Short Communications

Utilization of L-Threonine by a *Pseudomonad*: a Catabolic Role for L-Threonine Aldolase

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Several species of aerobic bacteria owe their ability to grow on L-threonine as sole source of carbon atoms and energy to their possession of an L-threonine dehydrogenase; the α -amino- β -oxobutyrate that is the product of the reaction catalysed by this enzyme is thereafter utilized via one of two pathways. In some bacteria, including some pseudomonads (Higgins, Pickard & Turner, 1968; Lessie, 1969), aminoacetone is produced by spontaneous decarboxylation of the α -amino- β -oxobutyrate and is further metabolized, probably via methylglyoxal, to yield pyruvate (Elliott, 1959; Higgins, Turner & Willetts, 1967). In *Arthrobacter* sp. L-threonine-derived α -amino- β -oxobutyrate yields acetyl CoA plus glycine in a reaction catalysed by a specific CoA ligase, so that L-threonine is metabolized as though it were an equimolar mixture of acetate and glycine (McGilvray & Morris, 1969).

This communication reports that certain pseudomonads, when they grow on L-threonine as sole source of carbon atoms and energy, utilize the threonine, not as the precursor of aminoacetone, but as the source of an equimolar mixture of acetate plus glycine produced by the sequential action of an L-threonine aldolase (i.e. L-threonine acetaldehyde-lyase) and an acetaldehyde dehydrogenase. There is a very real danger that this new route of threonine catabolism could be mistaken for that followed in *Arthrobacter* sp., since in crude cell extracts collaborative action of the aldolase and aldehyde dehydrogenase is manifested as an L-threonine-dependent reduction of NAD⁺ that could be falsely construed as L-threonine dehydrogenase activity.

The two organisms in which this L-threonine aldolase-initiated pathway has been discovered are probably both strains of *Pseudomonas putida*; they are:

(1) *Pseudomonas* TG2T, derived from *Pseudomonas* TG2 by serial subcultivation in threonine medium (Morris, 1969).

(2) *Pseudomonas putida* T, a similarly 'threonine-trained' substrain of *Pseudomonas putida* (N.C.I.B. 9304).

All organisms used in this study were grown in shaken liquid cultures at 37° in a minimal salts

medium supplemented with 15 mM-L-threonine (McGilvray & Morris, 1969), and were maintained at room temperature on slopes of this medium solidified with agar (1%). To prepare cell extracts harvested organisms were washed and suspended in 0.05 M-potassium phosphate buffer, pH 7, and disrupted by 3 min. ultrasonication; otherwise experimental procedures (including methods employed for enzyme assay) were as reported in Table 1 or as described by McGilvray & Morris (1969).

Extracts of L-threonine-grown *Pseudomonas* TG2T contained significantly high activities of isocitrate lyase and malate synthase (specific activities 11.4 and 4.8 μ moles/hr./mg. of protein respectively), and when the 'L-threonine dehydrogenase' activity of these extracts was spectrophotometrically assayed by following L-threonine-dependent reduction of NAD⁺ at pH 7.5 a specific activity of 6.8 μ moles/hr./mg. of protein was measured. These extracts of *Pseudomonas* TG2T further resembled those of threonine-grown *Arthrobacter* sp. (McGilvray & Morris, 1969) in possessing substantial tetrahydropteroylglutamate-dependent glycine-cleavage ability and an inducible L-serine dehydratase (specific activity 11.3 μ moles/hr./mg. of protein), even though, unlike *Arthrobacter* sp., *Pseudomonas* TG2T was not able to grow on glycine as sole source of carbon and nitrogen atoms.

The results of short-term incorporation studies in which [U-¹⁴C]threonine or [2-¹⁴C]acetate or [2-¹⁴C]-glycine was supplied to cultures of *Pseudomonas* TG2T growing on L-threonine were consistent with the view that this organism, like *Arthrobacter* sp., catabolized L-threonine to a mixture of acetate and glycine, which was subsequently metabolized via the tricarboxylic acid cycle, glyoxylate cycle and folate-dependent serine pathway (McGilvray & Morris, 1969). Yet there was no α -amino- β -oxobutyrate-Co Aligase activity in extracts of threonine-grown *Pseudomonas* TG2T, and when their 'L-threonine dehydrogenase' was assayed by colorimetric measurement of aminoacetone production no activity was detectable.

The paradox was resolved by the discovery that extracts of L-threonine-grown *Pseudomonas* TG2T

Table 1. *Activities of key enzymes of L-threonine catabolism in Pseudomonas TG2T, Pseudomonas putida T and Pseudomonas multivorans grown on various sources of carbon atoms*

Crude cell extracts prepared from exponentially growing organisms were assayed for activities of the listed enzymes by the following methods. (1) L-Threonine dehydrogenase was assayed at pH 7.5 by colorimetric measurement of the rate of aminoacetone production (McGilvray & Morris, 1969). (2) L-Threonine aldolase was assayed spectrophotometrically at pH 10 by coupling with excess of alcohol dehydrogenase and following the decrease in E_{340} consequent upon the oxidation of NADH by acetaldehyde. The reaction mixture in a 1.5 ml. silica cuvette (1 cm. light-path) contained in 1 ml. final volume: 20 μ moles of borate-NaOH buffer, pH 10, 0.2 μ mole of NADH, 0.1 mg. of alcohol dehydrogenase (Boehringer) and cell extract (about 0.2 mg. of protein). A reference cuvette contained the same mixture and the reaction was started by adding 30 μ moles of L-threonine (adjusted to pH 10) to the test cuvette. (3) Acetaldehyde dehydrogenase was assayed spectrophotometrically at pH 7.5 by following the increase in E_{340} consequent upon the reduction of NAD⁺ by acetaldehyde. The reaction mixture in a 1.5 ml. silica cuvette (1 cm. light-path) contained in 1 ml. final volume: 200 μ moles of potassium phosphate buffer, pH 7.5, 0.5 μ mole of NAD⁺ and cell extract (about 0.2 mg. of protein). A reference cuvette contained the same mixture and the reaction was started by adding 15 μ moles of redistilled acetaldehyde to the test cuvette. Specific activities were calculated as μ moles/hr./mg. of protein (assuming ϵ_{340} of NADH to be $6.2 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$).

Carbon source for growth (15 mm)	Organism <i>Pseudomonas</i> TG2T				<i>Pseudomonas putida</i> T		<i>Pseudomonas multivorans</i>
	L-Threonine	Acetate	Acetate + glycine	Pyruvate	L-Threonine	Pyruvate	L-Threonine
L-Threonine dehydrogenase	Nil	Nil	Nil	Nil	Nil	Nil	2.7
L-Threonine aldolase	24.3	23.2	19.2	21.1	4.9	0.6	Nil
Acetaldehyde dehydrogenase	7.2	< 0.1	< 0.1	0.3	11.5	0.7	Nil

possessed very considerable L-threonine aldolase activity plus a potent acetaldehyde dehydrogenase that catalysed NAD⁺ reduction (Table 1).

The L-threonine aldolase was assayed both (a) by the method of Greenberg (1962), wherein acetaldehyde is distilled into semicarbazide and the product assayed spectrophotometrically at 224 nm., and (b) by coupling the enzyme with an excess of alcohol dehydrogenase (EC 1.1.1.1) (C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany) and following the oxidation of NADH spectrophotometrically. High-voltage paper electrophoresis and radioautography of the assay mixture after 2 min. incubation with L-[U-¹⁴C]threonine demonstrated the simultaneous production of glycine. Preliminary studies with crude cell extracts showed that the aldolase had a pH optimum at about pH 10–10.5, did not act on D-threonine, but did catalyse acetaldehyde production from DL-allothreonine. In these crude extracts similar values of $V_{\max}$ and of K_m (6–10 mM) were measured for L-threonine and DL-allothreonine utilization. Again, in crude extracts the acetaldehyde dehydrogenase could utilize either NAD⁺ or, less well, NADP⁺; with Sephadex G-25-treated extracts no CoA requirement was detectable and the optimum pH for activity was pH 7–7.5.

When the apparently key enzymes of L-threonine utilization were assayed in extracts of *Pseudomonas* TG2T grown on (a) L-threonine, (b) acetate, (c) acetate plus glycine and (d) pyruvate, the findings

(summarized in Table 1) revealed that, although the L-threonine aldolase was constitutively synthesized, the acetaldehyde dehydrogenase was inducibly produced, presumably in response to acetaldehyde formation from L-threonine. In bacteria L-threonine aldolase has hitherto only been reported in anaerobes, e.g. *Clostridium pasteurianum* (Dainty & Peel, 1966), where it seems to serve the biosynthetic function of producing glycine from L-threonine. It is perhaps not surprising therefore that the catabolic L-threonine aldolase of *Pseudomonas* TG2T is produced at a much higher specific activity, and that it possesses very different properties from the clostridial enzyme, which displays a pH optimum of 6.8 and an absolute specificity for L-threonine (Dainty, 1967).

L-Threonine-grown *Pseudomonas putida* T resembled *Pseudomonas* TG2T in possessing both L-threonine aldolase (in this case inducibly synthesized) and acetaldehyde dehydrogenase; it, too, contained no L-threonine dehydrogenase (Table 1). Isocitrate lyase, malate synthase and L-serine dehydratase were also present at specific activities of 3.7, 10.8 and 5.3 μ moles/hr./mg. of protein respectively. Neither organism excreted aminoacetone into the culture medium during its growth on L-threonine.

According to Stanier, Palleroni & Doudoroff (1966), all strains of *Pseudomonas multivorans* are able to grow on L-threonine. Accordingly, the enzymic constitution of an authentic strain of *Pseudo-*

monas multivorans (no. 382 of Stanier *et al.* 1966) was examined after growth on L-threonine. Amino-acetone was produced by this organism during its growth on L-threonine, and extracts of the threonine-grown cells contained a genuine L-threonine dehydrogenase but no L-threonine aldolase or acetaldehyde dehydrogenase (Table 1); the specific activities of isocitrate lyase and malate synthase were conspicuously low in these extracts (0.9 and 0.7 μ mole/hr./mg. of protein respectively). This confirms the findings of Lessie (1969) with an unidentified strain of *Pseudomonas multivorans*, and means that among pseudomonads there exist at least two mechanisms for the utilization of L-threonine.

It is of interest that alternative means are available to aerobic bacteria for effecting oxidative catabolism of L-threonine to acetyl-CoA plus glycine. These are: (1) the *Arthrobacter* pathway (McGilvray & Morris, 1969), in which the sequential action of L-threonine dehydrogenase and α -amino- β -oxobutyrate-CoA ligase effects oxidation before cleavage, and (2) the *Pseudomonas* TG2T pathway,

in which the sequential action of L-threonine aldolase, acetaldehyde dehydrogenase and, presumably, an acetyl-CoA synthetase effects cleavage of the threonine molecule before oxidation.

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