JOURNAL OF BACTERIOLOGY, June 1979, p. 731-738
0021-9193/79/06-0731/08$02.00/0

Vol. 138, No. 3

Apparent Involvement of Purines in the Control of Expression of *Salmonella typhimurium pyr* Genes: Analysis of a Leaky *guaB* Mutant Resistant to Pyrimidine Analogs

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Received for publication 21 March 1979

A leaky *guaB* mutant of *Salmonella typhimurium* LT-2 was obtained during a selection for mutants resistant to a combination of the two pyrimidine analogs, 5-fluorouracil and 5-fluorouridine. In the absence of exogenous guanine compounds, the growth rate of this mutant is limited by the endogenous supply of guanine nucleotides due to a defective inosine 5'-monophosphate dehydrogenase. Under these conditions the guanosine 5'-triphosphate pool is about 20% of normal, the cytidine 5'-triphosphate pool is reduced to below 60%, and the uridine 5'-triphosphate pool is slightly elevated. Simultaneously, levels of the pyrimidine biosynthetic enzymes are abnormal: aspartate transcarbamylase, orotate phosphoribosyltransferase, and orotidylic acid decarboxylase levels are increased 4-, 11-, and 3-fold, respectively. Levels of dihydroorotase and dihydroorotate dehydrogenase are decreased to 10 and 20%, respectively. The pyrimidine metabolism of the *guaB* mutant is restored completely by addition of guanine (or xanthine) to the growth medium. The data indicate purine nucleotide involvement in the regulation of expression of the *pyr* genes of *S. typhimurium*.

In *Salmonella typhimurium* the enzymes of the pyrimidine de novo synthesis have been divided into two groups on a regulatory basis: (i) aspartate transcarbamylase (EC 2.1.3.2, *pyrB*), orotate phosphoribosyltransferase (EC 2.4.2.10, *pyrE*), and OMP decarboxylase (EC 4.1.1.23, *pyrF*), whose synthesis seems to be repressed by a uridine compound which is not UMP, but rather UTP, UDP, or a uridine sugar nucleotide (21); and (ii) dihydroorotase (EC 3.5.2.3, *pyrC*) and dihydroorotate dehydrogenase (EC 1.3.3.1, *pyrD*), whose synthesis seems to be repressed (primarily) by a cytidine compound which is not CMP, but rather CDP or CTP (21). However, it has been observed that the enzyme levels do not correlate well with the pool of any individual pyrimidine nucleotide (13), and it was suggested that other nucleotide derivatives (than di- or triphosphates) or ratios must be considered (13). Figure 1 shows the pathway for the synthesis of pyrimidine nucleotides. None of the structural genes is closely linked on the bacterial chromosome (1).

Attempts have been made to isolate regulatory mutants with constitutive levels of the pyrimidine biosynthetic enzymes by selecting for resistance to pyrimidine analogs (19). However, the mutants obtained proved to be defective in UMP kinase (*pyrH*). They had high levels of the enzymes as a consequence of reduced ability to metabolize UMP (9, 12; see Fig. 1).

I have repeated the search for regulatory mutants by selecting for resistance to the pyrimidine analog 5-fluorouracil plus 5-fluorouridine (9), using a strain of *S. typhimurium* that is limited in the ability to degrade pyrimidine nucleosides (KP-1469, *cdd-9 cod-8 deoD201 udp-11*). One of the mutants obtained has a defective IMP dehydrogenase (EC 1.2.1.14), i.e., is a leaky *guaB* mutant. When grown under conditions of guanine limitation, this mutant has abnormal levels of the pyrimidine de novo enzymes. In the presence of exogenous guanine, however, the mutant shows normal pyrimidine metabolism. This paper presents a characterization of this *guaB* mutant. The results obtained suggest that a purine nucleotide is involved in regulation of expression of the *pyr* genes in *S. typhimurium*.

MATERIALS AND METHODS

Bacterial strains. The strains employed in this work are all derivatives of *S. typhimurium* LT-2 and are listed in Table 1.

Growth conditions. Bacteria were grown with shaking in the Tris-buffered medium of Irr and Gallant (10), containing 0.3 mM inorganic phosphate. The medium was supplemented with glucose (0.2%) as a carbon source and with thiamine (1 μg/ml). Cell

growth was monitored in an Eppendorf photometer, model 1011 M, at 436 nm. An optical density of 1 corresponds to about 4×10^8 cells per ml.

Preparation of bacterial extracts. Cells were grown exponentially for several generations at the temperature indicated (usually 37°C). At an optical density of 1 they were harvested by centrifugation (6,000 × *g* for 10 min), suspended in 5 ml of sodium chloride (0.9%), and centrifuged again. Pellets were stored at −20°C overnight. Cells were resuspended in 0.10 M Tris-hydrochloride (pH 7.6)-2 mM EDTA to an optical density of 30 to 40, were disrupted by sonication for 1 min at 0°C with an MSE Ultrasonic disintegrator, and were used either without further treatment (for assays of orotate phosphoribosyltransferase, dihydroorotate dehydrogenase, and OMP decarboxylase) or after centrifugation to remove cell debris (for other analyses).

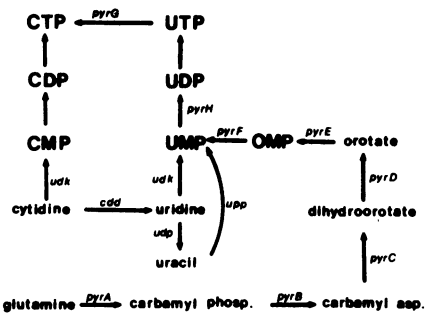

FIG. 1. *Pathways for the biosynthesis of pyrimidine nucleoside triphosphates in S. typhimurium. The enzymes are identified by their corresponding gene designations as follows: cdd, cytidine deaminase (EC 3.5.4.5); pyrA, carbamylphosphate synthetase (EC 2.7.2.5); pyrB, aspartate transcarbamylase (EC 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.-); udk, uridine kinase (EC 2.7.1.48), udp, uridine phosphorylase (EC 2.4.2.3); and upp, uracil phosphoribosyltransferase (EC 2.4.2.9).*

Enzyme assays. All assays were carried out at 37°C. One unit is defined as the amount of enzyme that utilizes 1 nmol or produces 1 nmol of product per min. Specific activities are given as units per milligram of protein.

Aspartate transcarbamylase was assayed as described by Gerhard and Pardee (4) except that the assays were performed at 37°C. Orotate phosphoribosyltransferase, OMP decarboxylase, dihydroorotase, and dihydroorotate dehydrogenase were assayed as described by Schwartz and Neuhard (21). IMP dehydrogenase was assayed as described by Magasanik (17). XMP aminase was assayed (at 37°C) by the method of Magasanik (17), modified as follows. A total volume of 0.45 ml contained Tris-hydrochloride (pH 8.8) (0.17 M), magnesium chloride (0.014 M), ammonium sulfate (0.28 M), ATP (3.8 mM), and XMP (0.5 mM). The reaction was started by addition of 25 μl of bacterial extract, and absorbancy at 290 nm was continuously recorded. A decrease of 2.56 absorbancy units corresponds to a decrease of 1 mM in the XMP concentration. Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was assayed as described (14).

Protein concentrations. Protein concentrations were determined by the method of Lowry et al. (16), using bovine serum albumin as a standard.

Nucleoside triphosphate pools. To exponential cultures at an optical density of 0.2, carrier-free [^{32}P]orthophosphate was added to give a specific activity of about 33 Ci/mol. The cells were extracted at an optical density of about 0.8 by pipetting samples (0.5 ml) into 0.10 ml of 2 M formic acid. After standing at 0°C for 15 to 60 min, the extracts were centrifuged. The supernatants were used for chromatographic analysis either on the same day or after storage at −20°C overnight. For determination of guanosine tetraphosphate, samples (100 μl) were chromatographed unidimensionally on polyethyleneimine-impregnated cellulose thin-layer plates (PEI plates) as described by Cashel et al. (3). For nucleoside triphosphate analyses, samples (25 μl) were chromatographed two-dimensionally on PEI plates; the system of Jensen et al. (K. F. Jensen, U. Houlberg, and P. Nygaard, Anal. Biochem., in press) was used since this system gives a particularly good separation of CTP and ATP and also allows determination of phosphoribosylpyrophosphate pools. For analysis of monophosphates, samples

TABLE 1. *Strains used*

Strain no.	Genotype	Nutritional requirements	Source or reference
KP-1001	*S. typhimurium* LT-2	None	
KP-1065	*pyrA81 deoD201*	Uracil, arginine	(9)
KP-1193	*pyrC1502 cdd-9 cod-8 tpp-1 udp-11*	Uracil (at 42°C)	
KP-1235	*trp guaA pyrE125*	Uracil, guanine, tryptophan	C. Ginther
KP-1362	*metB406 guaB231*	Methionine, guanine	J. Neuhard
KP-1468	*pyrC1502 cdd-9 cod-8 deoD201 udp-11*	Uracil (at 42°C)	This work
KP-1469	*cdd-9 cod-8 deoD201 udp-11*	None	This work
KP-1470	*cdd-9 cod-8 deoD201 udp-11 guaB*	Guanine (leaky)	This work
KP-1471[a]	*trp guaA*	Guanine, tryptophan	This work
KP-1476	*cdd-9 cod-8 deoD201 udp-11 guaB*	Guanine (at 42°C)	This work

[a] From KP-1235 by transduction to pyrimidine prototrophy with P22 (L3) grown on KP-1001.

(10 μl) were chromatographed two-dimensionally as described (11). Determination of radioactivity in the spots of the chromatograms was performed as described (11).

Transductions. For transductions with the *Salmonella* phage P22, the integration-deficient derivative L3 was used. Preparation of lysates and transductions were performed as described by Beck and Ingraham (1).

Construction of KP-1469 (*cdd-9 cod-8 deoD201 udp-11*). KP-1193 (*pyrC1502 cdd-9 cod-8 tpp-1 udp-11*) was transduced to utilize thymidine as a carbon source with P22 phage grown on KP-1065 (*pyrA81 deoD201*). One transductant (KP-1468), which had simultaneously lost the ability to utilize deoxyadenosine as a carbon source, was subsequently transduced to pyrimidine prototrophy at 42°C with P22 grown on *S. typhimurium* LT-2.

Isolation of KP-1476 [*cod-9 cod-8 deoD201 udp-11 guaB*(Ts)]. KP-1469 was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (5) and phenotypically expressed in the presence of guanine, followed by penicillin counterselection in the presence of hypoxanthine at 42°C, and plated on minimal agar supplemented with guanine. Colonies that required guanine at 42°C but were prototrophic at 32°C were picked. KP-1476 could satisfy its purine requirement at 42°C by either guanine, guanosine, or xanthine. Enzymatic assays revealed that it has a defective IMP dehydrogenase and had highly derepressed levels of XMP aminase when grown with guanine limitation (see Table 5).

Chemicals. [^{32}P]orthophosphate was obtained from AEC (Risø, Denmark). Other chemicals were analytical or reagent grade.

RESULTS

Isolation of a leaky *guaB* mutant (KP-1470) by resistance to pyrimidine analogs. During a search for pyrimidine regulatory mutants, samples (10^8 cells) of a nonmutagenized culture of KP-1469 were plated on agar plates containing glucose (0.2%), thiamine (1 μg/ml), Casamino Acids (0.2%), and 5-fluorouracil and 5-fluorouridine (5 μg of each per ml). Plates were incubated for 3 days at 37°C. Resistant mutants were screened for their content of ribonucleoside triphosphates by using the unidimensional chromatographic system of Cashel et al. (3). One mutant, KP-1470, which grows slowly (generation time, 120 min), has a very low GTP pool. The GTP pool and the growth rate were normalized by the addition of guanine, guanosine, or xanthine to the growth medium. Addition of guanosine (guanine and xanthine were not tested) simultaneously resulted in loss of resistance to the pyrimidine analogs (Table 2). When grown in the absence of guanine compounds, KP-1470 contained about 10% of the normal level of IMP dehydrogenase and was highly derepressed (30- to 40-fold) for XMP aminase

TABLE 2. *Growth characteristics and levels of GMP biosynthetic enzymes in KP-1469 and KP-1470*

Strain	Guanosine (30 μg/ml)	Generation time (min)	Resistance to FU + FUR[a]	IMP dehydrogenase (*guaB*) (units/mg)	XMP aminase (*guaA*) (units/mg)
KP-1469	−	58	s	0.7	3
KP-1469	+	58	s	0.7	1-2
KP-1470	−	120	r	0.1	103
KP-1470	+	58	s	NM[b]	3

[a] Tested on agar plates supplemented with Casamino Acids (0.2%) and containing 5-fluorouracil (FU) and 5-fluorouridine (FUR) (5 μg/ml each) and ± guanosine. s, Sensitivity; r, resistance.

[b] NM, Not measurable.

synthesis. Addition of guanosine to the culture normalized the level of XMP aminase. Under these conditions IMP dehydrogenase of the mutant was undetectable (Table 2). Genes coding for IMP dehydrogenase (*guaB*) and XMP aminase (*guaA*) are closely linked on the *Salmonella* chromosome (20) and have been shown to form an operon in *Escherichia coli* (22).

Nucleotide pools in KP-1469 and KP-1470. Table 3 shows the pools of the nucleoside triphosphates IMP, 5-phosphoribosyl-α-1-pyrophosphate, and guanosine tetraphosphate when KP-1469 and the *guaB* mutant KP-1470 were grown with various additions to the growth medium. By comparing experiments 1 and 5 of Table 3, it appears that KP-1470 grown with no addition to the medium has (i) a low GTP pool (less than 20% of normal), (ii) very high pools of IMP and ATP, (iii) a significantly reduced CTP pool (about 60% of normal), and (iv) a slightly increased UTP pool. (This slight increase in UTP is significant since it has been observed in more than 10 independent experiments.) Guanosine tetraphosphate and phosphoribosylpyrophosphate appear relatively normal. By comparing experiments 2 and 6 of Table 3 it can be seen that all pools of KP-1470 were nearly completely brought to normal upon addition of guanosine to the growth medium.

Levels of pyrimidine biosynthetic enzymes in KP-1469 and KP-1470. Table 4 gives the content of five pyrimidine de novo enzymes in KP-1469 and KP-1470 under different growth conditions.

In KP-1469, exogenous cytidine represses synthesis of dihydroorotase and dihydroorotate dehydrogenase, but has little effect on the synthesis of aspartate transcarbamylase and orotate phosphoribosyltransferase (Table 4, experiments 1 and 3). Exogenous uridine represses the

TABLE 3. *Pools of ribonucleotides in KP-1469 and KP-1470*

Strain	Expt.	Addition to growth medium (μg/ml)	Pools[a]						
			GTP	ATP	CTP	UTP	PRPP	IMP	ppGpp
KP-1469	1	None	2.35	4.85	1.07	1.58	1.11	<2	0.14
	2	Guanosine (30)	2.48	4.93	1.02	1.38	ND	<2	ND
	3	Cytidine (20)	2.08	4.42	1.65	1.36	ND	ND	ND
	4	Uridine (20)	2.00	4.11	0.90	1.74	ND	ND	ND
KP-1470	5	None	0.49	12.03	0.58	1.67	1.09	>10	0.13
	6	Guanosine (30)	2.23	7.92	1.03	1.52	ND	<2	0.10
	7	Cytidine (20)	0.48	13.22	1.09	1.87	ND	ND	ND
	8	Uridine (20)	0.49	11.83	0.62	1.80	ND	ND	ND

[a] Pools are given as micromoles per gram (dry weight) of bacteria. The determination of nucleoside triphosphates in experiments 1 and 5 has been repeated more than 10 times with similar results. PRPP, 5-Phosphoribosyl-α-1-pyrophosphate; ppGpp, guanine tetraphosphate. IMP migrates close to phosphate in the monophosphate chromatograms. In the mutant with no addition an IMP spot was clearly visible in the autoradiogram, whereas no distinct IMP spot could be seen in the other cases. The value of line 5 was obtained by subtraction of the radioactivity present in IMP of line 8. ND, Not determined.

TABLE 4. *Relative specific activities of pyrimidine biosynthetic enzymes in KP-1469 and the guaB mutant KP-1470*

Strain	Expt.	Addition to growth medium (μg/ml)	Sp act[a]				
			ATCase (*pyrB*)	OMPppase (*pyrE*)	OMPdecase (*pyrF*)	DHOase (*pyrC*)	DHOdehase (*pyrD*)
KP-1469	1	None	1.00 (20)	1.00 (30)	1.00 (23)	1.00 (52)	1.00 (28)
	2	Guanosine (30)	1.23	0.97	0.77	1.07	1.07
	3	Cytidine (20)	0.76	0.99	ND	0.46	0.42
	4	Uridine (20)	0.39	0.24	ND	0.66	0.58
KP-1470	5	None	4.63 [2.9–5.3]	11.00 [7.8–11.0]	2.61	0.12 [0.08–0.12]	0.23 [0.2–0.3]
	6	Guanosine (30)	1.15	1.15	1.15	0.81	0.98
	7	Cytidine (20)	5.59	10.82	ND	0.09	0.20
	8	Uridine (20)	4.10	8.72	ND	0.10	0.16
KP-1470	9	Guanine (15)	1.0	1.4	ND	1.1	1.0
	10	Guanine (15) + Cytidine (20)	0.9	0.8	ND	0.3	0.5
	11	Guanine (15) + Uridine (20)	0.4	0.2	ND	0.5	0.6

[a] ATCase, Aspartate transcarbamylase; OMPppase, orotate phosphoribosyltransferase; OMPdecase, OMP decarboxylase; DHOase, dihydroorotase; and DHOdehase, dihydroorotate dehydrogenase. The numbers given are means of two determinations in the same extract. Numbers in parentheses give the absolute specific activity in units per milligram of protein. The numbers in brackets give the outer limits observed in more than 10 independent experiments of KP-1470 relative to KP-1469 in unsupplemented medium. ND, Not determined.

synthesis of the two latter enzymes, but has less effect than cytidine on the synthesis of the first two enzymes (Table 4, experiments 1 and 4). This agrees with the observations of Schwartz and Neuhard (21). Guanosine has only minor effects upon the synthesis of the pyrimidine de novo enzymes in KP-1469 (Table 4, experiments 1 and 2).

In unsupplemented medium (Table 4, experiment 5) KP-1470 has elevated levels of aspartate transcarbamylase, orotate phosphoribosyltransferase, and OMP decarboxylase, and has depressed levels of dihydroorotase and dihydroorotate dehydrogenase, relative to the parent strain (KP-1469). However, all pyrimidine de novo enzymes of KP-1470 are normalized when guanosine or guanine is added to the growth medium (Table 4, compare experiments 2, 6, and 9). Exogenous cytidine and uridine have only marginal repressive effects on the synthesis of the pyrimidine de novo enzymes in KP-1470 grown in the absence of guanine compounds (Table 4, experiments 5, 7, and 8). On the other hand, when KP-1470 is grown in the presence of guanine, cytidine and uridine show normal repressive effects (Table 4, experiments 9, 10, and 11).

The low growth rate of KP-1470 is not

the cause of the disturbed levels of the *pyr* enzymes per se. The possibility existed that the low growth rate (generation time, 120 min) of the *guaB* mutant (KP-1470 was causing the disturbed levels of the pyrimidine de novo enzymes per se. This was ruled out by growing the parent strain (KP-1469) with glucose (0.2%) as a carbon source in the presence or absence of α-methylglucoside (2.5%) (7). In the absence of the glucoside the generation time of KP-1469 was 58 min; in the presence of glucoside the generation time was 156 min. Levels of aspartate transcarbamylase and dihydroorotase were identical in the two conditions.

The phenotype of KP-1470 is caused by the *guaB* mutation. P22 phage were grown on KP-1470, and the progeny phage were used to transduce a tight *guaA* mutant (KP-1471) to grow with xanthine as a guanine source. When the colonies that appeared on the xanthine-containing plates were tested for growth on plates with no purine source, 14% (12 colonies) grew rapidly and 86% (73 colonies) grew slowly, as with KP-1470. This is in good agreement with a reported 90% frequency of cotransduction between two point mutations in *guaA* and *guaB* (20). All slow-growing colonies were resistant to 5-fluorouracil plus 5-fluorouridine, whereas all rapidly growing colonies were sensitive to the analogs. Three of each class of transductants were further characterized: the slowly growing colonies had a generation time in liquid culture of 120 min at 37°C, and aspartate transcarbamylase and dihydroorotase were present in similar specific activities as in KP-1470. On the other hand, the rapidly growing cultures were normal in all respects (data not shown).

The P22 phage grown on KP-1470 was used to transduce a tight *guaB* mutant (KP-1362) to grow on plates containing no purine. About 95% of the transductants grew slowly; they were all found to be resistant to fluorouracil plus fluorouridine. Three of these transductants were analyzed more carefully. They were found to be identical with KP-1470 with regard to growth rate, pools of nucleoside triphosphates, and levels of aspartate transcarbamylase and dihydroorotase. A small number (about 5%) of the transductants grew rapidly. They were all sensitive to fluorouracil plus fluorouridine. Three of them were characterized with regard to growth rate, pools of nucleoside triphosphates, and content of the two pyrimidine enzymes mentioned above, and were found to be like wild-type *S. typhimurium* (data not shown). I believe that these latter transductants arise from recombinations within the *guaB* gene.

Another leaky *guaB* mutant behaves like KP-1470. To test whether the abnormal pyrimidine metabolism of KP-1470 is due to the particular nature of the *guaB* mutation harbored in KP-1470, I have isolated another leaky *guaB* mutant (KP-1476). This mutant was isolated by virtue of a temperature-sensitive guanine requirement which could be satisfied by xanthine. Table 5 gives the ratio of the pyrimidine biosynthetic enzymes in this mutant (KP-1476) when grown in the absence and presence of guanine. As seen from the table, the levels of the pyrimidine biosynthetic enzymes are disturbed as in KP 1470, and this effect becomes more severe the higher the temperature, i.e., the stronger the guanine limitation. Table 5 also gives the ATP/GTP and CTP/UTP ratios of KP-1476 grown in the absence of guanine. These ratios are similar to those observed in KP-1470.

DISCUSSION

KP-1470 was isolated as a mutant resistant to a combination of the pyrimidine analogs 5-fluorouracil and 5-fluorouridine.

Three lines of evidence suggest that KP-1470 contains a leaky mutation in *guaB* (coding for IMP dehydrogenase) (see Fig. 2) and that this mutation is responsible for the phenotype of the mutant.

Physiological and biochemical analysis of KP-1470. (i) Addition of guanosine, guanine, or xanthine normalizes the growth rate of KP-1470 and simultaneously results in loss of resistance to the pyrimidine analogs. Hypoxanthine has no effect. (ii) Purine nucleotide pools (low GTP and high ATP and IMP) of KP-1470 resemble those found when tight *guaB* mutants are starved for guanine (unpublished data). (iii) XMP aminase activity is strongly elevated and becomes normal upon addition of guanine, whereas IMP dehydrogenase activity is low in KP-1470 grown in the absence of guanine compounds and becomes unmeasurable upon addition of guanine compounds. In fact, the low IMP dehydrogenase activity is the only abnormality of KP-1470 that does not become normal upon addition of guanine.

Genetic evidence. The mutation responsible for the resistance of KP-1470 to pyrimidine analogs and for the unusual enzyme levels follows the *guaB* gene upon transduction into recipients harboring tight *guaB* and *guaA* mutations.

Behavior of other *guaB* mutants. A leaky *guaB* mutant (KP-1476) that was isolated by virtue of a temperature-sensitive guanine requirement shows a phenotype similar to that of KP-1470 (Table 5).

It appears plausible that a mutant with a low GTP pool might be resistant to 5-fluorouracil and 5-fluorouridine, since (i) uridine kinase

TABLE 5. *Analysis of pyrimidine metabolism in KP-1469 and a temperature-sensitive guaB mutant (KP-1476)*[a]

Strain	Temp (°C)	Generation time (min)[b] (−guanine)	Sp act[c]					Pool ratios (−guanine)	
			ATCase (*pyrB*)	OMPppase (*pyrE*)	DHOase (*pyrC*)	DHOdenase (*pyrD*)	XMP aminase (*guaA*)	ATP/GTP	CTP/UTP
KP-1469	39	58	0.9	0.9	1.02	1.04	2	2.1	0.75
KP-1476	39	97	2.5	7.8	0.27	0.33	29	24	0.42
	36	72	1.2	3.5	0.75	0.74	10	ND[d]	ND

[a] The cells were grown at the indicated temperature in the absence or presence of guanine. The specific activities of pyrimidine de novo enzymes and XMP aminase were determined in each culture. The table gives the specific activity in the culture (− guanine) divided by the specific activity of the same enzyme in the culture (+ guanine). A portion of the cultures grown in the absence of guanine was withdrawn, labeled with [^{32}P]orthophosphate, and used for determination of nucleoside triphosphates.

[b] The generation time in the absence of guanine is given; in the presence of guanine the strains grow equally fast.

[c] See footnote to Table 4 for abbreviations of enzyme names.

[d] ND, Not determined.

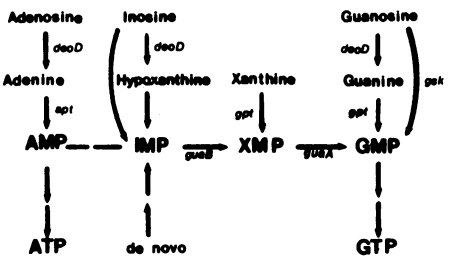

FIG. 2. *Relevant steps in the synthesis of guanine nucleotides in S. typhimurium. The enzymes are identified by the corresponding gene designations as follows: apt, adenine phosphosyltransferase (EC 2.4.2.7); gpt, guanine phosphoribosyltransferase (EC 2.4.2.8); gsk, guanosine kinase; guaA, XMP aminase (EC 6.3.5.2); guaB, IMP dehydrogenase (EC 1.2.1.14); and deoD, purine nucleoside phosphorylase (EC 2.4.2.1).*

(from *E. coli*) utilizes GTP much better than ATP as phosphate donor (23); and (ii) uracil phosphoribosyltransferase needs GTP as a positive allosteric effector both in *E. coli* (18) and also in *S. typhimurium* (J. Neuhard, personal communication). The low CTP pool of KP-1470 and the slightly increased UTP pool are probably also the direct result of the low GTP pool, since CTP synthetase (see Fig. 1) requires GTP for activity (15).

Schwartz and Neuhard (21) have proposed a division of the *pyr* genes into two groups on a regulatory basis. Synthesis of aspartate transcarbamylase (*pyrB*), orotate phosphoribosyltransferase (*pyrE*), and OMP decarboxylase (*pyrF*) was found to be repressed by a uridine compound (21), whereas synthesis of dihydroorotase (*pyrC*) and dihydroorotate dehydrogenase (*pyrD*) was found to be repressed (primarily) by a cytidine compound (21). However, the levels of the enzymes seem not to correlate well with the pool of any individual pyrimidine nucleotide (13). The results presented in Table 4 support the division proposed by Schwartz and Neuhard (21), as follows. (i) KP-1469 and KP-1470 (grown in the presence of guanine) respond to addition of pyrimidine nucleosides as described (21; see Table 4, experiments 1, 3, 4, 9, 10, and 11). (ii) The unusual distribution of levels of pyrimidine de novo enzymes in KP-1470 grown in the absence of guanine shows the same grouping, since aspartate transcarbamylase, orotate phosphoribosyltransferase, and OMP decarboxylase are elevated while dihydroorotase and dihydroorotate dehydrogenase are depressed (Table 4, experiments 1, 5, 7, and 8).

However, from nucleotide pool studies on KP-1470 grown in the absence of guanine it was expected that levels of the three uridine-regulated enzymes would be slightly depressed because of the slightly elevated UTP pool and that levels of the two cytidine-regulated enzymes would be elevated due to the low CTP pool. The opposite was observed.

Addition of exogenous uridine did not cause significant repression of aspartate transcarbamylase and orotate phosphoribosyltransferase in KP-1470 grown in the absence of guanine (Table 4, experiments 5 and 8); neither did the low levels of dihydroorotase and dihydroorotate dehydrogenase decrease appreciably upon addition of cytidine (Table 4, experiments 5 and 7). However, the pyrimidine nucleotides swelled to the same extent in KP-1470 as they do in KP-1469 (Table 3). Addition of guanine compounds, which restore the GTP pool (Table 3, experiments 5, 6, and 9), did repress synthesis of the two elevated enzymes (Table 4, experiments 5,

6, and 9). Furthermore, the enzymes responded normally to pyrimidine nucleosides in KP-1470 when the mutant was grown in the presence of guanine (Table 4, experiments 9, 10, and 11). Thus it appears that normal purine nucleotide pools are essential for the pyrimidine compounds to exert their normal repressive effects. This leads to the conclusion that a purine nucleotide is involved in the regulation of expression of the *pyr* genes in *S. typhimurium*.

Schwartz and Neuhard (21) studied the effects of exogenously added cytidine and uracil on the levels of pyrimidine de novo enzymes and nucleoside triphosphate pools in various mutants of *S. typhimurium* that harbored mutations affecting the interconversion of pyrimidine nucleotides. On the basis of this study the authors tended to emphasize UTP (or UDP) as the repressing metabolite for the synthesis of the *pyrB*, *pyrE*, and *pyrF* gene products and CTP (or CDP) as the repressing metabolite for the synthesis of the *pyrC* and *pyrD* gene products (21). On the other hand, based on similar experiments, Kelln et al. (13) tended to rule out CTP and UTP as repressing metabolites. The main reason for this conclusion was the observation that the strain KD-1104 (*pyrH11 cdd-4*) contains about twice as much dihydroorotase and dihydroorotate dehydrogenase as strain KD-1109 (*cdd-4*), even though KD-1104 had a slightly higher CTP pool (112%) than KD-1109. However, KD-1104 also contains about twice as much GTP as KD-1109. This is in agreement with the hypothesis that both pyrimidine and purine nucleotides are involved in the regulation of expression of the *pyr* genes. Thus the data of Kelln et al. (13) may well be explained on the basis of pyrimidine nucleoside triphosphates being the corepressors, if it is assumed that the purine nucleotides also are involved in the regulation. It could be suggested that the CTP/GTP ratio determines the repression of synthesis of *pyrC* and *pyrD* gene products. Thus in the study of Kelln et al. (13) this ratio (CTP/GTP) was 0.40 in KD-1104, whereas it was 0.55 in KD-1109. In the experiments reported herein the CTP/GTP ratio was 0.46 in KP-1469 and 1.18 in KP-1470 grown in the absence of guanine (Table 3). In this latter strain the synthesis of dihydroorotase and dihydroorotate dehydrogenase was strongly repressed in spite of a reduced (to 60%) CTP pool (Tables 3 and 4). Similarly, the UTP/ATP ratio may determine the degree of repression of *pyrB*, *pyrE*, and *pyrF*. This will explain why KP-1470 grown in the absence of guanine contains derepressed levels of aspartate transcarbamylase, orotate phosphoribosyltransferase, and OMP decarboxylase in spite of a slightly increased UTP pool. In this situation ATP is strongly increased (Tables 3 and 4).

The hypothesis may also explain the observation of Ginther and Ingraham (5) that a cold-sensitive mutant of *S. typhimurium* with a defective nucleoside diphosphokinase (*ndk*, EC 2.4.7.6) at the nonpermissive temperature contains slightly repressed levels of aspartate transcarbamylase and dihydroorotase (other enzymes were not measured), even though the pyrimidine nucleoside triphosphates did decrease without a concomitant increase in the diphosphates. In this experiment the purine nucleoside triphosphates also decreased and the pyrimidine/purine ratios remained relatively constant (5).

Although in the preceding paragraph the CTP/GTP and UTP/ATP ratios have been proposed as factors determining the expression of the *pyr* genes in *S. typhimurium*, it is (at present) not possible to conclude what purine nucleotides are involved in the regulation. Also, it is possible that the purine effect observed may not be (exclusively) specific for pyrimidine de novo enzymes. However, the effect is not nonspecific, since (i) the de novo enzymes responded in opposite directions to guanine limitation and (ii) glucose-6-phosphate dehydrogenase did not respond at all (data not given).

ACKNOWLEDGMENTS

I thank Lise Schack for excellent technical assistance, and B. Jochimsen and J. Neuhard for helpful discussions and for bacteriological advice. Thanks are also due to J. Neuhard for reading of the manuscript.

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