CRYSTALLINE ASPARTIC β-DECARBOXYLASE OF PSEUDOMONAS DACUNHAE

Ichiro Chibata, Toshio Kakimoto, Jyoji Kato, Takeji Shibatani, and Noriyuki Nishimura

Department of Applied Microbiology, Chemical Research Laboratory, Tanabe Seiyaku Co., Ltd., Higashi-yodogawa-ku, Osaka, Japan.

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Since the first report by Mardashev and Gladkova (1948) on L-aspartic β-decarboxylase [L-aspartate 4-carboxy-lyase (E.C.4.1.1.12)] in species of Pseudomycobacterium, its occurrence and enzymatic properties have been reported in a number of microorganisms such as Clostridium perfringens (Meister, et al., 1951; Nishimura, et al., 1962), Desulfovibrio desulfuricans (Senez and Cattanêo-Lacombe, 1956; Cattanêo-Lacombe, et al., 1958), Nocardia globerula (Crawford, 1958), Pseudomonas reptilivora (Seaman, 1960), Acetobacter sp. (Cooksey and Rainbow, 1962), Achromobacter d-15 (Wilson, 1963; Wilson and Kornberg, 1963), and Alcaligenes faecalis (Novogrodsky, et al., 1963; Novogrodsky and Meister, 1964).

As reported in our previous paper (Chibata, et al., 1965), we found Pseudomonas dacunhae produced markedly high activity of L-aspartic β-decarboxylase and applied the activity for production of L-alanine. This communication describes the crystallization and some of the properties of aspartic β-decarboxylase of Pseudomonas dacunhae.

Pseudomonas dacunhae was cultured under aerobic condition at 30° for 20 hr in 10 l of the medium containing 0.5% ammonium fumarate, 1.0% sodium fumarate, 0.9% peptone, 0.2% casein hydrolysate, 0.05% KH_2PO_4 and 0.01% $MgSO_4 \cdot 7H_2O$ (pH 5.5). The harvested cells (40 g dry wt.) were suspended in 500 ml of 0.1M potassium phosphate buffer (pH 6.8), soni-

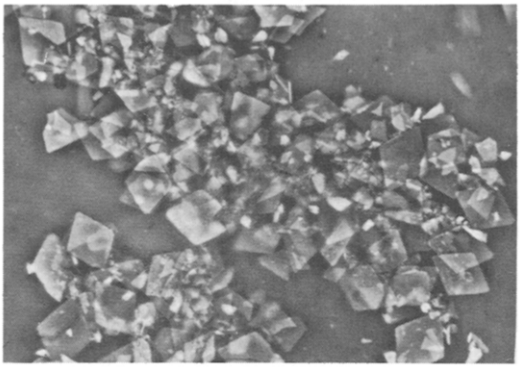

Fig.1. Crystalline aspartic β-decarboxylase of Pseudomonas dacunhae (x 800)

The crystalline enzyme possessed the highest specific activity ever reported (113,000 μl CO_2/hr/mg). Besides column chromatography, the homogeneity of the enzyme was confirmed by criteria such as ultracentrifugation ($S^0_{20,\,w}$= 22.1S) and electrophoresis. The molecular weight of this enzyme was determined as 820,000 by sedimentation equilibrium.

The stimulation of enzymic activity by pyridoxal phosphate and α-ketoglutarate with the known aspartic β-decarboxylase was observed also with the crystalline enzyme from Pseudomonas dacunhae. Besides these activators, several metal ions such as Fe^{++}, Fe^{+++}, Co^{++}, Ni^{++}, Mn^{++} and Al^{+++} also activated this enzyme in presence of pyridoxal phosphate (Table 2). The activity of the enzyme was increased 14-fold by the addition of pyridoxal phosphate and more than 30-fold by the either addition of α-ketoglutarate or metal and pyridoxal phosphate. The effects of these three factors are not cumulative.

cated for 15 min at 10 Kc, and the supernatant solution was collected by centrifugation (Step 1). To the solution ammonium sulfate was added, and the fraction precipitated between 30 and 50% saturation was collected, dissolved in a small volume of 0.1M potassium phosphate buffer (pH 6.8) and dialyzed overnight against the same buffer (Step 2). The dialyzed solution was kept at 50° for 1 h, cooled and centrifuged (Step 3). The supernatant solution was run into a DEAE-Sephadex column equilibrated with 0.2M potassium phosphate buffer (pH 6.8). The column was washed with the same buffer until no more material having absorption at 280 mμ appears, and then subjected to stepwise elution. The enzyme activity was discovered only in the fraction eluted by 0.3M buffer (Step 4). The active fraction was concentrated with ammonium sulfate, dissolved in a small volume of the buffer and dialyzed overnight against distilled water at 5°. This solution was then adjusted to the concentration of 0.04M potassium phosphate buffer (pH 6.8) containing 0.1M potassium chloride and passed into a Sephadex G-150 column equilibrated with the buffer. The column was eluted with the same buffer and the active fraction was collected (Step 5). The fraction was passed into a hydroxyl apatite column equilibrated with 0.14M potassium phosphate buffer (pH 6.8). The column was subjected to stepwise elution. The enzyme is eluted as a well-defined peak with a sharp leading edge by 0.3M buffer (Step 6). To prepare the enzyme saturated with pyridoxal phosphate, the above fraction was treated with pyridoxal phosphate and dialyzed against 0.1M potassium phosphate buffer (pH 6.8) to eliminate excess pyridoxal phosphate. Ammonium sulfate was added until the solution became slightly turbid and insoluble matter was discarded by centrifugation. The enzyme was crystallized from the supernatant solution by standing overnight at 5°. Fig. 1 is a photomicrograph of the crystalline enzyme. This procedure resulted in a 68-fold purification with an overall yield of 32% (Table 1).

Table 1. Summary of purification procedure

L-aspartic β-decarboxylase was assayed manometrically at 30°. Evolution of carbon dioxide was measured over 30 min after addition of L-aspartate. The main compartment contained, in a final volume of 3.2 ml, 100 μmoles of L-sodium aspartate, 600 μmoles of sodium acetate buffer (pH 5.3) and 5 μmoles of α-ketoglutarate. Enzyme, 4 μmoles of pyridoxal phosphate and 0.2 ml of 1% bovine serum albumin was added from the side-arm after 10 min equilibration. Specific activity of enzyme is defined as μl carbon dioxide released per hour per mg of protein under these conditions.

Step	Material	Volume	Total protein	Specific activity	Total activity	Yield
		ml	mg		x 10^5	%
1	Initial extract	470	18,400	1,670	307	100.0
2	Ammonium sulfate, 30-50% fraction	117	8,990	3,180	286	93.2
3	Heat treated fraction	81.5	1,810	11,800	214	69.7
4	DEAE-Sephadex fraction	200	280	59,400	166	54.1
5	Sephadex G-150 fraction	74.5	181	86,200	156	50.8
6	Hydroxyl apatite fraction	210	124	113,000	140	45.6
7	Crystals	-	87.1	113,000	98	31.9

The enzyme exhibited an absorbance maximum at 278 mμ and another maximum at about 360 mμ. Determination of the pyridoxal phosphate content of the enzyme by the phenylhydrazone procedure (Wada and Snell, 1961) gave a value of 16 mol of pyridoxal phosphate per mol of protein.

Details of results on the properties of the crystalline aspartic β-decarboxylase will be published elsewhere.

Table 2. Effects of pyridoxal phosphate, α-ketoglutarate and metal ions.

The reaction mixture contained L-sodium aspartate, 100 μmoles; pyridoxal phosphate, 4 μmoles; α-ketoglutarate, 5 μmoles; metals, 3.2 μmoles; sodium acetate buffer (pH 5.3), 600μmoles; 1% bovine serum albumin, 0.2 ml and the enzyme, 10μg, in a final volume of 3.2 ml.

Reaction mixture	CO_2 evolution	Relative activity
	μl CO_2/hr/mg	%
Enzyme	3,700	3.3
Enzyme + pyridoxal phosphate	51,500	45.6
Enzyme + α-ketoglutarate	110,000	97.3
Enzyme + pyridoxal phosphate + α-ketoglutarate	113,000	100.0
Enzyme + Fe^{++}	3,700	3.3
Enzyme + pyridoxal phosphate + Fe^{++}	107,000	94.7
Enzyme + pyridoxal phosphate + Fe^{+++}	108,000	95.6
Enzyme + pyridoxal phosphate + Co^{++}	94,700	83.8
Enzyme + pyridoxal phosphate + Ni^{++}	101,000	89.4
Enzyme + pyridoxal phosphate + Mn^{+++}	97,500	86.3
Enzyme + pyridoxal phosphate + Al^{+++}	91,200	80.7
Enzyme + pyridoxal phosphate + α-ketoglutarate + Fe^{++}	112,000	99.1

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