CRYSTALLINE LYSINE DECARBOXYLASE

Kenji Soda and Mitsuaki Moriguchi

Laboratory of Microbial Biochemistry, Institute for Chemical Research
Kyoto University, Uji, Kyoto-Fu, Japan

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Lysine decarboxylase (L-lysine carboxy-lyase, E.C. 4.1.1.18) was discovered and partially purified from *Bacterium cadaveris* and *Escherichia coli* by Gale and his coworker (Gale and Epps, 1944; Gale, 1945). Despite the widespread use of this enzyme for the manometric determination of L-lysine, it has not been enzymologically well-characterized. This communication describes the purification, crystallization and some of the properties of lysine decarboxylase from *Bacterium cadaveris*.

Purification and Crystallization.

Bacterium cadaveris IFO 3731 was grown in 7 l of a medium containing 0.2 % L-lysine, 1.0 % glycerine, 0.5 % peptone, 0.4 % $(NH_4)_2SO_4$, 0.2 % KH_2PO_4, 0.1 % K_2HPO_4, 0.01 % $MgSO_4 \cdot 7H_2O$ and 0.01 % yeast extract (pH 6.8). Cultures were carried out in a 10 l jar fermentor at 28° for 17 to 20 hours under aeration. The harvested cells were washed twice with 0.85 % sodium chloride solution. The yield of cells was approximately 5 g (wet weight) per 1 of the medium. All subsequent operations, unless otherwise stated, were performed at 0-5°.

Step I. The washed cells (600 g wet weight) were suspended in 2.5 l of 0.01 M potassium phosphate buffer, pH 6.2, containing 0.01 % 2-mercaptoethanol and subjected, in 200 ml portions, to sonication in a 19 kc oscillator for

30 minutes. The cells and cell debris were removed by centrifugation. The supernatant was dialyzed overnight against 100 volumes of 0.01 M potassium phosphate buffer, pH 6.2, containing 0.01 % 2-mercaptoethanol and 10^{-5} M pyridoxal 5'-phosphate.

Step II. The dialyzed enzyme was supplemented with pyridoxal 5'-phosphate and 2-aminoethylisothiouronium (final concentration, 10^{-5} M). To the enzyme solution was added 1 M acetate buffer, pH 4.4, under stirring to bring its final concentration to 0.02 M. After standing for 10 minutes, the enzyme solution was kept at 50° for 5 minutes, cooled rapidly and centrifuged.

Step III. The clear supernatant was brought to 30 % saturation with ammonium sulfate. After 30 minutes, the precipitate formed was removed by centrifugation. Ammonium sulfate was added to the supernatant to 55 % saturation ; the precipitate collected by centrifugation, was dissolved in 0.01 M potassium phosphate buffer, pH 6.2, containing 0.01 % 2-mercaptoethanol, 10^{-5} M pyridoxal 5'-phosphate and 0.2 M sodium chloride. The enzyme solution was dialyzed overnight against the same buffer. The insoluble materials formed during dialysis were removed by centrifugation.

Step IV. The dialyzed supernatant (149 ml) was placed on a DEAE-Sephadex A-50 column (3.5 x 41 cm) equilibrated with 0.01 M potassium phosphate buffer, pH 6.2, containing 0.01 % 2-mercaptoethanol, 10^{-5} M pyridoxal 5'-phosphate and 0.2 M sodium chloride. After the column was washed with the same buffer, the enzyme was eluted with 0.01 M potassium phosphate buffer, pH 6.2, containing 0.01 % 2-mercaptoethanol, 10^{-5} M pyridoxal 5'-phosphate and 0.6 M sodium chloride at a flow rate of 50 ml per hour and 10-ml aliquots of the eluate were collected. The active fractions were combined and precipitated by addition of ammonium sulfate (70 % saturation). The precipitate was dissolved in 0.01 M potassium phosphate buffer, pH 6.2, containing 0.01 % 2-mercaptoethanol and 10^{-5} M pyridoxal 5'-phosphate.

Step V. The enzyme (47 ml) was applied to a column of Sepharose 4B (3.5 x 140 cm) equilibrated with 0.01 M potassium phosphate buffer, pH 6.2,

containing 0.01 % 2-mercaptoethanol and 10^{-5} M pyridoxal 5'-phosphate, and eluted with the same buffer. A flow rate of 10 ml per hour was maintained and fractions of 5 ml were collected. The active fractions were pooled and concentrated by addition of ammonium sulfate (60 % saturation). The protein precipitated was dissolved in 0.01 M potassium phosphate buffer, pH 6.2, containing 0.01 % 2-mercaptoethanol, 10^{-5} M pyridoxal 5'-phosphate and 0.2 M sodium chloride and dialyzed against the same buffer.

Table I. Purification of Lysine Decarboxylase

The enzyme was assayed by determining the initial rate of carbon dioxide evolution from L-lysine manometrically. In the main compartment of a Warburg vessel, 1.2 ml of 0.1 M acetate buffer, pH 5.8, and 0.9 ml of enzyme were placed. The side arm contained 0.1 ml of 0.1 M L-lysine and 0.3 ml of water. L-Lysine was replaced by water in a blank. After equilibrium at 30° for 20 minutes, the reaction was initiated by addition of the side arm contents and reading were made at 2-minute intervals. One unit of enzyme activity is expressed in terms of μmoles of carbon dioxide released per minute. Specific activity is defined as units per mg of protein, determined by the method of Lowry et al.,(1951) or is estimated from the absorbance at 280 mμ

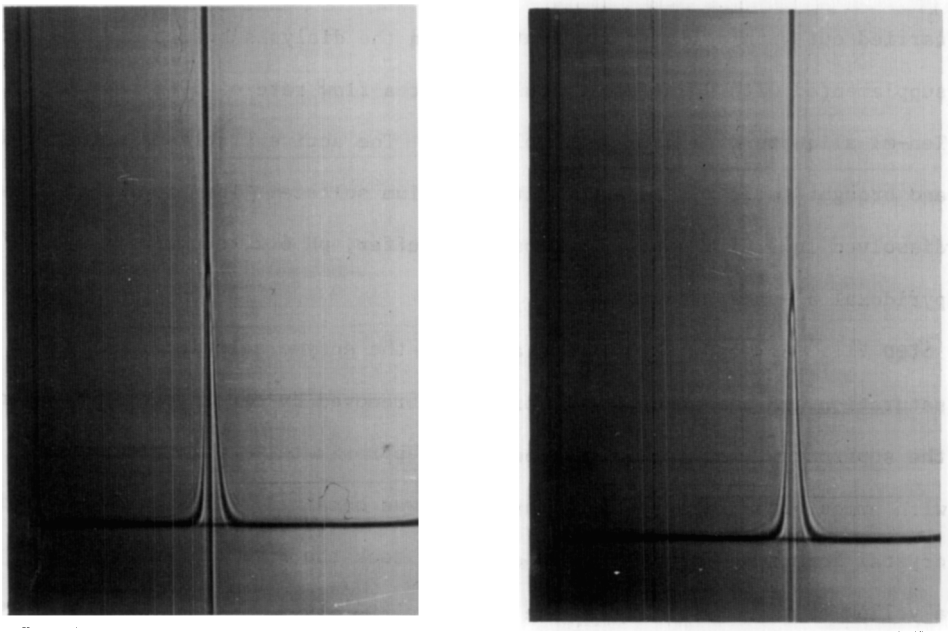
	Step	Total Protein	Total Units	Specific Activity
I.	Crude extract	87,345 mg	122,283	1.4
II.	Heat treatment	10,225	58,283	5.7
III.	Ammonium sulfate fractionation	5,364	44,521	8.3
IV.	First DEAE-Sephadex chromatography	1,974	35,137	17.8
V.	Sepharose 4B chromatography	1,160	30,970	26.7
VI.	Second DEAE-Sephadex chromatography	595	25,407	42.7
VII.	Crystallization	84	7,199	85.7

Step VI. The dialyzed enzyme (128 ml) was placed on a column of DEAE-Sephadex A-50 (3.5 x 40 cm) equilibrated as described in Step IV. After the column was washed with the aforementioned dialysis buffer, elution was carried out with a linear gradient between the dialysis buffer and the buffer supplemented with 0.5 M sodium chloride at a flow rate of 50 ml per hour. Ten-ml aliquots of eluate were collected. The active fractions were combined and brought to 70 % saturation with ammonium sulfate. The precipitate was dissolved in 0.01 M potassium phosphate buffer, pH 6.2, containing 10^{-5} M pyridoxal 5'-phosphate.

Step VII. Ammonium sulfate was added to the enzyme solution to 10 % saturation and the precipitate formed was removed by centrifugation. When the supernatant was gradually brought to approximately 20 % saturation with ammonium sulfate, a faint turbidity was obtained. On standing overnight, crystal formation occurred. The crystals took the form of fine rods with a yellow color. A summary of the purification procedure is given in Table I.

Properites.

The crystalline enzyme is homogeneous using the criterion of ultracentrifugation (Fig. 1), although it shows trace amounts of impurities on disc gel electrophoresis. An $S_{20,w}$ value of 21.1 S was calculated from the sedimentation rate for zero protein concentration. The molecular weight of the enzyme determined by the sedimentation equilibrium method (Van Holde and Baldwin, 1958), is 1,000,000 ± 50,000, assuming a partial specific volume of 0.74. The spectrum of the enzyme exhibits two absorption maxima at 280 mμ and 425 mμ ; these give an absorbance ratio of 12 : 1 (Fig. 2). No appreciable spectral shifts occurred when pH (5.8 - 9.0) was varied. 5-Hydroxylysine (DL and DL-allo) and S-(2-aminoethyl)-L-cysteine are decarboxylated by the enzyme at a rate of 35 % and 49 % that for L-lysine, respectively. The enzyme, when examined in the presence of acetate and phthalate buffers, has an optimum reactivity at pH 5.8. The K_m values are 3.7×10^{-4} M for L-lysine



(A) (B)

Fig. 1. Sedimentation pattern of lysine decarboxylase. Protein concentration, 8 mg per ml in 0.01 M potassium phosphate buffer, pH 6.2. Pictures were taken at bar angle of 75°. (A) and (B), 17 and 25 min, respectively, after achieving top speed (42,040 rpm).

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and 4.5×10^{-3} M for S-(2-aminoethyl)-L-cysteine. The activation of L-aspartate 4-decarboxylase by such α-keto acids as pyruvate and α-ketoglutarate was reported (Meister et al., 1951 ; Novogrodosky and Meister, 1964). However, the activity of L-lysine decarboxylase was not influenced by addition of these α-keto acids. The enzyme was found to contain 10 moles of pyridoxal 5'-phosphate per mole of enzyme, when examined by the phenylhydrazine method (Wada and Snell, 1961) and the cyanohydrin method (Bonavita, 1960). Many attempts were made to resolve the enzyme with little success.

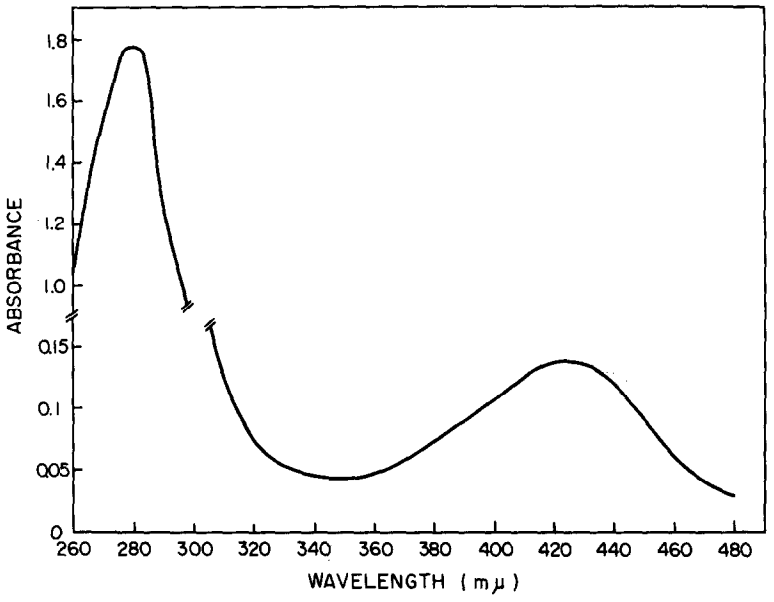

Fig. 2. Absorption spectrum of lysine decarboxylase (1.77 mg per ml) in 0.01 M potassium phosphate buffer, pH 6.2.

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