Purification and Properties of 4-Aminobutanal Dehydrogenase from a *Pseudomonas* Species*

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**SUMMARY**

4-Aminobutanal dehydrogenase has been purified from a *Pseudomonas* species. The enzyme has high activity toward aminomaldehydes (4-aminobutanal or 3-aminopropanal) and low activity toward succinic semialdehyde as substrate. The kinetic constants, effect of p-hydroxymercaptobenzoate, and pH profile with different substrates are documented. The enzyme has a molecular weight of 228,000 as determined by gel filtration and polyacrylamide gel electrophoresis with reference proteins. Electrophoresis in sodium dodecyl sulfate gave a subunit molecular weight of 75,000 indicating that this is a three-subunit enzyme.

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required concentrations. All buffer solutions contained 5 mM each of ethylene diaminetetraacetic acid and mercaptoethanol and 30% (v/v) glycerol unless otherwise stated (glycerol was required for maximum stability).

Most of the isolation procedures were performed with a 10 mM phosphate buffer which will be referred to hereafter as the standard buffer.

The pH optima of the enzyme (toward succinic semialdehyde, 4-aminobutanal, and 3-aminopropanal) were determined in three buffer systems. Between pH 7.0 and 9.0, a 0.1 M sodium phosphate buffer was used, while between pH 9.0 and 11.0, a 0.1 M carbonate-bicarbonate buffer system was used. For pH 6 or below, 0.1 M acetate buffer was used. The pH of the reaction mixture was checked before and after reaction to ensure the correct pH of the solutions.

The cells were grown at 30°C in a 100-liter New Brunswick fermentor in 0.2% putrescine as the sole carbon and nitrogen source a salt medium described previously (1).

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**RESULTS**

**Purification of 4-Aminobutanal Dehydrogenase**—Approximately 300 g of the putrescine-grown cells were used to obtain a soluble fraction by means of a previously reported procedure (2). The soluble fraction was dialyzed against several changes of 10 mM standard buffer.

The dialyzed solution was then applied to a column of DEAE-cellulose (5 × 80 cm) previously equilibrated with the same buffer (6 liters), and the wash which had no enzymatic activity was discarded. The column was then eluted with the same buffer with a linear gradient of NaCl from 0 to 0.5 M. The effluent was assayed for protein and activity toward 4-aminobutanal, 3-aminopropanal, and succinic semialdehyde as substrates (Fig. 1). The tubes that contained activity were pooled (Fractions A and B in Fig. 1). Fraction A was frozen under liquid N₂ and stored at −60°C. Fraction B was dialyzed against several changes of cold standard buffer and applied to a column of DEAE-Sephadex A-50 (5 × 60 cm). The column was washed with 2 liters of the same buffer and then eluted with the same buffer with a linear gradient of NaCl from 0 to 0.4 M. The effluent was monitored for protein and activity (toward 4-aminobutanal, 3-aminopropanal, and succinic semialdehyde). Three different fractions containing dehydrogenase activities were pooled (Fractions BⅠ, BⅡ, and BⅢ in Fig. 2). Fractions BⅠ and BⅡ were frozen under liquid N₂ for storage at −60°C. Fraction BⅢ was dialyzed against several changes of cold standard buffer and concentrated (see "Experimental Procedure"). The concentrated protein solution (41 ml) was dialyzed against cold standard buffer and chromatographed through a Sephadex G-200 column (5 × 90 cm) that was previously equilibrated with the
is active toward 4-aminobutanal, 3-aminopropanal, or succinic semialdehyde. Standard buffer that contained 0.15 M NaCl. The summary of a typical purification. Previously equilibrated with standard buffer containing 0.15 M NaCl, concentrated, and dialyzed against cold standard buffer, centrifuged, and plotted as in Fig. 1. 4-Aminobutanal dehydrogenase activity (not shown) follows that of 3-aminopropanal dehydrogenase. Tubes 160 to 190, 200 to 230, and 245 to 278 were collected to give Fractions B1, BII, and BIII, respectively. Fractions B1 and BII were frozen under liquid N2. Fraction BIII was used immediately.

Fig. 3 (right). Gel filtration of Fraction BIII on Sephadex G-200. Forty-one milliliters of concentrated Fraction BIII obtained from the experiment shown in Fig. 2 was applied to a Sephadex G-200 column (5 × 90 cm) and eluted with the standard buffer. After 620 ml were collected as one fraction and discarded (see text), 15-ml fractions were collected. Protein (—), 3-aminopropanal dehydrogenase (Δ—Δ), and succinic semialdehyde dehydrogenase (□—□) are plotted as in Fig. 1. 4-Aminobutanal dehydrogenase activity follows that of 3-aminopropanal dehydrogenase and was not plotted here. Tubes 14 to 25 were pooled as one fraction and Tubes 26 to 45 were pooled as a separate fraction. The fraction containing Tubes 14 to 25 had the highest specific activity and were used immediately. The second fraction (Tubes 26 to 45) was stored at 4°C for further use.

Fig. 1 (left). DEAE-cellulose chromatography of soluble fraction. Of soluble fraction containing 17.4 mg of protein per ml and 0.9 unit of 4-aminobutanal dehydrogenase per ml, 1.87 units of 3-aminopropanal dehydrogenase per ml, and 2.90 units of succinic semialdehyde dehydrogenase per ml, 1300 ml were applied to the column. After a 6-liter wash, not shown, the 0 to 0.5 M NaCl gradient (— — ) was started; 20-ml fractions were collected. Tubes 77 to 105 and 106 to 140 were pooled to give Fractions A and B, respectively. Fraction B was dialyzed and used immediately. Fraction A was frozen under liquid N2. Protein (——) was determined by absorbance at 280 nm; the units on the left ordinate represent absorbance as defined in the legend to Table I. Enzyme activities (3-aminopropanal dehydrogenase, Δ—Δ; succinic semialdehyde dehydrogenase, □—□) are given in units of enzyme per ml of each fraction, each unit representing 1 pmole of NADH formed per min under standard assay conditions described under "Experimental Procedure." Activity toward 4-aminobutanal follows the same profile of that for 3-aminopropanal and is not shown here. This and all subsequent chromatography was performed at 4°C.

Fig. 2 (center). Separation of Fraction B into three fractions on DEAE-Sephadex A-50. Seven hundred milliliters of Fraction B from the previous step containing 1.00 units of 4-aminobutanal dehydrogenase per ml, 2.42 units of 3-aminopropanal dehydrogenase per ml, and 0.71 unit of succinic semialdehyde dehydrogenase per ml were applied to a column of DEAE-Sephadex A-50 (5 × 60 cm). After a 2-liter wash, not shown, the NaCl gradient (— — — ) was started; 20-ml fractions were collected and protein (——), 3-aminopropanal dehydrogenase (Δ—Δ), and succinic semialdehyde dehydrogenase (□—□) activities were determined and plotted as in Fig. 1. 4-Aminobutanal dehydrogenase activity (not shown) follows that of 3-aminopropanal dehydrogenase. Tubes 160 to 190, 200 to 230, and 245 to 278 were collected to give Fractions BI, BII, and BIII, respectively. Fractions B1 and BII were frozen under liquid N2, Fraction BIII was used immediately.

The results are shown in Fig. 6.

pH and Enzyme Activities—The activity of the purified enzyme toward the three aldehydic substrates was determined. The results are shown in Fig. 7.

Kinetic Parameters—the Kmax and Vmax values of the purified enzyme were determined in pyrophosphate buffer (pH 8.5). The results are summarized in Table II. It may be noted that although the use of 3-aminopropanal gave the highest Vmax, it also resulted in a much higher Kmax. These results confirm and extend results obtained previously with a relatively crude enzyme fraction (4).

Stabilizing Effects of Mercaptoethanol and Glycerol—Early attempts at purification of this enzyme were unsuccessful due to its lability, even in the presence of mercaptoethanol or di-thiothreitol. Following the report by Jakoby and Bradbury (5) that 30% glycerol stabilized an aldehyde dehydrogenase from bakers' yeast, we found that our 4-aminobutanal dehydrogenase was stable in 30% glycerol with 5 mM each of mercaptoethanol and EDTA. To examine the mode of action of these agents, we dialyzed the purified enzyme against phosphate buffer either with or without glycerol and mercaptoethanol, incubated the enzyme for different lengths of time with p-hydroxymercuribenzoate, and then assayed the enzyme with or without mercaptoethanol (5 mM) in the assay mixture. Some of the results are shown in Fig. 8. The enzyme is much more sensitive toward this sulfhydryl reagent in the absence than in the presence of glycerol, as indicated both by a more rapid loss of enzymatic activity and less recovery of enzyme activity by the presence of mercaptoethanol in the assay mixture.

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Also, acrylamide gel electrophoresis in the presence of sodium dodecyl sulfate showed only one protein band. These results are shown in Fig. 6.

Criteria of Purity—Acrylamide gel electrophoresis (at pH 7.3 or 9.0) of the final purified enzyme showed one protein band which is active toward 4-aminobutanal, 3-aminopropanal, or succinic semialdehyde.

Also, acrylamide gel electrophoresis in the presence of sodium dodecyl sulfate showed only one protein band. These results are shown in Fig. 6.
TABLE I
Summary of purification of 4-aminobutanal dehydrogenase

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume</th>
<th>Total protein</th>
<th>Total units of 4-aminobutanal dehydrogenase</th>
<th>Recovery</th>
<th>Specific activity</th>
<th>3APA/ABA/SSA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>1,500</td>
<td>49,700</td>
<td>1,250</td>
<td>100</td>
<td>0.025</td>
<td>2.14/1.28</td>
</tr>
<tr>
<td>Soluble</td>
<td>1,300</td>
<td>22,600</td>
<td>1,170</td>
<td>94</td>
<td>0.052</td>
<td>2.08/1.32</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>700</td>
<td>2,600</td>
<td>700</td>
<td>55</td>
<td>0.269</td>
<td>2.42/1.07</td>
</tr>
<tr>
<td>DEAE-Sephadex A-50</td>
<td>425</td>
<td>712</td>
<td>237</td>
<td>18</td>
<td>0.333</td>
<td>2.64/0.12</td>
</tr>
<tr>
<td>Sephadex G-200</td>
<td>175</td>
<td>102</td>
<td>70</td>
<td>5.6</td>
<td>0.690</td>
<td>2.5/1.01</td>
</tr>
<tr>
<td>Hydroxylapatite + concentration</td>
<td>25</td>
<td>20.5</td>
<td>18.2</td>
<td>1.5</td>
<td>0.785</td>
<td>3.1/0.04</td>
</tr>
<tr>
<td>Sephadex G-200 in 0.15 M NaCl</td>
<td>6</td>
<td>18</td>
<td>3.87</td>
<td>0.3</td>
<td>0.22</td>
<td>2.9/0.34</td>
</tr>
</tbody>
</table>

*a A unit is the amount of enzyme that reduces 1 amole of NAD per min with 4-aminobutanal as substrate under standard assay conditions.
*b Protein was determined by biuret reaction for the first three steps and by A_{280} thereafter using a value of E\text{\,}_{1\text{ cm}}^{1\text{ cm}} = 10. Values determined by this method agreed well when compared with the biuret method.
*c Relative activities with 3-aminopropanal (3 APA), 4-aminobutanal (4 ABA), and succinic semialdehyde (SSA) as substrate.
*d This represents the units recovered in this fraction (IIIa) as pooled (see text). The remainder of the units were recovered in Fraction IIIb.
*e This low specific activity is due to inactivation during storage.

**Fig. 4 (left).** Hydroxylapatite chromatography of 4-aminobutanal dehydrogenase. The enzyme obtained from the previous step (175 ml) was dialyzed against 1 mM phosphate (see text) applied to a hydroxylapatite column (2.5 X 20 cm) previously equilibrated with the same buffer. The vertical arrows indicate the points at which buffer changes were made as follows: 40 mM, 50, 60, 80, and 0.1 M phosphate (left to right); 3-ml fractions were collected, and protein ( ), 3-aminopropanal dehydrogenase ( ), and succinic semialdehyde dehydrogenase ( ) activities were determined and plotted as in Fig. 1. 4-Aminobutanal dehydrogenase activity (not shown) follows that of 3-aminopropanal dehydrogenase. Tubes 155 to 320 showed similar patterns on polyacrylamide electrophoresis (see text) and were pooled. The rest of the activity containing fractions were pooled and stored at 4°C for further use.

**Fig. 5 (right).** Gel filtration of 4-aminobutanal dehydrogenase on Sephadex G-200 in the presence of 0.15 M NaCl. Fifteen milliliters of concentrated enzyme obtained from the experiment shown in Fig. 4 were applied to a Sephadex G-200 column (5 X 90 cm) equilibrated with 10 mM phosphate buffer, pH 7.0, containing 0.15 M NaCl and eluted with the same buffer. After the void volume (approximately 600 ml), 15-ml fractions were collected. Protein ( ) and 3-aminopropanal dehydrogenase activity ( ) were monitored. Fractions 72 to 80 were pooled and shown to have one major enzymatically active protein band.

**Fig. 6.** Polyacrylamide gel electrophoresis of purified 4-aminobutanal dehydrogenase at pH 9.0 in 6.0% gels. A, 4-aminobutanal dehydrogenase after Sephadex G-200 chromatography in 0.15 M NaCl stained for protein (left) and activity toward 3-aminopropanal (right). Ten micrograms of enzyme protein with a specific activity of 0.22 units per mg were used. B, sodium dodecyl sulfate electrophoresis of 10 μg of purified 4-aminobutanal dehydrogenase. The arrows indicate the band where dehydrogenase activity was observed. Although not shown here, the active band was also active toward all three aldehydic substrates.

**Molecular Weight and Number of Subunits**—The molecular weight of this enzyme was determined by Sephadex G-200 chromatography and by polyacrylamide gel electrophoresis according to Hendrick and Smith (6). Subunit weight was determined by electrophoresis in the presence of sodium dodecyl sulfate (7). The values of the molecular weight of the enzyme and of its subunit thus determined are summarized in Table III. It is clear that the enzyme consists of three presumably identical subunits with a molecular weight of approximately 75,000.

**DISCUSSION**

We have reported previously (1, 2, 8) in a series of papers that a Pseudomonas species grown on spermidine as the sole
source of carbon and nitrogen contains, besides the expected three dehydrogenases specific for succinic semialdehyde, 4-aminobutanal, and 3-aminopropanal, many identifiable dehydrogenase fractions with varying ratios of activity toward the aldehydic substrates. Cells grown on 4-aminobutyrate were used to purify succinic semialdehyde dehydrogenase and were therefore used to purify the latter enzyme. This enzyme proved to be very labile and was purified only after adoption of the 30% glycerol method of Jakoby and Bradbury (5).

The purified enzyme has been shown to consist of three apparently identical subunits of 75,000 daltons. It is very sensitive to sulphydryl reagents. Under standard assay conditions (pH 8.5, 5 mM aldehydic substrate, 0.5 mM NAD\(^+\)), it is most active toward 3-aminopropanal, somewhat less active toward 4-aminobutanal, and least active toward succinic semialdehyde. The ratios of \(V_{\text{max}}\) for 3-aminopropanal, 4-aminobutanal, and succinic semialdehyde are approximately 1:0.22:0.13. However, although this enzyme has the highest \(V_{\text{max}}\) for 3-aminopropanal, it also has a much higher \(K_m\) for 3-aminopropanal (8.3 \times 10^{-4} M) than for 4-aminobutanal and succinic semialdehyde (2.8 and 5.7 \times 10^{-3} M, respectively). This high \(K_m\) for 3-aminopropanal, together with the induction of this enzyme by putrescine (presumably via 4-aminobutanal), establishes this enzyme as 4-aminobutanal dehydrogenase even though it shows higher activity toward 3-aminopropanal under the standard assay conditions used throughout its purification.

The enzyme is maximally active toward the three different aldehydic substrates at pH near 8 to 9. With 3-aminopropanal as substrate, the activity drops off at pH above 8. Although the exact explanation for this is not known, it seems reasonable to suggest that, due to the enzyme-substrate "misfit" (as indicated by the high \(K_m\)), a large effect may result from the change from a positively charged ammonium ion to an uncharged amine at higher pH. At pH below 7.5, the enzyme activity toward the aldehydes drops off sharply while its activity toward succinic semialdehyde drops off much more gradually. Again, one cannot be certain about the relative contribution of conformational changes of the enzyme, changes in charge of side chains of the enzyme, or a change of succinic semialdehyde from a carboxylate ion to a carboxylic acid. However, the changes in the ratios of aminodehydrogenase activities to succinic semialdehyde dehydrogenase activity indicate some
TABLE III

Molecular weight of 4-aminobutanal dehydrogenase

<table>
<thead>
<tr>
<th>Method</th>
<th>Solvent</th>
<th>Species</th>
<th>Observed value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sephadex G 200 filtration°</td>
<td>10 mM phosphate, pH 7.0, 0.15 M NaCl</td>
<td>Trimer*</td>
<td>Mol wt = 232,000 ± 20,000</td>
</tr>
<tr>
<td>Polyacrylamide electrophoresis°</td>
<td>34 mM Tris-Asn, pH 7.3</td>
<td>Trimer</td>
<td>D_{20, w} = 4.08 × 10^{-7} cm^2 s^{-1}</td>
</tr>
<tr>
<td>Sodium dodecyl sulfate electrophoresis°</td>
<td>0.1 M sodium phosphate, pH 7.2</td>
<td>Subunit</td>
<td>Mol wt = 228,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

° Five to ten milligrams of phosphorylase a, catalase, fumarase, aldolase, bovine serum albumin, and ovalbumin were run separately as standards and gave a straight line plot for elution volume versus both log molecular weight and 10^{-4}D.

* Native enzyme.

The slope characteristics of four standard proteins and 4-aminobaldehyde dehydrogenase were obtained with gel concentrations of 9, 7, 6, and 3%. The proteins were dissolved in standard buffer (without mercaptoethanol) and applied to the gels (approximately 25 μg per gel). Protein bands were located with Coomassie blue, and the dye front was marked with a section of 26-gauge copper wire. A linear plot is obtained for molecular weight versus the slope characteristic for the standard proteins. The monomer, dimer, and trimers of bovine serum albumin, ovalbumin, catalase, and phosphorylase were used as standards.

The mobility of each polypeptide chain was calculated relative to that of bromphenol blue dye, the position of which was marked with a 26-gauge copper wire. Approximately 10 μg of each protein was applied to gels after dissociation for 2 hours at 38° in 20 mM sodium phosphate, pH 7.0, containing 1% sodium dodecyl sulfate and 1% mercaptoethanol. The standards, bovine serum albumin (monomer and dimer), phosphorylase, catalase, fumarase, ovalbumin, sperm whale myoglobin and lysozyme gave a linear plot for relative mobility versus log molecular weight.

REFERENCES

Purification and Properties of 4-Aminobutanal Dehydrogenase from a Pseudomonas Species
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