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Diaminopimelate decarboxylase from *Lactobacillus arabinosus*

Diaminopimelate decarboxylase (*meso*-2,6-diaminopimelate carboxy-lyase, EC 4.1.1.20), the terminal enzyme of the diaminopimelate pathway of lysine biosynthesis[1,2], has been studied in a number of bacteria[3] and has been purified 200-fold from *Escherichia coli*[4]. The first reaction in lysine biosynthesis is mediated by the enzyme aspartokinase; in *E. coli* a lysine-sensitive aspartokinase, aspartokinase III (lysine repressed and inhibited), has been extensively studied[5].

The mode of biosynthesis of lysine in *Lactobacillus arabinosus* 17-5 has been in doubt. That the organism can synthesize lysine is evidenced by the fact that it can be cultured in media devoid of lysine. Studies in this laboratory (unpublished) have indicated that in *L. arabinosus* 17-5 lysine is indeed derived from aspartic acid; however, all attempts to detect aspartokinase activity have been unsuccessful. A further clouding of the problem is the report that *L. arabinosus* 17-5 is without diaminopimelate decarboxylase activity[6]. In the present study we have found that when *L. arabinosus* 17-5 is cultured in certain growth media, diaminopimelate decarboxylase activity can be detected. The enzyme has been purified approx. 170-fold and some of its properties have been determined.

The 2,6-diaminopimelate employed in this study was found by descending paper chromatography to be 90–95% *meso* isomer and 5–10% LL isomer. Cells of *L. arabinosus* 17-5 were cultured in a previously described defined amino acid medium[7].

Potassium phosphate buffer, pH 6.8, at various concentrations, was used throughout the enzyme purification; β-mercaptoethanol (0.01 M) and EDTA (0.001 M) were added to all buffer solutions. All steps were conducted at 0–5°. After the cells were harvested by centrifugation, they were washed, suspended in 0.1 M phosphate buffer, and disrupted by use of a B. Braun mechanical homogenizer. Cell-free extracts were obtained by high-speed centrifugation. The extracts were adjusted in volume to give a protein concentration of approx. 20 mg/ml.

Nucleoproteins were removed from the crude extracts by use of protamine sulfate and streptomycin sulfate according to the method of WHITE AND KELLY[4]. Solid $(NH_4)_2SO_4$ was then added to give 55% saturation; after stirring for 1 h the precipitate was removed by centrifugation and redissolved in 0.5 vol. of 0.001 M phosphate buffer. The $(NH_4)_2SO_4$ fraction was then stirred for 30 min with calcium phosphate gel (80 mg dry wt. of gel per ml of enzyme solution). The gel was removed by centrifugation, and was washed with increasing concentrations of phosphate buffer (15 ml/g gel at 0.001 M, 0.01 M, and 0.1 M). Most of the desired enzyme activity was eluted by the 0.1 M phosphate buffer. Further purification was accomplished by use of DEAE-Sephadex; a 1 cm × 12 cm column was employed for 2–3 mg protein fractions eluted from the gel. Diaminopimelate decarboxylase activity was eluted from the column with successive 4-ml portions of 0.1 M phosphate buffer containing NaCl at concentrations of 0.0 M, 0.1 M, 0.15 M, and 0.2 M. The active fraction was usually found in the 0.1 M NaCl fraction. The enzyme was purified approx. 170-fold; pertinent data are given in Table I.

The composition of the medium employed for the culturing of *L. arabinosus* greatly affects the levels of diaminopimelate decarboxylase found in the cell-free

TABLE I

ENZYME PURIFICATION

The reaction mixtures contained the following: 90–95% *meso*-diaminopimelate, 400 μg; pyridoxal phosphate, 0.2 μmole; potassium phosphate buffer, 100 μmoles, pH 6.0; and a rate-limiting amount of enzyme in a total volume of 1.3 ml. The incubation temperature was 37°. Samples were removed (0.13 ml) periodically for up to 4 h, and the rate of disappearance of diaminopimelate was determined by the method of CHINARD[8]. Units of enzyme are defined as the disappearance of diaminopimelate in μmoles/h.

Purification data	*Enzyme fraction*				
	Crude extract	*Strepto-protamine*	*55% $(NH_4)_2SO_4$*	*Calcium phosphate gel*	*DEAE-Sephadex*
Enzyme units	462	459	250	240	210
Specific activity (units/mg protein)	3.3	5.9	23	80	570

extracts. Greatest specific activities were obtained from a defined amino acid medium[7]. As shown in Table II, when the defined medium was supplemented with peptone, yeast extract, or casein, there was a significant repression in enzyme specific activity. Although no definitive explanation for the effects of these substances can be given at the present time, these findings may explain in part the inability of others to demonstrate diaminopimelate decarboxylase in *L. arabinosus*[6]. As also seen in Table II, lysine does not apparently repress the formation of diaminopimelate decarboxylase, nor is the enzyme induced by the addition of diaminopimelate to the growth medium.

The purified enzyme has an absolute requirement for pyridoxal phosphate for activity. This requirement is interesting, as previously no completely inactive purified apoenzyme preparation of diaminopimelate decarboxylase has been obtained[4,9]. The optimum pH for activity of the purified enzyme was found to be 6.0, and the optimum temperature was 37°.

The rates of decarboxylation were measured with various concentrations of substrate, diaminopimelate. From a reciprocal plot of the data, the apparent K_m

TABLE II

EFFECTS OF VARIOUS MIXTURES AND COMPOUNDS ON THE SYNTHESIS OF DIAMINOPIMELATE DECARBOXYLASE IN *L. arabinosus* 17-5

Supplement to defined medium[7]	*Enzyme specific activity**
None	0.20
Yeast extract, 1.5 g/l	0.13
Casein, 8.0 g/l	0.11
Peptone, 8.0 g/l	0.09
Yeast extract, 1.5 g/l; peptone, 8.0 g/l	0.03
L-Lysine, 1.0 g/l	0.20
90–95% *meso*-diaminopimelase, 0.1 g/l	0.20

* Specific activity is defined as the disappearance of diaminopimelate in μmoles/h per mg protein. The components of the reaction mixture are given in Table I.

was found to be approx. 2.7 mM (Fig. IA). This value is in close agreement with that estimated by DEWEY *et al.*[9] with the enzyme from *Aerobacter aerogenes* (2.8 mM), but is somewhat higher than that obtained by WHITE AND KELLY[4] with the enzyme from *E. coli* (1.7 mM).

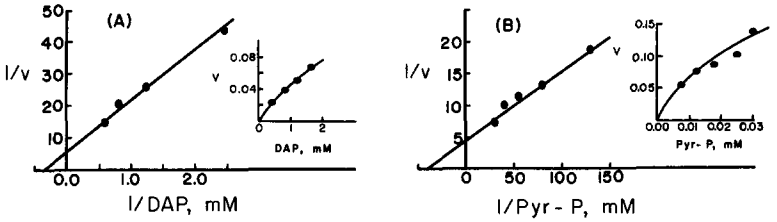

Fig. 1. Kinetics of diaminopimelate decarboxylase. A. Response curve and Lineweaver–Burk plot of the effect of diaminopimelate (DAP) concentration on reaction velocity. B. Response curve and Lineweaver–Burk plot of the effect of pyridoxal phosphate (Pyr-P) concentration on reaction velocity. Reaction velocities (v) are in terms of μmoles of diaminopimelate consumed per h per ml of reaction mixture. The components of the reaction mixture and the method of assay are given under Table I. A rate-limiting amount of enzyme purified through the step involving adsorption on calcium phosphate gel was used.

The effect of various concentrations of cofactor, pyridoxal phosphate, on the rate of diaminopimelate decarboxylation was also determined. As shown in Fig. 1B a reciprocal plot of the data give an apparent K_m of about 0.025 mM. This relatively high value indicates a rather weak association of the coenzyme with the enzyme protein, and offers an explanation for our being able to isolate a completely inactive apoenzyme.

We have demonstrated that cells of *L. arabinosus* 17-5 do possess diaminopimelate decarboxylase activity; this activity has been purified approx. 170-fold. We have not, however, been able to demonstrate aspartokinase activity in this organism. It may be that very low aspartokinase activity is present in *L. arabinosus*, and is not easily detected by the assay procedures employed (aspartate-dependent hydroxamate formation and β-aspartyl phosphate formation). Further investigation regarding lysine biosynthesis in this organism should be undertaken.

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