Microbial Metabolism of Amino Ketones: D-1-Aminopropan-2-ol and Aminoacetone Metabolism in *Escherichia coli*

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SUMMARY

Aminoacetone was formed from D- or L-1-aminopropan-2-ol, or both, by a variety of micro-organisms. An oxidoreductase capable of oxidizing D-1-aminopropan-2-ol to aminoacetone was purified 38-fold from *Escherichia coli*. It was inactive with L-1-aminopropan-2-ol, L-threonine and DL-glycerol-1-phosphate. It was highly active with a variety of diols and hydroxyketones and not narrowly specific as reported by other workers (Decker & Swain, 1968). The effect of growth conditions on activity suggested involvement in mono- or di-hydroxyacetone metabolism. Although D-1-aminopropan-2-ol oxidation was demonstrated in crude extracts of a number of other bacteria, a relationship between L-threonine and D-1-aminopropan-2-ol dehydrogenases and vitamin B₁₂ biosynthesis does not appear likely.

INTRODUCTION

A previous study with *Escherichia coli* showed that NAD-dependent oxidoreductase activities towards L-threonine, and both L- and D-1-aminopropan-2-ol were present in extracts and the properties of the first two enzymes were described (Turner, 1967). Aminoacetone was the product in both cases. The present paper describes the properties of a partially purified oxidoreductase acting on D-1-aminopropan-2-ol also to form aminoacetone. Interest in this enzyme arises because of its possible involvement in the reduction of aminoacetone to give D-1-aminopropan-2-ol which then may be incorporated into vitamin B₁₂ (Turner, 1966), although it still remains to be demonstrated that it is the D-isomer which is the product of aminoacetone reduction in this organism. Some support for this hypothesis comes from the work of Krasna, Rosenblum & Sprinson (1957), who showed that the incorporation of isotope from [¹⁵N]-L-threonine into the D-1-aminopropan-2-ol fragment of vitamin B₁₂ occurred in *Streptomyces griseus*.

Some of this work has been summarized previously in a preliminary note (Lowe & Turner, 1968).

METHODS

Micro-organisms and media. *Escherichia coli*, type I (National Collection of Industrial Bacteria (NCIB) no. 8114) was maintained and cultured as described previously (Turner, 1966). *Pseudomonas* strains 8/IX and DL8A were isolated by enrichment

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culture on DL-1-aminopropan-2-ol as the main carbon and nitrogen source, as described by Higgins, Pickard & Turner (1968). Other micro-organisms were obtained from the NCIB, Aberdeen, Scotland, the National Collection of Plant Pathogenic Bacteria (NCPBP), Hatching Green, Harpenden, Hertfordshire, or were laboratory strains. All micro-organisms were maintained as slope or stab cultures on nutrient agar (2.5 %, w/v). Some vitamin B₁₂-producing micro-organisms (see Table 7) were grown on peptone-yeast extract medium, modified from that described by Hall, Benedict, Wiesen, Smith & Jackson (1953) and consisting of 10 g. glucose, 3 g. yeast extract, 3 g. soya peptone, 1 g. CaCO₃, 1.5 g. K₂HPO₄, 1 g. KH₂PO₄ and 10 mg. CoSO₄·7H₂O made up to 1 l. with glass-distilled water. Other micro-organisms were grown on nutrient broth medium (Oxoid no. 2) or simple basal salts media containing 7 g. K₂HPO₄, 3 g. KH₂PO₄, 1.2 g. Na₂SO₄·10H₂O, 0.1 g. MgSO₄·7H₂O, 1 g. (NH₄)₂SO₄ and 5 g. of various carbon sources per litre. Synthetic media were adjusted to pH 7.0 prior to sterilization. Carbohydrates were autoclaved separately.

Preparation of enzyme extracts. Bacteria were harvested in the exponential growth phase and extracts prepared as described by Turner (1966).

Measurement of aminoacetone production and utilization by washed suspensions of bacteria. Aminoacetone production and utilization were measured as described by Higgins *et al.* (1968). The partially resolved amino alcohols were used as substrates for aminoacetone production.

Uptake of radioactive [3-¹⁴C]-DL-1-aminopropan-2-ol by growing and resting bacteria. Washed suspensions of *Escherichia coli* were incubated at 4 mg. dry wt./ml. with 0.3 mg. of [3-¹⁴C]-DL-1-aminopropan-2-ol (0.085 mCi/mmol) and 0.5 mmole K₂HPO₄ + KH₂PO₄ buffer, pH 7.0, in 5 ml. at 30° with shaking. Samples of culture or bacterial suspensions were then filtered through 0.45 µ Millipore membrane filters (Millipore Filter Corporation, Bedford, Massachusetts, U.S.A.). The bacteria were washed with 0.1 M-phosphate buffer before attaching the filters to planchets and drying. Samples of the combined filtrates and washings were also dried on planchets and examined for radioactivity. Radioactivity was measured using a Nuclear Chicago Model D 47 Gas Flow Detector (Nuclear Chicago, Des Plaines, Illinois, U.S.A.), which had a calculated counting efficiency of 31 %.

Measurement of L-threonine, D- and L-1-aminopropan-2-ol dehydrogenase activities in enzyme extracts. D- and L-1-aminopropan-2-ol dehydrogenase activities were assayed in reaction mixtures containing 50 µmole D- or L-1-aminopropan-2-ol, either as the optically pure bases or partially resolved tartrates, 500 µmole diethanolamine + HCl buffer pH 9.6, 10 µmole NAD⁺, and 0.5 mg. protein (dialysed crude extract) or 50 µg. of partially purified enzyme preparation, in a total volume of 1.6 ml. Unless otherwise indicated, 30 µmole (NH₄)₂SO₄ was included in the D-1-aminopropan-2-ol dehydrogenase assay mixture. Reactions were started by addition of enzyme to the otherwise complete reaction mixtures equilibrated at 37°. All incubations were for 20 min. at 37°. Reactions were stopped with 0.3 ml. 25 % (w/v) trichloroacetic acid. After centrifuging, 1.0 ml. of supernatant fluid was assayed for aminoacetone. When assaying for L-threonine dehydrogenase, 100 µmole of L-threonine was used as substrate with 500 µmole tris + HCl buffer pH 9.0.

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

Spectrophotometric enzyme assays. D-1-Aminopropan-2-ol and secondary alcohol-

dependent reduction of NAD^+ was measured in reaction mixtures containing 750 μmole diethanolamine + HCl buffer pH 9.6, 10 μmole NAD^+ , 50 μmole secondary alcohol substrate, 50 μmole $(\text{NH}_4)_2\text{SO}_4$ and 0.02 to 0.50 mg. protein in a total volume of 2.5 ml. Aminoacetone and hydroxyketone reductase activities were carried out using 750 μmole sodium acetate + acetic acid (or 3,3'-dimethylglutaric acid + NaOH) buffer pH 6.0, 10 μmole aminoacetone or 2.5 μmole hydroxyketone, 0.75 μmole NADH or NADPH, 50 μmole $(\text{NH}_4)_2\text{SO}_4$ and 0.02 to 0.50 mg. protein in 2.5 ml. total volume. Substrate or cofactor was omitted from the compensating cells and the reactions were started by the addition of enzyme to otherwise complete reaction mixtures equilibrated at 37°.

Enzymic activities are expressed as m μmoles of NADH produced, or NADH/NADPH oxidized/mg. protein/min. at 37°.

Purification of D-1-aminopropan-2-ol-aminoacetone oxidoreductase activity

(a) *Heat treatment.* Crude extract was adjusted to 8 mg. protein/ml., heated for 10 min., and precipitated protein was removed by centrifuging at 10,000g to give a clear supernatant fluid.

(b) *Molecular exclusion chromatography.* Portions of heat-treated enzyme were further purified by gel filtration on Bio-Gel P-300 as described by Turner (1967). Enzyme activity was eluted immediately after the void volume ($V_e/V_o = 1.3$). The active fractions were combined for further purification.

(c) *Ion-exchange cellulose chromatography.* The enzyme was next adsorbed on 30 g. DEAE cellulose 'Chromedia DE 52' (pre-swollen) previously equilibrated with 0.02M-phosphate buffer pH 7.0 at 4°. Protein was eluted from the cellulose by a gradient of 0 to 1.0M-NaCl in 0.02M-phosphate buffer pH 7.0, at a flow rate of about 30 ml./h. Under these conditions enzyme activity was eluted by 0.35 to 0.40M-NaCl.

Preparation of optically active 1-aminopropan-2-ols

(a) *Partially resolved D-(−)-1-aminopropan-2-ol D-(−)-tartrate.* This was prepared by the procedure of Sullivan (1963) as described by Turner (1967), except that L(+) tartaric acid was replaced by D(−) tartaric acid. The optical rotation of the recrystallized D-(−)-1-aminopropan-2-ol D-(−)-tartrate dihydrate, was $[\alpha]_D^{22.5} = -31.5^\circ$ (C. 5 and 10 in water).

(b) *Optically pure D- and L-1-aminopropan-2-ol.* The method used was a modification of that described by Chatelus (1964). For the preparation of D-1-aminopropan-2-ol, 10 g. of L-threonine and 66 g. of freshly distilled acetophenone (mole ratio 3:20) were heated with constant stirring at 130° for 7 h. The resultant deep-red solution (Schiff's base) was hydrolysed by shaking with four 50 ml. volumes of 3N-HCl at 50° to release the base. The combined aqueous fractions were dried. The hygroscopic residue was dissolved in the minimum of warm dry absolute ethanol. After cooling the solution, D-1-aminopropan-2-ol HCl was precipitated by adding 7 vol. dry diethyl ether and removed by filtration. The crystals were dissolved in ethanol and re-precipitated by ether to yield a flakey white product which was stored under vacuum over a desiccant. The product had a m.p. of 97°, cf. authentic DL-1-amino-propan-2-ol m.p. 96°; and exhibited $[\alpha]_D^{22.5} = -58^\circ$ (C. 2 and 5 in water) and $[\alpha]_D^{22.5} = -33.6^\circ$ (C. 1 and 3 in methanol), cf. $[\alpha]_D^{25} = -31.5^\circ$ (C. 1 in methanol), Clark,

Jones, Raich & Folkers (1954), and $[\alpha]_D = -58^\circ$ (unknown solvent), Karrer & Klarer (1925), reported for synthesized D-1-aminopropan-2-ol HCl.

Similarly, L-1-aminopropan-2-ol HCl, prepared from D-threonine, exhibited $[\alpha]_D^{22.5} = +38^\circ$ (C. 5 in water). Optical rotation values were determined as described previously (Turner, 1967).

Detection of 1-aminopropan-2-ol formation. Aminoacetone (10 mM) and NADH (0.4 mM) were incubated at 37° with purified enzyme (0.3 mg./ml.) in phosphate buffer, pH 6 (50 mM), in the presence of $(\text{NH}_4)_2\text{SO}_4$ (20 mM). After 1 to 2 h., reaction mixtures were deproteinized and applied to a column of BioRex 70 cation exchange resin. Basic compounds were eluted with 0.1 N-HCl and freeze-dried. Concentrated solutions were streaked on to thin-layer cellulose plates and chromatographed in butan-1-ol + propionic acid + water (47 + 22 + 31, by vol.). Aminopropanol was detected as a distinct ninhydrin-positive band running slightly ahead of aminoacetone (R_F values 0.4 and 0.3 respectively).

Chemicals. The sources of chemicals were those described previously by Turner (1967) and Higgins *et al.* (1968). In addition, D-tartaric acid, *meso*-butan-2,3-diol, DL-propan-1,2-diol, hydroxyacetone and DL-acetoin were obtained from British Drug House Ltd, Poole, Dorset; D-threonine, DL-1-bromopropan-2-ol and DL-1-fluoropropan-2-ol from K and K Laboratories Inc., Plainview, New York, U.S.A.; soya peptone L 44 and yeast extract L 21 from the Oxoid Division of Oxo Ltd, London; dihydroxyacetone from Calbiochem. Inc., Los Angeles, California, U.S.A.; DE 52 Chromedia ion-exchange cellulose from H. Reeve Angel and Co. Ltd, London. $[\text{U-}^{14}\text{C}]$ -L-Threonine was purchased from The Radiochemical Centre, Amersham, Buckinghamshire. All chemicals were of analytical reagent grade, or of highest quality available commercially.

RESULTS

Aminopropanol and aminoacetone metabolism by intact bacteria

Previous results had shown that *Escherichia coli* was unable to utilize the nitrogen of DL-1-aminopropan-2-ol for growth on a variety of carbon sources (Turner, 1967). The same results were obtained in the present study using aminoacetone. The ability of washed suspensions of *E. coli* to form the amino ketone from DL-1-aminopropanol was shown to be due to the oxidation of only the L-isomer (Table 1). Some micro-organisms among those tested were found, however, to produce aminoacetone from both stereo-isomers (Table 1).

The possible utilization of aminopropanols for biosynthetic rather than catabolic purposes by *Escherichia coli* was studied by adding $[\text{3-}^{14}\text{C}]$ -DL-1-aminopropan-2-ol (0.66 μCi , 4.2 μmole) to freshly inoculated 10 ml. cultures of *E. coli*, growing either on nutrient broth or on succinate basal salts media. Only about 6 % of the radioactivity was incorporated into bacterial substance within 36 to 60 h. In contrast, micro-organisms capable of growth on DL-1-aminopropan-2-ol, e.g. *Achromobacter* sp. P6 (Gottlieb & Mandel, 1959) and *Pseudomonas* sp. NCIB8858 (Higgins *et al.* 1968), incorporated about 86 % of the radioactivity under the same conditions. Washed suspensions of *E. coli*, grown on a variety of media, utilized $[\text{3-}^{14}\text{C}]$ -DL-1-aminopropan-2-ol only after a lag of about 10 h. Virtually all the radioactivity was taken up by the organism within 24 h. and no trace of radioactive products was detectable in suspension media. Suspensions of broth-grown bacteria utilized aminoacetone immediately at

rates of 1 to 2 μ moles/mg. dry wt/min. at 30° and pH 7. Aminoacetone uptake was decreased 50 % by an equimolar amount of L-I-aminopropan-2-ol whereas the D-isomer lowered uptake by only 5 %. Cultures growing on broth also utilized aminoacetone at slow rates.

Table 1. *Aminoacetone production from D- and L-I-aminopropan-2-ol by washed-cell suspensions of micro-organisms*

Micro-organisms were grown on nutrient broth, or DL-I-aminopropan-2-ol medium (values in parentheses), and aminoacetone production by suspensions was measured as described in the Methods section.

Micro-organism	Strain	Aminoacetone production (μ moles/mg. dry wt/min. at 30°) from	
		D-Aminopropanol*	L-Aminopropanol†
<i>Achromobacter anitratum</i>	Laboratory	0	1.2
<i>Aeromonas liquefaciens</i>	Laboratory	0	3.1
<i>Erwinia carotovora</i>	NCPPB 1280	5.0	2.6
<i>Escherichia coli</i> , type I	NCIB 8114	0	3.2
<i>Klebsiella aerogenes</i> , type I	NCIB 418	6.1	5.2
<i>Proteus vulgaris</i>	Laboratory	0	1.2
<i>Pseudomonas aeruginosa</i>	NCIB 8293	6.2	3.2
<i>Pseudomonas</i> sp.	NCIB 8194	0.4	0.7
<i>Pseudomonas</i> sp.	NCIB 8858	0.7 (0.2)	0.9 (8.9)
<i>Pseudomonas</i> sp.	8/IX‡	0.2 (—)	1.2 (13.2)
<i>Pseudomonas</i> sp.	DL 8A‡	7.5 (0.6)	3.2 (10.4)

* Composition 82 % D-I-aminopropan-2-ol, 18 % L-I-aminopropan-2-ol.

† Composition 88 % L-I-aminopropan-2-ol, 12 % D-I-aminopropan-2-ol.

‡ Isolated in this laboratory for ability to grow on DL-I-aminopropan-2-ol.

Table 2. *Effect of activators and inhibitors on D-I-aminopropan-2-ol dehydrogenase activity*

Activity was measured colorimetrically as described in the Methods section, except that $(\text{NH}_4)_2\text{SO}_4$ was omitted from reaction mixtures in Expt. 1.

Addition	Concentration (mM)	Activity (%)
Experiment 1		
None	—	100
Thioglycollate	5	83
Cysteine	5	81
2-Mercaptoethanol	5	48
$(\text{NH}_4)_2\text{SO}_4$	10	200
Na_2SO_4	10	140
K_2SO_4	10	103
Experiment 2		
DL-Serine	30	91
DL-1-Dimethylaminopropan-2-ol	30	90
Ethan-1,2-diol	30	86
DL-3-Fluoro-1-aminopropan-2-ol	30	58
Glycerol	30	58
DL-1-Fluoropropan-2-ol	30	57
Propan-1,3-diol	30	52
meso-Butan-2,3-diol	30	43
DL-1-Bromopropan-2-ol	30	17
DL-Propan-1,2-diol	30	9

D-I-Aminopropan-2-ol oxidation by cell-free extracts

The NAD⁺-dependent oxidation of D-I-aminopropan-2-ol to aminoacetone was assayed colorimetrically (see Methods section). Under optimum conditions, activities of 12 to 15 mμmoles aminoacetone formed/mg. protein/min. at 37° were found in fresh extracts. Aminoacetone reductase activity could not be measured accurately in crude extracts due to the presence of high NADH oxidase activity.

Fig. 1. Heat-treatment of crude cell-free extracts of *Escherichia coli*. Aliquots of crude cell-free extract, adjusted to 8 mg. protein/ml., were held at various temperatures for 10 min. After removing the precipitated protein by centrifuging, supernatants were assayed for D-I-aminopropan-2-ol dehydrogenase activity (○) colorimetrically, DL-propan-1,2-diol dehydrogenase activity (●) ($\times \frac{1}{8}$ given scale) spectrophotometrically, and protein content (■) as described in Methods. Enzyme activities are expressed in relation to the original protein concentration of untreated extract.

Table 3. *Partial purification of D-I-aminopropan-2-ol dehydrogenase*

Extracts from *Escherichia coli* grown on nutrient broth were fractionated as described in Methods.

Fraction	Volume (ml.)	Protein (mg./ml.)	Enzyme activity (mμmoles/mg. protein/min.)	Total units (IU)	Activity ratio D-I-aminopropan-2-ol DL-propan-1,2-diol
Crude extract	20	19	12	4560	0.15
Heat-treated	20	4.7	46	4300	0.15
BioGel P-300	70	0.45	144	4530	0.14
DE 52 cellulose	25	0.14	178	590	0.14
Peak fraction	3	0.12	450	162	0.14

The enzyme was activated by a number of monovalent cations, but inhibited by sulphhydryl compounds and more markedly by substrate analogues (Table 2). Substances with little or no effect at 5 mM included AMP, ADP, ATP, iodoacetate and glutathione.

D-I-Aminopropan-2-ol dehydrogenase was remarkably stable to heat: heating at 70° for 10 min. caused an increase in activity, as well as purification due to protein precipitation (Fig. 1).

Purification and properties of an oxidoreductase acting on D-I-aminopropan-2-ol and aminoacetone

Purification was carried out as a preliminary to studies on substrate specificity and content of aminoacetone reductase activity (Table 3). The relative activities of D-I-aminopropan-2-ol and DL-propan-1,2-diol remained constant during 38-fold purification. During exclusion chromatography on Bio-Gel P-300, the oxidoreductase was completely separated from L-threonine and L-I-aminopropan-2-ol dehydrogenases. The enzyme was eluted immediately after the initial nucleoprotein indicating a mol. wt of about 300,000 (see Turner, 1967).

Fig. 2. Effect of pH and buffers on D-I-aminopropan-2-ol and DL-propan-1,2-diol dehydrogenase activity. Enzyme activities in partially purified preparations were measured spectrophotometrically as described in Methods, although in the absence of $(\text{NH}_4)_2\text{SO}_4$: D-I-aminopropan-2-ol dehydrogenase activity (○), DL-propan-1,2-diol dehydrogenase activity (●) $\times \frac{1}{2}$ given scale. 0.3 M-Sodium acetate+acetic acid buffers were used at pH 5.1 to 5.9, 0.3 M- K_2HPO_4 - KH_2PO_4 buffers at pH 6.0 to 7.5, 0.3 M-tris+HCl buffers at pH 7.4 to 9.0 and 0.3 M-diethanolamine+HCl buffers at pH 8.5 to 10.2.

Fig. 3. D-I-Aminopropan-2-ol dehydrogenase activation by monovalent cations. Activity in partially purified enzyme preparation was assayed colorimetrically as described in Methods, except that $(\text{NH}_4)_2\text{SO}_4$ in the incubation mixtures was replaced by Li_2SO_4 (●), $(\text{NH}_4)_2\text{SO}_4$ (○), Na_2SO_4 (□), K_2SO_4 (■) or Rb_2SO_4 (△).

PH optima. Optimum activity for the oxidation of either D-I-aminopropan-2-ol or DL-propan-1,2-diol with NAD^+ occurred at pH 9.8 in diethanolamine+HCl buffer. Dehydrogenase activities were markedly lower in tris+HCl buffer (Fig. 2), particularly in the absence of NH_4^+ . For the reduction of aminoacetone and hydroxyacetone with either NADH or NADPH, optimum activity was at pH 6.0.

Cation activation. The activity of the enzyme was stimulated by NH_4^+ , Li^+ and Na^+ at about 10, 50 and 100 mM respectively (Fig. 3). These effects, which were not additive, occurred with all substrates tested (see below). Cations were equally effective in both directions of the oxidoreductase.

Substrate and cofactor specificities. Broad substrate specificity was found (Table 4). Inactive as substrates were L-threonine, L-serine, DL-2-hydroxy-2-phenylethylamine and DL-glycerol-1-phosphate. In the alcohol oxidation direction, activity with NADP⁺ was only 3 % of that with NAD⁺. With the reduction of aminoacetone and other ketones, NADPH was at least 60 % as active as NADH at the optimum concentrations of 0.3 to 0.4 mM, but its relative activity was higher at higher (inhibitory) cofactor

Fig. 4. Double reciprocal plots showing the effect of NH_4^+ on D-1-aminopropan-2-ol dehydrogenase activity. Activity in partially purified enzyme preparation from *Escherichia coli* was assayed colorimetrically as described in Methods except where noted. (a) Incubation mixtures contained 2 μmole NAD^+ with D-1-aminopropan-2-ol from 1.0 to 20.0 μmoles in presence of $(\text{NH}_4)_2\text{SO}_4$ at 1 mM ($\square$) and 20 mM ($\circ$). No $(\text{NH}_4)_2\text{SO}_4$ control ($\bullet$). (b) Incubation mixtures contained 20 μmole D-1-aminopropan-2-ol with NAD^+ from 0.1 to 2.0 μmoles in the presence of $(\text{NH}_4)_2\text{SO}_4$ at 1 mM ($\square$) and 20 mM ($\circ$). No $(\text{NH}_4)_2\text{SO}_4$ control ($\bullet$).

Table 4. *Substrate specificity of enzyme acting on D-1-aminopropan-2-ol*

Substrate-dependent reduction of NAD^+ and oxidation of NADH was measured spectrophotometrically at 340 nm. as described in the Methods section. Activities are expressed relative to those with D-1-aminopropan-2-ol, or aminoacetone, measured under appropriate optimum conditions.

Substrate	Activity (%)
Oxidation	
D-1-Aminopropan-2-ol	100
DL-Propan-1,2-diol	680
DL-1-Bromopropan-2-ol	350
meso-Butan-2,3-diol	320
Propan-1,3-diol	320
DL-1-Fluoropropan-2-ol	240
Glycerol	220
DL-1-Aminopropan-2,3-diol	90
L-1-Aminopropan-2-ol*	3
Reduction	
Aminoacetone	100
Dihydroxyacetone	1840
Hydroxyacetone	1200
Methylglyoxal	960
DL-Acetoin	250

* Composition 88 % L-1-aminopropan-2-ol; 12 % D-1-aminopropan-2-ol, used as their L-tartrates.

concentrations. Activities with each cofactor were not additive. 1-Aminopropan-2-ol formation by reduction of aminoacetone was demonstrated chromatographically (see Methods), but the identity of the stereoisomer was not established.

Table 5. *Michaelis constants for substrates and cofactors of enzyme acting on D-1-aminopropan-2-ol and aminoacetone*

K_m values were obtained using method of Lineweaver & Burk (1934). The effects of substrate concentrations were determined in the presence of saturating amounts of cofactors, and vice versa.

Limiting component	Michaelis constant (mM)
D-1-Aminopropan-2-ol	2.0 to 5.0
DL-Propan-1,2-diol	0.3
NAD ⁺	0.2 to 0.5
Aminoacetone	3.0 to 4.5
Hydroxyacetone	0.1 to 0.2
NADH	0.07
NADPH	0.12

Kinetic behaviour. The effect of D-1-aminopropan-2-ol and NAD⁺ on dehydrogenase activity, at various NH₄⁺ concentrations, are shown as double reciprocal plots in Fig. 4. Ammonium ions increased V_{max} and decreased K_m values for the substrate. Michaelis constants for various substrates and cofactors are given in Table 5. Activities in the direction of alcohol oxidation were unaffected by high substrate or cofactor concentration and were not influenced by nucleotides. In contrast, ketone reduction was extremely sensitive to high substrate and cofactor concentrations and was inhibited by ATP but not by ADP or AMP. With NADH at the optimum concentration (0.4 mM), excess substrate inhibition with aminoacetone and hydroxyacetone occurred above about 10 and 1.2 mM respectively. Aminoacetone was reduced twice as rapidly as hydroxyacetone when each was tested at 10 mM. With aminoacetone at 10 mM, NADH and NADPH were both inhibitory at above about 0.4 mM.

Inhibition by ATP. Whereas ketone reduction with NADPH was inhibited by 84 % by ATP at 0.3 mM, reduction with NADH was inhibited only 10 %. Inhibition by ATP was non-competitive. When the ketone concentration was varied, with NADPH saturating at 1 mM, the K_i for ATP was 0.07 mM. When the cofactor was varied, with ketone constant, the corresponding K_i was 0.60 mM.

Inhibition by substrate analogues and related compounds. Aminoacetone production from D-1-aminopropan-2-ol was inhibited by substrate analogues (Table 2), many of which were more effective substrates (Table 4). DL-Propan-1,2-diol, *meso*-butan-2,3-diol or DL-1-bromopropan-2-ol (ignoring isomeric forms) caused 91, 83 and 57 % inhibition of amino alcohol oxidation when added in equimolar amounts. Inhibition by DL-propan-1,2-diol was found to be competitive (K_i about 0.10 mM). DL-3-Fluoro-1-aminopropan-2-ol also acted as both substrate and inhibitor of D-1-aminopropan-2-ol oxidation. L-1-Aminopropan-2-ol was inactive as inhibitor or substrate.

Effect of growth conditions on dehydrogenase activities

Growth of *Escherichia coli* on simple synthetic media, containing glucose, succinate, fumarate, malate, aspartate, glycerol, lactate, pyruvate or acetate as sole source of carbon and energy, yielded extracts with low D-1-aminopropan-2-ol dehydrogenase activity. Activity towards all substrates tested was about one-fifth that found in

extracts of broth-grown organisms. DL-1-Aminopropan-2-ol added as a supplement to either broth or glucose-mineral salts media had no effect on enzyme activity. Of other alternative substrates tested, only hydroxyacetone induced enzyme formation markedly. Six- to tenfold increases occurred when washed suspensions of bacteria grown on glucose were incubated with hydroxyacetone at 150 mM. Dihydroxyacetone and aminoacetone stimulated 2.5- and 1.6-fold respectively. Similar results were obtained when inducers were added as 0.4 % supplements to cultures of *E. coli* growing on glucose. *Escherichia coli* was incapable of growth on the hydroxy and

Table 6. *Dehydrogenase activities in crude extracts of micro-organisms*

Micro-organisms were grown on nutrient broth and dehydrogenase activities were assayed spectrophotometrically as described in the methods section.

Micro-organism	Strain	Dehydrogenase activity (mμmoles/mg. protein/min. at 37°) with substrate shown			
		D-1-Amino-propan-2-ol	L-1-Amino-propan-2-ol	meso-Butan 2,3-diol	DL-Propan-1,2-diol
<i>Achromobacter</i> sp. P6	NCIB 10431	2.8	0.7	32.0	13.0
<i>Arthrobacter globiformis</i>	NCIB 9759	1.5	0.1	n.d.	n.d.
<i>Bacillus licheniformis</i>	NCIB 8061	2.6	0.3	136.0	170.0
<i>B. polymyxa</i>	NCIB 8524	21.0	4.0	850.0	700.0
<i>B. subtilis</i>	Laboratory	1.0	3.0	20.0	31.0
<i>Enterobacter cloacae</i>	NCIB 8529	1.0	0.3	185.0	166.0
<i>Erwinia carotovora</i>	NCPPB 1280	4.1	9.9	n.d.	n.d.
<i>Escherichia coli</i> , type I	NCIB 8114	9.0	10.0	35.0	58.0
<i>E. coli</i> , K12	NCIB 9481	11.4	n.d.	n.d.	150.0
<i>E. coli</i> , K12	NCIB 9482	8.5	n.d.	n.d.	125.0
<i>E. coli</i> , K12	NCIB 9483	7.7	n.d.	n.d.	95.0
<i>Klebsiella aerogenes</i> , type I	NCIB 418	2.0	8.0	n.d.	n.d.
<i>K. aerogenes</i> , type II	NCIB 8017	0.5	0.3	125.0	90.0
<i>Proteus vulgaris</i>	Laboratory	2.0	1.7	n.d.	n.d.
<i>Pseudomonas ovalis</i>	NCIB 9229	2.0	0.3	n.d.	n.d.
<i>Pseudomonas</i> sp.	NCIB 8858	0.3	1.0	n.d.	n.d.
<i>Pseudomonas</i> sp.	DL8A	8.0	2.0	34.0	64.0

* n.d. = not determined.

Table 7. *L-Threonine and D-aminopropanol dehydrogenases in micro-organisms used for production of vitamin B₁₂*

Micro-organisms were grown on peptone-yeast extract medium and enzymes in extracts assayed colorimetrically as described in the Methods section.

Micro-organism	Strain (NCIB no.)	Growth conditions	Dehydrogenase activity (mμmoles/mg. protein/min. at 37°)	
			D-Aminopropanol	L-Threonine
<i>Flavobacterium devorans</i>	8195	Aerobic	0.4	1.1
<i>Nocardia rugosa</i>	8926	Aerobic	3.1	17.0
<i>Propionibacterium freudenreichii</i>	5959	Anaerobic	0	0
<i>P. shermanii</i>	5964	Aerobic	3.2	19.0
<i>P. shermanii</i>	5964	Anaerobic	0	0
<i>P. shermanii</i>	8099	Anaerobic	0	0
<i>Pseudomonas dentrificans</i>	8376	Aerobic	1.1	3.7
<i>Streptomyces griseus</i>	9004	Aerobic	0.8	14.0
<i>S. olivaceus</i>	8238	Aerobic	0.8	2.0

amino ketones tested, or on the corresponding alcohols. Dehydrogenase activity towards L-1-aminopropan-2-ol was unaffected by hydroxy ketones and analogues, which reduced L-threonine dehydrogenase activity when included in growth media.

Dehydrogenase activities of various micro-organisms

Extracts of micro-organisms grown on broth were examined for dehydrogenase activities towards several secondary alcohols, including D-1-aminopropan-2-ol. Results are shown in Table 6.

A number of micro-organisms known to produce relatively large amounts of vitamin B₁₂ were examined for L-threonine and D-1-aminopropan-2-ol dehydrogenase. Two of the propionibacter species would grow only anaerobically, although a third was capable of aerobic growth. The results are shown in Table 7.

DISCUSSION

The small and slow uptake of radioactivity from [3-¹⁴C]-DL-1-aminopropan-2-ol by growing cultures of *Escherichia coli* does not allow us to make any unequivocal statement regarding a biosynthetic role for the D-isomer of the amino alcohol. Neither DL-1-aminopropan-2-ol nor aminoacetone act as a growth substrate or nitrogen source for the *E. coli* in synthetic media, although L-1-aminopropan-2-ol can be readily oxidized to aminoacetone by several intact micro-organisms including *E. coli*. However, the enzyme responsible for oxidation of L-aminopropanol in *E. coli* has been studied (Turner, 1967) and its metabolic significance is uncertain.

The enzyme responsible for D-1-aminopropan-2-ol oxidation in cell-free extracts of *Escherichia coli* is concluded to be an oxidoreductase of broad specificity in each direction. Evidence includes the constant activity for D-1-aminopropan-2-ol relative to DL-propan-1,2-diol, throughout purification; the inhibition of amino alcohol oxidation by alternative substrates; and induction of activity by hydroxyacetone or dihydroxyacetone but not aminoacetone or 1-aminopropan-2-ol. In a comparative study, the highest D-1-aminopropan-2-ol dehydrogenase activity was found in *Bacillus polymyxa*, known to possess only the D-specific butan-2,3-diol dehydrogenase (Taylor & Juni, 1960), whereas negligible activity was found in species with the L-specific enzyme, e.g. *Enterobacter cloaca* (Taylor & Juni, 1960).

The metabolic function of the enzyme studied in *Escherichia coli* is not clear, although specificity studies indicate involvement in diol and hydroxyketone metabolism. The acetoin fermentation, however, yielding butan-2,3-diol, is characteristic of *Aerobacter aerogenes* rather than *E. coli*, although the butan-2,3-diol-NAD⁺ oxidoreductase of *A. aerogenes* studied by Taylor & Juni (1960) was stereospecific for the D-enantiomorphs of the diol substrates tested. Alternatively, the heat-stability of the enzyme, its activation by monovalent cations, pH optimum and K_m for NAD⁺, all suggests close similarity to the glycerol-NAD⁺ oxidoreductases studied in *E. coli* (Asnis & Brodie, 1953) as well as *A. aerogenes* (Burton, 1955; Lin & Magasanik, 1960; Strickland & Miller, 1968). It should be noted that glycerol but not glycerol-1-phosphate was a substrate for the enzyme described here (Table 4). The failure of suspensions of *E. coli* to produce aminoacetone when incubated with D-1-aminopropan-2-ol may well be due to competition by endogenously generated substrates for the oxidoreductase found in extracts. The K_m for glycerol found by Asnis &

Brodie (1953) was 10.7 mm, compared with that of 2.0 to 5.0 mm for D-I-aminopropan-2-ol reported here.

The significant activity of NADPH as a cofactor for ketone reduction, in contrast to the inactivity of NADP⁺ in the oxidation direction, is noteworthy. It is possible that the oxidoreduction between NADPH and hydroxy- or aminoacetone may be catalysed by a separate contaminating enzyme. Work to establish this has not been carried out. Whether aminoacetone reduction, by either NADH or NADPH, yields the D- or L-stereoisomer has not been determined.

The enzyme from *Escherichia coli* K12, claimed by Dekker & Swain (1968) to be specific for the reversible oxidoreduction reaction between aminoacetone and D-I-aminopropan-2-ol, appears to differ from the enzyme described here. The enzyme was optimally active with the D-amino alcohol in tris+HCl buffer between pH 8.0 and 8.6, and inactive with all of a large number of substrate analogues. No activation by monovalent cations or activity with NADPH was reported. The activity with D-I-aminopropan-2-ol in crude extracts was about 14.0 to 14.6 m μ moles/mg. protein/min. at 37° and pH 8.4 (Dekker & Swain, 1968). This activity is about the same as that of the non-specific enzyme described here, and found to be present not only in *E. coli* type I, but also in *E. coli* K12 strains.

No convincing evidence for the joint involvement of L-threonine and D-I-aminopropan-2-ol dehydrogenases in the biosynthesis of the vitamin B₁₂ was found, in that *Propionibacterium* species, which are known to produce good yields of the vitamin during anaerobic growth (Mervyn & Smith, 1964), did not possess either enzymes when grown under such conditions. It now seems clear that in addition to the non-specific nature of the oxidoreductase acting on D-I-aminopropan-2-ol and aminoacetone, L-threonine dehydrogenase may be involved in catabolism yielding glycine and acetyl-CoA (McGilvray & Morris, 1969) as an alternative to aminoacetone.

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REFERENCES

- ASNIS, R. E. & BRODIE, A. F. (1953). A glycerol dehydrogenase from *Escherichia coli*. *Journal of Biological Chemistry* **203**, 153–159.
- BURTON, R. M. (1955). Glycerol dehydrogenase from *Aerobacter aerogenes*. *Methods in Enzymology* **1**, 397–400.
- CHATELUS, G. (1964). La décarboxylation thermique des acides α -aminés. *Bulletin de la Société Chimique de France* **1**, 2523–2532.
- CLARK, R. L., JONES, W. H., RAICH, W. J. & FOLKERS, K. (1954). Vitamin B₁₂. XXIII. Resolution of DL-I-amino-2-propanol. *Journal of the American Chemical Society* **76**, 3995–3997.
- DEKKER, E. E. & SWAIN, R. R. (1968). Formation of D₂-I-amino-2-propanol by a highly purified enzyme from *Escherichia coli*. *Biochimica et Biophysica Acta* **158**, 306–307.
- GOTTLIEB, S. F. & MANDEL, M. (1959). Utilization of I-amino-2-propanol by soil bacterium. *Canadian Journal of Microbiology* **5**, 363–368.
- HALL, H. H., BENEDICT, R. G., WIESEN, C. F., SMITH, C. E. & JACKSON, R. W. (1953). Studies on vitamin B₁₂ production with *Streptomyces olivaceus*. *Applied Microbiology* **1**, 124–135.

- HIGGINS, I. J., PICKARD, M. A. & TURNER, J. M. (1968). Aminoacetone formation and utilization by pseudomonads grown on DL-1-aminopropan-2-ol. *Journal of General Microbiology* **54**, 105–114.
- KARRER, P. & KLARER, W. (1925). Abbau der 1- β -Oxybuttersäure. *Helvetica Chimica Acta* **8**, 393–395.
- KRASNA, A. I., ROSENBLUM, C. & SPRINSON, D. B. (1957). The conversion of L-threonine to the D₂-1-amino-2-propanol of vitamin B₁₂. *Journal of Biological Chemistry* **225**, 745–750.
- LIN, E. C. C. & MAGASANIK, B. (1960). The activation of glycerol dehydrogenase from *Aerobacter aerogenes* by monovalent cations. *Journal of Biological Chemistry* **235**, 1820–1823.
- LINWEAVER, H. & BURK, D. (1934). The determination of enzyme dissociation constants. *Journal of the American Chemical Society* **56**, 658–663.
- LOWE, D. A. & TURNER, J. M. (1968). Enzymic oxidation of D-1-aminopropan-2-ol by diol dehydrogenases of microbial origin. *Biochimica et Biophysica Acta* **170**, 455–456.
- MCGILVRAY, D. & MORRIS, J. G. (1969). Utilization of L-threonine by a species of *Arthrobacter*. A novel catabolic role for 'aminoacetone synthase'. *Biochemical Journal* **112**, 657–671.
- MERVYN, L. & SMITH, E. L. (1964). The biochemistry of vitamin B₁₂ fermentation. *Progress in Industrial Microbiology* **5**, 153–201.
- STRICKLAND, J. E. & MILLER, O. N. (1968). Inhibition of glycerol dehydrogenase from *Aerobacter aerogenes* by dihydroxyacetone, high ionic strength and monovalent cations. *Biochimica et Biophysica Acta* **159**, 221–226.
- SULLIVAN, R. H. (1963). Resolution of racemic aminoisopropanol. *U.S. Patent* **3**, 116, 332 (C1-260-584).
- TAYLOR, M. B. & JUNI, E. (1960). Stereoisomeric specificities of 2,3-butanediol dehydrogenases. *Biochimica et Biophysica Acta* **39**, 448–457.
- TURNER, J. M. (1966). Microbial metabolism of amino ketones. Aminoacetone formation from 1-aminopropan-2-ol by a dehydrogenase in *Escherichia coli*. *Biochemical Journal* **99**, 427–433.
- TURNER, J. M. (1967). Microbial metabolism of amino ketones. L-1-Aminopropan-2-ol dehydrogenase and L-threonine dehydrogenase in *Escherichia coli*. *Biochemical Journal* **104**, 112–121.