Microbial Metabolism of Amino Ketones

L-1-AMINOPROPAN-2-OL DEHYDROGENASE AND L-THREONINE DEHYDROGENASE IN *ESCHERICHIA COLI*

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1. A wide range of intermediary metabolites and substrate analogues have no effect on the oxidation of DL-1-aminopropan-2-ol to aminoacetone by washed-cell suspensions of *Escherichia coli*. Only DL-2-hydroxy-2-phenylethylamine, DL-1,3-diaminopropan-2-ol, DL-serine and L-1-(3,4-dihydroxyphenyl)-2-aminoethanol act as inhibitors. 2. Dialysed cell-free extracts of *E. coli* exhibit an NAD⁺-dependent DL-1-aminopropan-2-ol-dehydrogenase activity of approx. 8 μ moles of aminoacetone formed/mg. of protein/min. at the pH optimum of approx. 10. The K_m values for the coenzyme and DL-amino alcohol are approx. 0.4 and 10.0 mM respectively. A smaller peak of activity occurs at pH 7.0–7.2, the K_m for NAD⁺ at pH 7 being approx. 0.05 mM. 3. Enzyme activity in cell-free extracts is inhibited by DL-2-hydroxy-2-phenylethylamine, DL-1-aminopropan-2,3-diol and DL-serine. DL-Phenylserine and DL-1-aminobutan-2-ol are oxidized to compounds reacting as amino ketones. 4. In fresh cell-free extracts L(+)-1-aminopropan-2-ol preparations are oxidized more rapidly than racemic or *laevo*-rotatory material, the D(–)-enantiomorph appearing to act as a competitive inhibitor. The K_m for L(+)-1-aminopropan-2-ol appears to be approx. 1.5 mM when highly resolved substrate preparations are used, either in the free base form or as the L(+)-tartrate salt. 5. L(+)-1-Aminopropan-2-ol dehydrogenase is a labile enzyme, and in appropriately treated extracts activity towards the D-enantiomorph is detectable and relatively higher than that towards the L-enantiomorph. 6. Optimum activity of L-threonine-dehydrogenase in cell-free extracts is exhibited at pH 9.6 in the presence of NAD⁺. The K_m values for coenzyme and amino acid substrate are approx. 0.08 and 5.0 mM respectively. This enzyme is distinct from L-aminopropan-2-ol dehydrogenases on the basis of kinetic evidence, and the separation of activities by gel filtration. 7. Both L-threonine and DL-1-aminopropan-2-ol dehydrogenases are markedly inhibited by 8-hydroxyquinoline and *p*-chloromercuribenzoate, but only slightly by other chelating and thiol reagents. 8. *E. coli* is incapable of growth on simple synthetic media, containing a variety of carbon sources, when DL-1-aminopropan-2-ol is supplied as the sole source of nitrogen. It appears unlikely that the micro-organism can deaminate aminoacetone. 9. The metabolic roles of L-threonine dehydrogenase, aminoacetone and L-aminopropan-2-ol dehydrogenases are discussed.

The ability of cell-free extracts of *Escherichia coli* to form aminoacetone from L-threonine and DL-1-aminopropan-2-ol respectively has been reported previously, and some properties of an aminopropanol dehydrogenase with optimum activity at pH 7 in such extracts have been described (Turner, 1965, 1966). Synthesis of aminopropanol dehydrogenase, but not of threonine dehydrogenase, was markedly repressed by growing *E. coli* on media containing glucose, fructose or other common catabolic repressors. The properties of the L-

threonine dehydrogenase, previously detected in this micro-organism by Green & Elliott (1964), were not studied in detail.

The metabolic roles of threonine and aminopropanol dehydrogenases are uncertain. It has been suggested, however, that the coupled activities of these enzymes could result in the conversion of L-threonine into the D-aminopropanol fragment present in the vitamin B₁₂ molecule (Neuberger & Tait, 1960; Neuberger, 1961; Turner, 1966), or to L-aminopropanol possibly functioning as a phos-

phatidyl base (Turner & Willetts, 1967). A role for threonine dehydrogenase in the degradation of the amino acid to acetyl-CoA by a series of reactions analogous to those of the succinate-glycine cycle (Shemin & Russell, 1953) has also been proposed (Elliott, 1959, 1960a; Green & Elliott, 1964).

The present paper describes experiments with washed suspensions of *E. coli* extending previous work on the microbial oxidation of aminopropanol. The specificity of the reaction and the further metabolism of aminoacetone were of particular interest. Some properties, including the stereo-specificity, of an aminopropanol dehydrogenase having optimum activity at pH 10 in cell-free extracts are described, and are compared with those of the enzyme with optimum activity at approx. pH 7. Some properties of the L-threonine dehydrogenase of *E. coli*, shown to be a different and distinct enzyme, are also described.

The characterization of aminopropanol and threonine dehydrogenase was judged to be an important preliminary to the examination of control processes regulating any pathway of L-threonine metabolism involving aminoacetone as an intermediate.

EXPERIMENTAL

The materials and methods used in the present work were the same as those described by Turner (1966), except where noted.

Growth of E. coli. *E. coli*, type I (N.C.I.B. no. 8114), was grown on nutrient broth as described by Turner (1966). Simple synthetic media contained 5.4g. of KH_2PO_4 , 0.4g. of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 8.0g. of the carbon source and 1.7g. of $(\text{NH}_4)_2\text{SO}_4$ per l. When DL-1-aminopropan-2-ol was tested as a source of nitrogen, 1.8g. replaced the $(\text{NH}_4)_2\text{SO}_4$ and 1.4g. of anhydrous Na_2SO_4 was added. Synthetic media were adjusted to pH 7 with 5N-NaOH and sterilized by autoclaving. When the carbon source was a sugar, an aqueous solution was sterilized separately and added to a concentrated solution of the mineral salts.

Measurement of threonine- and aminopropanol-dehydrogenase activities of cell-free extracts. Extracts of washed cells were prepared, and their enzyme activities measured, by the procedures described by Turner (1966), except that diethanolamine-HCl was used as the buffer system at pH 9.6 and 10.0, and optically active 1-aminopropan-2-ol preparations (see below) were used as the substrate where indicated. Additional details are given in the legend to Fig. 1.

Gel filtration. Exclusion chromatography was carried out on polyacrylamide gel. To 250ml. of 0.1M-potassium phosphate buffer, pH 7, was added 5.5g. of Bio-Gel P-300, with stirring. After soaking for 48hr., the gel was packed under low hydrostatic pressure in a glass column (2.54cm. $\times$ 36.0cm.), and washed with a continuous flow of buffer for a further 48hr. Preparation of the gel, and operation of the column, was carried out in a cold room at 0–5°. Samples of cell-free extract applied to the column contained approx. 50mg. of protein in a volume of 5ml., elution was effected with 0.1M-potassium phosphate buffer, pH 7, and

Fig. 1. Effect of pH on 1-aminopropan-2-ol- and threonine-dehydrogenase activities in cell-free extracts. Reaction mixtures contained 0.2m-mole of buffer, 0.15m-mole of sodium pyruvate, 10μ moles of NAD^+ , 0.1mg. of lactate dehydrogenase, 0.5m-mole of KCl, approx. 0.3mg. of protein (dialysed cell-free extract) and 0.2m-mole of DL-1-aminopropan-2-ol, adjusted to the appropriate pH with HCl, in a total volume of 1.56ml. Reactions were started by the addition of enzyme preparation, and incubation was for 30min. at 37°. Reactions were stopped by the addition of 0.3ml. of 25% (w/v) trichloroacetic acid, protein was removed by centrifugation and 0.5ml. of supernatant used for aminoacetone assay as previously described (Turner, 1966). When L-threonine was substituted for DL-aminopropanol, incubation was for 15min. and 0.1ml. of the deproteinized reaction mixture was used for aminoacetone assay. For measurement of DL-aminopropan-2-ol-dehydrogenase activity the buffers used were potassium phosphate (○), tris-HCl (●) and diethanolamine-HCl (△). For activity with L-threonine as the substrate the buffers used were tris-HCl (■) and diethanolamine-HCl (□).

5ml. samples were collected automatically at flow rates of approx. 12ml./hr. Absorption spectra of eluate samples were scanned between 225 and 300m μ , and extinction values at 260 and 280m μ were noted. Additional details are given in the legend to Fig. 2.

Preparation of optically active aminopropanol. L-(+)-Aminopropanol tartrate was prepared by the procedure of Sullivan (1963). Thus, in 100ml. of water was dissolved 77g. of L-(+)-tartaric acid, the solution was cooled in an ice bath, and 38.5g. of DL-1-aminopropan-2-ol was added slowly with stirring, care being taken to prevent a rise in temperature of the mixture above 50°. On cooling to room temp., i.e. approx. 22°, crystals of L-(+)-1-aminopropan-2-ol hydrogen L-(+)-tartrate dihydrate separated from the mother liquor. These were filtered off and dried as completely as possible on a sintered-glass funnel, and rapidly washed with ice-cold absolute ethanol. The yield was approx. 16g. After drying in warm air, some of the crystalline material was used to seed the crystallization of a larger batch. Large, perfectly formed crystals were obtained when required by slow cooling of the seeded solution. Values of

$[\alpha]_D^{25} + 33.4 \pm 1^\circ$ to $34.3 \pm 1^\circ$ (*c* 5 in water) were observed for such crystalline material. Partially resolved L-(+)-aminopropanol was obtained as the free base from preparations of the tartrate salt by the method of Sullivan (1963). Thus, to an aqueous solution of the crystalline tartrate was added $\text{Ca}(\text{OH})_2$ until the pH was 10.6. The resulting calcium tartrate was removed by filtration, and water removed by thin-film evaporation at reduced pressure. The free amine was finally distilled at $70\text{--}75^\circ/25\text{ mm}$. Hg. Such preparations exhibited $[\alpha]_D^{25} + 11 \pm 1^\circ$ to $+15 \pm 1^\circ$ (*c* 10 in water). Partially resolved D(-)-aminopropanol preparations were obtained by removing successive crops of aminopropanol tartrate during crystallization at temperatures down to 0° , and working-up such material and the mother liquor for the free amine as described above. Preparations exhibiting $[\alpha]_D^{25}$ values as low as $-5 \pm 1^\circ$ (*c* 10 in water) were obtained in this way.

Measurement of optical rotation and determination of optical resolution. Optical-rotation measurements were made on partially resolved L-aminopropan-2-ol preparations with an ETL-NPL Automatic Polarimeter, type 143A (National Physical Laboratory, Radlett, Herts.). Readings were converted into specific rotation values by calculation. Values obtained for the pure amino alcohol and aqueous solutions (10–100%, w/w, amino alcohol) were observed to be constant. The proportion of enantiomorphs present in optically active preparations was calculated from the value $[\alpha]_D^{25} - 25.5^\circ$ for the D(-)-enantiomorph (Karrer & Klarer, 1925), and the confirmatory data of Sullivan (1963), indicating that the enantiomorphs pairs exhibited equal and opposite optical rotations.

Assay of aminopropanol. The amino alcohol concentration of aqueous solutions of L-aminopropan-2-ol, containing approx. 1% (w/v), was determined by potentiometric titration against standard 0.1N-HCl. Aminopropanol is a moderately strong base, having pK_a approx. 9.5, the titration curve exhibiting a sharp inflexion at about pH 7. Dilute aqueous solutions of the amino alcohol, containing 0.5–3.0 $\mu\text{moles/ml}$, were assayed colorimetrically as the 2,4-dinitrophenyl derivative (A. D. Smith & J. B. Jepson, personal communication). To 1 ml. of aminopropanol solutions was added 1 ml. of reagent (0.25M-2,4-dinitrobenzenesulphonic acid in sat. sodium tetraborate, washed four times with chloroform), and the mixtures were heated for 40 min. at 100° in stoppered tubes. After cooling, the dinitrophenyl derivative was extracted into 4 ml. of benzene and the extinction read at $350\text{ m}\mu$ against a blank. Calibration curves showed that in the above procedure 0.1 μmole of aminopropanol gave $E_{350\text{ m}\mu}^{1\text{ cm}}$ 0.320, corresponding to molar extinction coefficient (ϵ) 1.28×10^4 .

Chemicals. DL-1-Aminopropan-2-ol, a number of aminopropanol analogues, L-(+)-tartaric acid and L-serine were obtained from British Drug Houses Ltd., Poole, Dorset. Other aminopropanol analogues, and ox-heart L-lactate-NAD oxidoreductase (EC 1.1.1.27) (lactate dehydrogenase) (75 units/mg.) as a suspension in aq. 50%-saturated $(\text{NH}_4)_2\text{SO}_4$, were obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks. NAD^+ and NADP^+ were supplied by C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany; DL-2-hydroxy-2-phenylethylamine hydrochloride by California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A.; 2'-aminoacetophenone hydrochloride by Aldrich Chemical Co. Inc., Milwaukee, Wis., U.S.A.; Bio-Gel P-300 by Bio-Rad Laboratories, Richmond, Calif.,

U.S.A.; 2,4-dinitrobenzenesulphonic acid (sodium salt) by Eastman Organic Chemicals, Rochester, N.Y., U.S.A. DL-1-Aminobutan-2-ol (oxalate salt) was a gift from Dr A. D. Smith. All other chemicals were of A.R. grade, or the highest quality available commercially.

RESULTS

Experiments with washed cell-suspensions

Examination of *E. coli* for evidence indicating the further metabolism of aminoacetone. The micro-organism was found to be incapable of growth on simple synthetic media (see the Experimental section) in which DL-aminopropanol served as sole source of nitrogen, and glycerol, lactate, pyruvate, succinate, malate or fumarate served as major sources of carbon. With fumarate and succinate media, aminopropanol concentrations ranging from 0.1 to 3.0 g./l. were tested. With others the concentration used was 1.8 g./l., and the concentration of the major carbon source was 8 g./l. When ammonium sulphate replaced aminopropanol in these media, vigorous growth was observed in all cases, and washed-cell suspensions were capable of oxidizing aminopropanol to aminoacetone (see Table 1). The presence of up to 3 g. of DL-aminopropanol/l. did not inhibit the growth of *E. coli* on nutrient broth, and such growing cultures rapidly oxidized the amino alcohol to aminoacetone.

Table 1. *Effect of growth on simple synthetic media on aminopropanol oxidation by washed-cell suspensions*

E. coli was grown on mineral salts medium plus 0.8% (w/v) of the compounds indicated. Media were prepared as described in the Experimental section. After growth, cells were harvested, washed and resuspended in phosphate buffer, pH 6.4. Oxidation of aminopropanol to aminoacetone was measured by incubating approx. 3 mg. dry wt. of cells with 0.5 m-mole of DL-1-aminopropan-2-ol in a total volume of 10 ml. in 0.1M-potassium phosphate buffer, pH 6.4. After 90 min. incubation, with shaking, 1 ml. of 25% (w/v) trichloroacetic acid was added, cells were removed by centrifugation, and 0.5 ml. of supernatant was used for aminoacetone assay (Turner, 1966).

Source of carbon	Aminoacetone production ($\mu\text{moles/mg. dry wt. of cells/min.}$)
Fumaric acid	4.50
DL-Malic acid	4.47
DL-Lactic acid	4.05
Succinic acid	4.00
Pyruvic acid	3.20
Glycerol	2.48
Maltose	1.07
Glucose	0.94
Lactose	0.89

Table 2. *Effect of some metabolic inhibitors, substrate analogues and alcohols on 1-aminopropan-2-ol oxidation by cell suspensions*

Aminopropanol oxidation to aminoacetone was measured as described for Table 1. Additions were made to cell suspensions in buffer approx. 15 min. before aminopropanol. Additions were omitted from control reaction mixtures. Cells were grown on nutrient broth in all cases.

Addition	Concn. of addition (mm) ...	Aminoacetone production (% of control)			
		125	25	10	2
Sodium arsenite	—	—	76	81	86
Sodium arsenate	—	—	89	92	108
Sodium fluoride	—	—	97	97	108
Phlorrhizin	—	—	—	93	97
Fluoroacetate	—	—	50	78	78
Iodoacetate	—	—	34	32	30
2,4-Dinitrophenol	—	—	46	52	86
DL-2-Hydroxy-2-phenylethylamine	—	—	73	—	—
DL-1,3-Diaminopropan-2-ol	—	—	75	—	—
DL-Serine	—	—	85	—	—
L-1-(3,4-Dihydroxyphenyl)-2-aminoethanol	—	—	86	—	—
Ethanol	115	113	—	—	—
Propan-1-ol	117	115	—	—	—
Propan-2-ol	110	107	—	—	—
Butan-1-ol	101	110	—	—	—
Butan-2-ol	70	82	—	—	—
Pentan-1-ol	66	83	—	—	—

A number of metabolic inhibitors were tested for their possible stimulatory effect on the yield of aminoacetone formed from aminopropanol by washed suspensions of cells grown on nutrient broth. It was known that each of the inhibitors tested had such an effect when suspensions of a pseudomonad grown on aminopropanol were used, due to the inhibition of aminoacetone utilization (I. J. Higgins & J. M. Turner, unpublished work). The results obtained (see Table 2) showed no such effect, but suggested in some cases that either the permeability of the intact cells or the activity of the aminopropanol dehydrogenase involved was decreased by the inhibitor.

Effect of intermediary metabolites and substrate analogues on aminopropanol oxidation by washed-cell suspensions. None of 34 common intermediary metabolites tested, including sugars, amino acids and mono-, di- and tri-carboxylic acids, affected the rate of aminoacetone formation from aminopropanol. Additions were present at the same concentration as aminopropanol, i.e. 25 mm. L-Threonine was tested over the range 10–50 mm.

Of a range of aminopropanol analogues tested, only DL-2-hydroxy-2-phenylethylamine, DL-1,3-diaminopropan-2-ol, DL-serine and L-1-(3,4-dihydroxyphenyl)-2-aminoethanol (noradrenaline) were inhibitory when used at the same concentration as aminopropanol (see Table 2). Inactive analogues were DL-1,2-diaminopropane, DL-propane-1,2-diol, DL-4-amino-3-hydroxybutyrate, 3-aminopropan-1-

ol, 1-aminopropane, 2-aminoethanol, DL-1-amino-propane-2,3-diol, propan-2-ol, L-2-aminopropan-1-ol, DL-3-hydroxybutyrate and DL-3-phenylserine. None of the amino alcohols tested was active as a substrate on the basis of amino ketone formation.

To test the possibility that aminopropanol-dehydrogenase activity was due to an alcohol dehydrogenase known to be present in *E. coli* (Still, 1940; Dawes & Foster, 1956), and possibly of broad substrate specificity, a series of primary and secondary alcohols was tested at two concentrations for inhibitory action. The results (see Table 2) showed that lower alcohols, including propan-2-ol, increased rather than inhibited aminopropanol oxidation by cell suspensions. Higher alcohols, including butan-2-ol, caused some inhibition.

Experiments with cell-free extracts

Effect of pH on aminopropanol- and threonine-dehydrogenase activities. The previous observation that growth in the presence of various metabolic repressors had less effect on the aminopropanol-dehydrogenase activities of washed cells than on the activities of cell-free extracts measured at pH 7 (Turner, 1966) prompted a re-examination of activity in such extracts. The results are shown in Fig. 1. It can be seen that two activity peaks were found, the smaller at pH 7.0–7.2 corresponding to that previously reported (Turner, 1966), and a second at pH 10. Threonine-dehydrogenase activity

was found to be optimum at pH 9.6 (Fig. 1). With both enzymes, activity in the alkaline region was inhibited by sodium carbonate-bicarbonate buffers.

Relative rates of aminoacetone formation from aminopropanol and threonine. To obtain (kinetic) evidence on the identity or otherwise of the enzymes responsible for aminopropanol and threonine oxidation at pH 9.6–10.0, relative rates of activity with each substrate and a mixture of the two were measured at saturating substrate concentrations. Measurements were carried out at pH 9.6 and at a range of extract concentrations to ensure that values representing initial rates were available. In all cases activities with mixed substrates were additive rather than intermediate. The average relative rates of aminoacetone formation from aminopropanol, threonine and a mixture of the two were approx. 5.7, 50.0 and 63.5 $\mu\text{moles/mg. of protein/min.}$ respectively. These results suggested that distinct and specific enzymes were involved.

Separation of amino alcohol dehydrogenases by gel filtration. Gel filtration of crude cell-free extracts on Bio-Gel P-300 (exclusion limit for proteins approx. 300 000 mol. wt.), under the conditions described in the Experimental section, permitted almost complete separation of aminopropanol- and threonine-dehydrogenase activities measured at pH 10. The results of one experiment are shown in Fig. 2. The aminopropanol dehydrogenase detected appeared to be an enzyme of relatively high molecular weight, emerging from the gel immediately after the nucleoprotein peak, whereas threonine dehydrogenase was concluded to be a somewhat smaller molecule. Gel filtration alone resulted in appreciable purification of both enzymes. Specific activities for the peak fractions shown in Fig. 2 were approx. 107 and 500 $\mu\text{moles of aminoacetone formed/mg. of protein/min.}$ from DL-aminopropanol and L-threonine respectively.

Effect of substrate concentration on dehydrogenase activities. Michaelis constants for DL-aminopropanol were obtained at both pH 9.6 and 10.0, by using the graphical method of Lineweaver & Burk (1934). Values of approx. 10 and 5 mM respectively were obtained. Aminopropanol concentration was calculated on the basis of single enantiomorphs, it being assumed that the dehydrogenase was stereospecific for one isomer (see below). The K_m values for L-threonine were found to be approx. 5 mM at both pH 9.6 and 10.0.

Effect of coenzyme concentration on aminopropanol and threonine dehydrogenases. Preliminary experiments established that NAD^+ was more active than NADP^+ for both enzymes at pH 9.6. Initial rates of aminoacetone formation from both amino alcohol substrates were measured at NAD^+ concentration 0.06–12.40 mM. The K_m values for NAD^+ , obtained by the graphical procedure of Lineweaver & Burk

Fig. 2. Separation of 1-aminopropan-2-ol and threonine dehydrogenases by gel filtration. The column of gel was prepared, the sample of cell-free extract applied and samples of eluate were collected as described in the Experimental section. Extinction values of eluate samples were measured at 260 $\text{m}\mu$ (▲) and 280 $\text{m}\mu$ (Δ), and are indicated by broken lines. Dehydrogenase activities with DL-1-aminopropan-2-ol and L-threonine were measured at pH 10, as described for Fig. 1 except that incubations were for 60 and 15 min. respectively. Activities are expressed as the extinction values recorded during the colorimetric assay of aminoacetone formed from DL-1-aminopropan-2-ol (●) and L-threonine (○).

(1934), were 0.40 and 0.08 mM in the aminopropanol- and threonine-dehydrogenases systems respectively. The fivefold difference between these values for the coenzyme supported the finding that different apoenzymes were involved. At pH 7, the K_m for NAD^+ with aminopropanol as substrate was found to be approx. 0.05 mM.

Effect of chelating and thiol reagents on dehydrogenase activities. The pattern of inhibition of aminopropanol and threonine dehydrogenases by a variety of chelating agents and thiol reagents was remarkably similar. Whereas EDTA and 2,2'-bipyridyl, at 5.0 and 0.2 mM respectively, were non-inhibitory, 8-hydroxyquinoline at 0.2 mM inhibited both enzymes by approx. 50% and at 2.5 mM caused 90–100% inhibition. Iodoacetate at 5.0 mM inhibited both enzymes by 20–25% (cf. 70% inhibition of aminopropanol oxidation by intact cells, Table 2) and 3-chloromercuribenzoate at concentrations above 0.05 mM caused complete

Table 3. *Activity and inhibitory effects of 1-aminopropan-2-ol analogues with cell-free extracts*

Reaction mixtures contained the components listed for Fig. 1, diethanolamine-HCl, pH 10.0, being the buffer system, plus an NAD⁺-regenerating system consisting of 0.1 mg. of lactate dehydrogenase and 0.15 m-mole of sodium pyruvate. When additions were racemic mixtures of the type indicated, 0.2 m-mole was present. With non-racemic compounds, 0.1 m-mole was added. Activities were assayed as described for Fig. 1, and are expressed relative to that obtained with DL-1-aminopropan-2-ol in the absence of any addition. With DL-phenylserine and DL-1-aminobutan-2-ol, relative activities are expressed on the basis of the colour yields observed during the colorimetric assay of amino ketones as for aminoacetone.

Substrate	Addition	Activity (%)
DL-1-Aminopropan-2-ol	None	100
	DL-2-Hydroxy-2-phenylethylamine	52
	DL-1-Aminobutan-2-ol	74
	DL-1-Aminopropane-2,3-diol	75
	DL-Serine	75
	DL-Phenylserine	85
	L-Aminopropane	90
	3-Aminopropan-1-ol	93
	Propan-2-ol	93
	L-2-Aminopropan-1-ol	94
	DL-4-Amino-3-hydroxybutyric acid	95
	DL-3-Hydroxybutyric acid	98
	Ethanolamine	99
DL-Phenylserine	None	109
DL-1-Aminobutan-2-ol	None	46

inhibition in both cases. These results were obtained after preincubation of the reagents with standard reaction mixtures, complete except for the amino alcohol substrate and the NAD⁺-regenerating system, for approx. 5 min. at 37°. Incubations were carried out for an additional 15 min. after the addition of aminopropanol or threonine.

Effect of substrate analogues on aminopropanol-dehydrogenase activity at pH 10. In view of the inhibitory effect of some substrate analogues on aminopropanol oxidation by intact cells (see Table 2), their effect on aminopropanol-dehydrogenase activity in cell-free extracts at pH 10 was investigated. Those analogues potentially capable of giving rise to α -amino ketones were also tested for activity as substrates. The results are given in Table 3. DL-2-Hydroxy-2-phenylethylamine and DL-serine were both more inhibitory in the cell-free system than with intact cells. DL-1-Aminobutan-2-ol also appeared to be inhibitory. Whereas DL-1-aminopropane-2,3-diol was non-inhibitory in the washed-cell system, it was effective as an inhibitor of activity in the cell-free extract. Of the compounds tested for activity as substrates, only DL-3-phenylserine and DL-1-aminobutan-2-ol gave rise to products reacting as amino ketones. In the former case this was in contrast with the result obtained with washed-cell suspensions. On the basis of the colour yield, phenylserine was as active as aminopropanol, although it was not determined whether the compound was acting as the substrate of aminopropanol rather than threonine dehydro-

genase. It was assumed that the product of the reaction was 2-amino-3-oxo-3-phenylpropionate, and that this compound, by analogy with 2-amino-3-oxobutyrate and 2-amino-3-oxoadipate (Laver, Neuberger & Scott, 1959*a,b*), decarboxylated spontaneously and rapidly to 2'-aminoacetophenone. If this assumption was correct, then rates with phenylserine and aminopropanol were comparable. It was found that substitution of 2'-aminoacetophenone for aminoacetone in the amino ketone assay procedure gave virtually the same colour yield. It was also assumed (see Table 3) that 1-aminobutan-2-one gave the same colour yield as aminoacetone in the assay.

Stereospecificity of aminopropanol dehydrogenase. Partially resolved preparations of aminopropanol, obtained by the method of Sullivan (1963) as described in the Experimental section, were compared for activity as substrates. Preliminary experiments with racemic DL-aminopropanol and an optically active preparation containing approx. 75% of the L-enantiomorph ($[\alpha]_D^{25} + 12.6^\circ$, *c* 10 in water) revealed that the latter was more active. At 20 mm with respect to total aminopropanol (irrespective of the relative amounts of each enantiomorph present), the predominantly L-form was 45% more active than the racemic mixture at pH 7, and 30% more active at pH 10.

The substrate activities of a number of partially resolved aminopropanol preparations were compared, over the range 3.3–33.3 mm, with respect to both enantiomorphs at pH 10. The results are

Fig. 3. Effect of the concentration of optically active aminopropanol preparations on dehydrogenase activity of cell-free extracts. Aminopropanol-dehydrogenase activities were measured at pH 10, and partially resolved aminopropanol preparations were obtained, as described in the Experimental section. The effect of substrate concentration, expressed as total aminopropanol (irrespective of the relative amounts of each enantiomorph present), was measured by using optically active preparations having the following $[\alpha]_D^{25}$ (c 10 in water) values: (a) $-4.6 \pm 1^\circ$ (41% L-), $\circ$; (b) $-3.3 \pm 1^\circ$ (43% L-), Δ ; (c) $-1.65 \pm 1^\circ$ (47% L-), $\bullet$; (d) 0° (50% L-), ∇ ; (e) $+9.5 \pm 1^\circ$ (69% L-), $\blacksquare$; (f) $+15.2 \pm 1^\circ$ (80% L-), $\square$. Activities are expressed in arbitrary units, based on readings taken during the colorimetric assay of aminoacetone. The apparent K_m values for aminopropanol calculated from the plots shown, and expressed with respect to the concentration of the L-enantiomorph present, were 6.5, 3.0, 2.0, 1.5, 1.5 and 1.4 mm respectively.

shown as double-reciprocal plots (Lineweaver & Burk, 1934) in Fig. 3. It can be seen that dehydrogenase activities with the various aminopropanol preparations varied widely, the activity increasing as the ratio of the L- to the D-enantiomorph increased, but that the V_{max} values in each case were approximately the same. When initial rates of aminoacetone formation were measured at the low aminopropanol concentration 3.3 mm, i.e. where the enzyme was not saturated with substrate and where activity was roughly proportional to substrate concentration, a twofold increase in the concentration of the L-enantiomorph resulted in a 3.7-fold increase in the rate of aminoacetone formation. Calculation of apparent Michaelis constants from the data included in Fig. 3 gave the values quoted in the legend. It can be seen that apparent K_m values for L(+)-aminopropanol decreased markedly as the concentration of the D(-)-enantiomorph decreased. These results suggested that not only was the enzyme stereospecific for the L-enantiomorph, but that the D-enantiomorph inhibited competitively. Similar results were obtained with selected crystals of L(+)-1-aminopropan-2-ol hydrogen L(+)-tartrate dihydrate as a direct source of highly resolved L-aminopropanol. Preliminary experiments showed that activity towards racemic aminopropanol was unaffected by potassium L(+)-tartrate at concentrations up to 150 mm. With L-aminopropanol tartrate as the substrate a Michaelis constant of approx. 1.5 mm was obtained by the procedure of Lineweaver & Burk (1934). This compared with the value of 1.4 mm obtained with a preparation of the free base containing approx. 80% of L-enantiomorph (see Fig. 3).

Evidence for the presence of D(-)-amino-

Table 4. Effect of storage and preincubation on the apparent stereospecificity of aminopropanol dehydrogenase

Non-dialysed cell-free extracts were stored and preincubated as indicated. Aminopropanol-dehydrogenase activities were measured at pH 10 as described in the Experimental section, except as noted. The 1-aminopropan-2-ol preparations used were optically inactive DL-racemate (12 mm with respect to single enantiomorphs), *dextro*-rotatory L-aminopropanol tartrate, having $[\alpha]_D^{25} + 33 \pm 1^\circ$ (c 5 in water) (12 mm with respect to aminopropanol), and *laevo*-rotatory D-aminopropanol, $[\alpha]_D^{25} - 4.6 \pm 1^\circ$ (c 10 in water), i.e. 60% of D-enantiomorph (12 mm). In all cases L(+)-tartrate was present at 12 mm, and substrate solutions were adjusted to pH 10 before use.

Storage conditions of cell-free extract	Aminoacetone formation from the aminopropanol preparations indicated (μ moles/mg. of protein/min.)		
	DL-	L-	'D-'
Frozen at -15° for 6 days	16.0	23.2	14.5
Dialysed overnight at 0° , then frozen at -15° for 5 days	8.0	9.7	7.5
Dialysed overnight at 0° , then held at $0-5^\circ$ for 5 days	2.3	0.3	3.0
Dialysed overnight at 0° , then frozen at -15° for 5 days but preincubated at 37° for 15 min, immediately before addition of substrate	2.0	0.4	2.5

propanol-dehydrogenase activity, albeit at low levels, was obtained after the accidental use of a sample of cell-free extract which had been stored for several days at 0° rather than -15°. It was observed that the apparent stereospecificity of the enzyme preparation had become reversed, i.e. that activity was greater with D(-)- rather than L(+)-aminopropanol. A similar effect was noted when fresh enzyme preparations were preincubated at pH 10 in the absence of amino alcohol substrate for 15 min. at 37°. The results of one experiment are shown in Table 4. It can be seen, by comparing the extreme cases, that overnight dialysis at 0°, freezing to -15° followed by preincubation at 37° for 15 min. caused an eightfold fall in extract activity towards racemic DL-aminopropanol, whereas activity towards a D-preparation fell only 5.8-fold but activity towards the L-preparation fell nearly 60-fold. The results also suggested that appreciable enzyme activity was lost by overnight dialysis of freshly prepared extracts. Similar experiments showed that threonine-dehydrogenase activity was unaffected by overnight dialysis.

DISCUSSION

The work described above demonstrates conclusively that the oxidations of DL-1-aminopropan-2-ol and L-threonine are reactions catalysed by distinct and specific enzymes. Although the properties of the aminopropanol and threonine dehydrogenases described are similar in some respects, e.g. coenzyme specificity, pH optimum and pattern of inhibition by thiol and chelating agents, they differ with respect to the coenzyme concentration required for half-maximum velocity and their molecular weights. Widely different Michaelis constants for NAD⁺ with DL-aminopropanol and L-threonine as substrates suggests that different apoenzymes are involved, and additional kinetic evidence showed that activities with mixed amino alcohol substrates were additive rather than intermediate. The almost complete separation of the two dehydrogenases by gel filtration suggests a significant difference in their molecular weights, that for aminopropanol being greater than that for threonine, and demonstrates unequivocally their distinct and separate nature.

A high degree of specificity for the enzymic oxidation of DL-1-aminopropan-2-ol is evidenced by the fact that a wide variety of substrate analogues, with the exception of DL-2-hydroxy-2-phenylethylamine, had little or no inhibitory effect when either intact cells or cell-free extracts were examined. DL-1-Aminobutan-2-ol was the only analogue found to be active as a substrate. In aminopropanol oxidation by washed cells, optimum at pH 6.4 (Turner, 1966), the question arises

whether the reaction is mediated by the enzyme in cell-free extracts previously studied at pH 7 (Turner, 1966) or by the enzyme of optimum activity at pH 10, and whether or not there are two distinct enzymes. Apart from preliminary evidence, based on the effect of growth conditions on enzyme activities suggesting that two enzymes are involved (Higgins, Pickard, Turner & Willetts, 1966), it may be noted that K_m values for NAD⁺ 0.40 and 0.05 mM were found for the enzymes active at pH 9.6 and 7.0 respectively. Together with the very wide pH range over which aminopropanol is rapidly oxidized by cell-free extracts, this supports the suggestion that two different enzymes are responsible, although further studies are required before this can be asserted with complete confidence.

The stereospecificity of 1-aminopropan-2-ol oxidation is of particular interest in view of the natural occurrence of the D-enantiomorph as part of the molecule of vitamin B₁₂ (Wolf, Jones, Valient & Folkers, 1950). From the present work it is clear that the L-enantiomorph is highly active as a substrate for oxidation to aminoacetone by cell-free extracts of *E. coli* at both pH 7 and pH 10. Detailed kinetic evidence obtained at pH 10 with a variety of partially resolved aminopropanol preparations, and freshly prepared cell-free extracts, indicates that the oxidation of aminopropanol is stereospecific in favour of the L(+)-enantiomorph, that D(-)-aminopropanol inhibits this reaction and that this inhibition is competitive. These results are complicated, however, by the finding that relatively low D(-)-aminopropanol-dehydrogenase activity is also exhibited by cell-free extracts, and that such activity is relatively important in 'aged' preparations. The possibility exists that the L- and D-stereospecific aminopropanol dehydrogenases observed are both competitively inhibited by each other's substrates. Although the main conclusions are clear-cut, rigorous kinetic studies of the enzymes involved must await the availability of optically pure preparations of the amino alcohol substrate.

Useful comparisons can be made between the 1-aminopropan-2-ol-dehydrogenase activity exhibited by crude extracts of *E. coli* at pH 10 and a highly active enzyme preparation purified from a species of pseudomonad grown on DL-aminopropanol as sole source of carbon and nitrogen (Higgins *et al.* 1966; Pickard, Higgins & Turner, 1966). The latter enzyme has optimum activity at pH 9.6, is slightly more active with NADP⁺ than NAD⁺, is stereospecific for L(+)-aminopropanol, and is virtually inactive with substrate analogues including L-threonine but excepting DL-1-aminobutan-2-ol and DL-2-hydroxy-2-phenylethylamine. The last-named compound, found to be an inhibitor but not a substrate of the enzyme studied in *E. coli*,

is readily oxidized by the pseudomonad enzyme. The oxidation of aminopropanol and hydroxyphenylethylamine by this enzyme is readily reversible, both aminoacetone and 2'-aminoacetophenone being reduced at optimum approx. pH 5 (Pickard *et al.* 1966). Thus, although differences exist, the L-aminopropanol dehydrogenases of *E. coli* and the *Pseudomonas* sp. appear to be similar with regard to amino alcohol substrate specificity.

Turning to the L-threonine dehydrogenase present in *E. coli*, the properties of this enzyme can be compared with those of the enzymes examined in two other micro-organisms. The pH optimum of 9.6 is somewhat higher than the reported values of 8.8 for *Rhodospseudomonas spheroides* (Neuberger & Tait, 1962) and 8.4 for *Staphylococcus aureus* (Green & Elliott, 1964). The K_m for L-threonine of 5 mM contrasts with the value 74 mM found by Neuberger & Tait (1962) but is similar to that of 8.7 mM reported by Green & Elliott (1964). In all three cases the K_m for NAD⁺ was similar, being approx. 0.1 mM. Threonine-dehydrogenase activity has also been detected in cell-free extracts of *Saccharomyces cerevisiae* (Krauze, Kagan, Yakovleva & Kretovich, 1965), *Corynebacterium erythrogenes*, *Bacillus subtilis* and *Streptococcus faecalis* (Green & Elliott, 1964). The conversion of L-threonine into aminoacetone by washed-cell suspensions of a variety of micro-organisms, including *Aeromonas*, *Sarcina* and a number of *Bacillus* species, has also been reported (Turner, 1965).

The linked metabolic roles of L-threonine and DL-1-aminopropan-2-ol dehydrogenases, whereby aminopropanol may be formed from threonine via aminoacetone, have been considered and discussed previously (Neuberger & Tait, 1960; Neuberger, 1961; Neuberger & Tait, 1962; Turner, 1966; Turner & Willetts, 1967). The finding in the present study that D(-)-aminopropanol-dehydrogenase activity is present in fresh extracts of *E. coli* supports the suggestion put forward by these authors that aminoacetone is an intermediate in the observed incorporation of isotopically labelled L-threonine into the D(-)-aminopropanol fragment of vitamin B₁₂ (Krasna, Rosenblum & Sprinson, 1957). Mutant strains of *Nocardia rugosa* have been isolated by DiMarco, Marnati, Migliacchi, Rusconi & Spalla (1962) which introduce the aminopropanol group into the vitamin molecule, and currently available evidence suggests that aminopropanol is incorporated as such by linkage to a cohyrnic acid amide moiety (see Wagner, 1966). The possibility that threonine is incorporated and later decarboxylated has been excluded for *E. coli* and *Propionibacter shermanii*, with the threonine analogue of cobinamide, by Bernhauer & Wagner (1961). Both this and the threonine O-phosphate analogue (Bernhauer & Wagner, 1962)

were shown to be inactive as precursors of vitamin B₁₂.

The finding of appreciable L(-)-aminopropanol-dehydrogenase activity in microbial extracts, together with dehydrogenase activity towards DL-aminopropanol in rat liver preparations (Higgins *et al.* 1966; Turner & Willetts, 1967), suggests that L-aminopropanol has a role other than in the biosynthesis of vitamin B₁₂, although the L-aminopropanol analogue of vitamin B₁₂ is fully active for *Ochromonas malhamensis* and has 60% of the activity of the natural form for *E. coli* (Heinrich, Friedrich & Riedel, 1961). It has been suggested that the L-amino alcohol may function as a phosphatidyl base of minor occurrence (Turner & Willetts, 1967), although there appears to be no evidence of its presence in phospholipids of microbial origin.

No indication has been obtained in the present study that aminoacetone is further catabolized by *E. coli*, e.g. via methylglyoxal, D-lactate, pyruvate and acetyl-CoA, by the route proposed by Elliott (1959, 1960a). The fact that the micro-organism is incapable of growth on media containing aminopropanol as sole source of nitrogen suggests that aminoacetone is not deaminated, although the possibility exists that any methylglyoxal produced might cause growth inhibition. The generation of aminoacetone *in vitro*, which occurs when *E. coli* is grown on nutrient broth in the presence of aminopropanol, does not inhibit such growth, however. Whereas aminoacetone is readily deaminated to methylglyoxal by mammalian blood plasma (Elliott, 1960b; Buffoni & Blaschko, 1963) and other tissues (Urata & Granick, 1961, 1963; Green & Elliott, 1964), it has not been established until recently that aminoacetone catabolism occurs in micro-organisms. Green & Elliott (1964) were unable to detect any disappearance of aminoacetone during its incubation with extracts of a variety of micro-organisms, including *E. coli*, although fresh homogenates of *Streptococcus faecalis* and *Corynebacterium erythrogenes* had slight activity. It is now known, however, that washed suspensions of pseudomonads grown on DL-1-aminopropan-2-ol rapidly utilize aminoacetone (Higgins & Turner, 1966) and are capable of growth on the amino ketone (Pickard *et al.* 1966). The finding that metabolic inhibitors, which prevent utilization of aminoacetone by such pseudomonads, had no stimulatory effect on aminoacetone formation from aminopropanol by *E. coli*, strengthens the conclusion that the further catabolism of this compound does not occur. It thus seems likely, in *E. coli* at least, that the sole metabolic function of aminoacetone is as a precursor of D- and L-aminopropanol. The report that a variety of aminoacetone analogues of the type R·CO·CH₂·NH₂ are potent inhibitors

of microbial growth, including the growth of *E. coli* (Cheng, Jonsson & Semeniuk, 1962), adds additional weight to the suggestion that the amino ketone is of biosynthetic importance.

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