Microbial Metabolism of Amino Alcohols

1-AMINOPROPAN-2-OL AND ETHANOLAMINE METABOLISM VIA PROPIONALDEHYDE AND ACETALDEHYDE IN A SPECIES OF *PSEUDOMONAS*

By A. JONES* and J. M. TURNER

Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

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1. Growth and manometric experiments showed that a *Pseudomonas* sp. P6 (N.C.I.B. 10431), formerly known as *Achromobacter* sp. P6, was capable of growth on both stereoisomers of 1-aminopropan-2-ol, and supported the hypothesis that assimilation involved metabolism to propionaldehyde, propionate and possibly 2-hydroxyglutarate. A number of alternative intermediary metabolites were ruled out. 2. Accumulation of propionaldehyde from 1-aminopropan-2-ol by intact cells occurred only during active growth, was transitory and was accompanied by morphological changes in the pseudomonad. 3. Enzymic and radioactive tracer evidence showed that 1-aminopropan-2-ol *O*-phosphate was the intermediate between amino alcohol and aldehyde. The operation of an inducibly formed ATP-amino alcohol phosphotransferase was established by measuring substrate disappearance, ADP formation and amino alcohol *O*-phosphate formation. This novel kinase had two activity peaks at about pH 7 and 9. It acted on both L- and D-isomers of 1-aminopropan-2-ol, and also on L-threonine and ethanolamine, but had only low activity towards choline. The enzyme was partially purified by ion-exchange chromatography. 4. An amino alcohol *O*-phosphate phospho-lyase (deaminating) produced propionaldehyde from DL- and D-1-aminopropan-2-ol *O*-phosphate, and also formed acetaldehyde less rapidly from ethanolamine *O*-phosphate. It had optimum activity at about pH 8 in Tris-HCl buffers. The enzyme was partially purified and evidence was obtained that a single enzyme was responsible for both activities. Apparent K_m values for the substrates were determined. Activity was inhibited by DL-threonine *O*-phosphate, DL-serine *O*-phosphate, choline *O*-phosphate and P_i . Enzyme formation was induced by growth with either amino alcohol substrate. 5. Radioactive tracer experiments with DL-1-amino[3- ^{14}C]propan-2-ol confirmed the operation of the amino alcohol kinase and demonstrated coupling with the phospho-lyase enzyme *in vitro* to produce [^{14}C]propionaldehyde. 6. An aldehyde dehydrogenase, found in extracts of the pseudomonad after growth on 1-aminopropan-2-ol, was characterized and concluded to be responsible for propionaldehyde and acetaldehyde oxidation. The enzyme was inactive with methylglyoxal. 7. Propionate and acetate were concluded to be metabolized via propionyl-CoA and acetyl-CoA, and studies were made of a CoA ester synthase found in extracts. 8. Studies of a strain of *Pseudomonas putida* N.C.I.B. 10558 suggested that 1-aminopropan-2-ols were metabolized via their *O*-phosphates, propionaldehyde and propionate. Amino alcohol kinase activity was detected and extracts contained a phospho-lyase showing higher activity with the 1-aminopropan-2-ol *O*-phosphate than with ethanolamine *O*-phosphate.

D-1-Aminopropan-2-ol occurs in Nature as a fragment of the vitamin B₁₂ molecule. It is known that both the nitrogen atom (Krasna *et al.*, 1957) and the carbon skeleton (Lowe & Turner, 1970b) are derived from L-threonine. The amino alcohol also occurs free in a variety of biological materials (Smith & Jepson, 1963; Van Rheen, 1963).

Previous studies suggested that 1-aminopropan-2-ol may be degraded via aminoacetone by *Pseudomonas*

* Present address: Department of Microbiology, University of Alberta, Edmonton 7, Alberta, Canada.

sp. N.C.I.B. 8858 (Higgins *et al.*, 1968), although the only oxidoreductase found was stereospecific for the L-isomer (Pickard *et al.*, 1968). An oxidoreductase capable of acting on the D-isomer, as found in *Escherichia coli* (Lowe & Turner, 1970a), could not be detected, although both isomers of the amino alcohol were utilized during growth of the pseudomonad. In contrast, it was shown that DL-1-aminopropan-2-ol was completely metabolized to propionaldehyde by *Erwinia carotovora* growing with the amino alcohol as a source of nitrogen. Ethanolamine gave rise to

acetaldehyde (Jones & Turner, 1971). No evidence that aminoacetone was an intermediate in propionaldehyde formation by the *Erwinia* sp. could be obtained.

A second species of *Pseudomonas*, originally isolated by Gottlieb & Mandel (1959) for studies of vitamin B₁₂ degradation and believed to be an *Achromobacter* sp., was also known to be capable of growth on DL-1-aminopropan-2-ol. The catabolic routes for each stereoisomer of the amino alcohol by this micro-organism and the possible roles of aminoacetone and propionaldehyde are the subjects of the present paper.

Methods

Growth of micro-organisms

Media. The pseudomonad used in most experiments was originally isolated by Gottlieb & Mandel (1959), who tentatively identified it as a species of *Achromobacter* (strain P6). A culture of the micro-organism, a generous gift from Dr. Manley Mandel, was deposited with the National Collection of Industrial Bacteria (N.C.I.B.), Aberdeen, U.K., and given the accession number N.C.I.B. 10431. Subsequently, as a result of electron microscopy and other studies, the microbe was identified as a pseudomonad (Dr. J. M. Shewan, personal communication), and is referred to as *Pseudomonas* sp. P6. The G+C content in the DNA of the pseudomonad was 59.7mol/100mol, based on the mean density in CsCl of 1.7185g/cm³ (Dr. M. Mandel, personal communication).

The strain of *Pseudomonas putida* used was the strain TG2 described by Morris (1969), originally isolated by serial cultivation in L-threonine medium. This micro-organism is available as N.C.I.B. 10559.

Pseudomonads were maintained and cultivated as described for *Pseudomonas* sp. N.C.I.B. 8858 by Higgins *et al.* (1968). Simple basal salts media contained (per l): 3.5g of K₂HPO₄, 1.5g of KH₂PO₄, 1.0g of Na₂SO₄, 0.2g of MgSO₄·7H₂O, 1g of (NH₄)₂SO₄ and 3g of the major carbon source. When the carbon compound also supplied nitrogen, 1.0g of Na₂SO₄ replaced the (NH₄)₂SO₄. Synthetic media were adjusted to pH7.0 before sterilization. Unless otherwise stated, DL-1-aminopropan-2-ol served as the sole source of carbon and nitrogen in media.

Growth in liquid media was measured turbidimetrically as $E_{540}^{1\text{cm}}$ in a Hilger and Watts Spectrophotometer. A calibration curve showed that a microbial density of 0.23mg dry wt. of organisms/ml gave $E_{540}^{1\text{cm}}$ 1.0.

Detection and isolation of volatile aldehydes formed. Conical flasks (250ml), each equipped with a cylindrical centre well (6cm high × 2.1cm internal diam.) and sealed with a Suba-Seal silicone rubber closure,

were used for routine comparative purposes. Growth medium (50ml) was placed in the outer compartment of such flasks, which were plugged with cotton-wool and sterilized by autoclaving. Similarly, sterilized carbonyl-trapping reagent (2,4-dinitrophenylhydrazine or semicarbazide) was placed in the centre well and the growth medium was inoculated with a loopful of the appropriately cultured micro-organism. The cottonwool plug was replaced aseptically with a sterile Suba-Seal closure and the flask was incubated with shaking at 30°C.

Volatile aldehyde formation was detected and assayed semi-quantitatively with 2,4-dinitrophenylhydrazine (5ml of 0.1% solution in 2M-HCl) as the carbonyl-trapping agent. Relative amounts of precipitated hydrazone were scored visually.

A modification of the above procedures was used for trapping volatile aldehydes formed under highly aerobic conditions. This involved passing the airstream from an otherwise sealed culture vessel through a condensation trap into a Dreschel bottle containing 2,4-dinitrophenylhydrazine reagent. The component vessels of the train were sterilized separately and assembled aseptically.

Biologically formed aldehyde was identified by analysis of the 2,4-dinitrophenylhydrazone obtained by filtration and then recrystallized twice from aqueous ethanol.

Quantitative measurement of aldehyde formation was based on the method of Burbridge *et al.* (1950), with 5ml of 0.2M-semicarbazide in 0.1M-potassium phosphate buffer, pH7, as the carbonyl-trapping reagent in place of 2,4-dinitrophenylhydrazine. Samples containing up to 2μmol of aldehyde were appropriately diluted and the E_{226} values read against a reagent blank. The calibration curve was linear for 50–1500nmol of propionaldehyde in a final volume of 10ml, and the molar extinction coefficient $\epsilon = 5.74 \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$ was used to calculate aldehyde concentration.

Measurement of O₂ uptake by washed bacterial suspensions. This was done manometrically with conventional equipment and procedures (Umbreit *et al.*, 1964). Incubation mixtures in Warburg flasks contained 100μmol of potassium phosphate buffer, pH7, 30μmol of substrate and 1.0–1.5mg dry wt. of bacteria in a final volume of 1.8ml. Manometer readings were taken at 10min intervals over a period of not less than 90min.

Distribution of radioactivity from DL-1-amino-[3-¹⁴C]propan-2-ol. Radioactivity in bacteria, culture filtrates and trapped propionaldehyde was measured as described by Jones & Turner (1971).

Enzyme extracts and enzyme assays

Extracts. Bacteria were harvested in the exponential growth phase and washed, and extracts were pre-

pared as previously described for *Escherichia coli* and *Pseudomonas* sp. N.C.I.B. 8858 (Turner, 1966; Pickard *et al.*, 1968).

Enzyme assays. These were carried out at 37°C unless otherwise stated.

(a) Amino alcohol *O*-phosphate phospho-lyase (deaminating). Aldehyde formation was measured either enzymically (Fleishood & Pitot, 1970) with alcohol dehydrogenase or colorimetrically with *N*-methylbenzothiazolone hydrazone (Paz *et al.*, 1965). In the former case incubation mixtures contained 100 μ mol of Tris-HCl buffer, pH 7.8, 10 μ mol of amino alcohol *O*-phosphate, 0.4 μ mol of pyridoxal phosphate, 0.01–1.0 mg of protein, 20 units of yeast alcohol dehydrogenase (C. F. Boehringer und Soehne G.m.b.H., Mannheim, West Germany) and 0.4 μ mol of NADH, in a total volume of 1 ml. Controls lacked either substrate or NADH, and the reaction was started by the addition of extract to both cuvettes. Changes in E_{340} were followed continuously. Initial rates were calculated by using the molar extinction coefficient for NADH $\epsilon = 6.22 \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$ (Horecker & Kornberg, 1948).

For colorimetric assays, incubation mixtures were as above except that alcohol dehydrogenase and NADH were omitted. Morpholinopropanesulphonic acid-HCl or Tris-HCl buffers prepared from specially purified Tris (Trizma grade from Sigma Chemical Co., St. Louis, Mo., U.S.A.) were used in later experiments to avoid the interference with development of colour found with some batches of Tris. Controls lacked substrate and incubations were done in a total volume of 1 ml, usually for 10 min. Reactions were stopped by the addition of an equal volume of aldehyde reagent (0.4% *N*-methylbenzothiazolone hydrazone in 0.1 M-3,3-dimethylglutaric acid-NaOH buffer, pH 4). The reaction mixtures were then heated for 3 min at 100°C, or for 30 min at 37°C, and cooled. To 1 ml samples were added 2.5 ml volumes of 0.2% FeCl_3 solution. The colour was allowed to develop for 5 min and then 6.5 ml of acetone was added to each and E_{670} values were read. Molar extinction coefficients of $\epsilon = 4.6 \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$ and $6.0 \times 10^3 \text{ M}^{-1} \cdot \text{cm}^{-1}$ were used to calculate concentrations of propionaldehyde and acetaldehyde respectively.

(b) ATP-amino alcohol phosphotransferase (amino alcohol kinase). Activity was assayed by measuring the formation of either ADP or amino alcohol *O*-phosphate.

In the former case activity was coupled via added pyruvate kinase and lactate dehydrogenase to NADH oxidation. Incubation mixtures contained 100 μ mol of Tris-HCl buffer, pH 7.8, 10 μ mol of ATP, 40 μ mol of MgCl_2 , 5 units of pyruvate kinase (Boehringer), 0.5 μ mol of phosphoenolpyruvate, 0.75 μ mol of NADH, 10 μ mol of amino alcohol, 2 μ mol of KCl, 6 units of lactate dehydrogenase (Boehringer) and

0.2–1.0 mg of cell-free-extract protein, in a total volume of 1 ml. Some preincubation of the first five components was desirable to eliminate traces of ADP in ATP preparations. The amino alcohol was omitted from controls, and reactions were started by the addition of extract. The coupled assay system was tested by observing the effect of added ADP on the E_{340} value due to NADH.

The second method of assay for kinase activity depends on the measurement of amino alcohol *O*-phosphate formation by linkage with amino alcohol *O*-phosphate phospho-lyase and alcohol dehydrogenase. Reaction mixtures contained 50 μ mol of Tris-HCl buffer, pH 7.8, 10 μ mol of ATP, 20 μ mol of MgSO_4 , 5 μ mol of KCl, 10 μ mol of amino alcohol, 0.4 μ mol of pyridoxal phosphate, 0.1–1.0 mg of extract protein, 20 units of yeast alcohol dehydrogenase and 0.4 μ mol of NADH, in a total volume of 1 ml. In some cases partially purified preparations of phospho-lyase were added to column fractions containing kinase activity (see Fig. 1). For routine purposes, however, kinase activity was assayed colorimetrically by a procedure utilizing endogenous or added phospho-lyase and the *N*-methylbenzothiazolone hydrazone method for aldehyde measurement. Alcohol dehydrogenase and NADH were omitted in this case.

Amino alcohol kinase activity was also demonstrated by measuring substrate disappearance. Incubation mixtures containing buffer, substrate, ATP, MgCl_2 , KCl and extract (as above) were stopped after incubation by the addition of an equal volume of 1 M-HCl. After removal of precipitated protein, 0.1 ml samples of supernatant were made up to 1 ml, added to 1 ml of 2,4-dinitrobenzenesulphonate reagent (a filtered 0.24 M solution in saturated borax) and heated in stoppered tubes at 100°C for 40 min. After cooling, the mixture was extracted with 4 ml of benzene and the E_{350} value of the benzene extract measured against a reagent blank (Smith & Jepson, 1967). A calibration curve was linear over the range 0–400 nmol of 1-aminopropan-2-ol and gave a molar extinction coefficient $\epsilon = 1.22 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$. 1-Aminopropan-2-ol *O*-phosphate, in amounts up to 400 nmol/sample, did not interfere with the assay.

(c) Aldehyde dehydrogenase. Activity was assayed spectrophotometrically by measuring NAD^+ reduction (King & Cheldelin, 1956; Jakoby, 1958). Incubation mixtures contained 375 μ mol of diethanolamine-HCl buffer, pH 9.1, 4 μ mol of NAD^+ , 0.5 μ mol of aldehyde and 0.2–1.0 mg of extract protein in a total volume of 3 ml. Substrate or cofactor was omitted from controls and reactions were usually started by the addition of extract. The E_{340} value due to NADH formation was followed.

(d) Acyl-CoA synthetase. Activity was assayed by a colorimetric method, essentially that described by Jones *et al.* (1953). Reaction mixtures contained

100 μ mol of potassium phosphate buffer, pH 7.5, 0.1 μ mol of CoA, 10 μ mol of ATP, 10 μ mol of $MgCl_2$, 50 μ mol of KF, 10 μ mol of GSH, 20 μ mol of carboxylic acid, 20 μ mol of neutral hydroxylamine and 1–2 mg of extract protein in a total volume of 1 ml. After incubation 2 ml of acid $FeCl_3$ reagent [5% (w/v) $FeCl_3$, 12% (w/v) trichloroacetic acid and 3M-HCl, in equal volumes] was added. After centrifugation, the E_{540} value was measured. A molar extinction coefficient of $\epsilon = 5.3 \times 10^2 M^{-1} \cdot cm^{-1}$ was used. The colour produced was linear for up to 4 μ mol of succinohydroxamic acid in samples.

(e) Propionyl-CoA carboxylase. The radioisotopic method of Tietz & Ochoa (1959) was used. The propionate-dependent fixation of $^{14}CO_2$ into acid-soluble material was followed. Reaction mixtures had a volume of 0.5 ml and were stopped with 0.2 ml of ethanol–acetic acid (1:1, v/v) (Olsen & Merrick, 1968).

(f) 2-Hydroxyglutarate synthase. This was assayed by a modification of the method of Wegener *et al.* (1965) and depended on the measurement of disappearance of [^{14}C]glyoxylate. Glyoxylate *p*-nitrophenylhydrazone was separated from other deriva-

tives by t.l.c. on silica gel in the solvent system butan-1-ol–water–ethanol (4:1:1, by vol.). The radioactivity of the material removed from chromatograms was counted by scintillation techniques.

(g) Other enzyme assays. Standard methods were used to assay L-threonine dehydratase (Datta, 1966), 1-aminopropan-2-ol dehydrogenase (Pickard *et al.*, 1968), ethanolamine deaminase (Kaplan & Stadtman, 1968a), ethanolamine oxidase (Narrod & Jakoby, 1966) and diol dehydratase (Lee & Abeles, 1964). For the last-named, extracts were prepared as described by Abeles (1966). Acetate kinase and phosphotransacetylase were assayed by the methods of Hodgson & McGarry (1968).

Separation of amino alcohol kinase and amino alcohol O-phosphate phospho-lyase activities. Crude extracts were prepared in 0.05M Tris–HCl buffer, pH 7.5, containing 10mM- $MgSO_4$. Approx. 5 ml of extract, containing 80mg of protein, was applied to a column of Whatman DE 52 DEAE-cellulose (15 cm $\times$ 1.5 cm diam.) equilibrated with Tris–HCl– $MgSO_4$ mixture. Protein was eluted with an increasing concentration gradient of 0–0.5M-NaCl over a total volume of 500 ml, and 5 ml fractions of eluate were

Fig. 1. Separation of *Pseudomonas sp. P6* enzymes by ion-exchange chromatography

Extract was chromatographed on DEAE-cellulose and enzymes were located in eluate fractions as described in the Methods section. Protein was detected automatically by measuring light-absorption at 253.7 nm (○) with an LKB Uvicord I Ultraviolet Absorptiometer. The activities of DL-1-aminopropan-2-ol kinase (●) and DL-1-aminopropan-2-ol O-phosphate phospho-lyase (▲) were measured semi-quantitatively, by a colorimetric assay, and are expressed arbitrarily as E_{670}^{15m}/ml of eluate. Peak-activity fractions were used for quantitative measurements of enzyme activity as described in the text. The linear concentration gradient of NaCl (□) used to elute protein is indicated.

collected. The presence of DL-1-aminopropan-2-ol *O*-phosphate phospho-lyase in fractions was determined initially, by the colorimetric assay method. Peak-activity samples of phospho-lyase were then used analytically to locate kinase activity by coupling of the two enzymes and colorimetric assay of the aldehyde produced. The results of anion-exchange chromatography are shown in Fig. 1. Specific-activity measurements showed approx. 10- and 20-fold purification of the kinase and phospho-lyase activities respectively.

Chemical methods

Aldehyde formation by cell-free extracts. The formation of volatile aldehydes by enzyme systems was detected by a procedure similar to that described for growing cultures (see above). Reaction mixtures were placed in the outer well of two-compartment flasks with carbonyl-trapping reagents in the centre well.

Characterization of 2,4-dinitrophenylhydrazones. Hydrazone derivatives of both authentic and biologically formed aldehydes were examined by melting-point analysis, mass spectrometry with an AEI MS-12 instrument, and by u.v.- and visible-light spectrophotometry of solutions in spectroscopically pure ethanol. T.l.c. on silica gel was done in two dimensions with light petroleum (b.p. 60–80°C)–ether (3:4, v/v) (solvent system I) and benzene–tetrahydrofuran (93:7, v/v) (solvent system II) as solvents (Byrne, 1965).

Chemicals. Optically active 1-aminopropan-2-ols were prepared either by resolution of the racemic compound (Sullivan, 1963; see Turner, 1967) or by thermal decarboxylation of L- or D-threonine (Chatelus, 1964; see Lowe & Turner, 1970b). DL-1-Amino[3-¹⁴C]propan-2-ol was a generous gift from Pfizer Ltd. (Sandwich, Kent, U.K.). 1-Aminopropan-2-ol *O*-phosphates were prepared by the method of Cooley *et al.* (1953). Aminoacetone hydrochloride was either synthesized by the method of Gabriel & Colman (1902) or prepared from its commercially available derivative 1-aminopropan-2-one semicarbazide hydrochloride (Eastman-Kodak Co., Rochester, N.Y., U.K.). All other chemicals were from commercial sources.

Results

Growth and manometric experiments

Growth of *Pseudomonas* sp. P6 (N.C.I.B. 10431) on DL-1-aminopropan-2-ol (Gottlieb & Mandel, 1959) was suspected to involve propionaldehyde as a catabolic intermediate. A strong aldehydic odour was noted during growth on the amino alcohol, and *Erwinia carotovora* had been found to convert DL-1-

aminopropan-2-ol into propionaldehyde quantitatively during growth on glycerol as the carbon source (Jones & Turner, 1971). The route whereby the amino alcohol was metabolized to aldehyde was therefore of interest.

The results of Gottlieb & Mandel (1959) on the use of alternative growth substrates by *Pseudomonas* sp. P6 were extended. Good growth of the pseudomonad occurred on citrate, succinate, lactate, glycerol and pyruvate, with DL-1-aminopropan-2-ol, ethanolamine or (NH₄)₂SO₄ as nitrogen sources. Growth densities were virtually the same with each nitrogen source when their concentrations were growth-limiting. In separate experiments, deamination of L-threonine, L-serine, 3-aminopropan-1-ol and DL-1,3-diaminopropan-2-ol permitted moderate growth on glycerol or pyruvate as the carbon source. Slow growth with *n*-propylamine, used as source of carbon and nitrogen or as nitrogen source with glycerol as carbon source, occurred after a 48 h lag. Of the other possible metabolites of 1-aminopropan-2-ol tested, DL-propane-1,2-diol, with (NH₄)₂SO₄ as nitrogen source, supported growth only after a 24 h lag, and allylamine was inactive as a source of carbon or nitrogen. The suspicion that 1-aminopropan-2-ol assimilation involved deamination, to yield propionaldehyde and then propionate, was supported by the fact that these compounds readily served as carbon sources for growth. Relatively low concentrations (30 mM) were required for optimum growth rates, and with propionate small amounts of HCO₃⁻ (3 mM) and vitamin B₁₂ (0.3 mg/l) shortened the lag period and stimulated growth rates. In contrast, HCO₃⁻ prolonged the lag before growth on DL-1-aminopropan-2-ol. Of the possible metabolites of propionate tested, L-2-hydroxyglutarate (5–25 mM) supported growth comparable with that on propionate (20 mM) and DL-1-aminopropan-2-ol (10–40 mM). Methylmalonate, malonate, acrylate and 3-hydroxypropionate did not support growth with (NH₄)₂SO₄ as the nitrogen source. Growth of the pseudomonad on the D-isomer of 1-aminopropan-2-ol was rapid compared with growth on the L-stereoisomer (Fig. 2). Experiments with DL-1-amino[3-¹⁴C]propan-2-ol confirmed the evidence from growth experiments that both stereoisomers were completely utilized during growth (see Fig. 3).

Washed suspensions of the micro-organism grown on DL-1-aminopropan-2-ol rapidly oxidized racemic, D- and L- preparations of the substrate. Similar results were obtained after growth on the individual stereoisomers (Table 1). In all cases propionate was rapidly oxidized. Aminoacetone, known to be a product of L-1-aminopropan-2-ol metabolism by *Pseudomonas* sp. N.C.I.B. 8858 growing on this compound (Higgins *et al.*, 1968), was not oxidized by *Pseudomonas* sp. P6. *n*-Propylamine was oxidized by *Pseudomonas* sp. P6 only after growth on this substrate, but

Fig. 2. Growth of *Pseudomonas* sp. P6 on racemic, D- and L-1-aminopropan-2-ol

Growth was on mineral salts medium containing the DL-isomer (Δ), D-isomer ($\square$) or L-isomer ($\circ$) of 1-aminopropan-2-ol as sole source of carbon and nitrogen. Samples of media were removed at the times indicated and cell density was measured as described in the Methods section.

Fig. 3. Fate of DL-1-amino[3- 14 C]propan-2-ol during growth of *Pseudomonas* sp. P6

DL-1-Aminopropan-2-ol + mineral salts medium, containing $0.42 \mu\text{Ci}$ of DL-1-amino[3- 14 C]propan-2-ol in 50 ml, was placed in the outer well of a 250 ml conical flask fitted with a centre well. The latter contained 5 ml of 0.2 M-semicarbazide-HCl in 0.1 M-phosphate buffer, pH 7, to trap propionaldehyde. Growth of the pseudomonad ($\bullet$), and radioactivity in medium ($\circ$), in microbial material ($\blacktriangle$) and in trapped propionaldehyde ($\square$), were measured as described previously (Jones & Turner, 1971).

not after growth on DL-1-aminopropan-2-ol; *n*-propylamine-grown organisms oxidized propionate as rapidly as *n*-propylamine. It thus appeared that *n*-propylamine was not a product of 1-aminopropan-2-ol catabolism, but that both were metabolized via propionate. Allylamine was not oxidized. L-2-Hydroxyglutarate was oxidized at a significant rate by washed suspensions of the micro-organism grown on DL-1-aminopropan-2-ol or on propionate with $(\text{NH}_4)_2\text{SO}_4$ (Table 1). Acrylate, 3-hydroxypropionate and malonate were not oxidized in either case.

Aldehyde accumulation during growth

An early finding was that propionaldehyde formation from 1-aminopropan-2-ol occurred only during growth of the pseudomonad. Washed suspensions were incapable of forming detectable amounts of aldehyde when incubated with the amino alcohol in phosphate buffer at the same concentration and pH as in growth media. The addition of alternative energy sources, variations of pH and the application of highly sensitive methods for aldehyde detection failed to reveal aldehyde formation.

Propionaldehyde accumulation during growth was detected by using sealed culture flasks, each equipped with a centre well containing 2,4-dinitrophenylhydrazine or semicarbazide reagent (see the Methods section). The identity of the aldehyde trapped in such experiments was established by examination of its 2,4-dinitrophenylhydrazone, together with those of authentic propionaldehyde, acetaldehyde, acetone and lactaldehyde. Melting-point analysis, t.l.c. in two solvent systems, visible-light spectroscopy and mass spectrometry all indicated the identity of the aldehyde formed from DL-1-aminopropan-2-ol with propionaldehyde. Experimental details are given in the Methods section, and the results in Table 2(a). Propionaldehyde accumulated only during growth of the pseudomonad on DL- or L-1-aminopropan-2-ol. None accumulated during growth on the D-isomer. The addition of $(\text{NH}_4)_2\text{SO}_4$, or D-1-aminopropan-2-ol, to cultures growing on the racemic or L-amino alcohol did not prevent propionaldehyde formation. Kinetic studies of growth on DL-1-amino[3- 14 C]propan-2-ol medium gave results indicating significant production of ^{14}C -labelled propionaldehyde during the incorporation of radioisotope into bacterial substance (Fig. 3). These results were complicated by the fact that the rate of 'trapping' of the propionaldehyde produced was slow and measurement of residual aldehyde in sealed vessels showed that propionaldehyde accumulation was transitory during growth on either DL- or L-1-aminopropan-2-ol (see Fig. 4). It should be noted that not all cultures of *Pseudomonas* sp. P6 accumulated aldehyde. Accumulation was favoured by high initial concentrations of DL-1-aminopropan-2-ol (2.5 g/l) and by high aeration.

Table 1. *Oxidation of possible catabolites of DL-1-aminopropan-2-ol by washed suspensions of Pseudomonas sp. P6 grown on different substrates*

Pseudomonas sp. P6 was grown on the substrates listed, in media otherwise described in the Methods section. Uptake of O₂ was measured by normal manometric procedures. Centre wells contained 720 μmol of KOH and the outer wells 100 μmol of potassium phosphate buffer, pH 7.0, 30 μmol of substrate and 1.0–1.5 mg dry wt. of bacteria in a final volume of 1.8 ml. Readings were taken at 10 min intervals over a period of not less than 90 min. Initial oxidation rates were corrected for endogenous respiration, which was approx. 0.40–0.60 μmol of O₂ absorbed/h per mg dry wt. n.d., Not determined.

		O ₂ absorbed (μmol/h per mg dry wt. of bacteria)				
	Growth substrate	...	DL-1-Aminopropan-2-ol	D-1-Aminopropan-2-ol	L-1-Aminopropan-2-ol	(NH ₄) ₂ SO ₄ + pyruvate
Oxidation substrate						
Expt. A						
	DL-1-Aminopropan-2-ol		18.6	12.6	17.7	0.98
	L-1-Aminopropan-2-ol		20.3	14.3	18.9	0.98
	D-1-Aminopropan-2-ol		12.2	9.43	17.7	0.41
	Aminoacetone		0.45	0.00	2.88	1.96
	D-Lactate		3.93	5.17	2.51	0.98
	Propionate		8.53	8.80	12.7	0.98
	Pyruvate		9.67	8.09	10.05	5.88
	L-Lactate		0.00	6.22	1.04	0.98
	Allylamine		0.00	0.50	n.d.	0.00
	n-Propylamine		0.50	0.60	0.40	0.00
Expt. B						
	DL-1-Aminopropan-2-ol		21.10 (4.15)*	n.d.	n.d.	n.d.
	Propionate		7.65 (22.4)*	n.d.	n.d.	n.d.
	L-2-Hydroxyglutarate		1.70*(2.17)*	n.d.	n.d.	n.d.

* Values in parentheses were obtained after growth on propionate+(NH₄)₂SO₄ medium.

Table 2. *Identification of aldehyde formed by growing cultures and cell-free extracts of Pseudomonas sp. P6*

The pseudomonad was grown on DL-1-aminopropan-2-ol + mineral salts medium for each experiment. With growing cultures the aldehyde was trapped as its 2,4-dinitrophenylhydrazone as described in the Methods section. With cell-free extracts 3 ml of standard incubation mixture used for the colorimetric assay of phospho-lyase activity (see the Methods section) was placed in a 25 ml conical flask equipped with a centre well containing 1 ml of the semicarbazide reagent. After 2 h incubation the semicarbazone was converted into the 2,4-dinitrophenylhydrazone, which was then extracted into ethyl acetate. In both cases the derivative was recrystallized and characterized as described in the Methods section. Derivatives of authentic compounds were examined for comparison. n.d., Not determined.

Aldehyde	Melting point (°C)	Molecular ion (<i>M</i> ⁺)	Chromatographic <i>R_F</i>		Spectroscopic λ _{max} . (nm)	
			Solvent I	Solvent II	No addition	KOH (1%, w/v) added
(a) Growth experiment						
Growth product*	154–156	238	0.38	0.58	359	520
Propionaldehyde*	155–156	238	0.37	0.59	359	520
Acetaldehyde	144–147	224	0.27	0.53	358	520
Acetone	131–132	224	0.32	0.54	351	532
Lactaldehyde	306–309	474	0	0.32	370	565
(b) Cell-free experiment						
Enzyme product	n.d.	238	n.d.	0.74	357	520
Propionaldehyde	155–156	238	n.d.	0.74	356	520

* Mixed m.p. 154–156°C.

Fig. 4. Enzymic formation and utilization of propionaldehyde during growth of *Pseudomonas* sp. P6 on DL-1-aminopropan-2-ol

Samples of cultures growing on DL-1-aminopropan-2-ol + mineral salts medium were removed at the times shown for measurement of growth (Δ) and propionaldehyde ($\blacktriangle$) by standard methods. Bacteria were then harvested, cell-free extracts were prepared and the activities of DL-1-aminopropan-2-ol *O*-phosphate phospho-lyase ($\circ$) and propionaldehyde dehydrogenase ($\bullet$) were measured by spectrophotometric procedures as described in the Methods section. Enzyme activities are expressed as nmol of NADH formed/min per mg of protein.

Even so, aldehyde accumulation was erratic and appeared to depend on a fine balance between rates of production and utilization.

Growth of the pseudomonad on DL-1-aminopropan-2-ol was accompanied by morphological changes related to aldehyde accumulation. When the propionaldehyde content of the medium increased, the micro-organism elongated severalfold to give filaments up to $10\mu\text{m}$ in length. Growth on propionaldehyde itself (1.5g/l) yielded similarly elongated organisms. No propionaldehyde accumulated in the medium during growth of the pseudomonad on *n*-propylamine.

Enzymic evidence for the role of propionaldehyde in 1-aminopropan-2-ol metabolism

Consideration of possible routes from 1-aminopropan-2-ol to propionaldehyde led to a search in cell-free extracts for enzymes capable of producing

aldehydes. Activities analogous to ethanolamine deaminase, diol dehydratase and L-threonine dehydratase could not be detected by standard assay procedures (see the Methods section) in extracts of the pseudomonad grown on DL-1-aminopropan-2-ol. In contrast, an enzyme analogous to 'O-phosphoryl-ethanolamine' phospho-lyase (Fleishood & Pitot, 1969, 1970) was highly active. A novel amino alcohol kinase, capable of forming the substrate of the phospho-lyase, and an aldehyde dehydrogenase capable of oxidizing the product were also found. The roles of these and other enzymes in 1-aminopropan-2-ol metabolism are shown in Scheme 1. Their properties are described below.

ATP-amino alcohol phosphotransferase. 1-Aminopropan-2-ol kinase activity was demonstrated by a variety of methods (see the Methods section). Colorimetric measurement of disappearance of amino alcohol from reaction mixtures with 2,4-dinitrobenzenesulphonic acid reagent (Smith &

Scheme 1. Pathway of metabolism of 1-aminopropan-2-ol in *Pseudomonas* sp. P6

Key to enzymes: (1) ATP-amino alcohol phosphotransferase (kinase); (2) amino alcohol *O*-phosphate phospho-lyase; (3) aldehyde dehydrogenase (EC 1.2.1.3); (4) acyl-CoA synthetase (EC 6.2.1.1).

Jepson, 1967) showed dependence on ATP and Mg^{2+} . When ADP formation was detected spectrophotometrically by coupling via added pyruvate kinase and lactate dehydrogenase, NADH oxidation depended on the addition of 1-aminopropan-2-ol, Mg^{2+} , ATP and phosphoenolpyruvate. Cysteine increased kinase activity by about 25%. A third detection method for the kinase involved coupling with endogenous amino alcohol *O*-phosphate phospho-lyase. The propionaldehyde thus formed was measured either colorimetrically with *N*-methylbenzothiazolone hydrazone or spectrophotometrically by following NADH oxidation in the presence of added alcohol dehydrogenase. In all cases activity was proportional to extract concentration and incubation time. Full details of assay conditions are given in the Methods section.

Rather than a single pH optimum at pH 7.8 (Jones & Turner, 1971), detailed studies showed two pH-activity peaks at approx. pH 7.0 and 9.0, irrespective of the assay method used (Fig. 5). Similar biphasic curves have been observed with the enzyme from other pseudomonads (A. Faulkner & J. M. Turner, unpublished work). Substrate-specificity studies, with the spectrophotometric assay system for ADP formation, showed that DL-1-aminopropan-2-ol (100%) and D-1-aminopropan-2-ol (92%) were more active than ethanolamine (26%) or choline (10%), but that L-threonine (86%) had relatively high activity. In a separate experiment, where propionaldehyde formation was assayed spectrophotometrically, the relative activities found were 100% for DL-1-aminopropan-2-ol, 59% for optically pure D-isomer and 115% for partially resolved L-isomer. The addition of L-threonine at 50 mM did not inhibit aldehyde formation from the racemic amino alcohol. Specific activities of crude extracts in the range 100–200 nmol/min per mg of protein at 37°C were found with each assay method under optimum conditions. Crude extracts lost 50% and 93% of their kinase activity after storage at –20°C for 16h and 40h respectively. Anion-exchange chromatography on DEAE-cellulose resulted in the partial purification of the kinase, although recoveries were low (see Fig. 1). No kinase activity could be detected in extracts of *Pseudomonas* sp. P6 grown on succinate- NH_4^+ medium.

Fig. 5. Effect of pH on the activity of *Pseudomonas* sp. P6 DL-1-aminopropan-2-ol

Enzyme activity was measured colorimetrically by following the disappearance of DL-1-aminopropan-2-ol (●) or spectrophotometrically by coupling ADP formation via pyruvate kinase and lactate dehydrogenase to NADH oxidation (○) as described in the Methods section. The two curves were obtained on different occasions. The buffers used were 0.5M-3,3'-dimethylglutaric acid-NaOH at pH 6.0–7.4, 0.5M-Tris-HCl at pH 7.5–8.4, 0.5M-diethanolamine-HCl at pH 8.5–10.0 and 0.5M-glycine-NaOH at pH 10.5. Points on the margins of these ranges were obtained by using mixed buffers.

Additional radioisotopic evidence for the operation of DL-1-aminopropan-2-ol kinase in crude extracts of the pseudomonad grown on the amino alcohol is given below.

Amino alcohol O-phosphate phospho-lyase (deaminating). Phospho-lyase activity towards DL-1-aminopropan-2-ol *O*-phosphate was detected enzymically in extracts by the method of Fleshood & Pitot (1970), but with the substrate of interest being used. A modification in which aldehyde formation was measured colorimetrically was also developed (see the Methods section). The identity of the enzymic product as propionaldehyde was concluded from examination of the 2,4-dinitrophenylhydrazone by t.l.c., visible-light spectroscopy and mass spectrometry (see Table 2b).

A sharp pH optimum at about pH 8 was found in Tris-HCl buffer. In Trizma-HCl buffer the optimum was later found to be at pH 8.5-9.0. Activity was low in phosphate buffer. The phospho-lyase activity of preparations partially purified by exclusion chromatography on Bio-Gel P-300 was increased 2.5-fold by adding pyridoxal phosphate (0.1 mM). Treatment with hydroxylamine followed by gel filtration yielded a completely resolved preparation, the activity of which was fully restored by addition of cofactor. Some additional properties of the enzyme are given in Table 3.

Substrate-specificity studies showed that DL-1-aminopropan-2-ol *O*-phosphate (100), D-1-aminopropan-2-ol *O*-phosphate (93), L-1-aminopropan-2-ol *O*-phosphate (113) and ethanolamine *O*-phosphate (30) were active at the relative rate values shown. The free bases, DL-serine *O*-phosphate, choline phosphate, DL-threonine *O*-phosphate and β -glycerophosphate were all inactive as substrates, but acted as inhibitors of activity towards DL-1-aminopropan-2-ol *O*-phosphate (Fig. 6). Inactive as inhibitors or activators were AMP, ADP, ATP, CTP and phospho-

enolpyruvate. The effect of metal ions on activity is shown in Table 4.

Phospho-lyase activity was some 20-fold greater in

Fig. 6. Effect of substrate analogues on the activity of *Pseudomonas* sp. P6 DL-1-aminopropan-2-ol *O*-phosphate phospho-lyase

Phospho-lyase activity was measured under optimum conditions by using the colorimetric assay procedure described in the Methods section. Reaction mixtures contained DL-choline *O*-phosphate (○), DL-threonine *O*-phosphate (■), DL-serine *O*-phosphate (△) or β -glycerophosphate (●) at the concentrations shown. In each case the concentration of DL-2-aminoisopropyl phosphate used was 10 mM.

Table 3. Some properties of amino alcohol *O*-phosphate phospho-lyases in *Pseudomonas* sp. P6 and *Ps. putida* TG2

Pseudomonads were grown on DL-1-aminopropan-2-ol + mineral salts medium and phospho-lyase activities in crude extracts of the bacteria were measured as described in the Methods section. Aldehyde production was measured enzymically for *Pseudomonas* sp. P6 and colorimetrically with *Ps. putida* TG2. n.d., Not determined.

Property	<i>Pseudomonas</i> sp. P6	<i>Ps. putida</i> TG2
pH optimum	7.8 (Tris-HCl) 8.5-9.0 (Trizma-HCl)	7.3 and 8.3
Apparent K_m values (mM)		
(i) DL-2-Aminopropan-2-ol <i>O</i> -phosphate	1.0	1.3*
(ii) Ethanolamine <i>O</i> -phosphate	1.5	2.0*
(iii) Pyridoxal phosphate	0.006	n.d.
K_i value for P_i (mM)	2.0	n.d.
Substrate specificity (relative activities)		
DL-1-Aminopropan-2-ol <i>O</i> -phosphate	100	100
D-1-Aminopropan-2-ol <i>O</i> -phosphate	93	92
L-1-Aminopropan-2-ol <i>O</i> -phosphate	113	100
Ethanolamine <i>O</i> -phosphate	30	20

* Substrate inhibition occurred above 5 mM.

Table 4. *Effects of cations on the amino alcohol O-phosphate phospho-lyase activity of Pseudomonas sp. P6*

DL-1-Aminopropan-2-ol *O*-phosphate phospho-lyase activity in crude extracts was measured colorimetrically as described in the Methods section, in the presence of the cations shown. Sulphate or chloride salts were used.

Cation added	Concn. of added cation	...	Relative activity	
			1mM	10mM
None			100	100
K ⁺			130	136
Na ⁺			130	152
Li ⁺			130	143
Rb ⁺			136	86
NH ₄ ⁺			98	73
Mn ²⁺			100	150
Mg ²⁺			87	176
Pb ²⁺			110	100
Zn ²⁺			107	44
Ca ²⁺			102	123
Co ²⁺			153	70
Cd ²⁺			102	40
Ag ⁺			20	18

extracts of the pseudomonad grown on DL-1-aminopropan-2-ol rather than succinate-NH₄⁺ medium (Table 5). Addition of NH₄⁺ or succinate or both to amino alcohol medium only partly repressed phospho-lyase biosynthesis. Growth on ethanolamine also resulted in enzyme formation. Growth on optically pure D-1-aminopropan-2-ol gave extracts with significantly high activity.

Specific activities varied according to the phase of growth when cultures were harvested (see Fig. 4). Extracts of micro-organisms grown on DL-1-aminopropan-2-ol and harvested in early exponential phase exhibited activities of 600–900 nmol of aldehyde formed/min per mg of protein. Overnight storage of crude extracts at either 0° or –20°C led to the loss of about 60% activity, unless 1.0 mM-dithiothreitol was added, when 50 and 0% was lost respectively. Overnight dialysis against 0.1 M-Tris-HCl buffer, pH 7.5, led to 78% activity loss. The addition of cysteine, EDTA, 2-mercaptoethanol or dithiothreitol (each at 0.1 mM) resulted in activity losses of 75, 57, 47 and 15% respectively.

The problem of whether activities towards DL-1-aminopropan-2-ol *O*-phosphate and ethanolamine *O*-phosphate were due to one or two enzymes was considered. With either substrate the pH optimum, *K_m* for substrate and relative inhibitory effects of substrate analogues were similar. Heat treatment of

crude extracts over the temperature range 20–50°C for 10 min resulted in 0–100% activity loss with each substrate. The ratio of activities remained essentially constant. Threefold purification of the enzyme by gel filtration in Bio-Gel P-300 or 18-fold purification by anion-exchange chromatography on DEAE-cellulose (Fig. 1) also had no effect on the activity ratio in any fraction. Growth of *Pseudomonas* sp. P6 on media containing ethanolamine rather than 1-aminopropan-2-ol also had no effect on the ratio of phospho-lyase activities (Table 5). It was concluded that a single amino alcohol *O*-phosphate phospho-lyase was responsible for both activities.

Phospho-lyase activity was also found in extracts of *Pseudomonas putida* N.C.I.B. 10558 (strain TG2 described by Morris, 1969) and some properties of the enzyme from this pseudomonad are shown in Table 3.

Evidence for the coupled action of the amino alcohol kinase and amino alcohol *O*-phosphate phospho-lyase *in vitro* is presented below.

Aldehyde dehydrogenase. Extracts of *Pseudomonas* sp. P6 grown on DL-1-aminopropan-2-ol medium possessed high propionaldehyde dehydrogenase activity. The enzyme was located entirely in the high-speed supernatant. Activity was followed spectrophotometrically, NAD⁺ reduction being dependent on the presence of enzyme plus aldehyde but unaffected by the addition of CoA.

Some properties of the dehydrogenase are given in Table 6. The activity was maximal in diethanolamine-HCl buffer but somewhat less so in Tris-HCl buffer, which inhibited markedly in the lower pH range relative to potassium phosphate buffer. Inhibition by excess of substrate was a marked feature of the enzyme. The cofactor for the enzyme was NAD⁺, there being no activity with NADP⁺. A number of univalent cations at 100 mM inhibited by the percentages shown: Li⁺, 64%; NH₄⁺, 64%; Rb⁺, 45%; Na⁺, 27%; K⁺, 10%; Cs⁺, 10%. Mg²⁺ inhibited by 42% and the anions H₂PO₄⁻ and HAsO₄²⁻ had no effect at the same concentration.

Substrate-specificity studies showed that only non-substituted aliphatic aldehydes were active (see Table 6); methylglyoxal, glyoxal, DL-lactaldehyde, glyceraldehyde, glyceraldehyde 3-phosphate and glyoxylate were inactive.

Aldehyde dehydrogenase activity in extracts differed markedly according to the growth phase (see Fig. 4). Variation of the activities of enzymes responsible for aldehyde formation and utilization explained the transient accumulation of propionaldehyde during growth. Propionaldehyde dehydrogenase activities in extracts of the pseudomonad harvested during exponential growth on different substrates are shown in Table 6. The enzyme was completely stable to dialysis for 16 h at 4°C against 0.1 M-Tris-HCl buffer, pH 7.5, in the presence of 1.0 mM-dithiothreitol.

Table 5. *Effect of growth conditions on the amino alcohol O-phosphate phospho-lyase activity of Pseudomonas sp. P6 and Ps. putida TG2*

The organisms were grown on the substrate(s) shown and extracts prepared as described in the Methods section. Phospho-lyase activities were assayed colorimetrically as described in the Methods section, except that for *Ps. putida* TG2 the substrate concentration was 5 mM. Expts. A and B were done on separate occasions. n.d., Not determined.

Growth substrate	Enzyme substrate	...	<i>Pseudomonas</i> sp. P6 phospho-lyase activity (nmol of NADH oxidized/min per mg of protein)		<i>Ps. putida</i> TG2 phospho-lyase activity (nmol of propionaldehyde formed/min per mg of protein)
			DL-1-Aminopropan-2-ol <i>O</i> -phosphate	Ethanolamine <i>O</i> -phosphate	DL-1-Aminopropan-2-ol <i>O</i> -phosphate
Expt. A					
	DL-1-Aminopropan-2-ol		627	217	2580
	DL-1-Aminopropan-2-ol + (NH ₄) ₂ SO ₄		835	n.d.	2900
	DL-1-Aminopropan-2-ol + (NH ₄) ₂ SO ₄ + succinate		373	n.d.	3200
	DL-1-Aminopropan-2-ol + succinate		450	n.d.	1390
	Succinate + (NH ₄)SO ₄		31	n.d.	0
	D-1-Aminopropan-2-ol		194	68	0
	Nutrient broth		18	n.d.	n.d.
Expt. B					
	Ethanolamine		261	88	n.d.
	Ethanolamine + (NH ₄) ₂ SO ₄		311	118	n.d.
	Ethanolamine + (NH ₄) ₂ SO ₄ + succinate		55	20	n.d.
	Ethanolamine + succinate		7	5	n.d.
	Succinate + (NH ₄) ₂ SO ₄		10	0	n.d.

Conversion of DL-1-amino[3-¹⁴C]propan-2-ol into its labelled O-phosphate derivative and propionaldehyde in vitro. The operation of the amino alcohol kinase in crude extracts was confirmed by incubation with 3-¹⁴C-labelled substrate under optimum conditions for product accumulation. Stopped-reaction mixtures were deproteinized, freeze-dried and extracted with ethanol to remove the bulk of the unused substrate, and the residual 1-aminopropan-2-ol O-phosphate was analysed by t.l.c. on cellulose with butan-1-ol-acetic acid-water (8:1:1, by vol.). Radioactive product, located by radioautography, was formed only when all reaction components were present. None was detected when ATP was omitted or when the extract was boiled. Pyridoxal phosphate prevented accumulation of the phosphorylated product, probably by stimulating its metabolism to propionaldehyde. P_i was also inhibitory, affecting kinase activity to a greater extent than that of the phospho-lyase.

Coupling of the kinase and phospho-lyase enzymes, already exploited for the assay of amino

alcohol kinase activity, was confirmed by radiotracer experiments. Incubation of DL-1-amino[3-¹⁴C]propan-2-ol with extract together with the appropriate cofactors under standard conditions and trapping the radioactive propionaldehyde as its 2,4-dinitrophenylhydrazone demonstrated coupling unambiguously. A conversion of 1.3% during a 2h incubation was calculated, on the basis of propionaldehyde formation, without making the incubation conditions fully optimum.

Examination of alternative pathways for 1-aminopropan-2-ol metabolism

A role for aminoacetone, which appeared unlikely from growth and manometry experiments, was ruled out by additional work. Inhibitors of 1-aminopropan-2-ol metabolism, which markedly stimulated aminoacetone accumulation by cultures of some pseudomonads growing on the amino alcohol (Higgins *et al.*, 1968), had no such effect on cultures

of *Pseudomonas* sp. P6. No induced formation of L- or D-1-aminopropan-2-ol dehydrogenases occurred by growing the organism on the amino alcohol rather than pyruvate + $(\text{NH}_4)_2\text{SO}_4$ or nutrient broth. Both enzyme activities were uniformly low (see Table 7). Specificity studies, albeit with crude extracts, suggested that the enzyme acting on the D-isomer was a non-specific alcohol dehydrogenase similar to that found previously in a pseudomonad and in *Escherichia coli* (Lowe & Turner, 1970a). Rates of aminoacetone reduction by the enzyme were not measured.

DL-1-Aminopropan-2-ol was inactive as a substrate in assays for ethanolamine oxidase by the lactaldehyde-detection method of Narrod & Jakoby (1966) after growth of *Pseudomonas* sp. on the amino alcohol. The ethanolamine oxidase of an *Arthrobacter* sp. was known to be slightly active with 1-aminopropan-2-ol (Narrod & Jakoby, 1966). No diol dehydratase activity (Lee & Abeles, 1964), i.e. propionaldehyde formation from propane-1,2-diol, could be detected. Thus lactaldehyde and propane-1,2-diol were not credible intermediates between 1-aminopropan-2-ol and propionaldehyde.

Table 6. *Some properties of aldehyde dehydrogenase from Pseudomonas sp. P6*

Enzyme activity in crude extracts was measured spectrophotometrically as described in the Methods section. Activity at pH 7.5 was measured in potassium phosphate buffer. n.d., Not determined.

Property		
pH optimum	9.0–9.1 (diethanolamine–HCl)	
Apparent K_m values (mM)		
Propionaldehyde at pH 7.5	0.02*	
Propionaldehyde at pH 9.1	0.20*	
NAD ⁺ at pH 9.1	0.15	
Substrate specificity (relative activities at pH 9.1)		
Acetaldehyde	85	
Propionaldehyde	100	
n-Butyraldehyde	150	
	Activity (nmol of NAD ⁺ reduced/min per mg of protein)	
	pH 7.5	pH 9.1
Growth substrate		
DL-1-Aminopropan-2-ol	129	203
Propionaldehyde + $(\text{NH}_4)_2\text{SO}_4$	n.d.	300
n-Propylamine	47	n.d.
DL-Propane-1,2-diol	n.d.	16
Propionate + $(\text{NH}_4)_2\text{SO}_4$	13	n.d.
Succinate + $(\text{NH}_4)_2\text{SO}_4$	14	0
D-1-Aminopropan-2-ol	n.d.	0

* Substrate inhibition occurred above 0.16 mM at pH 7.5 and above 0.36 mM at pH 9.1.

Table 7. *Activity of 1-aminopropan-2-ol dehydrogenases in extracts of Pseudomonas sp. P6 grown on various media*

Extracts of *Pseudomonas* sp. P6 were prepared after growth on the media shown and assayed for dehydrogenase activity towards DL-, L- and D-1-aminopropan-2-ol by a previously described (Lowe & Turner, 1970a) colorimetric method.

Growth medium	Substrate ...	Dehydrogenase activity (nmol of aminoacetone formed/min per mg of protein)		
		DL-1-Aminopropan-2-ol	L-1-Aminopropan-2-ol	D-1-Aminopropan-2-ol
DL-1-Aminopropan-2-ol		2.73	0.96	7.97
$(\text{NH}_4)_2\text{SO}_4$ + pyruvate		2.92	1.29	6.82
Nutrient broth		2.33	0.64	4.80

Further metabolism of propionate and acetate

Growth, manometric and enzymic results suggested that propionate was metabolized via propionyl-CoA to derivatives of either 2-hydroxyglutarate or perhaps methylmalonate. The pathway was not studied in detail.

Acyl-CoA synthetase activity towards propionate, but no activity analogous to acetate kinase or phosphotransacetylase, was detected in extracts. Optimum conditions for the assay of the synthetase were worked out. Maximum activity was at about pH 7.5 in Tris-HCl buffer. Omission of $MgCl_2$, KF or GSH from standard acyl-CoA synthetase incubation mixtures (see the Methods section) decreased activity by 20, 17 and 12% respectively. The apparent K_m for propionate was 1.1 mM. Inhibition by excess of substrate occurred above 100 mM. Relative rates were assessed with propionate (100), acetate (88), acrylate (28), L-2-hydroxyglutarate (16) and methylmalonate (11). The enzyme was stable overnight in 0.1 M-potassium phosphate buffer, pH 7.0, at 0° and -20°C. Under optimum assay conditions specific activities of about 20 nmol of propionyl-CoA formed/min per mg of protein were found in fresh extracts of the pseudomonad grown on DL-1-aminopropan-2-ol. Although HCO_3^- had been found to stimulate growth on propionate, it had no effect on labelled propionate uptake by washed suspensions of the organism grown on DL-1-aminopropan-2-ol or propionate media. Experiments with $H^{14}CO_3^-$ failed to provide clear evidence for propionyl-CoA carboxylase activity. Low 2-hydroxyglutarate synthase activity was found in crude extracts (5 nmol/min per mg of protein), but the standard assay conditions used (Wegener *et al.*, 1965) were not necessarily optimum.

DL-1-Aminopropan-2-ol metabolism by *Ps. putida* TG2 (N.C.I.B. 10558)

This pseudomonad grew rapidly on DL- or D-1-aminopropan-2-ol media, but in neither case was propionaldehyde formation detected on any occasion. Propionate was rapidly oxidized by washed suspensions of the microbe grown on either the DL- or the D-amino alcohol. After growth on the racemic substrate very high DL-1-aminopropan-2-ol *O*-phosphate phospho-lyase activity was found in extracts. At the pH optimum of 8.2 in Tris-HCl buffer, activities of 6000-7500 nmol of propionaldehyde formed/min per mg of protein were found. Ethanolamine *O*-phosphate had only about 20% of this activity. Relative activities remained constant during exclusion chromatography on Bio-Gel P-300, and during gradual but eventually almost complete loss of activities when stored for 48 h at 0°C. Analogues inactive as substrates but causing the percentage inhibition shown were choline *O*-phosphate (59%), L-threonine *O*-phosphate (45%), α -glycerophosphate (64%) and

DL-serine *O*-phosphate (35%). The concentration of these inhibitors was equimolar to that of the substrate, namely 5 mM. Substrate inhibition occurred above this concentration. The addition of DL-1-aminopropan-2-ol to cultures of *Ps. putida* TG2 growing on succinate-(NH_4)₂SO₄ medium induced phospho-lyase synthesis. Enzyme formation during growth on DL-1-aminopropan-2-ol was not repressed by either (NH_4)₂SO₄ or succinate.

Discussion

The growth of *Pseudomonas* sp. P6 on 1-aminopropan-2-ol was found to result in transient aldehyde production. Propionaldehyde was concluded to be an obligatory intermediate formed via 1-aminopropan-2-ol *O*-phosphate by a novel pathway involving two enzymes, the aminomethyl group giving rise to the aldehyde group (see Scheme 1).

The mechanism of aldehyde formation, from either 1-aminopropan-2-ol or ethanolamine, did not involve a cobamide coenzyme-dependent enzyme analogous to ethanolamine deaminase (Miyaki *et al.*, 1959a; Bradbeer, 1965). It is known that the hydroxymethyl group of ethanolamine gives rise to the aldehydic group of acetaldehyde in the deaminases from both *Proteus morganii* (Miyaki *et al.*, 1959b) and a *Clostridium* sp. (Kaplan & Stadtman, 1968b). Other possible routes involving deamination, oxidoreductase and dehydration-hydration steps, in various sequences, were ruled out on the basis of growth, manometric and enzymic experiments. The inability of aminoacetone to act as a substrate for growth or oxidation and the low activities of 1-aminopropan-2-ol dehydrogenases in extracts of the pseudomonad contrasted with the findings for *Pseudomonas* sp. N.C.I.B. 8858 (Higgins *et al.*, 1968).

The amino alcohol kinase catalysing the first step in 1-aminopropan-2-ol and ethanolamine catabolism was virtually inactive with choline and is a newly described enzyme. Evidence for an ethanolamine kinase distinct from that for choline in mammalian and insect tissues exists, but is indirect (Sung & Johnstone, 1967; Shelley & Hodgson, 1971a,b). Amino alcohol kinase activity in bacteria has not been described previously. 1-Aminopropan-2-ol kinase activity was demonstrated unambiguously by a variety of assay methods and the enzyme was partially purified. Radioisotopic experiments showed clearly that the conversion of 1-aminopropan-2-ol into its *O*-phosphate ester and into propionaldehyde occurred by an ATP-dependent process. The amino alcohol kinase had relatively low activity towards ethanolamine.

The phospho-lyase acting on the *O*-phosphate esters of both 1-aminopropan-2-ol and ethanolamine, also partially purified, was similar in most respects to

the 'O-phosphorylethanolamine' phospho-lyase found in mammalian liver (Fleishood & Pitot, 1969, 1970). Similarities included pH optimum, K_m values for ethanolamine O-phosphate and coenzyme, K_i for the inhibitor P_i and inhibition by heavy-metal ions. The activity of the mammalian enzyme towards 1-aminopropan-2-ol O-phosphate was not tested. The microbial enzyme was narrowly specific in the sense that the substrate analogues tested were inactive as substrates, although active as inhibitors. The kinetic properties of the enzyme favoured activity with the C_3 substrate under optimum conditions. The further metabolism of propionaldehyde, initially oxidized to propionate by an NAD^+ -dependent dehydrogenase, was concluded to proceed via propionyl-CoA and possibly 2-hydroxyglutarate. The further metabolism of propionate was not of prime interest in these studies and was not pursued. The balance of aldehyde-producing phospho-lyase and aldehyde-utilizing dehydrogenase activities probably accounted for variable 'leakage' of aldehyde from cultures of the pseudomonad, and offered an explanation for the non-accumulation of aldehyde by suspensions of the micro-organism. Acetaldehyde appeared to be oxidized to acetate by the NAD^+ -dependent dehydrogenase. The further metabolism of acetate, probably via acetyl-CoA, was not studied.

The general metabolic significance of the kinase and phospho-lyase enzymes is uncertain. It is tempting to suggest that the amino alcohol kinase is normally involved in the biosynthesis of the D-1-aminopropan-2-ol O-phosphate fragment of vitamin B_{12} . Although studies with *Streptomyces olivaceus* failed to demonstrate that D-1-aminopropan-2-ol was a precursor (Lowe & Turner, 1970a), studies with *Propionibacterium shermanii* have demonstrated specific incorporation of the compound into the amino alcohol moiety of the vitamin B_{12} molecule (Müller *et al.*, 1971). Alternatively the kinase may be concerned with ethanolamine in some aspect of phospholipid metabolism (see Hill & Lands, 1970). The regulation of amino alcohol kinase synthesis in *Pseudomonas* sp. P6 suggests that it normally functions catabolically. A role for ethanolamine O-phosphate phospho-lyase in mammalian metabolism was suggested from radioisotopic evidence (Sprinson & Coulon, 1954) that carbon atoms of ethanolamine were incorporated into fatty acids preferentially, via acetate. The carboxyl group of acetate was shown to be derived from the aminomethyl group of ethanolamine (Sprinson & Weliky, 1954, 1969). It is likely that the phospho-lyase functions catabolically in mammals as part of a salvage route for the ethanolamine fragment of phospholipids. In the pseudomonad studied the enzyme also functions catabolically but, although active with ethanolamine O-phosphate, 1-aminopropan-2-ol O-phosphate breakdown would probably be favoured.

Growth and enzyme studies with *Pseudomonas* sp. P6 suggested that both D- and L-1-aminopropan-2-ol were catabolized by the same pathway (Fig. 5). Both the kinase and phospho-lyase enzyme were active with each enantiomorphous form, although rates varied, and further work may reveal some degree of stereospecificity at low substrate concentrations. It may be noted that the rat degrades erythro-5-hydroxy-L-lysine by a series of reactions analogous to that for amino alcohol metabolism in *Pseudomonas* sp. P6. In the rat, however, the initial kinase is inactive with other stereoisomers of the amino alcohol substrate and is specific for GTP as the co-substrate (Hiles & Henderson, 1972).

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