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Microbial Metabolism of Amino Alcohols

AMINOACETONE METABOLISM VIA 1-AMINOPROPAN-2-OL IN *PSEUDOMONAS* SP. N.C.I.B. 8858

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1. *Pseudomonas* sp. N.C.I.B. 8858 grew well on D- and L-1-aminopropan-2-ol and on aminoacetone. 2. Cell-free extracts possessed high activities of inducibly formed L-1-aminopropan-2-ol–NAD^+ oxidoreductase, amino alcohol–ATP phosphotransferase, DL-1-aminopropan-2-ol *O*-phosphate phospho-lyase and aldehyde–NAD^+ oxidoreductase, but no 1-aminopropan-2-ol racemase or D-1-aminopropan-2-ol–NAD^+ oxidoreductase. 3. The amino alcohol kinase (activated by ADP) was non-stereospecific towards 1-aminopropan-2-ol and was one-third as active with ethanolamine. The phospho-lyase was active with L- and D-1-aminopropan-2-ol *O*-phosphate, but ethanolamine *O*-phosphate was only one-tenth as active as its higher homologues. The purified aldehyde dehydrogenase was active with propionaldehyde, acetaldehyde and also with methylglyoxal. The previously observed 2-oxo aldehyde dehydrogenase activity was considered to be due to the broadly specific aldehyde dehydrogenase. 4. Mutants of *Pseudomonas* sp. N.C.I.B. 8858 deficient in 1-aminopropan-2-ol kinase, 1-aminopropan-2-ol *O*-phosphate phospho-lyase, aldehyde dehydrogenase or an enzyme involved in propionate metabolism were incapable of growth on aminoacetone or 1-aminopropan-2-ol as carbon source, although all except the kinase- or phospho-lyase-deficient mutants could use these compounds and ethanolamine as nitrogen sources. The aldehyde dehydrogenase-deficient mutants produced copious amounts of propionaldehyde and acetaldehyde during growth on the corresponding amino alcohols. 5. The path of aminoacetone metabolism in *Pseudomonas* sp. N.C.I.B. 8858 was concluded to involve L-1-aminopropan-2-ol, the *O*-phosphate ester of this compound, propionaldehyde and propionate as obligatory intermediates. D-1-Aminopropan-2-ol was metabolized by the same route as the L-isomer, gratuitously inducing formation of the stereospecific L-1-aminopropan-2-ol dehydrogenase. 6. Extracts of the pseudomonad grown with ethanolamine as the nitrogen source were devoid of 1-aminopropan-2-ol dehydrogenase, the kinase and the phospho-lyase, but exhibited cobamide coenzyme-dependent deaminase activity. Mutants deficient in kinase or phospho-lyase (deaminating) grew well on ethanolamine as the nitrogen source. Ethanolamine deaminase was inactive with, but inhibited by, 1-aminopropan-2-ol.

Aminoacetone has been known as a microbial metabolite of L-threonine for some time (Elliott, 1958). It was also postulated to be an intermediate in a cyclic theme for glycine oxidation (Elliott, 1959), although the operation of such an 'aminoacetone cycle' has never been demonstrated. The possibility that aminoacetone may be metabolized via methylglyoxal, D-lactate and pyruvate as acetyl-CoA, as proposed by Elliott (1959, 1960), was first conveniently investigated when microbes capable of rapidly metabolizing aminoacetone were isolated. A pseudomonad obtained by enrichment culture on DL-1-aminopropan-2-ol was also capable of growth on aminoacetone and appeared to catabolize the amino alcohol via the amino ketone. More detailed work with a stock strain, *Pseudomonas* sp. N.C.I.B. 8858, supported this conclusion (Higgins *et al.*, 1968), although enzymic evidence suggested that only the L-isomer of 1-aminopropan-2-ol was catabolized via aminoacetone (Pickard *et al.*, 1968). Recent evidence that another pseudomonad, unable to grow on aminoacetone, metabolized both D- and L-1-aminopropan-2-ol via their *O*-phosphate esters and propionaldehyde (Jones & Turner, 1971, 1973) prompted a re-examination of *Pseudomonas* sp. N.C.I.B. 8858. The metabolism of aminoacetone and D-1-aminopropan-2-ol was of particular interest.

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Evidence is presented here that aminoacetone is metabolized via L-1-aminopropan-2-ol, its *O*-phosphate ester and propionaldehyde. D-1-Aminopropan-2-ol is broken down by an analogous route but is not an intermediate of aminoacetone metabolism.

Materials and Methods

Micro-organisms and media

Pseudomonas sp. N.C.I.B. 8858 was maintained on nutrient broth and cultivated on simple synthetic media by procedures similar to those described previously (Higgins *et al.*, 1968). Basal salts medium contained (per litre) 8g of KH_2PO_4, 0.1g of $MgSO_4$, $7H_2O$ and 0.3g of Na_2SO_4 (anhydrous). This was supplemented with 3g of DL-1-aminopropan-2-ol/l for routine purposes. Otherwise, media contained 3g of carbon source/l, and 1g of $(NH_4)_2SO_4$/l of medium replaced the Na_2SO_4. Media were adjusted to pH7 with HCl or NaOH and sterilized by autoclaving. Media containing aminoacetone were adjusted to pH6.6 to minimize polymerization and sterilized by membrane filtration. Liquid cultures were usually incubated in 1-litre batches in 2-litre conical flasks on a rotary shaker at 30°C. Continuous culture on the same media was carried out in a BioTec FL 103-05 (working volume 31) Laboratory Fermentor, equipped with standard accessories for agitation, aeration, foam, pH and temperature control and for addition of medium (BioTec AB, Stockholm 16, Sweden). Cell density during growth was measured as the extinction, E_{540}^{1cm}, and cultures were harvested as previously described (Higgins *et al.*, 1968).

Washed bacterial suspensions

Suspensions were prepared and used for measurements of O_2 uptake, and aminoacetone production and uptake, as described previously (Higgins *et al.*, 1968) unless stated otherwise.

Production and isolation of mutants

The procedure used was a modification of those described for mutants of a pseudomonad by Heptinstall & Quayle (1970) and Harder & Quayle (1971). A culture of wild-type *Pseudomonas* sp. N.C.I.B. 8858 was grown on DL-1-aminopropan-2-ol medium, harvested, washed once with sterile 50mM-Tris–maleate buffer, pH6.0, and resuspended in this buffer. To 1ml portions of the suspension was added 1.0ml of a solution (4mg/ml) of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and the whole incubated on a shaker at 30°C for 2h. The bacteria were centrifuged, washed twice with sterile water and resuspended in the appropriate enrichment medium in which both wild-type and the required mutants would grow (see Table 5). The bacterial culture was incubated on a shaker for 20h at 30°C to express mutations. After centrifugation the organisms were washed twice with sterile water and resuspended in 40ml of the appropriate selective medium in which wild-type, but not mutant strains, could grow (see Table 4). After incubation for 4h to exhaust the growth potential of mutants and to allow growth of wild-types to commence, sterile benzylpenicillin and D-cycloserine were added to give concentrations of 250 units/ml and 5mg/ml respectively, and further incubation was carried out for 20h at 30°C. Bacteria were then centrifuged, washed twice with sterile water to lyse the growing, i.e. wild-type, cells and resuspended in 10ml of enrichment medium. After incubation for 20h, as before, cells were washed twice and resuspended in selective medium, antibiotics were added at double the original concentration, and cells were allowed to grow and were washed again with water to cause lysis. A third enrichment cycle again involved treatment with antibiotics at four times the original concentration. After washing with water, bacteria were resuspended in 0.1M-potassium phosphate buffer, pH7.0, and serial tenfold dilutions were made. Samples (0.1ml) of the appropriate dilution were spread on plates of appropriate enrichment medium (see Table 4). Plates were incubated for 2 days at 30°C and the most suitable were used for replication on to selective and enrichment media. Replica plates were incubated for 2 days at 30°C and patterns of colony growth were compared. Presumptive mutants were picked off, resuspended in potassium phosphate buffer, and streaked on to fresh plates for further examination and for single-colony isolation. By using this general method, ratios of wild-type to mutants of about 10:1 were obtained.

Aldehyde production during growth or by washed-cell suspensions

Volatile aldehydes were detected and isolated as their 2,4-dinitrophenylhydrazones. Sealed flasks were used with centre wells containing the carbonyl-trapping reagent. Propionaldehyde or acetaldehyde derivatives were filtered off, recrystallized and analysed by t.l.c. in two solvent systems (Jones & Turner, 1973; Jones *et al.*, 1973).

Preparation of enzyme extracts

Micro-organisms suspended in buffer, usually 0.1M-potassium phosphate, pH7, were disrupted ultrasonically by using an MSE 100W ultrasonic disintegrator at full power. Suspensions were treated for 10s intervals, with cooling at 0–4°C, for a total of about 2min. Disrupted cells were centrifuged

at 100000*g* for 90min at 0°C to remove cell debris. Supernatants were used for enzyme purification and assays.

Enzyme assays

These were carried out at 37°C under the optimum conditions for activity unless otherwise stated. Protein was measured by a modified biuret procedure described previously (Turner, 1966).

L-1-*Aminopropan-2-ol–NAD*$^+$ *oxidoreductase*. A modification of the colorimetric method described by Turner (1966) was used. Reaction mixtures contained 100μmol of diethanolamine–HCl buffer, pH9.5, 10μmol of D- or L-1-aminopropan-2-ol, 10μmol of NAD$^+$ and 0.1–1.0mg of protein in a total volume of 1ml. Reactions were usually started by the addition of NAD$^+$ to the other components. After incubation for 5–30min, 0.5ml of 25% (w/v) trichloroacetic acid was added, protein was removed by centrifugation and 1ml samples of supernatants were assayed for aminoacetone (Pickard *et al.*, 1968).

'Aminoacetone reductase' activity was measured by following the aminoacetone-dependent oxidation of NAD(P)H at 340nm. Incubation mixtures contained 100μmol of Tris–HCl buffer, pH8.0, 5μmol of aminoacetone adjusted to pH8.0 immediately before use, 0.2μmol of NADH or NADPH and 0.01–0.1mg of protein in a total volume of 1ml. Reactions were started by the addition of aminoacetone. The enzyme was also fully active at pH8.0 in tris(hydroxymethyl)methylaminopropanesulphonic acid–NaOH buffer.

ATP–amino alcohol phosphotransferase (*amino alcohol kinase*). Activity was assayed by a modification of the colorimetric method of Jones *et al.* (1973), which depends on the enzymic conversion of the amino alcohol *O*-phosphate product into an aldehyde. Reaction mixture components were 100μmol of Tris–HCl buffer, pH7.8, 2μmol of amino alcohol, 5μmol of ATP, 10μmol of $MgSO_4$, 5μmol of ADP and 0.01–1.0mg of protein, in a volume of 1ml. Reactions were started by the addition of ATP and were stopped after 15–30min by heating at 100°C for 10min. Protein was removed by centrifuging and 0.5ml samples of supernatant were assayed for amino alcohol *O*-phosphate by incubation with 1–2 units of purified phospho-lyase enzyme (i.e. that catalysing the transformation of 1–2μmol of substrate/min) (see below) and 0.1μmol of pyridoxal phosphate in a total volume of 1ml for 2h. Reactions were stopped by the addition of 0.5ml of *N*-methylbenzothiazolone hydrazone reagent, which is required for the colorimetric assay of aldehydes (Jones & Turner, 1973).

For the detection of amino alcohol kinase in eluate fractions, phospho-lyase and pyridoxal phosphate were included in the initial incubation mixtures and the whole was incubated for 30–90min before colorimetric assay of aldehyde (Jones & Turner, 1973).

Amino alcohol O-phosphate phospho-lyase (*deaminating*). Aldehyde formation was measured either colorimetrically with *N*-methylbenzothiazolone hydrazone reagent (Paz *et al.*, 1965) or enzymically (Fleshood & Pitot, 1970*a*) as previously described (Jones & Turner, 1973; Jones *et al.*, 1973). Incubations were done at the pH optimum of 8.5 in Tris–HCl buffer, with either DL-1-aminopropan-2-ol *O*-phosphate or ethanolamine *O*-phosphate as substrate unless stated otherwise.

Aldehyde–NAD$^+$ *oxidoreductase*. A modification of the spectrophotometric method described by Jones & Turner (1973) was used. Propionaldehyde, acetaldehyde or methylglyoxal were used as substrates (see the text) and NAD$^+$ reduction was measured. Aldehyde dehydrogenase activity was commonly assayed at pH9.5 in diethanolamine–HCl buffer and methylglyoxal (2-oxo aldehyde) dehydrogenase activity (Higgins & Turner, 1969) at pH8.5 in Tris–HCl buffer.

Ethanolamine deaminase. The coenzyme B_{12}-dependent production of acetaldehyde from ethanolamine (Kaplan & Stadtman, 1971) was determined colorimetrically. Incubation mixtures contained 100μmol of Tris–HCl buffer, pH8.5, 10μmol of ethanolamine, 10nmol of 5′-deoxyadenosyl-cobamide coenzyme and 0.1–1.0mg of protein in a volume of 1ml. Reactions were started by the addition of protein and stopped after 15–30min by the addition of 0.5ml of *N*-methylbenzothiazolone hydrazone reagent (Jones & Turner, 1973).

1-*Aminopropan-2-ol racemase*. The conversion of one stereoisomer of 1-aminopropan-2-ol into another was assayed by using stereospecific 1-aminopropan-2-ol dehydrogenases. Optically pure D- or L-1-aminopropan-2-ol was incubated with extracts of the pseudomonad in the presence and absence of pyridoxal phosphate. A range of buffers, pH values and substrate concentrations was tested. After incubation for about 1h, reaction mixtures were adjusted to pH9.5 with excess of buffer and protein was removed by heating and centrifuging. Portions of supernatants were assayed for the alternative isomer of 1-aminopropan-2-ol by incubation for about 1h with NAD$^+$ (10μmol) and the appropriate stereospecific dehydrogenase. Reaction mixtures were examined for aminoacetone as described above. L-1-Aminopropan-2-ol–NAD$^+$ oxidoreductase was purified from *Pseudomonas* sp. N.C.I.B. 8858 by the procedure of Pickard *et al.* (1968) and the enzyme specific for the D-isomer was prepared from *Escherichia coli* as described by Lowe & Turner (1968). Model mixtures of stereoisomers confirmed the potential value of the assay procedures, which were also used to determine the stereospecificity of aminoacetone reduction (see below).

Stereospecificity of aminoacetone reductase activity of L-1-*aminopropan-2-ol–NAD*$^+$ *oxidoreductase.* The enzyme preparation (Pickard *et al.*, 1968) was incubated with 5μmol of aminoacetone, 10μmol of NADH or NADPH and 100μmol of Tris–HCl buffer, pH8, in a total volume of 1ml for about 1h. The reaction mixture was then adjusted to pH9.5 with 500μmol of diethanolamine–HCl buffer and protein was removed by heating at 100°C for 40min followed by centrifugation. This treatment also destroyed most of the non-reduced aminoacetone, less then 40nmol remaining. Samples of supernatant were assayed for the presence of D- or L-1-aminopropan-2-ol as described above. Several experiments showed that whereas 40–50nmol of aminoacetone was formed by incubation with the enzyme specific for the D-isomer, 700–1270nmol of aminoacetone was formed with L-1-aminopropan-2-ol dehydrogenase.

Partial purification of amino alcohol O-phosphate phospho-lyase and ATP–amino alcohol phosphotransferase

Solid $(NH_4)_2SO_4$ was added to extracts of the pseudomonad to give 40% satn., i.e. 22.6g/100ml (Dixon, 1953). The preparation was stirred for 30min at 4°C and precipitated material was removed by centrifuging at 4°C and discarded. To the supernatant was added more $(NH_4)_2SO_4$, to give 60% satn., i.e. 13.6g/100ml. Precipitated material was collected by centrifuging and resuspended in 0.05M-Tris–HCl buffer, pH7.5. This material was applied to a column of Whatman DE 52 DEAE-cellulose equilibrated with 0.05M-Tris–HCl buffer, pH7.5, and 5μM-pyridoxal phosphate and eluted with an increasing gradient of NaCl (see Fig. 3). Maximum activities for the phospho-lyase of about 30μmol of aldehyde formed/min per mg of protein were obtained, corresponding to 30–40-fold purification. About half of the total activity was recovered. For amino alcohol kinase, 18–23-fold purification was obtained by the above procedure, yielding fractions with specific activities of about 3μmol of phosphate ester formed/min per mg of protein. About 40% of the original activity was recovered.

In some experiments extracts of the pseudomonad were applied directly to the DEAE-cellulose column without preliminary fractionation with $(NH_4)_2SO_4$. In either case the chromatographic procedures previously described for extracts of another pseudomonad were used (Jones & Turner, 1973).

Chromatographic examination of aldehyde dehydrogenase and 2-oxo aldehyde dehydrogenase

In addition to fractionation on DEAE-cellulose, extracts were fractionated by molecular-exclusion chromatography on polydextran gel. A column of Sephadex G-200 gel, equilibrated with 0.1M-potassium phosphate buffer, pH7.0, was loaded with extract (about 200mg of protein). Protein was eluted with the same buffer and 6ml samples were collected. Samples were assayed for both propionaldehyde dehydrogenase (Jones & Turner, 1973) and methylglyoxal dehydrogenase (Higgins & Turner, 1969) activities by the methods outlined above.

Incorporation of radioactivity from ^{14}*C-labelled aminoacetone*

The procedures used to study the incorporation of ^{14}C from amino[U-^{14}C]acetone into constituents of the ethanol-soluble fraction of bacterial suspensions were similar to those described for a pseudomonad metabolizing acetate by Kornberg (1958). Samples were chromatographed by t.l.c. on cellulose, in two dimensions. The solvent systems were butan-1-ol–propionic acid–water (47:22:31, by vol.) and ethanol–pyridine–1M-acetic acid–water (95:3:10:3, by vol.). Chromatograms were left in contact with X-ray film for 2 weeks before photographic development.

Chemicals

The sources of most chemicals were those previously reported by Jones & Turner (1973) and Jones *et al.* (1973). Radioactive D-1-amino[U-^{14}C]-propan-2-ol was prepared from L-[U-^{14}C]threonine by the thermal decarboxylation method (Chatelus, 1964; see Lowe & Turner, 1970), which was also used to prepare the optically pure non-radioactive isomers. Partially resolved L-1-aminopropan-2-ol was prepared by fractional crystallization of the L(+)-tartrate salt of the racemic compound (Sullivan, 1963; see Turner, 1967); the 80%-pure isomer is referred to as 'L'-1-aminopropan-2-ol. Racemic 1-aminopropan-2-ol was redistilled at 159–161°C/750mmHg (100kPa) before use. Radioactive amino-[U-^{14}C]acetone was prepared enzymically from L-[U-^{14}C]threonine, with L-threonine dehydrogenase partially purified from *Pseudomonas oxalaticus* (N.C.I.B. 8642) grown on the amino acid as sole source of carbon and nitrogen (Blackmore & Turner, 1971). Aminoacetone was isolated from deproteinized reaction mixtures by t.l.c. on cellulose (0.5mm thick) in ethanol–1M-acetic acid–pyridine–water (93:10:3:3, by vol.). Radioactive product was located with an end-window counter, at the same position as authentic aminoacetone, and the band of cellulose scraped off the plate. Amino-[U-^{14}C]acetone was extracted with water and concentrated by freeze-drying. Unused L-[U-^{14}C]threonine was recovered by analogous procedures and re-used. Glyoxalase I, prepared from yeast, was obtained from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany.

Results

Growth of Pseudomonas sp. N.C.I.B. 8858

The pseudomonad grew well on DL-, D- and 'L'- (approx. 80% L-isomer, see the Materials and Methods section) 1-aminopropan-2-ol as carbon, nitrogen and energy sources. With the D-isomer, a lag period of 10–20h was observed before exponential growth commenced from an inoculum grown on succinate+$(NH_4)_2SO_4$ medium. A lag of only 3–4h occurred when racemic or L-1-aminopropan-2-ol was the substrate. The previously observed accumulation of aminoacetone, during the lag and early exponential phases of growth on the racemic amino alcohol (Higgins *et al.*, 1968), was also evident when the L-isomer, but not the D-isomer, was the growth substrate (Fig. 1). Growth on racemic 1-aminopropan-2-ol in the presence of ^{14}C-labelled DL- or D-aminopropan-2-ol confirmed that both isomers were utilized for growth, although preferential use of the L-isomer occurred initially (Fig. 2). Incorporation of the D-isomer commenced after significant utilization of L-1-aminopropan-2-ol had occurred, although diauxic growth was not observed. The pseudomonad grew relatively slowly on aminoacetone as source of both carbon and nitrogen, but grew well on propionate+$(NH_4)_2SO_4$ medium. Aminoacetone served as a good source of nitrogen for growth with succinate as the major carbon source.

Experiments with washed cell suspensions

Aminoacetone production and utilization. Aminoacetone was rapidly formed from L-1-aminopropan-2-ol by washed suspension of the pseudomonad grown on DL-, D- or 'L'-1-aminopropan-2-ol or aminoacetone itself (Table 1). Its production was optimal at pH9. None was formed from the D-amino alcohol even in the presence of inhibitors known to stimulate production from the racemic compound (Higgins *et al.*, 1968), or from either stereoisomer after growth on succinate+$(NH_4)_2SO_4$ or nutrient broth media.

Incubations of suspensions of the pseudomonad, grown on 1-aminopropan-2-ol or on aminoacetone, with the amino ketone resulted in its rapid utilization, maximally at pH7.5, and the rate of utilization was again dependent on the growth substrate (Table 1).

Oxidation of possible metabolites of 1-aminopropan-2-ol. O_2 uptake by suspensions of the pseudomonad was stimulated by various substrates as shown in Table 2. Growth on DL-, D- or 'L'-1-aminopropan-2-ol resulted in essentially the same oxidation pattern as that seen after growth on

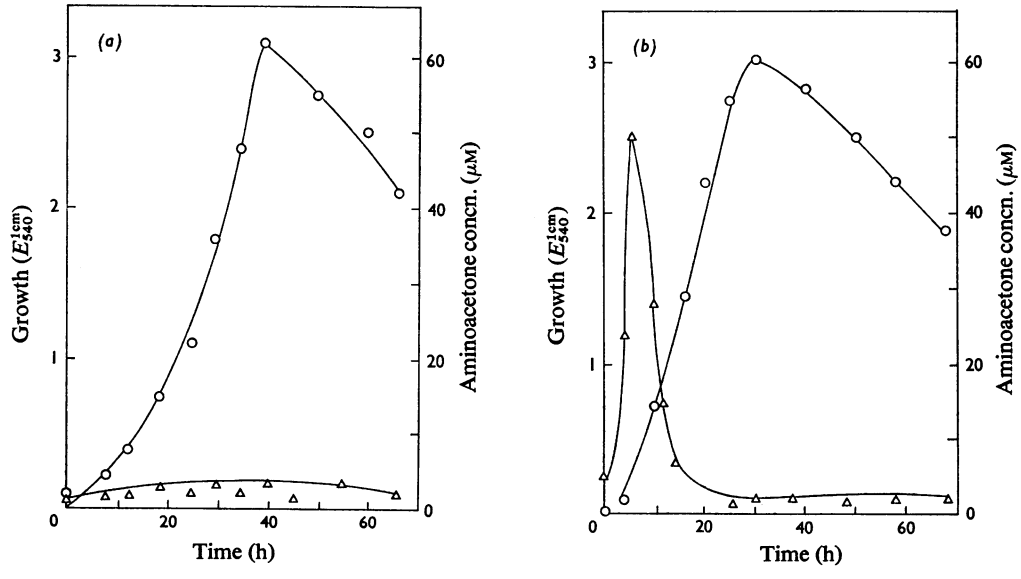


Fig. 1. *Aminoacetone formation and re-utilization during the growth of Pseudomonas sp. N.C.I.B.* 8858 *on each stereoisomer of 1-aminopropan-2-ol*

The pseudomonad was grown on media containing either (*a*) D-1-aminopropan-2-ol or (*b*) L-1-aminopropan-2-ol as sole source of carbon and nitrogen as described in the Materials and Methods section. △, Aminoacetone concentration in the medium (determined colorimetrically as described by Higgins *et al.*, 1968). ○, Growth (followed as E_{540}^{1cm} of appropriately diluted samples).

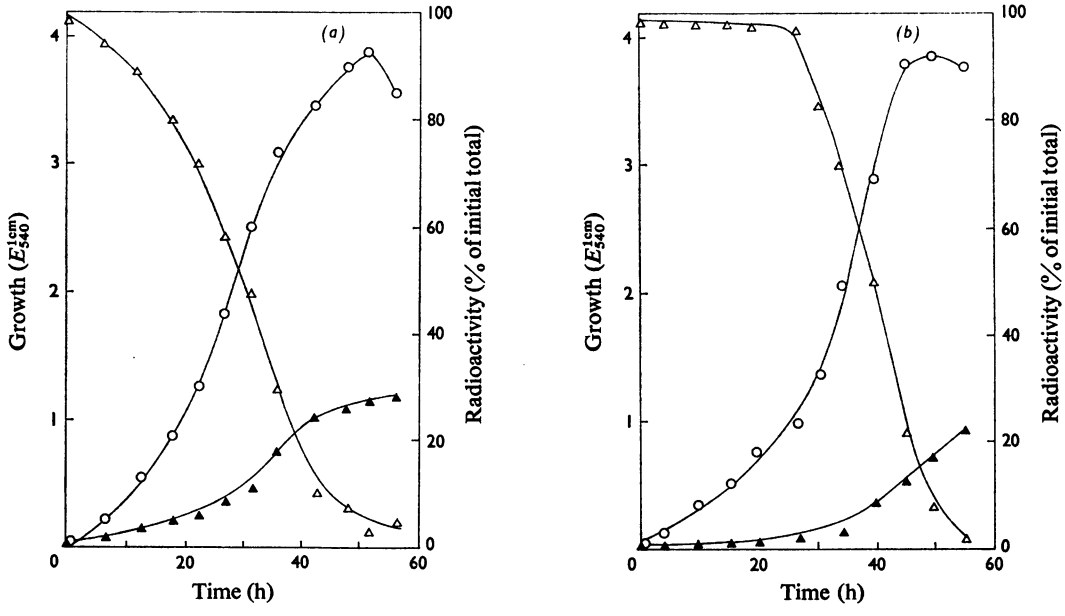


Fig. 2. *Utilization of* DL- *and* D-1-*aminopropan-2-ol during growth of Pseudomonas sp. N.C.I.B.* 8858

The pseudomonad was grown on basal salts + DL-1-aminopropan-2-ol medium containing 10 μCi of (*a*) DL-1-amino[3-^{14}C]-propan-2-ol or (*b*) D-1-amino[U-^{14}C]propan-2-ol per l. Samples were removed at the times indicated and growth was followed by measuring E_{540}^{1cm} after appropriate dilution. The distribution of radioactivity between medium and microbial material was measured by membrane filtration as described previously (Jones & Turner, 1971). ○, Growth; △, radioactivity in medium; ▲, radioactivity in microbial material.

Table 1. *Effect of growth conditions on aminoacetone formation and utilization by suspensions of Pseudomonas sp. N.C.I.B.* 8858

Aminoacetone formation from 1-aminopropan-2-ol isomers was measured colorimetrically by the method of Higgins *et al.* (1968) except that 0.1M-diethanolamine–HCl buffer at the optimum pH 9.0 was used. Aminoacetone uptake by suspensions in 0.1M-potassium phosphate buffer, pH 7.0, was also followed colorimetrically.

	Aminoacetone production from stereoisomer shown (nmol/h per mg dry wt.)		Aminoacetone utilization (nmol/h per mg dry wt.)
Growth substrates	L-1-Aminopropan-2-ol	D-1-Aminopropan-2-ol	
DL-1-Aminopropan-2-ol	413	5	8200
D-1-Aminopropan-2-ol	427	9	7900
L-1-Aminopropan-2-ol	365	8	8100
Aminoacetone	292	7	7500
Succinate + $(NH_4)_2SO_4$	19	9	510
Succinate + L-threonine	28	12	480
Nutrient broth	23	7	550

aminoacetone. The ability to oxidize the amino alcohol, aminoacetone, propionaldehyde and propionate was induced during growth on either stereoisomer of 1-aminopropan-2-ol. The oxidation of the amino alcohol was strongly inhibited by carbonyl reagents.

Aldehyde production. In contrast with *Pseudomonas* sp. P6 (N.C.I.B. 10431) no volatile aldehyde could be detected during growth, or after prolonged incubation of cell suspensions with 1-aminopropan-2-ol under either aerobic or anaerobic conditions (see the Materials and Methods section). Methylglyoxal could not be detected after incubation of suspensions with aminoacetone. Propionaldehyde formation from both amino alcohols and aminoacetone by mutants of *Pseudomonas* sp. N.C.I.B. 8858 is described below.

From these results it seemed likely that L- and D-1-aminopropan-2-ol were metabolized by analogous routes, that although the uptake or metabolism

Table 2. *Oxidation of possible metabolites of 1-aminopropan-2-ol by washed suspensions of Pseudomonas sp. N.C.I.B. 8858 grown on various media*

The pseudomonad was grown on the compounds shown as sole sources of carbon and nitrogen as described in the Materials and Methods section. O_2 uptake by washed suspensions was measured manometrically by using conventional procedures (Umbreit *et al.*, 1964) as described previously (Higgins *et al.*, 1968). O_2-absorption rates are corrected for endogenous values of 0.5–0.8 μmol of O_2 absorbed/h per mg dry wt. of bacteria.

Oxidation substrate (20mM)	O_2 absorbed (μmol/h per mg dry wt. of bacteria) after growth on: DL-1-Amino-propan-2-ol	D-1-Amino-propan-2-ol	L-1-Amino-propan-2-ol	Amino-acetone	Succinate+ $(NH_4)_2SO_4$
DL-1-Aminopropan-2-ol	8.8	7.9	8.4	7.2	1.2
D-1-Aminopropan-2-ol	7.8	7.5	7.3	6.8	1.0
L-1-Aminopropan-2-ol	8.6	7.8	8.9	7.1	0.7
Aminoacetone*	8.7	7.9	8.3	7.4	2.0
Methylglyoxal	3.5	2.1	3.2	2.4	2.0
Succinate	8.9	8.1	9.2	7.7	12.5
Propionaldehyde*	11.5	10.9	11.2	9.0	7.2
Propionate	10.5	10.2	10.7	8.2	4.6
DL-Lactaldehyde	7.5	7.3	7.8	6.9	1.2

* Used at concn. of 2mM.

of the D-isomer was slow either isomer induced enzyme formation, and that propionaldehyde and propionate were possible intermediates but that only L-1-aminopropan-2-ol and aminoacetone were interconvertible (see Scheme 1). The possibility that a racemase converted L-1-aminopropan-2-ol into the D-isomer, or vice versa, was not ruled out, however.

Metabolism of aminoacetone and its derivatives by cell-free extracts

Extracts were prepared and enzyme activities were assayed as described in the Materials and Methods section.

L-1-*Aminopropan-2-ol-NAD*$^+$ *oxidoreductase*. Previous work had shown that enzyme formation was induced by growth on the racemic amino alcohol or on aminoacetone (Higgins *et al.*, 1968). Individual stereoisomers of 1-aminopropan-2-ol were also active as inducers (Table 3), in spite of the fact that only the L-isomer was a substrate. Enzyme formation occurred when either L- or D-1-aminopropan-2-ol was added to cultures growing exponentially on succinate+$(NH_4)_2SO_4$ medium. The function of the enzyme as an aminoacetone reductase catalysing the initial step in aminoacetone metabolism by the pseudomonad is described below. The elution of the enzyme from a column of DEAE-cellulose is shown in Fig. 3.

1-*Aminopropan-2-ol racemase*. No conversion of the D- into the L-isomer, and hence into aminoacetone, could be detected in extracts under a variety of conditions, e.g. over the pH range 5.5–10.0. Stereospecific enzymes were used in the search for racemase activity (see the Materials and Methods section). No conversion of the L- into the D-isomer was detected in parallel experiments.

DL-1-*Aminopropan-2-ol O-phosphate phospho-lyase*. Phospho-lyase activity towards the *O*-phosphate esters of both L- and D-isomers was detected in crude extracts of the pseudomonad grown on DL-, D- or 'L'-1-aminopropan-2-ol or aminoacetone media. Values of 500–1000nmol/min per mg of protein were obtained at the optimum pH8.5, depending on the growth phase at harvesting. Only very low activities were found after growth on succinate +$(NH_4)_2SO_4$, succinate+ethanolamine or succinate +threonine media. In contrast with species of *Erwinia* (Jones *et al.*, 1973) the pseudomonad formed the enzyme when media contained $(NH_4)_2SO_4$ in addition to 1-aminopropan-2-ol (Table 3). The properties of the partly purified enzyme (see Fig. 3) were similar to those of the enzyme in some pseudomonads incapable of growth on aminoacetone (Jones & Turner, 1973). Both relative rates of activity and K_m values favoured DL-1-aminopropan-2-ol *O*-phosphate rather than ethanolamine *O*-phosphate as the physiological substrate. The K_m values were 0.4 and 1.0mM respectively and the higher homologue was tenfold more active than the lower. Both stereoisomers of 1-aminopropan-2-ol *O*-phosphate, and the racemic compound, were equally active. Analogous compounds, e.g. DL-threonine *O*-phosphate, were inactive as substrates but were often effective inhibitors. Iodoacetamide at 1mM inhibited by 67%. Pyridoxal phosphate stimulated activity in crude extracts 3–4-fold (K_m 0.01mM) and protected against loss of activity during dialysis and chromatography.

ATP–amino alcohol phosphotransferase. 1-Aminopropan-2-ol kinase was found in extracts of the

Table 3. *Effect of growth substrates on enzyme activities in extracts of Pseudomonas sp. N.C.I.B.* 8858

The pseudomonad was grown on the media shown, cell-free extracts were prepared and enzyme activities measured colorimetrically as described in the Materials and Methods section. N.D., not determined.

	Enzyme activities (nmol/min per mg of protein) in crude extracts			
Growth substrates	L-1-Aminopropan-2-ol dehydrogenase	DL-1-Aminopropan-2-ol kinase	DL-1-Aminopropan-2-ol *O*-phosphate phospho-lyase	Propionaldehyde dehydrogenase
DL-1-Aminopropan-2-ol	110	150	1012	132 (45)†
D-1-Aminopropan-2-ol	96	138	970	140 (49)†
'L'-1-Aminopropan-2-ol	108	147	830	142
Aminoacetone	85*	107	802	112 (39)†
Succinate+aminoacetone	81	105*	584*	98
Succinate+ethanolamine	1	29	20	120 (42)†
Succinate+L-threonine	3	18	15	10
Succinate+$(NH_4)_2SO_4$	1	11	12	12 (5)†
Succinate+$(NH_4)_2SO_4$+DL-1-aminopropan-2-ol	92	131	951	126 (41)†
Succinate+$(NH_4)_2SO_4$+aminoacetone	N.D.	93	327	N.D.
Nutrient broth	1	13*	25	20 (7)†

* Mean of two values determined on separate occasions.

† Values in parentheses were obtained with methylglyoxal as substrate, i.e. they correspond to 2-oxo aldehyde dehydrogenase activities.

pseudomonad grown on the amino alcohol or aminoacetone, but not after growth on succinate+$(NH_4)_2SO_4$ or succinate+ethanolamine media (see Table 3). Activity in dialysed extracts was found with either D- or L-1-aminopropan-2-ol as substrate and required ATP and Mg^{2+} ions. Cysteine (10mM) increased activity by about 30%. The addition of ADP at 5mM increased activity fourfold. Under optimum conditions activities of about 670nmol/min per mg of protein were found. The enzyme was separated from the phospho-lyase in crude extracts by chromatography on DEAE-cellulose (see Fig. 3). Ethanolamine was only 35% as active as substrate as DL-1-aminopropan-2-ol.

Aldehyde and 2-oxo aldehyde dehydrogenase activities. Growth of the pseudomonad on DL-1-aminopropan-2-ol or aminoacetone gave extracts with propionaldehyde dehydrogenase activity, maximal at pH9.5, of about 140nmol of propionaldehyde utilized/min per mg of protein. The enzyme was labile and such values may be underestimates. Enzyme formation was induced about tenfold during growth on either stereoisomer of the amino alcohol but not during growth on succinate+$(NH_4)_2SO_4$ or on nutrient broth (see Table 3). K_m values of 1.6 and 0.5mM were obtained for propionaldehyde and NAD^+ respectively under optimum conditions.

Conditions favouring aldehyde dehydrogenase formation also led to increases in 2-oxo aldehyde dehydrogenase activity (see Table 3), which had been detected in *Pseudomonas* sp. N.C.I.B. 8858 previously (Higgins & Turner, 1969). K_m values of 2.5 and 0.6 mM were found for methylglyoxal and NAD^+ respectively at pH9.5. Activity towards methylglyoxal was lost during storage under a variety of conditions. A constant ratio of activities with propionaldehyde and methylglyoxal in all preparations suggested that both activities were due to a single enzyme. Both were inhibited to a similar extent by *p*-hydroxymercuribenzoate (100% at 1mM), iodoacetamide (50% at 20mM) and iodosobenzoate (60% at 0.2mM) but were unaffected by EDTA, 2,2'-bipyridyl or 8-hydroxyquinoline at 1mM. Chromatography on DEAE-cellulose or Sephadex gave identical elution patterns, the ratio of activities remaining constant. The time-course of enzyme induction (Fig. 4) was consistent with a single enzyme, and mutants deficient in one enzyme were also devoid of the other (see below).

Induction of enzyme synthesis during adaptation to growth on DL-1-*aminopropan-2-ol*

When sterile DL-1-aminopropan-2-ol was supplied to the pseudomonad growing in continuous culture on succinate+$(NH_4)_2SO_4$, enzymes involved in aminoacetone and 1-aminopropan-2-ol metabolism were induced (Fig. 4). The concentration of amino alcohol in the culture was maintained during

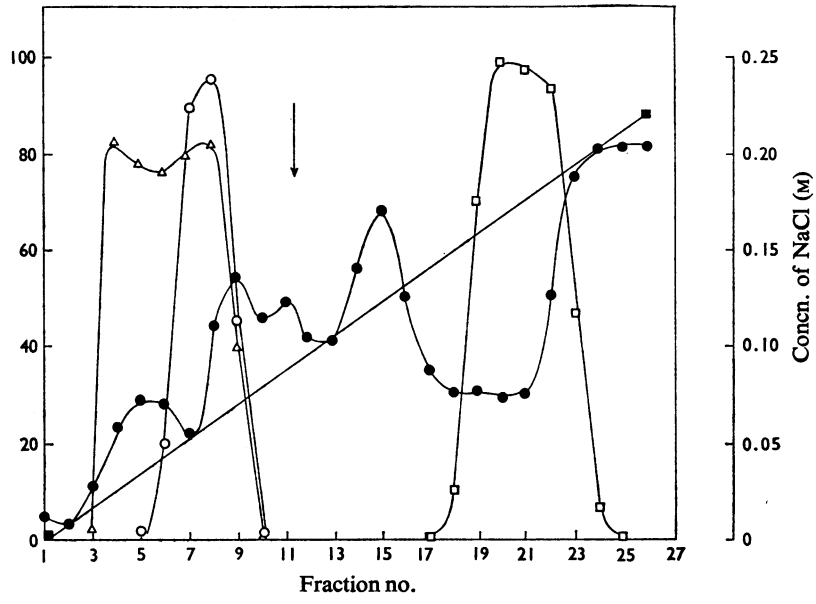


Fig. 3. *Separation and partial purification of enzymes involved in aminoacetone metabolism by Pseudomonas sp. N.C.I.B.* 8885

Crude extract was chromatographed on DEAE-cellulose, fractions (5ml) were collected and DL-1-aminopropan-2-ol kinase and DL-1-aminopropan-2-ol *O*-phosphate phospho-lyase were located by colorimetric assays as described previously (Jones & Turner, 1973). DL-1-Aminopropan-2-ol dehydrogenase was also located by a previously described colorimetric assay (Pickard *et al.*, 1968). The relative position of propionaldehyde dehydrogenase (indicated by the arrow) was determined in separate experiments by the spectrophorometric assay described in the Materials and Methods section. Protein was detected automatically by measuring absorption at 253.7nm with an LKB Uvicord I Ultraviolet Absorptiometer. The linear gradient in concentration of NaCl present in the Tris–HCl buffer is indicated. Enzyme activities were found only in the regions shown; points indicating absence of a particular enzyme in other fractions are omitted for clarity. ○, DL-1-Aminopropan-2-ol kinase; □, DL-1-aminopropan-2-ol *O*-phosphate phospho-lyase; △, DL-1-aminopropan-2-ol dehydrogenase; ●, protein and ■, NaCl concentration.

adaptation by dilution with the appropriate medium (see the Materials and Methods section). The results showed a sequential formation of kinase, phospho-lyase and aldehyde dehydrogenase enzymes. Separate experiments showed that induction of the L-1-aminopropan-2-ol dehydrogenase, the kinase and the phospho-lyase was more rapid when the L-isomer rather than D-1-aminopropan-2-ol was added to batch cultures in the mid-exponential phase of growth on succinate+$(NH_4)_2SO_4$ medium.

Studies with mutants of Pseudomonas sp. N.C.I.B. 8858

The detection of specific enzymes in cell-free extracts, and the effect of growth conditions on their formation, was consistent with the metabolism of both 1-aminopropan-2-ol isomers via their *O*-phosphate esters and propionaldehyde. The finding that growth on D-1-aminopropan-2-ol induced formation of an oxidoreductase stereospecific for the interconversion of the L-isomer and aminoacetone rationalized results obtained with washed cell suspensions. It seemed likely that aminoacetone was metabolized by initial reduction to L-1-aminopropan-2-ol and thence to the *O*-phosphate and aldehyde. The possibility that L-1-aminopropan-2-ol was metabolized, perhaps in part, via aminoacetone, methylglyoxal, D-lactate and pyruvate could not be discounted, however. In view of the possible existence of alternative routes for the metabolism of L-1-aminopropan-2-ol and aminoacetone in the pseudomonad, mutants deficient in enzymes of interest were isolated (see the Materials and Methods section). Mutants of three types, deficient in DL-1-aminopropan-2-ol kinase (and phospholyase) (type PND), deficient in aldehyde dehydrogenase (type DSD) or deficient in an enzyme involved in propionate metabolism (type NPD), were obtained by selecting strains incapable of growth on DL-1-aminopropan-2-ol medium. Enrichment media contained alternative sources of carbon, nitrogen or both, as shown in Table 4. The nature of each lesion (see Scheme 1) was deduced by enzyme assay (Table 5) and other observations. No

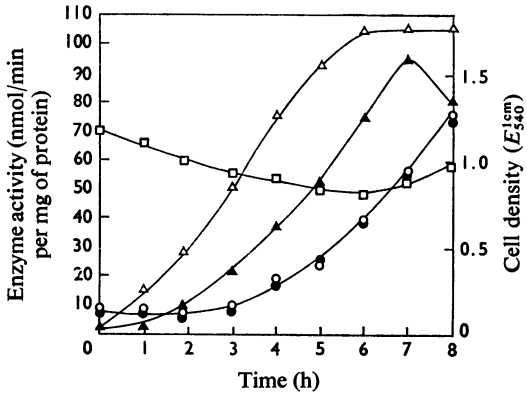


Fig. 4. *Sequence of induced enzyme formation during adaptation of Pseudomonas sp. N.C.I.B.* 8858 *to growth on* DL-1-*aminopropan-2-ol medium*

The pseudomonad was initially grown on succinate+$(NH_4)_2SO_4$ medium in continuous culture (see the Materials and Methods section). At zero time, sterile DL-1-aminopropan-2-ol solution at pH7 was added to the culture vessel to give a concentration of 3g/l, and dilution with DL-1-aminopropan-2-ol medium was commenced at a dilution rate of approx. 0.15/h. Samples of culture were collected at intervals of about 1h and cell density was measured as E_{540}^{1cm}. Cell-free extracts were prepared and enzyme activities were assayed as described in the Materials and Methods section. △, DL-1-Aminopropan-2-ol kinase; ▲, DL-1-aminopropan-2-ol *O*-phosphate phospho-lyase; ○, propionaldehyde dehydrogenase; ●, methylglyoxal dehydrogenase. Phospholyase activities are shown decreased by a factor of 10 and methylglyoxal dehydrogenase activities are shown increased by a factor of 3.

mutant incapable of growth on DL-1-aminopropan-2-ol medium was able to grow on aminoacetone or either stereoisomer of 1-aminopropan-2-ol as a source of carbon, although all had normal amounts of L-1-aminopropan-2-ol dehydrogenase when grown on succinate+$(NH_4)_2SO_4$ medium in the presence of either isomer (see Table 5). In type PND mutants, no oxidation of either 1-aminopropan-2-ol isomer or aminoacetone occurred after growth on succinate+$(NH_4)_2SO_4$+DL-1-aminopropan-2-ol medium. This contrasted with the wild-type micro-organism (Table 2). In the aldehyde dehydrogenase-deficient mutants (type DSD) aminoacetone and either isomer of the amino alcohol could be utilized as a source of nitrogen, but not of carbon, for growth. In each case a volatile aldehyde was produced. This was identified as propionaldehyde by chromatography of the 2,4-dinitrophenylhydrazone derivatives (see the Materials and Methods section). Mutants of the type NPD were also capable of growth on either isomer of 1-aminopropan-2-ol as a nitrogen source only, but were unable to oxidize propionate after growth in the presence of the amino alcohol. These mutants were unable to grow on propionate as a carbon source.

A fourth type of mutant was able to grown on the L- but not the D-isomer of 1-aminopropan-2-ol (type APD). After growth on the L-isomer, normal amounts of all the enzymes investigated were found. Further, neither the kinase nor the phospho-lyase was stereospecific for the L-isomer. Propionate oxidation was normal. Growth on succinate+$(NH_4)_2SO_4$ medium supplemented with D-1-aminopropan-2-ol produced enzyme activities equivalent

Table 4. *Growth characteristics of mutant strains of Pseudomonas sp. N.C.I.B.* 8858

Mutants were produced and isolated as described in the Materials and Methods section. Their ability to grow on the substrates shown was tested by inoculation into liquid media containing these compounds as sole carbon and nitrogen sources and incubation at 30°C with shaking for 48h. Growth was recorded visually. (p) indicates the production of propionaldehyde during growth, identified chromatographically as its 2,4-dinitrophenylhydrazone. The nature of the mutant strains was concluded to be as shown in Scheme 1.

	Strain of *Pseudomonas* sp. N.C.I.B. 8858				
Carbon and nitrogen source for growth	Wild-type	PND	DSD	NPD	APD
Aminoacetone*	±	−	−	−	±
D-1-Aminopropan-2-ol	+	−	−	−	−
DL-1-Aminopropan-2-ol	+	−	−	−	+
Succinate+D-1-aminopropan-2-ol	+	−	+ (p)	+	−
Succinate+DL-1-aminopropan-2-ol	+	−	+ (p)	+	+
Succinate+aminoacetone	+	−	+ (p)	+	+
Propionate+$(NH_4)_2SO_4$	+	+	+	−	+
Succinate+$(NH_4)_2SO_4$	+	+	+	+	+

* Growth on aminoacetone as the source of carbon and nitrogen was slow even with the wild-type and this is indicated appropriately.

Table 5. *Enzyme activities of mutant strains of Pseudomonas sp. N.C.I.B.* 8858

Mutants were grown on succinate + $(NH_4)_2SO_4$ medium supplemented with DL-1-aminopropan-2-ol (redistilled DL-1-aminopropan-2-ol was used, as the commercially supplied compound contains an impurity which induces formation of aldehyde dehydrogenase), or with the D-isomer (values shown in parentheses). After growth, cell-free extracts were prepared and enzymes assayed as described in the Materials and Methods section.

Enzyme	Enzyme activity (% of activity in wild type) in strain shown				
	Wild-type	PND	DSD	NPD	APD
L-1-Aminopropan-2-ol dehydrogenase	100 (100)	81 (88)	83 (89)	99 (87)	87 (15)
DL-1-Aminopropan-2-ol kinase	100 (100)	0 (0)	98 (89)	93 (91)	89 (10)
DL-1-Aminopropan-2-ol *O*-phosphate phospho-lyase	100 (100)	4 (5)	87 (74)	72 (82)	75 (6)
Propionaldehyde dehydrogenase	100 (100)	0 (0)	0 (0)	67 (85)	93 (0)

to growth on unsupplemented medium. It was concluded from these results that the mutation affected entry of the D-isomer, but not of the L-isomer, into the micro-organism. Evidence for a lag in D-1-aminopropan-2-ol utilization during growth, and the preferential utilization of the L-isomer (Fig. 2) was consistent with the possibility of two stereospecific uptake systems for aminopropanol in this pseudomonad. Type APD mutants had presumably lost the uptake system for the D-isomer. Metabolic routes consistent with the properties of the mutants are shown in Scheme 1.

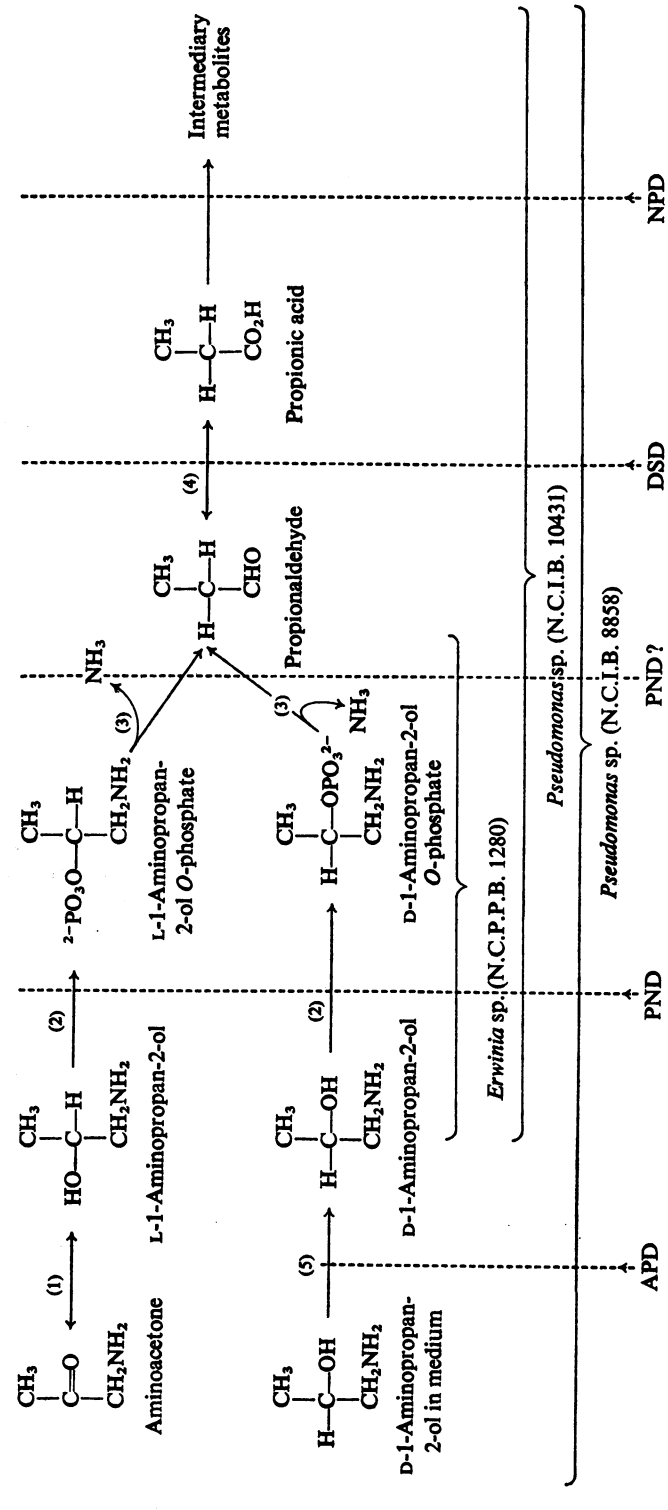
Aminoacetone reduction to L-1-*aminopropan*-2-*ol*

The finding that aminoacetone was unable to support the growth of mutants deficient in kinase and phospho-lyase suggested that 1-aminopropanol-2-ol and its *O*-phosphate ester were obligatory metabolites of aminoacetone. Previous work had shown that the L-1-aminopropan-2-ol dehydrogenase of the pseudomonad was readily reversible, and optimum conditions for aminoacetone reductase activity were worked out (Pickard *et al.*, 1968). At pH 7 the enzyme was about 4 times more active with NADPH than with NADH. With NADPH as the coenzyme (K_m 0.02mM) 3mM-ATP stimulated activity twofold at pH7, whereas with NADH (K_m 0.3mM) ATP inhibited by 70% under the same conditions (I. J. Higgins & J. M. Turner, unpublished work). The present study showed that aminoacetone reduction at the pH optimum of 8, with either NADH or NADPH as cofactor, produced L-1-aminopropan-2-ol stereospecifically. The product was identified enzymically (see the Materials and Methods section).

Aminoacetone utilization by crude extracts of *Pseudomonas* sp. 8858 had been observed previously and found to be stimulated by 2-oxo acids, e.g. 2-oxoglutarate, and NAD^+. The results were consistent with a mechanism involving a coupled aminoacetone–2-oxo acid aminotransferase and 2-oxo aldehyde–NAD^+ oxidoreductase system (Higgins *et al.*, 1967). The second enzyme was postulated to remove methylglyoxal, which was known to inhibit strongly aminoacetone disappearance. It has now been shown, however, that the addition of GSH, or purified yeast glyoxalase I plus GSH (added to catalyse the removal of methylglyoxal) did not similarly stimulate aminoacetone disappearance from reaction mixtures. In addition, a variety of potential hydrogen donors were found to replace 2-oxo acids. Ethanol, DL-isocitrate and DL-malate were highly active in the presence of NAD^+. The most effective stimulant of aminoacetone disappearance was NADH, which was almost 3 times more active than optimum concentrations of 2-oxoglutarate plus NAD^+. Finally, experiments with 2-oxo[5-^{14}C]glutarate failed to demonstrate the production of radioactive glutamate after incubation with aminoacetone under a variety of conditions (I. J. Higgins & J. M. Turner, unpublished work). This unpublished work strongly indicated that in systems containing 2-oxoglutarate and NAD^+ the disappearance of aminoacetone was due to its reduction to 1-aminopropan-2-ol, the reaction being brought about by the coupling of 2-oxoglutarate dehydrogenase and L-1-aminopropan-2-ol dehydrogenase.

Radioisotopic evidence on the route of aminoacetone metabolism

Incubation of 1μCi of amino[U-^{14}C]acetone with a suspension of the pseudomonad grown on DL-1-aminopropan-2-ol medium, with rapid sampling of incubation mixtures and radioautographic analysis (see the Materials and Methods section) revealed the formation of at least four radioactive products. Samples obtained at zero time, or when boiled bacterial suspensions were used, contained only unused aminoacetone. Two of the products were concluded to be 1-aminopropan-2-ol and its *O*-phosphate ester by two-dimensional co-chromatography with authentic compounds. The identity of the other two products, one of which contained very little radioactivity, was not established. In separate experiments, where samples were added to



Scheme 1. *Microbial metabolism of aminoacetone and 1-aminopropan-2-ols*

The numbers in parentheses refer to enzymes named in the key. The dashed lines represent enzyme lesions in the mutants of *Pseudomonas* sp. N.C.I.B. 8858 where indicated. The sections of the pathway known to operate in some other bacteria are shown. Key to enzymes: (1) L-1-aminopropan-2-ol–NAD(P) oxidoreductase (aminoacetone reductase); (2) DL-1-aminopropan-2-ol–ATP phosphotransferase; (3) DL-1-aminopropan-2-ol *O*-phosphate phospho-lyase; (4) propionaldehyde–NAD oxidoreductase; (5) 'permease', or uptake system, for D-1-aminopropan-2-ol (see the text).

2,4-dinitrophenylhydrazone reagent (0.2% in 2M-HCl), ethyl acetate extracts contained a compound chromatographing as a single spot with the same R_F value as propionaldehyde. Methylglyoxal and lactaldehyde were not detected as labelled products of aminoacetone metabolism by these experiments.

Ethanolamine metabolism by the pseudomonad

Good growth occurred with ethanolamine as the sole nitrogen source, but only poor growth occurred when it also served as the carbon and energy source. Suspensions of the pseudomonad grown on succinate+ethanolamine medium rapidly oxidized ethanolamine, acetaldehyde and acetate. Type PND mutants devoid of phospho-lyase (deaminating) grew rapidly with ethanolamine as nitrogen source suggesting an alternative deamination mechanism. Mutants deficient in aldehyde dehydrogenase (type DSD) also grew well with ethanolamine, producing a volatile aldehyde, identified as acetaldehyde by characterization of its 2,4-dinitrophenylhydrazone. Cell-free extracts of wild-type or mutant strains of the pseudomonad grown on succinate+ethanolamine medium contained an enzyme which produced acetaldehyde from the amino alcohol. This enzyme was independent of ATP, Mg^{2+} ions or pyridoxal phosphate for activity and was activated twofold by 5'-deoxyadenosyl-cobamide coenzyme. No activity was detected with DL-1-aminopropan-2-ol as substrate, although this compound inhibited activity with ethanolamine as substrate. The enzyme was concluded to be an ethanolamine deaminase. Growth on succinate+ethanolamine medium also led to the formation of aldehyde dehydrogenase (see Table 3).

Discussion

A pathway for aminoacetone metabolism other than deamination to methylglyoxal (Elliott, 1960; Urata & Granick, 1961, 1963; Buffoni & Blaschko, 1963) has been shown to operate in *Pseudomonas* sp. N.C.I.B. 8858. Growth, manometric, enzymic and genetic evidence showed that aminoacetone was metabolized via L-1-aminopropan-2-ol, L-1-aminopropan-2-ol *O*-phosphate, propionaldehyde and propionate (Scheme 1). The operation of this route for 1-aminopropan-2-ol had been shown earlier in a pseudomonad unable to metabolize aminoacetone (Jones & Turner, 1971, 1973) and in species of *Erwinia* also unable to oxidize propionaldehyde (Jones *et al.*, 1973). D-1-Aminopropan-2-ol was metabolized by the same pathway in all cases but was not a metabolite of aminoacetone in *Pseudomonas* sp. N.C.I.B. 8858. That the growth of *Pseudomonas* sp. N.C.I.B. 8858 on aminoacetone involved initial reduction and ATP-dependent phosphorylation reactions went some way to explain the characteristically slow rates of growth observed.

The fact that methylglyoxal was a substrate for the propionaldehyde dehydrogenase synthesized by *Pseudomonas* sp. N.C.I.B. 8858 during growth on aminoacetone or 1-aminopropan-2-ol explained the previous observation that methylglyoxal was metabolized by the micro-organism after growth on such media (Higgins *et al.*, 1968).

The elucidation of the route for aminoacetone metabolism shed little light on its possible role in L-threonine breakdown. *Pseudomonas* sp. N.C.I.B. 8858 was known to be incapable of growth on L-threonine as the sole carbon and nitrogen source (Higgins *et al.*, 1968) but grew well on succinate+L-threonine medium. Although the formation of L-1-aminopropan-2-ol dehydrogenase occurred when cells grown on succinate+$(NH_4)_2SO_4$ were suspended in media containing L-threonine (Pickard *et al.*, 1968), it was found that growth on succinate+L-threonine neither increased the ability of cells to utilize aminoacetone (Table 1) nor induced formation of the enzymes concerned (Table 3). It seems clear that aminoacetone is not an intermediate in the catabolism of L-threonine by this particular pseudomonad but that exogenous aminoacetone, a product of L-threonine degradation by other organisms, can be utilized. Growth of *Pseudomonas* sp. N.C.I.B. 8858 on either aminoacetone or succinate+aminoacetone media induced the enzymes required for aminoacetone catabolism (Table 3). A large number of bacteria capable of growth on L-threonine have been isolated recently, representing at least eight genera including *Pseudomonas* (Bell *et al.*, 1972), and are of obvious interest. A strain of *Bacillus subtilis* is believed to catabolize L-threonine partly via aminoacetone (Willetts & Turner, 1970).

The ability of L-1-aminopropan-2-ol–NAD^+ oxidoreductase to function as aminoacetone reductase in *Pseudomonas* sp. N.C.I.B. 8858 has now been clearly demonstrated. The low K_m for NADPH relative to NADH (0.02 and 0.30mM respectively) and the fact that aminoacetone reduction was four times faster with NADPH, together with the stimulating effect of ATP, initially suggested a role for the enzyme in the biosynthesis of 1-aminopropan-2-ol rather than in its degradation. The product of aminoacetone reduction by the stereospecific enzyme was confirmed to be L-1-aminopropan-2-ol with either cofactor. The amino alcohol moiety of vitamin B_{12} is known to be the D-isomer, however (Wolf *et al.*, 1950), and D-1-amino[U-^{14}C]propan-2-ol has been shown to be its precursor in *Propionibacterium shermanii* (Müller *et al.*, 1971). Although amino alcohol kinase was also initially considered for a role in the biosynthesis of the D-1-aminopropan-2-ol *O*-phosphate fragment of vitamin B_{12}, its mode of regulation by ADP and its substrate-induced formation indicated a catabolic function. The phospho-lyase may also be catabolic, as part of a salvage route

for the D-amino alcohol fragment of vitamin B_{12}. No role for the L-isomer is known except as an aminoacetone metabolite. Aminoacetone occurs in nature as a catabolite of L-threonine, and may be further catabolized via L-1-aminopropan-2-ol and propionate as described above, even though no single microbe has yet been found to utilize the entire pathway as a major catabolic route for threonine.

In *Pseudomonas* sp. N.C.I.B. 8858 the formation of kinase and phospho-lyase enzymes was not induced during growth with ethanolamine. Ethanolamine and its *O*-phosphate were relatively poor substrates for these enzymes. The role in ethanolamine catabolism ascribed to the analogous phospho-lyase of mammalian tissues (Fleshood & Pitot, 1969, 1970*a*,*b*) and to the kinase and phospho-lyase enzymes of *Erwinia* species (Jones *et al.*, 1973) is therefore inappropriate to the pseudomonad.

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