Specific Repression of Phosphoribosylpyrophosphate Synthetase by Uridine Compounds in *Salmonella typhimurium*

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Mutants of *Salmonella typhimurium* with specific pyrimidine requirements were used to demonstrate that only uridine compounds exert repressive control over synthesis of phosphoribosylpyrophosphate synthetase.

Evidence has been presented recently that, of the various end products biosynthetically derived from 5-phosphoribosyl-α-pyrophosphate (PRPP), only pyrimidine nucleotides function as repressing metabolites for PRPP synthetase in *Salmonella typhimurium* (4). The techniques used did not indicate whether uridine nucleotides, cytidine nucleotides, or both, were responsible for repression of PRPP synthetase. The isolation and characterization by Neuhard and Ingraham of mutant strains of *S. typhimurium*, in which cytidine and uridine nucleotide pools can be manipulated independently (3), provided a means of answering this question.

In one of the mutant strains, JL1045 (formerly DP45), the capacity to deaminate cytidine and cytosine is lost, and the ability to convert uridine triphosphate (UTP) to cytidine triphosphate (CTP) is blocked (2, 3). This strain specifically requires cytidine for growth. During starvation of the organisms for cytidine, CTP pools have been shown to drop to undetectable levels, while UTP pools increase by as much as 15-fold (2). PRPP synthetase is not derepressed during cytidine starvation (Table 1). The same result was obtained whether starvation was accomplished by transferring the cells into medium containing no cytidine or by allowing the bacteria to exhaust a limiting quantity of cytidine. A small derepression of aspartic transcarbamylase (ATCase), as previously reported (3), was observed under these conditions, a result that demonstrates that cytidine was growth-limiting and that the cells were capable of protein synthesis during starvation. The results suggest that cytidine nucleotides do not function as repressing metabolites for PRPP synthetase.

Another mutant strain, JL1055 (formerly DP55), is not only blocked in reactions for the interconversion of cytidine and uridine compounds, but is also incapable of de novo synthesis of pyrimidines (2, 3). This strain can be specifically starved for either cytidine or uridine nucleotides. As was the case with strain JL1045, starvation of strain JL1055 for cytidine (with uridine in excess) did not elicit significant derepression of PRPP synthetase (Table 1). Starvation of the same strain for uridine in the presence of excess cytidine brought about a fourfold derepression of PRPP synthetase, a value that was greater than was obtained by starving for both pyrimidines. Identical results were obtained when the bacteria were starved for uracil instead of uridine. Under these conditions, Neuhard has shown UTP pools to be very low and CTP pools to be expanded by as much as 10-fold (2). The parent strain (*pyrA81*) of strain JL1055 is derepressed about threefold by starvation for pyrimidines (4), a condition that must result in depletion of both uridine and cytidine pools, since strain *pyrA81* lacks carbamylphosphate synthetase (3). These findings demonstrate that pools of uridine compounds, presumably a uridine nucleotide or nucleotides, exert regulatory control over the synthesis of PRPP synthetase in the presence of very high pools of cytidine nucleotides. Since derepression of PRPP synthetase is no greater when both uridine and cytidine pools are depleted in strain *pyrA81*, it is most reasonable to propose that only uridine compounds can act as repressing metabolites for PRPP synthetase. If any repressive effects of cytidine compounds exist, they must be completely overcome by the effects of uridine nucleotide pools.

TABLE 1. *Repression and derepression of PRPP synthetase in mutants with specific pyrimidine requirements*

Strain[a]	Additions to minimal medium[b] (μg/ml)	Specific activity	
		PRPP synthetase[c]	ATCase[d]
		±SD	
JL1045 (cod, cdd, pyrG)	None	0.076 ± 0.010	9.2
	Cytidine, 30	0.079 ± 0.008	2.2
JL1055[e] (cdd, udp, pyrA81, pyrG)	None	0.110 ± 0.014	12.6
	Uridine, 30 Cytidine, 30	0.065 ± 0.026	0.35
	Uridine, 10 Cytidine, 0	0.087 ± 0.014	3.95
	Uridine, 0 Cytidine, 10	0.234 ± 0.058	40.0

[a] Genotype designations are: *cod*, cytosine deaminase; *cdd*, cytidine deaminase; *udp*, uridine phosphorylase; *pyrA*, carbamylphosphate synthetase; *pyrG*, cytidine triphosphate synthetase.

[b] The bacteria were grown in minimal medium (4) plus 0.5% glucose supplemented with 30 μg/ml of required supplements, harvested during logarithmic growth and resuspended in the same medium containing the indicated supplements. The cells were collected during logarithmic growth, or, in the case of starvation, 1.0 to 1.5 hr after resuspending. Sonic extracts were assayed for PRPP synthetase with the ³²P transfer assay and for ATCase as previously described (4).

[c] Results are expressed as micromoles per minute per milligram of protein.

[d] Results are expressed as micromoles per 20 min per milligram of protein.

[e] Arginine (100 μg/ml) was included in all media for growth of this strain.

These findings also eliminate the possibility that derepression of PRPP synthetase during pyrimidine starvation is the indirect consequence of purine accumulation under these conditions (4). Purine nucleotides do indeed accumulate during pyrimidine starvation, but they accumulate to the same extent during either uridine or cytidine starvation (2). Thus, the results eliminate the possibility that purine nucleotide accumulation brings about derepression, e.g. by lowering PRPP pools through feedback inhibition.

Through the use of very similar techniques, Neuhard and Ingraham (2) have demonstrated that the aspartic transcarbamylase of *S. typhimurium* is repressed by both uridine and cytidine nucleotides, and Abd-El-Al and Ingraham (1) have shown that carbamylphosphate synthetase is repressed only by cytidine nucleotides (and arginine). Thus, three possible patterns of repression for governing enzymes of pyrimidine biosynthesis have been found in the same organism.

LITERATURE CITED

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