

## Bacterial Catabolism of Threonine

### THREONINE DEGRADATION INITIATED BY L-THREONINE ACETALDEHYDE-LYASE (ALDOLASE) IN SPECIES OF *PSEUDOMONAS*

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1. The route of L-threonine degradation was studied in four strains of the genus *Pseudomonas* able to grow on the amino acid and selected because of their high L-threonine aldolase activity. Growth and manometric results were consistent with the cleavage of L-threonine to acetaldehyde+glycine and their metabolism via acetate and serine respectively. 2. L-Threonine aldolases in these bacteria exhibited pH optima in the range 8.0–8.7 and  $K_m$  values for the substrate of 5–10 mM. Extracts exhibited comparable allo-L-threonine aldolase activities,  $K_m$  values for this substrate being 14.5–38.5 mM depending on the bacterium. Both activities were essentially constitutive. Similar activity ratios in extracts, independent of growth conditions, suggested a single enzyme. The isolate *Pseudomonas* D2 (N.C.I.B. 11097) represents the best source of the enzyme known. 3. Extracts of all the L-threonine-grown pseudomonads also possessed a CoA-independent aldehyde dehydrogenase, the synthesis of which was induced, and a reversible alcohol dehydrogenase. The high acetaldehyde reductase activity of most extracts possibly resulted in the underestimation of acetaldehyde dehydrogenase. 4. L-Serine dehydratase formation was induced by growth on L-threonine or acetate+glycine. Constitutively synthesized L-serine hydroxymethyltransferase was detected in extracts of *Pseudomonas* strains D2 and F10. The enzyme could not be detected in strains A1 and N3, probably because of a highly active 'formaldehyde-utilizing' system. 5. Ion-exchange and molecular exclusion chromatography supported other evidence that L-threonine aldolase and allo-L-threonine aldolase activities were catalysed by the same enzyme but that L-serine hydroxymethyltransferase was distinct and different. These results contrast with the specificities of some analogous enzymes of mammalian origin.

The ability of L-threonine to serve as the sole source of carbon and nitrogen for the growth of a wide variety of bacteria has been reported (Yamada *et al.*, 1971; Bell *et al.*, 1972; Halvorson, 1972). The breakdown of the amino acid is unusual in that several catabolic routes are known to operate. Detailed studies of a number of soil isolates suggest that breakdown initiated by L-threonine 3-dehydrogenase (EC 1.1.1.103) is the most common route (Bell & Turner, 1976). The initiation of L-threonine catabolism by L-threonine dehydratase (L-threonine hydro-lyase, EC 4.2.1.16) is uncommon among micro-organisms capable of growth on the amino acid, but has been described in a species of *Corynebacterium* (Bell & Turner, 1977).

Although L-threonine acetaldehyde-lyase (L-threonine aldolase, EC 4.1.2.5) has been detected in a wide variety of micro-organisms (Yamada *et al.*, 1971),

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its physiological significance is mostly uncertain. L-Threonine aldolase-initiated catabolism of threonine has been demonstrated in two strains of *Pseudomonas putida* only (Morris, 1969). A survey of 75 soil isolates capable of growth on the amino acid revealed that only six possessed aldolase activity (Bell *et al.*, 1972). Of these, all pseudomonads, only four had high L-threonine aldolase and low threonine 3-dehydrogenase and threonine dehydratase activities (S. C. Bell, T. R. G. Gray & J. M. Turner, unpublished work). Evidence for the route of threonine degradation and the separate identity of threonine aldolase and L-serine hydroxymethyltransferase enzymes in species of *Pseudomonas* is reported below.

#### Materials and Methods

##### *Micro-organisms and media*

The *Pseudomonas* spp. isolated from soil, and found to possess high L-threonine aldolase activity, were identified and allocated the strain designations A1,

D2, F10 and N3 (S. C. Bell, T. R. G. Gray & J. M. Turner, unpublished work). The strains D2 and N3 have now been deposited with the National Collection of Industrial Bacteria and given the accession numbers N.C.I.B. 11097 and N.C.I.B. 11096 respectively. The two strains of *Pseudomonas putida* used, T and TG2T (Morris, 1969), substrains of N.C.I.B. 9034 and N.C.I.B. 10558 respectively, were kindly given by Professor J. G. Morris, University College of Wales, Aberystwyth.

All pseudomonads were maintained on nutrient broth and cultivated on basal salts medium supplemented with 2 g of L-threonine/litre as described for similar soil isolates (Bell & Turner, 1976). Some media contained alternative carbon sources, replacing L-threonine, and were supplemented with 1 g of  $(\text{NH}_4)_2\text{SO}_4$ /litre as the nitrogen source.

#### Preparation of cell-free extracts

Bacteria, harvested by centrifugation at 5000 g for 20 min, were suspended in 0.1 M-potassium phosphate, pH 7, and disrupted ultrasonically in an MSE 100 W ultrasonic disintegrator. Suspensions were treated for periods of 10 s at full power, with cooling at 0–4°C, for a total of about 2 min. Cell debris was removed by centrifugation, as previously described (Bell & Turner, 1976), and high-speed supernatants were used for enzyme assays after minimum delay. In some cases, extracts were prepared in 0.1 M-Tris/HCl buffer, as indicated in the text.

#### Enzyme assays

All enzyme assays were done at 37°C. Protein determinations were made as outlined previously (Bell & Turner, 1976).

**L-Threonine aldolase (EC 4.1.2.5).** Activity was measured spectrophotometrically, the acetaldehyde produced being reduced to ethanol by NADH in the presence of alcohol dehydrogenase (Morris, 1969). The optimum conditions for assay, and some properties of the enzyme in different bacteria, are given in the text. Activity was measured colorimetrically by a method for the detection and measurement of acetaldehyde (Paz *et al.*, 1965) as previously described (Bell & Turner, 1976), by using the value  $\epsilon_{670} = 6.0 \times 10^4 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ .

**Alcohol dehydrogenase (EC 1.1.1.1).** The ethanol-dependent reduction of  $\text{NAD}^+$  was followed at 340 nm in reaction mixtures containing 100  $\mu\text{mol}$  of potassium phosphate buffer, pH 7.5, 5  $\mu\text{mol}$  of  $\text{NAD}^+$ , 10  $\mu\text{mol}$  of the alcohol and extract, in a total volume of 1 ml. Activity in the reverse direction was measured as the aldehyde-dependent oxidation of NADH followed at 340 nm. Reaction mixtures contained 100  $\mu\text{mol}$  of potassium phosphate buffer, pH 7.5, 0.25  $\mu\text{mol}$  of NADH, 1  $\mu\text{mol}$  of acetaldehyde and extract, in a total volume of 1 ml.

**Aldehyde dehydrogenase (EC 1.2.1.3).** Activity

was assayed by following the acetaldehyde-dependent reduction of  $\text{NAD}^+$  at 340 nm. Reaction mixtures contained 100  $\mu\text{mol}$  of potassium phosphate buffer, pH 7.5, 0.25  $\mu\text{mol}$  of  $\text{NAD}^+$ , 1  $\mu\text{mol}$  of the aldehyde and extract protein in a total volume of 1 ml.

**Other enzymes.** Standard methods were used to assay glyoxylate carbo-ligase (EC 4.1.1.47) (Kornberg & Gotto, 1961), erythro-3-hydroxyaspartate dehydratase (EC 4.2.1.38) (Kornberg & Morris, 1965), L-serine hydroxymethyltransferase (EC 2.1.2.1), L-threonine dehydratase (EC 4.2.1.16) and L-threonine 3-dehydrogenase (EC 1.1.1.103) (Bell & Turner, 1976).

#### Column chromatography

The fractionation of cell-free extracts, to investigate the identity or otherwise of related enzymes, was effected by either ion-exchange or molecular exclusion chromatography on columns of the appropriate support.

Columns (1.5 cm  $\times$  25 cm) of Whatman DE 52 DEAE-cellulose, equilibrated with the appropriate buffer, were used for anion-exchange chromatography. Crude extract prepared in the same buffer (50–100 mg of protein in approx. 3 ml of buffer) was applied to the column, proteins were eluted as indicated in the text, and 5 ml fractions of eluate collected automatically.

Columns (2.5 cm  $\times$  36 cm) of Sephadex G-200, dry bead diameter 40–120  $\mu\text{m}$ , equilibrated with the appropriate buffer, were used for molecular exclusion chromatography. Elution was by the upward-flow technique. Samples (80–100 mg of protein) of crude extract were applied to the column and eluted with buffer at a flow rate of approx. 15 ml/h. Fractions (approx. 4 ml) were collected automatically. The measurement of column void volumes and the elution volumes for proteins of known molecular weight allowed estimations of enzyme molecular weights to be made (Andrews, 1965).

Protein in eluate samples was detected by its  $A_{280}^{1\text{cm}}$  or  $A_{254}^{1\text{cm}}$ .

#### Chemical determinations

Samples of cell-free growth media were analysed for acetaldehyde (Paz *et al.*, 1965), ethanol (enzymically, under the conditions described for the assay of alcohol dehydrogenase), 2-oxobutyrate (Ning & Gest, 1966) and aminoacetone (Pickard *et al.*, 1968).

The production of acetaldehyde throughout growth was also assayed by using carbonyl-trapping reagents, as described by Jones & Turner (1973).

#### Chemicals

DL-allo-Threonine was obtained from Koch-Light Laboratories, Colnbrook, Bucks., U.K.; L-allo-threonine was from Calbiochem, Los Angeles, CA, U.S.A.; D-threonine was from Sigma Chemical Co.,

St. Louis, MO, U.S.A.; L-threonine was from SAS Scientific Chemicals, London WC1B 4DR, U.K.

## Results

### *Growth and manometric studies*

All four pseudomonads isolated from soil grew well on basal salts medium supplemented with L-threonine, acetate+glycine, acetate, glycine, DL-1-aminopropan-2-ol or succinate as carbon and energy sources (except isolate F10, which grew poorly on glycine). None of the bacteria grew on aminoacetone or propionate. Isolate D2 grew well on DL-allo-threonine.

During growth on L-threonine+mineral salts medium, none of the pseudomonads excreted detectable amounts of aminoacetone, acetaldehyde, ethanol or 2-oxobutyrate. Samples of medium were taken during the early, mid and late phases of exponential growth.

O<sub>2</sub> uptake by suspensions of each L-threonine-grown pseudomonad was stimulated by the potential intermediate as shown in Table 1. A similar pattern of oxidation was seen in each case. Only low oxidation rates (0–0.5 μmol/h per mg dry wt. of bacteria) were observed with 2-oxobutyrate, aminoacetone, DL-1-aminopropan-2-ol or glyoxylate. O<sub>2</sub> uptake with glycine+acetate was greater than with glycine alone and was comparable with that for L-threonine oxidation.

These results were consistent with the catabolism of L-threonine via acetaldehyde and glycine, the former being metabolized via acetate and the latter via serine to pyruvate.

### *Key enzymes of threonine metabolism*

Although preliminary surveys had shown that L-threonine aldolase was the most active of the enzymes known to initiate L-threonine catabolism in

all cases, it was possible that non-optimum assay conditions had been used. The properties of these, and other key enzymes of threonine metabolism, were therefore studied in crude extracts as a preliminary to investigating the effect of growth conditions on their activity.

*L-Threonine aldolase.* The activity of this enzyme was assayed by the spectrophotometric and colorimetric methods as outlined in the Materials and Methods section. The optimum pH values for assay of the individual enzymes in Tris/HCl buffer were distinct but different, varying from pH 8.0 to 8.7 depending on the strain of pseudomonad (Table 2). Plots of reaction velocity versus substrate concentration gave normal hyperbolic curves and linear double-reciprocal plots (Lineweaver & Burk, 1934), indicating apparent  $K_m$  values in the range 5–10 mM. Pyridoxal phosphate (0.5 mM) enhanced enzyme activity in crude extracts in all cases, whereas low concentrations of hydrazine derivatives substantially inhibited aldolase activity (Table 2). When AMP, ADP, ATP, phosphoenolpyruvate and acetyl-CoA were tested as potential effectors of enzyme activity, no effect was apparent at 1 or 5 mM, with saturating or non-saturating concentrations of L-threonine. The only stereoisomer of L-threonine also active as a substrate was L-allo-threonine, which was 63–137% as active depending on the source of the extract.  $K_m$  values of 14.5–38.5 mM were obtained for L-allo-threonine (Table 2). Fractionation of cell-free extracts by ion-exchange chromatography suggested that both substrates were acted on by the same enzyme (see below). D-Threonine and D-allo-threonine were tested, but found to be inactive. The effect of growth conditions on threonine aldolase activities, measured under the conditions found to be optimum in these preliminary studies, is shown in Table 3.

*L-Threonine dehydratase and L-threonine 3-dehydrogenase.* Results indicated that the L-threonine dehydratase activity detected in crude extracts was

Table 1. *Oxygen uptake by pseudomonads in the presence of L-threonine and its possible catabolites*

The pseudomonads were grown on L-threonine+salts medium as outlined in the Materials and Methods section. O<sub>2</sub> uptake by washed suspensions was measured manometrically by conventional procedures (Umbreit *et al.*, 1964) under the conditions described previously (Higgins *et al.*, 1968). Rates of O<sub>2</sub> uptake are corrected for endogenous values of 0.3–0.8 μmol of O<sub>2</sub> absorbed/h per mg dry wt. of bacteria.

Substrate oxidized (7 mM)	O <sub>2</sub> absorbed (μmol/h per mg dry wt. of bacteria) by various pseudomonads during incubations with substrates shown			
	A1	D2	F10	N3
L-Threonine	6.2	7.2	5.9	8.0
Acetate	5.3	6.5	6.1	7.8
Glycine	5.7	6.1	5.7	6.1
Acetate+glycine (each 7 mM)	6.9	8.5	6.3	9.4
L-Serine	5.2	5.8	5.5	6.3
Pyruvate	5.3	6.2	4.8	5.8
Propionate	0.9	0.8	0.8	1.0

Table 2. *Properties of L-threonine aldolase in extracts of pseudomonads*

Enzyme activity was assayed colorimetrically, as described by Bell & Turner (1976). Reaction mixtures contained 20  $\mu$ mol of Tris/HCl buffer, pH as appropriate, 100  $\mu$ mol of L-threonine or L-allo-threonine, and cell-free extract, in a total volume of 1 ml. Reactions were started by the addition of substrate and terminated by the addition of 1 ml of aldehyde reagent.  $V_{max}$  values are given as nmol of product formed/min per mg of protein.

Enzyme property	<i>Pseudomonas</i> strain					
	A1	D2	F10	N3	<i>Ps. putida</i> TG2T	<i>Ps. putida</i> T
pH optimum	8.0	8.4	8.7	8.7	8.7	8.6
$K_m$ for L-threonine (mM)	8.4	10.0	6.5	10.7	7.0	5.0
$K_m$ for L-allo-threonine (mM)	14.5	38.5	20.0	25.8	55.0	12.3
$V_{max}$ for L-threonine	78	1100	53	48	685	94
$V_{max}$ for L-allo-threonine	48	1800	69	29	1065	131
Activation by 0.5 mM-pyridoxal phosphate (%)	62	26	75	112	18	88
Inhibition by 1.0 mM-isonicotinic acid hydrazide (%)	25	29	35	51	19	27
Inhibition by 1.0 mM-N-(3-hydroxy-benzyl)-N-methylhydrazine phosphate (%)	36	11	47	62	14	29

biosynthetic in function. The enzyme was strongly inhibited by L-isoleucine (95–100% inhibition at 2 mM) in each case, and appeared to be formed constitutively. L-Threonine 3-dehydrogenase was not detectable in any of the pseudomonads. The effects of different pH values, enzyme-protecting compounds, metal ions and potential effectors were tested in the search for activity.

#### *Enzymic evidence for the further catabolism of glycine and acetaldehyde*

High L-threonine aldolase activity in extracts of L-threonine-grown pseudomonads prompted a search for enzymes involved in the further metabolism of its reaction products acetaldehyde and glycine.

*Aldehyde dehydrogenase.* Substantial CoA-independent acetaldehyde dehydrogenase activity was detected in extracts of all four isolates, the activity in *Pseudomonas* N3 being the highest. In this case a pH optimum of 8.0 was observed in Tris/HCl buffer and activity in potassium phosphate buffer was 4-fold greater at this pH. Activity was also found with formaldehyde and propionaldehyde, although the relationship between activity and time was not linear with these substrates. The enzyme was specific for NAD<sup>+</sup> and the  $K_m$  value for acetaldehyde was less than 5  $\mu$ M.

*Alcohol dehydrogenase.* High alcohol dehydrogenase activity was discovered during studies of NAD<sup>+</sup>-dependent aldehyde oxidation. The alcohol-NAD<sup>+</sup> oxidoreductase, present in extracts of all four isolates, was assayed in each direction. In extracts of *Pseudomonas* N3, the rate of ethanol-dependent NAD<sup>+</sup> reduction was maximum at pH 8.6 in Tris/

HCl buffer. The dehydrogenase was specific for NAD<sup>+</sup> and the  $K_m$  value for ethanol was 0.55 mM. The pH optimum for acetaldehyde-dependent NADH oxidation (acetaldehyde reductase activity) was pH 6.5 in potassium phosphate buffer. Activities with the substrates acetaldehyde, formaldehyde and propionaldehyde were 1510, 82 and 1225 nmol of NADH oxidized/min per mg of protein respectively. The enzyme was specific for NADH and the  $K_m$  for acetaldehyde was about 5  $\mu$ M.

To determine whether alcohol dehydrogenase and aldehyde reductase activities were associated with a single enzyme, cell-free extract was fractionated by ion-exchange chromatography (Fig. 1). Both activities were eluted as a single peak, the activity ratio remaining constant. The ratio was also constant in extracts of each pseudomonad when grown on different media (Table 3).

*L-Serine dehydratase.* Substantial activity was found in extracts of all L-threonine-grown bacteria (Table 3).

*L-Serine hydroxymethyltransferase.* Activity measured in the direction of serine formation was followed as the glycine-dependent disappearance of formaldehyde (Scrimgeour & Huennekens, 1962). Although found in extracts of the isolates D2 and F10, enzyme activity could not be measured in extracts of strains A1 and N3 owing to the presence of a highly active formaldehyde-utilizing system (S. C. Bell & J. M. Turner, unpublished work).

No significant *erythro*-3-hydroxyaspartate dehydratase or glyoxylate carbo-ligase activities, characteristic of the  $\beta$ -hydroxyaspartate and glycerate pathways of glycine metabolism (Kornberg & Gotto, 1961; Kornberg & Morris, 1965), were

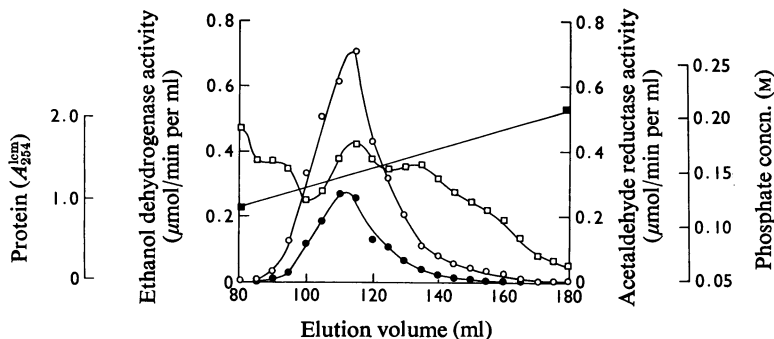


Fig. 1. Partial purification of acetaldehyde-metabolizing enzymes of *Pseudomonas sp. N3*

Crude extract was applied to a column of DEAE-cellulose equilibrated with 0.05M-potassium phosphate buffer, pH7.5, eluted with a linear gradient of 0.05–0.40M buffer (■) at the same pH, and enzyme activities were assayed spectrophotometrically as described in the Materials and Methods section. Alcohol dehydrogenase (●) and aldehyde reductase (○) activities were found only in the fractions indicated. Protein (□) was detected spectrophotometrically.

Table 3. Effect of growth conditions on enzyme activities in extracts of pseudomonads

The bacteria were grown on basal salts medium supplemented with the carbon sources shown, cell-free extracts were prepared and enzyme activities measured, as outlined in the Materials and Methods section. Enzymes were assayed under the conditions found to be optimum for their activity in cell-free extracts. Enzyme activity is given as nmol of product formed (or substrate utilized)/min per mg of protein. N.D., Not done.

Enzyme	Growth substrate	<i>Pseudomonas</i> strain			
		A1	D2	F10	N3
L-Threonine aldolase	L-Threonine	67	915	55	46
	Acetate+glycine	26	942	39	28
	Succinate	21	1002	37	31
L-allo-Threonine aldolase	L-Threonine	52	1160	75	33
	Acetate+glycine	23	1620	57	29
	Succinate	29	1550	35	21
L-Threonine dehydratase	L-Threonine	42	112	65	47
	Succinate	36	60	N.D.	N.D.
L-Serine dehydratase	L-Threonine	435	200	155	310
	Acetate+glycine	560	630	81	280
	Succinate	59	140	21	73
L-Serine hydroxymethyltransferase	L-Threonine	N.D.*	122	54	N.D.*
	Acetate+glycine	N.D.*	167	87	N.D.*
	Succinate	N.D.*	149	63	N.D.*
Acetaldehyde dehydrogenase	L-Threonine	67	119	55	145
	Succinate	1	4	3	2
Ethanol dehydrogenase	L-Threonine	521	15	144	483
	Succinate	236	19	62	148
Acetaldehyde reductase	L-Threonine	1650	38	462	1590
	Succinate	760	63	195	760

\* Highly active formaldehyde-utilizing system present.

detected in extracts when assayed under a variety of conditions.

#### Effect of growth conditions on metabolic activity

Enzyme activities in extracts of the pseudomonads grown on selected media were measured under the conditions found to be optimal for their assay. The

results are shown in Table 3. Of the enzymes acting on L-threonine, L-threonine aldolase had its activity partially increased by the growth of *Pseudomonas* strains A1, F10 and N3 on the amino acid. The aldolase of strain D2, however, was synthesized constitutively at high activity. Of the acetaldehyde-utilizing enzymes, synthesis of the aldehyde dehydro-

genase was induced dramatically by growth on L-threonine but not succinate media. The reversible 'aldehyde reductase' (alcohol dehydrogenase), although highly active in most extracts, was partially constitutive, its formation being induced only moderately by growth on L-threonine. Any coupling of aldehyde dehydrogenase and reductase activities may have led to their underestimation by the assay methods used. L-Serine dehydratase formation was induced by growth on media containing L-threonine or glycine+acetate only, whereas L-serine hydroxymethyltransferase formation appeared to be constitutive.

The effect of growth conditions on the ability of the pseudomonads to oxidize L-threonine and its catabolites is shown in Table 1.

#### Possible identity of threonine aldolase and serine hydroxymethyltransferase

In view of the finding that the L-threonine aldolase, L-allo-threonine aldolase and L-serine hydroxymethyltransferase reactions are catalysed by the same enzyme in rabbit liver (Schirch & Gross, 1968; Akhtar & El-Obeid, 1972), the specificity of the enzymes catalysing these reactions in pseudomonads was of interest. In addition to the isolates A1, D2, F10 and N3, the two strains of *Pseudomonas putida* (Morris, 1969) were included in the study.

Preliminary studies had suggested that L-threonine aldolase and L-allo-threonine aldolase were the same enzyme. When extracts of each pseudomonad were fractionated by ion-exchange chromatography on DEAE-cellulose, the two activities were not separated. A typical elution pattern is shown in Fig. 2. From *Pseudomonas* A1 the enzymes were purified

10–13-fold, with about 50% recovery. Similar results were obtained with the other pseudomonads. The preparation with highest activity, obtained by ion-exchange chromatography of isolate D2, had a specific activity of about 18  $\mu\text{mol}$  of acetaldehyde formed/min per mg of protein for L-threonine aldolase. Ratios of aldolase activities remained constant not only during ion-exchange chromatography but also after partial inactivation by heat treatment (Bell & Turner, 1973), in extracts of the pseudomonads grown on different media (Table 3) and during gel-filtration on Sephadex G-200. The use of molecular exclusion chromatography (see the Materials and Methods section) suggested mol.wts. for threonine aldolase from the different pseudomonads of 220000–270000.

The separation of L-serine hydroxymethyltransferase and threonine aldolase enzymes by DEAE-cellulose chromatography of extracts of isolates A1, F10, N3 and *Pseudomonas putida* T has been reported (Bell & Turner, 1973). About 15–20-fold purification was found for both enzymes, with recoveries of 85–95% in each case. In contrast, the enzymes from isolate D2 and *Ps. putida* TG2T, although purified in good yields, were not separated. Gel filtration on Sephadex G-200, however, resulted in partial separation of the enzymes. L-Threonine aldolase was eluted before L-serine hydroxymethyltransferase in both cases, the ratio of enzyme activities differing markedly in different fractions (Fig. 3). The mol.wts. of L-threonine aldolase and L-serine hydroxymethyltransferase were estimated to be approx. 245000 and 120000 respectively in each of the two pseudomonads. Heat treatment of crude extracts (20 mg of protein/ml of 0.1M-potassium phosphate buffer, pH 7), at 25–60°C for 10 min, also markedly affected enzyme

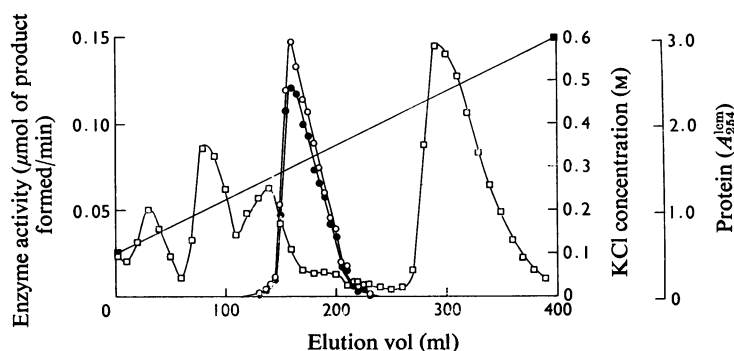


Fig. 2. Partial purification of threonine aldolase of *Pseudomonas* sp. A1

Crude extract prepared in 0.1M-Tris/HCl buffer, pH 7.5, was applied to a column of DEAE-cellulose equilibrated with the same buffer. Proteins were eluted with an increasing gradient of  $\text{Cl}^-$  (■) in the same buffer, as shown. L-Threonine aldolase (○) and L-allo-threonine aldolase (●) activities were assayed colorimetrically. Additional details are given in the Materials and Methods sections. Protein (□) was detected spectrophotometrically. The peak fractions had specific activities for L-threonine aldolase of 680–880 nmol of product formed/min per mg of protein.

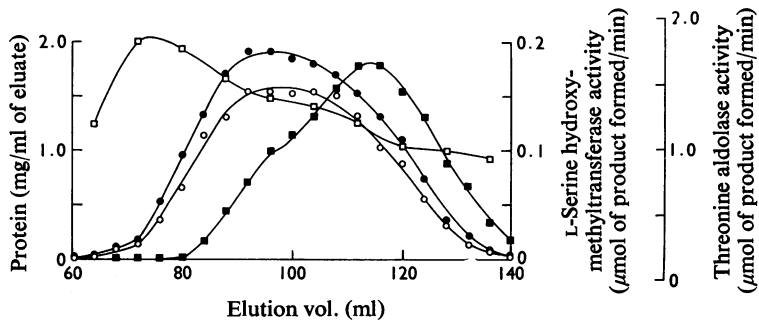


Fig. 3. Separation of threonine aldolase and serine hydroxymethyltransferase enzymes by gel filtration

Crude extract (90mg of protein) of *Pseudomonas* sp. D2 was applied to a column packed with approx. 180ml of pre-swollen Sephadex G-200 equilibrated with 0.1M-potassium phosphate buffer, pH7.5, containing pyridoxal phosphate (25  $\mu$ M) and 2-mercaptoethanol (5mm). Proteins were eluted with the same buffer and the volumes of eluate fractions were measured. L-Threonine aldolase (○) and DL-allo-threonine aldolase (●) were measured spectrophotometrically and L-serine hydroxymethyltransferase (■) was measured colorimetrically as described in the Materials and Methods section. Protein (□) was detected spectrophotometrically.

activity ratios, the L-serine hydroxymethyltransferase being more susceptible to heat denaturation.

### Discussion

For the pseudomonads studied, the growth substrate spectrum, the pattern of substrate oxidation and the effect of growth conditions on enzyme formation were consistent with L-threonine catabolism initiated by its cleavage to glycine+acetaldehyde. Glycine appeared to be further metabolized by the L-serine pathway (Morris, 1963), and acetaldehyde via acetate. The catabolic pathway in each case was the same as that previously deduced for two other pseudomonads, probably both strains of *Pseudomonas putida* (Morris, 1969). The isolate N3 described here is now believed to be a strain of *Pseudomonas putida* (I. J. Bousfield, personal communication). The similarities between L-threonine degradation initiated by L-threonine 3-dehydrogenase (McGilvray & Morris, 1969; Bell & Turner, 1976) and by L-threonine aldolase, particularly the identity and regulation of the glycine- and acetaldehyde-metabolizing enzymes, are apparent from our results. The inducible acetaldehyde reductase activity found in three of the pseudomonads may represent a mechanism for disposing of the aldehyde under anaerobic conditions.

Although L-threonine aldolase has been detected in a variety of micro-organisms, its physiological significance is mostly uncertain in view of the low activities found (Yamada *et al.*, 1971). It is also possible for the aldolase to be confused with L-threonine dehydrogenase under certain circumstances (Newman *et al.*, 1976). Apart from certain pseudomonads only the yeast *Candida humicola* has been shown to possess a catabolic L-threonine aldolase (Kumagai *et al.*, 1972). The survey by Bell *et al.* (1972)

suggested that aldolase-initiated L-threonine catabolism was uncommon. The L-threonine aldolase purified 200-fold from extracts of *Clostridium pasteurianum* (Dainty, 1970) was formed constitutively and appeared to play a biosynthetic role in the formation of glycine from L-aspartate via L-threonine (Dainty & Peel, 1970).

The finding that L-serine hydroxymethyltransferase could be separated from threonine aldolase activities during the fractionation of extracts of four pseudomonads (Bell & Turner, 1973), now extended to the remaining two, was of some interest. In rabbit liver, a single enzyme had been shown to be responsible for the hydroxymethyltransferase reaction, with tetrahydrofolate as an acceptor for formaldehyde, and for the cleavage of L-threonine and its L-allo-isomer (Schirch & Gross, 1968). Both aldolase activities were attributed to the broad specificity of the higher-specific-activity L-serine hydroxymethyltransferase by the Commission on Biochemical Nomenclature (1973). The observation that purified rat liver mitochondrial L-serine hydroxymethyltransferase was unable to catalyse the aldol cleavage of L-threonine or L-allo-threonine (Palekar *et al.*, 1973) supported our findings with the enzymes from pseudomonads. The presence of a single enzyme in certain pseudomonads acting on both L-threonine and its L-allo-isomer parallels the properties of the inducibly formed enzyme purified to homogeneity from *Candida humicola* (Yamada *et al.*, 1970; Kumagai *et al.*, 1972). In contrast, the aldolase purified from *Clostridium pasteurianum* was highly specific for L-threonine (Dainty, 1970), and a similar enzyme has been detected in rat liver cytoplasm (Riario-Sforza *et al.* 1969; Palekar *et al.*, 1973). It is now clear that L-serine hydroxymethyltransferase and L-threonine aldolase can be distinct and different

enzymes, but that the similarity of their reaction mechanisms (Jordan & Akhtar, 1970) allows overlapping specificity in some cases. In either case the enzyme may also be (fortuitously) active with L-allo-threonine. The Commission on Biochemical Nomenclature (1976) have reinstated the deleted entry EC 4.1.2.5, threonine aldolase, to the enzyme nomenclature list. It is notable that the isolate *Pseudomonas* sp. D2 described here is the best known source of L-threonine aldolase. The enzyme in crude extracts was about 30% as active as the enzyme purified 510-fold from *Candida humicola* (Kumagai *et al.*, 1972) and after 16-fold purification on DEAE-cellulose had a specific activity about 6-fold greater.

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