaldehyde consumption, being 30 to 90% of the latter. The addition of rotenone to the assay medium used to measure formaldehyde consumption inhibited the rate of disappearance of formaldehyde, giving a value in agreement with the 340 nm assay. Since in the presence of rotenone one obtains a lower rate of formaldehyde metabolism, and since in its absence one cannot measure the endogenous aldehyde dehydrogenase activity by the 340 nm spectrophotometric assay, it was felt that the Nash procedure (30) gave a more valid measurement of aldehyde dehydrogenase activity.

**NAD+ Dependence of Formaldehyde Oxidation**—The requirement of the partially purified enzyme for oxidized pyridine nucleotide was first reported by Racker (35) and confirmed by subsequent investigators (5, 8, 17). In the absence of exogenous NAD+, the rate of formaldehyde consumption by rat liver mitochondria was linear for about 2 min. As shown in Fig. 1A, addition of 1.0 mM NAD+ stimulated the initial rate about 2-fold, and prolonged the period of linearity to about 5 min. NAD+ permeability was ensured by the presence of 50 mM phosphate buffer. At 1 mM NAD+, the formaldehyde consumption rate was linear (Fig. 1B) with mitochondrial protein up to at least 2 mg/ml when the incubation time was 2 min. Although the formaldehyde oxidation rate was lower in the absence of exogenous NAD+, it was also linear with protein.

By "endogenous mitochondrial aldehyde (formaldehyde) dehydrogenase activity," we mean the rate of formaldehyde oxidation by a mitochondrial preparation which contains only endogenous pyridine nucleotide, exogenously added NAD+ is omitted from the assay medium.
The dependence of formaldehyde consumption on NAD$^+$ is shown in Fig. 2. Increasing amounts of NAD$^+$, in the assay medium containing 50 mM phosphate buffer, caused an increase in the aldehyde dehydrogenase activity, which plateaued at about 1 mM NAD$^+$. The activity in the absence of exogenous NAD$^+$ corresponded well with the endogenous NAD$^+$ level of about 5 nmol/mg. The $K_m$ for NAD$^+$, obtained from absissa intercepts of double reciprocal plots, was 0.33 mM NAD$^+$, and the $V_{max}$ Activities averaged 80 nmol of formaldehyde consumed/min/mg of mitochondrial protein. Although Tottmar et al. (14) reported NADP$^+$ to support acetaldehyde consumption, this pyridine nucleotide did not support formaldehyde consumption. The addition of NADP$^+$ to the NAD$^+$-supported reaction was also without effect.

Whether this indicates the presence of another enzyme, as suggested by other studies (14), which can metabolize acetaldehyde with the support of NADP$^+$ is at present not clear.

In studies utilizing exogenous NAD$^+$, phosphate was always added to assure permeation of the NAD$^+$ into the mitochondria. The role of phosphate in the stimulation of aldehyde dehydrogenase activity is, however, not fully understood. As seen in Fig. 3, increasing P$_i$ concentrations resulted in a large stimulation of enzymatic activity, a phenomenon also observed by Marjanen (9) using rat liver homogenate and acetaldehyde. The stimulation plateaued at a P$_i$ concentration of 50 mM. This enhanced activity cannot be completely explained by increased membrane permeability to the pyridine nucleotide, since P$_i$ concentrations below 10 mM do not induce swelling (35). In addition, P$_i$-stimulated aldehyde dehydrogenase activity occurs even in the absence of added NAD$^+$, although total enzyme activity is much lower. In agreement with this observation, crude enzyme obtained from mitochondria (no membrane
present) was also stimulated by P, (16). All subsequent experiments were performed with the assay medium containing 50 mM P,

The absolute requirement for NAD+ can be seen from the data in Fig. 4 and Table I. When mitochondria were depleted of NAD+ by the method of Hunter et al. (35), no measurable formaldehyde oxidation could be observed (Fig. 4). The depleted mitochondria contained only 1.5% of the NAD+ present in the control mitochondrial preparation (Table I). Reincorporation of NAD+ into the depleted mitochondria restored aldehyde dehydrogenase activity as well as the level of NAD+. Although the reincorporation phenomenon requires the presence of ATP, its addition to control mitochondria was found to have no effect on HCHO metabolism (Fig. 4).

Properties of Aldehyde Dehydrogenase with Formaldehyde as the Substrate—Substrate dependence plots indicated aldehyde dehydrogenase to be saturated with formaldehyde at 1 mM level. Using a substrate range of 50 μM to 2 mM (Fig. 5), the Kₘ for formaldehyde was found to be 0.38 mM, a value close to that for the other substrate, NAD+. The Kₘ value for formaldehyde was essentially the same in the absence of exogenous NAD+, hence, it is not clear why Smith and Packer (20) obtained the higher value of 0.8 mM with this substrate. Perhaps the answer lies in their assay, where formaldehyde consumption was measured indirectly by oxygen consumption. The assay procedure of Tottmar et al. (14) was also indirect, based on the 340 nm absorption of NADH. The latter investigators, using acetaldehyde, obtained curved double reciprocal plots, which suggested to them the presence of more than one enzyme, one with a Kₘ of less than 10 mM and one with a Kₘ of 1.5 mM, for acetaldehyde. A single Kₘ value for acetaldehyde similar to the lower one of Tottmar et al. (14) was obtained in rat liver homogenates by Marjanen (9). Although Grunnet (22) was unable to demonstrate two aldehyde dehydrogenase enzymes with acetaldehyde, in rat liver mitochondria, two Kₘ values were obtained with a partially purified preparation (22). Interestingly, Erwin and Dietrich (10) also observed curved double reciprocal plots with acetaldehyde as a substrate with bovine brain mitochondria, but not with other aldehyde substrates.

The pH optimum for formaldehyde oxidation by mitochondria was approximately 8.0 (Fig. 6), a value similar to that obtained by Glenn and Vanko (5) with choline and betaine aldehydes as substrates. Wallenstein and Weinhouse (4) observed a pH optimum between 7.4 and 7.8 for mitochondrial aldehyde dehydrogenase when acetaldehyde was employed as

![Fig. 4. Effect of mitochondrial depletion and reincorporation of NAD+ on formaldehyde oxidation. Mitochondria (5 mg/ml) were depleted (DEPL. MIT. II. - - -) of NAD+ by incubating with 20 mM phosphate for 20 min at 23°, as described by Hunter et al. (35); following incubation, the mitochondria were washed with 250 mM sucrose, centrifuged at 8060 x g for 20 min, and resuspended in 250 mM sucrose. For reincorporation (MIT. REINCORP.), an aliquot of the depleted mitochondria was incubated with 2 mM NAD+, 2 mM ATP, 250 mM sucrose, 5 mM phosphate (pH 7.5), and 15 mM Tris-Cl (pH 7.5) at 0° for 20 min; mitochondria were then washed and centrifuged as indicated above and resuspended in 250 mM sucrose. Formaldehyde oxidation was measured in an assay medium containing mitochondria (1.0 mg/ml), 1.0 mM formaldehyde, 250 mM sucrose and 50 mM phosphate, pH 7.5. Control mitochondria (O—O), were incubated with only 250 mM sucrose for 20 min at 23°; control mitochondria plus 20 mM ATP (MIT. + ATP, Δ—Δ).

![Fig. 5. Lineweaver-Burk plot of the reaction catalyzed by mitochondrial formaldehyde dehydrogenase as a function of the concentration of formaldehyde. Incubation conditions were as described in Fig. 1B.](http://www.jbc.org/)

![Fig. 6. Activity of rat liver mitochondrial formaldehyde dehydrogenase as a function of pH. Measurements were carried out in 50 mM phosphate buffer adjusted to appropriate pH with 1 N HCl or 1 N NaOH, 250 mM sucrose, 1.0 mM formaldehyde, 1.0 mM NAD+, and 1 mg/ml of mitochondrial protein; incubation period was for 2 min.](http://www.jbc.org/)
the substrate. In contrast, a pH optimum near 9.0 was reported by Tottmar et al. (14) with acetaldehyde as substrate. Grunnet (22) also reported a pH optimum with acetaldehyde between 8.5 and 9.0 with a partially purified preparation, as did Marjanen (16) for the mitochondrial enzyme using, presumably, acetaldehyde. The latter three groups used the spectrophotometric procedure, whereas the other two groups (4, 5) measured oxygen uptake. This may account for the discrepancy in the pH optima.

As reported by Marjanen (9), no difference was seen in aldehyde dehydrogenase activity under O2 from that under air. Anaerobiosis (Table II), however, lowered the activity by 50%. The presence of exogenous NAD+ decreased the extent of inhibition. The requirement for O2 presumably arises from the need to oxidize the accumulated NADH back to NAD+. The partial inhibition observed with anaerobiosis in the presence of 1 mM NAD+, may be attributed to inhibition of the phosphate-induced swelling of mitochondria, since anaerobiosis has been shown (39) to inhibit the swelling phenomenon. This would decrease membrane permeability to pyridine nucleotides, and thereby lower enzyme activity.

Localization of Mitochondrial Aldehyde Dehydrogenase Activity—Inner and outer mitochondrial membrane fractions were prepared with digitonin by the method of Schnaitman et al. (28). The inner membrane pellet, obtained by 8000 × g centrifugation, consisted of mitochondria essentially devoid of their outer membranes. However, less than half of the preparation remained in the condensed state typical of freshly prepared mitochondria. (Electronmicrographs and their interpretation were courtesy of Dr. P. Goldblatt.) Total monoamine oxidase activity in the 8000 × g pellet was only 5% of the activity found in intact mitochondria, indicating low contamination with outer membrane. Malate dehydrogenase activity in the mitoplasts (inner membrane matrix marker) was 75% of intact mitochondria. Approximately 85% of the aldehyde dehydrogenase activity was found in the mitoplast fraction. Electron micrographs of the outer membrane obtained by centrifugation at 145,000 × g consisted entirely of small, clear vesicles, similar to those of liver microsomes. Most of the monoamine oxidase activity was concentrated in this fraction. Less than 10% of the total mitochondrial formaldehyde-oxidizing activity was found in the outer membrane fraction. This latter observation is in agreement with the report of Grunnet (22), who used acetaldehyde as the substrate. Smith and Packer (20), however, employing the method of Parsons to separate inner and outer mitochondrial membrane, reported high activity in the inner membrane, but 2 times more activity in the outer membrane fraction. Based on the aldehyde dehydrogenase requirement for NAD+, and the alteration of its activity by certain Krebs’ cycle substrates and respiratory chain inhibitors (to be discussed below), it would appear that the enzyme-metabolizing formaldehyde is localized to the inner membrane or matrix compartment. The mitoplasts (inner membrane plus matrix) were subfractionated by the method of Greenawalt (29) into matrix components and inner membrane. The results shown in Table III indicate a distribution of formaldehyde dehydrogenase similar to malate dehydrogenase which has been reported to be a matrix marker enzyme (28, 29). However, like the earlier report (28), appreciable amounts of malate dehydrogenase were found in the inner membrane fraction (Table III), making a firm statement of location of these enzymes difficult. The use of Lubrol WX to extract the matrix components cannot be considered definitive, since this detergent is known to solubilize enzymes of the hepatic microsomal membrane (40).

Role of Succinate and Inhibitors of the Mitochondrial Respiratory Chain—We have recently shown1 that the apparent stimulation of microsomal N-demethylation reactions by succinate in the presence of mitochondria was due to inhibition of mitochondrial oxidation of formaldehyde. Fig. 7 shows the inhibition of formaldehyde dehydrogenase activity of mitochondria with increasing succinate concentrations. Accompanying the inhibition is a fall in the level of intramitochondrial NAD+ and a corresponding rise in the level of intramitochondrial NADH (Table IV); the changes in the reduced and oxidized forms resulted in a ratio of NAD+/NADH equal to 1.7/1; a 65% decrease from the initial value. Malonate, a competitive inhibitor of succinate dehydrogenase, prevented the succinate inhibition of aldehyde dehydrogenase activity (Table V). Succinate also inhibited formaldehyde oxidation by the inner mitochondrial membrane fraction, presumably by altering the fraction of the pyridine nucleotide in the oxidized form. Preliminary experiments with the solubilized enzyme, obtained by sonication of the inner mitochondrial fraction,

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**Table II**

**Effect of anaerobiosis on mitochondrial aldehyde dehydrogenase activity**

The assay medium consisted of 0.25 M sucrose containing 50 mM phosphate buffer (pH 7.5), 1 mM HCHO, 1 mM NAD+ when added, and 1.5 mg of mitochondrial protein/ml in a total volume of 0.1 ml; reaction was initiated with mitochondria and incubated at 37°. The data were obtained from a 4-min time point. The flasks used in this experiment contained a center well to which were added the mitochondria; the assay medium was added to the main chamber. The flasks were stoppered and purified nitrogen (38) or oxygen was introduced via 23-gauge needles and PE 60 tubing for 15-min prior to mixing.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>HCHO consumption</th>
<th>Monoamine oxidase</th>
<th>Malate dehydrogenase</th>
<th>Aldehyde dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specfic activity</td>
<td>(nmol/mg mitochondrial protein)</td>
<td>(μmol/min)</td>
<td>(μmol/kat)</td>
</tr>
<tr>
<td>Aerobic (NA+ omitted)</td>
<td>80</td>
<td>110 (100)</td>
<td>39 (100)</td>
<td></td>
</tr>
<tr>
<td>Aerobic (+ NAD+)</td>
<td>135</td>
<td>4.5 (1)</td>
<td>3.5 (13)</td>
<td></td>
</tr>
<tr>
<td>Anaerobic (NA+ omitted)</td>
<td>33</td>
<td>127 (88)</td>
<td>62 (81)</td>
<td></td>
</tr>
<tr>
<td>Anaerobic (+ NAD+)</td>
<td>99</td>
<td>400 (100)</td>
<td>18 (100)</td>
<td></td>
</tr>
<tr>
<td>Whole mitochondria</td>
<td>115</td>
<td>635</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Outer membrane</td>
<td>106</td>
<td>4.5 (1)</td>
<td>3.5 (13)</td>
<td></td>
</tr>
<tr>
<td>Mitoplast</td>
<td>0.6</td>
<td>127 (88)</td>
<td>62 (81)</td>
<td></td>
</tr>
<tr>
<td>Inner membrane</td>
<td>—</td>
<td>400 (100)</td>
<td>18 (100)</td>
<td></td>
</tr>
<tr>
<td>Matrix</td>
<td>—</td>
<td>635</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

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* Nanomoles/min/mg of protein.
* Percentage of the total activity (specific activity × total protein).
* Dashes indicate below measurable levels.
* Malate dehydrogenase activity is known to be stimulated by Lubrol WX (28).
* Lubrol WX was found to inhibit aldehyde dehydrogenase activity by 40 to 60%.
Rat Liver Mitochondrial Aldehyde Dehydrogenase

**FIG. 7.** Effect of sodium succinate on mitochondrial formaldehyde oxidation. Increasing concentrations of succinate (adjusted to pH 7.5, prior to addition) were added to the incubation assay described in Fig. 1B.

**TABLE IV**

Effect of succinate and inhibitors of mitochondrial respiratory chain on pyridine nucleotide levels in rat liver mitochondria during HCHO oxidation

<table>
<thead>
<tr>
<th>Addition</th>
<th>Pyridine nucleotide levels (nmol/mg of protein)</th>
<th>NAD+/NADH ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAD⁺</td>
<td>NADH</td>
</tr>
<tr>
<td>None</td>
<td>4.77 ± 0.34</td>
<td>1.01 ± 0.35</td>
</tr>
<tr>
<td>Rotenone (1 µM)</td>
<td>2.81 ± 0.23</td>
<td>2.13 ± 0.33</td>
</tr>
<tr>
<td>Antimycin A (2 µM)</td>
<td>3.45 ± 0.21</td>
<td>1.72 ± 0.12</td>
</tr>
<tr>
<td>Succinate (10 mM)</td>
<td>3.50 ± 0.15</td>
<td>2.05 ± 0.31</td>
</tr>
<tr>
<td>Succinate &amp; antimycin A</td>
<td>1.43 ± 0.17</td>
<td>4.68 ± 1.21</td>
</tr>
</tbody>
</table>

- Values represent the mean of four different rat liver mitochondrial preparations, each run in duplicate ± S.E.

**TABLE V**

Effect of succinate on formaldehyde oxidation and pyridine nucleotide levels in rat liver mitochondria

<table>
<thead>
<tr>
<th>Addition</th>
<th>HCHO metabolism (% of control)</th>
<th>Pyridine nucleotide levels (nmol/mg of protein)</th>
<th>NAD⁺</th>
<th>NADH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NAD⁺</td>
<td>NADH</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Succinate</td>
<td>65</td>
<td>74</td>
<td>194</td>
<td></td>
</tr>
<tr>
<td>Malonate &amp; succinate</td>
<td>90</td>
<td>40</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>Malonate</td>
<td>100</td>
<td>40</td>
<td>180</td>
<td></td>
</tr>
</tbody>
</table>

- HCHO consumption in control mitochondria was 130 nmol/mg of protein/4 min.
- The levels of NAD⁺ and NADH were 4.78 and 0.85 nmol/mg of protein, respectively.

indicate a lack of succinate inhibition of formaldehyde oxidation. Thus, the succinate effect depends upon a functional respiratory chain and is not an action on the enzyme itself. Qualitatively, the respiratory chain inhibitors exerted similar effects as succinate on formaldehyde oxidation (Fig. 8), and in addition, each inhibitor caused a lowering of the intramitochondrial NAD⁺ with a concomitant elevation of the NADH (Table IV). However, quantitatively, there were apparent differences. When the formaldehyde-containing incubation medium contained both 10 mM succinate and 2 µM antimycin A, the lowest level of intramitochondrial NAD⁺ was attained, with a ratio to less than 0.5 (Table IV). Inhibition of formaldehyde oxidation (Fig. 8), however, was always slightly less than the rotenone-induced block, despite the fact that NAD⁺ levels were diminished more severely in the presence of succinate and antimycin A. To test whether rotenone exerted a direct effect on formaldehyde dehydrogenase, the enzyme was isolated from mitochondria and found to be sensitive to rotenone.

Acetaldehyde Oxidation by Rat Liver Mitochondria—Acetaldehyde, a product of various drugs oxidized by the mixed function oxidase system and of ethanol metabolism, has been shown to be metabolized largely in the mitochondria (9, 24). Using the same incubation conditions employed to measure formaldehyde oxidation, the rate of acetaldehyde metabolism was similar (Fig. 9), and in the presence of NAD⁺, acetaldehyde oxidation was also stimulated (not shown); succinate also inhibited oxidation of acetaldehyde.

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**Fig. 8.** Effect of succinate and respiratory chain inhibitors on mitochondrial oxidation of formaldehyde. The incubation assay contained 250 mM sucrose, 50 mM phosphate (pH 7.5), and 0.1 mM HCHO (O--O); plus 10 mM succinate (●●●●●); plus 2.0 µM antimycin A (A—A); both 10 mM succinate and 2.0 µM antimycin A (A—–A), and rotenone (■■■); mitochondrial protein was 1 mg/ml.

**Fig. 9.** Rate of acetaldehyde oxidation as a function of the amount of mitochondrial protein in the presence and absence of succinate. Incubation medium was the same as that described in Fig. 1B except that formaldehyde was replaced by 0.2 mM acetaldehyde; (O--O) absence and (●—●) presence of 10 mM succinate. Oxidation of acetaldehyde was measured by the procedure of Gupta and Robinson (33).
Conclusions and Significance—The present study was undertaken to characterize the biochemical properties of intact mitochondrial NAD⁺-linked aldehyde dehydrogenase, utilizing formaldehyde as the substrate. This aldehyde is important because it is one of the products of methanol oxidation (41-43) as well as a product of hepatic microsomal dealkylations. Toxicities associated with it (e.g., Ref. 44), as well as the propensity of aldehydes to react with proteins enhance the significance of an enzyme capable of oxidizing formaldehyde (as well as other aldehydes). In a recent report, we found that aldehydes produced in endoplasmic reticulum fragments during oxidative dealkylation of drugs were transferred directly to the mitochondria without accumulating in the assay medium. This would imply that a direct transfer process occurs intracellularly, as by membrane to membrane alignment.

Earlier suggestions that the mitochondrial aldehyde dehydrogenase is linked to the electron transport chain have been confirmed with our demonstration that inhibitors of this chain block the consumption of formaldehyde. Similarly, the mechanism of succinate interference with mitochondrial aldehyde oxidation has been determined and shown to be due to reverse electron transport. The ramifications of this finding are clear. The presence of large amounts of succinate intracellularly can cause the accumulation of potentially toxic aldehydes. A scheme which may hold promise for aiding in depletion of toxic aldehydes from the body would be to make oxalacetate available to aid in rapid reoxidation of mitochondrial NADH.

REFERENCES

Biochemical properties of rat liver mitochondrial aldehyde dehydrogenase with respect to oxidation of formaldehyde.

D L Cinti, S R Keyes, M A Lemelin, H Denk and J B Schenkman


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