Bull. Inst. Chem. Res., Kyoto Univ., Vol. 58, No. 3, 1980

Purification and Properties of L-Methionine Decarboxylase of *Streptomyces* sp.

Haruo MISONO*, Yoshiyasu KAWABATA**, Mitsuyoshi TOYOSATO***, Tatsuo YAMAMOTO, and Kenji SODA****

Received March 12, 1980

L-Methionine decarboxylase has been purified 580-fold from the crude extract of *Streptomyces* sp. 590 that was grown in the medium containing 1.0 % L-methionine as an inducer. The purification was carried out by the several steps including protamine treatment, ammonium sulfate fractionation, and DEAE-cellulose, hydroxyapatite, Sephadex G-150 and DEAE-Sephadex A-50 column chromatography. The purified enzyme still contained a small amount of impurities, and has a molecular weight of approximately 130,000. The enzyme has a maximum reactivity at pH 6.4. The enzyme activity was increased by addition of pyridoxal 5′-phosphate and was inhibited strongly by the typical inhibitors of pyridoxal 5′-phosphate enzymes. In addition to L-methionine, L-norleucine, *S*-ethyl-L-cysteine, L-norvaline, L-isoleucine, DL-homocysteine, L-ethionine, L-leucine, *S*-methyl-L-methionine and *S*-methyl-L-cysteine were decarboxylated. The Michaelis constant for L-methionine, *S*-ethyl-L-cysteine and L-norleucine were determined to be 5, 44 and 16 mM, respectively. D-Methionine inhibited L-methionine decarboxylation competitively (Ki=10 mM). The enzyme was also inhibited by $AgSO_4$, $HgCl_2$ and $CuSO_4$.

KEY WORDS: L-Methionine decarboxylase/ Pyridoxal 5′-phosphate enzyme/ Decarboxylation of L-methionine/

INTRODUCTION

Various amino acid dehydrogenases have been demonstrated in animals, plants and microorganisms,[1] and used as an excellent analytical tool for the specific determination of their substrate L-amino acids.[2] Some of them have been purified to homogeneity and characterized extensively as reviewed by Soda and Misono.[3]

Decarboxylation of methionine was first reported by Mazelis with an enzyme system of cabbage leaves[4,5] and horse raddish peroxidase.[6,7] In this reaction, pyridoxal 5′-phosphate and Mn^{2+} ion were required and the product was identified as 3-methylthiopropylamide, which is the oxidative decarboxylation product of methionine. Thus, the decarboxylation of methionine catalyzed by peroxidase is different from the usual amino acid decarboxylase reaction. Methionine is decarboxylated after activation to *S*-adenosylmethionine to yield *S*-adenosylpropylamine, which is a precursor of spermidine and spermine in *Escherichia coli*[8] and rat liver.[9]

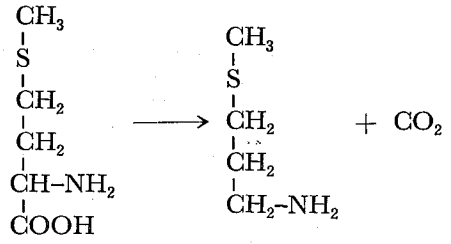
* Present address: Laboratory of Applied Microbiology, Department of Agricultural Chemistry, Kochi University, Nankoku, Kochi 783.

** Present address: Safety Evaluation Laboratory, The Green Cross Corporation, Fukuzaki-cho, Kanzaki-gun, Hyogo 214-1.

*** Present address: Research Laboratory, Nihon Shoji Co. Ltd., Ibaragi, Osaka 567.

**** 味園春雄，川畑好之康，豊里満良，山本龍男，左右田健次：Laboratory of Microbial Biochemistry, Institute for Chemical Research, Kyoto University, Uji, Kyoto 611.

In 1967, Hagino and Nakayama showed the occurrence of L-methionine decarboxylase (EC 4.1.1.57) in strains of *Streptomyces* sp., which catalyzes the decarboxylation of L-methionine to form 3-methylthiopropylamine[10,11] as follows:



Little effort, however, has been devoted to the purification and characterization of the enzyme.

We here report the induction of L-methionine decarboxylase by L-methionine, its purification and some of its properties.

EXPERIMENTAL PROCEDURES

Materials DEAE-cellulose was purchased from Serva, Heidelberg, Germany; Sephadex G-150 and DEAE-Sephadex A-50 from Pharmacia, Uppsala, Sweden; *S*-methyl-L-cysteine and *S*-ethyl-L-cysteine from Fluka AG, Buchs; Pyridoxal 5'-phosphate (pyridoxal-P) from Kyowa Hakko Kogyo, Tokyo; D-cycloserine from Shionogi Seiyaku Co., Osaka; D and L-penicillamine from Calibiochem, California; L-methionine and the other amino acids from Ajinomoto Co., Tokyo. Hydroxyapatite was prepared according to the method of Tiselius *et al.*[12] The other chemicals were analytical grade reagents.

Microbiological Methods Three strains of *Streptomyces* sp. 590, 591 and 592 were kindly supplied by Dr. K. Nakayama, Kyowa Hakko Kogyo Co. The organisms were grown in a medium containing 1.0 % glycerol, 0.1 % peptone, 0.1 % K_2HPO_4, 0.1 % KH_2PO_4, 0.3 % NaCl, 0.2 % ammonium sulfate, 0.01 % $MgSO_4 \cdot 7H_2O$, 0.01 % yeast extract and 1.0 % L-methionine. The pH of the medium was adjusted to 7.0–7.2 with 2 N NaOH.

In a large scale cultivation, *Streptomyces* sp. 590 was grown in two 30-l Marubishi fermentor jars containing 25 l of the medium at 28° for 24 h and transferred into a 200-l Marubishi fermentor jar containing 110 l of the medium containing 1.0 % L-methionine. The culture was carried out at 28° for 14–17 h with aeration. The cells harvested by centrifugation was wahsed twice with 0.85 % NaCl solution.

Enzyme Assay L-Methionine decarboxylase activity was assayed by measurement of CO_2 evolved from L-methionine manometrically. The main compartment of a vessel contained 375 μmol of potassium phosphate buffer (pH 6.8), 0.25 μmol of pyridoxal-P, and enzyme in a final volume of 1.7 ml. The side arm contained 50 μml of L-methionine in a volume of 0.5 ml. The other side arm contained 0.3 ml of 8 N H_2SO_4. After equilibration at 37° for 15 min, the reaction was started by addition of L-methionine. After incubation for 10–15 min, H_2SO_4 was tipped

into a main compartment to stop the reaction and the reading was made after further incubation for 5 min to release CO_2 completely from the reaction mixture. One unit of enzyme is defined as the amount of enzyme that catalyzes the formation of 1.0 μmol of CO_2 per min. The specific activity is expressed as units per mg of protein. Protein was determined by the method of Lowry *et al.*[13] with crystalline bovine serum albumin as a standard; with most column fractions, protein elution pattern was estimated by the 280 nm absorption.

RESULTS

L-Methionine Decaboxyalse Activity of Streptomyces sp. L-Methionine decarboxylase activity of *Streptomyces* sp. 590, 591 and 592 was tested with the crude extracts which were obtained by sonication or by grinding cells in a mortar with levigated aluminum oxide. As shown in Table I, the enzyme activity is most aboundant in *Streptomyces* sp. 590, and this strain was used in the following experiments.

Table I. L-Methionine Decarboxylase Activity of Three Strains of *Streptomyces* sp.
Cultivation were carried out at 27° in 2-liters flasks containing 700 ml of the medium without L-methionine. After the cells were grown for 24 h, L-methionine (final 0.5 %) was added to the medium and cultivation was continued for further 7 h. The crude extract was prepared by sonication of the cells (A) or by grinding the cells in a mortar with levigated aluminum oxide (B). (A), The washed cells were suspended in 0.01 M potassium phosphate buffer (pH 7.2) containing 0.01 % 2-mercaptoethanol and 10 μM pyridoxal-P and subjected to sonication in a 19-kc Kaijo Denki Oscillator at 0–5° for 10 min. After centrifugation, the supernatant solution was dialyzed overnight against 0.01 M potassium phosphate buffer (pH 7.2) containing 0.01 % 2-mercaptoethanol and 1 μM pyridoxal-P. (B), The washed cells were disrupted by grinding the cells in a mortar with levigated aluminum oxide and extracted with 0.1 M potassium phosphate buffer (pH 7.2) containing 0.01% 2-mercaptoethanol and 10 μM pyridoxal-P. The supernatant solution obtained by centrifugation was dialyzed as described above. The enzyme activity was determined manometrically.

Microorganisms	Crude Extract	Specific Activity
Streptomyces sp. 590	A	0.0100
	B	0.0060
Streptomyces sp. 591	A	0.0025
	B	0.0010
Streptomyces sp. 592	A	0.0023
	B	0.0010

Effect of Added L-Methionine in the Growth Medium on Enzyme Activity Fig. 1 presents data concerning the inducibility of L-methionine decarboxylase. Cells grown in the medium without L-methionine had no activity. L-Methionine served as a good inducer. The activity increased with an increase in the concentration of L-methionine in the medium. The highest activity was obtained in the medium supplemented with 1.0 % L-methionine. The changes in L-methionine decarboxylase activity during the growth of *Streptomyces* sp. 590 is shown in Fig. 2. The highest specific activity was obtained 14–18 h after feeding of L-methionine.

Purification of Enzyme All procedures, unless otherwise specified, were carried out

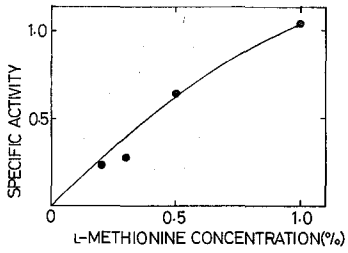


Fig. 1

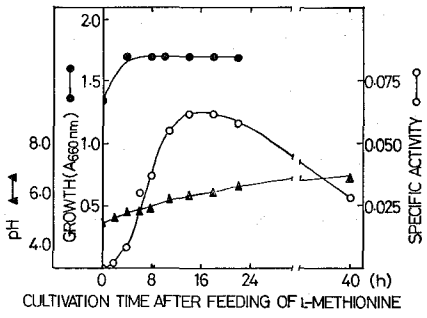


Fig. 2

Fig. 1. Effect of L-methionine concentration in medium on activity.
Streptomyces sp. 590 were grown in 500-ml flasks containing 120 ml of the medium without L-methionine at 27°. After the cells were grown for 24 h, various concentrations of L-methionine was added to the medium and the cultivation was carried out for further 10 h. The crude extract was prepared by sonication of the cells as described in Table I.

Fig. 2. Changes of L-methionine decarboxylase activity during growth of *Streptomyces* sp. 590.
Streptomyces sp. 590 was grown at 28° in a 30-l Marubishi fermentor jar containing 20-l of the medium without L-methoinine. After growth of the cells for 24 h, L-methionine (final 1.0 %) was fed to the medium and the cultivation was continued at 28°. (●), Growth of the cells, (▲), pH, and (○), specific activity.

at 0–5°. The buffers used here contained 0.01 % 2-mercaptoethanol and 10 μM pyridoxal-P.

Step 1. The washed cells (4.5 kg, wet weight) were suspended in 4.0 l of 0.01 M potassium phosphate buffer (pH 7.2) and subjected, in 100-ml portions, to sonication in a 19-kc Kaijo Denki Oscillator for 10 min. After centrifugation, the supernatant solution was dialyzed overnight against 0.01 M potassium phosphate buffer (pH 7.2). The precipitate formed during dialysis was discarded and the supernatant solution was used as the crude extract.

Step 2. To the crude extract, 1.0 ml of 2 % protamine sulfate solution (pH 7.2) was added per 100 mg of the protein with stirring. After 20 min, the bulky precipitate was removed by centrifugation.

Step 3. The supernatant solution was brought to 30 % saturation with ammonium sulfate. After the precipitate was removed, ammonium sulfate was added to the supernatant solution to 55 % saturation. The precipitate was collected by centrifugation and dissolved in a small volume of 0.01 M potassium phosphate buffer (pH 7.2). The enzyme solution was dialyzed overnight against the same buffer. The insoluble materials formed during dialysis were removed.

Step 4. The supernatnat solution was placed on a DEAE-cellulose column (6.0×80 cm) which was previously equilibrated with 0.01 M potassium phosphate buffer. After the column was washed thoroughly with the same buffer containing 0.2 M NaCl, the enzyme was eluted with the buffer supplemented with 0.24 M NaCl. The active fractions were pooled and concentrated by addition of ammonium sulfate (60 % saturation). The precipitate was dissolved in 0.01 M potassium

phosphate buffer (pH 7.2) and dialyzed against the same buffer.

Step 5. The enzyme solution was re-chromatographed on a DEAE-cellulose column (5.5×40 cm) as described in Step 4. The active fractions were pooled and concentrated by addition of ammonium sulfate (60 % saturation). The precipitate was dissolved in 0.01 M potassium phosphate buffer (pH 7.2) and dialyzed against the same buffer.

Step 6. The enzyme solution was applied to a hydroxyapatite column (3.7× 50 cm) equilibrated previously with 0.01 M potassium phosphate buffer (pH 7.2). After the column was washed thoroughly with the same buffer and then with 0.02 M potassium phosphate buffer (pH 7.2), the enzyme was eluted with 0.04 M potassium phosphate buffer (pH 7.2). The active fractions were pooled and concentrated by addition of ammonium sulfate (60 % saturation). The precipitate was dissolved in a small volume of 0.01 M potassium phosphate buffer (pH 7.2) containing 0.1 M KCl.

Step 7. The enzyme was applied to a Sephadex G-150 column (4.7×120 cm) equilibrated with 0.01 M potassium phosphate buffer (pH 7.2) containing 0.1 M KCl and eluted with the same buffer. The active fractions were pooled and concentrated by addition of ammonium sulfate (60 % saturation). The precipitate was dissolved in a small volume of 0.01 M potassium phosphate buffer (pH 7.2) containing 0.1 M KCl.

Step 8. The enzyme was re-chromatographed on a Sephadex G-150 column (2.5×110 cm) as described above. The active fractions were pooled and concentrated by addition of ammonium sulfate (60 % saturation). The precipitate was dissolved in a small volume of 0.02 M sodium pyrophosphate buffer (pH 8.3) and dialyzed against the same buffer containing 0.1 M NaCl.

Step 9. The enzyme solution was placed on a DEAE-Sephadex A-50 column (1.3×15 cm) equilibrated with 0.02 M sodium pyrophosphate buffer (pH 8.3)

Table II. Summary of Purification of L-Methionine Decarboxylase

Steps	Total Protein (mg)	Total Units	Specific Activity	Yield (%)
1. Crude extract	41,000	2,400	0.051	100
2. Protamine treatment	33,200	2,640	0.079	110
3. Ammonium Sulfate fractionation	17,200	2,600	0.151	108
4. First DEAE-cellulose chromatography	4,230	2,020	0.479	84
5. Second DEAE-cellulose chromatography	760	1,050	1.380	44
6. Hydroxyapatite chromatography	156	785	5.040	33
7. First Sephadex G-150 chromatography	35.7	355	9.970	15
8. Second Sephadex G-150 chromatography	20.0	250	12.30	10
9. DEAE-Sephadex A-50 chromatography	4.5	135	29.70	5.6

containing 0.1 M NaCl. The column was washed with the same buffer and with the buffer containing 0.2 M NaCl. The elution of the enzyme was carried out with a linear gradient between the buffers containing 0.2 M NaCl and 0.35 M NaCl. The active fractions were pooled and concentrated by addition of ammonium sulfate (60 % saturation). A summary of the purification procedure of the enzyme is presented in Table II.

Properties of Purified Enzyme

Purity and Molecular Weight The enzyme purified as described above still contains some impurities by the criterion of disc-gel electrophoresis.[14] The molecular weight of L-methionine decarboxylase was determined to be approximately 130,000 by the Sephadex G-150 gel filtration method of Andrews[15] (Fig. 3).

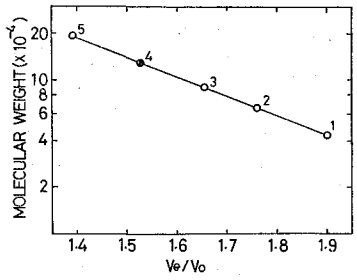


Fig. 3

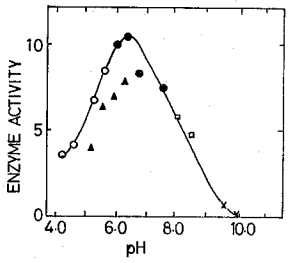


Fig. 4

Fig. 3. Molecular weight determination of L-methionine decarboxylase by Sephadex G-150 gel filtration. A Sephadex G-150 column (1 × 140 cm) was standardized with egg albumin (*Mr*; 45,000), bovine serum albumin (*Mr*; 67,000), kynureninase of *Pseudomonas marginalis* (*Mr*; 91,000)[16] and bovine liver catalase (*Mr*; 195,000). The prostein were eluted with 0.01 M potassium phosphate buffer (pH 7.2) containing 0.01 % 2-mercaptoethanol, 20 μM pyridoxal-P and 0.1 M KCl. 1, Egg albumin; 2, bovine serum albumin; 3, bacterial kynureninase; 4, L-methionine decarboxylase and 5, catalase.

Fig. 4. Effect of pH on activity. The activity was determined by manometry with following buffers (final concentration of 0.15 M); ○ acetate buffer; ▲, malate-NaOH buffer; ●, potassium phosphate buffer; □, pyrophosphate buffer and ×, glycine-KOH buffer.

Effect of pH on the Enzyme Activity The activity of enzyme was measured at various pH values (Fig. 4). The maximum reactivity was obtained at pH 6.4 in potassium phosphate buffer.

Effect of pH and Temperature on Stability of the Enzyme The enzyme was found to be stable in the pH range of 4.5—6.0, and was more labile in the alkaline region than in the acidic pH, when kept at 50° for 10 min (Fig. 5). The thermal stability of the enzyme was examined at pH 5.5 and 7.2 in the presence of pyridoxal-P (Fig. 6). The enzyme was stable at up to 45° at pH 7.2 and 50° at pH 5.5. A slight activation of the enzyme was observed at 37°–50° at pH 5.5.

Substrate Specificity The ability of the enzyme to catalyze decarboxylation of various amino acids is presented in Table III. In addition to L-methionine, which is the best substrate, several derivatives of L-methionine and L-cysteine were decarboxylated. *S*-Ethyl-L-cysteine was a good substrate, whereas *S*-methyl-L-cysteine, L-methionine, *S*-methyl-L-methionine and DL-homocysteine were rather poor substrates. D-

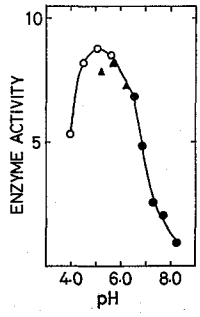


Fig. 5

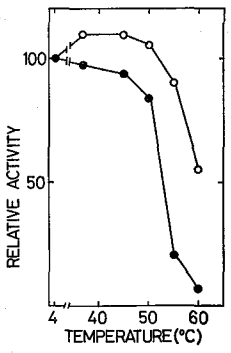


Fig. 6

Fig. 5. Effect of pH on stability of enzyme. The purified enzyme was preincubated at 50° for 10 min in 0.1 M buffer solution of various pH values containing 0.1 mM pyridoxal-P and the remaining activity was assayed. The buffers used are acetate (○), succinate-NaOH (▲) and potassium phosphate (●).

Fig. 6. Effect of temperature on the enzyme stability. The enzyme was preincubated in 0.1 M potassium phosphate buffer (pH 7.2) (●) and acetate buffer (pH 5.5) (○) containing 0.1 mM pyridoxal-P at various temperature for 7 min. The enzyme activity was determined by manometry.

Table III. Substrate Specificity

The enzyme activity was determined manometrically. The final concentration of substrate was 22 mM, unless otherwise specified.

Substrate[a)]	Relative Activity
L-Methionine	100
S-Ethyl-L-cysteine	61
S-Methyl-L-cysteine	13
L-Ethionine	19
S-Methyl-L-methionine	13
DL-Homocystein[b)]	24
L-Norleucine	63
L-Norvaline	34
L-Leucine	15
L-Isoleucine	29

a) Inert: D-methionine, D-isoleucine, D-leucine, D-valine, L-alanine, L-valine, L-α-aminobutyrate, DL-α-aminocaprylate, L-cysteine, DL-methionine sulfone, DL-methionine sulfoxide, DL-methionine sulfoximine, *S*-benzyl-L-cysteine, DL-homocysteine, α-methyl-L-methionine, L-lysine, L-ornithine, L-glutamine, L-glutamate, L-asparatate, L-α-aminoadipate, γ-aminobutyrate, ε-aminocaproate, L-serine, L-threonine, L-tryptophan, L-histidine, L-phenylalanine and L-tyrosine.
b) A final concentration: 12.5 mM.

Methionine, DL-methionine sulfone, DL-methionine sulfoxide and DL-methionine sulfoximine were inert. L-Norleucine, L-leucine, L-isoleucine and L-norvaline were also decarboxylated by the enzyme. L-Norleucine, in which a methylene is substituted for sulfur atom of methionine, was a good substrated. L-Alanine, L-valine, aromatic amino acids, basic amino acids and acidic amino acids tested were not substrates.

Effect of Substrate Concentration on the Decarboxylase Activity Plots of the reciprocal of

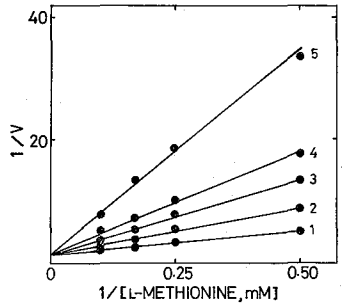


Fig. 7. Effect of L-methionine concentration on L-methionine decarboxylase in the absence or presence of D-methionine. The initial rate of L-methionine decarboxylation at various concentrations of L-methionine was determined by manometry. The reaction mixture contained 375 μmol of potassium phosphate buffer (pH 6.8), 0.25 μmol of pyridoxal-P, L-methionine and D-methionine, and enzyme in a final volume of 2.5 ml. The concentrations of D-methionine are: 1, 0 mM; 2, 4 mM; 3, 10 mM; 4, 20 mM and 5, 40 mM.

the reaction velocity against the reciplocal of the substrate concentrations are shown in Fig. 7. The Michaelis constant for L-methionine was calculated to be 5 mM according to the method of Lineweaver and Burk.[17] The Michaelis constants for *S*-ethyl-L-cysteine and L-norleucine also were estimated to be 44 and 16 mM, respectively.

Effect of Non-substarte Amino Acids Non-substrate amino acids were investigated for their inhibitory effects on the enzyme activity (Table IV). D-Methionine inhibited

Table IV. Effect of Various Amino Acids on the Enzyme Activity

The reaction mixture was composed of 375 μmol of potassium phosphate buffer (pH 6.8), 0.25 μmol of pyridoxal-P, 50 μmol of various amino acids, 50 μmol of L-methionine and enzyme in a final volume of 2.2 ml.

Amino Acids	Relative Activity
None	100
L-Alanine	100
L-Valine	100
DL-α-Aminobutyrate	100
DL-α-Aminocaprylate	100
L-α-Aminoadipate	100
α-Methyl-L-methionine	100
DL-Methionine sulfone	100
DL-Methionine sulfoximine	100
S-Benzyl-L-cysteine	100
D-Methionine	60

Table V. Effect of Various Compounds on the Enzyme Activity

After the enzyme was preincubated with various compounds for 20 min, the enzyme reaction was initiated by addition of L-methionine.

Compounds	Concentration (mM)	Relative Activity
None	—	100
p-Chloromercuribenzoate	0.1	100
Iodoacetate	0.1	100
DTNB[a]	1.0	100
Sodium Fluoride	1.0	100
α, α'-Dipyridyl	1.0	100
EDTA[b]	1.0	100
$AgSO_4$	1.0	2
$HgCl_2$	1.0	1
$CuSO_4$	1.0	25
$FeSO_4$	1.0	67
$MnCl_2$	1.0	94
$MgCl_2$	1.0	96

a) DTNB, 5,5′-dithiobis(2-nitrobenzoate).
b) EDTA, ethylenediaminetetraacetate.

the decarboxylation of L-methionine. D-Methionine inhibited the reaction competitively against the L-enantiomer (Fig. 7). The Ki value was calculated to be 10 mM.

Effect of Various Inhibitors Various compounds were tested for their inhibitory effects on L-methionine decarboxylase activity (Table V). The enzyme was strongly inhibited by heavy metal ions: Ag^{2+}, Hg^{2+} and Cu^{2+}. Fe^{2+} slightly inhibited the reaction, but sulfhydryl reagents such as *p*-chloromercuribenzoate, iodoacetate and 5, 5′-dithiobis(2-nitrobenzoate), and chelators such as EDTA and α, α'-dipyridyl showed no effect.

Effect of Pydrdoxal-P and Inhibitors of Pyridoxal-P-dependent Enzymes L-Methionine decarboxylase which was dialyzed against 0.01 M potassium phosphate buffer (pH 7.2) showed only low activity in the absence of added pyridoxal-P. The activity was stimulated by addition of pyridoxal-P (Table VI). The enzyme was inhibited strongly by the typical inhibitors of pyridoxal-P-dependent enzymes: hydroxylamine, phenylhydrazine, cycloserine, penicillamine and cysteine (Table VII), and the activity was recovered by addition of pyridoxal-P.

Table VI. Effect of Pyridoxal-P on Activity of L-Methionine Decarboxylase

The enzyme was dialyzed overnight against 0.01 M potassium phosphate buffer (pH 7.2). The reaction mixture containing 375 μmol of potassium phosphate buffer (pH 6.8), various concentrations of pyridoxal-P and enzyme in a final volume of 2.0 ml was incubated at 37° for 20 min. The reaction was started by addition of 50 μmol of L-methionine.

Concentration of Pyridoxal-P (M)	Enzyme Activity
0	0.069
10^{-8}	0.085
10^{-7}	0.152
10^{-6}	0.250
10^{-5}	0.297
10^{-4}	0.292

Table VII. Effect of Inhibitors of Pyridoxal-P-dependent Enzymes

The reaction mixture contained 375 μmol of potassium phosphate buffer (pH 6.8), enzyme and inhibitors of pyridoxal-P-dependent enzymes in a final volume of 2.0 ml. After preincubation of the reaction mixture for 15 min, the enzyme reaction was initiated by addition of 50 μmol of L-methionine.

Inhibitors (1 mM)	Relative Activity
None	100
D-Cycloserine	5.7
L-Cycloserine	2.8
Hydroxylamine	3.5
D-Penicillamine	4.3
L-Penicillamine	5.0
D-Cysteine	8.5
L-Cysteine	4.3
Phenylhydrazine	0

DISCUSSION

Most of bacterial amino acid decarboxylases are inducibly formed by the substrate amino acids,[18] except several biosynthetic amino acid decarboxylases including α, ε-diaminopimelate decarboxylase[19] and *S*-adenosylmethionine decarboxylase.[8] L-Methionine decarboxylase also is an inducible enzyme. When *Streptomyces* sp. 590 was grown in the absence of added L-methionine, no activity of L-methionine decarboxylase was found. The enzyme activity increased with an increase in the concentration of L-methionine in the medium.

L-Methionine decarboxylase purified 580-fold from the crude extract still contained impurities. The enzyme has an optimum reactivity at pH 6.4. The inducible enzymes have the optimum pH in the acid range,[3] and the constitutive enzymes such as aromatic amino acid decarboxylase of *Micrococcus percitreus*[20] and arginine decarboxylase of *Escherichia coli*[21] have it in the alkaline region.

Although most of amino acid decarboxylases have high substrate specificity,[1,3] except aromatic amino acid decarboxylases,[20,22] L-methionine decarboxylase has the comparatively low substrate specificity. In addition to L-methionine, several analogs of L-methionine and L-cysteine and several straight-chain and branched-chain amino acids were decarboxylated. However, the enzyme has a strict optical specificity as well as the other amino acid decarboxylases. Replacement of sulfur of methionine by CH_2 resulted in a decrease in the reactivity. Substitution of sulfoxide, sulfone or sulfoximine for thioether in methionine leads to a loss of the susceptibility to the enzyme, although *S*-methyl-L-methionine is a poor substrate. α-Methyl-L-methionine is not a substrate, indicating that the occurrence of α-hydrogen in the substrate is required to bind to the enzyme. The length of straight chain of L-amino acids also significantly influences the susceptibility to the enzyme; the C_6, C_5 and C_7 straight chain amino acids increased in the reactivity in this order and the C_4 and C_3 amino acids are inert. Either positively or negatively charged group in the side chain, ω-amino or carboxyl group, probably prevent the amino acid from binding with the enzyme, because L-lysine, L-ornithine and L-α-aminoadipate were neither substrates nor inhibitors.

Amino acid decarboxylases contain pyridoxal-P as a coenzyme, except histidine decarboxylase of *Lactobacillus* 30a,[33,24] *S*-adenosylmethionine decarboxylase of *E. coli*[8] and a constitutive aspartate α-decarboxylase of *E. coli*,[25] which do not contain pyridoxal-P, but a pyruvoyl residue in the active site. L-Methionine decarboxylase was unstable in the absence of pyridoxal-P. The enzyme activity was stimulated by added pyridoxal-P and was almost completely inhibited by inhibitors of pyridoxal-P-dependent enzymes. These suggest that L-methionine decarboxylase is a pyridoxal-P-dependent enzyme, although the pyridoxal-P content has not been investigated.

ACKNOWLEDGMENTS

We wish to thank Dr. K. Nakayama, Kyowa Hakko Kogyo, for his kind supply of three strains of *Streptomyces* sp.

REFERENCES

(1) B.M. Guirard and E.E. Snell, "Comprehensive Biochemistry" Vol. 15, Elsevier, Amsterdam, 1964, pp. 138–199.
(2) V.A. Najjar, "Methods in Enzymology" Vol. 3, Academic Press, New York, 1957, p. 462.
(3) K. Soda and H. Misono, "Seikagaku Jikken Koza, Vol. 11, Aminosan Taisha to Seitai Amin, part A" Japanese Society of Biochemistry Ed., Tokyo Kagaku Dojin, Tokyo, 1976, p. 179.
(4) M. Mazelis, *Biochem. Biophys. Res. Commun.*, **1**, 59 (1959).
(5) M. Mazelis, *Federation Proc.*, **19**, 4 (1960).
(6) M. Mazelis, *J. Biol. Chem.*, **237**, 104 (1962).

(7) M. Mazelis and L.L. Ingraham, *J. Biol. Chem.*, **237**, 190 (1962).
(8) R.B. Wickner, C.W. Tabor, and H. Tabor, *J. Biol. Chem.*, **245**, 2132 (1970).
(9) A.E. Pegg, *Biochem. J.*, **141**, 581 (1974).
(10) H. Hagino and K. Nakayama, *Agric. Biol. Chem.*, **31**, 1367 (1967).
(11) H. Hagino and K. Nakayama, *Agric. Biol. Chem.*, **32**, 727 (1968).
(12) A. Tiselius, S. Hjerén, and Ö. Levin, *Arch. Biochem. Biophys.*, **65**, 132 (1965).
(13) O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
(14) B.J. Davis, *Ann. N.Y. Acad. Sci.*, **121**, 404 (1964).
(15) P. Andrews, *Biochem. J.*, **91**, 222 (1964).
(16) M. Moriguchi, T. Yamamoto, and K. Soda, *Biochemistry*, **12**, 2969 (1973); *Bull. Inst. Chem. Res., Kyoto Univ.*, **51**, 333 (1973).
(17) H. Lineweaver and D. Burk, *J. Amer. Chem. Soc.*, **56**, 658 (1934).
(18) E.F. Gale, "Advanced in Enzymology" Vol. 5, Interscience, New York, 1945, p. 67.
(19) P.J. White and B. Kelley, *Biochem. J.*, **96**, 75 (1965).
(20) N. Nakazawa, H. Kumagai, and H. Yamada, *Biochem. Biophys. Res. Commun.*, **61**, 75 (1974).
(21) H.W. Wen and D.R. Morris, *J. Biol. Chem.*, **248**, 1687 (1973).
(22) G.A. Lancaster and T.L. Sourkens, *Can. J. Biochem.*, **50**, 791 (1972).
(23) G.W. Chang and E.E. Snell, *Biochemistry*, **7**, 2005 (1968).
(24) W.D. Riley and E.E. Snell, *Biochemistry*, **7**, 3520 (1968).
(25) J.M. Williamson and G.M. Brown, *J. Biol. Chem.*, **254**, 8074 (1979).