Purification and Properties of L-1-Aminopropan-2-ol: NAD Oxidoreductase from a Pseudomonad Grown on DL-1-Aminopropan-2-ol

By M. A. PICKARD, I. J. HIGGINS AND J. M. TURNER

Department of Biochemistry, University of Liverpool

(Accepted for publication 11 June 1968)

SUMMARY

Two pseudomonad strains grown on DL-1-aminopropan-2-ol as sole source of carbon + nitrogen provided rich sources of L(+)-1-aminopropan-2-ol:NAD⁺ oxidoreductase. The activity of this enzyme in crude extracts of *Pseudomonas* sp. strain NCIB 8858 was about 400 mμmoles aminoacetone formed/mg. protein/min., under optimum conditions. Growth on media containing other carbon sources commonly repressed enzyme formation. Enzyme formation was induced by incubating succinate-grown bacteria in media containing DL-1-aminopropan-2-ol, aminoacetone or L-threonine; but induction was inhibited by DL-2-hydroxy-2-phenylethylamine or DL-2-phenylserine. The enzyme was purified twenty-fold by treatment with protamine sulphate and ammonium sulphate followed by gel-filtration. The molecular weight of the enzyme was estimated to be 70 to 80 thousand. The partly purified enzyme was optimally active at pH 9.5. The K_m values for DL-1-aminopropan-2-ol and NAD⁺ were about 0.1 and 0.3 mM, respectively. Activity with NADP⁺ as the coenzyme was about the same as that with NAD⁺, the K_m being about 0.2 mM. The enzyme was inactive with α -NAD⁺. Activity was unaffected by a variety of thiols, thiol-alkylating and metal-chelating agents. The enzyme was virtually inactive with twenty-one potential substrates but oxidized DL-1-aminobutan-2-ol, DL-2-hydroxy-2-phenylethylamine and DL-phenylserine at significant rates and DL-1,3-diaminopropan-2-ol and DL-3-hydroxybutyrate slowly. Enzyme activity towards 1-aminopropan-2-ol was stereospecific for the L(+) isomer, which was oxidized about six times more rapidly than the racemic substrate. The D(–)-enantiomorph was a competitive inhibitor of the reaction, K_i = about 0.3 mM, and no dehydrogenase activity towards this isomer was observed with either purified enzyme preparations or crude cell-free extracts. Aminoacetone-dependent oxidation of NADH occurred optimally at pH 5 in acetate buffer; a second peak of activity was found at pH 8.4 in diethanolamine + HCl buffers. Several buffers appeared to inhibit activity at pH 7.5. 2'-Aminoacetophenone was active as a substrate, but 5-aminolaevulate had little activity. NADPH was also active as coenzyme.

INTRODUCTION

Several micro-organisms are capable of oxidizing DL-1-aminopropan-2-ol to aminoacetone (Turner, 1965) and dehydrogenase activities towards this compound have been studied in cell-free extracts of *Escherichia coli* (Turner, 1966*b*; 1967). Evidence has been obtained that when various pseudomonads are grown on the amino alcohol, as sole source of carbon and nitrogen, aminoacetone is the metabolic intermediate formed initially and can serve as an alternative growth substrate (see

Higgins, Pickard & Turner, 1968). The work described in the present paper shows that certain pseudomonads grown on DL-1-aminopropan-2-ol possess large concentrations of an L(+)-1-aminopropan-2-ol:NAD oxidoreductase. The properties of this enzyme are of interest in comparison with those of the similar enzyme in *Escherichia coli* (Turner, 1966, 1967). Interest in the metabolism of the amino alcohol via the amino ketone stems from the fact that the route of aminoacetone catabolism by micro-organisms is uncertain. The amino ketone is known to be a microbial metabolite of L-threonine (Elliott, 1958; see Turner, 1966, 1967; Higgins *et al.* 1968). Some of this work has been described in preliminary communications (Higgins, Pickard, Turner & Willetts, 1966; Pickard, Higgins & Turner, 1966).

METHODS

Micro-organisms. The sources, maintenance and growth of the micro-organisms used were as described by Higgins *et al.* (1968). Additional details are given here in the text.

Cell-free extracts. Extracts were prepared by using an Aminco-French pressure cell (American Instrument Co. Inc., Silver Spring, Md., U.S.A.) as previously described for extracts of *Escherichia coli* (Turner, 1966).

Measurement of aminopropanol-dehydrogenase activity by colorimetric methods. These were modifications of the method described by Turner (1966). In the case of partly purified enzyme, reaction mixtures contained 0.1 m-mole diethanolamine + HCl buffer (pH 9.5), 0.15 m-mole sodium pyruvate, 2 μ moles NAD⁺, 0.1 mg lactate dehydrogenase, 10 μ g. protein (enzyme preparation) and 20 μ mole SDL-1-aminopropan-2-ol, adjusted to pH 9.5 with HCl, in a total volume of 1.0 ml. The racemic amino alcohol substrate was replaced by partly resolved preparations where indicated in the text. When crude cell-free extracts were used, the reaction mixture was modified as shown in Table 1. Reactions were started by the addition of enzyme preparation to the other components in thin-walled tubes equilibrated at 37°. Incubations were for 10 and 30 min. for purified enzyme and crude extracts, respectively. Reactions were stopped by adding 0.25 ml. of 25 % (w/v) trichloroacetic acid. After centrifugation to remove precipitated material, 1.0 ml. of the supernatant fluid was used for aminoacetone assay.

Measurement of aminopropanol-dehydrogenase activity by a spectrophotometric method. The 1-aminopropan-2-ol-dependent reduction of NAD⁺ was measured by following the change in extinction of reaction mixtures at 340 m μ by using a Unicam SP. 800 Recording Spectrophotometer (Unicam Instruments Ltd., Cambridge) equipped with the necessary accessories for enzyme kinetic work. The scale expansion unit was linked to a Kent Mark 3 potentiometric slave recorder (George Kent Ltd., Luton, Beds.). Reaction mixtures contained 0.3 m-mole diethanolamine + HCl buffer (pH 9.5), 6 μ moles NAD⁺, 0.1 mg. protein (partially purified enzyme), and 75 μ moles of amino alcohol substrate, in a total volume of 3 ml. The concentration of substrates was calculated for single enantiomorphs. Reactions were started by adding enzyme to otherwise complete reaction mixtures equilibrated at 25°. For the measurement of the aminoacetone-dependent oxidation of NADH, the reaction mixture contained 66 μ moles of sodium acetate + acetic acid buffer (pH 5), 0.3 μ moles NADH, 0.2 mg. protein (partially purified enzyme) and

2 μ moles aminoacetone, in a total volume of 3 ml. Changes in extinction at 30° were followed by using the equipment described above. Extinction changes were related to changes in coenzyme concentration by using the value 6.22×10^3 for the molar extinction coefficient, ϵ , for NADH (Horecker & Kornberg, 1948).

Enzyme activities are expressed as m μ moles of aminoacetone or NADH produced, or NADH oxidized, per mg. protein/min.

Assay of aminoacetone and protein. Assays were made as described previously (Turner, 1966).

Purification of L-I-aminopropan-2-ol-dehydrogenase. All purification steps were done at 0–5°.

(a) *Protamine sulphate treatment.* To 100 ml. cell-free extract containing 25 mg. protein/ml. were slowly added 80 ml. 1% (w/v) aqueous solution of protamine sulphate, with gentle stirring which was continued for 15 min. after the final addition. The 'stringy' precipitate was removed by centrifugation at 10,000g for 15 min. in a refrigerated centrifuge and discarded.

(b) *Ammonium sulphate fractionation.* To 100 ml. of extract treated with protamine sulphate was slowly added 20.3 g. solid (NH₄)₂SO₄ with gentle stirring. The amount of salt added corresponded to about 30% of saturation at 0°. After 15 min. further stirring, precipitated material was removed by centrifugation in the cold and discarded. More (NH₄)₂SO₄ (6.2 g.) was then added, as described above, to give about 40% of saturation. Precipitated material was collected by centrifugation, and dissolved in 10 ml. 0.1 M-potassium phosphate buffer (pH 7); the supernatant fluid was discarded.

(c) *Gel filtration.* Exclusion chromatography was done on polyacrylamide gel. To 250 ml. of 0.1 M-potassium phosphate buffer (pH 7) was added 5.5 g. Bio-Gel P-300. After vigorous stirring, followed by equilibration at 0–5° for 24 hr, the gel was packed under low hydrostatic pressure in a glass column (2.54 cm. $\times$ 36.0 cm.). The column of gel was then washed with a continuous flow of buffer for a further 48 hr. Samples of (NH₄)₂SO₄-fractionated extract (about 130 mg. protein in 10 ml. buffer) were applied to the column; elution was effected with 0.1 M-potassium phosphate buffer (pH 7), and 5 ml. samples of effluent collected. Fractions containing enzyme activity were pooled, unless otherwise stated, and protein was precipitated by adding an equal volume of saturated (NH₄)₂SO₄ solution. The precipitate was collected by centrifugation, dissolved in buffer to about 1.0 mg. of protein/ml. and stored in 0.5 ml. portions at –15° until required.

Other details of the purification are given in Table 3 and Fig. 3.

Estimation of molecular weight. Gel-filtration was done by the method of Andrews (1965) with Sephadex G. 200 polydextran gel. The column of gel was calibrated with catalase, bovine serum albumin, cytochrome-c and alcohol dehydrogenase. Detection of reference proteins in column eluate samples was by the methods of Chance & Maehly (1955) for catalase, Racker (1955) for alcohol dehydrogenase, and also by the spectrophotometric measurement of extinction at 280 m μ .

Chemicals. The sources of all chemicals were those previously described by Turner (1967) and Higgins *et al.* (1968), except for α -NAD⁺ which was obtained from C. F. Boehringer and Soehne G.m.b.H., Mannheim, Germany, and protamine sulphate (from salmon milt) which was bought from British Drug Houses Ltd., Poole, Dorset.

RESULTS

l-Aminopropan-2-ol dehydrogenase activity in cell-free extracts of *pseudomonads*

Extracts of three *pseudomonads* previously found to be capable of growth on DL-1-aminopropan-2-ol (Higgins *et al.* 1968) were examined for dehydrogenase activity towards this compound after growth on various media. The results are shown in Table 1*a*. It can be seen that in the cases of *Pseudomonas* sp. strains NCIB 8858

Table 1. *Effect of growth conditions on l-aminopropan-2-ol and threonine dehydrogenase activities in cell-free extracts of pseudomonads*

The bacteria were grown in nutrient broth (2.5 %, w/v, Oxoid No. 2) or in simple defined media (Higgins *et al.* 1968) containing DL-1-aminopropan-2-ol, or succinic acid (5 g./l.) + (NH₄)₂SO₄ (1.7 g./l.), as carbon and nitrogen sources. The results in the second part of the table were obtained by using simple defined media containing the compounds shown (4 g./l.) as major sources of carbon, with DL-1-aminopropan-2-ol (1 g./l.) as sole source of nitrogen.

Enzyme activities in extracts were measured by using reaction mixtures of the following composition: 0.2 m-mole diethanolamine + HCl buffer (pH 9.6); 0.15 m-mole sodium pyruvate; 5 μ moles NAD⁺; 0.1 mg. lactate dehydrogenase; equiv. 1.0 mg. protein of cell-free extract; 125 μ moles either DL-1-aminopropan-2-ol (pH 9.6) or L-threonine; total volume of 1.4 ml. The results shown were obtained in two separate experiments.

<i>Pseudomonas</i> strain	Growth medium	Enzyme activity with substrate indicated (m μ moles aminoacetone formed/mg. of protein/min.)	
		Aminopropanol	Threonine
(a) NCIB 8858	Nutrient broth	1.0	0.7
	Succinate + NH ₄ ⁺	0.2	0.5
	Aminopropanol	111.0	51.3
8/IX	Nutrient broth	0.6	0.2
	Succinate + NH ₄ ⁺	0.3	0.5
	Aminopropanol	97.5	22.4
NCIB 9397	Nutrient broth	0.4	0.1
	Succinate + NH ₄ ⁺	0.3	0.3
	Aminopropanol	1.3	0.9
(b) NCIB 8858	Aminopropanol	45.4	—
	Sucrose*	52.2	—
	Succinic acid*	24.8	—
	Fructose*	24.1	—
	2-Oxoglutaric acid*	23.4	—
	Pyruvic acid*	21.5	—
	DL-Lactic acid*	17.4	—
	Citric acid*	17.4	—
	Maltose*	16.4	—

* DL-1-aminopropan-2-ol as sole nitrogen source.

and 8/IX enzyme activity after growth on the amino alcohol was at least 100-fold greater than after growth on succinate + NH₄⁺ or nutrient broth. When *Pseudomonas* sp. NCIB 8858 was grown on simple defined media in which DL-1-aminopropan-2-ol served as sole source of nitrogen but other compounds served as the major source of carbon, the enzyme activity was decreased (see Table 1*b*). When succinate was used as the major carbon source and the amino alcohol was used at growth-limiting concentrations, enzyme activity was further decreased.

The effect of the growth phase of the culture when bacteria were harvested on the enzyme activity in cell-free extracts is shown in Fig. 1. Maximum activity was attained during the early logarithmic growth phase.

Induction of 1-aminopropan-2-ol dehydrogenase formation

When succinate-grown organisms were suspended in an equal volume of DL-1-aminopropan-2-ol containing medium and incubated overnight at 30°, dehydrogenase formation was induced before appreciable growth occurred. The effect of replacing the amino alcohol by various analogues and derivatives in a similar experiment is shown in Table 2. Of the compounds tested, only aminoacetone and L-threonine caused appreciable induction of enzyme formation. Inactive compounds were: 2-aminoethanol, DL-1-2-diaminopropane, DL-1-aminopropan-2,3-diol, 3-aminopropan-1-ol, DL-2-hydroxy-2-phenylethylamine, DL-3-phenylserine, propan-2-ol, DL-4-amino-3-hydroxybutyrate, L-2-aminopropan-1-ol, 3-aminopropan-1-ol.

Table 2. *Aminopropanol-dehydrogenase formation by Pseudomonas sp. NCIB 8858 in the presence of amino alcohols*

The micro-organism was grown overnight on succinate (NH₄)₂SO₄ medium (see Table 1), harvested, washed, and suspended in an equal volume of a similar defined medium containing aminopropanol, or on the amino alcohol, as sole source of carbon + nitrogen. Microbial suspensions were incubated for about 18 hr. at 30° with shaking, and the final population density measured. The micro-organism was then harvested, washed, and the aminopropanol-dehydrogenase activity in cell-free extracts measured as described for Table 1. Activities are expressed in relation to the activity found in bacterial extracts incubated with aminopropanol. The results given were obtained in two experiments. Compounds found to be inactive as inducers are listed in the text.

Amino alcohol or derivative (5 g./l.)	Addition	Conc. of addition (g./l.)	Aminopropanol- dehydrogenase activity (%)
DL-1-Aminopropan-2-ol	—	—	100
Aminoacetone*	—	—	52
L-Threonine	—	—	38
DL-3-Phenylserine	—	—	7
DL-2-Hydroxy-2-phenylethylamine	—	—	1
Aminopropanol {	Hydroxyphenylethylamine	—	100
		0	100
		0.01	64
		0.05	37
		0.10	22
	Phenylserine	0.25	0
		0.10	23
		0.50	26
		1.00	33
		5.00	6

* The concentration of aminoacetone was 0.1 g./l.

Several compounds inhibited the induction of enzyme synthesis when succinate-grown organisms were incubated in DL-1-aminopropan-2-ol medium together with the analogue or derivative. DL-2-Hydroxy-2-phenylethylamine and DL-3-phenylserine, the phenyl analogues of aminopropanol and threonine respectively, were particularly effective. As can be seen from Table 2, about equal concentrations of DL-1-aminopropan-2-ol and the two analogues completely prevented enzyme synthesis. In both cases, the phenyl compounds prevented growth of *Pseudomonas* sp. NCIB 8858 on DL-1-aminopropan-2-ol.

Properties of L-aminopropan-2-ol dehydrogenase in crude cell-free extracts

Optimum enzyme activity was at pH 9.5 (see Fig. 2). At the pH optimum, the Michaelis constant for DL-L-aminopropan-2-ol was about 0.3 mM (calculated for one enantiomorph) and for the coenzyme NAD⁺, about 0.4 mM. The graphical procedure of Lineweaver & Burk (1934) was used to determine K_m values.

Partial purification of L-aminopropan-2-ol dehydrogenase from extracts of Pseudomonas sp. NCIB 8858

Cell-free extract was prepared from organisms grown on DL-L-aminopropan-2-ol medium. Initial experiments showed that preliminary treatment with protamine sulphate facilitated purification by precipitation with ammonium sulphate. Molecular exclusion chromatography on polyacrylamide gel resulted in further purification.

Table 3. *Partial purification of aminopropanol-dehydrogenase*

Cell-free extracts of *Pseudomonas* sp. NCIB 8858 grown on aminopropanol medium, were fractionated as described in Methods. The results given were obtained by using a preparation of L(+)-L-aminopropanol (82 % L-isomer) as substrate. Activities were otherwise assayed colorimetrically as described in Methods. The total number of units of activity present in a fraction was calculated by multiplying the protein content by the enzyme activity.

Fraction	Volume (ml.)	Protein (mg./ml.)	Enzyme activity (mμmoles) aminoacetone formed/mg. protein/min.)	Total units (× 10 ⁻⁶)	Yield (%)	Purification
Cell-free extract	100	20	410	814	100	1
Protamine sulphate-treated	160	5.8	840	782	96	2
(NH ₄) ₂ SO ₄ -precipitate	10	19	2910	555	68	7
Pooled fractions from gel-filtration	45	1.7	4600	351	43	11
Peak fraction from gel-filtration	4.5	1.6	8500	61	7.5	21

Details of the procedure finally adopted are given in methods and in Table 3. The behaviour of the partly purified enzyme on gel-filtration is shown in Fig. 3, and is compared with that of a similar enzyme present in an extract of *Escherichia coli* (Turner, 1966, 1967). The elution patterns were obtained in consecutive experiments with the same column and fraction-collecting equipment. The enzyme from each micro-organism was eluted as a single well-defined peak, but the enzyme from the pseudomonad behaved as a protein of relatively lower molecular weight.

Properties of the partially purified enzyme

Samples of enzyme preparation obtained by gel-filtration (see Fig. 3 and Table 4) were used in the following investigations.

(a) *Effect of pH value.* With potassium phosphate (pH 5.8 to 8.0) and diethanol-amine HCl (pH 8.0 to 10.5) buffers, optimum enzyme activity was found to occur at pH 9.5 (see Fig. 2).

(b) *Effect of substrate and coenzyme concentrations.* The Michaelis constant for DL-L-aminopropan-2-ol was 0.12 mM with respect to one enantiomorph. Both NAD⁺

and NADP^+ were active as coenzymes. When used at the optimum concentration of 5 mM, in the absence of lactate dehydrogenase and pyruvate, they were equally active. Michaelis constants of 0.3 mM and 0.2 mM were obtained for NAD^+ and NADP^+ , respectively. In the presence of NAD^+ at a concentration of less than 1 mM, the addition of lactate dehydrogenase + pyruvate increased enzyme activity up to 50%. The enzyme was completely inactive with $\alpha\text{-NAD}^+$, tested at concentrations to 5 mM. Kinetic constants were obtained by using the graphical method of Lineweaver & Burk (1934).

Fig. 1

Fig. 2

Fig. 1. Aminopropanol-dehydrogenase activity in cell-free extracts during growth of *Pseudomonas* sp. NCIB 8858. A simple defined medium containing DL-I-aminopropan-2-ol as carbon + nitrogen source (Higgins *et al.* 1968), was inoculated with the succinate-grown pseudomonad to give an initial population density equiv. 0.25 mg. dry wt organism/ml. The culture was incubated at 30° with shaking, and samples withdrawn at intervals. Population density (○) was measured as previously described (Higgins *et al.* 1968), and aminopropanol-dehydrogenase activity (●) in extracts of the harvested organisms was measured as described for Table 1.

Fig. 2. Effects of pH value on aminopropanol-dehydrogenase activity. The enzyme activities of cell-free extracts (●) and partially-purified enzyme preparations (○) were measured colorimetrically, as described in Table 1 and Methods. Potassium phosphate buffers were used at pH 5.8 to 7.9, and diethanolamine HCl buffers at pH 8.0 to 10.4.

Effect of thiols, thiol-alkylating and metal-chelating reagents. Enzyme activity was unaffected by incubation or pre-incubation (15 min.) with the thiol compounds L-cysteine, 2-mercaptoethanol, reduced glutathione or dithiothreitol at concentrations 0.01 to 1.00 mM. Enzyme activity was not affected by the thiol-alkylating reagents iodoacetate and iodoacetamide, or the chelating agents EDTA and 2,2'-bipyridyl, all at 0.1 to 10.0 mM. The results with iodoacetate contrasted with the inhibitory effect of this compound on DL-I-aminopropan-2-ol dehydrogenase activity in *Escherichia coli* extracts (Turner, 1967).

Effect of inorganic cations and dialysis. Overnight dialysis against 0.1 M-potassium phosphate buffer (pH 7) at 0 to 4° caused no loss of activity. In contrast to the enzyme from *Escherichia coli* (Turner, 1967) potassium ions were not required for optimum activity. No effect was exerted by K^+ , Na^+ , NH_4^+ or Li^+ ions at 1 mM; high concentrations (200 to 400 mM) were inhibitory. Activity was unaffected by dialysis against buffers containing these ions at 1 mM.

Fig. 3. Behaviour of aminopropanol-dehydrogenase preparations during gel-filtration. Exclusion chromatography of enzyme preparations on Bio-Gel P-300 was done as described in Methods. The presence of protein in eluate samples was detected by measuring extinction values at 280 $m\mu$. Protein elution patterns with preparations from both *Escherichia coli* (▲) and *Pseudomonas* sp. NCIB 8858 (Δ) are indicated by broken lines. Aminopropanol-dehydrogenase activities were measured colorimetrically as described in Methods, and are expressed as the extinction values at 551 $m\mu$. Elution patterns for the enzyme from *E. coli* (●) and the pseudomonad (○) were obtained during successive experiments using the same column. Additional details are given in the text.

Stability. The enzyme was extremely stable. When stored at 1 mg. protein/ml. phosphate buffer (pH 7) at -15° , partly purified preparations retained at least 90% of their original activity for longer than one year.

Substrate specificity. Of the twenty-six analogues and derivatives of 1-aminopropan-2-ol tested, only three compounds showed appreciable activity: the substrate homologue DL-1-aminobutan-2-ol, and the phenyl analogues of the substrate and threonine, i.e. DL-2-hydroxy-2-phenylethylamine and DL-3-phenylserine (see Table 4). Compounds inactive as substrates were: DL-propane-1,2-diol, DL-4-amino-3-hydroxybutyrate, DL-serine, 3-aminopropan-1-ol, 2-aminoethanol, DL-1-aminopropan-2,3-diol, propan-2-ol, L-2-aminopropan-1-ol, DL-N,N-dimethylaminopropan-2-ol, L-threonine, DL-malate, DL-lactate, glycerol, DL-5-hydroxylysine, DL-3-hydroxyvaline, DL-1-bromopropan-2-ol, DL-1-chloropropan-2-ol and DL-1-fluoropropan-2-ol. The Michaelis constant for DL-1-aminobutan-2-ol was about 0.13 mM. The maximum velocity of this compound was 75% of that observed for DL-1-aminopropan-2-ol. Both phenyl analogues gave K_m values of about 0.7 mM.

Stereospecificity towards amino alcohol substrate. The optical isomers of DL-1-aminopropan-2-ol were partially resolved by the method of Sullivan (1963) as described by Turner (1967). These optically active preparations were tested as substrates for preparations to the partly purified enzyme. A preparation of the L(+)-enantiomorph, containing about 82 % L-isomer and 18 % D-isomer, was oxidized about six times faster than the racemic mixture. In each case the total concentration of both enantiomorphs present was 2 mM. A Michaelis constant of about 0.2 mM for the L(+)-amino alcohol, and a maximum velocity of about 4600

Fig. 4

Fig. 4. Effect of the concentration of optically active aminopropanol preparations on dehydrogenase activity. Aminopropanol-dehydrogenase activities were measured colorimetrically as described in Methods. Samples of optically active aminopropanol were prepared via the hydrogen tartrate dihydrate salts (Turner, 1967). The preparations of D(–)- and L(+)-1-aminopropan-2-ol contained 75 and 82 %, respectively, of the enantiomorphs indicated. The results shown illustrate the effect of the concentration of the 'L'-enantiomorph at three different concentrations of the 'D'-isomer, i.e. 2.5 (Δ), 5.0 (●) and 10.0 (○) mM. All concentrations are expressed in terms of the total concentration of both enantiomorphs present.

Fig. 5

Fig. 5. Effect of pH on aminoacetone reduction by partially purified aminopropanol-dehydrogenase. Enzyme activity was measured spectrophotometrically, with aminoacetone and NADH as the substrates, as described in Methods. The results of two experiments (○, Δ) were obtained with sodium acetate+acetic acid buffer, pH 4.0 to 5.0; potassium phosphate buffers, pH 5.5 to 8.0; diethanolamine+HCl buffers, pH 8.3 to 10.2. The results of a third experiment were obtained with 2,2-dimethylglutaric acid+NaOH buffers, pH 5.0 to 8.0 (●). All results are expressed as the % activity relative to that found in each experiment with sodium acetate+acetic acid buffer (pH 5.0).

μmoles aminoacetone formed/mg. protein/min., were found with the highly resolved substrate preparation. A sample of predominantly D(–)-1-aminopropan-2-ol (75 % D-isomer) was completely inactive as a substrate. Experiments with mixtures of *dextro-laevo*-rotatory and optically-inactive preparations of the amino alcohol showed that the D(–)-enantiomorph acted as a competitive inhibitor of L(+)-1-aminopropan-2-ol oxidation (see Fig. 4). At 'D' (–)-enantiomorph concentrations of 2.5, 5.0 and 10.0 mM, the apparent Michaelis constants, K_p , for the L (+)-isomer were 1.7, 5.9 and 7.6 mM, respectively. From these results the inhibitor constant, K_i , was calculated

to be about 0.3 mM. No oxidation of D (-)-1-aminopropan-2-ol to aminoacetone could be detected using crude cell-free extracts.

Reversibility of 1-aminopropan-2-ol oxidation. The ability of the partially purified enzyme to effect the aminoacetone-dependent oxidation of NADH was studied spectrophotometrically. Optimum activity occurred at pH 5.0 in acetate buffers, with a second peak at pH 8.4 in diethanolamine + HCl buffers. Phosphate inhibited aminoacetone reduction. Potassium phosphate buffer at 0.1 M inhibited reduction by 40% when compared with activity in sodium cacodylate buffer also at pH 7.5 (see Fig. 5). The reduced cofactors NADH and NADPH were equally effective over a range of

Table 4. *Substrate specificity of partially purified enzyme*

The activity of samples of partially purified enzyme, $(\text{NH}_4)_2\text{SO}_4$ -precipitate (see Table 3), with the compounds shown below, was measured by the spectrophotometric method described in Methods. The preparation of L-aminopropanol used contained 82% L-isomer. Potential substrates found to be inactive are listed in the text.

Substrate tested	Enzyme activity (m μ moles NADH formed/mg. protein/min.)
DL-1-Aminopropan-2-ol	490
'L'-1-Aminopropan-2-ol	3000
DL-1-Aminobutan-2-ol	365
DL-2-Hydroxy-2-phenylethylamine	174
DL-3-Phenylserine	125
DL-3-Hydroxybutyrate	50

concentrations, with Michaelis constants of 0.3 mM at pH 5. At pH 8.4, the K_m for NADH was 0.4 mM. 2'-Aminoacetophenone and 5-aminolaevulate had 47% and 6%, respectively of the activity found with aminoacetone at pH 5. With NADH as cofactor, the Michaelis constants for aminoacetone and 2'-aminoacetophenone were 0.3 and 0.2 mM, respectively, at pH 5. Under optimum conditions, the aminoacetone-dependent oxidation of NADH occurred at a rate of 500 m μ moles/mg./min. at 30°. All kinetic constants in this work were obtained by the graphical method of Lineweaver & Burk (1934).

Estimation of molecular weight. The weight-average molecular weight of the enzyme was determined by gel-filtration on Sephadex G. 200 as described in Methods. The simultaneous elution from the calibrated column of the enzyme and of a sample of bovine serum albumin confirmed previous results, suggesting a molecular weight of $70,000 \pm 5000$.

DISCUSSION

The induced formation of L-1-aminopropan-2-ol dehydrogenase provides additional evidence that the enzyme-catalyzed formation of aminoacetone represents the initial catabolic step involved in the growth of *Pseudomonas* on the amino alcohol (see Higgins *et al.* 1968). Of the other potential inducers tested, only aminoacetone and threonine were effective. In view of the observation that growth on aminopropanol leads to high threonine dehydrogenase activity in cell-free extracts (see Table 1), and the demonstrated reversibility of aminopropanol dehydrogenation, it is possible that aminopropanol formed from aminoacetone, or from threonine via the amino ketone,

was the actual inducer. The rapid oxidation of aminopropanol by intact bacteria grown on aminoacetone has been previously observed (Higgins *et al.* 1968).

The narrow substrate specificity of aminopropanol-dehydrogenase purified from *Pseudomonas* sp. NCIB 8858 is of interest in relation to the specificity of the enzyme in *Escherichia coli* (Turner, 1967). In the latter case narrow specificity was deduced from the observation that substrate analogues had little or no inhibitory effect on aminopropanol oxidation by crude cell-free extracts. The exception, DL-2-hydroxy-2-phenylethylamine, which was an inhibitor but not a substrate of the enzyme in *Escherichia coli*, was found to be a substrate of the purified *Pseudomonas* enzyme described here. This phenyl derivative, together with DL-3-phenylserine, although active as substrates did not act as inducers and indeed inhibited the induction of enzyme formation by aminopropanol. It appears certain that aminopropanol-dehydrogenase activity is due to an enzyme which is specific for the L(+) -enantiomorph of this amino alcohol. It seems appropriate that the *Pseudomonas* enzyme should be given the systematic name L-1-aminopropan-2-ol:NAD oxidoreductase, although it should be noted that, in contrast to the enzyme in *E. coli*, NADP⁺ can also act as the coenzyme.

The highly specific functioning of aminopropanol, not only as a substrate but also as an effective inducer of enzyme formation, raises again the question of a metabolic function for the amino alcohol. At the present time several possibilities have been considered (see Turner, 1967). The effect of growth conditions on enzyme activity in cell-free extracts indicates a catabolic role in *Pseudomonas*. The fact that less metabolically versatile micro-organisms, grown on rich broth media, are capable of oxidizing aminopropanol to aminoacetone (Turner, 1966*a*), and the discovery of aminopropanol dehydrogenase activity in rat liver preparations (Turner & Willetts, 1967), suggest that the amino alcohol may be of general metabolic significance.

The interest and support of Professor T. W. Goodwin in this work is gratefully acknowledged. The authors thank Miss E. Duggan for expert technical assistance. The work of I.J.H. and M.A.P. was supported by grants from the Medical Research Council and the University of Liverpool, respectively. Part of this work was aided by a research grant from the Medical Research Council.

REFERENCES

- ANDREWS, P. (1965). The gel filtration behaviour of proteins related their molecular weights over a wide range. *Biochem. J.* **96**, 595.
- CHANCE, B. & MAEHLY, A. C. (1955). Assay of catalases and peroxidases. *Meth. Enzymol.* **2**, 764.
- ELLIOTT, W. H. (1958). A new threonine metabolite. *Biochem. biophys. Acta* **29**, 446.
- HIGGINS, I. J., PICKARD, M. A. & TURNER, J. M. (1968). Aminoacetone formation and utilization by pseudomonads grown on DL-1-aminopropan-2-ol. *J. gen. Microbiol.* **54**, 105.
- HIGGINS, I. J., PICKARD, M. A., TURNER, J. M. & WILLETTS, A. J. (1966). 1-Aminopropan-2-ol; NAD oxidoreductase. *Biochem. J.* **99**, 27*P*.
- HORECKER, B. J. & KORNBERG, A. (1948). The extinction coefficients of the reduced band of pyridine nucleotides. *J. biol. Chem.* **175**, 385.
- LINWEAVER, H. & BURK, D. (1934). The determination of enzyme dissociation constants. *J. Am. chem. Soc.* **56**, 658.
- PICKARD, M. A., HIGGINS, I. J. & TURNER, J. M. (1966). Microbial metabolism of 1-aminopropan-2-ol. *J. gen. Microbiol.* **45**, i.

- RACKER, E. (1955). Alcohol dehydrogenase from baker's yeast. *Meth. Enzymol.* **1**, 500.
- SULLIVAN, R. H. (1963). Resolution of racemic aminoisopropanol. *U.S. Patent* 3, 116, 332 (Cl. 260-584).
- TURNER, J. M. (1966*a*). Aminoacetone production by micro-organisms. *Biochem. J.* **98**, 7*P*.
- TURNER, J. M. (1966*b*). Microbial metabolism of amino ketones: aminoacetone formation from 1-aminopropan-2-ol by a dehydrogenase in *Escherichia coli*. *Biochem. J.* **99**, 427.
- TURNER, J. M. (1967). Microbial metabolism of amino ketones: L-1-aminopropan-2-ol and L-threonine dehydrogenase in *Escherichia coli*. *Biochem. J.* **104**, 112.
- TURNER, J. M. & WILLETTS, A. J. (1967). Amino ketone formation and aminopropanol-dehydrogenase activity in rat liver preparations. *Biochem. J.* **102**, 511.