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Microbial Metabolism of Amino Ketones

AMINOACETONE FORMATION FROM 1-AMINOPROPAN-2-OL BY A DEHYDROGENASE IN *ESCHERICHIA COLI*

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1. Washed-cell suspensions of *Escherichia coli*, incubated at the optimum pH of 6·4 and with a saturating substrate concentration of approx. 10mM, convert DL-1-aminopropan-2-ol into aminoacetone at a rate of approx. 4·0mμmoles/mg. dry wt. of cells/min. at 30°. 2. Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Ca^{2+}, K^+ and NH_4^+, as sulphates, and EDTA have no effect on this rate, although Cu^{2+} inhibits and Fe^{2+} activates to some extent. 3. Conditions of growth markedly affect the rate of aminoacetone production by cell suspensions. 4. Dialysed cell-free extracts of *E. coli* exhibit 1-aminopropan-2-ol-dehydrogenase activity, the enzyme having optimum activity at pH 7·0, a requirement for NAD^+ and K^+, and a K_m for the amino alcohol substrate of 0·8mM, calculated for a single enantiomorph. 5. Under optimum conditions 1-aminopropan-2-ol dehydrogenase forms aminoacetone at rate of approx. 3·0mμmoles/mg. of protein/min. at 37°. The enzyme is only slightly inhibited by DL-3-hydroxybutyrate and DL-2-hydroxy-2-phenylethylamine. 6. L-Threonine-dehydrogenase activity is exhibited by both whole cells and cell-free extracts. Whole cells produce aminoacetone from L-threonine more slowly than they do from DL-1-aminopropan-2-ol, whereas the situation is reversed in cell-free extracts. Both kinetic evidence, and the fact that synthesis of 1-aminopropan-2-ol dehydrogenase, but not of threonine dehydrogenase, is repressed by compounds such as glucose and pyruvate, provide evidence that the amino alcohol is oxidized by a specific enyme. 7. The metabolic role of 1-aminopropan-2-ol dehydrogenase is discussed.

Aminoacetone was first detected as a microbial metabolite by Elliott (1958), who demonstrated the formation of this amino ketone from threonine, or glycine plus glucose, by washed-cell suspensions of *Staphylococcus aureus*. The compound was later isolated and identified (Elliott, 1959, 1960*b*). Factors affecting aminoacetone formation by *Staph. aureus* cells were investigated by using both threonine and glycine as precursors, and mechanisms involving dehydrogenation and a synthetase reaction with acetyl-CoA respectively were suggested (Elliott, 1960*b*). The formation of aminoacetone by *Rhodopseudomonas spheroides*, and the presence of an enzyme in this micro-organism that converts L-threonine into aminoacetone by oxidative decarboxylation, using NAD^+, was reported by Neuberger & Tait (1960, 1962). These workers described factors affecting aminoacetone formation by washed-cell suspensions of *R. spheroides* from both L-threonine and a mixture of glycine, α-oxoglutarate and fumarate, and described the properties of an L-threonine dehydrogenase that was purified approximately fivefold from extracts of the organism (Neuberger & Tait, 1962). A sevenfold purification of the enzyme from *Staph. aureus*, by Green & Elliott (1964), revealed that it was similar in many respects to the enzyme partially purified from *R. spheroides* by Neuberger & Tait (1962), although differences in non-nucleotide substrate specificity were apparent. In both cases, however, the mechanism of aminoacetone formation was shown not to involve 1-aminopropan-2-ol as an intermediate, and this compound was found to be completely inactive as a substrate for both enzyme preparations. In addition, the amino alcohol was found to be only slightly inhibitory towards the threonine dehydrogenase of *Staph. aureus* (Green & Elliott, 1964), and to be completely inactive in this respect with the *R. spheroides* enzyme (Neuberger & Tait, 1962).

The present paper describes the rapid conversion of 1-aminopropan-2-ol into aminoacetone by washed-cell suspensions of *Escherichia coli*, and presents evidence that a dehydrogenase distinct from

L-threonine dehydrogenase is responsible. The characteristic ability of a variety of micro-organisms to produce aminoacetone with greater facility from 1-aminopropan-2-ol rather than from L-threonine is noted.

Interest in the metabolic role of aminoacetone was based on the suggestion (Elliott, 1959, 1960*b*) that an aminoacetone cycle, analogous to the succinate–glycine cycle proposed by Shemin & Russell (1953), may operate for the oxidation of glycine in Nature. A new precursor, or product, of an intermediate of such a cycle may be of significance in elucidating its possible significance. The interconversion of aminoacetone and 1-aminopropan-2-ol by an oxidoreductase mechanism may also be of significance in the formation of the D-1-aminopropan-2-ol moiety of vitamin B_{12} from L-threonine by *Streptomyces griseus* (Krasna, Rosenblum & Sprinson, 1957).

EXPERIMENTAL

Growth and harvesting of E. coli. *E. coli*, type I (National Collection of Industrial Bacteria no. 8114), was maintained on nutrient agar slopes, and grown on 2·5% (w/v) nutrient broth (Oxoid no. 2) unless otherwise stated. Cultures were incubated for approx. 16hr. at 30° with agitation in 2l. conical flasks containing 500ml. of growth medium. Organisms were harvested by centrifuging (5000*g* for 20min.), washed once and resuspended in the appropriate buffer by homogenization in a Potter-type homogenizer.

Preparation of cell-free extracts. Organisms suspended in 0·1M-potassium phosphate buffer, pH7, were disrupted in a cold Aminco–French pressure cell (American Instrument Co. Inc., Silver Spring, Md., U.S.A.) operated at 20000lb./in.^{2} and the resulting cell-crush was centrifuged at 100000*g* for 90min. in an MSE Superspeed 50 preparative ultracentrifuge. The clear supernatant was dialysed overnight against a large volume of 0·1M-potassium phosphate buffer, pH7, containing 2-mercaptoethanol (0·1mM). Extracts were stored at −18° until required.

Measurement of aminoacetone production by washed-cell suspensions. Organisms were suspended in 0·1M-potassium phosphate buffer, pH6·4, and diluted with the same buffer so that the $E^{1\,cm.}_{540\,m\mu}$ for the suspension was 1·50 (i.e. 0·6mg. dry wt. of cells/ml.). Incubation mixtures contained 1m-mole of phosphate buffer, 3·0mg. dry wt. of cells and 0·5m-mole of DL-1-aminopropan-2-ol in a total volume of 10ml. Incubations were started by the addition of substrate to the other components contained in 50ml. conical flasks equilibrated at 30° and plugged with cotton wool. Incubation mixtures were shaken for 90min. at 30° on a rotary shaker, and incubation was terminated by the addition of 1ml. of 25% (w/v) trichloroacetic acid. Cells were removed from suspension by centrifuging, and 0·5ml. of supernatant was used for aminoacetone assay.

Measurement of aminopropanol-dehydrogenase activity of cell-free extracts. The method used was a modification of that described for threonine dehydrogenase by Green & Elliott (1964). Reaction mixtures contained 0·2m-mole of tris–HCl buffer, pH7·05, 0·15m-mole of sodium pyruvate, 10μmoles of NAD$^+$, 0·1mg. of lactate dehydrogenase, 0·5m-mole of KCl, 0·6mg. of protein (dialysed cell-free extract) and 0·25m-mole of DL-1-aminopropan-2-ol in a total volume of 1·56ml. Reactions were started by the addition of enzyme preparation to the other components in thin-walled tubes equilibrated at 37° in a water bath. Incubation was for 30min. at 37°. Reactions were stopped by the addition of 0·3ml. of 25% (v/v) $HClO_4$. After centrifuging to remove precipitated material, 1·0ml. of supernatant was used for aminoacetone assay.

Measurement of threonine-dehydrogenase activity of cell-free extracts. The method used was similar to that described for aminopropanol dehydrogenase except that the substrate was L-threonine (0·125m-mole), approx. 0·1mg. of extract protein was used and incubation was usually for 15min. at 37°.

Assay of aminoacetone. Aminoacetone was determined colorimetrically after condensation with acetylacetone by the method of Mauzerall & Granick (1956). The sample containing aminoacetone was adjusted to pH4·6 where necessary, made up to 1ml. and added to an equal volume of 2·0M-sodium acetate buffer, pH4·6. After the mixture had been heated with 0·1ml. of acetylacetone, the colour due to pyrrole was developed with 2ml. of Ehrlich's reagent. Calibration curves showed that in the above procedure 10mμmoles of aminoacetone gave an $E^{1\,cm.}_{551\,m\mu}$ value of 0·130, corresponding to a molecular extinction coefficient, ϵ, of $5{\cdot}33 \times 10^4$.

Assay of protein. Protein was measured by a modified biuret procedure. To 0·5ml. of a sample, containing 2–10mg. of protein, was added 3·5ml. of water, followed by 0·9ml. of 20% (w/v) NaOH. After mixing, 0·15ml. of 25% (w/v) $CuSO_4,5H_2O$ was added, the mixture shaken vigorously and the precipitate removed by centrifuging. The biuret colour was read at 560mμ. Calibration curves were constructed with crystalline bovine plasma albumin.

Units of enzyme activity. Where enzyme activity is related to the amount of protein present in cell-free extracts, specific activities are expressed as mμmoles of aminoacetone produced/mg. of protein/min.

Chemicals. DL-1-Aminopropan-2-ol was obtained from British Drug Houses Ltd., Poole, Dorset. Aminoacetone hydrochloride was purchased from California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A. NAD$^+$ and NADP$^+$ were supplied by C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany, and ox-heart lactate dehydrogenase (75 units/mg.) as a suspension in aq. 50%-saturated $(NH_4)_2SO_4$ by Koch–Light Laboratories Ltd., Colnbrook, Bucks. Powdered nutrient broth was obtained as Nutrient Broth no. 2 (code no. CM67) from the Oxoid Division of Oxo Ltd., London. All other chemicals were of A.R. grade, or the highest quality available commercially.

RESULTS

The preliminary results of a systematic search for micro-organisms capable of producing amino ketones from a variety of known and possible precursors revealed that washed suspensions of *E. coli* formed aminoacetone from DL-1-aminopropan-2-ol at a rate approx. 20 times that from equimolar amounts of DL-threonine. Whereas similar results were obtained for some other species in the Order Eubacteriales, e.g. *Achromobacter*

anitratus, and for the actinomycete *Strep. griseus*, most of the micro-organisms tested produced aminoacetone more readily from threonine than aminopropanol (J. M. Turner, unpublished work). In view of the facts that aminoacetone formation from aminopropanol had not been previously reported, and that the metabolism of *E. coli* has been extensively studied, it was decided to investigate the reaction with this micro-organism.

Factors affecting aminoacetone formation from 1-aminopropan-2-ol by cell suspensions

Cell density. Initial experiments, with incubation mixtures at pH 7 and shaken for 16hr. but otherwise under the conditions described in the Experimental section, showed that aminoacetone production was proportional to cell density up to approx. 0·6mg. dry wt./ml. Above this value there was an increasingly large deviation from linearity. With an incubation time of 90min. and at pH 6·4, aminoacetone production was proportional to cell density up to at least 1·3mg. dry wt./ml.

pH. The optimum pH for aminoacetone formation was 6·4 (see Fig. 1). The fall in activity above pH 7·5 may be due in part to the known instability of aminoacetone under alkaline rather than acid conditions (Elliott, 1960*b*).

Substrate concentration. At pH 6·4, the system was fully saturated at an aminopropanol concentration of 10mM with respect to single isomers. The apparent K_m, determined from a double-reciprocal plot according to the method of Lineweaver & Burk (1934), was approx. 0·3mM.

Inorganic ions. Of the cations tested, as their sulphates, Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Ca^{2+}, K^+ and NH_4^+ had no effect at 1mM concentration. With Cu^{2+}, 20% inhibition was observed, and Fe^{2+} caused 10% stimulation at this concentration. EDTA (1mM) had no effect. No inhibition by 100mM-SO_4^{2-} was detectable.

Substrate analogues. No detectable inhibition of aminoacetone formation was found in the presence of 10mM-DL-serine, -L-threonine or -DL-3-phenylserine. Less than 10% inhibition was observed with DL-3-hydroxybutyrate or DL-2-hydroxy-2-phenylethylamine at 10mM concentration. Aminoacetone production from L-threonine alone was less than 5% of that from aminopropanol under the conditions employed. Analogue concentrations were calculated on the basis of single isomers of the type indicated, but *allo*/*threo* isomers were not taken into account.

Growth conditions. Micro-organisms harvested shortly after cultures had entered the stationary phase of growth were as active as cells harvested in the exponential growth phase. Storage of suspensions of washed cells in 0·1M-potassium phosphate buffer, pH 6·4, for several hours at less than 5° had no detectable effect on their activity.

When micro-organisms were grown on nutrient broth in which 40% of the powdered broth was

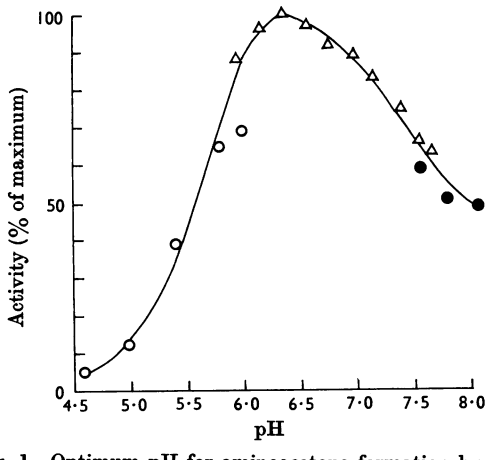

Fig. 1. Optimum pH for aminoacetone formation by cell suspensions. Incubation mixtures contained 1·3m-moles of buffer, 0·25m-mole of DL-1-aminopropan-2-ol and approx. 7mg. dry wt. of cells in a total of 10ml. Incubation at 30° was for 90min. Aminoacetone formation was measured as described in the Experimental section. The buffers used were phthalate–NaOH (○), potassium phosphate (△) and tris–HCl (●).

Table 1. *Effect of growth conditions on aminoacetone production by whole-cell suspensions*

E. coli was grown on media containing 1·5% (w/v) nutrient broth plus 1% (w/v) of the compounds indicated. Media were adjusted to pH 7 with 5N-NaOH where necessary, and sugars were sterilized separately. After inoculation and growth, cells were harvested, washed and resuspended in buffer, and their ability to produce aminoacetone was measured as described in the Experimental section.

Addition to growth medium	Aminoacetone production (mμmoles/mg. dry wt. of cells/min.)	(%)
Nutrient broth	4·23	100
Acetic acid	3·95	94
DL-Lactic acid	3·95	94
Succinic acid	3·80	90
Sucrose	3·75	89
Maltose	3·16	75
DL-Malic acid	2·99	71
Citric acid	2·79	67
Pyruvic acid	2·42	57
Fumaric acid	2·35	56
Glycerol	2·00	47
Fructose	1·43	34
Glucose	0·93	22

Table 2. *Effect of omitting various components of the reaction mixture, and also the effect of* K^+ *concentration, on the* 1-*aminopropan*-2-*ol-dehydrogenase activity of crude extracts*

Complete reaction mixtures contained 0·2m-mole of tris–HCl buffer, pH7·05, 0·15m-mole of sodium pyruvate, 10μmoles of NAD^+, 0·1mg. of lactate dehydrogenase, 0·5m-mole of KCl, 0·75mg. of bacterial protein and 0·25m-mole of DL-aminopropanol in a total volume of 1·56ml. Enzyme activity was measured as described in the Experimental section. The effect of K^+ concentration was determined on a separate occasion.

Component omitted	Specific activity (mμmoles of aminoacetone/mg./min.)
None	1·86
Aminopropanol	0·05
KCl	1·16
NAD^+ (10μmoles)	1·48
Pyruvate	1·66
Lactate dehydrogenase	1·68
Pyruvate and lactate dehydrogenase	1·42
Pyruvate, NAD^+ and lactate dehydrogenase	0·54
Extract	0·10
NAD^+ (4·5μmoles)	1·64
NAD^+ (6·0μmoles)	1·29
NAD^+ (9·0μmoles)	0·97
NAD^+ replaced by $NADP^+$	1·49
None	2·30
KCl (0·1m-mole)	2·38
KCl (0·2m-mole)	2·18
KCl (0·3m-mole)	1·90
KCl (0·5m-mole)	0·95

replaced by an equal weight of various intermediary metabolites, e.g. pyruvate or glucose, the ability of cell suspensions to form aminoacetone from aminopropanol was markedly affected in some cases. The results are shown in Table 1. In no case did a modification of medium composition give rise to washed-cell suspensions having increased aminoacetone-producing activity.

Examination of incubation product. Aminoacetone was concluded to be the amino ketone produced from aminopropanol on the following evidence. The absorption spectrum of the colour obtained by the procedure used for assay showed a peak at 550mμ, with a shoulder at approx. 520mμ, and was identical with that obtained with a sample of authentic aminoacetone. The coloured complex formed with picric acid and alkali (Shuster, 1956; Elliott, 1960*a*) had a similar spectrum in both cases ($\lambda_{max.}$ approx. 440mμ). When cell-suspension supernatants were heated with acetylacetone at 100° for 10min., and the resulting pyrrole was extracted with ether, paper chromatography of the pyrrole in butan-1-ol–acetic acid–water (63:10:27, by vol.) and butan-1-ol–1·5N-ammonia

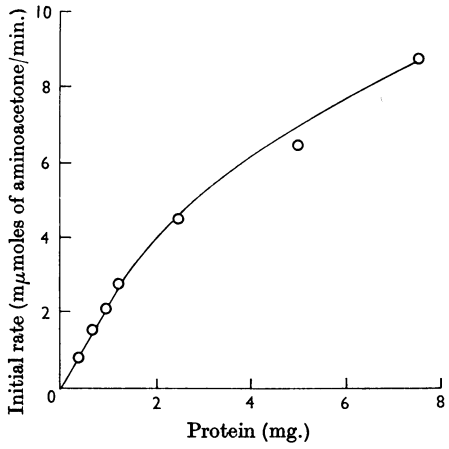

Fig. 2. Effect of extract protein concentration on enzyme reaction rate. 1-Aminopropan-2-ol-dehydrogenase activity was measured as described in the Experimental section, with the amounts of bacterial protein shown, in a total volume of 1·56ml.

(1:1, v/v) gave R_F 0·86 in both cases (Neuberger & Tait, 1962). Although the amino ketone was not isolated and fully characterized, the nature of the substrate, and the nature of the enzyme system described below, provide strong evidence for its identity as aminoacetone.

1-*Aminopropan*-2-*ol-dehydrogenase activity in extracts*

A search was made for aminopropanol-dehydrogenase activity in soluble extracts of *E. coli* by using the assay system described in the Experimental section. The effect of omitting various components of this system, also the effect of NAD^+ concentration and the substitution of coenzyme by $NADP^+$, is shown in the first part of Table 2. The effect of various factors on enzyme activity is described below.

Protein concentration. The rate of enzymic formation of aminoacetone was proportional to bacterial protein concentration up to approx. 1·0mg./ml. of reaction mixture, as shown in Fig. 2.

Optimum pH. The effect of pH on enzymic oxidation of aminopropanol is shown in Fig. 3. A higher than usual NAD^+ concentration was used in this case to counteract the possible effect of pH on the efficiency of the coenzyme-regenerating system. The pH optimum was approx. 7·0.

Michaelis constant for aminopropanol. The effect of substrate concentration on the activity of the enzyme was determined, and the results were plotted as a double-reciprocal plot (Lineweaver & Burk, 1934). The results indicated a K_m value of 0·8mM, calculated on the basis of a single enantiomorph of DL-aminopropanol.

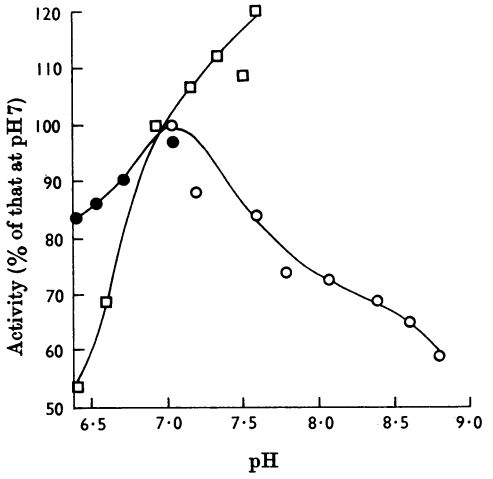

Fig. 3. Effect of pH on 1-aminopropan-2-ol-dehydrogenase and threonine-dehydrogenase activities. Aminopropanol-dehydrogenase activity was measured as described in the Experimental section, except that 15 μmoles of NAD^+ were present, and buffers of pH between 6·4 and 8·8 were used at a concentration of 0·14M. The buffers were sodium phosphate (●) and tris–HCl (○). The plot illustrating the effect of pH on threonine-dehydrogenase activity was constructed from data obtained on a separate occasion. In this case L-threonine replaced DL-aminopropanol in the assay procedure, and other details were as described in the Experimental section. The buffer used was 0·14M-potassium phosphate (□).

Effect of univalent cations. Green & Elliott (1964) found that L-threonine dehydrogenase of *Staph. aureus* was both activated and protected against thermal denaturation by K^+. Further investigation by Green (1964) suggested that a K^+–apoenzyme complex is essential for enzyme activity. In the present case, K^+ was found to be essential for maximum activity of aminopropanol-dehydrogenase activity. The effect of K^+ is shown in the second part of Table 2.

Effect of substrate analogues. The effects of DL-serine, L-threonine, DL-3-hydroxybutyrate and DL-2-hydroxy-2-phenylethylamine on aminoacetone formation, together with the amount of aminoacetone formed from L-threonine alone under the same conditions, are shown in the first part of Table 3. The presence of DL-3-hydroxybutyrate or DL-2-hydroxy-2-phenylethylamine caused significant inhibition, and the amount of aminoacetone produced from threonine alone was fivefold greater than that formed from aminopropanol under similar conditions. Aminoacetone formation from a mixture of aminopropanol and threonine was greater than that from either substrate alone, i.e. activites were additive.

Evidence for the separate identity of aminopropanol dehydrogenase and threonine dehydrogenase

The effect of substrate concentration on the L-threonine-dehydrogenase activity detected in

Table 3. *Activity and inhibitory effects of 1-aminopropan-2-ol analogues*

Aminopropanol-dehydrogenase activity was measured as described in the Experimental section. Additions were made at the concentrations shown. Threonine replaced, or was present in addition to, aminopropanol where indicated. The concentration of additions was calculated for single isomers of the type indicated.

Substrate	Addition	Specific activity (mμmoles of aminoacetone/mg./min.)
DL-Aminopropanol (40mM)	None	2·10
DL-Aminopropanol (40mM)	DL-Serine (40mM)	2·08
DL-Aminopropanol (40mM)	DL-Serine (80mM)	1·75
DL-Aminopropanol (40mM)	DL-3-Hydroxybutyrate (40mM)	1·65
DL-Aminopropanol (40mM)	DL-3-Hydroxybutyrate (80mM)	1·55
DL-Aminopropanol (40mM)	DL-2-Hydroxy-2-phenylethylamine (40mM)	1·15
DL-Aminopropanol (40mM)	DL-2-Hydroxy-2-phenylethylamine (80mM)	1·07
DL-Aminopropanol (40mM)	L-Threonine (40mM)	14·50
DL-Aminopropanol (40mM)	L-Threonine (80mM)	15·90
L-Threonine (40mM)	None	11·10
L-Threonine (80mM)	None	15·10
L-Threonine (58mM)	None	36·20
L-Threonine (72mM)	None	36·70
DL-Aminopropanol (58mM)	None	2·96
DL-Aminopropanol (72mM)	None	2·96
L-Threonine (58mM)	DL-Aminopropanol (58mM)	42·10
L-Threonine (72mM)	DL-Aminopropanol (72mM)	39·76

Table 4. *Effect of 1-aminopropan-2-ol concentration on dehydrogenase activities with single and mixed substrates*

Conditions were as described for Table 3.

Substrate	Specific activity at the concn. of aminopropanol shown (mμmoles of aminoacetone/mg./min.)				
	0	12·5 mM	25·0 mM	50·0 mM	100·0 mM
DL-Aminopropanol	0	2·90	2·90	2·74	3·06
DL-Aminopropanol + L-threonine (62·5 mM)	31·6	32·90	32·50	34·50	32·90

Table 5. *Effect of growth conditions on dehydrogenase activities for 1-aminopropan-2-ol and threonine*

Organisms were grown on nutrient broth supplemented with the compounds shown, as described for Table 1. Cell-free extracts were prepared and their dehydrogenase activities measured immediately, as described in the Experimental section.

Supplement to growth medium	Specific activity with substrate indicated (mμmoles of aminoacetone/mg./min.)	
	DL-Aminopropanol	L-Threonine
None	2·12	59·0
Glycerol	0·30	65·0
Fumarate	0·23	53·0
Pyruvate	0·20	53·0
Glucose	0·05	63·0
Fructose	0·03	70·0

extracts was determined at pH 7·05 by the method of Lineweaver & Burk (1934). The K_m value of 10·5 mM for L-threonine was calculated from the results. The fact that this value was over tenfold greater than that obtained under identical conditions for aminopropanol was at variance with the velocity measurements for single and mixed substrates (see the first part of Table 3), if a single dehydrogenase was involved in the oxidation of both substrates. The redetermination of relative reaction rates, at two substrate concentrations, is shown in the second part of Table 3. In addition to the fact that the activity values for mixed substrates do not lie between the corresponding values for single substrates, in this case the activity with threonine was approx. 12-fold that with aminopropanol. The effect of the concentration of aminopropanol used both singly and in mixtures containing a constant concentration of threonine is shown in Table 4. Once again activities were additive, the aminopropanol concentration being saturating at all concentrations.

When organisms were grown on media supplemented with compounds found to depress the rate of aminoacetone production from aminopropanol by whole cells (see Table 1), the aminopropanol-dehydrogenase activity of cell-free extracts was lowered. In contrast, threonine-dehydrogenase activity was virtually unaffected. The results are shown in Table 5.

DISCUSSION

The conversion of 1-aminopropan-2-ol into aminoacetone by an oxidoreductase mechanism accounts for the appearance of the amino ketone in the incubation medium when washed suspensions of *E. coli* are incubated with aminopropanol. The microbial formation of aminoacetone via this route has not been reported previously. The fact that the relative rates of aminoacetone formation observed with threonine and aminopropanol as substrate are reversed when whole cells are compared with soluble extracts suggests that permeability factors limit the uptake of the amino acid.

Although a number of similarities exist when the aminopropanol dehydrogenase described above is compared with the microbial threonine dehydrogenases described by Neuberger & Tait (1962) and Green & Elliott (1964), e.g. a requirement for K^+ and maximum activity with NAD^+ rather than $NADP^+$, differences are also apparent. Whereas these threonine dehydrogenases have pH optima at 8·8 and 8·4 respectively, and that of a similar enzyme present in frog-liver mitochondria (Hartshorne & Greenberg, 1964) has optimum activity at pH 7·8, the activity of the aminopropanol dehydrogenase of *E. coli* is maximal at 7·0. Whereas crude extracts of *R. spheroides* produced no aminoacetone from aminopropanol (Neuberger & Tait, 1962), and the amino alcohol was similarly inactive with *Staph. aureus* preparations (Green & Elliott, 1964), aminoacetone production from aminopropanol in fresh extracts of *E. coli* occurs at an appreciable rate. More direct evidence that distinct dehydrogenases are responsible for aminoacetone formation are the findings that activities with mixed substrates are additive when compared with those for single substrates both used at saturating concentrations, that mixed substrate

activities are inconsistent with the relative K_m values if a single enzyme is involved, and that activity ratios appear to vary during storage of extracts. Whereas aminopropanol dehydrogenase is repressed by growth of *E. coli* on media containing glucose etc., threonine-dehydrogenase activities are virtually unaffected. Although final proof depends on the purification and separation of the two activities, it appears certain that the dehydrogenases for aminopropanol and threonine in *E. coli* are distinct enzymes.

The occurrence of 1-aminopropan-2-ol in Nature was first discovered by Wolf, Jones, Valiant & Folkers (1950), who identified the compound as a product of vitamin B_{12} hydrolysis. More recently the compound has been detected as a constituent of urine from both rats and man (Smith & Jepson, 1963), and in the faeces of normal dairy cattle (Van Rheenan, 1963). The fact that aminopropanol can also be further metabolized is demonstrated by the fact that a soil bacterium, tentatively identified as an *Achromobacter* species, has been shown to utilize DL-aminopropanol as sole source of carbon, energy and nitrogen (Gottlieb & Mandel, 1959). After the discovery of the compound as a component of vitamin B_{12}, aminopropanol was incorporated in synthetic culture media used for studies of the biosynthesis of the vitamin by actinomycetes (Bray & Shemin, 1958), and several strains of *Nocardia rugosa* have been shown by DiMarco, Marnati, Migliacchi, Rusconi & Spalla (1962) to introduce aminopropanol into the structure of vitamin B_{12}.

The metabolic origin and role of aminopropanol is uncertain. The conversion of L-threonine into the D-1-aminopropan-2-ol moiety of vitamin B_{12}, demonstrated isotopically with *Strep. griseus* by Krasna *et al.* (1957), was interpreted by these authors to be the first evidence for the enzymic decarboxylation of threonine. Gale (1946), however, had previously reported that a survey of some 200 coliform organisms, 800 streptococci, 30 clostridia and representative strains of a variety of other organisms had not yielded any evidence for the decarboxylation of any common amino acid other than lysine, ornithine, arginine, tyrosine, histidine and glutamate. More recent work, reviewed by Guirard & Snell (1964), has failed to extend this range of specificity to threonine for any biological material. On the basis of the present work on *E. coli*, it appears more likely that aminopropanol is derived from threonine via aminoacetone. The coupling of threonine dehydrogenase with an aminopropanol dehydrogenase was a possibility considered by Neuberger (1961) when considering the biogenesis of the aminopropanol moiety of vitamin B_{12} by *Strep. griseus*. It is recognized that the stereospecificity of the reaction is of importance in this case, and that this has not been determined in the present work. It has been noted, however, that *Strep. griseus* readily converts aminopropanol into aminoacetone. The formation of aminopropanol from parenterally administered threonine by mammals, shown isotopically by Smith & Jepson (1963), could also be accounted for by a coupled enzyme system. Aminoacetone formation from threonine by mammalian tissues has been demonstrated by Urata & Granick (1961, 1963). Aminopropanol-dehydrogenase activity in mammalian tissues has not yet been reported.

The interconversion of aminoacetone and aminopropanol by an oxidoreductase mechanism suggests a new function for the key intermediate in the aminoacetone cycle proposed by Elliott (1959). Evidence for the operation of such a cycle has been reviewed by Green & Elliott (1964).

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