Purification and Properties of 3-Aminopropanal Dehydrogenase from a *Pseudomonas* Species†

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ABSTRACT: 3-Aminopropanal dehydrogenase has been purified from a *Pseudomonas* species. The enzyme has high activity toward 3-aminopropanal and low inherent activity toward 4-aminobutanal and succinic semialdehyde as substrate. The enzyme has a molecular weight of 213,000–226,000 as determined by gel filtration and polyacrylamide gel electrophoresis with reference proteins. Electrophoresis in sodium dodecyl sulfate gave a subunit molecular weight of 74,000 indicating that this is a three subunit enzyme. Purified 3-aminopropanal dehydrogenase does not cross-react with antisera to succinic semialdehyde dehydrogenase but does cross-react with antisera to 4-aminobutanal dehydrogenase. While 3-aminopropanal dehydrogenase and 4-aminobutanal dehydrogenase are similar in size and antigenicity, their induction patterns and kinetic constants differ significantly. It is suggested that there is a close evolutionary relationship between these two enzymes.

Work in this laboratory, in part reported earlier (Padmanabhan and Tchen, 1972), suggested that a *Pseudomonas* species grown on polyamines as the sole source of C and N contains not only NAD^+-specific dehydrogenases for succinic semialdehyde, 4-aminobutanal, and 3-aminopropanal, but also hybrids of these enzymes. The purification and properties of succinic semialdehyde dehydrogenase and 4-aminobutanal dehydrogenase have been reported (Callewaert *et al.*, 1973, 1974; Rosemblatt *et al.*, 1973). Evidence supporting the presence of *in vivo* hybrids at the subunit level of these two enzymes and the evolutionary significance of such an occurrence has also been documented (Rosemblatt *et al.*, 1974).

In this paper we wish to report the purification and properties of 3-aminopropanal dehydrogenase. We shall show that this enzyme is similar but not identical with 4-aminobutanal dehydrogenase. The evolutionary significance of these findings is discussed.

Experimental Section

The common reagents and methods, including enzyme assays, homogeneous succinic semialdehyde dehydrogenase, and 4-aminobutanal dehydrogenase, and the immunochemical methods used in this paper are essentially as described in the preceding papers (Callewaert *et al.*, 1973; 1974; Rosemblatt *et al.*, 1973) unless otherwise stated.

The phosphate buffer used throughout was prepared as described previously (Callewaert *et al.*, 1974) and contained 30% (v/v) of glycerol unless otherwise stated (glycerol was required for maximal stability). Most of the isolation procedures were performed in a 10 mM phosphate buffer containing 5 mM each of ethylenediaminetetraacetic acid and mercaptoethanol, which will be referred to hereafter as the standard buffer.

The cells were grown at 30° in a 100-l. New Brunswick fermentor in 0.2% bis(3-aminopropyl)amine in a salt medium described previously (Padmanabhan and Tchen, 1969).

Analytical isoelectric focusing in polyacrylamide gels was performed essentially according to the method of Hayes and Wellner (1969).

Results

Purification of 3-Aminopropanal Dehydrogenase. Approximately 350 g of the bis(3-aminopropyl)amine grown cells was used to obtain a soluble fraction by a previously reported procedure (Callewaert *et al.*, 1973). The soluble fraction was then dialyzed against several changes of 10 mM cold standard buffer.

The dialyzed solution was then applied to a column of DEAE-cellulose (5 × 90 cm) previously equilibrated with the same buffer. The wash (9 l.) had little enzymatic activity and 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 dehydrogenase activity toward 3APA, 4APA, and SSA[1] as substrates. The major peak of activity was pooled, dialyzed against several changes of cold standard buffer containing 0.1 M NaCl, and applied to a column of DEAE-Sephadex A-50 (5 × 80 cm). The column was washed with the same buffer and then eluted with a linear gradient of NaCl from 0.1 to 0.4 M. The effluent was monitored for protein and dehydrogenase activities (toward 3APA, 4ABA, and SSA). The major protein and activity peak was pooled, dialyzed against several changes of cold standard buffer, and concentrated. The concentrated protein solution (20 ml) was dialyzed against cold standard buffer containing 0.15 M NaCl and chromatographed through a Sephadex G-200 column (5 × 90 cm) that was equilibrated with the same buffer. The effluent was monitored for protein and dehydrogenase activities. Two fractions containing activity were pooled into fractions A and B. Fraction A was dialyzed against cold standard buffer and applied to a hydroxylapatite column (2.5 × 20 cm) previously equilibrated with the same buffer. The column was eluted in a stepwise manner with the following: 10 mM, 30 mM, 50 mM, 70 mM, and 0.1 M phosphate buffer (pH 7.0). The eluent was assayed for protein and activity. Polyacrylamide gel electrophoresis was also performed on selected tubes and the

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[1] Abbreviations used are: 3APA, 3-aminopropanal; 4ABA, 4-aminobutanal; SSA, succinic semialdehyde; 3APADH, 4ABADH, SSADH, the NAD^+-linked dehydrogenases for 3APA, 4ABA, and SSA, respectively; SDS, sodium dodecyl sulfate.

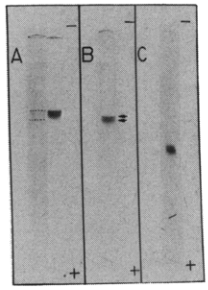

FIGURE 1: Polyacrylamide gel electrophoresis of purified 3APADH. (A) 3-APADH (10 μg) (fraction A-II of text) was subjected to electrophoresis on 7.5% gels at pH 9.0 and stained for protein (left) and 3-APADH activity (right). The arrows indicate the band(s) where dehydrogenase activity was observed. Although not shown here, the active bands were active toward all three aldehydic substrates. (B) 3APADH (10 μg) was subjected to electrophoresis as above, but for a longer period of time in a higher percentage (9%) gel and stained for 3APADH activity. Two to three bands were observed which also stained for protein and activity toward 4ABA and SSA (not shown). (C) Sodium dodecyl sulfate electrophoresis of 10 μg of purified 3APADH on 5% gels.

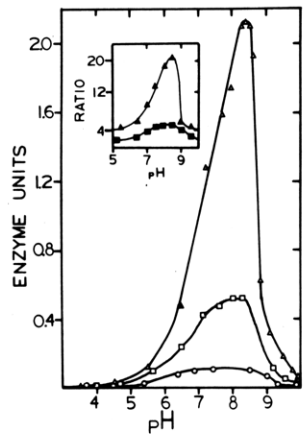

FIGURE 2: pH and dehydrogenase activities of 3APADH. Dehydrogenase activities of the purified enzyme toward 3APA (△), 4ABA (□), and SSA (○) are plotted against pH. The units are in μmol of NADH min^{-1} 0.236 mg of the purified $enzyme^{-1}$. The insert shows the ratios of activities toward 3APA:SSA (▲) and 4 ABA:SSA (■) at different pH. The buffers used were: 0.1 M acetate buffer at pH 6 and below; 0.1 M phosphate buffer for pH 7–9; and 0.1 M bicarbonate buffer at pH 9–11. The pH of the reaction mixtures was checked both before and after assay.

gels were stained for protein and enzyme activity. Those tubes which gave one protein and enzyme band were pooled (A-II) and dialyzed against cold standard buffer. A second enzyme peak (A-I) was not homogeneous and was pooled and frozen at −60° for storage. Table I shows the summary of a typical purification.

Criteria of Purity. Acrylamide electrophoresis (at pH 7.3 or 9.0) of the final purified enzyme showed an apparently broad protein and activity band over a range of gel concentrations (Figure 1A). Closer inspection reveals the presence of two, probably three protein bands, all of which stained for activity (Figure 1B). SDS electrophoresis of the purified protein gave only one protein band (Figure 1C).

pH and Enzyme Activities. The activity of the purified enzyme toward the three aldehydic substrates was determined. The results are shown in Figure 2.

Kinetic Parameters. The K_m and V_{max} values of the enzyme were determined in pyrophosphate buffer (pH 8.5). The results are summarized in Table II. It may be noted that the K_m's for 3APA and 4ABA are approximately equal, while the V_{max} for 3APA is much higher. This is in contrast to the 4-aminobutanal dehydrogenase which was previously isolated from this organism (Callewaert *et al.*, 1974) which had a 30-fold lower K_m for 4ABA than for 3APA.

Molecular Weight and Number of Subunits. The molecular weight of this enzyme was determined by Sephadex G-200 chromatography and by polyacrylamide electrophoresis according to Hendrick and Smith (1968). Subunit weight was determined by electrophoresis in the presence of sodium dodecyl sulfate (Weber and Osborn, 1969). The values of the molecular weight of the enzyme and its subunit thus determined are summarized in Table III. It is clear that the enzyme consists of three subunits with a molecular weight of approximately 74,000.

Comparative Immunodiffusion. 3-Aminopropanal dehydrogenase cross-reacts in immunodiffusion experiments with antisera prepared against 4-aminobutanal dehydrogenase purified from this organism (Callewaert *et al.*, 1974). However, the precipitin line formed by 4-aminobutanal dehydrogenase against its own antisera crosses that formed by 3-aminopropanal dehydrogenase (Figure 3), indicating similarity but nonidentity of the two enzymes. Anti-succinic semialdehyde dehydrogenase (Rosenblatt *et al.*, 1973) does not cross-react with 3-aminopropanal dehydrogenase.

TABLE I: Summary of Purification of 3-Aminopropanal Dehydrogenase.

Step	Volume (ml)	Total Protein (mg)	Total Units[a] (3APA)	Recovery (%)	Specific Activity[b]	3APA/4ABA/SSA[c]
Crude extract	1500	22,000	2640	100	0.12	9.2:1.2:1
Soluble	1400	9,200	2350	89	0.26	7.0:0.9:1
DEAE-cellulose	440	1,150	1170	44	1.02	15:2.1:1
DEAE-Sephadex A-50	125	375	985	37	2.62	13.7:1.6:1
Sephadex G-200 pool A	200	164	577	22[d]	3.52	39:4.0:1
Hydroxylapatite pool A-II	45	10.6	72.4	2.7[e]	6.76	68:6.8:1

[a] One unit of activity is the amount of enzyme that reduces 1 μmol of NAD^+/min with 3-aminopropanal as substrate under standard assay conditions (pH 8.5, 0.1 M pyrophosphate buffer, 1.2 mM 3APA, 0.5 mM NAD^+, and 5.0 mM mercaptoethanol). [b] Protein was determined by biuret reaction for the first three steps and by OD_{280} thereafter using a value of $E_{1\,cm}(1\%) = 10$. Values determined by this method agreed well when compared with the biuret method. [c] Relative activities with 3-aminopropanal, 4-aminobutanal (1.4 mM), and succinic semiadlehyde (0.4 mM) as substrate. [d] This represents the units recovered in this fraction (A) as pooled (see text). The remainder of the units were recovered in fraction B. [e] Units recovered in fraction A-II as pooled (see text). An equivalent amount was present in fraction A-I.

TABLE II: Kinetic Constants of 3-Aminopropanal Dehydrogenase.

Variable Substrate	Fixed Substrate	$K_m \times 10^5$	V_{max} (μmol/min^{-1} ml^{-1})
3-Aminopropanal	NAD^+ [a]	51	2.00
4-Aminobutanal	NAD^+ [b]	59	0.35
Succinic semialdehyde	NAD^+ [c]	13.5	0.26
NAD^+	3-Aminopropanal[d]	8.3	2.00
	4-Aminobutanal[e]	8.3	0.35
	Succinic semialdehyde[f]	8.4	0.26

The different concentrations of fixed substrate used are: (a) 0.4 mM, 0.04 mM, 0.02 mM, 0.01 mM, 0.008 mM, and 0.004 mM; (b) 0.4 mM, 0.3 mM, 0.2 mM, 0.1 mM, 0.08 mM, 0.06 mM, 0.04 mM, and 0.03 mM; (c) 0.4 mM, 0.2 mM, 0.08 mM, 0.06 mM, 0.04 mM, and 0.03 mM; (d) 0.9 mM, 0.6 mM, 0.45 mM, 0.3 mM, and 0.24 mM; (e) 1.5 mM, 0.75 mM, 0.6/mM, 0.45 mM, 0.3 mM, and 0.15 mM; (f) 0.4 mM, 0.3 mM, 0.2 mM, 0.1 mM, 0.08 mM, 0.06 mM, 0.04 mM, and 0.02 mM. The enzyme preparation used contained 0.236 mg of protein/ml with a specific activity of 6.76 with 3APA as substrate.

Titration with Anti-4-aminobutanal Dehydrogenase. When 3-aminopropanal dehydrogenase is titrated with antisera prepared against 4-aminobutanal dehydrogenase, the loss of enzyme activity closely parallels that obtained with the original antigen, 4-aminobutanal dehydrogenase (Figure 4). At high concentrations of antibody, the activity of both enzymes is partially recovered.

Analytical Isoelectric Focusing. The tendency for 3-aminopropanal dehydrogenase to separate into two to three bands during electrophoresis in acrylamide gels (Figure 1B) and the similarities of this enzyme in weight and electrophoretic mobility (Figure 5A) to 4-aminobutanal dehydrogenase prompted us to investigate the behavior of these two enzymes with analytical isoelectric focusing. Figure 5B shows the patterns obtained for these enzymes in a pH 3–10 gradient. While 3APADH band B is clearly different from 4ABADH band B, the top band (A) may be common to both preparations.

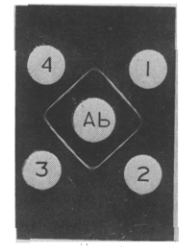

FIGURE 3: Comparative immunodiffusion with antisera to 4ABADH. The center well of an agar plate in phosphate buffer, pH 7.0, without glycerol but with 0.15 M NaCl was filled with anti-4ABADH. Wells 1 and 3 contained purified 4ABADH. Wells 2 and 4 contained purified 3APADH. Note that the lines formed by the original antigen (wells 1 and 3) cross those formed by 3-aminopropanal dehydrogenase (for explanation see text). Similar experiments using anti-SSADH showed no precipitin line with purified 3APADH (data not shown).

Discussion

We have reported previously in a series of papers that a *Pseudomonas* species grown on spermidine as the sole source of C and N 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 (Padmanabhan and Tchen, 1969, 1972; Callewaert *et al.*, 1973). Cells grown on 4-aminobutyrate were used to purify SSADH and study its properties (Callewaert *et al.*, 1973; Rosemblatt *et al.*, 1973). Cells grown on putrescine were used to purify 4ABADH and to study its properties (Callewaert *et al.*, 1974). From these studies, evidence was obtained that two minor dehydrogenase fractions from putrescine grown cells are *in vivo* hybrids of subunits of 4ABADH and SSADH (Rosemblatt *et al.*, 1974). These results represent an unusual situation with no known precedent and indicate an evolutionary relationship between these enzymes (4ABADH and SSADH).

TABLE III: Molecular Weight of 3-Aminopropanal Dehydrogenase.

Method	Solvent	Species	Observed Value
G-200 filtration[a]	10 mM Phosphate (pH 7.0); 0.15 M NaCl	Monomer	Mol wt = 213,000 ± 20,000
			$D_{20,w} = 4.17 \times 10^{-5}$ cm^2 sec^{-1}
Polyacrylamide electrophoresis[b]	34 mM Tris-Asn (pH 7.3)	Monomer	Mol wt = 226,000 ± 15,000
SDS electrophoresis[c]	0.1 M Sodium phosphate (pH 7.2)	Subunit	Mol wt = 74,000 ± 1,000

[a] 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 *vs.* both log molecular weight and $10^{-6}/D$. [b] The slope characteristics of four standard proteins and 3-aminopropanal dehydrogenase were obtained with gel concentrations of 9, 7, 5, and 3%. The proteins were dissolved in standard buffer (without mercaptoethanol) and applied to the gels (approximately 25 μg/gel). A linear plot was obtained for molecular weight *vs.* the slope characteristic for the standard proteins. The monomer, dimer, and trimers of bovine serum albumin, ovalbumin, catalase, and phosphorylase were used as standards. [c] The mobility of each polypeptide chain was calculated relative to that of Bromophenol 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 hr 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 *vs.* log molecular weight.

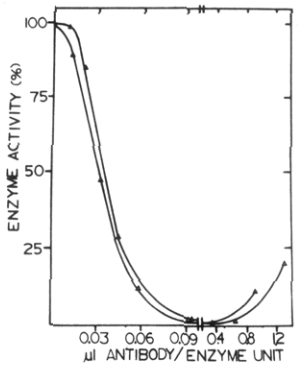

FIGURE 4: Inhibition of 3APADH by antiserum toward 4ABADH. Purified 3APADH and 4ABADH were incubated with various dilutions of the immunoglobulin fraction of an antiserum to 4ABADH. Following incubation for 4 hr, the tubes were centrifuged and 0.1 ml of supernatants were assayed for activity toward 3APA. The differences in specific activity of the two enzymes were normalized by plotting the percentage of remaining activity against the amount (μl) of the immunoglobulin fraction used per unit of the dehydrogenase being assayed. The curves represent (lower) 3APADH and (upper) 4ABADH.

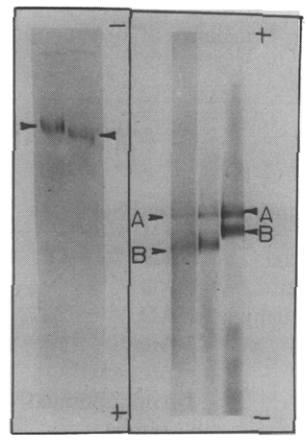

FIGURE 5: Comparison of 3APADH and 4ABADH by comparison of electrophoresis and isoelectric focusing. (A) Electrophoresis of 3APADH (left) and 4ABADH in parallel gels (10%) at pH 9.0. Gels were stained for activity toward 3APA. (B) Isoelectric focusing of 3APADH stained for protein (left) and activity toward 3APA (center). In a parallel gel, 4ABADH was stained for activity toward 3APA (right). The letters designate the bands of activity toward 3APA.

Cells grown on bis(3-aminopropyl)amine (an analog of spermidine) should contain high levels of 3APADH and were therefore used to purify this enzyme. It should be mentioned that cells grow very slowly in this medium and that these cells probably contain significant amounts of the other two dehydrogenases (4ABADH and SSADH) as this organism degrades putrescine constitutively (Michaels and Tchen, 1971). Similar to the 4ABADH, 3APADH proved to be very labile and was purified only after adoption of the 30% glycerol method of Jacoby and Bradbury (1972). The purified enzyme has been shown to consist of three subunits of molecular weight of 74,000. It is most active for 3-aminopropanal, less active toward 4-aminobutanal, and least active toward succinic semialdehyde. The ratios of V_{max} for the respective substrates are approximately 1:0.17:0.13, which is similar to that observed for 4ABADH (Callewaert *et al.*, 1974). However, the K_m's for 3-aminopropanal and 4-aminobutanal are approximately equal (5.1 and 5.9 $\times 10^{-4}$, respectively) in the case of 3APADH but differ by almost 30-fold (8.3 and 0.28 $\times 10^{-4}$, respectively) in the case of 4ABADH. These properties, together with the induction of the enzyme in the present study by bis(3-aminopropyl)amine (presumably *via* 3-aminopropanal), establish it as 3-aminopropanal dehydrogenase.

The enzyme is maximally active toward the three different aldehydic substrates at pH 8.3–8.5. This range of optimal pH is much sharper than that obtained for 4-ABADH. In contrast, the molecular weight of the enzyme and its subunits are virtually identical with those obtained for the 4ABADH from this organism (Callewaert *et al.*, 1974). As with the 4ABADH (Rosemblatt *et al.*, 1974), 3APADH does not cross-react with antisera to SSADH. It does cross-react on immunodiffusion with antisera to BADH, although the results suggest antigenic similarity but not identity. 3APADH can also be completely inactivated by antibodies to 4ABADH, closely following the titration curve of the original antigen.

3-APADH can also be separated into two to three active species by prolonged electrophoresis in high concentrations of acrylamide or by isoelectric focussing. In both experiments, one band is very similar to that of 4ABADH. It is not clear at present if the multiple bands are due to alteration during purification, or to hybrid formation between the two enzymes. The latter explanation is consistent with the similarity of immunochemical properties and molecular weight of these two enzymes which strongly suggest a close evolutionary relationship. Further investigation into the nature and extent of the evolutionary relationship of these dehydrogenases to each other and to succinic semialdehyde dehydrogenase could lead to a better understanding of the process of enzyme evolution.

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