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Determining a novel NAD^+-dependent amine dehydrogenase with a broad substrate range from *Streptomyces virginiae* IFO 12827: purification and characterization

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Abstract

A novel NAD^+-dependent amine dehydrogenase (AMDH) was screened from serinol-assimilating microorganisms and type cultures using a serinol as a substrate. The newly found enzyme from *Streptomyces virginiae* IFO 12827 was strictly dependent on NAD^+ or NADH, and did not require artificial electron acceptors such as phenazine methosulfate (PMS), on which all the previously reported AMDHs acted. The enzyme was purified from the cell-free extract of *S. virginiae* cells to homogeneity by a six-step purification procedure. The enzyme had a homodimeric structure consisting of 46 kDa subunits. It catalyzed the reversible oxidative deaminations of not only amines but also amino alcohols and amino acids. The production of 2-amino-1-propanol and aspartic acid by the reductive amination of the corresponding keto alcohol and keto acid in the presence of ammonium ions and NADH, and that of acetophenone from phenethylamine by the oxidative deamination in the presence of NAD^+ were confirmed by the AMDH reactions. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Amine dehydrogenase; NAD^+-dependent; *Streptomyces virginiae*; Amine; Amino alcohol; Amino acid

1. Introduction

Methylamine dehydrogenases, which catalyze the oxidative deamination of methylamine to formaldehyde and ammonium ion, were purified from methylotrophs and reported to be quinoproteins containing tryptophan tryptophylquinone (TTQ) [1–5]. Aromatic amine dehydrogenase (AMDH) from *Alkaligenes faecalis*, which also contains TTQ as a prosthetic group [6,7], and that from *Pseudomonas putida* [8] catalyze the oxidative deamination of some aromatic amines including tyramine, phenethylamine as well as some aliphatic amines to a lesser extent. The other type of AMDH is a quinohemoprotein containing not only quinone but also heme *c*, which was found in *P. putida*, catalyzing the deamination of some aliphatic amines such as *n*-propylamine and *n*-butylamine [9–12]. Polyamine dehydrogenase from *Citorbacter freundii* is a similar quinohemoprotein containing heme *b*, which mainly catalyzes the deamination of spermidine and spermine [13,14]. However, these enzymes have a rather

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strict substrate specificity, and require artificial electron acceptors such as phenazine ethosulfate or phenazine methosulfate without exception [15]. The physiological electron acceptor for the above AMDHs is a copper protein including amicyanin and azurin [12,15].

Biocatalysts, which can reduce ketones or keto alcohols accompanying amination with NAD(P)H and ammonium ions, have been desired for a long time, because such enzymes would easily produce amines, especially chiral amines, from the corresponding keto compounds with an established NAD(P)H recycling system in one step. Some amino acid dehydrogenases including alanine dehydrogenase, leucine dehydrogenase, etc., catalyze such reactions [16,17], however, their substrates are only 2-keto acids. In addition, some aminotransferases transfer amino groups from a donor, generally an amino acid, to an acceptor, generally a 2-keto acid, to yield amino acids. However, the applications of these enzymes have been limited to a few compounds including the L-phenylalanine derivatives [17,18] becasuse they require expensive donors and 2-keto acids.

In the course of our searching for a serinol-utilizing enzyme, we have found a very new AMDH serving NAD^+ as a cofactor. This paper describes the purification and some properties of the novel AMDH with a broad substrate range from *S. virginiae*, and discuss the application of this enzyme as a biocatalyst.

2. Materials and methods

2.1. Strain and culture conditions

The *S. virginiae* IFO (Institute for Fermentation Osaka, Japan) 12827 strain was used throughout this study. It was selected as an $NAD(P)^+$-dependent serinol dehydrogenase-producing strain from approximately 300 microorganisms consisting of 100 serinol-assimilating microorganisms, 100 actinomycetes isolated from soil samples and 100 type cultures maintained in our laboratory. The detailed procedures and results of the screening will be reported elsewhere. The *S. virginiae* IFO 12827 strain was pre-cultured in a 50-ml medium containing 1% (w/v) soluble starch, 0.2% yeast extract, 0.2% NZ amine (type A), 0.2% malt extract and 0.1% meat extract (pH 7.3) in a 300-ml flask at 30°C for 2 days by rotary shaking (200 rpm). The broth was transferred into 3 l of medium consisting of 1.5% soluble starch, 0.8% soytone, 0.3% glucose, 0.5% meat extract, 0.2% K_2HPO_4, 0.2% NaCl, 0.1% (v/v) of 10% $CaCl_2 \cdot 2H_2O$ solution, 0.1% TM solution as described by Okanishi et al. [19] and 0.13% antifoam A (Sigma) in a 5-l jar fermentor, and cultivation was performed at 30°C for 36 h at a constant aeration rate of 0.75 l/min and an agitation speed of 300 rpm. The cells (about 70 g wet weight) were collected by centrifugation (20,000 g × 10 min), suspended and then washed twice in 80 ml of 20 mM potassium phosphate buffer (KPB) (pH 7.0).

2.2. Enzyme and protein assay

AMDH activity was spectrophotometrically assayed at 25°C by measuring the increase in the absorption of formazane produced from 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H tetrazolium chloride (INT) at 490 nm ($\varepsilon = 15{,}000\ M^{-1}\ cm^{-1}$). The reaction mixture consisted of 150 μmol Tris–HCl buffer (pH 8.0), 0.75 mg (1.4 μmol) INT, 1.5 μmol NAD^+, 15 μmol serinol, and 10–50 μl of the enzyme solution in a total volume of 1.5 ml. The reverse reaction of AMDH was measured by the decrease of NADH at 340 nm in 1.5 ml of reaction mixture containing 150 μmol KPB buffer (pH 7.0), 15 μmol dihydroxyacetone, 300 μmol NH_4Cl, 0.15 μmol NADH ($\varepsilon = 6{,}220\ M^{-1}\ cm^{-1}$) and 10–50 μl of enzyme solution in a total volume of 1.5 ml. The blank contained buffer instead of substrate. One unit of enzyme in the oxidative reaction was defined as the

amount that formed 1 μmol of INT formazane in 1 min at 25°C under these conditions, unless otherwise indicated. For the reductive reaction, 1 unit of enzyme was defined as the amount that converted 1 μmol of NADH in 1 min at 25°C.

The protein concentration was estimated by measuring the absorbance at 280 nm, or by using the Bio-Rad Protein Assay kit with bovine serum albumin as the standard protein according to the manufacturer's protocol.

2.3. Enzyme purification

All purifications were performed at 0–5°C using 20 mM KPB (pH 7.0) supplemented with 1 mM EDTA, 1 mM NaN_3 and 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and centrifugation was carried out at 20,000 × g for 30 min, unless otherwise indicated. The cells (about 140 g wet weight) collected from the 6-l culture broth were suspended in the buffer (420 ml) and physically homogenized with a IKA Ultra-Turrax (Jank and Kunkel, Germany) for 3 min, then disrupted with a Kubota 201M ultrasonic oscillator (Tokyo, Japan) for 20 min. The cell debris was removed by centrifugation and the combined supernatant was treated with 0.01% (v/v) polyethylene imine (mean M_r:70,000) for 30 min to remove the nucleic acid followed by centrifugation.

The enzyme solution was mixed with about 100 ml of Blue–Sepharose CL-4B matrices with a gentle stirring for 1 h. The matrices were filtered off with suction and washed once with the buffer. AMDH activity appeared in the filtrate.

Ammonium sulfate was added to the filtrate up to 20% saturation, which had been concentrated by the ultrafiltration apparatus with a AIP-0013 hollow fiber (Asahikasei, Tokyo), and the slight precipitate was removed by centrifugation. The supernatant solution was applied to a Butyl–Toyopearl 650 (Tosho, Tokyo) column (6 × 8 cm) which had been equilibrated with 20% saturation ammonium sulfate in the buffer. The enzyme was eluted by linearly lowering the ionic strength from 20% to 0% saturation with ammonium sulfate in the buffer (600 ml). The fractions (8 ml) with high enzyme activity were collected, and concentrated with the above ultrafiltration system followed by replacing the buffer with 10 mM KPB (pH 7.0).

The enzyme solution was loaded on a Serine–Sepharose CL-4B column (2.5 × 22 cm) equilibrated with 10 mM buffer (pH 7.0), and then washed well with the same buffer. AMDH was eluted with a linear 0–1.0 M NaCl plus 20 mM serine gradient in the same buffer (500 ml). Fractions (7 ml) with high enzyme activity were collected, concentrated with an ultrafiltration system using a Centriprep-10 apparatus (Amicon, MA, USA).

The enzyme was subjected to an NAD^+–Agarose column (N-6 binding type, Sigma-Aldrich, MO, USA, 1.5 × 7 cm) equilibrated with the same buffer. After washing the column, AMDH was eluted with 1.0 mM NADH in the 10 mM buffer. The combined fractions were concentrated and dialyzed with the above ultrafiltration system against 20 mM Tris–HCl buffer (pH 8.0).

The enzyme solution was subjected to a FPLC system (Pharmacia Biotech, Japan) equipped with a Poros HQ column (4.6 × 100 mm, PerSeptive Biosystems, MA, USA) equilibrated with 10 mM Tris–HCl buffer (pH 8.0). The enzyme was eluted with a linear gradient of 0–1 M NaCl at a flow rate of 4 ml/min. The fractions (2 ml) with the enzyme activity were pooled, concentrated with the ultrafiltration system and stored at −20°C with 20% glycerol.

2.4. Polyacrylamide gel electrophoresis (PAGE)

SDS-PAGE was performed in a 10% polyacrylamide slab gel using the Tris–glycine buffer system described by Laemmli [20]. The molecular mass of the enzyme subunit was determined from the relative mobility of the standard proteins.

2.5. High-performance liquid chromatography (HPLC)

Analytical HPLC for protein proceeded using a Shimadzu GC-14A system (Kyoto, Japan) equipped with a TSK-Gel G3000SW$_{XL}$ column (7.8 mm × 30 cm, Tosoh, Japan) at a flow rate of 0.4 ml/min using 0.1 M KPB containing 0.1 M NaCl (pH 7.0) at 280 nm. The molecular mass of the native enzyme was determined by comparing the retention time with those of standard proteins.

2.6. Determination of products from ketones by AMDH reaction

The reaction mixture for determining the product from the keto alcohol consisted of 10 μmol of each substrate, 10 μmol NADH, 0.1 mmol KPB (pH 7.0), and 0.01 units of the partially purified AMDH after Serine–Sepharose chromatography in a total volume of 2 ml. The reaction proceeded for 17 h at 25°C, then the product was analyzed by gas chromatography (GC). GC was performed using a Shimadzu GC-14A system equipped with a coiled column (3 mm × 1 m) packed with TENAX TA (60–80 mesh, Shimadzu) with an FID (flame ionization detector) under the following conditions: column temperature was 150°C then raised to 180°C at 10°C min^{-1} and maintained at 180°C for 10 min; injection and detection temperatures were 250°C, at a flow rate of 50 ml/min N_2. The substrate and product were indicated by the following retention times (min): hydroxyacetone, 2.8; 2-amino-1-propanol, 3.7.

For determining the amino acid product, the sample was analyzed by the HPLC system described above equipped with a CAPCELL PAC C18 AG120 column (4.6 mm × 25 cm, Shiseido, Tokyo) at a flow rate of 1.0 ml/min at 340 nm using a linear mobile phase gradient from (A):10 mM sodium phosphate buffer (pH 6.8) to (B):10 mM sodium phosphate buffer (pH 6.8) plus CH_3CN (1:2 by volume) for 40 min at 45°C. The amino acid-containing sample (25 μl) was previously mixed with 350 μl of 0.1 M sodium borate buffer (pH 9.1) containing 0.03% β-mercaptopropionic acid and 350 μl of 0.1% *o*-phthalaldehyde (OPA) in CH_3CN to convert the amino acid into its OPA-derivative.

The reaction mixture for analyzing the ketone product from the amine consisted of 10 μmol of phenethylamine, 10 μmol NAD^+, 0.2 mmol Tris–HCl (pH 8.0), 1.9 μmol INT, 0.01 units of AMDH in a total volume of 2 ml. The reaction proceeded for 17 h at 25°C. The sample was analyzed by an HPLC system equipped with a TSK-Gel ODS-80TS column (4.6 mm × 15 cm, Tosoh) using a mobile phase containing 20 mM sodium phosphate buffer (pH 7.0) plus CH_3CN (20% by volume) at a flow rate of 1.0 ml/min at 35°C and detected at 220 nm. Retention times (min) of the phenethylamine and acetophenone were 8.2 and 17.1.

2.7. Chemicals

The marker protein kit for HPLC and glutamate dehydrogenase from beef liver were obtained from the Oriental Yeast, Japan. Alanine dehydrogenase from *Bacillus stearothermophilus* and microbial glutamate dehydrogenase were supplied by the Seikagaku and Toyobo, Japan, respectively. SDS-PAGE molecular weight standards (low molecular weight), DEAE–Sepharose CL-6B and Sepharose CL-4B were purchased from Pharmacia Biotech, Sweden, and NAD^+–Agarose (N-6 binding type) from Sigma. Blue–Sepharose CL-4B was prepared from epoxy-activated Sepharose CL-4B with Cibacron Blue 3GA (Sigma) according to the method of Itoh [21]. Serine–Sepharose was prepared with in a two-step reaction from epoxy-activated Sepharose CL-4B. Aminohexyl (AH)–Sepharose was first synthesized from epoxy-activated Sepharose CL-4B with 1,6-diaminohexane in the same manner as stated above [21], then it was coupled with serine using

1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (Sigma). AH–Sepharose (100 ml) was mixed with 70 ml of distilled water containing 150 mg serine, the pH was adjusted to 4.6 and solid carbodiimide (ca. 9 g) gradually added to the mixture for 1 h by adjusting the pH at 4.6–6.0, then incubated for 24 h with gentle stirring. The obtained Serine–Sepharose was rinsed well with 1 M NaCl and distilled water. All other reagents used were of analytical grade.

3. Results and discussion

3.1. Purification of AMDH

The enzyme was purified to be homogeneous from the cell-free extract by sequential column chromatography. Preliminary experiments showed that the general dialysis procedure using a dialysis membrane and ammonium sulfate fractionation caused the loss of AMDH activity, and the enzyme easily lost its activity through column chromatography. Therefore, we checked on its stabilizing conditions, and as a result, the addition of 1 mM EDTA, 1 mM NaN_3 and 0.5 mM PMSF to the buffer gave good results, suggesting that the enzyme was probably sensitive toward the contaminating proteases. The enzyme did not bind to Blue– and Red–Sepharose in spite of its NAD(H)-dependent oxidoreductase activity. However, Blue–Sepharose treatment was useful to eliminate the contaminating alanine dehydrogenase activity from the crude extract which is normally produced by various *Streptomyces* strains [22]. Hydrophobic chromatography of Butyl–Toyopearl and affinity chromatographies using Serine–Sepharose and NAD^+–Agarose were effective in purifying the enzyme. The elution profiles for these column chromatographies are shown in Fig. 1. The final chromatography using the FPLC system with a Poros HQ column effectively eliminated minor impurities from the enzyme sample. Purified AMDH (0.74 mg) showing about 0.12 units/mg protein was obtained from 140 g (wet weight) of cells with a yield of 0.06% (Table 1). We could not completely prevent the loss of activity during the purification procedures, which resulted in the low recovery of AMDH. Therefore, specific activity of the enzyme in vivo must be much higher than the purified one. The purity of the enzyme was checked by SDS-PAGE (Fig. 2) and was homogeneous according to the analysis.

3.2. Physicochemical properties of AMDH

According to the analytical HPLC on TSK-Gel $3000SW_{XL}$, the molecular mass of the enzyme was estimated to be 95 ± 5 kDa. SDS-PAGE revealed a single band, and the subunit molecular mass was 46 kDa. These data showed that AMDH was a dimeric protein consisting of identical molecular weight subunits.

3.3. Enzymatic reaction and kinetic properties of AMDH

AMDH catalyzed the reversible dehydrogenation of serinol in the presence of NAD^+ to give dihydroxyacetone, ammonium ion and NADH, while $NADP^+$ showed a slight activity (13%) as a cofactor. The enzyme showed no activity in the PMS and the 2,6-dichloroindophenol (DCIP) system generally used for quinoprotein AMDHs [11]. On the other hand, in the reductive amination reaction, NADPH showed a much lower activity (8.5%) than NADH. No enzyme activity was observed in the absence of ammonium ions. As described below, the enzyme catalyzed the reversible oxidation–reduction reactions of various amines or ketones and aldehydes accompanying the deamination or amination reactions. Therefore, the enzyme was defined as a novel AMDH (Amine: NAD^+ oxidoreductase (deaminating)).

The K_m values of AMDH toward substrates and cofactors were calculated from the Linew-

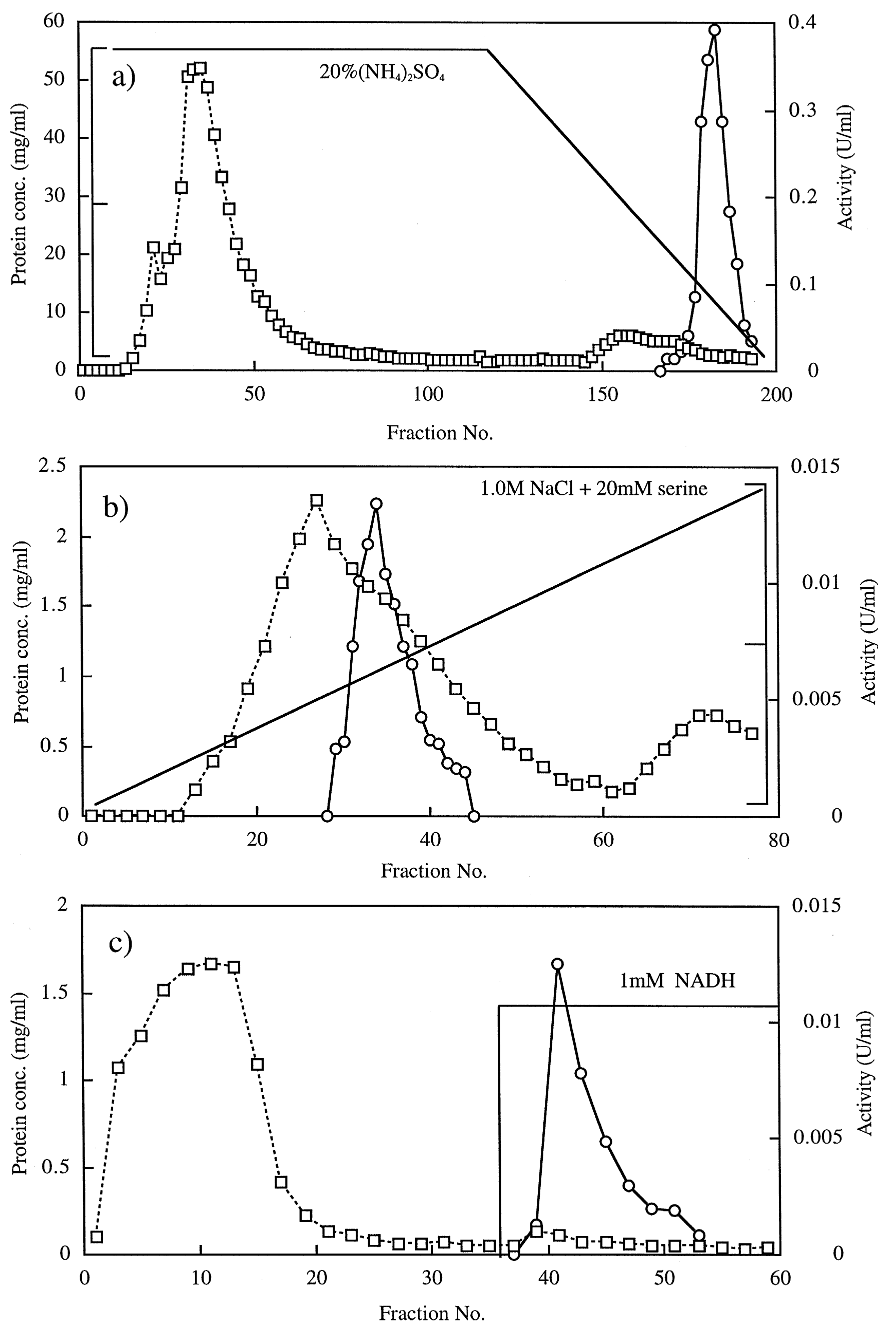

Fig. 1. Butyl–Toyopearl 650 M (a), Serine–Sepharose (b) and NAD^+–Agarose (c) column chromatography of AMDH from *S. virginiae* IFO 12827. Symbols: solid line, concentrations of $(NH_4)_2SO_4$ (a), NaCl and serine (b) and NADH (c) in the elution buffers; (□) dashed line, the absorbance at 280 nm of the protein; (○) solid line, AMDH activity.

ever–Burk plots. In the oxidative deamination reaction, the enzyme activities were measured using 100 mM Tris–HCl buffer (pH 9.0) instead of the buffer of pH 8.0 in the absence of INT. The K_m values for serinol and NAD^+ were 4.0 mM and 0.84 mM. In the reductive amination reaction at pH 7.0, the K_m values for dihydroxyacetone, NADH and NH_4^+ were 2.2 mM, 0.022

Table 1
Summary of the purification of AMDH from *S. virginiae* IFO 12827

Step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Yield (%)
Crude extract	4006	147	0.037	100
PEI[a] treatment	4332	263	0.061	179
Blue–Sepharose	3962	120	0.030	82
Butyl–Toyopearl	822	65	0.079	44
Serine–Sepharose	278	4.8	0.017	3.3
NAD^+–Agarose	15	1.5	0.01	0.1
Poros HQ	0.74	0.09	0.12	0.06

[a]Polyethylene imine.

mM and 26.5 mM, respectively. In all cases, regular saturation curves between the activity versus the substrate concentration were observed.

The effect of pH on the AMDH activity was measured in the some buffer systems. As shown in Fig. 3, the enzyme showed maximum activity at pH 10.0 in the deaminating reaction, while the optimum pH of the amination reaction was rather broad and between 6.5 and 7.0.

The enzyme activity of the deamination reaction was measured at various temperatures from 10°C to 45°C. The enzyme showed maximum activity at around 30°C. The enzyme was incubated in the buffer (pH 7.0) at various temperatures for 30 min and retained the following activities: 25°C, 100%; 30°C, 55%; 40°C, 25%; and 45°C, 0%. AMDH was not a thermo-stable enzyme.

3.4. Substrate specificity of AMDH

AMDH activity for the oxidative deamination of various compounds was examined and the results are shown in Table 2 compared with the well-known alanine and glutamate dehydrogenases. The latter two enzymes did not act on some of the listed substrates except for alanine or glutamate, respectively. Therefore, AMDH was concluded to be a very different enzyme from the general amino acid dehydrogenases. AMDH acted on a broad range of substrates

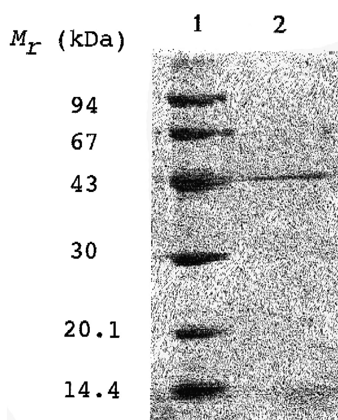

Fig. 2. SDS-PAGE of the purified AMDH. Lane 1, the standard molecular proteins for SDS-PAGE were: 1, phosphorylase *b*; 2, bovine serum albumin; 3, ovalbumin; 4, carbonic anhydrase; 5, soybean trypsin inhibitor; 6, lysozyme; Lane 2, the purified AMDH.

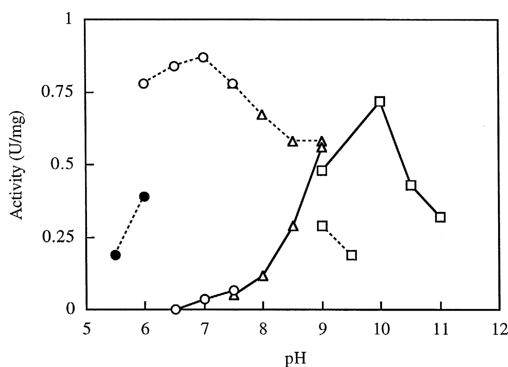

Fig. 3. AMDH activity as the function of pH. The activity in the oxidative deamination reaction (solid line) was measured in the following 0.1 M buffers: KPB (pH 6.5–7.5) (○), Tris–HCl (pH 7.5–9) (△), glycine–NaOH (pH 9–11) (□), and in the reductive amination reaction (dashed line) in the following 0.1 M buffers: acetate (pH 5.5–6) (●), KPB (pH 6–7.5) (○), Tris–HCl (pH 7.7–9) (△), glycine–NaOH (pH 9–9.5) (□).

Table 2
Substrate specificity of AMDH for the oxidative deamination compared with L-alanine (NAD^+) and L-glutamate (NAD^+) dehydrogenases

Substrate	Relative activity (%)		
	AMDH	AlDH[a]	GDH[b]
Amino alcohol			
Serinol (NAD^+)	100	0	0
Serinol ($NADP^+$)	13	–[c]	–
(*R,S*)-2-Amino-1-propanol	32	0	0
(*S*)-2-Amino-1-butanol	92	–	–
(*S*)-2-Amino-3-methyl-1-butanol	80	–	–
(*R,S*)-2-Amino-1-pentanol	105	–	–
(*S*)-Leucinol	89	–	–
(*S*)-Isoleucinol	107	–	–
(*S*)-Methioninol	100	–	–
2-Aminocyclohexanol	43	–	–
2-Amino-3-hydroxypyridine	34	–	–
Amino acid			
L-Alanine	95	100	0
D-Alanine	88	–	–
L-Aspartic acid	69	0	0
D-Aspartic acid	90	–	–
L-Glutamic acid	94	0	100
D-Glutamic acid	80	–	–
L-Serine	69	–	–
D-Serine	63	–	–
Amine			
Methylamine	105	–	–
Ethylamine	78	–	–
n-Propylamine	74	–	–
n-Butylamine	78	–	–
n-Hexylamine	118	–	–
n-Octylamine	104	–	–
(*R*)-*sec*-Butylamine	66	0	0
(*S*)-*sec*-Butylamine	107	–	–
2-Aminopentane	108	0	0
3-Aminopentane	116	–	–
(*R*)-2-Aminoheptane	66	–	–
(*S*)-2-Aminoheptane	89	–	–
Benzylamine	114	–	–
(*R*)-1-Phenethylamine	139	0	0
(*S*)-1-Phenethylamine	71	0	0

[a] *B. stearothermophilus* enzyme.
[b] Both enzymes from beef liver and microorganism showed the same results.
[c] Not tested.

including not only the aliphatic and aromatic aminoalcohols and amines but also the L- and D-amino acids, especially the 2-amino compounds. Primary alkylamines such as methylamine, *n*-propylamine and *n*-butylamine also served as a substrate. These results indicated that the NAD^+-dependent AMDH is quite different from the quinone-containing AMDHs in substrate specificity.

In the reductive amination reaction (Table 3), the enzyme indicated a broad substrate specificity that coincided with the oxidative deamination reactions. Especially, pyruvate showed higher activities than other substrates.

Stereospecificity of the enzyme is the most interesting issue of AMDH. Although the enzyme did not show high stereospecificity toward some compounds in the oxidative deamination, twice the activity was observed for (*R*)-phenethylamine compared with that for the (*S*)-form. Similar stereospecificity was observed

Table 3
Substrate specificity of AMDH for the reductive amination

Substrate	Relative activity (%)
Keto alcohol	
Dihydroxyacetone (NADH)	100
Dihydroxyacetone (NADPH)	8.6
Hydroxyacetone	108
3-Hydroxy-2-butanone	100
4-Hydroxy-2-butanone	122
3-Acetyl-1-propanol	100
4-Hydroxy-3-hexanone	94
5-Hydroxy-4-octanone	93
2-Hydroxyacetophenone	100
Keto acid	
Pyruvate	200
Oxalacetate	83
2-Oxoglutarate	107
Hydroxypyruvate	88
Ketone	
2-Butanone	118
2-Pentanone	108
2-Hexanone	100
Acetophenone	87
4-Phenyl-2-butanone	93
Aldehyde	
Formaldehyde	49
Acetaldehyde	118
n-Propionaldehyde	97
n-Butyraldehyde	118
n-Hexylaldehyde	100
Benzaldehyde	149

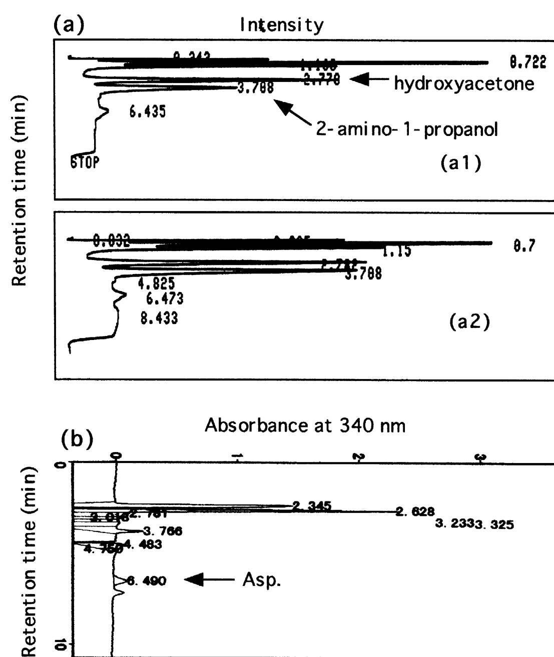

Fig. 4. Analyses of products by AMDH-catalyzed reactions. (a): gas chromatogram of the product from hydroxyacetone (a1) and that of co-chromatogram with an authentic 2-amino-1-propanol (a2); (b) HPLC elution profile of the product from oxalacetate; the retention time of the product was the same as that of L-aspartic acid.

for the (*S*)-*sec*- and (*R*)-*sec*-butylamines. Further experiments to determine the absolute configurations of the produced amine compounds by the AMDH reactions are in progress.

3.5. AMDH-catalyzed production of amines and amino acid

To clarify the products catalyzed by the AMDH reactions, hydroxyacetone, oxalacetate and phenethylamine were chosen as substrates. We determined using GC that 2-amino-1-propanol was produced from hydroxyacetone by the AMDH reaction, which was confirmed by co-chromatography with an authentic compound, as shown in Fig. 4a.

AMDH also catalyzed the reductive amination of oxalacetate to give aspartic acid. Fig. 4b displays the formation of this amino acid on HPLC compared with an authentic one. However, the absolute configuration of aspartic acid has not yet been determined.

Formation of acetophenone from phenethylamine by the AMDH-catalyzed deamination reaction was observed during HPLC in the presence of INT at pH 8.0 (data not shown).

The above results clarified that the AMDH would directly produce various amino alcohols and amine derivatives during the reductive amination reactions from the corresponding ketones. Such an enzyme might be a quite useful biocatalyst for producing amines in the near future.

4. Conclusion

Our findings reported here showed that the enzyme found in *S. virginiae* IFO 12827, which was first isolated as a serinol-dehydrogenating one, is a very novel NAD^+-dependent AMDH and would be a useful biocatalyst producing amine derivatives. Using the enzyme coupled with a NADH regeneration system, some chiral amines may be practically produced. To further study the enzyme's properties including stereospecificity, whether or not it contains a prosthetic group and its application to organic syntheses, etc., cloning and the expression of the enzyme gene is under way.

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