OCCURRENCE OF *meso*-α,ε-DIAMINOPIMELATE DEHYDROGENASE IN *BACILLUS SPHAERICUS*

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Summary: A new amino acid dehydrogenase catalyzing the oxidative deamination of *meso*-α,ε-diaminopimelate was found in the crude extract of *Bacillus sphaericus* IFO 3525. This dehydrogenase requiring NADP was specific for *meso*-diaminopimelate and the other isomers were not substrates. The enzyme was optimally active at about pH 10.5. NAD could not replace NADP.

α,ε-Diaminopimelate (DAP*) has been demonstrated in various bacteria, actinomycetes and blue-breen algae (1-3), since discovered by Work (4) as a constituent of the cell walls of *Corynebacterium diphtheriae*. DAP functions not only as a structural component of the cell, but also an immediate precursor of L-lysine (5-6). The naturally occurring *meso* and L-isomers are interconvertible by DAP epimerase (7). *meso*-DAP was shown by Burton (8) to be oxidized by L-amino acid oxidase of *Neurospora crassa*. Work (9) reported subsequently that L-DAP is oxidized by the *Neurospora* oxidase as well as the *meso* isomer, and that L-amino acid oxidase of rattlesnake venom also oxidatively deaminates both the *meso* and L-isomers, though very slowly. Oshima *et al.* (10) showed that NADP-specific L-glutamate dehydrogenase of *Brevibacterium* (= *Micrococcus*) *glutamicus* acts on DAP. The enzymatic transamination of DAP has been reported by Meadow and Work (11).

We present here evidence for the occurrence of a new bacterial amino acid dehydrogenase that catalyzes oxidative deamination of *meso*-DAP.

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*DAP: α,ε-Diaminopimelate.

MATERIALS AND METHODS

DAP was synthesized from glutaraldehyde according to the method of Roy and Karel (12). The three isomers of DAP were separated as described by Wade *et al.* (13). NAD and NADP, and sodium L-glutamate, L-leucine and L-alanine were products of Kyowa Hakko Kogyo Co., Tokyo, and Ajinomoto Co., Tokyo, respectively. The other chemicals were analytical grade reagents.

Bacillus sphaericus IFO 3525 was grown in a medium containing 1.5% peptone, 0.1% glycerol, 0.2% K_2HPO_4, 0.2% KH_2PO_4, 0.2% NaCl, 0.01% yeast extract and 0.01% $MgSO_4 \cdot 7H_2O$. The pH of the medium was adjusted to 7.2 with 2 N NaOH. The bacteria were grown in 2-liter flasks containing 700 ml of the medium, with vigorous shaking on a reciprocating shaker, at 28° for 18-20 hr. The cells were harvested by centrifugation and were washed twice with 0.85% NaCl.

The washed cells were suspended in 0.1 M potassium phosphate buffer (pH 7.4) containing 0.01% 2-mercaptoethanol, and subjected to sonication in a 19-kc Kaijo Denki Oscillator for 10 min. After centrifugation at 17,000 *g* for 30 min, the supernatant solution was dialyzed against 0.01 M potassium phosphate buffer (pH 7.4) containing 0.01% 2-mercaptoethanol and employed as the crude extract. The crude extract was brought to 40% saturation with solid ammonium sulfate, and the precipitate was removed by centrifugation. To the supernatant fraction was added ammonium sulfate to achieve 80% saturation. The precipitate obtained by centrifugation was dissolved in 0.01 M potassium phosphate buffer (pH 7.4) containing 0.01% 2-mercaptoethanol, and was dialyzed against the same buffer. The inactive precipitate formed during dialysis was removed by centrifugation. All operations were carried out at 0-5°. This enzyme preparation, unless otherwise specified, was used in the experiments.

The dehydrogenase activity was determined at 25° by measuring the rate of increase in absorbance at 340 nm with a Shimadzu spectrophotometer MPS 50L using a cuvette of 1.0-cm light path. One unit of enzyme is defined as the amount of enzyme that catalyzes the formation of 1 μmole of NADPH per min (ε: 6.2×10^3). Specific activity is expressed as units per mg of protein. Protein was determined by the method of Lowry *et al.* (14) using crystalline serum albumin as a standard.

RESULTS AND DISCUSSION

When *meso*-DAP was incubated with the crude extract of *Bacillus sphaericus* in the presence of NADP at pH 8.0, the reduction of NADP was observed (Figure 1). The reaction occurred only in the presence of *meso*-DAP and enzyme, and proceeded linearly as a function of amount of enzyme and incubation time. *meso*-DAP was oxidized with the liberation of equimolar ammonia as shown in Table I, but the consumpsion of oxygen was not observed when measured manometrically at 30° in a system (3.0 ml) containing 30 μmoles of *meso*-DAP, 3 μmoles of NADP, 100 μmoles of pyrophosphate buffer (pH 8.3) and 6.2 mg of enzyme. The reaction product from *meso*-DAP was reacted with

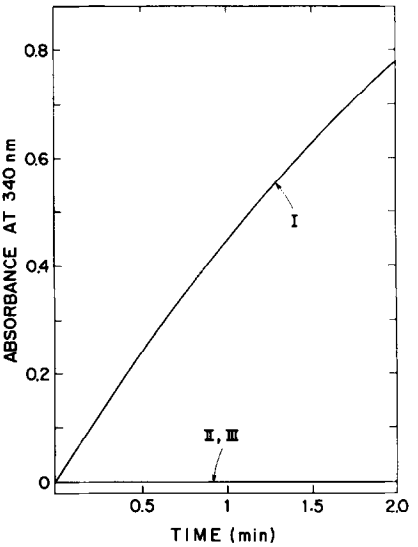

FIGURE 1. *meso*-DAP dehydrogenase reaction. The reaction mixture contained 10 µmoles of *meso*-DAP, 1 µmole of NADP, 200 µmoles of Tris-HCl buffer (pH 8.0) and 1.9 mg of enzyme in a final volume of 1.0 ml. I, complete system; II, *meso*-DAP was omitted; III, the enzyme was omitted.

TABLE I. Enzymatic formation of ammonia from *meso*-DAP.

Time	*meso*-DAP	Ammonia	Keto Acid
min	µmoles	µmoles	
0	5.0	0	-
60	3.78	1.36	+

The reaction mixture was composed of 5 µmoles of *meso*-DAP, 5 µmoles of NADP, 200 µmoles of potassium phosphate buffer (pH 7.2) and 0.4 mg of enzyme in a final volume of 1.0 ml. After incubation at 25° for 60 min, the reaction was terminated by addition of 0.05 ml of 50% trichloroacetic acid followed by centrifugation. *meso*-DAP was determined by the method of Soda *et al.* (19) using a solvent of methanol-water-pyridine (80:20:4). Ammonia was determined by Nessler's reagent (20). The formation of keto acid was examined by 2,4-dinitrophenylhydrazine.

2,4-dinitrophenylhydrazine, *o*-aminobenzaldehyde and ninhydrin to develope yellow, orange and violet colors, respectively, suggesting that the product has both carbonyl and amino groups in it and also that the product is dehydrated to yield a heterocyclic compound in part (15). 2,4-Dinitrophenylhydrazone of the reaction product showed the Rf value (0.43) closely similar to that of α-amino-ε-ketopimelate, which was prepared by oxidation of *meso*-DAP with L-amino acid oxidase of *Neurospora crassa* (8) or snake venom (9), on paper chromatography with the solvent of *n*-butanol-ethanol-water (7:1:2). This result suggests that *meso*-DAP is enzymatically converted into α-amino-ε-ketopimelate. This keto acid was formed only in the presence of *meso*-DAP, NADP and the enzyme.

DAP has been shown to be deaminated by NADP-specific L-glutamate dehydrogenase of *Brevibacterium glutamicus* (10). L-Glutamate dehydrogenase activity, however, was not detected in the crude extract of *Bacillus sphaericus* IFO 3525, when the activity was measured at pH 8.0 and 10.5 using NAD or NADP (Table II). This finding indicates that *meso*-DAP dehydrogenase

TABLE II. Activities of amino acid dehydrogenases of *Bacillus sphaericus*.

Enzymes	pH	Specific Activity	
		NAD	NADP
Leucine dehydrogenase	10.5	0.532	0
Alanine dehydrogenase	10.5	0.516	0
Glutamate dehydrogenase	8.0	0	0
	10.5	0	0
meso-DAP dehydrogenase	8.0	0	0.013
	10.5	0	0.340

The dehydrogenase activity was determined by measuring the rate of reduction of NAD or NADP. The reaction mixture contained 10 μmoles of amino acid, 1 μmole of NAD or NADP, 200 μmoles of Tris-HCl buffer (pH 8.0) or glycine-KCl-NaOH buffer (pH 10.5) and crude extract in a final volume of 1.0 ml.

activity is not attributable to the activity of glutamate dehydrogenase.

The crude extract of *B. sphaericus* IFO 3525 contains also the high activity of L-leucine and L-alanine dehydrogenases (Table II). Leucine and alanine dehydrogenases of bacilli have been purified to homogeneity and crystallized (16-17). Although leucine and alanine dehydrogenases have a relatively wide substrate specificity (16, 18), both the enzymes, which require NAD specifically, did not work on *meso*-DAP.

The enzyme was specific for *meso*-DAP, and L- and D-isomers were not substrates. The enzyme has an optimum reactivity at about pH 10.5. NAD could not replace NADP. Thus, good evidence has been presented for the occurrence of the new NADP-specific dehydrogenase catalyzing the oxidative deamination of *meso*-DAP in the crude extract of *B. sphaericus*. Further work is needed to elucidate which of both amino groups of *meso*-DAP with D and L configurations is deaminated, and also enzymological and physicochemical properties of the enzyme.

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