Short Communications

Metabolism of $\alpha\beta$ -Diaminopropionate in a *Pseudomonas* sp.

By D. RAJAGOPAL RAO, K. R. VIJAYALAKSHMI and K. HARIHARAN

Discipline of Biochemistry, Central Food Technological Research Institute, Mysore-2A, India

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L- $\alpha\beta$ -Diaminopropionate is a constituent of the polypeptide antibiotic viomycin (Haskell, Fusari, Frahrdt & Bartz, 1952) and also occurs in the free state in the seeds of some plants belonging to the family Mimosaceae (Gmelin, Strauss & Hassenmaier, 1958, 1959). D- $\alpha\beta$ -Diaminopropionate is present in the free amino acid pool of the digestive fluid of *Bombyx mori* (Wada & Toyata, 1965). β -Oxalyl-L- $\alpha\beta$ -diaminopropionate is found in *Lathyrus sativus* and is neurotoxic to chicks, young rats and monkeys (Rao, Malathi & Sarma, 1969).

Studies on the biosynthesis of L- $\alpha\beta$ -diaminopropionate indicate that serine is the precursor in a *Streptomyces* sp. (Dubus, Dyer, Floyd & Shaw, 1969), but not in *Lathyrus sativus* (Roy, 1969). Seneviratne & Fowden (1968) have studied the metabolism of L- $\alpha\beta$ -diaminopropionate in *Acacia* and *Phaseolus* seedlings and indicated that it predominantly undergoes acetylation, although a small proportion participates in transamination. We have studied the metabolism of $\alpha\beta$ -diaminopropionate in a *Pseudomonas* sp. and have found that it is metabolized to pyruvate by an ammonia-lyase reaction.

Materials and methods. DL- $\alpha\beta$ -Diaminopropionate was synthesized from $\alpha\beta$ -dibromopropionic acid by amination (Poduška, Rudinger & Šorm, 1955) and purified on two columns (each 50 cm $\times$ 3 cm) of Dowex 50W (X4; H⁺ form; 100–200 mesh). L- $\alpha\beta$ -Diaminopropionate was a product of Calbiochem, Los Angeles, Calif., U.S.A. D- $\alpha\beta$ -Diaminopropionate was a gift from Dr S. Wada of Takeda Chemical Industries, Osaka, Japan. N-Acetyldehydroalanine (2-acetamidoacrylic acid) was a product of Koch-Light Laboratories Ltd., Colnbrook, Bucks, U.K. Pyridoxal phosphate was a product of E. Merck A.-G., Darmstadt, Germany. DL-Isoserine was a product of Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

A *Pseudomonas* sp. was isolated from soil by using the basal salts solution of Goodhue & Snell (1966), containing 15 mM-DL- $\alpha\beta$ -diaminopropionate as sole source of carbon and nitrogen. Large-scale liquid cultures were grown in the same medium. The harvested cells were suspended in 4 vol. of

0.1 M-potassium phosphate buffer, pH 8, and sonicated in a Branson Sonifier (model B12A) set at 80 W for three periods of 3 min each, with intervals for cooling. After sonication, the suspension was centrifuged at 0–4°C at 10 000 g for 20 min to remove cell debris. The supernatant (20–30 mg of protein/ml) constituted the crude extract. The ammonia-lyase activity was determined at 37°C by following the appearance of pyruvate in a mixture similar to that described in Table 1, but containing 50 mM-potassium phosphate buffer, pH 8. Specific activity is defined as μ mol of pyruvate formed/30 min per mg of protein.

$\alpha\beta$ -Diaminopropionate was determined after paper-chromatographic separation (butan-1-ol-acetic acid-water, 2:1:1, by vol.) by the method of Giri, Radhakrishnan & Vaidyanathan (1952). Pyruvate was determined in the reaction mixtures, without extraction of the 2,4-dinitrophenylhydrazone, by an adaptation of the Friedemann & Haugen (1943) procedure. Ammonia was determined by nesslerization after diffusion (Seligson & Seligson, 1951).

Results and discussion. Whole cells of the pseudomonad oxidized $\alpha\beta$ -diaminopropionate and related compounds, the rate of oxidation decreasing in the order DL- $\alpha\beta$ -diaminopropionate, L-serine, glycine and glyoxylate. No new amino acids accumulated, in contrast with the observations with L- $\alpha\gamma$ -diaminobutyrate (Rajagopal Rao, Hariharan & Vijayalakshmi, 1969). Freeze-dried cells on incubation with $\alpha\beta$ -diaminopropionate in the presence or absence of arsenite yielded substantial amounts of pyruvate. Cell-free extracts of the pseudomonad did not catalyse transamination of $\alpha\beta$ -diaminopropionate under a variety of conditions with various keto acids (pyruvate, oxaloacetate, α -oxoglutarate and glyoxylate) in the presence or absence of pyridoxal phosphate. Instead, the extracts had an ammonia-lyase that catalysed the formation of nearly stoichiometric quantities of ammonia and pyruvate from L- and DL- $\alpha\beta$ -diaminopropionate (Table 1). Pyruvate was characterized as its 2,4-dinitrophenylhydrazone by paper chromatography and catalytic hydrogenolysis to alanine. Further,

Table 1. *Stoichiometry of $\alpha\beta$ -diaminopropionate ammonia-lyase reaction*

The reaction mixture contained the following components (final concentrations): potassium phosphate buffer, pH 8.0, 20 mM, L- or DL- $\alpha\beta$ -diaminopropionate, 20 mM, and partially purified enzyme (2 mg of protein). Separate reactions were run at 37°C for each time-interval. At the end of the incubation period, the reaction was terminated by addition of HClO₄ (final concn. 2%, w/v). Blanks without substrate and the complete system with boiled enzyme were run in all reactions. The buffer concentration was kept low, as higher concentrations interfered with the paper-chromatographic determination of $\alpha\beta$ -diaminopropionate. Higher concentrations of buffer (50–100 mM) do not affect the yields of pyruvate and ammonia. In Expt. 1, L- $\alpha\beta$ -diaminopropionate, and in Expt. 2, DL- $\alpha\beta$ -diaminopropionate, were used.

Expt. no.	Incubation time (min)	$\alpha\beta$ -Diaminopropionate	Pyruvate formed (μ mol)	Ammonia formed (μ mol)
		utilized (μ mol)		
1	15	1.89	1.80	3.60
	30	4.39	3.60	7.50
	45	6.19	5.30	10.70
2	15	1.59	1.60	3.20
	30	2.79	3.20	6.10
	45	4.39	4.75	8.95

the $\alpha\beta$ -diaminopropionate ammonia-lyase could be coupled to lactate dehydrogenase in the presence of NADH.

Fractionation of bacterial extracts by protamine sulphate treatment (200 mg of protamine/50 ml of crude extract), ammonium sulphate fractionation (40–60% saturation) and Sephadex G-75 chromatography (elution with 0.1 M-potassium phosphate buffer, pH 8.0) gave a tenfold increase in specific activity of the ammonia-lyase (15 μ mol of pyruvate formed/30 min per mg of protein). This fraction was more active with L- $\alpha\beta$ -diaminopropionate (100%) than with the D-isomer (30%). The partially purified ammonia-lyase was inhibited by carbonyl-binding agents such as sodium cyanide (40% inhibition at 1 mM), hydroxylamine (85% inhibition at 0.1 mM), semicarbazide (75% inhibition at 0.1 mM) and isonicotinic acid hydrazide (40% inhibition at 0.1 mM). An occasional slight stimulation (10–20%) of pyruvate formation from DL- and L- $\alpha\beta$ -diaminopropionate was observed on preincubation of the enzyme with 0.04 mM-pyridoxal phosphate.

was partially reversed (70%) by the addition of 2 mM-dithiothreitol. The enzyme was also inhibited (30%) under similar conditions by 0.1 mM-N-ethylmaleimide. The partially purified enzyme did not catalyse the formation of pyruvate from D- or L-serine or DL-isoserine. Bacterial extracts did not form pyruvate from N-acetyldehydroalanine, though rat kidney extracts had this activity, in agreement with the observations by Price & Greenstein (1947).

Non-enzymic deamination of $\alpha\beta$ -diaminopropionate at pH 4.6 at 100°C for 30 min in the presence of Al³⁺ and pyridoxal under conditions similar to those used by Metzler & Snell (1952a,b) yielded pyruvate exclusively (36% yield).

It is known that β -chloro-L-alanine is converted into pyruvate by both enzymic and non-enzymic reactions, but no intermediates have been detected in this system (Gregerman & Christensen, 1956). The formation of pyruvate from $\alpha\beta$ -diaminopropionate probably occurs by a concerted β -elimination reaction as follows:

Further purification and separation of enzymes acting on D- and L- $\alpha\beta$ -diaminopropionate is needed to ascertain whether pyridoxal phosphate is a cofactor of either a $\alpha\beta$ -diaminopropionate racemase or the ammonia-lyase reaction. The ammonia-lyase is probably a thiol enzyme as, on incubation with 0.1 mM-p-chloromercuribenzoate for 5 min in the presence of 50 mM-potassium phosphate buffer, pH 8, it was completely inhibited and the inhibition

This reaction is similar to that involved in the dehydration of serine and in the desulphydration of cysteine (Snell, 1958; Braunstein, 1960).

The present findings indicate that the metabolism of $\alpha\beta$ -diaminopropionate in the *Pseudomonas* sp. differs from that in plants.

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