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Escherichia coli Mutants Deficient in Guanine-Xanthine Phosphoribosyltransferase

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We studied the purine phosphoribosyltransferases (PRTases) of *Escherichia coli* and were able to isolate a mutant that is defective in its ability to convert guanine and xanthine to their respective ribonucleotides. The affected gene (*gpt*) lies between *metD* and *proA* and is 78.6% co-transducible with *proA*. Both this point mutant and a strain with a *pro-lac* deletion contain less than 2% of wild-type xanthine PRTase activity, yet still contain about 30% of wild-type guanine PRTase activity. Thus, the *gpt* gene is only one of at least two genes responsible for guanine PRTase activity in *E. coli*.

Purine phoshoribosyltransferases (PRTases) catalyze the synthesis of purine ribonucleotides from purine bases and phosphoribosyl pyrophosphate. In mammalian systems there are two distinct purine PRTases. One of these is specific for adenine, and the other is specific for hypoxanthine and guanine (2, 15, 26, 29). In some microorganisms there appear to be three distinct purine PRTases. Thus, *Streptococcus* (4) and *Lactobacillus* (19) possess an adenine PRTase, a hypoxanthine PRTase, and a xanthine PRTase. In the enteric bacteria, the problem as to the number of different purine PRTases is more complicated. Although it has been known that these organisms have a distinct adenine PRTase (11, 18), the relationship between their hypoxanthine, guanine, and xanthine PRTase activities is only now being realized.

The finding that certain *pro* deletion mutants of *Salmonella typhimurium* were also deleted for a gene responsible for guanine and xanthine PRTase activity suggested that the guanine and xanthine activities reside in a single enzyme (7). Since extracts of these mutants still retain normal hypoxanthine PRTase activity, it appeared that the guanine-xanthine enzyme is distinct from the hypoxanthine enzyme. However, the hypoxanthine enzyme also appears to have some activity with guanine, and thus, in effect, *Salmonella* contains two PRTases that are capable of using guanine as a substrate (3, 6, 8). Evidence has also been presented which suggests that *Escherichia coli* may also contain two guanine PRTases (23).

In this report we describe the properties of PRTase mutants of *E. coli* and demonstrate that the data which has been presented for *Salmonella* is also applicable to *E. coli*.

MATERIALS AND METHODS

Isolation of strain JW2. A stock of P1 virus that could transduce the *gpt* and *proA* genes at high frequency was isolated by following the procedure that Rae and Stodolsky used to obtain high frequencies of transduction for the *proA* gene (27). P1*kc* were grown on strain W3110 and used to transduce the *gpt-pro-lac* deletion strain 165 to the ability to produce colonies on minimal plates containing guanine. One transductant that produced P1 phage spontaneously was infected with a multiplicity of 10 P1*vir* phage per cell and incubated to lysis. This lysate transduced strain χ761 to *proA*$^+$ with a frequency of 10^{-2} per plaque-forming unit. The lysate was treated with hydroxylamine as previously described (14). χ761 was transduced at a multiplicity of 0.2 mutagenized P1 phage per cell, diluted 100-fold into minimal medium lacking proline and containing adenine, and grown overnight at 37 C. The cells were then centrifuged and suspended in an equal volume of 0.01 M $MgSO_4$. After dilution to 10^6 cells per ml in minimal medium containing guanine and 200 U of sodium penicillin G per ml (Eli Lilly & Co.), the cells were incubated for 6 h at 37 C. Samples of 0.1 ml were then spread on minimal plates lacking proline and containing adenine. Colonies growing on these plates were then tested for their ability to grow on guanine by transferring them to guanine-containing plates with sterile toothpicks. Out of 600 *proA*$^+$ transductants tested, six grew slowly on guanine; one was designated JW2 and was studied further.

P1 virus techniques. The methods for growing stocks of P1 virus and carrying out transductions are

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described by Wall and Harriman (30). The origins of the P1 strains are also given there.

Chemicals. [^{14}C]adenine, [^{14}C]hypoxanthine, and [^{14}C]guanine were purchased from New England Nuclear Corp. [^{14}C]xanthine was purchased from Schwarz/Mann. 5-Phosphoribose-1-pyrophosphate tetrasodium salt was obtained from Sigma Chemical Co. Its purity was determined by the procedure described by Hochstadt-Ozer and Stadtman (11). Unlabeled inosine, guanosine, and adenosine were purchased from P-L Biochemicals. All other unlabeled purines, purine nucleosides and purine ribonucleotides were purchased from Sigma Chemical Co. Epichlorohydrin triethanolamine (ECTEOLA) cellulose powder with an exchange capacity of 0.5 meq/g was obtained as Cellex-E from Bio-Rad Laboratories (control no. 12166).

Media. The compositions of the P1 broth, plating media, and diluent used are same as those described by Harriman (9). The compositions of the minimal plates and top agar (Vogel and Bonner minimal E) are described by Caro and Berg (5). VBC medium was used for testing growth on purines. This medium consists of medium E supplemented with vitamin-free Casamino Acids (Difco) at 0.2% and the described purine at 0.4 mM.

Bacterial strains. The origins and properties of the bacterial strains used are given in Table 1.

Preparation of crude extracts. Bacteria were grown in 200 ml of VBC medium supplemented with adenine, hypoxanthine, guanine, xanthine, and thymine each at a concentration of 0.4 mM, and arginine at a concentration of 50 μg per ml. When the cells reached stationary phase (2×10^9 to 4×10^9 cells/ml), they were harvested by centrifugation, washed once, and suspended in 2 ml of 0.03 M sodium phosphate buffer, pH 7.4. The cell suspension was treated for 90 s with periodic cooling intervals using a Branson W 185 sonifier and then centrifuged at 29,000 $\times$ *g* for 1 h. The supernatant fluid was dialyzed overnight against 0.03 M sodium phosphate buffer (pH 7.4) at 4 C. All enzymatic assays were performed on this dialyzed fraction, referred to as "crude extract."

Enzyme assays. Purine PRTase activities were assayed in a final volume of 0.4 ml containing: 50 mM tris(hydroxymethyl)aminomethane (Tris) (pH 8.0), 1 mM 5-phosphoribose-1-pyrophosphate, 5 mM $MgSO_4$, and 0.25 mM ^{14}C-labeled purine base (1 μCi/μmol). The reaction was started by the addition of enzyme. After incubation for 5 min at 37 C, the reaction was stopped by the addition of 0.1 ml of 0.4 M sodium ethylenediaminetetraacetic acid (EDTA), pH 8.0. EDTA was added prior to enzyme in control reactions. A 10-μl sample of the reaction mixture was applied to a cellulose thin-layer chromatogram (Eastman no. 6065 cellulose with fluorescent indicator) previously spotted with 6 μg each of purine ribonucleotide, ribonucleoside, and base. The chromatogram was developed in 1 M ammonium acetate (11) until the solvent front had migrated about 8 cm from the origin for guanine and xanthine PRTase assays and 11 cm from the origin for adenine and hypoxanthine PRTase assays (this took 15 to 30 min). After drying, areas of the chromatogram containing the purine ribonucleotide and ribonucleoside were located under ultraviolet light, cut out, immersed in scintillation fluid, and counted in a scintillation counter. Because of the phosphatase activity present in crude *E. coli* extracts, the radioactivity appearing as ribonucleoside (2 to 8%) was counted along with that appearing as ribonucleotide. This sum was compared with the radioactivity of an unchromatographed sample counted under similar conditions, to determine the amount of product formed. Specific activity is expressed as the number of nanomoles of product formed per minute per milligram of protein. Protein concentrations of crude extracts were determined by the Lowry method using bovine serum albumin as the standard (22). Different assays of the same extract varied by approximately 5%. All specific activities presented

TABLE 1. *Bacterial strains*

Strain designation	Genotype	Source
χ761	F$^-$ *argA, his, ile, metA or B, purE, thi, trp, ara, gal, lacZ, mtl, xyl, tonA, tsx, strA gpt+, proA, leu,* T5	Derived from W945 (R. Curtiss III)
JW2	*pro*$^+$, *gpt*$^-$ derivative of χ761	(As described in this paper)
W3110	prototroph	S. Luria
TJ13	F$^-$ *mal,*λ^r, *xyl, argI, metB, his, proA, metD1, lac* (del_{172}) *strA, gpt, purE* (*purE*$^-$ from 761 by P1 transduction)	W. Epstein and this laboratory
165	F$^-$ (*proA, B, lac*)Δ111, *str, purE* (Soll's E50141 transduced to *purE* using χ761 as donor)	Larry Soll and this laboratory
χ463	F$^-$ *ara, leu, azi, tonA, proA, lacZ,* T6^r, *purE, trp, lysA, str, yxl, metE, thi*	Derived from W945 (R. Curtiss III)
RE26	F$^-$ *proA, trp, his, lac, tsx*	E. C. R. Reeve

in this paper represent the averages of at least two independently grown and assayed cultures of the given genotype.

ECTEOLA cellulose chromatography. All procedures were carried out at 4 C. For column chromatography, cell extracts were dialyzed overnight against 9 mM Tris-hydrochloride, 0.2 mM $MgSO_4$ (pH 8.2). The dialyzed extract was then made 1% with streptomycin sulfate (Sigma), stirred for 40 min, and centrifuged at 49,000 × *g* for 10 min (25). A sample of the resulting supernatant containing a total of 20 to 40 mg of protein was applied to a column (0.8 by 8.0 cm) that had been equilibrated with Tris-$MgSO_4$ buffer. PRTase activities were eluted with a 0 to 0.125 M KCl gradient in Tris-$MgSO_4$ buffer. The flow rate was 12 ml/h, and 1.5-ml fractions were collected.

RESULTS

Isolation of a *gpt* mutant. In the enteric bacteria, purine PRTase are periplasmic proteins and are responsible for uptake of exogenous purines (12). In a purine auxotroph, the absence of a particular PRTase activity is manifested in the inability of such a cell to use the respective purine for growth. Thus, purine-requiring *Salmonella gpt* deletion mutants were found to be unable to grow using guanine or xanthine as a purine source, although they grow using hypoxanthine or adenine. When an *E. coli pro-lac* episome was introduced into these *Salmonella gpt* mutants, growth on guanine was restored. It appeared that the *E. coli* gene of guanine-xanthine PRTase resides on the episome and, by analogy with the mapping data for the *Salmonella* gene, would be closely linked to *pro*. This suggested to us that *E. coli* point mutants for guanine-xanthine PRTase could be readily obtained using localized mutagenesis (13) complemented with penicillin enrichment treatment.

Briefly, the procedure can be described as shown in Fig. 1. The *gpt* gene seemed to be closely linked to the *pro* region in both *E. coli* and *S. typhimurium* (7). Therefore P1 phage were grown on a $proA^+$ gpt^+ donor. The lysate was mutagenized with hydroxylamine and used to transduce a $proA^-gpt^+purE^-$ recipient to $proA^+$. Penicillin enrichment was then used to select for $proA^+$ transductants unable to grow well using guanine as a purine source. This procedure selected for $proA^+$ transductants, which were also co-transduced for a *gpt* gene mutated as a result of the hydroxylamine mutagenesis. We were able to isolate an *E. coli* guanine-xanthine PRTase mutant (designated JW2) from the pro^+ survivors of the penicillin treatment. We designate the mutant gene *gpt* using the same terminology as that used for the corresponding gene previously isolated in *Salmonella* (8).

Growth response to purines. Our *gpt* mutant was isolated because of its inability to grow well on guanine. However, further studies with this mutant indicated that it is capable of growth on guanine, though at a much slower rate than the wild type. It will not grow at all on xanthine (Table 2). We originally attempted to isolate *gpt* mutants by using penicillin to select against cells that could grow using guanine as an exogenous purine source. We were unsuccessful and resorted to localized muta-

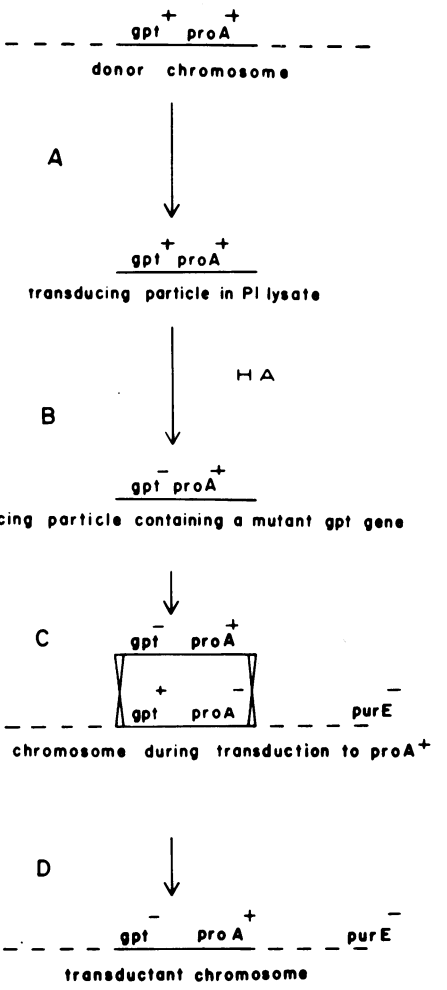

FIG. 1. *Isolation of a gpt mutant by localized mutagenesis. (A) A P1 lysate is prepared from a wild-type strain of E. coli. It contains $proA^+gpt^+$ transducing particles, providing that the two genes are co-transducible. (B) Some of the $proA^+gpt^+$ transducing particles will acquire a mutation in the gpt gene as a result of hydroxylamine mutagenesis of the transducing lysate. (C) The mutagenized phage are used to transduce a $proA^-gpt^+$ recipient. (D) The mutant gpt gene can be found among $proA^+$ transductants.*

genesis techniques. It is probable that slow growth on guanine killed many of the *gpt* mutants in the presence of penicillin, and selection against cells able to grow on xanthine should be a more successful technique for isolating further mutants.

Purine PRTase activities. Enzyme assays performed on extracts of our *gpt* mutant and its parent showed that, although the *gpt* mutant is greatly deficient in xanthine PRTase activity, it still retains about 30% guanine PRTase activity (Table 3). This remaining guanine PRTase activity is unrelated to the *gpt* enzyme as shown by the following experiment. The *gpt* gene is closely linked to *proA* and is therefore deleted in many strains carrying deletions of the *proA/B* region. We investigated enzyme levels in cells containing the *proA-lac* deletion Δ111 and found that this strain has a phenotype similar to that of the *gpt* point mutant. We presume that *gpt* is included in this deletion. These cells lacked almost all xanthine PRTase activity but still retained about 30% of wild-type guanine PRTase activity (Table 3). Thus, the *gpt* gene was responsible for 70% of the guanine PRTase activity measurable in crude extracts.

TABLE 2. *Division times (in minutes) of 761 pur⁻ and its gpt⁻ derivative on various purines*[a]

Strain	Purine		
	Hypoxanthine	Guanine	Xanthine
761 (*gpt*⁺)	34	58	77
JW2 (*gpt*⁻)	38	315	ND[b]

[a] Cells were grown overnight in broth and then diluted 1:50 into VBC minimal media with appropriate supplements (as described in Materials and Methods) minus purines. The appropriate purine was added to a final concentration of 0.4 mM. The cultures were incubated at 40 C and the absorbance was followed at 500 nm using a Coleman Junior II spectrophotometer.

[b] ND, Not detectable.

TABLE 3. *Purine phosphoribosyltransferase activities*[a]

Strain	Adenine	Hypoxanthine	Guanine	Xanthine
761(*gpt*⁺)	105.3	193.4	63.1	41.4
JW2(*gpt*⁻)	110.0	164.3	21.5	0.16
165(*gpt*⁻, Δ111 deletion)	97.5	176.6	18.2	0.80

[a] Activity is expressed as the number of nanomoles of product formed per minute per milligram of protein. For determination of xanthine PRTase activity in *gpt* mutants, reaction mixtures containing 0.1 to 0.2 mg of protein were incubated for 30 min.

A comparison of the PRTase activities for hypoxanthine, guanine, and xanthine (Table 3) with cell division times (Table 2) shows that PRTase levels in purine-requiring cells can limit the rate of cell division on exogenous purines.

Purine analogue resistance. Cells are sensitive to many purine analogues, but in order for the analogues to be effective they must first be converted to their respective nucleotides by purine PRTases. Cells resistant to the action of analogues are in many cases unable to carry out this conversion and show altered purine PRTases (1, 4, 17). Thus cells lacking functional purine PRTases are expected to be resistant to purine analogues. Our *gpt* mutant is resistant to the effects of azaguanine and thioguanine, as is shown in Fig. 2. It should be noted that the sensitivity and resistance is measured in cells capable of carrying out de novo purine synthesis. This property of the *gpt* mutant is useful in checking for the presence or absence of the PRTase activity in *pur*⁺ cells (7).

ECTEOLA cellulose separation. Hypoxanthine, guanine, and xanthine PRTase activities in wild-type *E. coli* can be separated into three

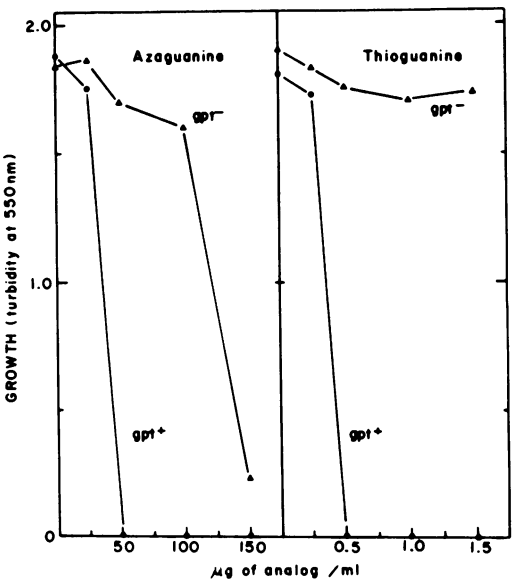

FIG. 2. *Growth of gpt⁺ and gpt⁻ bacteria in the presence of analogues. The cells used were pur⁺ transductants of 761 (gpt⁺) and JW2 (gpt⁻). They were grown overnight in VBC medium with appropriate supplements (and no purines). They were then diluted 10^4-fold for thioguanine resistance and 10^6-fold for azaguanine resistance, and 0.1 ml was added to 1.9 ml of minimal medium containing the indicated amount of analogue as the sole purine. After 18 to 20 h of growth at 37 C, the turbidity at 550 nm was determined in a Gilford spectrophotometer.*

distinct components on ECTEOLA cellulose columns. This procedure was first described for *E. coli* B (25). We have used this procedure to separate purine PRTase activities in *E. coli* K-12 and obtained the results shown in Fig. 3. Three distinct components were evident. Two of these components functioned predominately with guanine and xanthine as substrates. The other functioned mainly with hypoxanthine as a substrate, but did demonstrate significant activity towards guanine. When extracts of our *gpt* point mutant and our *gpt*$^-$, Δ111 deletion mutant were chromatographed in the same manner, the result shown in Fig. 4 was obtained. Both guanine-xanthine PRTase peaks were absent from the profiles. This suggested that the product of the *gpt* gene exists in two distinct molecular forms.

Location of the *gpt* gene. The finding that the *gpt* gene is carried by an *E. coli* F′ *pro-lac* episome and our ability to find a mutant *gpt* gene among *proA*$^+$ transductants indicated a linkage between *proA* and *gpt*. We quantitated this linkage by measuring co-transduction frequencies for the *gpt* and *proA* genes. Three different *proA* strains were used as either donors or recipients with JW2, and the results are presented in Table 4. A chi-square contingency test showed that there was no significant difference between the different co-transduction frequencies measured, and we therefore averaged the results from the five experiments. We find a co-transduction frequency of 78.6% for *proA* and *gpt*. This corresponds to a distance of 0.15 min on the Taylor and Trotter linkage map of *E. coli* (28). It was then necessary to determine the order of *gpt* and *proA* relative to the other genes of *E. coli* using three-factor crosses. The gene *metD*, co-transducible with *proA* and on the opposite side of *proA* from the *lac* operon (16), was used as an outside marker. The rationale for the experiment was as follows (Fig. 5). Selection was for cells that could grow on xanthine as a purine source (*gpt*$^+$) and did not require proline (*proA*$^+$). Cells that could grow under these conditions were tested for the presence of the *metD* marker of the donor strain. If the gene order is *metD gpt proA*, cross A would require a quadruple crossover event, whereas cross B would require only a double crossover event in order for recombinants to also receive the donor *metD* marker. Since quadruple crossover events are rarer than double crossover events, cross B should show a higher frequency of donor *metD* markers among the *gpt pro* recombinants than cross A. If, however, the order of genes is *metD proA gpt*, we would expect a higher frequency of donor *metD* markers among the recombinants of cross A than of cross B. The results were that, out of 452 recombinants tested in cross A, 12 (2.7%) carried the donor *metD*$^+$ marker. Out of 284 recombinants tested from cross B, 26 (9%) carried the donor *metD*$^-$ marker. Therefore, cross B produced a higher percentage of recombinants carrying the donor *metD* allele than cross A, and the gene order must be *metD gpt proA*.

DISCUSSION

Our experiments show that the *E. coli gpt* lies in the same relative region of the chromosome as the *Salmonella gpt* gene, which is near the *proA/B* region, on the threonine side (24). Hoppe and Roth (14) isolated specialized P22 transducing phages carrying genes from an *E. coli* F′ carried in a *Salmonella* strain. From the pattern of acquistion of *pro*, *gpt*, and *lac* trans-

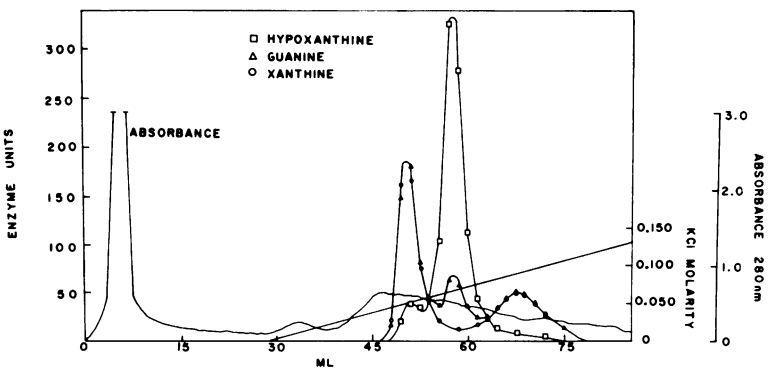

FIG. 3. *ECTEOLA cellulose chromatography of an extract from W3110 (gpt*$^+$*). An extract was applied to an ECTEOLA cellulose column and eluted with a linear KCl gradient. Individual fractions were then tested for hypoxanthine, guanine, and xanthine PRTase activities. PRTase activity is plotted as units per 1 ml for guanine and xanthine and units per 0.05 ml for hypoxanthine.*

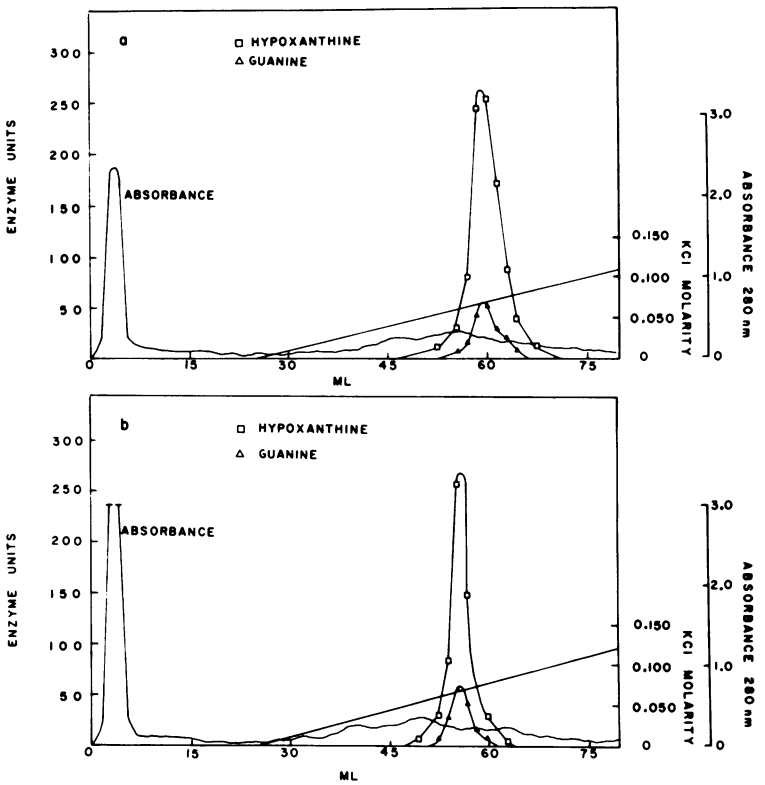

FIG. 4. *ECTEOLA cellulose chromatography of extracts from (a) the gpt⁻, Δ 111 deletion, strain 165, and (b) the gpt⁻ point mutant, strain JW2. Extracts were applied to ECTEOLA cellulose columns and eluted with linear KCl gradients. Individual fractions were then tested for hypoxanthine and guanine PRTase activities. PRTase activity is plotted as units per 1 ml for guanine and units per 0.5 ml for hypoxanthine.*

TABLE 4. *Linkage of gpt to proA*[a]

Donor	Recipient	Selected marker	Co-transduction (%)[b]
JW2	463	*pro*⁺	315/387 (81.4)
463	JW2	*gpt*⁺	85/107 (79.4)
JW2	761	*pro*⁺	206/264 (78.0)
761	JW2	*gpt*⁺	198/264 (75.0)
JW2	RE26	*pro*⁺	157/200 (78.5)

[a] Bacteriophage P1*vir* were grown on the donor strain and used to transduce the recipient strain for the indicated selected markers (*proA* or *gpt*) at a multiplicity of 0.2 plaque-forming units per cell. Colonies formed on selective plates were then tested for the presence of the other marker of the donor strain by transferring cells with sterile toothpicks to the appropriate selective plate.

[b] The numbers and percentages of cells transduced for both markers relative to the total number of tested transduced cells for the selected marker are given.

ducing phages they obtained, these workers suggested that the *gpt* gene was on the opposite side of the *proA/B* region from the *lac* genes. Our mapping of *E. coli* chromosomal genes confirms their suggestion. It has recently come to our attention that Livshitz (20) has isolated a mutant he calls *gpp* with growth properties similar to our *gpt* mutant. His *gpp* gene is 81% co-transduced with *proA* and lies on the opposite side of *proA* from the *lac* operon. It is very likely the same gene as the *gpt* gene, but we prefer the abbreviation *gpt* for the gene since it corresponds to current terminology in *Salmonella* (8). The gene product is currently named phosphoribosyltransferase, but at one time was called a pyrophosphorylase. Livshitz (21) has more recently isolated additional mutants with alterations in his *gpp* gene that seem to affect specifically the ability of cells to grow on xanthine.

Our studies with the *gpt* mutants have allowed us to reach some conclusions concerning the number of different enzymes involved in purine phosphoribosyltransfer in *E. coli*. The simplest interpretation of our results is that the *gpt* gene is the structural gene for a guanine-xanthine PRTase. Although this enzyme contains all the xanthine PRTase activity of the

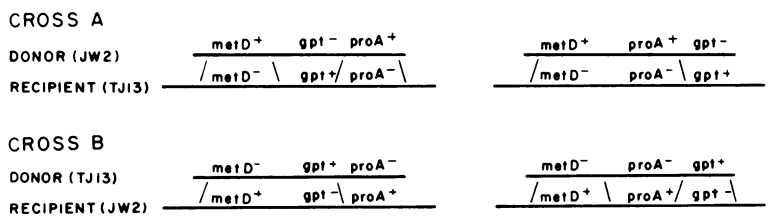

FIG. 5. *Schematic representation of crossover events necessary for gpt⁺, proA⁺ recombinants to acquire the metD allele of the donor strain.*

cells, it is only one of at least two PRTases capable of functioning with guanine as a substrate. This was shown when extracts of both our *gpt* point mutant and our *gpt⁻*, Δ111 deletion mutant still retained about 30% of wild-type guanine PRTase activity. Furthermore, both of these *gpt* mutants, which are purine auxotrophs, are able to grow slowly on guanine. It became apparent during ECTEOLA cellulose chromatography that this remaining guanine PRTase activity can most likely be attributed to the hypoxanthine enzyme. Both activities co-chromatographed. We believe that the hypoxanthine enzyme thus has some cross-specificity for guanine and that this is the origin of the guanine PRTase activity that we detect in our *gpt* mutants.

The *gpt* enzyme is probably also weakly active for hypoxanthine. This possibility was first suggested when we found that hypoxanthine PRTase levels seemed to be slightly lower in extracts from *gpt* mutants. We were also able to detect hypoxanthine PRTase activity accompanying the guanine-xanthine PRTase components that were resolved on ECTEOLA cellulose columns (Fig. 3). Furthermore, a partially purified guanine-xanthine PRTase from *E. coli* B was found to have activity toward hypoxanthine (23). In work to be published, we will show that mutants of the *E. coli* gene corresponding to the *Salmonella hpt* gene (6) still contain 6% of the original level of hypoxanthine PRTase activity, and this activity is due to the *gpt* gene.

Hochstadt-Ozer (10) has suggested that in *Salmonella* the cross-specificity of these enzymes is not present when the enzymes are associated with the membrane, but appears only when the enzymes are no longer membrane bound. However, we find that the growth response of our *E. coli* strains to exogenous purines parallels the enzyme levels measured in soluble extracts.

Our data support the concept of three distinct purine PRTases existing in the enteric bacteria. One of these functions only with adenine as a substrate, another can use hypoxanthine or guanine as substrates, whereas a third can use hypoxanthine, guanine, or xanthine as substrates.

In terms of the percentage of total activity measured in wild-type extracts, our results differ from those reported for the *Salmonella* enzymes (8). The *Salmonella* hypoxanthine PRTase is responsible for only 10% of the guanine PRTase activity in the cell, whereas the *E. coli* hypoxanthine enzyme is responsible for 30%. Also, the *Salmonella* guanine-xanthine enzyme contributes 25 to 35% of the total wild-type hypoxanthine PRTase activity, whereas the *E. coli* enzyme contributes about 6%. The data from extracts of both organisms show the remarkable cross-specificity of the 6-keto purine PRTases. In view of the finding that the substrate specificity of these enzymes can be altered by mutation (3), this may indicate a common genetic origin for these enzymes.

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