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Repression of Cytosine Deaminase by Pyrimidines in *Salmonella typhimurium*

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The synthesis of cytosine deaminase in *Salmonella typhimurium* is repressed by pyrimidines. This repression is mediated by both a uridine and a cytidine compound, indicating a distinct difference in the regulation of synthesis of cytosine deaminase from the regulation of the de novo pyrimidine pathway enzymes. A salvage role for the enzyme in pyrimidine metabolism is postulated.

Cytosine deaminase (cytosine aminohydrolase; EC 3.5.4.1) catalyzes the hydrolytic deamination of cytosine to uracil and NH_3 (2, 6, 14). In *Salmonella typhimurium*, the enzyme is encoded by the gene *cod* (Fig. 1), which is located at the 69-min position of the bacterial chromosome (15). Although numerous studies (1, 10, 16, 18) have examined the regulation of expression of the genes *pyrA* through *pyrF* (Fig. 1), which encode the six de novo pyrimidine pathway enzymes in *S. typhimurium*, it is uncertain whether such regulation exists for the *cod* gene in this microorganism. Cytosine deaminase synthesis has been found to be inducible in *Mycobacterium* (8), whereas its expression in *Escherichia coli* (11) is not under the control of the *cytR* gene or the *deoR* gene, and the four *deo* genes, along with *udp* and *cdd*, have been shown to be controlled by *cytR* and *deoR* (17). Whether the function of cytosine deaminase in pyrimidine metabolism is related to the anabolism of pyrimidine nucleotides or the catabolism of pyrimidine bases is the subject of this brief report. When the partial characterization of an enzyme activity capable of degrading CMP to cytosine was reported for *S. typhimurium* (3), an investigation of the regulation of cytosine deaminase synthesis seemed appropriate, since cytosine deaminase might be involved in a recycling pathway to replenish pyrimidine nucleotide levels. Here we present evidence that repression of cytosine deaminase synthesis by both a uridine and a cytidine compound occurs in *S. typhimurium*. This finding is consistent with the enzyme being involved in pyrimidine biosynthesis and enhances the possibility of the salvage pathway.

The *S. typhimurium* strains employed are listed in Table 1. Coomassie brilliant blue G-250 was purchased from Eastman Organic Chemicals, Rochester, N.Y., and the remaining reagents were obtained from Sigma Chemical Co., St. Louis, Mo.

To test for cytosine deaminase synthesis being induced by pyrimidines in *S. typhimurium*, the wild-type strain LT2 was grown in minimal medium with various pyrimidine supplements (Table 2). While not affecting the generation time of the strain (51 min), the presence of pyrimidines in the medium decreased cytosine deaminase activity, indicating no induction of synthesis by uracil, cytosine, or uridine. It can be concluded that cytosine deaminase is not a *cyt*-enzyme, since *cytR* gene expression can be induced by uridine in *S. typhimurium* (13).

The previous data indicated repression of cytosine deaminase rather than induction. One index of repression of enzyme synthesis by pyrimidine compounds would be the elevation of the enzyme activity in question after the starvation of a pyrimidine-requiring strain for uracil (4, 18). From the data shown in Table 3, it can be seen that the arginine- and uracil-requiring strain, S2 (51-min generation time), after being starved for uracil for 90 min, had a cytosine deaminase activity that was about 2.3-fold higher than the activity found when the strain was grown in the presence of uracil (under repressing conditions). This derepression of cytosine deaminase synthesis after pyrimidine limitation confirmed repression as the regulation involved. To differentiate between repression by a uridine and a cytidine compound, it was necessary to use the arginine-, uracil-, and cytidine-requiring strain JL1055. This strain, having been constructed specifically to allow the independent manipulation of uridine and cytidine nucleotide pool levels within *S. typhimurium* cells, enables the identification of a uridine or a cytidine compound as a corepressor of synthesis (12). The resultant activities that were found after a 90-

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TABLE 1. Strains employed

Strain	Genotype[a]	Source (reference)
LT2	Wild type	B. N. Ames
KD1109	*cdd-4*	(10)
HD11-AE2	*pyrH11 cdd-4 galE2*	R. A. Kelln
S2	*pyrA81*	(19)
JL1055	*pyrA81 pyrG cdd udp*	(12)

[a] All strains are derivatives of *S. typhimurium* LT2. Genetic nomenclature is that of Sanderson and Hartman (15).

min starvation of strain JL1055 cells (75-min generation time) for uridine nucleotides, cytidine nucleotides, or both types of nucleotides were compared with the fully repressed activity (grown in uracil and cytidine medium) of this strain (Table 3). A threefold derepression of cytosine deaminase synthesis was noted for all of the pyrimidine starvation conditions examined. This finding suggests that both a uridine and a cytidine compound are repressing metabolites of *cod* expression in *S. typhimurium*.

To ensure that the pyrimidine starvation conditions used were not causing nonspecific derepression of enzyme synthesis, cytosine deaminase was assayed in a UMP kinase (EC 2.7.4.4) mutant strain and in an isogenic strain harboring a functional UMP kinase gene (Table 2). As with previous studies in *S. typhimurium* (10, 16), a defective UMP kinase strain, HD11-AE2, was chosen for this analysis since a growing cell population of such a strain contains greatly increased (i.e., derepressed) activities of those enzymes repressed by pyrimidine compounds. This increase is due to the inability of *pyrH* strains to maintain wild-type (i.e., *pyrH*$^+$) levels of UDP (0.30 and 0.66 μmol/g [dry weight] for *pyrH* and *pyrH*$^+$, respectively) and hence, UTP (2.52 and 3.42 μmol/g [dry weight] for *pyrH* and *pyrH*$^+$, respectively). Thus, a partial starvation is set up within the bacterial cell. The isogenic *pyrH*$^+$ strain KD1109 serves as a control since its pyrimidine ribonucleotide levels are not altered by a *pyrH* mutation and thus contain normal levels of UDP and UTP (10).

Pyrimidine additions to minimal medium had little effect on the generation time of either strain HD11-AE2 (76 min) or strain KD1109 (62 min). By comparing cytosine deaminase levels in strains HD11-AE2 and KD1109, it is evident that the *pyrH* strain contained approximately four times the activity of its isogenic *pyrH*$^+$ strain (Table 2). Not only did this observation indicate derepression of enzyme synthesis, but it appeared that a uridine nucleotide might be the primary corepressor. Previous nucleotide pool studies with the *pyrH11* strain suggested that this may be the case, since the uridine nucleotide levels in the strain were significantly lower, whereas its cytidine nucleotide levels were normal (relative to levels in a *pyrH*$^+$ strain) (9, 10). In strain KD1109, the reduction of cytosine deaminase activity by 32% with uracil in the medium also suggests repression by a uridine nucleotide (Table 2). In strain HD11-AE2, uracil-containing medium had only a nominal effect on enzyme activity (Table 2), as might be expected, since uracil could not be converted beyond the monophosphate level in a *pyrH*

TABLE 2. Effect of pyrimidines on cytosine deaminase levels in strains LT2, KD1109, and HD11-AE2[a]

Strain (relevant genotype)	Addition to minimal medium[b]	Sp act[c]	Level of[d]: UTP	CTP
LT2 (wild type)	None	36.9		
	Uracil	29.6		
	Uridine	28.6		
	Cytosine	26.8		
KD1109 (*cdd*)	None	31.9	3.42	2.21
	Uracil	21.6	4.48	2.68
	Cytidine	27.8	3.54	4.10
HD11-AE2 (*pyrH cdd galE*)	None	120	2.52	2.48
	Uracil	142	2.75	2.70
	Cytidine	57.1	1.28	6.20

[a] Strains were grown in medium A (7) fortified with $FeCl_3 \cdot 6H_2O$ (0.8 μg/ml), $ZnSO_4 \cdot 7H_2O$ (5 μg/ml), and 0.2% glucose at 37°C to a concentration of approximately 10^9 bacterial cells per ml. The cells were harvested by centrifugation at 17,400 × *g* for 8 min at 4°C, washed with water, and resuspended in 2 ml of ice-cold 0.05 M Tris-hydrochloride buffer, pH 7.3. The cell suspension was subjected to ultrasonic disruption on a sonic oscillator (model SW185; Heat Systems-Ultrasonics, Plainview, N.Y.) at 0°C for 1 min, centrifuged for 30 min at 27,100 × *g* at 4°C, and assayed for enzyme activity without further treatment. Cytosine deaminase activity was determined at 37°C in a 1-ml assay mixture that contained 50 mM Tris-hydrochloride buffer (pH 7.3), cell extract, and 0.50 mM cytosine. The decrease in absorbancy at 285 nm was measured and the product was estimated by using a molar extinction of 1.038×10^3. Product formation in the assay was proportional to protein concentration and time. Protein concentration was determined by the method of Bradford (5), using lysozyme as the standard.

[b] Pyrimidine bases and nucleosides were present at a final concentration of 50 μg/ml when added to medium A.

[c] Specific activity is expressed as nanomoles of uracil formed per minute per milligram of protein at 37°C and represents the mean of three separate determinations.

[d] Data are from reference 10 and are expressed as micromoles of nucleotide per gram (dry weight) of bacteria.

TABLE 3. Effect of pyrimidine limitation on *cod* gene expression

Strain (relevant genotype)	Growth conditions[a]	Sp act[b]
S2 (*pyrA81*)	Repressing uracil	20.9
	Limiting uracil	47.8
JL1055 (*pyrA pyrG cdd udp*)	Repressing uracil and cytidine	24
	Limiting uracil, repressing cytidine	69
	Repressing uracil, limiting cytidine	75
	Limiting uracil and cytidine	78

[a] Strain S2 was grown at 37°C in medium A (7) to which $FeCl_3 \cdot 6H_2O$ (0.8 μg/ml), $ZnSO_4 \cdot 7H_2O$ (5 μg/ml), arginine (50 μg/ml), and 0.2% glucose were added with either a repressing (50 μg/ml) or a limiting (8 μg/ml) uracil concentration. Strain JL1055 was grown at 37°C in medium A (7) which contained added $FeCl_3 \cdot 6H_2O$ (0.8 μg/ml), $ZnSO_4 \cdot 7H_2O$ (5 μg/ml), arginine (50 μg/ml), and 0.4% glucose. The pyrimidine growth conditions of this strain are represented as follows: repressing uracil and cytidine (50 μg/ml each), limiting uracil (2 μg/ml) and repressing cytidine (10 μg/ml), repressing uracil (50 μg/ml) and limiting cytidine (5 μg/ml), and limiting uracil and cytidine (5 μg/ml each). Both strains S2 and JL1055 were starved for the limiting pyrimidine(s) for 90 min after growth had been arrested at approximately 10^9 bacterial cells per ml. The pyrimidine-repressed or -starved cells were harvested and assayed for enzyme activity as described in Table 2, footnote *a*, except protein was quantitated for strain JL1055 as previously described (18).

[b] Specific activity is expressed as nanomoles of uracil formed per minute per milligram of protein at 37°C, which represents the mean of three separate determinations for strain S2 or the average of two separate determinations for strain JL1055.

strain. With such elevated UMP levels (10), especially when grown with uracil (8.4 and 4.8 μmol of UMP per g [dry weight] for *pyrH* and *pyrH*$^+$, respectively), it can be concluded that UMP is not the repressing metabolite. The effect of cytidine metabolites on strain HD11-AE2, with its pronounced 53% decrease in enzyme activity, was in contrast with the negligible effect on the activity of the *pyrH*$^+$ strain KD1109 (Table 2). Still, a cytidine compound appeared to be more effective in repressing cytosine deaminase synthesis when the strain was partially limited for pyrimidine nucleotides (as in strain HD11-AE2). In contrast, a uridine compound was able to repress synthesis when pyrimidine ribonucleotide levels were normal (as in strain KD1109). Therefore, although both pyrimidine compounds repress synthesis, a uridine compound may be able to interact with the putative aporepressor with a greater specificity than a cytidine compound in preventing *cod* gene expression.

The pattern of repression of cytosine deaminase by pyrimidine compounds does not resemble that of the de novo pyrimidine pathway enzymes (encoded by *pyrA* through *pyrF*) in *S.*

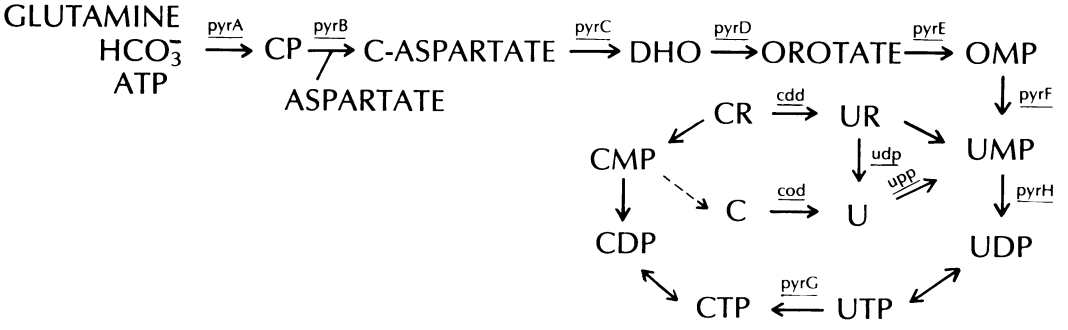

FIG. 1. Pathways for de novo and salvage biosynthesis of pyrimidine ribonucleotides in *S. typhimurium*. Abbreviations: CP, carbamoyl phosphate; C-ASPARTATE, carbamoyl aspartate; DHO, dihydroorotate; CR, cytidine; UR, uridine; C, cytosine; U, uracil. Underlined genetic symbols identify the following enzymes: *pyrA*, carbamoyl phosphate synthetase (EC 2.7.2.9); *pyrB*, aspartate carbamoyltransferase (EC 2.1.3.2); *pyrC*, dihydroorotase (EC 3.5.2.3); *pyrD*, dihydroorotate dehydrogenase (EC 1.3.3.1.); *pyrE*, orotate phosphoribosyltransferase (EC 2.4.2.10); *pyrF*, OMP decarboxylase (EC 4.1.1.23); *pyrG*, CTP synthetase (EC 6.3.4.2); *pyrH*, UMP kinase (EC 2.7.4.4); *cdd*, cytidine deaminase (EC 3.5.4.5); *cod*, cytosine deaminase (EC 3.5.4.1); *udp*, uridine phosphorylase (EC 2.4.2.3.); *upp*, uracil phosphoribosyltransferase (EC 2.4.2.9). The dashed line represents a partially characterized enzymatic reaction.

typhimurium (Fig. 1). It has been shown that the de novo enzymes are repressed by either a uridine or a cytidine compound (10, 16), whereas cytosine deaminase is repressed by both compounds. This difference in the pattern of repression of these enzymes in *S. typhimurium* may indicate that cytosine deaminase is regulated by a distinct regulatory element controlling only *cod* gene expression. With cytosine deaminase being repressed by pyrimidines and with CMP apparently being enzymatically converted to cytosine (3), greater credibility is lent to the existence of a biosynthetic salvage pathway for the conversion of cytidine nucleotides to uridine nucleotides in *S. typhimurium*. A possible function of the pathway might be to recycle RNA degradation products, using the combined activities of the CMP degrading enzyme, cytosine deaminase, and uracil phosphoribosyltransferase (EC 2.4.2.9) (Fig. 1), in an attempt to maintain pyrimidine ribonucleotides at appropriate levels for RNA synthesis during pyrimidine starvation.

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