CRYSTALLINE ASPARTATE AMINOTRANSFERASE FROM *PSEUDOMONAS STRIATA*

Toshiharu YAGI, Mitsuyoshi TOYOSATO and Kenji SODA
Laboratory of Microbial Biochemistry, Institute for Chemical Research Kyoto University, Uji, Kyoto-Fu 611, Japan

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1. Introduction

Aspartate aminotransferase (EC 2.6.1.1.) is ubiquitously distributed in nature and plays the most important role in amino acid metabolism. Two forms of aspartate aminotransferase are found in cytosol and mitochondria of mammalian and avian tissues, and their physicochemical, kinetic and protein chemical properties have been extensively studied as reviewed by Snell and Di Mari [1] and Braunstein [2]. Although the occurrence of microbial aspartate aminotransferase also was demonstrated many years ago [3], little effort has been devoted to the purification and the characterization of the enzyme. Recently, Mavrides and Orr [4] obtained the purified two aminotransferases from *Escherichia coli,* which were found homogeneous upon gel electrophoresis, and reported that one of them (enzyme A) is mainly an aspartate aminotransferase. In this communication, we describe the preparation of crystalline aspartate aminotransferase from *Pseudomonas striata* and some of its properties to compare with those of the mammalian enzymes.

2. Materials and methods

Pseudomonas striata IFO 12996 [5] was grown in a medium composed of 1.0% peptone, 0.1% glycerol, 0.5% NaCl, 0.5% meat extract and 0.01% yeast extract. The pH was adjusted to 7.2 with KOH. Cultures were carried out at 28°C for about 17 h under aeration. The cells harvested were washed twice with 0.85% NaCl solution.

DL-α-Aminoadipic acid was synthesized by the method of Waalkes et al. [6]. L-Cysteinesulfinic acid was obtained from Sigma, other amino acids and sodium α-ketoglutarate from Ajinomoto Co., Tokyo, and [5-^{14}C]sodium α-ketoglutarate from Daiichi Pure Chemicals, Tokyo.

Enzyme assay Method A. The enzyme was routinely assayed by method A. The reaction mixture contained 150 μmol of Tris–HCl buffer (pH 8.0), 15 μmol of L-cysteinesulfinate, 15 μmol of α-ketoglutarate, and 9.0 nmol of pyridoxal 5′-phosphate (pyridoxal-P) in a final vol of 1.5 ml. After incubation at 30°C for 10 min followed by centrifugation, sulfur dioxide formed was determined with acidic *p*-rosaniline by the method of West and Gaeke [7]. Method B. The reaction mixture contained 150 nmol of NADH and 5 μg of malate dehydrogenase in addition to the components described for method A. The decrease in absorbance at 340 nm was measured at 25°C with a Shimadzu MPS-50L recording spectrophotometer. Method C. The reaction system consisted of 0.5 μmol of [5-^{14}C]sodium α-ketoglutarate, 15 μmol of amino acids except tyrosine (1 μmol), kynurenine (1 μmol) and α-aminoadipate (10 μmol), 2 nmol of pyridoxal-P, 10 μmol of sodium pyrophosphate buffer (pH 8.3) and enzyme in a final vol of 0.2 ml. After incubation at 30°C for 3 to 12 min, the reaction was terminated by addition of 0.2 ml of trichloroacetic acid. The radioactivity of [^{14}C]glutamate separated by paper chromatography was determined with a Tri-Carb liquid scintillation 3320 spectrometer.

One unit of enzyme is defined as the amount of enzyme that catalyzes the formation of 1.0 μmol of sulfur dioxide (method A) or glutamate (method C), or the decrease of 1.0 μmol of NADH per min. The specific activity was expressed as units per mg of protein. Protein was determined by the method of Lowry et al. [8] using egg albumin as a standard; with

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most column fractions, protein elution patterns were estimated by the 280-nm absorption.

3. Results and discussion

3.1. *Purification and crystallization*

All operations were carried out at 0–5°C. Step 1. The washed cells (about 1.5 kg, wet weight) were suspended in 2 liters of 0.02 M potassium phosphate buffer (pH 7.2) containing 2×10^{-4} M pyridoxal-P and 0.01% 2-mercaptoethanol, and subjected in 500 ml portions to sonication for 30 min in a 19-KC oscillator followed by centrifugation. The supernatant solution was dialyzed against 0.01 M potassium phosphate buffer (pH 7.2) containing 10^{-5} M pyridoxal-P. Step 2. The cell-free extract was brought to 30% saturation with ammonium sulfate and the precipitate was removed by centrifugation. Ammonium sulfate was added to the supernatant to 70% saturation. The resultant precipitate was dissolved in 0.01 M potassium phosphate buffer (pH 7.2) containing 10^{-5} M pyridoxal-P and dialyzed against the same buffer. Step 3. The enzyme was applied to a DEAE-cellulose column (10 × 80 cm) equilibrated with the dialysis buffer. The enzyme was eluted with the buffer supplemented with 0.1 M sodium chloride. To the enzyme solution was added ammonium sulfate to 70% saturation. The precipitate was dissolved in 0.01 M potassium phosphate buffer (pH 7.2) containing 10^{-5} M pyridoxal-P, dialyzed and chromatographed again on a DEAE-cellulose column as described above. Step 4. The enzyme was applied to a Sephadex G-150 column (2.5 × 130 cm) buffered with 0.01 M potassium phosphate buffer (pH 7.2) containing 10^{-5} M pyridoxal-P, and eluted with the same buffer. The active fractions were concentrated by addition of ammonium sulfate (70% saturation) and dialyzed against 0.001 M potassium phosphate buffer (pH 7.2). Step 5. The enzyme was applied to a hydroxyapatite column (1.5 × 50 cm) equilibrated with 0.001 M potassium phosphate buffer (pH 7.2). The active fractions were concentrated by ammonium sulfate (60% saturation) and dissolved in a small volume of 0.01 M potassium phosphate buffer (pH 7.2) containing 10^{-5} M

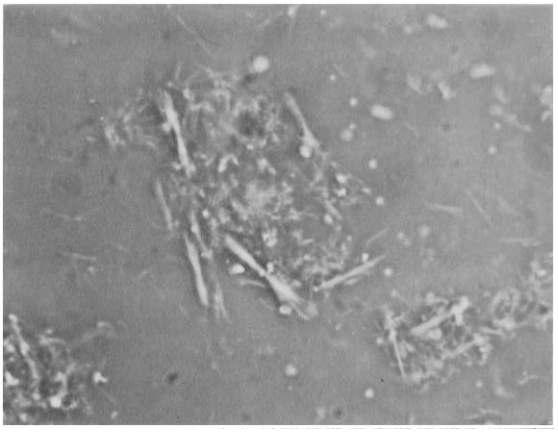

Fig.1. Crystals of aspartate aminotransferase.

Table 1
Purification of aspartate aminotransferase

Step	Fraction	Total protein	Total units	Specific activity	Yield
1.	Crude extract	206 000 (mg)	24 100	0.12	100
2.	Ammonium sulfate fractionation	62 000	19 500	0.31	81
3.	DEAE-cellulose chromatography	3600	13 700	3.81	57
4.	Sephadex G-200 chromatography	1100	8000	7.27	33
5.	Hydroxyapatite chromatography	68	1900	27.94	7.9
6.	1st crystallization	35	1700	48.57	7.1
	2nd crystallization	31	1400	45.15	5.8

Activity of aspartate aminotransferase was determined by the assay method A.

pyridoxal-P. Step 6. Ammonium sulfate was added to the enzyme solution until a faint turbidity was obtained. On standing overnight crystal formation occurred. The crystals took the form of fine needles (fig.1). A summary of the purification is presented in table 1.

3.2. *Properties*

The crystalline enzyme was shown to be homogeneous by the criteria of ultracentrifugation and disc gel electrophoresis (fig.2). The sedimentation coefficient ($S^0_{20,w}$) of the enzyme is 4.2 S. Assuming a partial specific volume of 0.74, a mol. wt. of 80 000 was obtained by the meniscus depletion sedimentation equilibrium method [9]. The spectrum of the bacterial aspartate aminotransferase is pH dependent. The enzyme exhibits absorption maxima at 280 (ϵ: 24 000) and 350 nm (ϵ: 2000) at pH 8.0, and 280 and 440 nm (ϵ: 1680) at pH 6.0. The spectral shift of the enzyme by varying the pH is closely similar to that of the mammalian enzyme [2]. The enzyme has a maximum reactivity in the pH range of 8.3–8.6. The K_m values determined by the assay method B were calculated to be 0.45 mM for L-aspartate, 0.29 mM for α-ketoglutarate, 12.5 mM for L-glutamate and 0.1 mM for oxalacetate. The K_m values of mammalian aspartate aminotransferases depend on the enzyme sources and the reaction conditions [2]. The value of mitochondrial aspartate aminotransferase of bovine liver for α-ketoglutarate is five-fold larger than that for aspartate, whereas the cytoplasmic enzyme has more than fifteen times higher affinity for α-ketoglutarate than for aspartate [10]. The affinities of both the mitochondrial and cytoplasmic enzymes of pig heart for α-ketoglutarate are much larger than those for aspartate [11]. But there is practically no differences in the magnitudes of K_m of the *Pseudomonas* aspartate aminotransferase for aspartate and α-ketoglutarate. The bacterial enzyme is similar to the mitochondrial and cytoplasmic enzymes in the low affinity for glutamate [11].

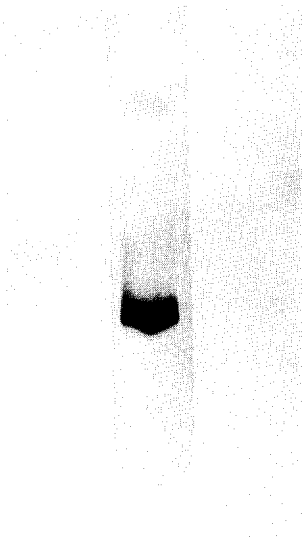

Fig.2. Disc gel electrophoresis of the enzyme. A sample of the crystalline enzyme preparation (50 μg) was electrophoresed under the conditions of Davis [14].

The activity of the bacterial enzyme with various amino acids and α-ketoglutarate as the amino acceptor is presented in table 2. Transamination of the natural amino acids, L-glutamate and L-aspartate, are significantly higher than those observed for the other amino donors. Aspartate is less actively transaminated than glutamate. This may be attributed to inhibition of the enzyme by the reaction product, oxalacetate, as reported for the mitochondrial aspartate aminotransferase of pig heart [11]. Cysteinesulfinate also is a good amino donor: this high reactivity is at least in part due to rapid breakdown of the keto product, β-sulfinylpyruvate to sulfur dioxide and pyruvate [12]. Phenylalanine shows about 29.5 and 2.7% reactivity with the mitochondrial and cytoplasmic aminotransferases, respectively, in comparison with glutamate [13]. The *Pseudomonas* enzyme is similar to the

Table 2
Amino donor specificity

Amino donor	Relative activity (%)
L-Glutamic acid	100
L-Aspartic acid	34
L-Cysteinesulfinic acid	21
L-Phenylalanine	6.9
L-Tyrosine	1.2
DL-α-Aminoadipic acid	0.4
L-Kynurenine	0.3
L-Alanine	0.1
L-Lysine	0.1

The enzyme activity was assayed by the method C.

cytoplasmic enzyme in this respect. α-Aminoadipate is far less susceptible to the enzyme, indicating that the enzyme can more effectively discriminate between 5-carbon and 6-carbon substrates than between a 4-carbon substrate and a 5-carbon substrate.

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