Note

Purification and Properties
of *meso*-Diaminopimelate
Dehydrogenase from
Brevibacterium sp.

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meso-Diaminopimelate dehydrogenase (EC 1.4.1.16) catalyzes the reversible oxidative removal of an amino group with the D-configuration in the substrate in the presence of NADP⁺ to yield L-2-amino-6-ketopimelate.¹⁾ The enzyme functions in lysine biosynthesis of *Bacillus sphaericus*²⁾ and *Corynebacterium glutamicum*.³⁾ It has been purified to homogeneity from *B. sphaericus* IFO 3525 and characterized enzymologically.^{4~6)} The enzyme is used for spectrophotometric measurement of *meso*-2,6-diaminopimelate and for assay of diaminopimelate epimerase.^{7,8)} The enzyme is also useful for the preparation and measurement of L-2-amino-6-ketopimelate,^{2,9)} an intermediate in bacterial lysine biosynthesis, and for the preparation of stereospecifically isotope-labeled *meso*-2,6-diaminopimelate.¹⁰⁾ We found a high activity of the enzyme in a lysine producer, *Brevibacterium* sp. ICR 7000, and purified the enzyme to homogeneity from these cells to compare its properties with those of the *B. sphaericus* enzyme.

We describe here an improved purification procedure and some properties of *meso*-diaminopimelate dehydrogenase from *Brevibacterium* sp. ICR 7000.

The dehydrogenase activity was measured at 30°C by the rate of increase in the absorbance at 340 nm with a Hitachi 124 spectrophotometer. The standard reaction mixture consisted of 10 μ mol *meso*-2,6-diaminopimelate, 1 μ mol of NADP⁺, 200 μ mol of glycine-KCl-KOH buffer (pH 10.5) and enzyme in a final volume of 1.0 ml. The assay system for the reductive amination contained 3.65 μ mol of L-2-amino-6-ketopimelate, 0.2 μ mol of NADPH, 100 μ mol of ammonium sulfate, 300 μ mol of Tris-HCl buffer (pH 7.5) and enzyme in a final volume of 1.0 ml. L-2-Amino-6-ketopimelate was prepared by oxidation of 2,6-diaminopimelate (a mixture of D, L, and *meso*) with *meso*-diaminopimelate dehydrogenase and purified by Dowex 50 $\times$ 8 (H⁺) column chromatography.¹⁾ Protein was measured by the procedure of Lowry *et al.*¹¹⁾ with bovine serum albumin as a standard. One unit of the

enzyme was defined as the amount of enzyme that catalyzes the formation of 1 μ mol of NADPH per min in the oxidative deamination of *meso*-2,6-diaminopimelate. Specific activity was expressed as units per mg of protein. *Brevibacterium* sp. ICR 7000 was grown in a medium (pH 7.2) containing 1.0% peptone, 0.2% K₂HPO₄, 0.2% KH₂PO₄, 0.2% NaCl, 0.01% MgSO₄·5H₂O and 0.01% yeast extract. The bacteria were grown aerobically in 2-liter flasks containing 500 ml of the medium on a reciprocating shaker at 28°C for 48 hr. The cells were harvested by centrifugation and washed twice with 0.85% NaCl. The enzyme was purified at 0~4°C and potassium phosphate buffer (pH 7.4) containing 0.01% 2-mercaptoethanol was used throughout, unless otherwise specified.

Step 1. The washed cells (about 360 g, wet weight) were disrupted by grinding in a mortar with levigated aluminum oxide and extracted with 0.1 M potassium phosphate buffer (pH 7.2) containing 0.02% 2-mercaptoethanol. The supernatant solution obtained by centrifugation was dialyzed against 0.01 M buffer.

Step 2. The dialyzed enzyme solution was brought to 50% saturation with ammonium sulfate. The pH was kept at 7.4 by addition of 14% ammonium hydroxide solution during the ammonium sulfate fractionation. The precipitate was removed by centrifugation and ammonium sulfate was added to the supernatant solution to 80% saturation. The precipitate was collected, dissolved in 0.01 M buffer, and dialyzed against 0.01 M buffer.

Step 3. The enzyme solution was put on a DEAE-cellulose column (4.5 $\times$ 45 cm) equilibrated with 0.01 M buffer. After the column was washed with the buffer containing 0.1 M NaCl, the enzyme was eluted with the buffer supplemented with 0.2 M NaCl. The active fractions were combined and concentrated with an Amicon membrane filter PM-10.

Step 4. The enzyme solution was placed on a hydroxyapatite column (3.2 $\times$ 15 cm) equilibrated with 0.01 M buffer. The column was washed with the same buffer and the enzyme was eluted with 0.03 M buffer. The active fractions were combined and concentrated with an Amicon membrane filter PM-10.

Step 5. The enzyme solution was then put on a 2',5'-ADP-Sepharose 4B column (1.2 $\times$ 7.0 cm) equilibrated with 0.1 M potassium phosphate buffer (pH 7.2) containing 0.01% 2-mercaptoethanol. After the column was washed with the same buffer, the enzyme was eluted with the buffer containing 0.3 M KCl. The active fractions were concentrated with an Amicon membrane filter PM-10 and stored at -20°C in the presence of 10% glycerol until use. Approximately 380-fold purification was achieved with an overall yield of 23%. A summary of the purification procedure is presented in Table I.

The purified enzyme showed a single band upon disc gel electrophoresis and sodium dodecyl sulfate-disc gel electrophoresis. The molecular weight of the enzyme was approximately 70,000 by the Sephadex G-150 gel filtration method of Andrews.¹²⁾ The molecular weight of the

TABLE I. PURIFICATION OF *meso*-DIAMINOPIMELATE DEHYDROGENASE FROM *Brevibacterium* sp. ICR 7000

Step	Total protein (mg)	Specific activity (units/mg)	Total units	Yield (%)	Purification
Crude extract	7,480	0.348	2,600	100	1
Ammonium sulfate fractionation	3,400	0.623	2,120	81.5	1.8
DEAE-cellulose	481	3.98	1,910	73.5	11.4
Hydroxyapatite	175	6.29	1,100	42.3	18.1
2',5'-ADP-Sepharose 4B	4.74	131.0	621	23.9	376

subunits was about 39,000 by sodium dodecyl sulfate-disc gel electrophoresis by the method of Weber and Osborn.¹³ These results suggest that the enzyme is composed of two subunits identical in molecular weight, although the discrepancy between the values of molecular weight thus obtained has not been elucidated. The isoelectric point of the enzyme was 5.0 by isoelectric focusing on a polyacrylamide gel.¹⁴

The enzyme was stable up to 48°C when incubated for 10 min in 0.01 M potassium phosphate buffer (pH 7.4). The enzyme was stable, when incubated at 40°C for 10 min, over the pH range 7.0 to 9.0. The enzyme had its maximal reactivity at about pH 10.5 for the oxidative deamination of *meso*-2,6-diaminopimelate and at pH 8.5 for the reductive amination of L-2-amino-6-ketopimelate. The enzyme deaminated exclusively the *meso* form of 2,6-diaminopimelate; neither the D nor L-isomer served as a substrate. L-Glutamate, L-alanine, L-leucine, L-valine, and L-lysine were inert as substrates for the enzyme. Ishino *et al.*³ reported that the partially purified enzyme from *C. glutamicum* uses NAD⁺ in addition to NADP⁺ as a coenzyme, though the relative rate was not shown. We investigated the reactivity of NAD⁺ with the *Brevibacterium* enzyme. NAD⁺ served as a coenzyme at 3% of the rate of NADP⁺ at the concentration of 1 mM. The enzyme was inhibited by iodoacetic acid (55% inhibition at 1 mM), *N*-ethylmaleimide (52% at 1 mM), HgCl₂ (100%, 0.01 mM), CuSO₄·5H₂O (68%, 0.01 mM), and NiSO₄ (21%, 1 mM). The enzyme activity was little affected by 1 mM EDTA, α,α' -dipyridyl, NaN₃, KCN, or sodium arsenate.

The enzyme also might function in the lysine biosynthesis of this bacterium as shown in *C. glutamicum*³ and *B. sphaericus*.² L-Lysine (10 mM), however, did not inhibit the enzyme. The enzyme is similar to the *B. sphaericus* enzyme in this respect.⁵

The Michaelis constants for *meso*-2,6-diaminopimelate and NADP⁺ were 6.25 and 0.14 mM, respectively, at pH 10.5 by the procedure described in our previous paper.⁵ The *K_m* values for NADPH, L-2-amino-6-ketopimelate, and ammonia were 0.23, 0.21 and 62.5 mM, respectively, at pH 7.5. The *K_m* value for NAD⁺ was 2.0 mM by

Lineweaver-Burk plots with the reaction system (pH 10.5) containing 10 mM *meso*-2,6-diaminopimelate.

The properties of this enzyme are similar to those of the *B. sphaericus* enzyme.^{4,5} However, the pH optimum for the reductive amination of L-2-amino-6-ketopimelate, the *K_m* value for ammonia, and the reactivity of NAD⁺ are different in the enzymes from *Brevibacterium* sp. and *B. sphaericus*.

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