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# BACTERIAL TRANSAMINATION OF THE STEREOISOMERS OF DIAMINOPIMELIC ACID AND LYSINE

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Two of the three stereoisomers of diaminopimelic acid are known to be metabolised by bacteria. The *meso* isomer is converted to L-lysine by diaminopimelic acid decarboxylase[1], while the *meso* and L,L-isomers are interconverted by diaminopimelic acid racemase[2]. We now present evidence that in certain bacteria all three stereoisomers may participate in transamination reactions with oxoglutaric, oxaloacetic or pyruvic acids. Transamination of both isomers of lysine was also observed.

Tests for transamination were carried out by incubating the keto acid and amino acid in the presence of pyridoxal phosphate with a cell suspension of acetone-dried bacteria at pH 8.5, and examining the reaction mixtures by paper chromatography (for method, see Fig. 1). Comparisons were also made of the ability of the cells to transaminate L- or D-glutamic acid. No investigations were made as to the stereoisomeric configuration of the products of transamination, nor was the possibility of indirect transamination eliminated. No reaction occurred in the absence of bacterial cells.

The transaminase activities of various representative organisms are shown in Table I. Only *Bacillus sphaericus*, lacking diaminopimelic acid racemase[3], was suitable for testing both *meso* and L,L-isomers, since with other organisms these two isomers were rapidly interconverted by the racemase. *Escherichia coli* mutant 26-26 and *Bacillus cereus*, both of which contain no diaminopimelic acid decarboxylase[4,2], were used to eliminate any possibility that the apparent transamination of *meso*-diaminopimelic acid was due, in reality, to a preliminary decarboxylation to L-lysine, followed by transamination of lysine.

*Meso*diaminopimelic acid actively transaminated with at least one keto acid in all the organisms tested. The rates varied, both absolutely, and with respect to glutamic acid transamination. In *B. sphaericus* the rate of transamination of *meso*-diaminopimelic acid was as high as that of glutamic acid, in *Sarcina lutea* it was lower,

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while in *Staphylococcus saprophyticus* and *Aerobacter aerogenes* glutamic acid was not detectably transaminated. In *B. sphaericus*, where diaminopimelic acid transaminated equally actively with all the three keto acids tested, the L,L and D,D-isomers behaved identically and were less active than the *meso* form. In *Sarcina lutea*, oxaloacetic acid was the most active amino group receptor, and D,D-diaminopimelic acid transaminated faster than the *meso* isomer. In *Aero. aerogenes*, where transamination was slow, the active amino-group receptors were pyruvic acid for the *meso* isomer but oxaloacetic and oxoglutaric acids for the D,D isomer.

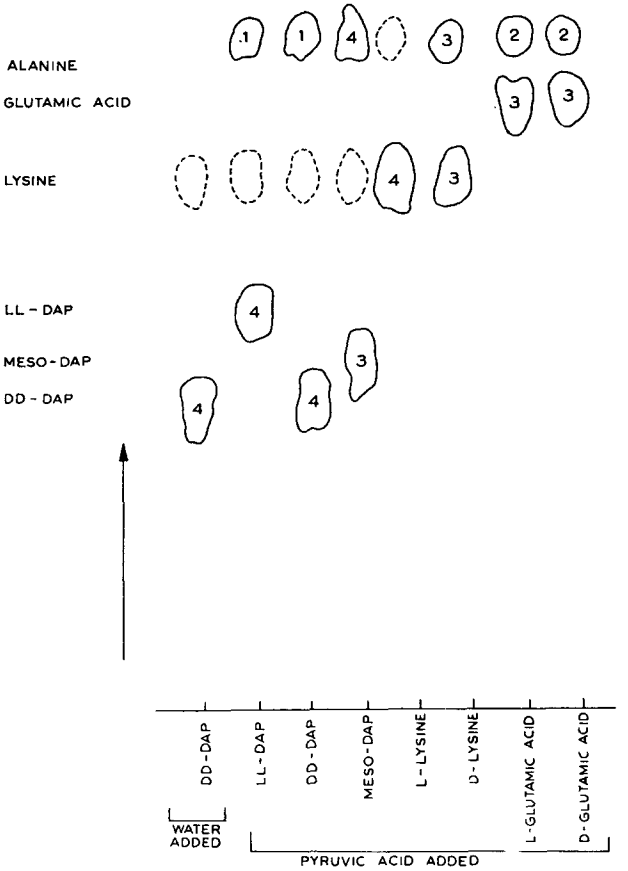


Fig. 1. Descending chromatogram showing effect of incubating acetone-dried *Bacillus sphaericus* (2.6 mg in 0.2 ml of 0.1 *M* borate buffer pH 8.5) at 37° for 1.5 h with 0.02 *M* pyruvic acid (0.1 ml), 0.01 *M* amino acid (0.1 ml) and 3 m*M* pyridoxal phosphate (25 $\mu$l). Samples (0.1 ml), were deproteinised with ethanol (0.2 ml) and the supernatant solution (0.1 ml) was examined for amino acids by chromatography on Whatman No. 1 paper, using methanol water pyridine 10 *N* HCl (80:17.5:10:2.5) as solvent[7]. Spots were developed by dipping paper in ninhydrin in acetone (1% w/v) and heating at 100° for 3 min. Numbers on spots indicate their colour strength (maximum 4); dotted spots were faint. Solvent irrigation was in direction of arrow; reaction mixtures indicated were applied at base line.

D,D-diaminopimelic acid has hitherto been found to be metabolically inert, but these experiments show that it can participate in enzymic reactions; however, it cannot support the growth of diaminopimelic acid-requiring mutants of *E.coli*[5].

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TABLE I

TRANSAMINASE ACTIVITIES OF CERTAIN BACTERIA

Acetone-dried cells were incubated with amino acid and keto acid in the presence of pyridoxal phosphate in the amounts shown in Fig. 1. Paper chromatography of reaction mixture after 1.5 h showed degree of transamination, *i.e.* strength of alanine, aspartic acid or glutamic acid spots from transamination with pyruvic acid, oxaloacetic acid or oxoglutaric acid respectively, compared with that produced in the absence of keto acid. 3, strong spot about equal intensity to substrate amino acid; 2, spot about half intensity of substrate amino acid; 1, weak spot; tr, spot barely visible. In all these cases spots were strong after 18 h incubation. 0, no spot after 18 h.

| *Organism* | *Enzyme content* | | *Transamination with* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Diaminopimelic acid decarboxylase* | *Diaminopimelic acid racemase* | *Keto acid* | *Amino acid* | | | | | |
| | | | | *Diaminopimelic* | | *Lysine* | | *Glutamic* | |
| | | | | *meso* | D, D | L | D | L | D |
| *Escherichia coli* mutant 26-26 | — | + | pyruvic | 1 | 1 | 0 | 0 | 1 | 0 |
| | | | oxaloacetic | 1 | 1 | 0 | 0 | 1 | 0 |
| | | | oxoglutaric | 2 | 2 | 2 | 2 | – | – |
| *Bacillus cereus* N.R.R.L. 569 | — | + | pyruvic | 2 | 2 | 1 | 1 | 2 | 2 |
| | | | oxaloacetic | 1 | tr*** | 1 | 1 | 2 | 1 |
| | | | oxoglutaric | 1 | 1 | 1 | 1 | – | – |
| *Bacillus sphaericus* (asporogenous) N.C.T.C. 7582) | + | — | pyruvic | 3 | 1* | tr | 2 | 2 | 2 |
| | | | oxaloacetic | 3** | 1*, ** | 1** | 2*** | 3 | 3** |
| | | | oxoglutaric | 3 | 1* | tr | tr | – | – |
| *Sarcina lutea* N.C.T.C. 576 | + | + | pyruvic | 1 | 2 | 1 | 1 | 3 | 1 |
| | | | oxaloacetic | 2 | 3 | 2 | 2 | 3 | tr |
| | | | oxoglutaric | 1 | 2 | tr | 2 | – | – |
| *Aerobacter aerogenes* A.T.C.C. 12409 | + | + | pyruvic | 1 | 0 | 0 | 0 | 0 | 0 |
| | | | oxaloacetic | 0 | 1 | 1 | 0 | 0 | 0 |
| | | | oxoglutaric | 0 | 1 | 1 | 0 | – | – |
| *Staphylococcus saprophyticus* N.C.T.C. 4297 | + | + | pyruvic | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | oxaloacetic | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | oxoglutaric | 1 | 1 | 1 | 1 | – | – |

* L,L-diaminopimelic acid behaved identically
** Product was aspartic acid plus alanine.
*** Product was alanine.

Since D,D-diaminopimelic acid is not enzymically racemised[2], its transamination must occur directly and not through intermediate formation of the *meso* isomer. In view of the various reaction rates of the *meso* and D,D isomers in different organisms and with different keto acids, it is unlikely that the same enzyme is involved in transaminating the two isomers.

Both isomers of lysine were also found to be transaminated by most organisms, but not always at the same rates, or with the same keto acids. In *B. sphaericus*, D-lysine was more effective than the L-isomer, suggesting that preliminary racemisation was unlikely. Transamination of D-lysine has not been previously reported, but since this isomer is known to support the growth of a lysine-requiring mutant of *E.coli*[5], the reaction may be of some metabolic significance.

The strain of *B.sphaericus* used was a variant which had no diaminopimelic

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acid decarboxylase activity in the absence of pyridoxal phosphate[3]. Under these conditions oxoglutaric, oxaloacetic and pyruvic acids (m$M$) induced decarboxylation of *meso*diaminopimelic acid in the Warburg apparatus ($Q_{CO_2}$ of the order of 3.0) but the rate was about 30% of that obtained in the presence of 100 $\mu M$ pyridoxal phosphate[6]. Transamination by this strain was slow with all three keto acids in the absence of pyridoxal phosphate, but the rate was increased progressively by graded additions of pyridoxal phosphate ($\mu M$ to 100 $\mu M$). The stimulation of decarboxylation by keto acids has not been explained[6].

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SUMMARY

The three stereoisomers of diaminopimelic acid transaminated with one or more of the ketoacids, pyruvic, oxoglutaric or oxaloacetic acid, when incubated with acetone-dried bacteria and pyridoxal phosphate. In certain cases, the DD-isomer was more active than the *meso* form. Transamination of D-lysine also occurred.

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# PENTOSE PHOSPHATE CLEAVAGE BY *LEUCONOSTOC MESENTEROIDES*

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The *Lactobacillus* species ferment pentoses such that the methyl and carboxyl groups of acetate arise from C-1 and C-2 of pentose, respectively, while lactate arises from carbon atoms 3, 4, and 5[1,2]. It has recently been shown that an enzyme preparation purified from *Lactobacillus pentosus* catalyzes a phosphorolytic cleavage of xylulose-5-phosphate, resulting in the production of acetyl P** and triose phosphate[3]. The

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** The following abbreviations are used: acetyl P, acetyl phosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; $P_i$, inorganic phosphate; DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide.

*References p. 602.*