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Original Scientific Paper

Purification and Properties of Diaminopimelate Decarboxylase from *Micrococcus Glutamicus*

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Diaminopimelate decarboxylase (E. C. 4.1.1.20) from *Micrococcus glutamicus* hom$^-$, lysine excreting strain, is purified 350-fold by ammonium sulphate precipitation, gel filtration on Sephadex G-150, and chromatography on hydroxylapatite and DEAE-Sephadex. The enzyme has a molecular weight of 53000, isoelectric point of 4.3, optimal pH for activity 7.7, energy of activation 11.1 kcal/mol, and K_m for substrate 1.26 mM. For its stability, the presence of pyridoxal phosphate and sulphydril reagent is necessary, and most catalytic activity is retained within a pH range of 5.5 to 8.5. Aminoacids, L-lysine, L-norleucine, L-α-aminoadipic, L-glutamic and L-aspartic acid, are inhibitors of diaminopimelate decarboxylase from *M. glutamicus* hom$^-$.

EXPERIMENTAL

Diaminopimelate (Dpm) decarboxylase (EC.4.1.1.20) is an enzyme which specifically decarboxylates the meso isomer of Dpm to yield L-lysine. It has been detected in various organisms synthesizing lysine via the dihydrodipicolinate — diaminopimelate pathway, including the bacteria, blue-green and green algae, some protozoa and plants.[1–8] Studies of this enzyme were mostly devoted to its distribution and biological role in microorganisms, while the data concerning its purification, physico-chemical and catalytic properties are limited.[4,8–13] Only the enzyme from *Escherichia coli* has been purified to some extent.[10]

Micrococcus glutamicus, a homoserine requiring mutant, is a lysine excretor, whose specific control mechanism of lysine biosynthesis has been described by Nakayama et al.[14] As far as Dpm decarboxylase from *M. glutamicus* is concerned, its in vivo behaviour has been studied,[15] but the data on the enzyme's other properties are lacking, except for certain characteristics obtained with the enzyme from closely related organism *Brevibacterium* 22.[12,13]

In order to study Dpm decarboxylase from *M. glutamicus* hom$^-$, and to learn more about Dpm decarboxylases in general, the purification of Dpm decarboxylase from *M. glutamicus* hom$^-$ has been undertaken and basic characteristics of the enzyme have been determined.

MATERIALS AND METHODS

Growth and Harvesting of Bacteria

As a source of Dpm decarboxylase, *Micrococcus glutamicus*, a homoserine-requiring auxotrophic mutant (ATCC 13286) was used. The bacteria were grown in 3 l conical flasks in a medium containing peptone (Difco, Detroit, USA), 2 g; casein hydrolysate enzymatic (S.A.G.E.C., Paris, France), 4 g; yeast extract (Difco), 3 g; glucose (autoclaved separately), 10 g; and distilled water to 1 liter. pH was adjusted to 7.0 before sterilization. Each flask, containing 800 ml of the medium, was inoculated with an overnight culture in the same medium, and incubated at 28 °C on a rotary shaker (200 RPM/4 cm). Bacterial cells were harvested when the culture had reached the stationary phase. For harvesting a Sorvall RC-2B centrifuge at 17000 x g with a continuous flow system at 100 ml/min was used. Harvested cells were washed with 0.1 M Na-phosphate buffer pH 6.8 and kept frozen at —20 °C until required.

Measurement of Enzyme Activity

For Dpm decarboxylase activity determination, the colorimetric method according to White and Kelly[10] was used. The reaction mixture contained 0.1 mM PyrP, 1.0 mM BAL, 8.4 mM *meso*-Dpm, 0.2 M Na-phosphate buffer pH 7.7 and 0.1 ml enzyme solution in a total volume of 0.5 ml. When Dpm decarboxylase activity in the suspension of bacteria was measured the reaction mixture also contained 0.6 mg of cetyltrimethyl ammonium bromide per ml. The reaction was initiated by the addition of the enzyme to the reaction mixture preincubated at 37 °C. Samples of 0.1 ml for Dpm assay[16] were taken at suitable time intervals and pipeted into 87% acetic acid.

Enzyme activity was also measured manometrically in a Warburg apparatus. Reaction mixtures at pH 6.8 of otherwise the same composition as above were used. To start the reaction, the substrate or enzyme solution was tipped from the side arm, and evolution of CO_2 was measured assuming the lack of retention of CO_2 by the buffer. It was assumed that decarboxylation of one mole of *meso*-Dpm evolved 22.4 l of CO_2.

The reaction rates were calculated from the initial rates and enzyme activity was expressed as μg Dpm decarboxylated per min per mg of protein. Each determination was performed in duplicate.

Other Determinations

The presence of L-lysine, L-norleucine, α-aminoadipic, L-glutamic and L-aspartic decarboxylase activities were checked manometrically under conditions similar to those in the Dpm decarboxylase assay. The concentration of proteins in enzyme preparations was determined by the method of Lowry et al.[17] using bovine serum albumin as a standard, or estimated by measuring uv absorbancy at 280 nm.

The isoelectric point was determined by isoelectric focusing according to Haglund[18] in LKB ampholine 110 ml column (Bromma, Sweden), using 2% ampholyte pH 3—6 stabilized by sucrose density gradient, at 0 °C. Protein samples of 2.5 mg were introduced into the preformed pH gradient, and electrofocusing continued for additional 30 hours. pH was determined at 0 °C with Radiometer pH meter 26.

Polyacrylamide Gel Electrophoresis

To monitor purification progress, disc polyacrylamide gel electrophoresis with a pH 8.9—7% and pH 7.5—7% system for separating gel was used[19]. Electrophoresis was performed in glass tubes (5 × 70 mm) at 4 °C and 2 mA per tube. Samples were applied on the top of the stacking gel in 20% sucrose. The run was terminated when bromphenol blue, added to the catode buffer as a marker, had reached the lower end of the gel. Gels were stained in 0.5% solution of Amido Black 10B in 7% acetic acid and destained in 7% acetic acid.

Electrophoresis at pH 7.5 preserved enzyme activity. The gels were frozen and cut into 1.5 mm pieces, which were placed directly into the reaction mixture described above for Dpm decarboxylase activity determinations, and incubated at 37 °C for 60 minutes.

Chemicals

meso-2,6-Diaminopimelic acid was prepared from a commercial mixture (Koch--Light, Colnbrook, Bucks, England) containing *meso*-, LL- and DD-Dpm by fractional crystallization from aqueous ethanol[20]. The purity was checked by chromatography[21], specific optical rotation and ir spectroscopy[22]. Pyridoxal-5-phosphate (PyrP), 2,3--dimercaptopropan-1-ol (BAL) and ninhydrin were obtained from E. Merck, Darmstadt, Germany; amino acids, 2-mercaptoetan-1-ol (2-ME), 1,4-dithiotreitol (DTT), and protamine sulphate were purchased from Calbiochem, Lucerne, Switzerland; hydroxylapatite from Clarkson Chem. Comp., Williamsport, Pa., U.S.A., as Hypatite C; Sephadex G-25, -100, -150, -200, and DEAE-Sephadex A-50 Medium from Pharmacia, Uppsala, Sweden; and unuversal buffer from BDH, Pool, England.

RESULTS

Purification of Dpm Decarboxylase

Throughout the purification procedure the enzyme was maintained at 0—4 °C and in the presence of PyrP and SH-groups protector. Sediments were collected by centrifugations of 20 min at 25 000 x g.

Preparation of Cell-free Extracts. — To obtain a soluble extract, bacteria were broken in a vibrational ball mill (E. Bühler, Tübingen): A suspension of washed organisms in 0.1 M Na-phosphate buffer, pH 6.8, containing 0.1 mM BAL and PyrP was mixed with glass beads (0.35 mm) in the ratio 1 : 2 (*v*/*v*) and treated for 20 minutes. During this time most of the enzyme activity originally present in the cells was released. Treatments with fewer or smaller glass beads or for shorter time periods, as well as ultrasonic disruption, were less efficient. Tre disruption of the cells was followed by filtration to remove the glass beads and centrifugation for 20 min at 20 000 x g. In the supernatant liquid 80—100% of enzyme activity present in total cell suspension was obtained.

Precipitation of Nucleic Acids. — Nucleic acids were removed as described by White and Kelly[10] for purification of Dpm decarboxylase from *E. coli*. Nucleic acids were precipitated by adding 2 ml of protamine sulphate solution (1% *w*/*v* in 0.1 M Na-phosphate buffer pH 6.8) and 12 ml of streptomycin sulphate solution (25% *w*/*v* in the same buffer) to each 100 ml of the cell extract. After standing for 1 h, the precipitate was removed by centrifugation. Supernatant liquid was subjected to the overnight dialysis against 0.05 M Na-phosphate buffer pH 6.8, containig 0.1 mM BAL and PyrP. The faint precipitate formed in the course of dialysis was removed by centrifugation. The loss of Dpm decarboxylase activity was negligible; in fact an increase in the specific activity was detected.

Fractionation with Ammonium Sulphate. — For protein fractionation, precipitations with acetone and ammonium sulphate were tested. Since acetone fractionation (—20 °C, 1 h) led to the severe loss of enzyme activity, the other reagent was chosen. The extract from the previous step was diluted to give 15—20 mg protein/ml, and pH was adjusted to 7.7. Solid ammonium sulphate was added to give 35% saturation, the solution stirred for 1 hr and then centrifuged. The precipitate was discarded since it was found to contain only 2—3% of the total enzyme activity.

Most of Dpm decarboxylase activity was precipitated at 35—55% ammonium sulphate saturation. After standing for 1 hr with stirring, the precipitate was collected by centrifugation as before and dissolved in 0.01 M Na-phosphate

buffer pH 7.7 containing 0.1 mM BAL and PyrP. The residual enzyme activity in the supernatant liquid was undetectable. The total recovery in this step was arroud 70%. The yield was smaller when precipitation was carried out without the presence of PyrP and at pH lower than 7.7, and when desalting of the precipitate was not undertaken immediately after dissolving it.

Gel Filtration. — For the further purification the enzyme solution from the previous step was adjusted to 20 mg/ml and centrifuged 60 min at 96 000 x g. To establish the optimal conditions for enzyme separation by gel filtration, Sephadex G-100, G-150 and G-200 were tested. The best separations were obtained on Sephadex G-150 in 0.01 M Na-phosphate buffer pH 6.8 containing 0.1 mM PyrP and BAL. Recoveries varied from 50 to 90% and specific activity increased 2—3 fold. (Figure 1).

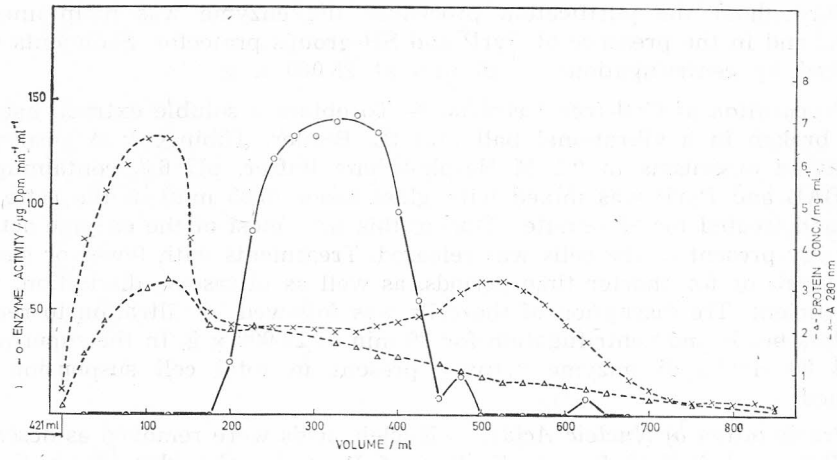

Figure 1. Gel filtration of 1.2 g of Dpm decarboxylase active ammonium sulphate precipitate, on 5 × 84 cm column of Sephadex G-150 in 0.01 M Na-phosphate buffer, pH 6.8, containing 0.1 mM BAL and PyrP. Flow rate was 48 ml/h, and each fraction contained 12 ml. Protein concentration was determined accoording to Lowry et al.[14]

Chromatography on Hydroxylapatite. — The Dpm decarboxylase fraction from the previous step was applied to the hydroxylapatite column equilibrated with 0.001 M Na-phosphate buffer pH 6.8, containing. 0.1 mM PyrP and 1 mM 2-ME, and adsorbed proteins were eluted by applying a linear concentration gradient (0.01 M — 0.1 M) of Na-phosphate buffer, pH 6.8. Dpm decarboxylase activity was eluted with 0.07—0.09 M phosphate, and the recovery was 71—83%. The specific activity of the enzyme was increased 2—3 times, since most of the proteins were adsorbed more strongly and eluted as a separate peak with 0.25 M buffer (Figure 2).

Chromatography on DEAE-Sephadex. — The last step in the purification of Dpm decarboxylase was its chromatography on a column of DEAE-Sephadex A-50 shown in Figure 3. Most of the inactive proteins were removed by elution with 0.4 M NaCl in 0.1 M Na-phosphate buffer, pH 6.8, containing 1 mM 2-ME and 0.1 mM PyrP. Dpm decarboxylase was eluted with linear concentration gradient between 0.4 and 0.6 M NaCl in the same buffer. The degree of purification was good (to 17-fold), but this chromatography usually led to the significant loss of enzyme activity and the yields were at best around 50%.

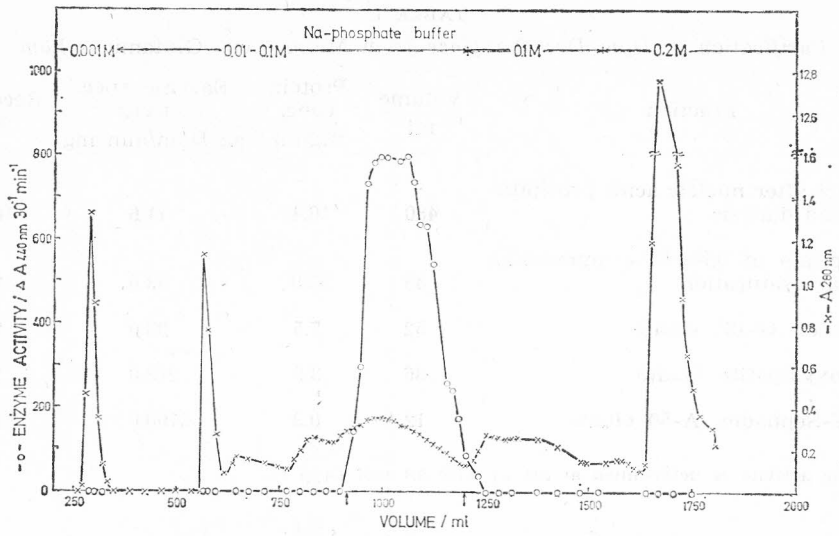

Figure 2. Chromatography of 372 mg of Dpm decarboxylase active fraction from Sephadex G-150 column, on 2.5 × 26 cm column of hydroxylapatite in Na-phosphate buffer, pH 6.8, containing 1 mM 2-ME and 0.1 mM PyrP. Flow rate was 30 ml/h and each fraction contained 10 ml.

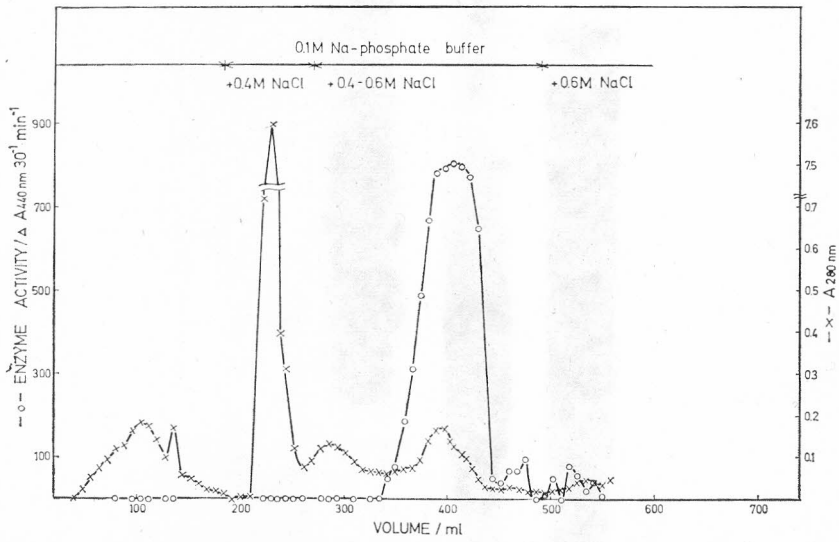

Figure 3. Chromatography of 107 mg of Dpm decarboxylase active fraction from hydroxylapatite column on 1.5 × 25 cm column of DEAE-Sephadex A-50 equilibrated in 0.1 M Na-phosphate buffer, pH 6.8, containing 1 mM 2-ME and 0.1 mM PyrP. Elution was performed as indicated with a combination of stepwise and linear NaCl gradients, composed of equal volumes of 0.4 ond 0.6 M NaCl in the same buffer. Flow rate was 12 ml/h and fractions of 4 ml were collected.

More stable enzyme and better recoveries were rendered when 2-ME was used instead of BAL in buffers throughout the procedure.

By the described procedure (summarized on Table I), Dpm decarboxylase of specific activity of 20 μmol/min mg protein was obtained. Although this represents the highest specific activity of Dpm decarboxylase thus far achieved,

TABLE I

Purification of Dpm Decarboxylase from Micrococcus Glutamicus *hom*

Fraction	Volume ml	Protein conc. mg/ml	Enzyme spec. activity µg Dpm/min mg	Recovery %
Extract after nucleic acids precipitation and dialysis	480	16.4	11.6	100.0
Precipitate at 35—55% ammonium sulphate saturation	35	55.0	33.6	71.5
Sephadex G-150 eluate	52	7.5	93.0	39.8
Hydroxylapatite eluate	36	3.8	218.0	33.3
DEAE-Sephadex A-50 eluate	12.4	0.3	3700.0	15.0

Enzyme activity is determined at pH 7.7 with 8.4 *mM* Dpm.

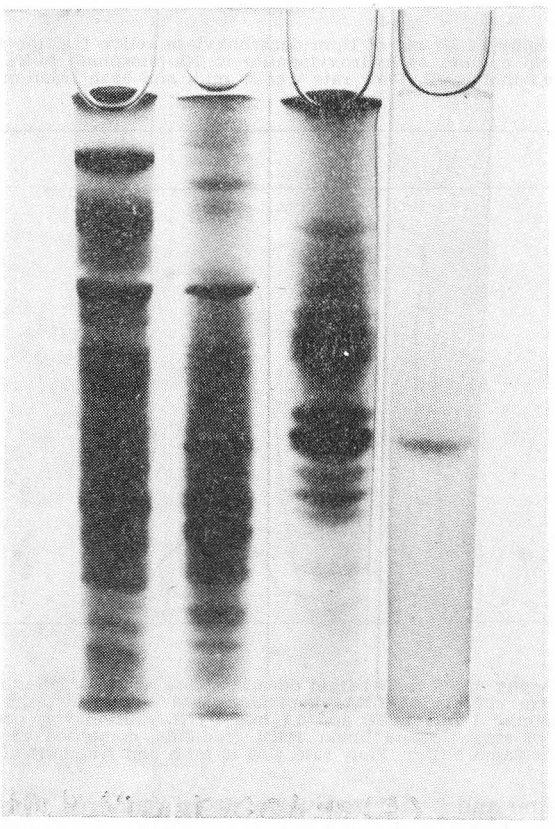

Figure 4. Polyacrylamide gel electrophoresis (pH 7.5, 7% gel) of
a) enzyme fraction after ammonium sulphate precipitation (300 µg),
b) enzyme fraction after Sephadex G-150 (300 µg),
c) enzyme fraction after Hypatite C (200 µg),
d) enzyme fraction after DEAE-Sephadex (100 µg).

the preparation was not electrophoretically homogenous. As shown in Figure 4, representing the electrophoretic patterns on polycrylamide gels of various enzyme fractions, the number of protein bands was reduced to four, and only one among them (the major) was found to exhibit Dpm decarboxylase activity.

Properties of Purified Dpm Decarboxylase

Stability Properties

Dpm decarboxylase from *M. glutamicus* hom⁻ for its full activity needs the presence of PyrP and SH-reducing agent. The presence of both these compounds is also needed for the stability of the enzyme. During preliminary isolation experiments, it was observed that Dpm decarboxylase from *M. glutamicus* is a rather unstable enzyme. When the crude enzyme preparation was incubated in 0.01 M Na-phosphate buffer, pH 6.8, at 37 °C for 1 hour, the enzyme lost 50% of its activity. The addition of NaCl, KCl or $(NH_4)_2SO_4$ in 0.2 M concentration enhanced this inactivation, while the presence of SH-reagent (1 mM) and PyrP (0.05 and 0.1 mM) prevented it, PyrP being much more efficient.

While White and Kelly[10] have recomended BAL as the best SH-protector for Dpm decarboxylase from *E. coli*, we have observed that our purest enzyme preparations completely lost their activity when stored in the presence of BAL. Therefore the protecting ability of three thiol compounds (BAL, 2-ME, and DTT) were compared. BAL, 2-ME, or DTT at 1 mM concentration were added to the purified enzyme in 0.1 M Na-phosphate buffer, pH 7.5, containing 0.1 mM PyrP, and the samples were kept at —20 °C for different time intervals. As can be seen from Figure 5. DTT is the most efficient protector, while BAL can even enhance the enzyme instability. The relatively high stability of the dialysed sample could be due to the incomplete removal of previously added DTT.

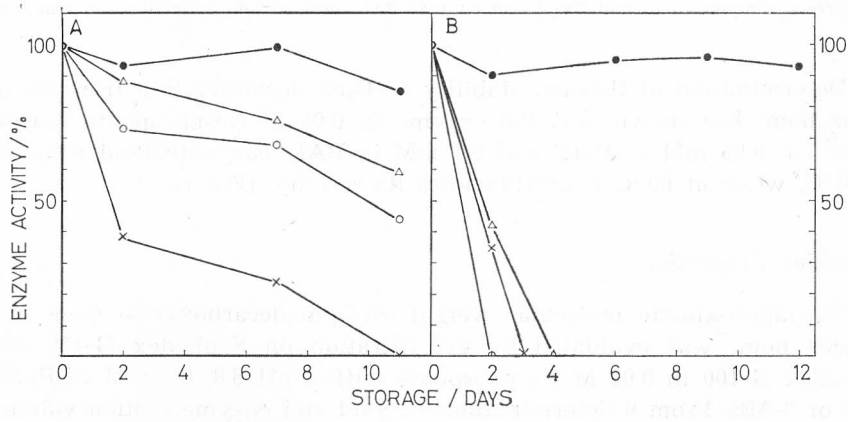

Figure 5. The influence of different thiol reagents on the stability of Dpm decarboxylase from *M. glutamicus* hom⁻ at — 20 °C in 0.1 M Na-phosphate buffer, pH 7.5, 0.1 mM in PyrP. Enzyme activity was determined by the colorimetric assay.

A — 0.76 mg/ml of enzyme preparation whose SH-protector has been removed by dialysis (3 h) and subsequent 5 fold dilution with the above buffer;

B — 1.2 mg/ml of enzyme preparation whose SH-protector has been removed by gel filtration Sephadex G-25 column (0.9 × 60 cm, 0.5 ml sample) in the same buffer. (○) without additions. (●) 1 mM DTT added, (△) 1 mM 2-ME added, (×) 1 mM BAL added.

The influence of pH on Dpm decarboxylase stability is presented on Figure 6. Dpm decarboxylase from *M. glutamicus* hom⁻ irreversibly loses its activity when kept in solutions of pH below 5.5 and above 8.5. The range between these values can be considered as the pH range of the greatest enzyme stability.

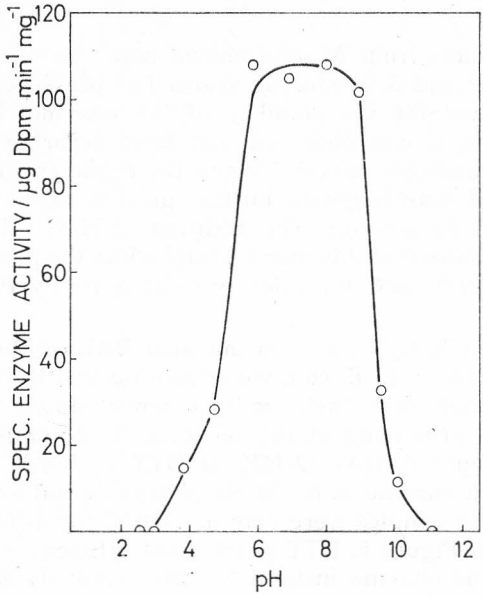

Figure 6. The influence of Ph on the stability of Dpm decarboxylase from *M. glutamicus* hom⁻. The enzyme activity was measured colorimetrically after 3 hours at 4 °C in universal buffer of different pH and subsequent overnight dialysis against 0.2 M Na-phosphate buffer pH 7.7. All the buffers contained 0.05 mM PyrP and 0.1 mM BAL, and protein concentration was 3 mg/ml.

Determination of thermal stability of Dpm decarboxylase from *M. glutamicus* hom⁻ has shown that the enzyme in 0.01 M Na-phosphate buffer, pH 6.8 or 7.7, 0.05 mM in PyrP and 0.1 mM in BAL, can withstand temperature of 50 °C, while at 60 °C it quickly loses its activity. (Figure 7).

Molecular Properties

The approximate molecular weight of Dpm decarboxylase from *M. glutamicus* hom⁻ was evaluated by gel filtration on Sephadex G-150 and on Sephadex G-100 in 0.01 M Na-phosphate buffer, pH 6.8, 0.1 mM in PyrP and BAL or 2-ME. From 9 determinations of void and enzyme elution volumes on different Sephadex G-150 columns, the molecular weight was calculated according to the equation of Kulkarni and Mehrotra[23], and the value of 53000 ± 2400 was obtained. The value in the same range was also obtained when molecular weight was calculated according to Determann and Michel[24] from gel filtration on Sephadex G-100, using ovalbumin as a standard.

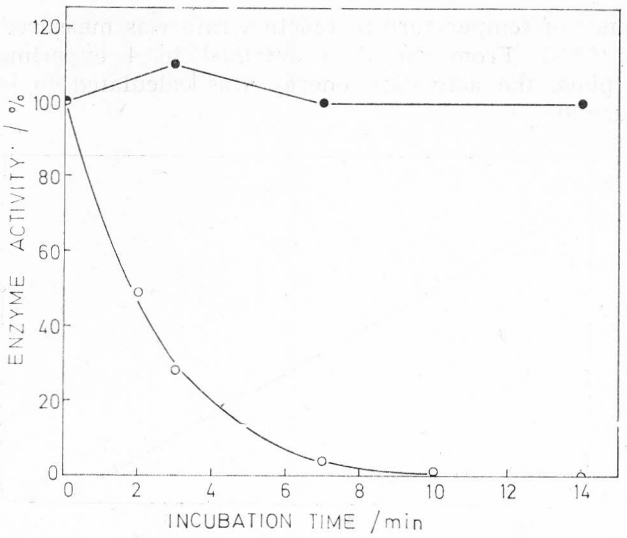

Figure 7. The stability of Dpm decarboxylase from *M. glutamicus* hom⁻ in 0.01 M Na-phosphate buffer, pH 7.7, 0.05 mM in PyrP and 0.1 mM in BAL at (●) 50 °C and at (○) 60 °C.

The isoelectric point of Dpm decarboxylase was determined by subjecting enzyme fraction after chromatography on hydroxylapatite to isoelectric focusing. After preliminary experiment performed with pH 3—10 ampholytes, pH gradient 3—6 was used. The enzyme activity was detected in several fractions within pH range 4.2 to 4.4. The enzyme activity maximum was at pH 4.3 at 0 °C.

Catalytic Properties

The optimal pH for the reaction of Dpm decarboxylation catalysed by Dpm decarboxlase from *M. glutamicus* hom⁻ was determined to be 7.7. (Figure 8).

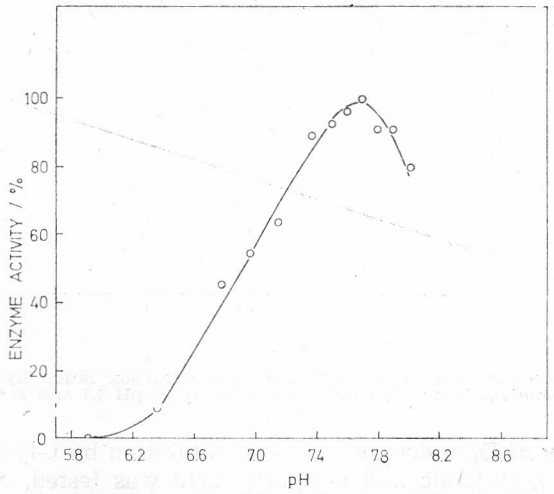

Figure 8. The influence of pH on the rate of Dpm decarboxylation by Dpm decarboxylase from *M. glutamicus* hom⁻. The colorimetric assay in Na-phosphate buffer, as specified in the text, was applied.

The influence of temperature in reaction rate was measured in the range from 26 °C to 50 °C. From the data obtained in 4 experiments analyzed by Arrhenius plots, the activation energy was calculated to be 11.15 ± 0.05 kcal/mol. (Figure 9).

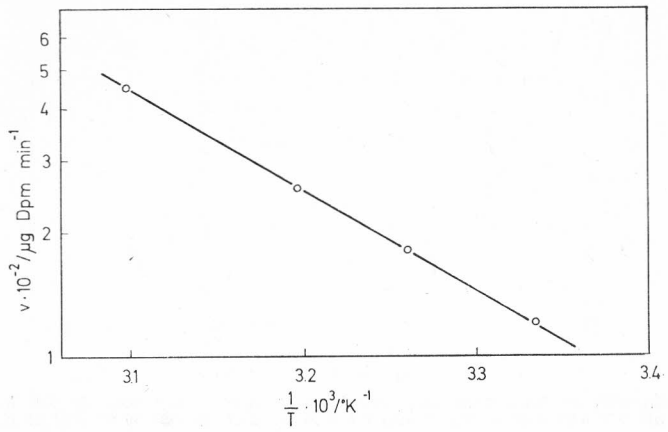

Figure 9. Temperature influence on the rate of Dpm decarboxylation by Dpm decarboxylase from *M. glutamicus* hom⁻ at pH 7.7. Enzyme activity was determined by the colorimetric assay.

The influence of substrate concentration on the reaction rate was evaluated by using Dpm concentrations from 0.06 to 8.40 mM at pH 7.7 and 37 °C. During the rate determinations at low concentrations, special precautions were made to take very short time intervals and to adjust aliquot volumes in such a way as to obtain a colour of an intensitiy suitable for measurement. The data presented in the Lineweaver-Burk double reciprocal plots[25] show that the enzyme follows Michaelis-Menten kinetics. (Figure 10). K_m graphically evaluated from 6 determinations was 1.26 ± 0.22 mM.

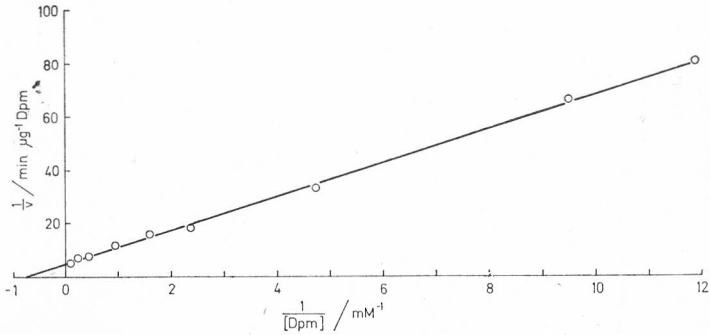

Figure 10. Lineweaver-Burk plot of the rates of Dpm decarboxylation by Dpm decarboxylase from *M. glutamicus* hom⁻. The colorimetric assay at pH 7.7 and 37 °C was used.

Susceptibility of Dpm decarboxylase to inhibition by L-lysine, L-norleucine, L-α-aminoadipic, L-glutamic and L-aspartic acid was tested, after it had been determined that none of them is the substrate of the enzyme. The reaction rates were determined manometrically at pH 6.8 with 5.1 mM Dpm and several

concentrations of inhibitor. Owing to the low solubility of some amino acids, the concentration range of inhibitor was limited to 0 to 25 mM. It was found that all examined amino acids inhibited to varying extent the decarboxylation of Dpm catalysed by Dpm decarboxylase from *M. glutamicus* hom⁻. From the results plotted in a modified Dixon plot [26] [(v_o/v_i) vs I] the amino acid concentrations necessary for 20% inhibition were determined and presented in Table II.

TABLE II

Inhibition of Dpm decarboxylase from M. glutamicus *hom⁻ by amino acids*

Amino acid	Conc. for 20% inhibition (mM)
L-norleucine	2.0
L-lysine	4.2
L-α-aminoadipic	8.2
L-glutamic	25.0
L-aspartic	39.0

The numbers represent mean values of three determinations

By comparison of the degrees of inhibition within the group of amino acids of similar size (the first 3 or 4) and within the group of similar acidity (the last 3), it can be seen that inhibitory effect depends both on the acidity of the ω-terminal group and on the size of the amino acid.

The enzyme was not affected by the presence of ATP and AMP in concentrations up to 2×10^{-3} M.

DISCUSSION

Our standard procedure for isolation of Dpm decarboxylase from *M. glutamicus* hom⁻ consists of standard protein separation methods combined and performed under conditions found to be appropriate for this enzyme. They differ from the conditions applied for the isolation of Dpm decarboxylase from other microorganisms.[4,9,10,12] Introduction of PyrP and 2-ME or DTT (instead of BAL) in all buffers and the use of Sephadex G-150 made gel filtration and hydroxylapatite chromatography applicable to Dpm purification. Additions to the buffers solved certain problems of enzyme instability but did not prevent its inactivation on DEAE-Sephadex, an effect reported also by White and Kelly[10]. A probable reason for the enzyme activity loss could be a possible appoenzyme — coenzyme resolution caused by adsorption to this ion exchanger. Nevertheless, DEAE-Sephadex chromatography is the most efficient step in Dpm decarboxylase purification from *M. glutamicus*.

According to the described purification procedure Dpm decarboxylase of the highest specific activity yet reported, was obtained with relatively good enzyme recovery. The enzyme was still not electrophoretically homogenous, and attempts to determine the satelite bands as multiple forms of Dpm decarboxylase were unsuccesful.

Like Dpm decarboxylase from other organisms, the enzyme from *M. glutamicus* needs for its activity reactive SH-groups and PyrP as a cofactor. There are some disagreements about the effects of PyrP and different sulfhydril

reagents on enzyme stability. Our data agree with findings of Grandgenett and Stahly[27] for the enzyme from *Bacillus licheniformis*, and not with observations of White and Kelly[10], who considered that the higher loss of *E. coli* Dpm decarboxylase is due to the presence of PyrP. Other disagreements are reported regarding the action of BAL ,which was the most potent protector for the *E. coli* enzyme and caused inactivation of purified *M. glutamicus* Dpm decarboxylase. However, these differences might be only apparent, since the deletorious effect coud be ascribed to the mixture of both reagents, BAL and PyrP, which might undergo a condensation reaction, leaving the enzyme without protection.

Pronounced differences were found in molecular weights and pH optima of the *E. coli* and *M. glutamicus* Dpm decarboxylases. While White and Kelly[10] reported a value of 200000 for the *E. coli* enzyme, and Rosner[11] observed a value of around 105 000 for the Bacillus subtilis enzyme, we have determined the molecular weight of the Dpm decarboxylase from *M. glutamicus* to be in the range of 53 000. We have no explanation for these differences, but if one compares the *E. coli* and *M. glutamicus* enzyme molar activities, whose ratio is 2 to 1 (assuming similar purity of preparation), one might consider that the *M. glutamicus* preparation is a monomeric from of Dpm decarboxylase.

The optimal pH for catalytic activity of purified Dpm decarboxylase from *M. glutamicus* was found to be 7.7, which is higher than the value reported for most other Dpm decarboxylases (*Aerobacter aerogenes*[9] and *E. coli* pH 6.7—6.8, *Lactobacillus arabinosus*[4] pH 6.0, *Bacillus cereus*[3] pH 7.0, *Brevibacterium*[13] pH 6.9) and lower than the pH optimum for the *B. subtilis* enzyme (pH 8.5) reported by Rosner[11]. This property might also be connected with the form of the enzyme. However, it remains unsolved what is the form of Dpm decarboxylase in the cell and whether it differs in vivo in different microorganisms.

Isoelectric point of 4.3 indicates that Dpm decarboxylase from *M. glutamicus* hom$^-$ is an acidic protein, what is in an agreement with its binding to basic Sephadex derivative at pH 6.8. There are no data for pI of other Dpm decarboxylases.

Other characteristics, like the Arrhenius energy of activation and Michaelis constant, are comparable with the data for the enzymes from other organisms[4,9,10,11], exept for the much higher K_m value of the *B. licheniformis*[27] and *Brevibacterium* 22[13] enzymes. The first one is ascribed by the authors to the impure preparation, while the other might be high partly due to the same reason and partly due to the mixture of Dpm acids and very long incubation periods employed in the assay procedure. One ought to take into account that, concomitantly with decarboxylation catalysis, enzyme inactivation takes place. Also, the influence of DD- and LL-isomers upon Dpm decarboxylase is still not quite clear. The same precautions must be taken in interpreting previously reported activating effect of Dpm on the *Brevibacterium* enzyme[12,13], which was not observed with any other Dpm decarboxylase irrespectively of its molecular weight. Likewise, AMP and ATP influences upon Dpm decarboxylase were also not observed with the *M. glutamicus* enzyme, either when tested with the crude extract or with its purified form, what is in agreement with observations for the *E. coli*[10] and the *B. licheniformis*[27] Dpm decarboxylase.

Like the enzyme from other sources Dpm decarboxylase from *M. glutamicus* hom$^-$ is competitively inhibited by L-lysine, as reported earlier[15]. Dpm decarboxylase is also inhibited by L-norleucine, L-α-aminoadipic, L-glutamic and

L-aspartic acid. Experiments are performed at phosphate buffer and PyrP concentrations high enough to exclude nonspecific inhibition and inhibition due to the lack of cofactor. The results on inhibition by amino acids are in agreement with the observations of White and Kelly[10] and Rosner[11], but not with those of Dewey et al.[9] and Hoare and Work[28] for other amino acids and the enzymes from other sources. They lead to the conclusion that, for inhibitory action both ends of the amino acid do not have to bind to the enzyme, or do not bind in the same manner, as has been proposed for the substrate[29], since this would enable only amino acids with both ends polar, to be inhibitors. Further, our results show that the degree of Dpm decarboxylase inhibition by aliphatic amino acids depends on their size and electrostatic properties, better inhibitors being amino acids more similar to the substrate in size and pI. This might be indicative of the inhibitor binding in the same region as the substrate.

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SAŽETAK

Izolacija i svojstva dekarboksilaze diaminopimelinske kiseline iz bakterije *Micrococcus glutamicus*

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Dekarboksilaza diaminopimelinske kiseline (E.C. 4.1.1.20) iz bakterije *Micrococcus glutamicus* hom⁻, producenta lizina, pročišćena je 350 puta taloženjem amonij-sulfatom, gel-filtracijom na Sephadexu G-150 i kromatografijom na hidroksilapatitu i DEAE-Sephadexu. Enzim ima molekularnu težinu od 53 000, izoelektričku točku kod pH = 4.3, optimalni pH djelovanja 7.7, energiju aktivacije 11.1 kcal/mol, a K_m za supstrat 1.26 mM. Za stabilnost mu je potrebna prisutnost piridoksalfosfata i sulfhidrilnog reagensa, a glavninu aktivnosti zadržava u pH-području od 5.5 do 8.5. Aminokiseline, L-lizin, L-norleucin, L-α-aminoadipinska, L-glutaminska i L-asparaginska kiselina inhibitori su dekarboksilaze diaminopimelinske kiseline bakterije *M. glutamicus* hom⁻.

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