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Biodegradative L-Threonine Deaminase of *Salmonella typhimurium*

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The threonine deaminase formed under anaerobic conditions by *Salmonella typhimurium* is induced by L-serine and L-threonine, is catabolite repressible, requires cyclic adenosine 3',5'-monophosphate for its synthesis and adenylic acid for optimal activity, and is immunologically different from biosynthetic threonine deaminase.

Threonine serves as a relatively efficient source of nitrogen for strains of *Salmonella typhimurium* possessing threonine deaminase activity which is not inhibited by L-isoleucine. We successfully used a minimal medium containing L-threonine as the sole source of nitrogen for selecting mutant strains of *S. typhimurium* containing end-product-insensitive biosynthetic threonine deaminase (G. Luginbuhl and R. O. Burns, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1974, p. 54). In this work it was important to know whether *S. typhimurium* would form a degradative L-threonine deaminase since genetic regulatory mutants derepressed for such an enzyme also would be expected to utilize L-threonine as the sole source of nitrogen.

It is known that *Escherichia coli*, which is genetically related to *S. typhimurium*, is capable of producing two apparently distinct threonine deaminases (9). One of these, termed biosynthetic, is formed aerobically under control of multivalent repression (3), and its activity is specifically inhibited by L-isoleucine. The other, termed biodegradative, is formed anaerobically, induced by threonine and serine, is subject to a cyclic adenosine 3'5'-monophosphate (cyclic AMP)-reversed glucose effect, and requires adenylic acid for optimal activity (6, 10).

It was conjectured that formation of biodegradative threonine deaminase by *S. thyphimurium* would require physiological conditions similar to those described for the synthesis of this enzyme by *E. coli*. Wild-type *S. typhimurium* strain LT2 and a biosynthetic threonine deaminase-negative strain (*ilv A-216*) were grown anaerobically and aerobically and assayed for isoleucine-inhibited and AMP-requiring threonine deaminase activity. Table 1 demonstrates that *S. typhimurium* formed an AMP-requiring enzyme under anaerobic but not aerobic conditions. The enzyme formed under aerobic conditions was totally inhibited

TABLE 1. *Threonine deaminase activity in S. typhimurium LT2 and strain Ilv A-216*[a]

Organism	Growth condition	Threonine deaminase activity (μMole product per mg per min)			
		Total	+Isoleucine (1 mM)	Charcoal absorbed	Charcoal absorbed +AMP (10 mM)
LT-2	Anaerobic	3.40	3.40	0.52	3.23
	Aerobic	0.03	0	0.03	0.03
ilv A-216	Anaerobic	4.00	4.00	0.67	3.67
	Aerobic	<0.01			

[a] Cells were grown in medium consisting of 2% N-Z-Case (Sheffield), 1% yeast extract (Difco), and 0.5% K_2HPO_4. A 25-ml amount of medium, in a 15 × 140 mm test tube, was inoculated with 0.1 ml of an overnight nutrient broth culture, and incubated standing at 37 C for 15 h. Cells were harvested by centrifugation and washed with the standard buffer described by Shizuta and Tokushige (8). The cells were resuspended in the same buffer and disrupted by sonic oscillation. Degradative threonine deaminase was assayed according to the procedure of Shizuta and Tokushige (8); biosynthetic threonine deaminase was assayed as described previously (1). Nucleotides were removed from the crude extracts by adding 0.5 g of charcoal (Norite A) to 3 ml of extract. The suspension was stirred in the cold for 10 min, and the charcoal was removed by centrifugation. Specific activity is expressed as micromoles of α-ketobutyrate formed per minute per milligram of protein.

by L-isoleucine and therefore is a biosynthetic enzyme. Absorption of crude extracts with charcoal (Table 1, footnote) is a rapid and convenient way of partially resolving the nucleotide cofactor from biodegradative threonine deaminase, and therefore provides a simple method for identifying nucleotide-requiring threonine deaminase.

The formation of biodegradative threonine deaminase was further investigated with *S. typhimurium* LT2 and an adenylcyclase-deficient strain (*cya-791*) which was isolated as a spontaneous mutant resistant to the antibiotic Fosfomycin (M. D. Alper and B. Ames, manuscript in preparation). Fosfomycin (phosphonomycin) was obtained through the courtesy of H. D. Brown, of Merck, Sharp and Dohme Research Laboratories, Rahway, N.J. Strain *cya-791* was isolated and characterized as follows. Approximately 2×10^8 cells of *S. typhimurium* LT2 were spread onto plates of modified Davis-Mingioli (1) medium containing filter-sterilized Fosfomycin (50 μg/ml). The plates were incubated at 27 C for 48 h, after which the resistant colonies were replica plated onto tetrazolium indicator plates (5) supplemented with 1% glycerol and 1% rhamnose. Those colonies which were unable to use glycerol and rhamnose, and therefore which appeared red on the indicator plates, were purified. Among 20 colonies tested, 4 were able to grow on minimal medium containing glycerol and rhamnose as carbon source only when cyclic AMP was present. The cyclic AMP-requiring character of one of these mutants (*cya-791*) was tested for linkage to the *ilv* region by phage P22-mediated transduction (4). The adenyl cyclase-minus character of mutant *cya-791* demonstrated 1% co-transduction with the *ilvA* gene, and it was therefore concluded that the cyclic AMP requirement of this strain was the consequence of a mutation in the *cya* locus (7).

TABLE 2. *Formation of biodegradative threonine deaminase activity by S. typhimurium LT2 and strain Cya-791*[a]

Additions to medium	Specific activity		
	LT2		Cya-791
	Expt 1	Expt 2	
None	1.13	1.72	<0.01
cAMP[b]	2.47	2.40	3.77
glucose	0.50	1.00	0.12
cAMP + glucose	1.78	1.53	1.58
L-Serine (.2%)	0.07	0.17	0.02
+cAMP	4.77	5.72	6.75
+glucose	0.02	0.02	0.15
+cAMP + glucose	1.58	2.13	1.93
L-Threonine (.2%)	2.32	3.47	0.05
+cAMP	15.62	14.80	19.33
+glucose	0.73	2.17	0.07
+cAMP + glucose	6.03	5.20	6.98
aerobic	<0.01	<0.01	<0.01
aerobic + cAMP	<0.01	<0.01	<0.01
L-Serine (.2%) + L-Threonine (.2%)	0.48	0.57	0.02
+cAMP	9.63	8.40	11.53
+glucose	<0.01	0.02	0.03
+cAMP + glucose	2.00	2.65	3.05

[a] The basal medium consisted of 2% polypeptone (BBL), 0.5% K_2HPO_4, pH 7.4. Supplements were present at 5 mM. All cultures were grown anaerobically unless otherwise specified. Anaerobic cultures were prepared in 29 × 103 polypropylene Sorvall centrifuge tubes containing 40 ml of medium. Cultures were deep inoculated with 0.2 ml of an overnight nutrient broth culture and incubated standing at 37 C for 15 h. Cells were grown aerobically by shaking 40 mls of culture in 250 ml flasks at 37 C. Cells were harvested in the late exponential growth phase. The cells were harvested by centrifugation, washed once in the standard buffer described by Shizuta and Tokushige (8), suspended in 1 ml of the same buffer, and disrupted by sonic oscillation. Enzyme was assayed as described in Table 1.

[b] cAMP = cyclic adenosine monophosphate.

Table 2 shows the specific activity of biodegradative threonine deaminase in *S. typhimurium* LT2 and *cya-791* grown under various conditions. We concluded that: (i) degradative threonine deaminase formation is subject to a cyclic AMP-reversed catabolite repression, (ii) serine induces enzyme formation but gives rise to severe catabolite repression; (iii) threonine induces enzyme formation; (iv) cyclic AMP is required for enzyme formation, even in the absence of catabolite repression; (v) the degradative enzyme is repressed by aerobic growth, and this repression is not overcome by cyclic AMP.

The disparate conditions required for synthesis of biosynthetic and biodegradative threonine deaminase, as well as the difference in properties of these activities in crude extracts, considered together with the observation that mutation in the *ilvA* gene (the structural gene for biosynthetic threonine deaminase) does not curtail production of the anaerobically formed threonine deaminase, suggests that these enzymes are distinct. Evidence for the lack of shared structure in these enzymes is provided in Table 3, which shows that columns of anti-biosynthetic threonine deaminase antibody-substituted Sepharose retained the biosynthetic but not the biodegradative enzyme.

TABLE 3. *Test for immunological cross-reactivity between biodegradative and biosynthetic threonine deaminase*[a]

Antibody column	Crude extract	Units			Activity of enzyme retained[b]		
		Applied	Off	Retained	Reaction mixture	Reaction mixture + L-isoleucine	Reaction mixture
1	Biodegradative TD	25.83	26.83	(−1)	—	—	—
2	Biosynthetic TD	10.40	7.67	2.73	+	—	+
1′	Biosynthetic TD	9.15	7.27	2.05	+	—	+

[a] Antiserum (rabbit) against purified (1) biosynthetic threonine deaminase was prepared by standard techniques and coupled to Sepharose 4B (Pharmacia) by the procedure of Cuatrecasas (2). Columns of the antibody-substituted Sepharose were prepared in Pasteur pipettes and equilibrated with appropriate buffers (1, 8). Crude extracts containing biodegradative and biosynthetic threonine deaminase were prepared and passed through the columns. Units of threonine deaminase retained were computed from the difference in units eluted and units added. Column 1′ is column 1 which was previously used to test retention of biodegradative threonine deaminase; retention of biosynthetic threonine deaminase by this column shows that antibody sites were not covered by undetected biodegradative enzyme.

[b] Retention of threonine deaminase by the columns was ascertained by passing a reaction mixture through the column and measuring the level of α-ketobutyrate in the effluent. The sequence shown, reading left to right, demonstrates activity, inhibition of activity, and regain of activity by the antibody-associated enzyme.

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