Microbial Metabolism of Amino Alcohols via Aldehydes

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Attempts to study the possible biosynthetic role of L-aminopropan-2-ol by Lowe & Turner (1970) were complicated as many micro-organisms metabolised 14C-labelled amino-propanol via common intermediary metabolic routes. The catabolism of the amino alcohol via aminoacetone by a pseudomonad has been described (Higgins, Pickard & Turner, 1962; Pickard, Higgins & Turner, 1968). We now report the catabolism of both L- and D-L-aminopropan-2-ol via propionaldehyde, also ethanolamine via acetaldehyde, by what is a new metabolic route.

Several species of Erwinia can grow on L-, D- or racemic L-aminopropan-2-ol as sole source of nitrogen but not carbon. When Erwinia carotovora (National Collection of Plant Pathogenic Bacteria, NCPPB 1280) was grown on glycerol, glucose, citrate, malate or pyruvate, L-aminopropan-2-ol was converted to propionaldehyde (see Fig. 1). The aldehyde was formed stoichiometrically, without dilution or incorporation into bacterial material, by a mechanism closely linked to growth. No detectable aldehyde was formed by non-growing suspensions of the micro-organism in 0.1 M-phosphate buffer at pH 7 or when alternative utilisable sources of nitrogen were supplied simultaneously during growth. Ethanolamine was similarly catabolized to acetaldehyde, the nitrogen atom being used for growth. Some radioactivity from [14C]ethanolamine was incorporated into cell substance, probably phospholipids. Both aldehydes were trapped as their 2,4-dinitrophenylhydrazones and identified by infrared and mass spectrometry.

The micro-organism tentatively identified by Gottlieb & Mandel (1959) as Achromobacter sp. P6 (NCIB 10431) grew on both L-aminopropan-2-ol enantiomorphs as sole sources of both carbon and nitrogen. Small but significant amounts of propionaldehyde were produced during growth on L- and DL-aminopropanol, but again the aldehyde was not formed in detectable amounts by non-growing suspensions of the micro-organism in phosphate buffer. Radioactivity from [3-14C]aminopropanol was rapidly incorporated into bacterial material by growing cultures (see Fig. 1). Aldehyde formation during growth on the amino alcohol was not prevented by the addition of NH4+. Ethanolamine could also be utilized by Achromobacter P6 with formation of acetaldehyde in cultures.

Growth, respirometric and enzymic studies suggested that propionaldehyde and acetaldehyde production by both micro-organisms proceeded by common enzyme-catalysed steps, but that the aldehydes were further metabolized only by Achromobacter P6. The catabolic pathway in the case of L-aminopropan-2-ol involves O-phospho-L-aminopropan-2-ol, propionaldehyde, propionate and propionyl-CoA as early intermediates. Some properties of the enzymes involved have been determined. An amino alcohol kinase, analogous to the choline kinase of bakers' yeast (Wittenberg & Kornberg, 1953) but virtually inactive with choline, is efficiently coupled in vivo to an O-phosphoamino alcohol phospho-lyase similar to that in mammalian liver described by Fleshood & Pitot (1969, 1970).

The amino alcohol kinases were of particular interest as enzymes specific for the phosphorylation of ethanolamine or L-aminopropan-2-ol have not been reported previously.
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Their activities were assayed spectrophotometrically either by a modification of the method for creatine kinase described by Tanzer & Gilvarg (1959) involving the addition of ATP, phosphoenol pyruvate, pyruvate kinase, lactate dehydrogenase and NADH, or by coupling via the phospho-lyase enzyme to the NADH-dependent reduction of aldehyde catalysed by added alcohol dehydrogenase (Fleshood & Pitot, 1969). The enzyme from *Achromobacter* p6 was optimally active at pH 7.8, had an obligatory requirement for ATP and Mg²⁺ (each used at 10 mM) and required DL-cysteine for activity. Under these conditions relative activities with DL-1-aminopropan-2-ol (100), D-1-aminopropan-2-ol (92), ethanolamine (26) and choline (10) were as indicated when substrates were used at 10 mM. (The amino alcohol kinase of *Erwinia carotovora* has not been studied in such detail.). The O-phosphoamino alcohol phospho-lyase activity in extracts of each micro-organism, assayed spectrophotometrically (Fleshood & Pitot, 1969), was about threefold greater with DL-1-aminopropan-2-ol phosphate than ethanolamine phosphate under optimum conditions, i.e. pH 7-8 in tris-HCl buffer, with substrate at approx. 10 mM. Activity was unaffected by NH₄⁺ but enzyme formation by *E. carotovora* was repressed when NH₄⁺ was present in addition to amino alcohols acting as nitrogen sources for growth.

Fig. 1. Distribution of radioactivity from [3-¹⁴C]-DL-1-aminopropan-2-ol during growth of Erwinia and Achromobacter species. Both micro-organisms were grown on a medium containing (g./l.): glycerol, 3 g; DL-1-aminopropan-2-ol, 1 g; (4±2 μCi); KH₂PO₄, 3 g.; K₃PO₄, 7 g.; MgSO₄.7H₂O, 0±1 g.; and Na₂SO₄, 1 g. adjusted to pH 7. Cultures were incubated at 32° in sealed conical shake-flasks each equipped with a centre well containing 0.2 M-semicarbazide in 0.1 M-phosphate buffer pH 7 to trap propionaldehyde. After inoculation with a culture grown on non-radioactive medium, samples of culture media and centre-well contents were taken aseptically at times indicated. Portions of culture samples were immediately filtered through Millipore bacterial filters and the filters washed with fresh medium. Filters with retained micro-organisms were dried and attached to planchets for end-window counting of radioactivity by standard procedures. Portions of culture filtrates plus washings, and centre-well contents, were counted by liquid-scintillation techniques. Microbial growth was followed by measuring the light extinction of culture samples at 540 nm. (□). Radioactivity in medium (○), microbial material (△) and trapped propionaldehyde (□) is expressed as d.p.m. x 10⁻³/ml. of culture, corrected for counting efficiency differences. Results obtained with *Erwinia carotovora* are shown in part (a); those with *Achromobacter* p6 in part (b).

Whereas the Achromobacter species grown with an amino alcohol as the nitrogen and carbon source possessed a highly active aldehyde dehydrogenase system (see King & Cheldelin, 1956; Jakoby, 1958) with a pH optimum of 9-0, *Erwinia carotovora* did not. This lack accounts for the copious production of aldehydes observed during growth of the latter organism. The subsequent metabolism of propionate by *Achromobacter* p6 involved an acyl-CoA synthetase (Berg, 1956) which was more active with propionate than acetate.
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Each of the enzyme systems investigated was active with metabolites of both \( \text{1-aminopropan-2-ol} \) and ethanolamine, although whether single or separate and specific enzymes were involved was not clear in all cases. In general, aminopropanol and its derivatives served as better substrates than the corresponding ethanolamine compounds.

The metabolism of \( \text{1-aminopropan-2-ol} \) to propionaldehyde via the \( O \)-phospho derivative was a new finding. The metabolism of \( O \)-phosphoethanolamine via acetaldehyde has, however, recently been reported in mammalian tissues (Fleshood & Pitot, 1969; 1970) although it is still a novel reaction for a micro-organism. The distribution of the new amino alcohol phosphokinase and \( O \)-phosphoamino alcohol phospholyase enzymes in micro-organisms, their detailed properties, regulation and possible function in vitamin \( \text{B}_{12} \) and phospholipid metabolism, is currently under investigation in these laboratories.

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