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Repression of the Tyrosine, Lysine, and Methionine Biosynthetic Pathways in a *hisT* Mutant of *Salmonella typhimurium*

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A comparison was made of the repressibility of certain enzymes in the tyrosine, methionine, and lysine biosynthetic pathways in wild-type *Salmonella typhimurium* and a *hisT* mutant. The results show that (i) tyrosine represses the synthesis of the tyrosine-sensitive 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate synthetase and the tyrosine aminotransferase to the same extent in a *hisT* mutant as in wild type and (ii) there is no detectable alteration in the extent to which methionine represses *O*-succinylhomoserine synthetase or in the extent to which lysine represses the lysine-sensitive β-aspartokinase as a result of the *hisT* mutation.

Previous studies on the regulation of amino acid biosynthesis in *Escherichia coli* and *Salmonella typhimurium* have indicated that there are several different mechanisms by which amino acids regulate the synthesis of the enzymes in their respective biosynthetic pathways (7, 25). In the histidine and branched-chain amino acid pathways, the aminoacyl-transfer ribonucleic acids (tRNA's) have been implicated as corepressors (5, 13, 26), whereas in the aromatic amino acid pathway, the evidence has indicated that the free amino acids function as corepressors (12, 14, 19, 20, 23). *hisT* mutants of *S. typhimurium* are of particular interest because they contain normal levels of the aminoacyl-tRNA's, and yet the enzymes of the histidine and isoleucine-valine pathways are derepressed (6, 8, 10, 21). These mutants lack the enzyme responsible for the conversion of uridine residues to pseudouridines in the anticodon region of tRNA (9, 22). A comparison of the chromatographic mobilities and the nucleotide sequences of $tRNA^{His}$ (22) and $tRNA^{Leu}$ (1) from wild-type *S. typhimurium* with those from a *hisT* mutant showed that the mutant tRNA's contain two uridine residues in the anticodon region instead of two pseudouridines. The data indicate that derepression of the histidine and isoleucine-valine pathways in *hisT* mutants is due to this alteration in the tRNA's (21, 22). Since *E. coli* $tRNA^{Tyr}$ and $tRNA_m^{Met}$ and yeast $tRNA^{Lys}$ contain a pseudouridine in the anticodon region (3) and it has been shown that $tRNA^{Tyr}$ (22) and $tRNA^{Lys}$ (9) are altered in *hisT* mutants, it was of interest to determine the effect of the *hisT* mutation on the ability of tyrosine, lysine, and methionine to repress the synthesis of certain enzymes in their respective biosynthetic pathways.

S. typhimurium LT2 and *hisT1504*, isogenic strains differing only in the *hisT* gene (8), were kindly supplied by R. F. Goldberger. Cells were grown to an absorbance of 1.0 at 660 nm (approximately 10 h) in a shaker incubator at 30°C in minimal media (10) containing 0.5% glucose and amino acid supplements, as indicated. The cells were harvested, washed twice with 10 mM potassium phosphate, pH 7 (unless indicated otherwise), and disrupted by sonic oscillation in a Raytheon 10-kc oscillator for 10 min. Cell debris was removed by centrifugation, and the extracts were stored in small portions at −70°C. Protein concentrations were determined by the method of Lowry et al. (18).

The data in Table 1 show that when either wild-type *S. typhimurium* or the *hisT* mutant is grown in minimal medium, about 50% of the 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP) synthetase (the first enzyme in the aromatic amino acid biosynthetic pathway) is sensitive to inhibition by tyrosine, i.e., is the tyrosine-sensitive isoenzyme. When wild-type cells are grown on minimal medium supplemented with tyrosine, the total amount of DAHP synthetase decreases only slightly. However, the amount of the tyrosine-sensitive enzyme is reduced about 70%, whereas the phenylalanine-sensitive enzyme is increased about 60%. The reason for this increase in the phenylalanine-sensitive enzyme is unclear at

TABLE 1. *Effects of tyrosine on the synthesis of DAHP synthetase in wild-type and a hisT mutant of S. typhimurium*

DAHP synthetase[a]	U/mg of protein[b]			
	Wild-type LT2		Mutant *hisT1504*	
	Minimal medium	Minimal medium + 0.5 mM tyrosine	Minimal medium	Minimal medium + 0.5 mM tyrosine
Total	27	26	23	22
Tyr sensitive	14	4	12	3
Phe sensitive	14	22	15	18

[a] DAHP synthetase (EC 4.1.2.15) activity was determined by a modification of the procedure of Srinivasan and Sprinson (24). Reaction mixtures (0.25 ml) contained 4 mM erythrose 4-phosphate, 8 mM phosphoenolpyruvate, 50 mM potassium phosphate, pH 7.5, 1 mM $CoCl_2$, and a rate-limiting amount of cell extract (25 to 100 μg of protein). Incubation was for 10 min at 37°C. Assays were performed in the absence of tyrosine and phenylalanine to measure total enzyme, in the presence of 0.5 mM phenylalanine to measure Tyr-sensitive enzyme, and in the presence of 0.5 mM tyrosine to measure Phe-sensitive enzyme.

[b] Micromoles of product formed per hour under the conditions described.

the present time but has been observed consistently. The *hisT* mutant responds in much the same way as the wild type, with the tyrosine-sensitive DAHP synthetase repressed about fourfold when the cells are grown in the presence of tyrosine.

A comparison of the ability of several amino acids to repress the synthesis of certain enzymes in their respective biosynthetic pathways in wild type and in a *hisT* mutant of *S. typhimurium* is shown in Table 2. Both the tyrosine-sensitive DAHP synthetase and the tyrosine aminotransferase (which catalyzes the last step in tyrosine biosynthesis) are repressed four- to fivefold when the cells are grown in the presence of tyrosine. The lysine-sensitive β-aspartokinase, one of the two isoenzymes that catalyze the first step in the biosynthesis of lysine, threonine, and methionine, is reduced more than sevenfold when either wild type or the *hisT* mutant is grown in the presence of lysine. *O*-succinylhomoserine synthetase, a biosynthetic enzyme specific for the methionine pathway, is repressed to the same extent by methionine in the *hisT* mutant as in wild type. It is of interest to note that the levels of the

TABLE 2. *Comparison of the repressibility of certain enzymes in wild-type and a hisT mutant of S. typhimurium*

Enzyme	U/mg of protein[a]			
	Wild-type LT2		Mutant *hisT1504*	
	Minimal medium	Minimal medium + amino acids	Minimal medium	Minimal medium + amino acids
DAHP synthetase (Tyr sensitive)[b]	16.0	4.0	12.0	3.0
Tyrosine aminotransferase[b]	6.7	1.4	7.3	1.4
β-Aspartokinase (Lys sensitive)[c]	1.7	0.2	1.4	0.2
O-Succinylhomoserine synthetase[d]	3.3	0.6	3.0	0.4
Histidinol phosphate phosphatase[e]	0.9	0.3	6.8	6.0
Acetohydroxy acid synthetase[e]	3.3	1.0	7.0	4.3

[a] Micromoles of product formed per hour under the conditions described.

[b] The medium contained 0.5 mM tyrosine when indicated. Tyrosine-sensitive DAHP synthetase activity was determined as described in the footnotes to Table 1. Tyrosine aminotransferase (EC 2.6.1.5) activity was determined by the procedure of Heinonen et al. (14). Reaction mixtures contained 5 to 50 μg of cell extract protein and were incubated for 10 min at 37°C.

[c] The medium contained 2 mM lysine when indicated. Lysine-sensitive β-aspartokinase (EC 2.7.2.4) activity was determined by the procedure of Lee et al. (16). Reaction mixtures contained 10 mM threonine and 0.75 to 1.5 mg of cell extract protein and were incubated for 30 min at 30°C.

[d] The medium contained 2 mM methionine when indicated. *O*-succinylhomoserine synthetase (EC 2.3.1.46) was determined by the procedure of Lee et al. (15). Reaction mixtures contained 150 to 500 μg of cell extract protein and were incubated for 10 min at 25°C.

[e] Cells were grown in minimal medium or in minimal medium supplemented with 1.0 mM histidine, 0.5 mM isoleucine, 0.5 mM leucine, and 0.5 mM valine. The cells were suspended and washed twice with 50 mM tris(hydroxymethyl)aminomethane-hydrochloride, pH 8, containing 80 μg of thiamine pyrophosphate, 10 mM $MgCl_2$, and 10% glycerol before sonic treatment. L-Histidinol phosphate phosphatase (EC 3.1.3.15) activity was determined by the procedure of Ames et al. (2). Reaction mixtures contained 2 to 50 μg of cell extract protein and were incubated for 30 min at 37°C. Acetohydroxy acid synthetase (EC 4.1.3.12) activity was determined by a modification of the procedure of Bauerle et al. (4). Reaction mixtures (0.5 ml) contained 50 mM tris(hydroxymethyl)aminomethane-hydrochloride, pH 8, 50 mM sodium pyruvate, 10 mM $MgCl_2$, 50 μg of thiamine pyrophosphate, 20 μg of flavin adenine dinucleotide, and 0 to 750 μg of cell extract protein. Incubation was for 30 min at 37°C.

enzymes cited above are not significantly greater in the *hisT* mutant grown in minimal medium than in the wild type grown in minimal medium.

Previous reports have shown that enzymes in the histidine and isoleucine-valine pathways are derepressed in *hisT* mutants (10, 17). For comparative purposes, Table 2 shows that the amount of histidinol phosphate phosphatase is seven- to eightfold higher in the *hisT* mutants than in wild type when the cells are grown in minimal medium. Growth of the cells in the presence of histidine decreases the amount of this enzyme about threefold in wild type, but does not decrease significantly the amount of enzyme in the *hisT* mutant. The amount of acetohydroxy acid synthetase is about twofold greater in the *hisT* mutants than in wild type when the cells are grown in minimal medium, and the amount of this enzyme is decreased threefold in wild type but only about 40% in the *hisT* mutant when the cells are grown in the presence of the branched-chain amino acids.

The results of this investigation indicate that although the *hisT* mutation reduces the repressibility of the histidine and branched-chain amino acid biosynthetic enzymes, it does not appear to alter the extent to which certain enzymes in the tyrosine, lysine, and methionine biosynthetic pathways are repressed by their respective amino acid end products.

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