**Carnitine Biosynthesis**

**PURIFICATION OF 4-N-TRIMETHYLAMINOBUTYRALDEHYDE DEHYDROGENASE FROM BEEF LIVER**

(Received for publication, June 18, 1979, and in revised form, October 3, 1979)

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- **4-N-Trimethylaminobutyrate** is formed during carnitine biosynthesis by the oxidation of 4-N-trimethylaminobutyraldehyde. The aldehyde dehydrogenase which catalyzes this reaction has been isolated from bovine liver. This enzyme was purified to homogeneity, as judged by sodium dodecyl sulfate polyacrylamide gel electrophoresis, using two affinity type columns. Blue dextran and 5'-AMP covalently linked to a Sepharose matrix were used to bind this NAD⁺-requiring enzyme. Two other aldehyde dehydrogenases with broader specificities were also purified to homogeneity using the same affinity columns. The three enzymes appear to be distinct as they are different with respect to subcellular locations, substrate specificity, behavior on the affinity columns, disulfiram inhibition, and esterase activity. The enzyme with preference for trimethylaminobutyraldehyde as substrate probably functions in carnitine biosynthesis.

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The formation of L-carnitine (3-hydroxy-4-N-trimethylamino butyrate) from 6-N-trimethyl-L-lysine has been studied extensively in recent years and the biosynthetic route appears to be well established. LaBadie et al. (1) indicated that at least in the rat, trimethyllysine arises from the turnover of proteins containing this amino acid. They showed that asialofetuin, which had been modified by methylation of the ε-amino groups of lysine, was taken up and degraded by a perfused liver and the freed trimethyllysine served as a precursor of carnitine. However, the same protein containing monomethyl- and dimethyllysyl residues, although degraded, did not give rise to carnitine. Thus, it appears that lysine must be fully methylated when part of the protein. On the other hand, Rebouche and Broquist (2) described an enzyme from *Neurospora crassa* that would methylate free lysine to yield trimethyllysine. Borum and Broquist (3) have isolated this enzyme and found it to sequentially methylate lysine, monomethyllysine, and dimethyllysine to yield trimethyllysine. In both systems the trimethyllysine produced serves as a precursor for carnitine.

The conversion of trimethyllysine to 4-N-trimethylaminobutyrate was postulated to occur via hydroxylation, aldol cleavage, and oxidation of the resulting aldehyde (4, 5). The evidence for the hydroxylation and aldol cleavage have been published (5) and this report deals with the enzyme which catalyzes the oxidation of 4-N-trimethylaminobutyraldehyde to the corresponding acid. Lindstedt and Lindstedt (6) have shown that the trimethylaminobutyrate so produced is hydroxylated to produce carnitine. The biosynthetic pathway for the formation of carnitine from lysine and methionine has thus been fully elucidated.

The dehydrogenase responsible for the oxidation of trimethylaminobutyraldehyde to trimethylaminobutyrate during this process has not previously been described. Kaufman and Broquist (7) have reported that the aldehyde is an intermediate in carnitine biosynthesis as a result of trapping experiments using *Neurospora*. The involvement of this aldehyde was also shown in our previous report (5). Described herein is the isolation and characterization of an aldehyde dehydrogenase from bovine liver that prefers trimethylaminobutyraldehyde as substrate. This enzyme has been shown to be distinct from two other aldehyde dehydrogenases that have broader substrate specificity. This aldehyde dehydrogenase was isolated from the cytoplasmic fraction of beef liver and was not detected in either the mitochondrial or microsomal fractions.

**EXPERIMENTAL PROCEDURES**

**Materials**

Bovine liver was a gift from the Bartusch Packing Co., St. Paul, Minn. The following were purchased from Sigma Chemical Co.: disulfiram, phenylmethylsulfonyl fluoride, 5'-AMP-Sepharose, bovine serum albumin, yeast aldehyde dehydrogenase, and the molecular weight markers for sodium dodecyl sulfate gels (albumin-bovine, albumin-egg, pepsin, trypsinogen, β-lactoglobulin, and lysozyme). Aldrich Chemical Co. was the supplier of iodomethane, 4-aminobutyraldehyde diethyl acetal, pyrazole, and the Bio-Rad Protein Assay Kit. The following were purchased from Bio-Rad Lab. Blue dextran, Sepharose 4B, and Sephadex G-200 were products of Pharmacia Fine Chemicals, and cyanogen bromide was purchased from Eastman Kodak Co. New England Nuclear was the supplier of [U-¹⁴C]leucine (25 μCi/μmol), and [methyl-¹⁴C]iodide (56 μCi/μmol) was purchased from Amersham Corp. The standards for gel filtration chromatography (cytochrome c, myoglobin, albumin-egg, albumin-bovine, γ-globulin, and asparaginase) were purchased from Schwarz-Mann. 4-N-Trimethylaminobutyraldehyde diethyl acetal was synthesized as previously described (5). All other chemicals used were reagent grade.

**Methods**

**Enzymatic Assays**

The aldehyde dehydrogenases were assayed either spectrophotometrically using a Beckman model 25 recording spectrophotometer or fluorometrically using a Perkin Elmer MPF-44A recording fluorescence spectrophotometer by following the appearance of NADH. For the spectrophotometric assay the increase in absorbance at 340 nm was measured. The 1.0-ml reaction mixture contained 0.25 mM NAD⁺, and enzyme sample in 0.1 M sodium pyrophosphate buffer, pH 9.0. The reaction was started by adding either 4-N-trimethylaminobutyraldehyde diethyl acetal or NAD⁺ to the reaction mixture.
aldehyde to a final concentration of 90 μM for the enzyme which preferred trimethylaminobutylaldehyde as substrate or propionaldehyde to a final concentration of 0.5 mM for the enzymes with the broad substrate specificity. The activity was calculated using the molar extinction coefficient of NADH as 6220. The fluorometric assay measured NADH using excitation at 340 nm and measuring the fluorescence at 460 nm. Standard solutions of NADH were used to calibrate the fluorometer. The assay mixture was identical with that used for the spectrophotometric assay. Disulfiram inhibition studies were done using the spectrophotometric assay. Disulfiram was dissolved in ethanol and then mixed with the pyrophosphate buffer such that the final concentration of disulfiram and ethanol were 40 μM and 25%, respectively.

Esterase activity of the aldehyde dehydrogenases was measured by a procedure similar to that reported by MacGibbon et al. (8). Cleavage of the ester, p-nitrophenyl acetate, was followed spectrophotometrically at 400 nm. The reaction mixture contained 50 μM p-nitrophenyl acetate, 0.25 mM NAD⁺, and enzyme, in a total volume of 1.0 ml using 0.1 M sodium pyrophosphate buffer, pH 7.3.

Protein Determination

Protein was estimated either from the absorbances at 260 and 280 nm using the method of Warburg and Christian (9), or by using a Bio-Rad Protein Assay Kit. The Bio-Rad assay is based on the binding of a dye to proteins (10). Bovine serum albumin was used to generate a standard curve.

Purification of Aldehyde Dehydrogenases

Fresh bovine liver was obtained from the slaughter house. Fifty grams of tissue were cut into small pieces and homogenized with 100 ml of a solution containing 0.25 M sucrose, 0.05% bovine serum albumin, and 20 mM 4-(2-hydroxethyl)-1-piperazineethanesulfonic acid buffer, pH 6.8, using a Potter-Elvejhem homogenizer. These and all subsequent steps were done at 4°C. The homogenate was made by first homogenizing with a pestle having 0.026 inch clearance and then with a pestle having 0.012 inch clearance as described by Fleischer and Kervina (11). The subcellular fractions were then isolated as previously described (5). The microsomes were not routinely separated from cytosolic enzymes, but separate experiments have shown that the aldehyde dehydrogenase is not located in the microsomes. The mitochondrial pellet was resuspended in 10 ml of a solution which contained 0.1% 2-mercaptoethanol, 1.0 mM EDTA, 30 mM sodium phosphate buffer, pH 6.0, and then glycerol was added to 20% (v/v) to stabilize the enzyme.

Blue dextran-Sepharose was prepared by covalently attaching blue dextran to a cyanogen bromide-activated Sepharose 4B matrix using the method described by Ryan and Vestling (12). The mitochondrial sample was placed on a column containing 14 ml of this modified Sepharose which had been equilibrated with the pH 6.0 buffer used to suspend the mitochondria. This column was developed with equilibration buffer containing 1) no additions, 2) 0.1 M NaCl, 3) 0.3 M NaCl, 4) 1.0 M NaCl, and 5) 1.0 mM NAD⁺. The column eluate was monitored for protein by measuring the absorbance at 280 nm using a Gilson 360-12 double beam chromatographic UV monitor. The 5-ml fractions were collected and every second fraction was assayed for aldehyde dehydrogenase activity using both propionaldehyde and trimethylaminobutylaldehyde as substrates. The activity present in the NAD⁺ eluate was pooled and concentrated using an Amicon ultrafiltration membrane. The column was recycled by washing it with 8 M urea and reequilibrating with buffer.

The cytosol fraction was placed on a 70 ml blue dextran-Sepharose column which was then developed using the same buffers as with the mitochondrial sample. The aldehyde dehydrogenase in the void volume of the column was concentrated 3-fold using the Amicon cell. The enzyme was then eluted with the 0.3 M buffer and concentrated to about 10 ml. The concentrated sample from the void volume was placed on a column of 5'-AMP-Sepharose (bed volume, 22 ml). The column was prepared and developed as described by Greenfield and Pietrusko (13). The enzymatic activity present in the pH 6.0 buffer eluted, concentrated as before, and stored at -80°C. This column was also recycled by washing it with 8 M urea and reequilibrating with buffer.

The enzyme from the 0.3 M NaCl eluate of the blue dextran-Sepharose column was repeatedly filtered in an Amicon cell until the final NaCl concentration was less than 3 mM. The sample was then placed on a 5'-AMP-Sepharose column which had been equilibrated with 30 mM sodium phosphate buffer at pH 8.0 containing 0.1% 2-mercaptoethanol and 1.0 mM EDTA. The column was developed with equilibration buffer then with the same buffer containing 1.0 M NaCl. The protein and enzyme activity were again monitored in the eluate, and the active fractions were pooled, concentrated, and stored at -80°C.

Characterization of the Dehydrogenases

Sephadex G-200 Gel Filtration—Sephadex G-200 was suspended in 0.1% 2-mercaptoethanol, 1.0 mM EDTA, 0.2 M NaCl, 30 mM sodium phosphate, pH 6.8, and allowed to swell overnight. A gel filtration column (1.8 x 92.5 cm) was equilibrated with the same buffer and standardized using blue dextran, [U-14C]leucine, cytochrome c, myoglobin, albumin (egg), albumin (bovine), γ-globulin, and apoferritin. The molecular weights of the samples were estimated from the calibration curve made by plotting Kᵥ versus log of the molecular weight as described by Andrews (14).

Polyacrylamide Gel Electrophoresis—High pH (about 9.5) gels (7.5%) were run at 30 mA/tube by the procedure of Ornstein (15) and Davis (16) and Dextran Blue dextran-Sepharose was prepared by covalently attaching blue dextran to Sepharose which had been equilibrated with the pH 6.0 buffer used to suspend the mitochondria. There was no activity toward trimethylaminobutylaldehyde, but not trimethylaminobutyraldehyde. A dehydrogenase which acts on trimethylaminobutylaldehyde was isolated from bovine liver. In the process two other aldehyde dehydrogenases with broader substrate specificities were also purified. The mitochondrial fraction exhibited no activity toward tri- methylaminobutyraldehyde, but there was some activity toward other aldehydes. Purification of the mitochondrial enzyme was accomplished using a blue dextran-Sepharose column (Fig. 1). Although a portion of the enzymatic activity was not bound to the column, the activity in the void volume (Fig. 1) was retained when rechromatographed on a second blue dextran column. There was no activity toward trimethylaminobutyraldehyde in any of the fractions from these columns. The mitochondrial enzyme was not released when 1.0 M NaCl was added to the elution buffer but it did elute from the column with 1.0 M NaCl. The cytosolic fraction from the bovine liver contained both non-specific and specific (towards trimethylaminobutyraldehyde) aldehyde dehydrogenases. Both of these activities had an absolute requirement for NAD⁺. These enzymes were separated on a blue dextran-Sepharose column (Fig. 2). The non-specific enzyme was not retained by the column, while the specific enzyme emerged after 0.3 M NaCl was added to the
buffer. A considerable amount of activity toward trimethylaminobutyaldehyde was also found in one tube after adding 1.0 M NaCl to the elution buffer. This was probably the result of stripping the column of any residual activity not released by the 0.3 M NaCl. No aldehyde dehydrogenase of either type was found in the 1.0 mM NAD⁺ eluate. Fractions 14 to 26 (Fig. 2), containing the nonspecific enzyme, were placed onto a 5'-AMP-Sepharose column (Fig. 3). The activity was retained by the column and eluted from it when the buffer was changed as indicated. Fractions 160 to 205 (Fig. 2), containing the specific enzyme, were also fractionated on a 5'-AMP-Sepharose column. When this sample was added to a column that had been equilibrated with pH 6.0 buffer, as with the previous enzyme, the activity appeared in the void volume along with a large peak of protein and no purification was achieved. When the column was equilibrated and developed at pH 8.0, the enzyme activity was largely separated from contaminating proteins (Fig. 4). It was also observed that a more purified sample of the same enzyme would bind to a column at pH 8.0. It eluted with 1.0 M NaCl, but no further purification was achieved.

A summary of the purification of the three aldehyde dehydrogenases is presented in Table I. There was no nonspecific aldehyde dehydrogenase activity found in the sucrose homogenates and in some cases a decrease in absorbance at 340 nm was observed. This oxidation of NADH was not observed

![Fig. 1. Isolation of the mitochondrial nonspecific aldehyde dehydrogenase using a blue dextran-Sepharose column.](http://www.jbc.org/)

![Fig. 2. Separation of the cytosolic specific and nonspecific aldehyde dehydrogenases using a blue dextran-Sepharose column.](http://www.jbc.org/)

![Fig. 3. Purification of the cytosol nonspecific enzyme on a 5'-AMP-Sepharose column.](http://www.jbc.org/)

![Fig. 4. Purification of the cytosol specific enzyme on a 5'-AMP-Sepharose column.](http://www.jbc.org/)

...after isolating mitochondria or in the dialyzed mitochondrial supernatant. The various stages of purification as visualized on high pH gels are shown in Fig. 5.

**Characterization of Aldehyde Dehydrogenases**—The purified samples represented in gels 3, 5, and 6 of Fig. 5 (Samples 7, 9, and 3, Table I) were used for further characterization of the enzymes. Several techniques, including DE52 column chromatography, a second blue dextran-Sepharose column, and Sephadex G-200 chromatography were used to try to eliminate the smear below the band on the gel of the cytosol, specific enzyme (gel 5, Fig. 5). However, none of these resulted in a preparation showing any change in gel pattern. Sample 9 (Table I) was assayed for proteolytic activity by the procedure of Gade and Brown (20), but none was detected after 24 h of incubation. High pH gels were run with substrate (NAD⁺, NADH, or trimethylaminobutyaldehyde) added, but the pattern remained the same. Varying the concentration of sample added to the gel also had no effect. Using the activity stain previously described for these gels, a single band was seen on each gel, which corresponded with the single protein band for
TABLE 1

| Sample and description | Activity on trimethylaminobutyraldehyde | Activity on Propionaldehyde |
|------------------------|------------------------------------------|----------------------------|
|                        | mg | units* | specific activity units/mg protein | mg | units* | specific activity units/mg protein |
| 1. 600 x g supernatant | 1538 | 63.5 | 0.041 | 0 | 0 |
| 2. Mitochondria | 233 | 0 | 0 | 2.64 | 0.016 |
| 3. Fractions 275-290 (Fig. 2) from blue dextran separation of Sample 2 | 2.21 | 0 | 0 | 1.28 | 0.58 |
| 4. 6100 x g supernatant (cytosol) | 1090 | 65.1 | 0.060 | 0 | 0 |
| 5. 40% (NH₄)₂SO₄ supernatant of Sample 4 | 1030 | 48.3 | 0.047 | 14.3 | 0.014 |
| 6. Fractions 14-26 (Fig. 2) from blue dextran separation of Sample 5 | 426 | 0.82 | 0.0019 | 5.75 | 0.0135 |
| 7. Fractions 48-60 (Fig. 3) from 5'-AMP separation of Sample 6 | 13.1 | 0.73 | 0.056 | 3.0 | 0.23 |
| 8. Fractions 60-205 (Fig. 2) from blue dextran separation of Sample 5 | 9.6 | 15.2 | 1.58 | 0 | 0 |
| 9. Fractions 14-39 (Fig. 4) from 5'-AMP separation of Sample 8 | 2.9 | 14.8 | 5.10 | 0.61 | 0.21 |

* Protein was measured with the Bio-Rad Assay Kit and by the A₂₈₀ method for these samples, protein for all other samples was measured by the A₂₈₀ method only.

** One unit equals the enzyme that will produce 1 μmol of NADH/min.

Fig. 5. Purification of aldehyde dehydrogenases shown by polyacrylamide gels. The gels were run at high pH (about 9.5) and stained for protein. Gel 1, 40% (NH₄)₂SO₄-treated cytosol fraction (Sample 5, Table 1); gel 2, pool of void volume from the cytosol fraction separated on the blue dextran-Sepharose (Sample 3, Table 1); gel 3, purified cytosol nonspecific dehydrogenase from 5'-AMP-Sepharose column (Sample 5, Table 1); gel 4, pool of 0.3 M NaCl wash from the cytosol fraction separated on the blue dextran-Sepharose column Sample 8, Table 1); gel 5, purified cytosol trimethylaminobutyraldehyde-specific dehydrogenase from 5'-AMP-Sepharose column (Sample 9, Table 1); gel 6, purified mitochondrial nonspecific dehydrogenase from a 5'-AMP-Sepharose column (Sample 3, Table 1).

Fig. 6. Sodium dodecyl sulfate gel electrophoresis of the aldehyde dehydrogenases. Sodium dodecyl sulfate gels of (1) the cytosol-specific, (2) cytosol nonspecific, and (3) mitochondrial nonspecific enzymes were run. A gel of standards (4) is shown with molecular weights given in parentheses.

the two nonspecific enzymes and with the band above the smear for the specific enzyme.

Sodium dodecyl sulfate gel electrophoresis of these enzymes indicated the presence of a single protein in each (Fig. 6). The same band was obtained for each individual sample whether they were treated with sodium dodecyl sulfate in the presence or absence of 2-mercaptoethanol. Another gel (not shown) was made for each sample with the standards present to facilitate the estimation of molecular weight. A calibration curve for these gels was generated using the mobilities of the standard proteins. The subunit molecular weights were estimated as 55,000, 56,500, and 55,100 for the cytosol-specific, cytosol nonspecific, and mitochondrial enzyme, respectively.

Molecular weights of the three proteins were also estimated using Sephadex G-200 chromatography. The calculated molecular weights were 160,000, 149,000, and 155,000 for the cytosol-specific, cytosol nonspecific, and mitochondrial enzymes, respectively. Each eluted from the gel filtration column in a sharp band with the absorbance at 280 nm corresponding with the enzymatic activity. Using various fractions spanning the protein peak of the cytosol-specific enzyme from the column, high pH gels were made and stained for protein. The resulting pattern was the same (i.e. a band and a smear) for each fraction regardless of its position in the peak.

Kinetic parameters of the three purified enzymes were determined using the spectrophotometric assay. Data obtained from Lineweaver-Burk (21) plots indicated that all three enzymes have high affinity for their aldehyde substrates as can be seen from the Kₘ values determined (Fig. 7). The activities were also determined at a variety of pH values as shown in Fig. 8. The activity of the three enzymes toward a variety of substrates was tested. The results are summarized in Table II along with other characteristics of the three enzymes. Pyridoxal and pyridoxal 5'-phosphate were not substrates for any of these enzymes.

Because of the report of Hinson and Neal (22), that alcohol dehydrogenases can also function as aldehyde dehydrogenases, these aldehyde dehydrogenases were assayed for alcohol dehydrogenase activity. The assays were done with the same aldehydes as substrates, but NADH was used in place of NAD⁺. No oxidation of NADH was detected. Hinson and Neal also found that the aldehyde dehydrogenase from horse liver which they studied, could be blocked with the alcohol...
dehydrogenase inhibitor, pyrazole. Attempts to inhibit the three aldehyde dehydrogenases described here with pyrazole at concentrations up to 0.1 M were unsuccessful. The reversibility of the specific enzyme was examined using trimethylaminobutyrate and NADH, but no aldehyde was produced. Trimethylaminobutyrate was also ineffective as an inhibitor of the forward reaction.

Disulfiram, a known inhibitor of some aldehyde dehydrogenases (13, 23), was tested as an inhibitor of these three enzymes at pH 9 (Table II). Of the activity of the specific enzyme of the cytosol, 70% remained when 40 μM disulfiram was present in the reaction mixture. Only 17% of the cytosol nonspecific enzyme activity remained with disulfiram at this concentration, but there was no inhibition of the mitochondrial enzyme by this compound. Another reported character-

istic of aldehyde dehydrogenases is their esterase activity, as displayed by their ability to cleave p-nitrophenyl acetate (8, 24). Esterase assays were conducted at pH 7.3 because the ester bond is weakened and will spontaneously hydrolyze at high pH values. Both of the nonspecific aldehyde dehydrogenases showed considerable esterase activity under these conditions, but the specific enzyme displayed no esterase activity (Table II).

DISCUSSION

In attempting to locate the aldehyde dehydrogenase that would convert trimethylaminobutyraldehyde to trimethylaminobutyrate, the commercially available, K⁺-activated, yeast aldehyde dehydrogenase was tested. Although this enzyme has been shown to have a broad substrate specificity (25), it would not act on trimethylaminobutyraldehyde. However, it was found that crude homogenates of bovine liver contain considerable activity toward this substrate. Purification of this activity showed that it was associated with the cytosol rather than the mitochondria. In addition to this specific enzyme, two nonspecific aldehyde dehydrogenases were detected. Crude preparations often showed a negative reaction rate during the assay (i.e. decrease in absorbance at 340 nm) which was probably due to the presence of alcohol dehydrogenases and sufficient endogenous NADH so that addition of propionaldehyde resulted in its reduction to propanol. Dialysis of such samples eliminated this activity. The aldehyde dehydrogenases were found to be quite unstable and unless 2-mercaptoethanol and glycerol were added to all of the buffers. With this treatment the activity remained fairly stable at 4°C, but storage for several weeks at this temperature resulted in loss of most of the activity. At -80°C the enzymes retained their activity for several months. Phenylmethysulfonylfluoride, a protease inhibitor, was of no value in stabilizing the specific dehydrogenase.

Two affinity chromatographic techniques, blue dextran-Sepharose and 5'-AMP-Sepharose column chromatography, were used to purify the three aldehyde dehydrogenases. These methods have been used for a number of NAD⁺-requiring enzymes (12, 13). They allowed rapid purification of these unstable enzymes. The mitochondrial enzyme binds very tightly to the blue dextran-Sepharose column (Fig. 1). This suggested that it has a high affinity for NAD⁺, which was supported by the fact that after the enzyme was eluted from the column with NAD⁺, the bound cofactor could not be

![Fig. 7. Lineweaver-Burk plot.](image)

![Fig. 8. pH optimum of the aldehyde dehydrogenases.](image)

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**Table II: Summary of aldehyde dehydrogenase properties**

| Blue dextran column | Relative activities | Molecular weight | Esterase activity | Summary of aldehyde dehydrogenase properties |
|---------------------|--------------------|------------------|------------------|---------------------------------------------|
| Blue dextran        | 0.3 M NaCl         | 340              | -                | Cytosol-specific enzyme                      |
| Relative activities | 1.0                | 55,000           | -                | Cytosol nonspecific enzyme                   |
| Propionaldehyde     | 1.00               | 55,100           | +                | Mitochondrial nonspecific enzyme             |
| Trimethylaminobutyraldehyde | 25.0 | 55,500 | +               |                                             |
| Acetaldehyde        | 1.00               | 56,500           | +                |                                             |
| Butyraldehyde       | 0.24               | 55,000           | +                |                                             |
| Benzaldehyde        | 0.15               | 55,100           | +                |                                             |
| Kₚ (μM)             | 4.17               | 55,500           | +                |                                             |
| Vₘ₉ₘ (units/mg)     | 5.77               | 55,100           | +                |                                             |
| pH optimum           | 9.5-9.8            | 155,000          | +                |                                             |
| Inhibition by disulfiram | 30%              | 150,000          | +                |                                             |
| Esterase activity   | -                  | 149,000          | +                |                                             |
| Molecular weight    | 160,000            | 55,000           | -                |                                             |
| Sephadex G-200      | 149,000            | 55,600           | +                |                                             |
| Sodium dodecyl sulfate | 155,000         | 55,100           | +                |                                             |

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**Notes:**

- **(A-A)**: Cytosolic trimethylaminobutyraldehyde (TMABA)-specific dehydrogenase.
- **B**: Cytosolic nonspecific aldehyde dehydrogenase.
- **C**: Mitochondrial nonspecific aldehyde dehydrogenase.
removed by exhaustive dialysis. The other nonspecific dehydrogenase, from the cytosol, displayed a much different behavior on the blue dextran-Sepharose column (Fig. 2). It was not retained when applied to the column under the same conditions as used for the mitochondrial enzyme. The specific dehydrogenase, like the mitochondrial enzyme, was retained on the blue dextran-Sepharose, but not as tightly. It was released with 0.3 M NaCl, whereas the mitochondrial enzyme was not released even with 1.0 M NaCl.

The specific enzyme did not bind to the 5'-AMP column equilibrated at pH 6.0, whereas both nonspecific enzymes were bound under these conditions. This is evidence that the activity on trimethylaminobutyraldehyde, found with the purified cytosol nonspecific enzyme from a 5'-AMP-Sepharose column (Fig. 3), resulted from activity of the nonspecific dehydrogenase, and not contaminating specific dehydrogenase. While the specific dehydrogenase would not bind at pH 6.0, equilibration of the 5'-AMP column with pH 8.0 buffer allowed the specific dehydrogenase to be retarded sufficiently to separate it from the contaminating protein (Fig. 4). In some cases, where the specific dehydrogenase was purified prior to being applied to the 5'-AMP-Sepharose column, it bound to the column at pH 8.0 and elution of a sharp peak of activity required buffer containing 1.0 M NaCl. Even the purified specific enzyme would not bind to the column at pH 6.0, as did the nonspecific dehydrogenases.

The two nonspecific dehydrogenases, isolated as described above, showed single bands on high pH gels (about 9.5) gels when stained either for protein or propionaldehyde activity. Neither gave a positive activity stain when trimethylaminobutyraldehyde was used in place of propionaldehyde. They also gave single bands on sodium dodecyl sulfate gels (Fig. 6), and the presence or absence of 2-mercaptoethanol, made no difference, indicating that there are no disulfide linkages between subunits. The specific dehydrogenase gave similar results on sodium dodecyl sulfate gels (Fig. 6), and a single band on high pH gels when stained for activity with trimethylaminobutyraldehyde. However, when the high pH gels of the specific enzyme were stained for protein (Fig. 5, gel 5), a band as well as a smear was observed. The smear was not the result of proteolytic activity in the sample, since tests for such activity were negative. A second blue dextran-Sepharose column using a 0.1 to 0.3 M NaCl gradient, a Sephadex DE52 column, and a Sephadex G-200 column were used in an effort to eliminate proteolytic activity in the sample, since tests for such activity were negative. A second blue dextran-Sepharose column using a 0.1 to 0.3 M NaCl gradient, a Sephadex DE52 column, and a Sephadex G-200 column were used in an effort to eliminate the smear of protein which migrated faster than the discrete band observed. Single protein peaks that coincided with the enzymatic activity were obtained from all three of these systems. When samples from the leading edges of the peaks, within the peaks, and the trailing edges of the peaks were used on high pH gels, the gel pattern remained equally diffuse. This is consistent with the view that this protein is homogeneous as suggested by the sodium dodecyl sulfate gel patterns.

The possibility of a dissociating-reassociating process of oligomeric forms of the enzyme was considered. However, changing the concentration of the sample electrophoresed or adding the substrates, NAD⁺, NADH, or trimethylaminobutyraldehyde, did not alter the gel pattern. A series of gels made from Sample 9 in Table I were sliced into segments. The segments of gel containing the top band were combined and segments containing the smear were combined. The protein was eluted from each using the same buffer in which it originally was applied to the gel and the two fractions were rerun on a second set of gels. The protein pattern for these gels showed major bands that was identical for both samples. The fraction that was eluted from the segments with the smear had some smear remaining on the second gel. However, the major band that was produced by each sample on the second gel was at a different location than previously observed. It ran almost three times as far as the original band. Incubations of the original enzyme sample with the various components of the gel also resulted in appearance of new smears on the gel. Therefore, it appears that the complexity of the protein pattern is caused by the interaction of a homogeneous protein with components of the gel, rather than heterogeneity of the protein.

Besides the difference in their behavior on the blue dextran and 5'-AMP-Sepharose columns and their subcellular locations, these three enzymes were shown to be distinct by various other parameters as summarized in Table II. The main differences are in their substrate specificities, their inhibition by disulfiram, and their esterase activity. It was reported (23) that a mitochondrial, nonspecific enzyme from horse liver was not inhibited by disulfiram, whereas the cytosolic enzyme was, and that the mitochondrial enzyme had a lower Kᵢ for acetalddehyde than the cytosolic enzyme. Similar results were reported for isozymes from human liver (13). The results of this study are in agreement with those findings. While the three enzymes described here are very similar by many criteria, they are sufficiently different to be considered distinct enzymes.

The dehydrogenase displaying specificity toward trimethylaminobutyraldehyde apparently functions in carnitine biosynthesis. Enzymes which catalyze all of the reactions in the biosynthesis of carnitine have now been identified, and all except one, trimethyllysine hydroxylase, are found in the cytosol fraction of the liver.

REFERENCES
1. LaBadie, J., Dunn, W. A., and Aronson, N. N. (1976) Biochem. J. 160, 85-95
2. Rebouche, C. J., and Broquist, H. P. (1976) J. Bacteriol. 126, 1207-1214
3. Borum, P. R., and Broquist, H. P. (1977) J. Biol. Chem. 252, 5651-5655
4. Hochalter, J. B., and Henderson, L. M. (1976) Biochem. Biophys. Res. Commun. 70, 364-366
5. Hulse, J. D., Ellis, S. R., and Henderson, L. M. (1978) J. Biol. Chem. 253, 1564-1569
6. Lindstedt, G., and Lindstedt, S. (1970) J. Biol. Chem. 245, 4178-4186
7. Kaufman, R. A., and Broquist, H. P. (1977) J. Biol. Chem. 252, 7437-7439
8. MacGibbon, A. K. H., Haylock, S. J., Buckley, P. D., and Blackwell, L. F. (1978) Biochem. J. 171, 533-538
9. Warburg, O., and Christian, W. (1942) Biochem. J. 280, 384-421
10. Bradford, M. M. (1976) Anal. Biochem. 72, 248-254
11. Fleischer, S., and Kervina, M. (1974) Methods Enzymol. 31, 3-41
12. Ryan, L. D., and Vestling, C. S. (1974) Arch. Biochem. Biophys. 109, 279-284
13. Greenfield, N. J., and Pietruszko, R. (1976) Biochim. Biophys. Acta 483, 35-45
14. Andrews, P. (1966) Biochem. J. 91, 222-233
15. Ornstein, L. (1964) Ann. N. Y. Acad. Sci. 121, 321-349
16. Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 404-427
17. Blakely, R. W., and Boezi, J. A. (1977) Anal. Biochem. 82, 580-582
18. Jacobson, M. K., and Bernofsky, C. (1974) Biochim. Biophys. Acta 350, 277-291
19. Weber, K., and Osborn, M. (1968) J. Biol. Chem. 244, 4406-4412
20. Rebouche, C. J., and Broquist, H. P. (1976) J. Biol. Chem. 251, 5013-5018
21. Segel, I. H. (1976) Biochemical Calculations, 2nd Ed, p. 234, John Wiley and Sons, Inc., New York
22. Hinson, J. A., and Neal, R. A. (1972) J. Biol. Chem. 247, 7106-7107
23. Eftestol, J., Mope, L., Takio, K., and Yonetani, T. (1976) J. Biol. Chem. 251, 236-240
24. Feldman, R. I., and Weiner, H. (1972) J. Biol. Chem. 247, 267-272
25. Lundquist, F. (1968) Biochem. J. 68, 172-177
Carnitine biosynthesis. Purification of 4-N'-trimethylaminobutyraldehyde dehydrogenase from beef liver.
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J. Biol. Chem. 1980, 255:1146-1151.

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