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Studies on L-Lysine Decarboxylase from *Bacterium cadaveris**

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Crystalline L-lysine decarboxylase was obtained from the cell-free extract of *Bacterium cadaveris* grown in the medium containing L-lysine as an inducer. The purification was carried out by the several steps including heat treatment, ammonium sulfate fractionation, the first DEAE-Sephadex chromatography, Sepharose 4B gel filtration, and the second DEAE-Sephadex chromatography. The purified enzyme was crystallized by addition of ammonium sulfate. The crystals took the form of small rod. The crystalline enzyme is homogeneous by the criteria of ultracentrifugation ($S^0_{20,w}$=21.1 S). The molecular weight is about 1,000,000, 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. No appreciable spectral shifts occurred when pH (5.8–9.0) was varied. δ-Hydroxylysine (DL and DL-allo) and *S*-(β-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 Km are 3.7×10^{-4} M for L-lysine and 4.5×10^{-3} M for *S*-(β-aminoethyl)-L-cysteine. The enzyme was found to contain 10 moles of pyridoxal 5′-phosphate per mole. The activity of enzyme was not influenced by the addition of α-keto acid.

INTRODUCTION

The amino acid decarboxylases are the enzymes which catalyze decarboxylation of L-amino acids to yield equimolecular carbon dioxide and the analogous amines, or ω or L-α amino acids.

In 1908, Ackerman *et al.*, first showed that if natural media consisting of certain amino acids are inoculated with putrefying material and the media are examined chemically after a period of some weeks, the amines corresponding to these amino acids can be isolated in a fair yield.[1~4] The biological production of such amines was later shown to be ascribed to the action of microbial amino acid decarboxylases.

The occurrence of various amino acid decarboxylases was demonstrated in animals, plant and microorganisms. In 1936, histidine decarboxylase (L-histidine carboxy-lyase, EC. 4.1.1.22) was discovered in animal tissues by Werle.[5] Glutamate decarboxylase (L-glutamate 1-carboxyl-lyase, EC 4.1.1.15) was found in several higher plants by Okunuki.[6] Gale and his collaborators reported the occurrence of the decarboxylases of arginine, histidine, lysine, tyrosine, glutamate and ornithine.[7]

These amino acid decarboxylases have been used as an excellent analytical tool for the specific determination of L-amino acids.

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In 1960, Shukuya and Schwert purified partially L-glutamate decarboxylase from *Escherichia coli* and described the spectrophotometric properties.[8]

Aspartate β-decarboxylase was purified and crystallized from the extract of *Achromobacter sp.* by Wilson.[9]

Recently, the crystalline histidine decarboxylase[10] and glutamate decarboxylase[11] were obtained.

The amino acid decarboxylase activity of *Streptococcus faecalis* was found to be dependent upon the presence of pyridoxine in the medium.[12,13] It was subsequently observed that pyridoxal plus adenosine triphosphate, or pyridoxal 5'-phosphate (PLP) produced considerable activation of the decarboxylase activity of the cells grown in the vitamin B_6-deficient media. A codecarboxylase preparation isolated from yeast[14] was reported to be identical with synthetic PLP in the amino acid decarboxylase reaction.[15~18]

Some of the amines produced from amino acids by the amino acid decarboxylase were shown to have the pharmacological activity in animals, though the physiological function of the decarboxylases has not yet been unambiguously elucidated. Histamine produced by histidine decarboxylase causes a fall in blood pressure.[19] γ-Aminobutyric acid formed by glutamate decarboxylase was found to play a metabolically important role in the brain.[20]

L-Lysine decarboxylase (L-lysine carboxy-lyase, EC. 4.1.1.18) was discovered in *Bacterium cadaveris* and *Escherichia coli* and partially purified by Gale and his co-worker.

This communication describes the purification, crystallization and some of the properties of lysine decarboxylase from *Bacterium cadaveris*.

EXPERIMENTAL PROCEDURES

Materials α-N-Acetyl-L-lysine[22] and S-(β-aminoethyl)-L-cysteine·HCl[23] were prepared by the methods given in the literatures. *O*-(β-Aminoethyl)-DL-serine·2HCl was prepared by the catalytic hydrogenation from ε-*N*-benzyl-oxycarbonyl-*O*-(β-aminoethyl)-DL-serine, which was kindly supplied by Dr. G. I. Tesser of Katholieke Universiteit, the Netherlands. ε-N-Acetyl-L-lysine was a gift from Dr. Shimpei Yamamoto of Kochi University, which was prepared from L-lysine·HCl through acetylation of the terminal amino group.[23] ε-N-Methyl-L-lysine·HCl was a gift from Dr. N. L. Benoiton, University of Ottawa, Canada. DL-(1-^{14}C)-Lysine·2HCl was purchased from New England Nuclear Corporation, Boston, Mass., U. S. A. The other materials were commercial products.

Microbiological Methods *Bacterium cadaveris* IFO 3731 was grown in a medium containing 0.2% L-lysine·HCl, 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 10–1 jar fermentor at 28° for 17 to 20 hours under aeration. The harvested cells were washed twice with 0.85% NaCl solution. The yield of cells was approximately 5 g (wet weight) per 1 of the medium.

Analytical Methods Protein was determined by the method of Lowry *et al.*,[24] and by measurement of absorbance at 280 mμ. The radioactivity of $^{14}CO_2$ absorbed in KOH solution in the center well of a Warburg vessel was determined with a Tri-Carb liquid scintillation 3320 spectrometer.

Determination of Enzyme Activity *Method I.* L-lysine decarboxylase activity was

followed by the manometric measurement of CO_2 released from L-lysine. The main compartment of a Warburg vessel contained 100 μmoles of acetate buffer (pH 5.8) and enzyme in a final volume of 1.9 ml. The center well contained 0.2 ml of water. The side arm contained 10 μmoles of L-lysine·HCl in 0.4 ml. After equilibration at 30° for 20 min, the reaction was started by the addition of L-lysine·HCl, and readings were made at 2-min intervals. When the pH values of the buffers used were above 6.0, after incubation for 5 or 10 min, 0.2 ml of 8 N sulfuric acid contained in the second arm was added to the main compartment to release completely CO_2 from the reaction mixture.

Methods II. When low concentration of the substrate was used, or the coexistence of S-(β-aminoethyl)-L-cysteine. HCl was required, the activity of enzyme was determined by measuring the radioactivity of $^{14}CO_2$ formed from DL-(1-^{14}C)-lysine·2HCl. The main compartment of a Warburg vessel consisted of 30 μmoles of acetate buffer (pH 5.8) and enzyme in a final volume of 0.5 ml. The first side arm contained 2 mμmoles (22,600 cpm) of DL-(1-^{14}C)-lysine·2HCl and the desired amount of unlabeled L-lysine·HCl in a final volume of 0.5 ml. After equilibration at 30° for 10 min, the substrate was added to the content of the main compartment to initiate the reaction. The reaction was terminated after 5 min by the addition of 0.2 ml of 8 N sulfuric acid from the second side arm, and was allowed to stand for 30 min. An aliquot of content of the center well was quantitatively transferred to a scintillation vial containing the toluene-dioxane-ethylcellosolve system.

The activity was calculated from the initial rate (5 to 10 min). One unit of the enzyme activity is expressed as the amount of the enzyme that gives rise to 1 μmole of CO_2 per hour. Specific activity is defined as units per mg of protein. Diamine oxidase was assayed by the method of Tabor.[25]

RESULTS

Relationship between the Condition of Growth and the Activity of the Cell-free Extract The effect of L-lysine concentration in a growth medium on the activity of L-lysine decarboxylase was investigated. The synthetic media containing various concentrations of L-lysine (0–2.0%) were used. The cell-free extract from the cells grown in an L-lysine-free medium had only a slight activity as shown in Fig. 1. The activity increased with increasing the concentration of L-lysine in a medium. The high activity was obtained when 0.5–1.0% L-lysine was employed. This finding shows that the enzyme is inducibly formed.

The effect of initial pH of the medium on the enzyme activity of the cell-free extract is shown in Fig. 2. The highest total units were obtained when the value of pH of a synthetic medium is adjusted to 7.0 (before autoclaving).

The change in L-lysine decarboxylase activity during the growth of *Bacterium cadaveris* is shown in Fig. 3. When the cells were harvested in the logarithmic phase and the early stage of the stationary phase, the decarboxylase activity was found remarkably high. The pH values of the medium decreased from about 6.6 to 4.2 during the growth. The cells grown for 45 hours have only a slight activity. The cells cultivated in the medium containing glycerine as a carbon source showed higher activity than those grown in the glucose medium. The presence of 1% peptone and 1% meat extract in the medium has almost no influence on production of the enzyme.

Activity of Cadaverine Oxidase The cells were cultivated on a medium containing 0.2% L-lysine, 1% glycerine, 0.2% KH_2PO_4, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$ and 0.01% yeast extract. The pH was adjusted to 6.8 by the addition of NaOH solution. The cell-free extract from the cells harvested had little activity of cadaverine oxidase.

Isolation and Identification of the Product from S-(β-aminoethyl)-L-cysteine The decarboxylation product from S-(β-aminoethyl)-L-cysteine was isolated as follows. The reaction mixture contained 500 mg of S-(β-aminoethyl)-L-cysteine and enzyme in a final volume of 100 ml. The pH of the reaction mixture was adjusted to about 5.8 with acetic acid. After incubation at 30° for 3 hours, the reaction was terminated by heating the

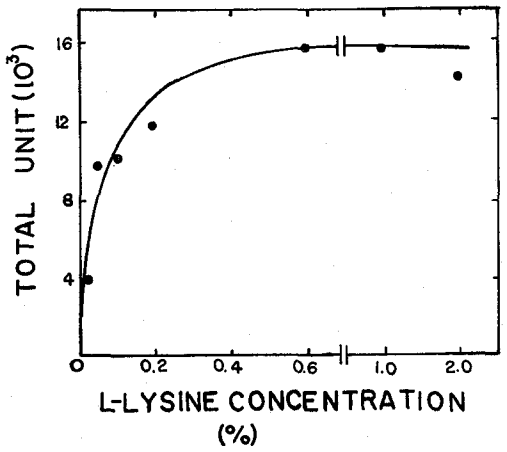

Fig. 1. Effect of L-lysine concentration in the medium on the activity.

The cultivation was carried out in a medium containing 1% glucose, 0.4% $(NH_4)_2SO_4$, 0.2% KH_2PO_4, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.01% yeast extract and various concentrations of L-lysine, (pH 7.0) at 30° for 15 hours. The activity was determined by Method I.

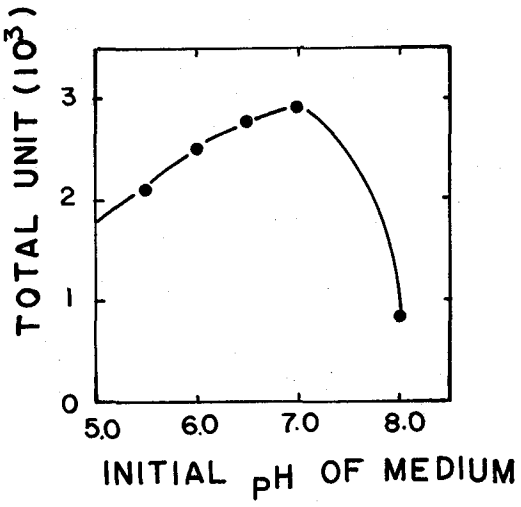

Fig. 2. Effect of initial pH of the medium on the activity.

The cells were grown in the medium containing 0.2% L-lysine. The activity was determined by Method I.

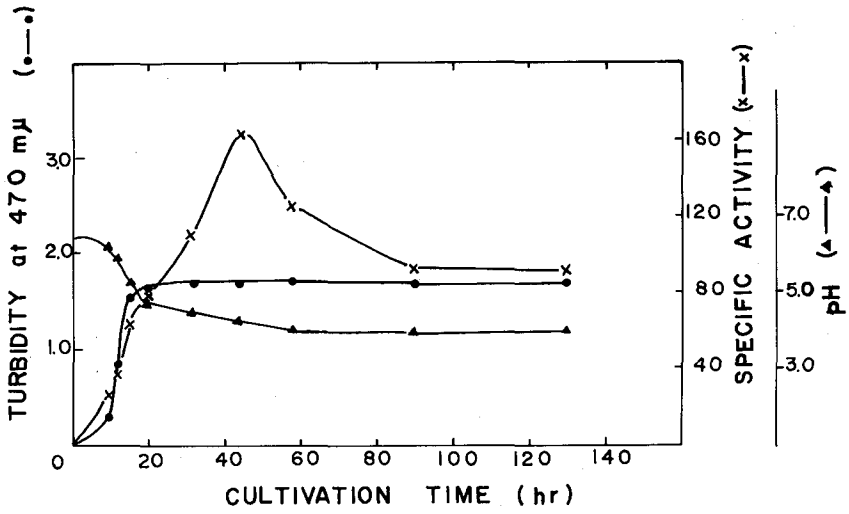

Fig. 3. Change of L-lysine decarboxlyase activity during growth of *bacterium cadaveris*.

The cultures were carried out on a medium containing 1% glycerine, 0.4% $(NH_4)_2SO_4$, 0.2% KH_2PO_4, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.01% yeast extract and 0.2% L-lysine at 28°. The pH of the medium was 6.6 after autoclaving. The enzyme activity was determined by Method I. (●) Growth, (×) Specific Activity, (▲) pH.

mixture at 100° for several minutes. The supernatant obtained by centrifugation was concentrated to a small volume at 40° under reduced pressure. The solution was placed on a column (4×10 cm) of Dowex 1×4 (OH^- form). The column was eluted with water. The eluate containing the product, which was positive to the ninhydrin test, was concentrated to dryness at 40°. The residue was dissolved in a small volume of ethanol. The pH value of the solution was adjusted to 6.0 with HCl. The mercuric chloride solution in ethanol was added to the solution until the precipitation ceased. After standing overnight, the precipitate was collected by centrifugation, and resuspended in about 10 ml of water. Hydrogen sulfide was thoroughly introduced to the suspension, which was then filtered. The filtrate was boiled to remove hydrogen sulfide, and adjusted to pH 6.0 followed by the addition of 10% picric acid in methanol. The solution was again boiled and allowed to cool slowly. The yellow crystal was formed, and recrystallized from methanol. The result of elemental analysis, and the infrared spectrum of the compound obtained show that this compound is the picrate of bis-(β-aminoethyl)-sulfide formed from *S*-(β-aminoethyl)-L-cysteine (Table I, and Fig. 4).

Purification of Enzyme All the operations, unless otherwise stated, were carried out at 0–5°. Every buffers employed here contained 0.01% 2-mercaptoethanol and 10μM. PLP.

Step 1. The washed cells (600 g wet weight) were suspended in 2.5 l of 0.01M potassium phosphate buffer (pH 6.2), and subjected, in 200-ml portions, to sonication in a 19 kc oscillator for 30 min. 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).

Step 2. The dialyzed enzyme was supplemented with PLP and 2-aminoethylisothiouronium (final concentrations, 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 min, the enzyme solution was kept at 50° for 5 min, cooled rapidly and centrifuged.

Step 3. The clear supernatant was brought to 30% saturation with ammonium sulfate. After 30 min, 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 0.2 M NaCl. The enzyme solution was dialyzed overnight against the same buffer. The insoluble materials formed during dialysis were removed by centrifugation.

Step 4. The dialyzed supernatant (149 ml) was placed on a DEAE-Sephadex A-50 column (3.5×41 cm) equilibrated with 0.01 M potassium phosphate buffer (pH 6.2) containing 0.2 M NaCl. After the column was washed with the same buffer, the enzyme was eluted with 0.01 M potassium phosphate buffer (pH 6.2) containing 0.6 M NaCl 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 the addition of ammonium sulfate (70% saturation). The precipitate was dissolved in 0.01 M potassium phosphate buffer (pH 6.2).

Step 5. The enzyme (47 ml) was applied to a column of Sepharose 4B (3.5×140 cm) equilibrated with 0.01 M potassium phosphate buffer (pH 6.2) 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 the addition of ammonium sulfate (60% saturation). The protein precipitated was dissolved in 0.01 M potassium phosphate buffer (pH 6.2) containing 0.2 M NaCl and dialyzed against the same buffer.

Step 6. The dialyzed enzyme (128 ml) was placed on a column of DEAE-Sephadex A-50 (3.5×40 cm) equilibrated as described in Step 4. 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 NaCl at a flow rate of 50 ml

Table I. The Elemental Analysis of the Picrate of Bis-(β-Aminoethyl)-sulfide.

		C%	H%	N%
Calculated	$C_{16}H_{18}O_{14}N_8S$	33.22	3.13	19.54
Found		33.06	3.06	19.04

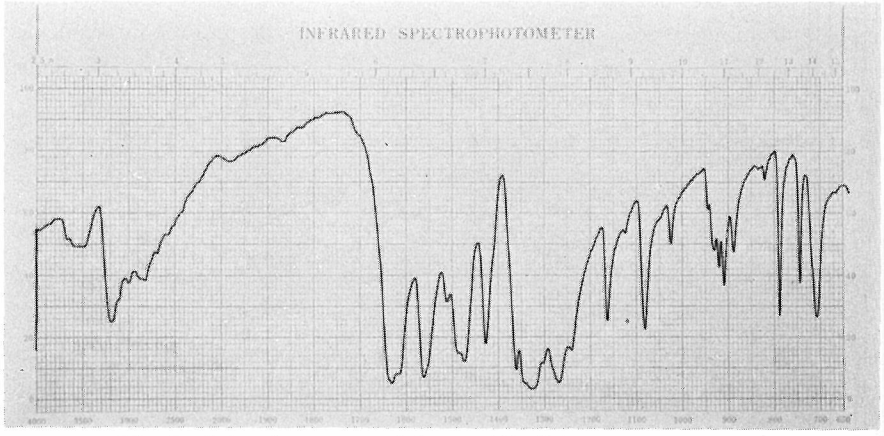

Fig. 4. Infrared spectrum of picrate of bis-(β-Aminoethyl)-sulphide.

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 0.01M potassium phosphate buffer (pH 6.2).

Step 7. 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 (Fig. 5). A summary of the purification procedure is given in Table II.

Properties of Crystalline Enzyme

Purity and Molecular Weight The crystalline enzyme is homogeneous by the criterion of ultracentrifugation (Fig. 6), although only trace amounts of impurities were shown 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 of Van Holde and Baldwin[26] is 1,000,000± 50,000, assuming a partial specific volume of 0.74.

Absorption Spectrum of the Enzyme The spectrum of the enzyme exhibits two absorption maxima at 280 and 425 mμ, these give an absorbance ratio of 12 : 1 (Fig. 7). No appreciable spectral shifts occurred when pH (5.8–9.0) was varied.

Effect of Enzyme Concentration and Incubation Time A linear relationship between

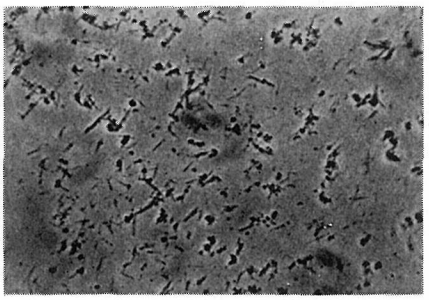

Fig. 5. Crystals of L-lysine decarboxylase.

Table II. Purification of L-Lysine Decarboxylase.

	Step	Total Protein	Total Units	Specific Activity	Yield
I.	Crude extract	87, 350 mg	7, 468, 180	1.4	100%
II.	Heat treatment	10, 230	3, 578, 750	5.7	48
III.	Ammonium sulfate fractionation	5, 360	2, 682, 000	8.3	36
IV.	First DEAE-Sephadex chromatography	1, 970	2, 115, 000	17.8	28
V.	Sepharose 4B chromatography	1, 160	1, 871, 360	26.7	25
VI.	Second DEAE-Sephadex chromatography	595	1, 654, 400	42.7	22
VII.	Crystallization	84	150, 400	85.7	2

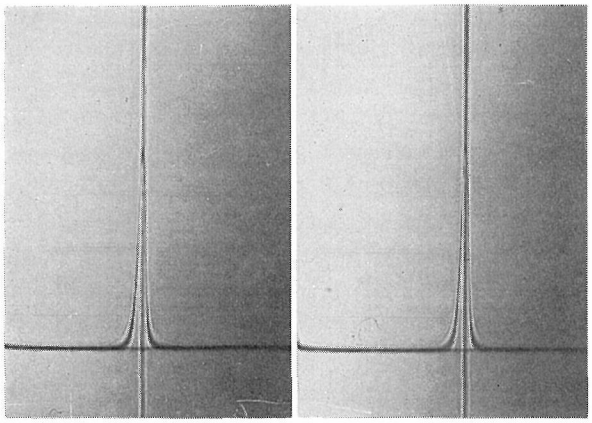

Fig. 6. Sedimentation pattern of crystalline L-lysine decarboxylase.
Protein concentration: 8 mg/ml in potassium phosphate buffer (pH 6.2).
Revolution: 59,780 r.p.m. Temperature: 10°, Bar angle: 75°,
Time: (A) 17 min, and (B) 25 min after achiving top speed.

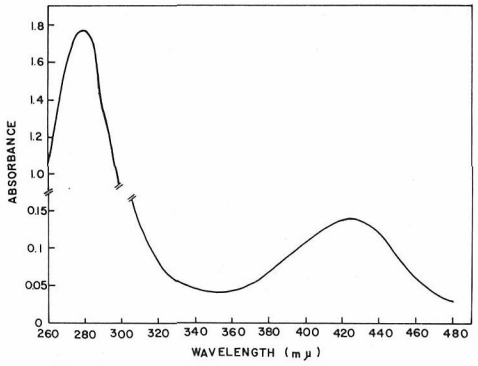

Fig. 7. Absorption spectrum of lysine decarboxylase (1.77 mg per ml) in 0.01 M potassium phosphate buffer, pH 6.2.

the enzyme activity and the concentration of the enzyme was obtained as shown in Fig. 8. The release of CO_2 from the substrate proceeded as a function of time within 10 min (Fig. 9).

Effect of pH on the Enzyme Activity The activity of enzyme was measured at various pH values (Fig. 10). The optimum pH was found to be at 5.8 in acetate and phthalate buffers. When pyridine buffer was employed, the activity declined above pH 5.5.

Effect of Heating on the Enzyme Activity The enzyme was heated at 50° for 5 min in 0.20 M buffer solution of various pH values, and the remaining activity was assayed. As indicated in Fig. 11, the enzyme was most stable at pH 5.3, and was more labile in the alkaline side than in the acidic pH range. The enzyme in pyridine buffer was very labile even at pH 5.3. After the enzyme preparation was heated at 50° for a given period and cooled rapidly, the enzyme activity was determined. Figure 12 shows that the enzyme activity was relatively stable against heating under the conditions employed. Figure 13 shows the relationship between the temperature of heat treatment and the enzyme activity. The enzyme was relatively stable at approximately 50°, but the activity was lost above 70° under the conditions. These results indicate that the enzyme is relatively stable when

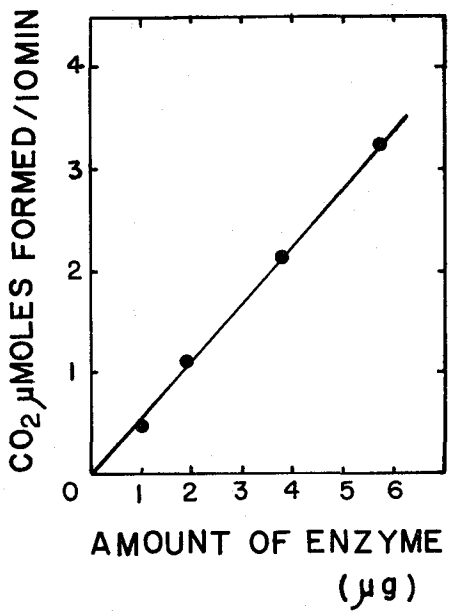

Fig. 8. Effect of concentration of enzyme on the activity. The enzyme activity was determined by Method I.

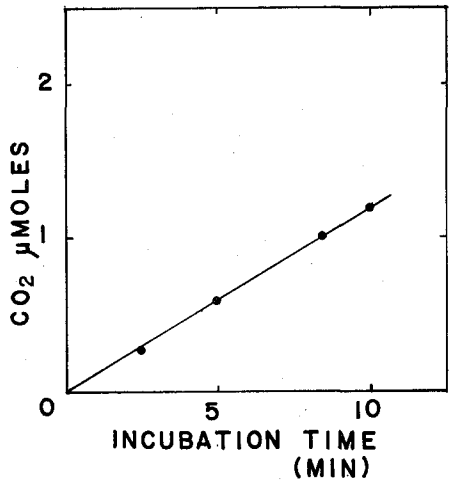

Fig. 9. Time course of L-lysine decarboxylase reaction. The crystalline enzyme (0.9 μg) was used.

heated in the pH range 5.0–6.0.

Substrate Specificity The substrate specificity of the enzyme is given in Table III. Of the various compounds tested, in addition to L-lysine, δ-hydroxylysine and *S*-(β-aminoethyl)-L-cysteine were shown to be decarboxylated with 35 and 49% of the reactivity, respectively, in comparsion with L-lysine. None of α-acetyl-, ε-acetyl-, ε-methyl- and ε-carbobenzoxy-derivatives of L-lysine was susceptible to the enzyme. The finding indicates that both the α- and ε-amino groups of the substrate are required to be free for the decarboxylase reaction. *O*-(β-Aminoethyl)-DL-serine, an oxygen analogue of lysine, D-lysine

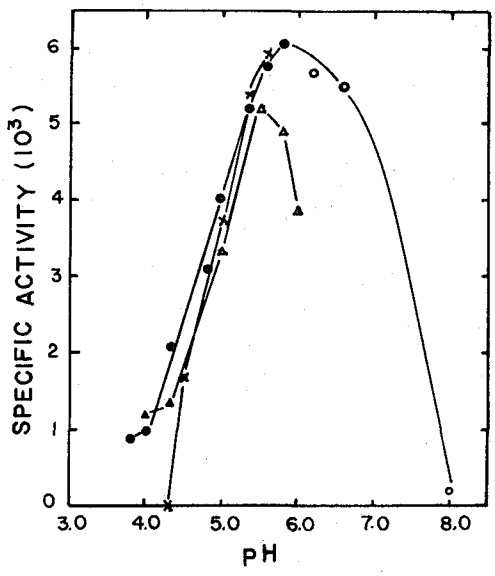

Fig. 10. Effect of pH on the activity. The activity was determined by Method I. The buffers used were acetate (●) for pH 3.8 to 5.8, phthalate (×) for pH 4.3 to 5.8, pyridine (△) for pH 4.0 to 6.0 and potassium phosphate (○) for pH 6.0 to 8.0.

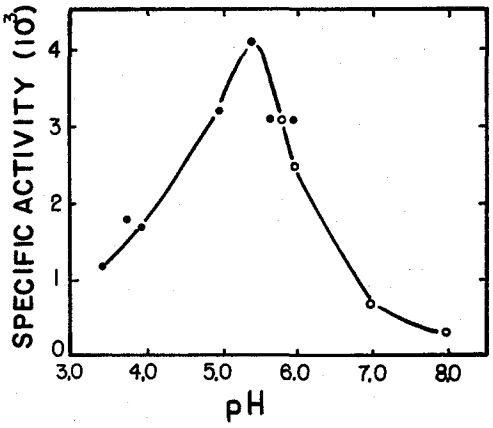

Fig. 11. Effect of pH on stability of the enzyme. The crystalline enzyme was heated at 50° for 5 min in 0.02 M buffer solution of various pH values before the incubation. The buffers used were acetate (●) for pH 3.5 to 5.8, and potassium phosphate (○) for pH 6.0 to 8.0. The remaining activity of the treated enzyme was assayed by Method I.

and the other amino acids tested were inert.

Effect of Related Compounds of L-Lysine on Enzyme Activity Table IV shows the effect of the several compounds related to L-lysine on the decarboxylase activity. The activity was markedly reduced by the addition of α-*N*-acetyl-L-lysine. L-Ornithine showed slightly inhibitory effect, but D-lysine and ε-*N*-acetyl-L-lysine had almost no influence.

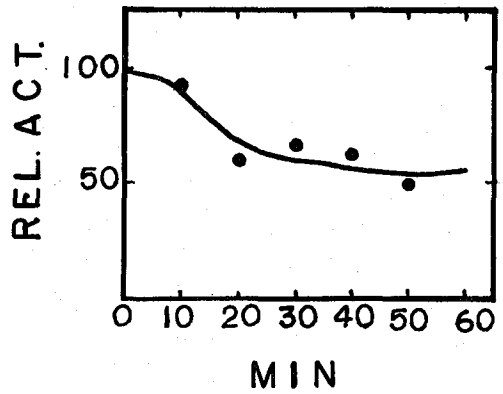

Fig. 12. Effect of heating period on the enzyme activity. The enzyme in 0.02 M acetate buffer (pH 5.3) was heated at 50° for various times. The treated enzyme was assayed by Method I.

Table III. Substrate Specificity of L-Lysine Decarboxylase.

The enzyme activity was determined by Method I. The reaction mixture contained 100 μmoles of acetate buffer, pH 5.8, the indicated amount of substrate and enzyme in a final volume of 2.3 ml.

Substrate	Relative Activity
L-Lysine (10 μmoles)	100
D-Lysine (10 μmoles)	0
α-*N*-Acetyl-L-Lysine (10 μmoles)	0
ε-*N*-Acetyl-L-Lysine (10 μmoles)	0
ε-*N*-Methyl-L-Lysine (20 μmoles)	0
ε-*N*-Carbobenzoxy-L-Lysine (20 μmoles)	0
δ-Hydroxyllysine (DL, DL-allo) (40 μmoles)	35
O-(β-Aminoethyl)-DL-serine (10 μmoles)	0
S-(β-Aminoethyl)-L-cysteine (20 μmoles)	49
L-Ornithine (20 μmoles)	0
L-α,γ-Diamino-*n*-butyric Acid (20 μmoles)	0

Table IV. The Effect of the Several Compounds Related to L-Lysine on L-Lysine Decarboxylase Activity.

The enzyme activity was determined by Method I. The reaction mixture contained 10 μmoles of L-lysine, 10 μmoles of the related compound of L-lysine, 100 μmoles of acetate buffer, (pH 5.8) and enzyme (5 μg) in a final volume of 2.3 ml.

Addition	Relative Activity
None	100
D-Lysine	92
α-*N*-Acetyl-L-Lysine	64
ε-*N*-Acetyl-L-Lysine	95
L-Ornithine	85

Effect of Substrate Concentration on the Decarboxylase Activity The decarboxylase activity was investigated with various concentrations of the substrates. A plot of the reciprocal of the reaction velocity against the reciprocal of the substrate concentration is given in Fig. 14. The Michaelis constant for L-lysine was calculated to be 3.7×10^{-4} M, according to the method of Lineweaver and Burk.[27] *S*-(β-Aminoethyl)-L-cysteine and α-*N*-acetyl-L-lysine, which is not the substrate, were the competitive inhibitors for L-lysine decarboxylase reaction with Ki values of 5.7×10^{-3} M and 7.1×10^{-4} M, respectively. The Km value for *S*-(β-aminoethyl)-L-cysteine was 4.5×10^{-3} M, which is approximately ten times higher than that for L-lysine (Fig. 15).

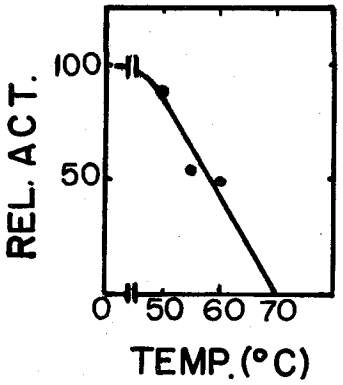

Fig. 13. Effect of heating temperature on enzyme activity. After the enzyme in 0.02 M acetate buffer (pH 5.3) was heated at the indicated temperature for 10 min, the enzyme activity was determined by Method I.

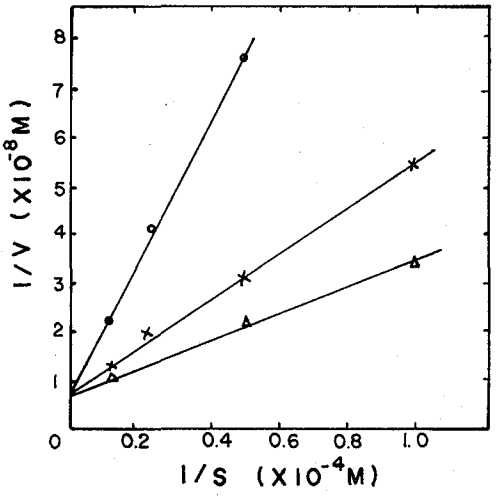

Fig. 14. Effect of L-lysine concentration on L-lysine decarboxylation in the absence (△) of, or in the presence of *S*-(β-Aminoethyl)-L-cysteine (○) or α-*N*-Acetyl-L-Lysine (×). The initial rate of L-lysine decarboxylation at various concentrations was determined by Method II. The reaction mixture contained 30 μmoles of acetate buffer, pH 5.8, 2 μmoles (22,600 cpm) of DL-(1-^{14}C)-lysine, unlabeled L-lysine, enzyme and 20 μmoles of *S*-(β-aminoethyl)-L-cysteine or 10 μmoles of α-*N*-acetyl-L-lysine in a final volume of 1.0 ml.

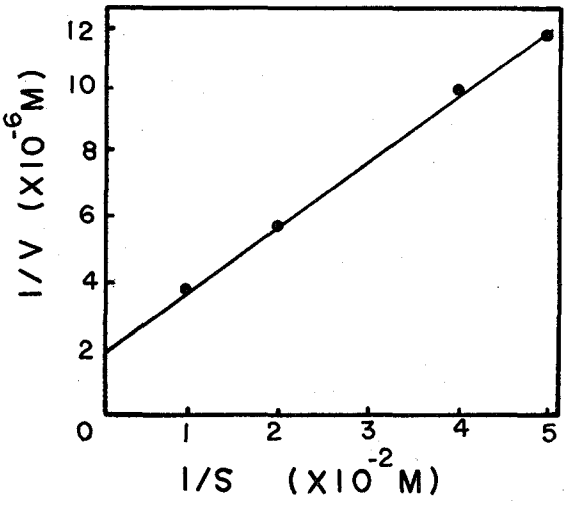

Fig. 15. Effect of *S*-(β-aminoethyl)-L-cysteine concentration on *S*-(β-aminoethyl)-L-cysteine decarboxylation. The initial rate of *S*-(β-aminoethyl)-L-cysteine decarboxylation at various concentrations was determined by Method I.

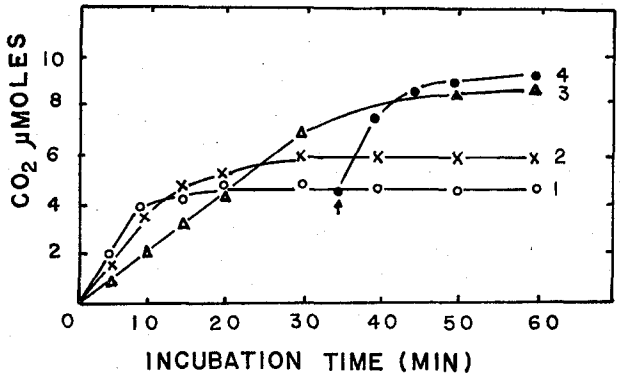

Fig. 16. Effect of PLP concentration on L-lysine decarboxylase. The enzyme activity was determined by Method I. Curve 1. None, Curve 2. PLP (10^{-5} M), Curve 3. PLP (10^{-3} M), Curve 4. PLP (10^{-3} M) was added to the reaction mixture of Curve 1 at the point indicated by the arrow.

Effect of Inhibitors The various compounds were investigated for their inhibitory effects on L-lysine decarboxylase activity (Table V). The enzyme activity was completely inhibited by such sulfhydryl reagents as *p*-chloromercuribenzoic acid (PCMB) and iodoacetamide, and also by the carbonyl reagents, *e.g.*, semicarbazide, isoniazide and hydroxylamine at a concentration of 1×10^{-3} M. Hydroxylamine and PCMB showed the inhibitory effects even at the lower concentrations, namely, 1×10^{-5} M, and 1×10^{-6} M, respectively. L-Penicillamine and D-penicillamine were slightly inhibitory at the higher concentration (1×10^{-3} M), but D-cycloserine showed much higher inhibition. The activity was little influenced by the chelating agents *e.g.*, α,α′-dipyridyl and ethylenediaminetetraacetic acid.

Effect of Thiol Compounds on L-Lysine Decarboxylase The decarboxylase activity

was not affected by thiol compounds, *e. g.*, L-cysteine, sodium thioglycollate, glutathione and aminoethylisothiouronic acid (Table VI). As shown in Table VII, the activity was completely inhibited by 10^{-6} M PCMB. The inhibition was recovered by the addition of the thiol compounds. These findings suggest that the SH-group of the enzyme plays an important role in the decarboxylase reaction.

Table V. Effect of Inhibitors on the Activity of L-Lysine Decarboxylase.

The activity was determined by Method I. The substrate was added to the reaction mixture after the inhibitor and the enzyme were preincubated for 20 min.

Compound (1 mM)	Relative Activity
None	100
Hydroxylamine	0
Semicarbazide	0
L-Penicillamine	84
D-Penicillamine	79
D-Cycloserine	17
p-Chloromercuribenzoic acid (0.1 mM)	0
Iodoacetamide	0
Arsenite	87
Sodium Fluoride	46
Etylenediaminetetraacetic acid	85
α,α′-Dipyridyl	98
Isoniazide	0

Table VI. Effect of Thiol Compounds on L-Lysine Decarboxylase Activity.

The enzyme activity was determined by Method I. After the preincubation of enzyme with the thiol compound for 5 min, the substrate was added.

Thiol Compound (1 mM)	Relative Activity
None	100
L-Cysteine	96
Sodium thioglycollate	93
Glutathione	94
Aminoethylisothiouronic acid	96

Table VII. Effect of PCMB and Thiol Compounds.

The enzyme activity was determined by Method I. The enzyme was preincubated with PCMB (10^{-6} M) for 20 min, and then with the thiol compounds (1 mM) for another 5 min before the substrate was added.

Addition	Relative Activity
None	100
PCMB	0
PCMB+L-Cysteine	20
PCMB+Sodium thioglycollate	0
PCMB+Aminoethylisothiouronic acid	39
PCMB+Glutathione	19

Effect of α-Keto Acids and PLP on Enzyme Activity The activation effects of PLP and α-keto acids, which were reported to activate L-aspartate-β-decarboxylase,[9,28,29] were investigated. Table VIII shows the effect of various α-keto acids on L-lysine decarboxylase. The activity was influenced by none of the α-keto acids tested. Figure 16 shows the effect of the concentration of PLP on the enzyme. The initial rate of the reaction in which PLP was not exogeneously added (curve 1) was very similar to that of the reaction performed in the presence of 10^{-5} M of PLP (curve 2). The evolution of CO_2 ceased with 45% of the theoretical value in the reaction mixture containing no exogeneous PLP, but went up to approximately the 55% value when PLP was added to the reaction mixture (a final concentration: 10^{-5} M). The presence of the higher concentration of PLP (a final concentration: 10^{-3} M) resulted in the decline of the initial reaction rate, though the enzymatic decarboxylation of L-lysine attained to about 90% of the theoretical value in 60 min after the reaction was initiated (curve 3). When PLP was added to the reaction mixture 35 min after the start of the reaction, a rapid increase in the reaction rate of the enzymatic decarboxylation was observed, and almost 90% of the substrate was decarboxylated (curve 4).

The effect of the keto acids on the activity was investigated in the presence of PLP (Table IX). When the keto acids were added to the reaction mixture containing PLP, stimulation of the enzyme activity was not observed, but rather inhibition was under the conditions employed.

L-Lysine decarboxylase was completely inhibited by hydroxylamine. This inhibition was partially diminished by the addition of PLP (Table VIII). The activity of the enzyme inhibited was more effectively restored by the addition of the low concentration of PLP rather than by the addition of the high concentration of it.

Table VIII. Effect of Various α-Keto Acids on L-Lysine Decarboxylase.
The enzyme activity was assayed by Method I.

α-Keto Acid (1 mM)	Relative Activity
None	100
Pyruvate	92
α-Ketoglutarate	96
Oxaloacetate	94

Table IX. Effect of α-Keto Acids on L-Lysine Decarboxylase in the Presence of PLP.
The enzyme activity was determined by Method I.

Addition	Relative Activity
None	100
PLP*	41
PLP+Pyruvate**	61
PLP+α-Ketoglutarate**	56
PLP+Oxaloacetic Acid**	87

* 10^{-3} M, ** 10^{-3} M

PLP Content The enzyme was found to contain 10 moles of PLP per mole of enzyme, when examined by the phenylhydrazine method[30] and Bonavita's method.[31]

DISCUSSION

Most bacterial amino acid decarboxylases are inducibly formed when the organisms are cultivated in media containing the substrate amino acids. It was reported that certain amino acid decarboxylases are produced by bacteria grown in an acid environment.[7,32] Hanke *et al.*[33] and Gale *et al.*[34] suggested that amine formation by bacteria might be a physiological mechanism to counteract such an acid environment.

When *Bacterium cadaveris* was grown on the synthetic medium containing no exogeneous L-lysine, only a slight activity of L-lysine decarboxylase was formed. L-Lysine decarboxylase activity increased with increasing the concentration of L-lysine in the synthetic medium. The highest activity was observed in the cells harvested after the cultivation for 45 hours.

The enzyme was purified to homogeneity from *Bacterium cadaveris*, and the crystalline enzyme was obtained by the addition of ammonium sulfate.

The optimum pH for the enzyme reaction was 5.8 when the activity was determined in acetate and phthalate buffers. Most of the bacterial decarboxylases have the optimum pH in the acid range, but the optimum reactivity of diaminopimelate decarboxylase[35] and aromatic amino acid decarboxylase[36] is at approximately 7 and in the weakly alkaline region, respectively.

L-Lysine decarboxylase of *Bacterium cadaveris* was found to catalyze the decarboxylation of both δ-hydroxylysine and *S*-(β-aminoethyl)-L-cysteine in addition to L-lysine, but none of α-acetyl-, ε-methyl- and ε-carbobenzoxy-derivatives of L-lysine was susceptible to the enzyme. Thus, it seems to be concluded that both α- and ε-amino groups of L-lysine are required to be free as the substrate. The decarboxylation of L-lysine was competitively inhibited by either *S*-(β-aminoethyl)-L-cysteine or α-*N*-acetyl-L-lysine.

L-Lysine decarboxylase activity was not stimulated by thiol compounds. The activity was completely inhibited by PCMB at concentration of 1×10^{-6} M. This inhibition was restored by the addition of the thiol compounds, except sodium thioglycollate. These findings suggest that the SH-group of the enzyme plays an important role in the catalytic process.

The enzyme was not stimulated, but rather inhibited by the keto acids in the presence of PLP. The addition of the higher concentration of PLP(10^{-3} M) resulted in a decrease of the initial rate. The high concentration of PLP may possibly react with also other free amino groups of L-lysine residue of the protein than those in the active center to cause some disadvantageous conformational change. L-Lysine decarboxylase exhibits absorbance maxima at 280 and 425 mμ in 0.01 M potassium phosphate buffer (pH 6.2). The 425-mμ peak is charasteristic of PLP enzymes as reported by Shukuya and Schwert.[8]

The absorption spectrum of L-glutamate decarboxylase[8] (pH 5.0) has a maximum at 415 mμ. Changing the pH of the enzyme solution from pH 5.0 to 6.5 results in a decrease in absorbance at 415 mμ, and in appearance of a new peak at 340 mμ. Anderson and Change[37] concluded, on the basis of experiments with sodium borohydride, that the peak at 415 mμ is due to an intramolecular PLP aldimine which is converted to a sub-

stituted aldamine upon a pH change from 5.0 to 6.5.

L-Aspartate β-decarboxylase catalyzes the removal of ω-carboxyl group of the substrate in contrast with the other amino acid decarboxylases. The observation that L-aspartate β-decarboxylase is activated by both PLP and a variety of α-keto acids[28,38] has been confirmed by studies on the enzyme obtained from various sources.[9,39,40,41,42] The decarboxylase activity decreases when the enzyme is incubated with the substrate. This inactivation is explicable as follows. The enzyme-bound PLP (λmax; 360 mμ) undergoes transamination with the substrate to yield the enzyme-bound pyridoxamine 5'-phosphate (PMP) (λmax; 325 mμ), which can not function as the coenzyme for the β-decarboxylase. The activation observed by the addition of the α-keto acids is due to the regeneration of the bound cofactor, PLP from the enzyme-bound PMP through the reverse transamination with the keto acids.

On the other hand, the activity of L-aspartate β-decarboxylase of *Ps. dacunhae*[43] was not stimulated by α-ketoglutarate. Although the addition of L-aspartate also led to decrease in absorbance at 360 mμ and to appearance of a new peak at 330 mμ, this peak was not affected by the addition of α-ketoglutarate. Inactivation of the decarboxylase by the preincubation with various amino acids was not observed.

Diamiopimelate decarboxylase activity was not increased by the addition of pyruvic acid or α-ketoglutaric acid (1 mM) in the absence or in the presence of PLP, at optimum (16 μM) or sub-optimum (3.2 μM) concentrations.[35]

The crystalline histidine decarboxylase of *Lactobacillus 30a*[10] is not dependent upon PLP, though this coenzyme is appearently required in histidine decarboxylase of mammalian cell,[44] indicating the occurrence of two mechanistically different routes for histidine decarboxylase reaction.

L-Lysine decarboxylase of *Bacterium cadaveris* has the largest molecular weight (approximately 1,000,000) among the PLP enzymes so far studied, and ten moles of PLP are bound per mole of the enzyme protein probably through an aldimine linkage between the formyl group of PLP, and the terminal amino group of lysine residue of the protein. It seems likely that the enzyme consists of several subunits. Further work is needed to elucidate the reaction mechanism and the subunit structure.

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