for the enzyme in vivo unless an alkaline micro-environment was postulated or conditions were present in vivo which could potentiate inhibition by UMP at near-neutral pH (Yon, 1972b). Substances such as deoxycholate which can bind to the hydrophobic site on the enzyme may provide such potentiation. Fig. 2 shows the effects of UMP and deoxycholate at pH values between 7 and 11. In the absence of detergent the enzyme is most active and most sensitive to UMP at pH 10.6 (Fig. 2); however, between pH 7 and 8.5 the enzyme is insensitive to UMP (50 μM) at the substrate concentrations used in this study. A similar result has been obtained previously under slightly different conditions of substrate concentration (Yon, 1972b). When the experiment is repeated in the presence of 0.5 mM-deoxycholate (Fig. 2) the pH-activity profile in the absence of UMP is unchanged, but inhibition by 50 μM-UMP is increased at all pH values. Of especial interest is the inhibition between pH 7 and pH 8.5; the relative increase in inhibition is much greater in this range than at more alkaline values.

These results suggest that end-product inhibition by UMP at physiological pH values may be dependent in vivo on the local concentrations of substances able to bind to the hydrophobic site, thereby producing effects similar to those produced by deoxycholate in these experiments. Such a control mechanism would, presumably, involve a three-way interaction between the hydrophobic site, the UMP-binding site and the active site. It will be of interest to determine the specificity of the hydrophobic site, as judged by the potentiating effect on inhibition by UMP of various biological lipid molecules and of natural- and artificial-membrane systems.

Yon, R. J. (1972b) Biochem. J. 128, 311–320

Bacterial Threonine Aldolase and Serine Hydroxymethyltransferase Enzymes

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A number of aerobic bacteria capable of growth on L-threonine as the major source of carbon and nitrogen has been isolated. Several strains possessed L-threonine aldolase (EC 4.1.2.5) but no significant amounts of other enzymes known to initiate threonine degradation (Bell et al., 1972). Four of these strains, together with two previously isolated strains of Pseudomonas putida (Morris, 1969) have been used to investigate the possible identity of L-threonine aldolase and L-serine hydroxymethyltransferase (EC 2.1.2.1).

Some properties of the bacterial threonine aldolases are given in Table 1. Maximum activity was found at pH 8.3–9.0, in contrast to the values of pH 7.3–7.7 reported for mammalian enzymes (Karasek & Greenberg, 1957; Malkin & Greenberg, 1964; Schirch & Gross, 1968). The pH optimum of the enzyme from P. putida TG2T was pH 8.5–8.8, i.e. different from the value of pH 10.0–10.5 reported by Morris (1969). Again in contrast to mammalian enzymes a comparison of $K_m$ values showed that L-threonine rather than D-L-allo-threonine was the favoured substrate. Under optimum conditions rates with the two isomeric substrates were similar in crude extracts.

Partial purification of L-threonine aldolases was carried out for studies of enzyme specificity. Separate enzymes for L-threonine and D-L-allo-threonine are known in sheep liver (Karasek & Greenberg, 1957) whereas both substrates are believed to be acted on by a single enzyme in rabbit liver (Schirch & Gross, 1968). In the case of rat liver, evidence for separate enzymes is contradictory (Malkin & Greenberg, 1964; Riario-Sforza et al., 1973).
Bacteria were grown in 1-litre portions of medium containing 7.0g of K2HPO4, 3.0g of KH2PO4, 1.2g of Na2SO4 (anhydrous), 0.1g of MgSO4·7H2O and 2g of L-threonine. The pH was adjusted to 7.0 before autoclaving. After growth for 24h at 30°C, bacteria were harvested by centrifuging and extracts prepared by using an Aminco-French pressure cell. Aldolase activities were assayed in 100000g supernatants as indicated in the text.

Table 1. Properties of threonine aldolases from micro-organisms

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>pH optimum</th>
<th>Specific activity with L-threonine (nmol of product/min per mg of protein)</th>
<th>Specific activity with DL-allo-threonine (nmol of product/min per mg of protein)</th>
<th>( K_m ) for L-threonine (mM)</th>
<th>( K_m ) for DL-allo-threonine (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. putida TG2T</td>
<td>8.5–8.8</td>
<td>630</td>
<td>89</td>
<td>7.0</td>
<td>10.5</td>
</tr>
<tr>
<td>P. putida T</td>
<td>8.4–8.8</td>
<td>89</td>
<td>70</td>
<td>5.0</td>
<td>21.0</td>
</tr>
<tr>
<td>Pseudomonas sp. A1</td>
<td>8.3–8.5</td>
<td>937</td>
<td>1190</td>
<td>190</td>
<td>30.3</td>
</tr>
<tr>
<td>Pseudomonas sp. D2</td>
<td>8.6–8.8</td>
<td>50</td>
<td>68</td>
<td>10.0</td>
<td>6.5</td>
</tr>
<tr>
<td>Pseudomonas* sp. (%)</td>
<td>8.6–8.8</td>
<td>43</td>
<td>31</td>
<td>20.0</td>
<td>37.0</td>
</tr>
<tr>
<td>Pseudomonas sp. N3</td>
<td>8.3–9.0</td>
<td>47.6</td>
<td></td>
<td></td>
<td>* Genus uncertain.</td>
</tr>
</tbody>
</table>
Microbial extracts were fractionated by anion exchange on DEAE-cellulose. Columns of Whatman DE-52 were equilibrated with 0.1 M-Tris buffer, pH 7.5, containing 0.1 M-Cl\(^-\). Crude extracts were applied and non-adsorbed proteins eluted with 1 column volume of buffer. Enzymes of interest were eluted with buffer containing a linear gradient of Cl\(^-\) increasing to 0.6 M. In the case of all micro-organisms both L-threonine aldolase and DL-allo-threonine aldolase activities were eluted at 0.23–0.37 M-Cl\(^-\) as a single peak. The ratio of aldolase activities remained constant during ion-exchange chromatography and also during heat-treatment of crude extracts at 25–55°C for 10 min. Both activities were decreased by 50% at 50°C and completely destroyed at 60°C in 0.1 M-Tris buffer, pH 8. Activities with mixed substrates at saturating concentrations were intermediate rather than additive in all cases. It was concluded that single enzymes catalysed the cleavage of both L-threonine and DL-allo-threonine.

The possibility was considered that L-threonine aldolase, particularly an enzyme acting on allo-threonine, was responsible for L-serine hydroxymethyltransferase activity (Schirch & Gross, 1968). The mechanism of the hydroxymethyltransferase is similar to that of the aldolase except that the former enzyme requires tetrahydrofolic acid to accept formaldehyde whereas free acetaldehyde is liberated from threonine (Jordan & Akhtar, 1970). Preliminary work with micro-organisms by using L-threonine aldolase to initiate threonine dissimilation suggested that the glycine fragment was further metabolized to L-serine by the action of L-serine hydroxymethyltransferase. The possibility thus arose that a single enzyme, acting on different substrates in opposite directions, was responsible for the first two metabolic steps. Crude bacterial extract was applied to a

![Fig. 1. Elution of L-threonine aldolase and L-serine hydroxymethyltransferase of Pseudomonas sp. A1 from DEAE-cellulose](image)

Crude extract (50–100 mg of protein) was applied to a column (1.5 cm × 25.0 cm) of Whatman DE-52 DEAE-cellulose and enzymes were eluted as described in the text. Fractions of 5 ml were collected. Threonine aldolase (○) was assayed spectrophotometrically (Morris, 1969) at pH 8 and serine hydroxymethyltransferase (○) colorimetrically (Scrimgeour & Huennekens, 1962) at pH 7.5. Enzyme activities are expressed in arbitrary units based on changes in E\(_{1cm}\) at the appropriate wavelengths. Amount of protein (□) and the potassium phosphate gradient (△) are shown.
DEAE-cellulose column equilibrated with 0.05M-potassium phosphate buffer, pH 7.5, and eluted with a gradient increasing to 0.4M buffer at the same pH. Pyridoxal phosphate (25µM) and 2-mercaptoethanol (5mM) were added to all buffers. L-Serine hydroxymethyltransferase was detected in eluate fractions by a colorimetric method for measuring the glycine-dependent disappearance of formaldehyde (Scrimgeour & Huennekens, 1962). L-Threonine aldolase was assayed spectrophotometrically by measuring NADH oxidation owing to acetaldehyde in the presence of alcohol dehydrogenase (Dainty, 1970). In the case of four micro-organisms, serine hydroxymethyltransferase and threonine aldolase enzymes were readily separated by ion exchange. The former enzyme was eluted between 0.11M- and 0.12M-potassium phosphate buffer, whereas the aldolase was eluted between 0.19M and 0.22M buffer (see Fig. 1). In contrast, the enzymes from *P. putida* TG2T (Morris, 1969) and *Pseudomonas* sp. D2 were inseparable. Both enzymes were eluted as single peak at 0.13–0.14M- and 0.18M-potassium phosphate buffer respectively. These results suggest that in the two pseudomonads either serine hydroxymethyltransferase and threonine aldolase behave identically on anion-exchange chromatography or that both reactions are catalysed by a single enzyme.

It is noteworthy that most of the bacteria known to degrade threonine by the aldolase reaction are pseudomonads or closely related species. Although L-threonine aldolase was found by Yamada *et al.* (1971) in a wide variety of micro-organisms grown on L-threonine medium, the activity of the enzyme in extracts was extremely low. The microbe with the highest activity found, a strain of *Candida humicola*, exhibited a specific activity of only 5.73nmol of product formed/min per mg of protein. After 510-fold purification to apparent homogeneity (Kumagai *et al.*, 1972) the specific activity of the enzyme towards L-threonine was only threefold greater than that in crude extracts of *Pseudomonas* sp. D2 (see Table 1).


**Electrophoretic Separation of Acid α-D-Glucosidases in Several Human Tissues**

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