4-N-Trimethylaminobutyraldehyde Dehydrogenase: Purification and Characterization of an Enzyme from *Pseudomonas* sp. 13CM

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4-N-Trimethylaminobutyraldehyde dehydrogenase from *Pseudomonas* sp. 13CM was purified 14-fold to apparent homogeneity by hydrophobic chromatography on a Phenyl-Toyopearl, and affinity chromatography was done on a 5'-AMP Sepharose4B in the presence of dithiothreitol. The enzyme was found to be a trimer with identical 55 kDa subunits. The isoelectric point was found to be 5.5. The optimum temperature and pH were 40°C and pH 10.0. The purified enzyme was further characterized with respect to substrate specificity, kinetic parameters, and analog inhibition. The *Km* values for 4-N-trimethylaminobutyraldehyde, 4-dimethylaminobutyraldehyde, and NAD*⁺* were 7.4, 51, and 125 μM respectively. The enzyme was inhibited by SH reagents, and by heavy metal ions.

**Key words:** aldehyde dehydrogenase; analog inhibitor; biosynthesis; carnitine; quaternary ammonium compound

Naturally occurring quaternary ammonium compounds constitute a class of metabolites with more than 100 reported examples, including choline and L-carnitine.11 Choline or its metabolites are required for synthesis of phospholipids in cell membranes, methyl group metabolism and cholineric neurotransmission, and transmembrane signaling, as well as for lipid-cholesterol transport and metabolism.21 L-Carnitine is a highly polar zwitterionic quaternary amine carboxylic acid present in some prokaryotes and all eukaryotes.3) During growth on D-carnitine, carnitine racemase and L-carnitine dehydrogenase activity were detected in the cell-free extract of *Pseudomonas* sp. AK1.14) *Acinetobacter calcoaceticus* is able to split the C-N bond of L- and D-carnitine to yield trimethylamine.15) Monoxyenase-catalyzed cleavage of L-carnitine resulting in the formation of trimethylamine and malic acid has been postulated.16)

To elucidate the structure and function of quaternary ammonium compound-degrading enzymes, we took up 4-N-trimethylaminol-1-butanol (TMA-Butanol), which shows a considerable structural resemblance to choline as a theme. A microorganism identified as *Pseudomonas* sp. 13CM was isolated from soil, and a new enzyme, NAD⁺*-*dependent 4-N-carnitine dehydrogenase (EC.1.1.1.108), which has been purified and characterized from various bacteria.6–9) 3-Dehydrocarnitine formed by L-carnitine dehydrogenase was degraded to betaine by the addition of NAD⁺, adenosine triphosphate, and coenzyme-A.10) Enterobacteriaceae such as *Escherichia coli*, *Salmonella typhimurium*, and *Proteus vulgaris* do not assimilate the carbon and nitrogen skeleton, but are able to convert L-carnitine via crotonobetaine to gamma-butyrobetaine in the presence of carbon and nitrogen sources under anaerobic conditions.11)

In contrast to the wide distribution of L-carnitine in nature, D-enantiomer does not exist in nature. Nevertheless, various microorganisms are able to catabolize D-carnitine. A NAD⁺*-*dependent D-carnitine dehydrogenase (EC.1.1.1.254) has been purified and characterized from various *Agrobacterium* species, which were able to utilize D-carnitine as sole source of carbon and nitrogen.12,13) During growth on D-carnitine, carnitine racemase and L-carnitine dehydrogenase were induced in *Pseudomonas* sp. AK1.14) *Acinetobacter calcoaceticus* is able to split the C-N bond of L- and D-carnitine to yield trimethylamine.15) Monoxyenase-catalyzed cleavage of L-carnitine resulting in the formation of trimethylamine and malic acid has been postulated.16)

Abbreviations: TMA-Butanol, 4-N-trimethylaminol-1-butanol; DMA-Butanol, 4-dimethylaminol-1-butanol; TMABaldehyde, 4-N-trimethylaminobutyraldehyde; DMBaldehyde, 4-dimethylaminobutyraldehyde; DTT, dithiothreitol

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In the L-carnitine biosynthetic pathway, aldolitic cleavage of beta-hydroxyxtrimethyllysine by beta-hydroxyxtrimethyllysine aldolase yields TMABaldehyde and glycine. Beta-hydroxyxtrimethyllysine aldolase might be identical to serine hydroxymethyltransferase (EC.2.1.2.1), because this enzyme catalyzes the reaction.\(^{17}\) Subsequently, TMABaldehyde is oxidized by TMABaldehyde-DH (EC.1.2.1.47) to form gamma-butyrobetaine. TMABaldehyde-DH has been purified to homogeneity from beef liver\(^{19}\) and rat liver.\(^{20}\) Rebouche and Engel\(^{21}\) showed that TMABaldehyde-DH activity is highest in the cytosolic fraction of human liver and kidney, but very low in brain, heart, and muscle homogenates. As compared with enzymatic studies of TMABaldehyde-DH in mammals, studies of microbial TMABaldehyde-DH are very limited. Kaufman and Broquist\(^{22}\) demonstrated that TMABaldehyde-DH is an intermediate in the L-carnitine biosynthesis of Neurospora crassa by isotope labeling experiments.

In this report, we describe the isolation and characterization of TMABaldehyde-DH, responsible for the conversion of TMABaldehyde to gamma-butyrobetaine from Pseudomonas sp. 13CM. Properties of the purified enzyme were compared with those of TMABaldehyde-DH, which was purified from rat liver and beef liver.

### Materials and Methods

**Materials.** 4-Dimethylamino-1-butanol (DMA-Butanol) and 4-aminobutyraldehyde dimethylacetal were purchased from Tokyo Kasei (Tokyo). Phenyl-Toyopearl and TSK-gel G3000SW were from Tosoh (Tokyo). Standard proteins for gel filtration and SDS–PAGE were from Bio-Rad (Hercules, CA). All other reagents were commercial products of analytical grade.

**Chemical synthesis.** TMA-Butanol iodide and TMA-Baldehyde iodide were prepared from DMA-Butanol and 4-aminobutyraldehyde dimethylacetal respectively, according to the method described in previous paper.\(^{17}\) 4-Aminobutyraldehyde dimethylacetal was hydrolyzed by 0.1 m HCl overnight at room temperature to produce 4-aminobutyraldehyde as a 90% solution. The same procedure was followed for hydrolysis of 4-dimethylaminobutyraldehyde dimethylacetal, resulting in 4-dimethylaminobutyraldehyde (DMABaldehyde).

**Microorganism and culture.** Pseudomonas sp. 13CM, isolated from soil, was used. Pseudomonas sp. 13CM was grown on TMA-Butanol medium as described previously.\(^{17}\) The washed cells were suspended in a small volume of potassium phosphate buffer (50 mM, pH 7.5) containing 1.0 mM DTT, and stored at 20 °C until use.

**Enzyme assay.** The standard reaction mixture (1.5 ml) contained 225 µmol glycine-NaOH buffer (pH 9.5), 0.15 µmol TMABaldehyde iodide, 3.0 µmol NAD\(^+\), and an appropriate amount of the enzyme. The reaction was started by the addition of NAD\(^+\). Activity was calculated using an extinction coefficient of 6,200 M\(^{-1}\)cm\(^{-1}\) for NADH. One unit of enzyme activity was defined as the amount of enzyme that catalyzes the formation of 1 µmol of NADH per min under the assay conditions. Specific activity was defined as units of enzyme activity per mg protein. Because of the instability of 4-aminobutyraldehyde at alkaline pH, measurement of the activity for 4-aminobutyraldehyde as substrate was performed at pH 7.5 using 450 mM Tris–HCl buffer.\(^{23}\)

**Protein measurement.** Protein was measured by the Lowry method\(^{24}\) using bovine serum albumin as the standard protein, or by the absorbance at 280 nm, and an E\(^{1%}\)\(_{1cm}\) value of 10.0 was used.

**Purification of TMABaldehyde-DH.** Purification of TMABaldehyde-DH was done at 4 °C. Potassium phosphate buffer (50 mM, pH 7.5) containing 1.0 mM DTT was used throughout the purification procedure. Centrifugation was done at 10,000 × g for 20 min at 4 °C. The washed cells, harvested from 0.8-liter of the medium, were suspended in 100 ml of the buffer and disrupted for 40 min with a sonicator. Cell-free extract was obtained by centrifugation as supernatant.

**Phenyl-Toyopearl 650M column chromatography.** To the cell-free extract, 0.3 M of ammonium sulfate solution in the buffer was added to a final concentration of 0.15 M. The resulting solution was loaded onto a Phenyl-Toyopearl 650M column (1.6 × 30 cm) equilibrated with the buffer, which contained 0.15 M of ammonium sulfate. The unabsorbed proteins with TMABaldehyde-DH activity were combined, and 1.0 M of ammonium sulfate solution in the buffer was added to a final concentration of 0.5 M. The resulting solution was loaded onto the same column equilibrated with the buffer, which contained 0.5 M of ammonium sulfate. After the column was washed with the same buffer, a linear gradient of ammonium sulfate (0.5–0 M) was done at a flow rate of 2.0 ml/min. Then the column was further eluted with the buffer for two column beds volume and the enzyme was eluted at this step. The combined fraction was concentrated with a Millipore-Ultrafree-15Centrifugal Filter device (fractional molecular weight, 10,000).

**5'-AMP Sepharose4B column chromatography.** The concentrated enzyme solution was put on a 5'-AMP Sepharose4B column (1.6 × 12 cm) equilibrated with the buffer. The column was washed for two column beds volume with the buffer, which contained 0.4 M KCl. The enzyme was eluted with the buffer, which contained 0.5 M KCl for two column beds volume. The flow rate was maintained at 1.0 ml/min. Fractions with enzyme activity were pooled and then stored at 20 °C.
Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis (native-PAGE) was done using 10% gel at pH 8.8. The protein was stained with Commassie brilliant blue (CBB) R-250 and checked for enzyme activity. The reaction mixture contained 150 mM of glycine-NaOH buffer (pH 9.5), 64 μM of 1-methoxy phenazine methosulfate, 0.24 mM of nitroblue tetrazolium, 0.1 mM of TMALdehyde, and 2 mM of NAD⁺. SDS polyacrylamide gel electrophoresis (SDS–PAGE) was done using 12.5% gel, following the method of Laemmli. SDS polyacrylamide gel electrophoresis (SDS–PAGE) was done using 12.5% gel, following the method of Laemmli. Phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.1 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa) were used as standard proteins.

Isoelectric focusing. The isoelectric point of the enzyme was determined by the Phast System of Pharmacia Biotech (Uppsala, Sweden) according to the manufacturer’s instructions. Phast Gel IEF was used to determine the isoelectric point.

Measurement of molecular mass. The molecular mass of the enzyme was estimated by gel filtration on a TSK-gel G3000SW column (0.78 × 30 cm) equilibrated with 0.2 M of potassium phosphate buffer (pH 7.5) containing 1.0 mM of DTT. The standard proteins used were thyroglobulin, γ-globulin, ovalbumin, myoglobin, and vitamin B₁₂.

Amino terminal sequence analysis. The amino terminus was sequenced by Edman’s method with an Applied Biosystems 491 Protein Sequencer.

Results

Purification of TMALdehyde-DH

The purification steps and their results are summarized in Table 1. The enzyme was purified 14.2-fold, giving a preparation with a specific activity at 12.4 unit·mg⁻¹. Since sulfhydryl-reducing agents were found to protect the enzyme activity, DTT was included throughout the isolation procedure. The purified enzyme gave a single protein band on native-PAGE, and the enzyme activity was detected at the same position on the gel (data not shown).

Protein characterization

The molecular mass of the native enzyme was estimated to be about 160 kDa by gel filtration. SDS–PAGE gave one band at a molecular mass of 55 kDa (Fig. 1). The N-terminal 13 amino acid residues were identified as PQLRDAAYWRAQS, and were compared against a protein sequence database. No sequence similarity with known proteins was found. The purified enzyme was subjected to isoelectric focusing and developed by CBB staining (data not shown). One band was visualized, with a pI value of 5.5.

Effects of temperature and pH

The residual activity of the enzyme was measured after heat treatment at various temperatures for 30 min in 1.0 mM of DTT containing 50 mM of potassium phosphate buffer (pH 7.5). The enzyme was relatively stable up to 30°C, but was rapidly inactivated at temperatures above 40°C.

Table 1. Summary of Purification of TMALdehyde-DH from Pseudomonas sp.13CM

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total activity (U)</th>
<th>Total protein (mg)</th>
<th>Specific activity (U·mg⁻¹)</th>
<th>Purification (fold)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-free extract</td>
<td>305</td>
<td>349</td>
<td>0.8</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>1st Phenyl-Toyopearl</td>
<td>265</td>
<td>221</td>
<td>1.2</td>
<td>1.4</td>
<td>87</td>
</tr>
<tr>
<td>2nd Phenyl-Toyopearl</td>
<td>148</td>
<td>12.8</td>
<td>11.6</td>
<td>13.3</td>
<td>49</td>
</tr>
<tr>
<td>5'-AMP Sepharose4B</td>
<td>49</td>
<td>4.0</td>
<td>12.4</td>
<td>14.2</td>
<td>16</td>
</tr>
</tbody>
</table>
above 35 °C (Fig. 2). The optimal temperature was found to be 40 °C (Fig. 2). The influence of pH on enzyme activity was studied in a pH range of 4.6 to 10.4 using various buffers. The optimum pH was found to be 10.0 (Fig. 3A). The pH stability of the enzyme was measured after pre-incubation for 15 min at 30 °C using various buffers. While the enzyme was stable between pH 8.0 and 9.0, it was completely inactivated at pH 9.6 (Fig. 3B).

**Effects of metal ions and various reagents**

The effects of various metal ions and other reagents on the enzyme activity were examined. The activity of the enzyme was measured after incubation of purified enzyme with various metal ions and inhibitors for 5 min at 30 °C. The enzyme was inhibited not only by heavy metal ions such as Hg$^{2+}$ and Cu$^{2+}$ (Table 2), but also by sulfhydryl agents such as p-chloromercuribenzoate, 5,5'-dithiobis-(2-nitrobenzoic acid), and iodoacetamide (Table 3). K$^+$, Zn$^{2+}$, Li$^+$, Ba$^{2+}$, Co$^{2+}$, and semicarbazide caused 10–30% inhibition. Rb$^+$, Na$^+$, Mg$^{2+}$, Ca$^{2+}$, and chelating agents such as potassium fluoride, sodium azide, 2, 2'-dipyridyl, and EDTA had no effect on the enzyme activity.

### Table 2. Effects of Metal Ions on TMABaldehyde-DH Activity

<table>
<thead>
<tr>
<th>Metal compound</th>
<th>Concentration (mM)</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>CuSO$_4$</td>
<td>0.05</td>
<td>64</td>
</tr>
<tr>
<td>HgCl</td>
<td>0.05</td>
<td>72</td>
</tr>
<tr>
<td>BaCl$_2$</td>
<td>1.0</td>
<td>70</td>
</tr>
<tr>
<td>CoSO$_4$</td>
<td>1.0</td>
<td>82</td>
</tr>
<tr>
<td>LiCl</td>
<td>1.0</td>
<td>87</td>
</tr>
<tr>
<td>ZnSO$_4$</td>
<td>1.0</td>
<td>89</td>
</tr>
<tr>
<td>KCl</td>
<td>1.0</td>
<td>95</td>
</tr>
</tbody>
</table>

**Fig. 2.** Effect of Temperature on TMABaldehyde-DH Activity and Stability.

Thermo stability (■): The enzyme solution in 50 mM of potassium phosphate buffer, pH 7.5, containing 1.0 mM of DTT was incubated for 30 min at each temperature. The enzyme activity was assayed as described in “Materials and Methods.” Optimal temperature (●): Enzyme activity was assayed as described in “Materials and Methods,” except that the reaction temperature was varied.

**Fig. 3.** Effects of pH on TMABaldehyde-DH Activity and Stability.

A. Enzyme activity was assayed under the standard assay conditions, except that the following buffers were used at a final concentration of 150 mM in the incubation mixture: potassium phosphate buffer (■), Tris–HCl buffer (●), and glycine-NaOH buffer (▲). B. The enzyme was incubated for 15 min at 30 °C in the following buffers which were used at a final concentration of 150 mM in the incubation mixture: citrate buffers (×), potassium phosphate buffers (■), Tris–HCl buffers (●), and glycine-NaOH buffers (▲).
Substrate specificity and Michaelis constants

The enzyme oxidized TMABaldehyde and DMABaldehyde. TMA-Butanol, betaine aldehyde, 4-aminobutyraldehyde, and other aliphatic aldehydes such as acetaldehyde, propionaldehyde, butyraldehyde, trimethylacetaldehyde, and 3-methylbutyraldehyde were not dehydrogenated. The enzyme did not react with NADP\(^+\), and was very specific for NAD\(^+\). The \(K_m\) values for TMABaldehyde, DMABaldehyde, and NAD\(^+\) were calculated to be 7.4, 51, and 125 \(\mu\)M respectively. Substrate inhibition appeared at 0.24 \(\mu\)M of TMABaldehyde. No reduction reaction was observed.

Inhibition by analogs of TMABaldehyde

A variety of compounds bearing resemblances to TMABaldehyde were tested as inhibitors of the enzyme (Table 4). Trimethylamine, tetramethylammonium chloride, and 3-methylbutyraldehyde strongly inhibited enzyme activity. Other aliphatic aldehydes, such as phenylacetaldehyde, 3-methylbutyraldehyde, and trimethylacetaldehyde, strongly inhibited enzyme activity.

Discussion

Previously, we purified a new enzyme, TMA-Butanol-DH from *Pseudomonas* sp. 13CM.\(^{17}\) In this study, we finally succeeded in purifying TMABaldehyde-DH to homogeneity from *Pseudomonas* sp. 13CM, which has never been reported before from a microorganism. TMABaldehyde-DH has been purified to homogeneity only from beef liver\(^{19}\) and rat liver.\(^{20}\) In the present study, TMABaldehyde-DH was purified by hydrophobic
chromatography on a Phenyl-Toyopearl and affinity chromatography on a 5'-AMP Sepharose4B. When cell-free extract was applied to a Phenyl-Toyopearl column equilibrated with the buffer, which contained 0.15 M of ammonium sulfate, most of the activity of TMA-Baldehyde-DH was detected in the washing fractions, while TMA-Butanol-DH activity was detected in absorbed fractions. Since most contaminant proteins, such as TMA-Butanol-DH, can be separated from the enzyme, this step is necessary in the purification of the enzyme. The specific activity of 12.4 unit/mg and 5.1 unit/mg and 0.77 unit/mg-1 and 0.77 unit/mg-1 respectively (Table 5).

A cytosolic TMA-Baldehyde-DH has been isolated from a cytoplasmic fraction of bovine liver, converting TMA-Baldehyde into gamma-butyrobetaine. The purification and characterization of TMA-Baldehyde-DH from rat liver cytosol, and the identification of the corresponding rat cDNA, was reported recently. Kaufman and Broquist found that TMA-Baldehyde is an intermediate in the L-carnitine biosynthesis of N. crassa, and they suggested that aldehyde dehydrogenase mediates its conversion to gamma-butyrobetaine. Betaine aldehyde dehydrogenase of E. coli, Xanthomonas translucens, and Arthrobacter globiformis is a homotetramer with 50–55 kDa subunits. On the other hand, betaine aldehyde from P. aeruginosa and plants is a homodimer of 61–63 kDa. A 4-Aminobutyraldehyde dehydrogenases (EC.1.2.1.19), which butyraldehyde dehydrogenases (EC.1.2.1.19), which require alpha-ketoglutarate, oxygen, ascorbic acid, and Fe²⁺ by gamma-butyrobetaine hydroxylase (EC.1.14.1.1). The pathways involved in the degradation of TMA-Butanol have been studied in Pseudomonas sp. 13CM. The findings presented herein, when taken together with our previous study on TMA-Butanol-DH in Pseudomonas sp. 13CM, appear to establish the complete pathway of TMA-Butanol degradation. TMA-Butanol degradation in Pseudomonas sp. 13CM is postulated to proceed as follows: TMA-Butanol → TMABaldehyde → gamma-butyrobetaine → L-carnitine.

The molecular mass of TMA-Baldehyde-DH of Pseudomonas sp. 13CM was found to be 160 kDa. TMA-Baldehyde-DH of Pseudomonas sp. 13CM is a trimer with three identical subunits, similar to TMA-Baldehyde-DH of B. taurus (Table 5). This is comparable with the figure of other aldehyde dehydrogenases. The rat amplicon contained an open reading frame (ORF) of 1,485 base pairs, coding for a polypeptide of 494 amino acids with a predicted molecular mass of 55 kDa. Betaine aldehyde dehydrogenase of E. coli, Xanthomonas translucens, and Arthrobacter globiformis is a homotetramer with 50–55 kDa subunits. On the other hand, betaine aldehyde from P. aeruginosa and plants is a homodimer of 61–63 kDa. A 4-Aminobutyraldehyde dehydrogenases (EC.1.2.1.19), which have been purified from Pseudomonas, E. coli, and mammalians also have been reported to act as dimers, trimers, or tetramers. Aldehyde dehydrogenases (EC.1.2.1.3) have been reported to act as dimers or tetramers.

TMA-Baldehyde-DH of Pseudomonas sp. 13CM lost 20% of its initial catalytic activity upon heat treatment at 35 °C for 30 min (Fig. 2). Its pH optimum of 10.0 is comparable to that of TMA-Baldehyde-DH of B. taurus (pH 9.5–9.8). Aldehyde dehydrogenase activities

### Table 5. Comparison of Specific Activity, Molecular Mass, and \( K_m \) Values among TMABaldehyde-DHs

<table>
<thead>
<tr>
<th>Origin</th>
<th>Pseudomonas sp. 13CM</th>
<th>Bos taurus (^{(9)} )</th>
<th>Rattus norvegicus (^{(20)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific activity (U·mg⁻¹)</td>
<td>12.4</td>
<td>5.1</td>
<td>0.77</td>
</tr>
<tr>
<td>Molecular mass (kDa)</td>
<td>160</td>
<td>160</td>
<td>—</td>
</tr>
<tr>
<td>( K_m ) value (µM)</td>
<td>7.4</td>
<td>4.2</td>
<td>1.4</td>
</tr>
<tr>
<td>4-N′Trimethylaminobutyaldehyde</td>
<td>7.4</td>
<td>4.2</td>
<td>1.4</td>
</tr>
<tr>
<td>4-Dimethylaminobutyraldehyde</td>
<td>51.0</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Betaine aldehyde</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Propionaldehyde</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Butyraldehyde</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pentanal</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hexanal</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Heptanal</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Octanal</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hexadecanil</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Trimethylacetaldehyde</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3-Methylbutyaldehyde</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>NAD⁺ (µM)</td>
<td>125.0</td>
<td>+</td>
<td>28.0</td>
</tr>
<tr>
<td>NADP⁺ (mM)</td>
<td>—</td>
<td>—</td>
<td>1.6</td>
</tr>
</tbody>
</table>

\( + \): Oxidized  
\( + \): Not tested.  
\( + \): Inert.
were greatly inhibited by $\text{Hg}^{2+}$.\textsuperscript{48} as well as by $p$-chloromercuribenzoate,\textsuperscript{38,51–53} 5,5’-dithiobis-(2-nitrobenzoic acid),\textsuperscript{46,48} and iodoacetamide.\textsuperscript{46,53,54} From these results, we suggest that the sulfhydryl group is important in the enzyme activity, as are other aldehyde dehydrogenases.

No reduction reaction was observed with TMABaldehyde-DH of \textit{Pseudomonas} sp. 13CM. The reversibility of TMABaldehyde-DH from \textit{B. taurus} was also checked using gamma-butyrobetaine and NADH, but no aldehyde was produced.\textsuperscript{19} The $K_m$ value of 7.4 $\mu$M for TMABaldehyde is higher than the $K_m$ values for TMABaldehyde-DH from \textit{B. taurus}\textsuperscript{19} and \textit{R. norvegicus}.\textsuperscript{20} 4.2 $\mu$M and 1.4 $\mu$M respectively. TMABaldehyde-DH of \textit{Pseudomonas} sp. 13CM and \textit{R. norvegicus}\textsuperscript{20} showed the lowest $K_m$ value for TMABaldehyde of the substrates (Table 5). It has been reported that TMABaldehyde-DH from \textit{B. taurus}\textsuperscript{19} and \textit{R. norvegicus}\textsuperscript{20} can oxidized a range of aliphatic aldehydes. Other than TMABaldehyde, TMABaldehyde-DH from \textit{B. taurus} can oxidize acetaldehyde, propionaldehyde, butyraldehyde, and benzaldehyde with very low relative activity.\textsuperscript{19} On the other hand, \textit{R. norvegicus} TMABaldehyde-DH also catalyzed the dehydrogenation of 4-aminobutyraldehyde, betaine aldehyde, aliphatic aldehydes in the C2-C8 range, and longer aldehydes, such as hexadecanal and octadecanal.\textsuperscript{20} Both NAD$^+$ and NADP$^+$ can be used as coenzymes in \textit{R. norvegicus}.\textsuperscript{20} but the enzyme of \textit{B. taurus}\textsuperscript{19} and the purified TMABaldehyde-DH from \textit{Pseudomonas} sp. 13CM were highly specific only for NAD$^+$.

The inhibitor dissociation constant ($K_i$ value) and mode of inhibition will be described elsewhere. Molecular cloning of the gene is also in progress.

References


23) Kurys, G., Ambroziak, W., and Pietruszko, R., Human


