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Selection for Purine Regulatory Mutants in an *E. coli* Hypoxanthine Phosphoribosyl Transferase-Guanine Phosphoribosyl Transferase Double Mutant

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Summary. We have studied the relationship between purine salvage enzymes, 6-mercaptopurine resistance, and the *purR* phenotype in *E. coli*. Mutants resistant to 6-mercaptopurine were found to have defects in HPRT, the *purR* repressor, or in both. Analysis of these mutants led to the isolation of a hypoxanthine phosphoribosyl transferase-guanine phosphoribosyl transferase double mutant (*hpt*$^-$ *gpt*$^-$) that is extremely sensitive to adenine. Two classes of adenine resistant mutants were isolated from this strain. The first class was deficient in APRT (*apt*$^-$) while the second class represented purine regulatory mutants (*purR*$^-$). There is thus selection for the *purR* phenotype in a *hpt*$^-$ *gpt*$^-$ background.

Introduction

In *E. coli* de novo purine biosynthesis is regulated by feedback inhibition (Gots 1971) and repression (Benson and Gots 1976; Gots et al. 1976). Feedback inhibition is mediated by purine mononucleotides which act allosterically on the first enzyme in the pathway, PRPP amidotransferase (Gots 1971). Repression involves the control of transcription of various de novo purine biosynthetic enzymes by the *purR* gene product (Benson and Gots 1976; Gots et al. 1976), and its corepressor(s) ATP and GTP (Koduri and Gots 1979). The *purR* designation is used strictly to identify a common phenotype. Whether or not a number of genetically distinct repressor molecules exist is unknown, but a single *purR* mutation has been shown to affect the synthesis of at least four independent de novo enzymes (Benson and Gots 1976).

Investigations by Dorfman (1969) and Armitt and Woods (1970) in yeast indicated that adenylosuccinate synthetase might act as a repressor of the *de novo* pathway. Benson and Gots (1976) studied the relationship of adenylosuccinate synthetase (*purA*) and de novo regulation in *Salmonella*. They found that a large number of independent *purA* mutants were also deficient in their ability to repress the de novo enzymes in the presence of high concentrations of adenine. Further analysis of these double mutants, however, indicated that the *purR* and *purA* genes were unlinked. These authors suggest that the sensitivity of *purA* mutants to adenine inhibition under certain conditions might confer a selective advantage on *purR* mutants appearing in the population.

We have been studying the relationship between the salvage and de novo pathway (Fig. 1) in *E. coli* mutants deficient in various salvage or de novo enzymes. In this paper we describe the isolation and preliminary characterization of a *hpt*$^-$ *gpt*$^-$ double mutant and the strong selection for purine regulatory mutants in this strain in the presence of adenine.

Abbreviations: FGAR = formyl glycinamide ribotide. HPRT = hypoxanthine phosphoribosyl transferase. GPRT = guanine phosphoribosyl transferase. APRT = adenine phosphoribosyl transferase. PRPP = 5 phosphoribosyl – 1 pyrophosphate. 6MP = 6-mercaptopurine. FA = 2-fluoroadenine

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Materials and Methods

Bacterial Strains and Growth Media

The *Escherichia coli* strains used in this study are listed in Table 1. The minimal salts medium of Vogel and Bonner (VB) (1956) was supplemented with glucose (0.5% w/v). Amino acids were added as needed to a final concentration of 40 μg/ml. Purines were added as described in the text. Phosphoribosyl transferase assays were performed on cells grown in L-broth (1% bacto tryptone, 0.5% yeast extract, 0.5% Nacl, 0.1% glucose, 10 mM $MgSO_4$· pH 7.0). TL471 was always grown in a purine-free medium unless otherwise noted.

Mutagenesis and Genetic Methods

For mutagenesis N methyl-N′-nitro-N-nitrosoguanidine (NTG) treatments were employed according to the procedure of Miller (1972). Transduction with phage P1*vir* and all mating experiments were likewise performed as described by Miller (1972).

Chemicals

The tetra sodium salt of 5-phosphoribosyl-1 pyrophosphate (PRPP) and 6-mercaptopurine (6MP) were purchased from Sigma Chemical Company (St. Louis, Missouri). ^3H purines and ^{14}C formate were obtained from Amersham (Arlington Heights, Illinois). ^{14}C 6-mercaptopurine was purchased from Research Products International (Mount Prospect, Illinois). 2-fluoradenine (NSC27364) was kindly provided by the Drug Synthesis and Chemistry Branch Division of Cancer Treatment, NCI. All other chemicals were obtained from commercial sources.

Preparation of Extracts and Enzyme Assays

For in vitro FGAR determinations, 200 ml of culture was grown overnight in VB medium supplemented with glucose, amino acids, and thiamine HCl. When strains were to be assayed for the *purR* phenotype 200 μg/ml adenine was added to the medium. Cells were washed three times in 10 mM $MgSO_4$ and resuspended in 1–2 ml of fresh tris buffer (20 mM Tris HCl pH 7.6, 3 mM $MgCl_2$) containing 2-mercaptoethanol (2 mM). The cells were ruptured by sonic treatment consisting of 2–30 s bursts in a Heat Systems-Ultrasonics, INC Sonicator (Model W185F)

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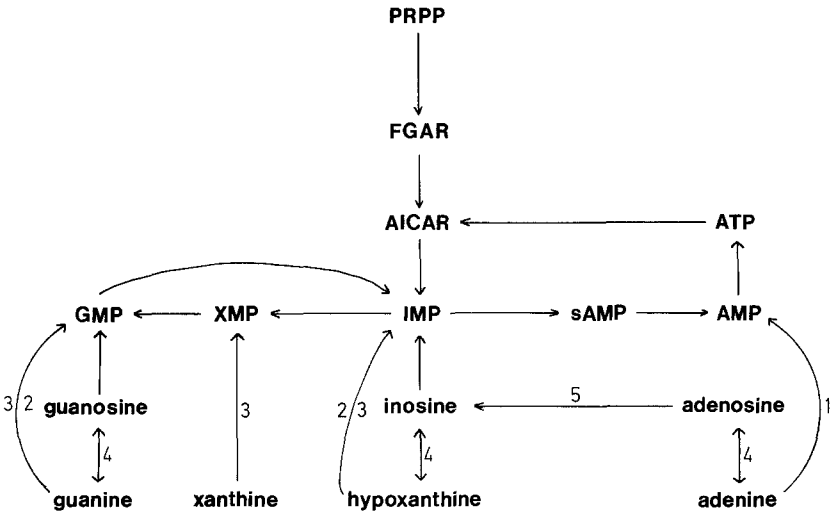

Fig. 1. Purine Salvage Pathways in *E. coli.* Preformed purine bases are utilized primarily via adenine phosphoribosyl transferase (1), hypoxanthine phosphoribosyl transferase (2), and guanine phosphoribosyl transferase (3). Purine nucleoside phosphorylase (4) catalyzes the conversion of nucleosides to their respective bases, and adenosine deaminase (5) converts adenosine to inosine

Table 1. Strains used

Strain No.	Relevant genotype	Preparation and/or source[a]
CSH 26	*Δ pro-gpt-lac*	CSH
Sϕ 606	*Δ pro-gpt-lac, hpt, purR*	isolated from CSH 26, resistance to 6MP (1 mM) (Jochimsen et al. 1975).
TL 343/F′128	wt	isolated from CSH 26.
TL 350	*Δ pro-gpt-lac, hpt, purR, apt*	isolated from CSH 26 resistance to 2-fluoroadenine
TL 352/F′128	*hpt, purR*	isolated from Sϕ 606.
X761	*purE, leu*	from J. Wall
JW2	*purE, gpt, leu*	from J. Wall
X761 pur$^+$	*wt, leu*	isolated from X761
JW2 pur$^+$	*gpt, leu*	isolated from JW2
TL 471	*gpt, hpt*	isolated from JW2 pur$^+$ by Plvir transduction
259	Hfr H	CGSG 259
259-2	Hfr H, *hpt*	isolated from 259, resistance to 6MP.
EZ-O/F′d25	F′d25: *purE-lac*	from J. Beckwith
CSH 18/F′	F′: *pro-gpt-lac*	CSH

[a] Abbreviations used: CSH=Cold Spring Harbor Laboratory; CGSC=*E. coli* Gen. Stock Ctr. Yale University, New Haven, Conn.

and the resulting crude extract was clarified by centrifugation at 27,000×*g* for 1 h. Protein concentrations were determined using the Bio-Rad protein binding assay (Bradford 1976).

The stock FGAR reaction mixture contained 200 mM Tris HCl (pH 8), 18 mM $MgCl_2$, 30.8 mM glutamine (pH 7), 2 mM glycine, 3.6 mM ATP (pH 7), 20 μM azaserine, 7.28 mM PRPP, 2 mM sodium formate, and 10 μCi ^{14}C formate (60.3 mCi/mM) per milliliter of reaction mix. Forty μl of cell extract containing 100–400 μg protein was added to an equal volume of reaction mix and incubated at 37° C for 15 min. Reaction mix was also added to a portion of the extract that had been boiled for 2 min as a measure of non-enzymatic background activity. The reaction was immediately stopped by adding 4.5 μl of a cold 50% TCA solution and the precipitated protein was removed by centrifugation at 800×*g* for 5 min. Ten μl of the supernatant fluid was spotted on Whatman 3 mm paper and chromatographed in isopropyl alcohol-H_2O-NH_4OH (70:30:1) as described by Westby and Gots (1969). The FGAR fractions (RF=0.18) were cut out and counted in PPO-toluene in a Beckman LS-150 scintillation counter.

Two methods were used to assay purine phosphoribosyl transferases. For quantitative measurements, 1 ml of culture was grown overnight in L broth, washed in 10 mM $MgSO_4$, and sonicated for 45 s at 0° C. Protein content of clarified extracts was determined and 5–10 μg of protein in 25 μl was added to an equal volume of stock reaction mix containing 100 mM Tris HCl (pH 7.4), 2 mg/ml BSA, 8 mM $MgSO_4$, 2 mM PRPP, 200 μM purine, and 20 μCi ^3H purine per 1 ml of reaction mixture. The combined mixture was incubated at 37° C for 10 min and enzyme activity stopped by the addition of 1 ml sodium acetate buffer (50 mM NaOAc·3 H_2O, 2 mM NaH_2PO_4 ·H_2O pH 5.0). LaCl was added to a final concentration of 0.1 M and the preciptiated nucleotides were collected on Whatman GF/A filters. The filters were dried and radioactivity measured as described above. For qualitative determination of phosphoribosyl transferase activities of a large number of samples, 0.2 ml of culture was grown overnight in microwells. Cells were lysed by the addition of 20 μl $CHCl_3$ and 10 μl 0.1% SDS as described by Miller (1972). Ten μl were removed and assayed for phosphoribosyl transferase activity as described above.

6-Mercaptopurine Uptake

Duplicate 5 ml cultures were grown in VB medium in the absence of purines to an OD_{540} of 0.6–0.75. Five μl (0.5 μCi) of ^{14}C 6MP (28 mCi/mM) was added to each tube and incubation was continued at 37° C with shaking. At 0, 1, 5, and 20 min a 1 ml sample was diluted into 5 ml of 10 mM $MgSO_4$ and immediately filtered through nitrocellulose filters. The filters were washed twice with 5 ml of 10 mM $MgSO_4$, dried, and radioactivity measured as described above. Control tubes without cells were used as a measure of the nonspecific adsorption to the filters. The relative amount of 6MP uptake after subtraction of background and 0 time values is given as cpm/OD_{540}.

Results

Occurrence of Purine Regulatory Mutants in 6MP Treated Cells

We have previously reported that inhibition of de novo purine biosynthesis by mutation or aminopterin had no effect on the synthesis of the purine phosphoribosyl transferases (Levine and

Taylor 1980). In order to determine the effect of phosphoribosyl transferase deficiencies on the de novo pathway we obtained and constructed a series of mutants described in Fig. 2. Synthesis of FGAR in cell free extracts was used as a measure of de novo purine enzyme transcription (Benson and Gots 1976). Our initial experiments indicated that extracts from Sϕ 606(*hpt*$^-$, *gpt*$^-$), TL 350(*hpt*$^-$, *gpt*$^-$, *apt*$^-$), and TL 352(*hpt*$^-$) grown in the absence of purines, synthesized at least twice as much FGAR as extracts from various wildtype strains (data not shown). *PurR* mutants that had been isolated in the absence of drug analogs by us and others (Benson and Gots 1976) have typically synthesized higher levels of FGAR in the in vitro system and so we were isterested in determining if Sϕ 606, TL 350, and TL 352 were *purR* mutants. Cells were grown overnight in the presence of 200 μg/ml adenine and extracts were prepared as described in Materials and Methods. The results presented in Table 2 indicate that whereas the synthesis of FGAR is repressed in extracts made from wildtype cells grown in the presence of adenine, no such repression is observed in extracts prepared from Sϕ 606 and its derivatives.

HPRT Base Substitution Mutants

Sϕ 606 was originally isolated as a 6MP (1 mM) resistant derivative of CSH 26 (Jochimsen et al. 1975). To confirm that a relationship exists between *hpt* and *purR* we mutagenized CSH 26 with NTG and isolated a number of independent 6MP resistant strains. Table 3 lists the percentage of wildtype HPRT activity and the specific activity of FGAR synthesized in extracts from these cells grown overnight in the presence of adenine. The results clearly indicate that there is a correlation between HPRT activity and the *purR* phenotype.

Separation of Hpt and PurR

To determine whether *hpt* and *purR* are physically linked we attempted to restore HPRT activity and then assay for the *purR* phenotype. A streptomycin resistant derivative of Sϕ 606 was mated with Hfr H 259 and *pro*$^+$ conjugants were selected. Isolates were assayed for HPRT activity and colonies having wildtype levels were assayed for in vitro FGAR synthesis under repressed conditions as described above. The results presented in Table 4 indicate that restoration of HPRT activity has no effect on the purR phenotype. *Hpt* and *purR* are therefore not closely linked on the *E. coli* chromosome.

Relationship of 6MP, HPRT, and PurR Mutants

Resistance to 6MP in *E. coli* has generally been attributed to the cells inability to transport or metabolize the analog. In most cases this involved the modification or absence of HPRT (Brockman 1963), but enhanced degradation of analog intermediates has also been reported to confer resistance to the drug (Coggin et al. 1966). In order to study this further we used an appropriate *E. coli* F′ strain carrying a *purE-lac* fusion (Jacob et al. 1965) (EZ-/OF′d25). In a *lac* deletion strain carrying this plasmid growth on melibiose at 42° C is dependent on the expression of the *lac Y* gene residing on the F factor. In a *purR*$^+$ strain the presence of high adenine concentrations will effectively repress the synthesis of lactose permease and prevent growth. Adenine resistant survivors of this selection typically have mutations in *purR* or in the operator region of the *purE* gene (Gots et al. 1976).

In our initial experiments EZ-O F′d25 derivatives resistant to 1 mM 6MP were selected and assayed for HPRT activity. Only 3 of 18 6MP resistant cells lacked wildtype HPRT activity.

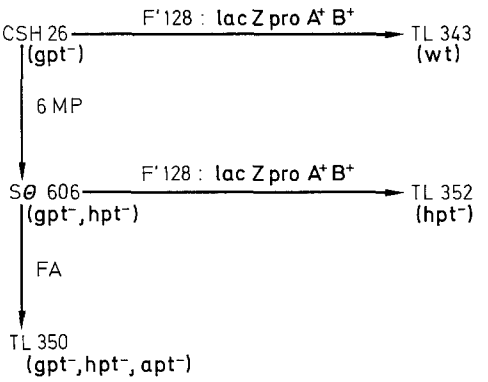

Fig. 2. CSH 26 derivatives

Table 2. Repressed levels of FGAR enzymes in CSH 26 derivatives

Strain	Relevant genotype	Repressed FGAR (nmoles/min/mg)[a]
TL 343	wt	0.10
CSH 26	*gpt*$^-$	0.08
Sϕ 606	*gpt*$^-$, *hpt*$^-$	2.47
TL 350	*gpt*$^-$, *hpt*$^-$, *apt*$^-$	3.01
TL 352	*hpt*$^-$	2.85

[a] FGAR is expressed as nmoles/min/mg protein in extracts from cells grown overnight in 200 μg/ml adenine

Table 3. HPRT activities and regulatory properties of 6MP resistant derivatives of CSH 26[a]

Strain	HPRT activity (% of CSH 26)	Repressed FGAR (nmoles/min/mg)	PurR phenotype[b]
CSH 26	100	0.09	+
TL 417	8.3	2.21	−
TL 418	30.6	1.44	−
TL 420	<0.1	2.36	−
TL 421	92.6	0.08	+
TL 422	85.0	0.04	+

[a] All assays were done in duplicate and repeated at least twice. HPRT activities are given as a percent of the level in CSH 26

[b] + = wildtype regulatory properties; − = *purR* mutant

Table 4. Effect of restoration of HPRT activity on the PurR phenotype

Strain	HPRT (%wt)	Repressed FGAR	PurR phenotype[a]	6MP[b]
Sϕ 606	<0.1	2.81	−	*r*
TL 462	108	2.17	−	*r*
TL 463	100	2.48	−	*r*

[a] − = *purR* mutant

[b] *r* = resistant

Similar results were obtained with other 6MP resistant strains although the percentage of *hpt*$^-$ mutants varied. These results support the idea that resistance to 6MP can occur independently of HPRT mutations.

In a second set of experiments we used EZ-O F′d25 to deter-

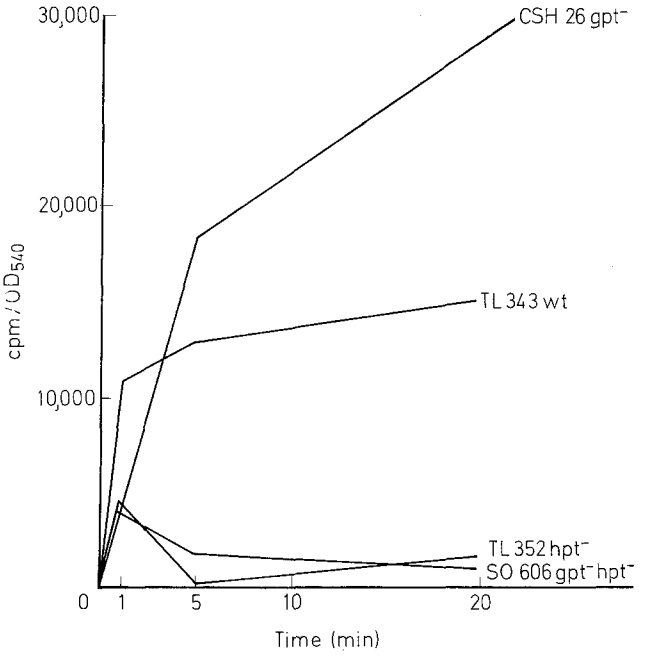

Fig. 3. Uptake of ^{14}C-6 MP in HPRT Deficient Strains. ^{14}C-6MP was added to exponentially growing duplicate cultures. Aliquots were removed at 0, 1, 5, and 20 min, filtered, and counted as described in Materials and Methods. 0 time values were in cpm/OD_{540}: TL 343, 846; CSH 26, 730, Sϕ 606 str, 517; TL 352, 472

Table 5. Relationship of 6MP resistance and adenine resistance

Selection	% of colonies resistant to	
	6MP	ade
6MP	100	87
ade	42	100

Strains carrying a fused *purE – lac* operon were made 6MP resistant or adenine resistant as described in the text. Resistant colonies were replica plated onto selective medium and the percentage of resistant colonies was determined

Table 6. Regulatory properties of adenine resistant strains that are 6MP sensitive or resistant

Strain	Selection phenotype[a]		Repressed FGAR (nmoles/min/mg)	PurR phenotype[b]
	adenine	6 MP		
EZ-OF'd25	*s*	*s*	0.04	+
TL 458	*r*	*s*	0.001	+
TL 459	*r*	*s*	0.02	+
TL 460	*r*	*r*	1.72	–
TL 461	*r*	*r*	2.25	–

[a] *s*=sensitive, *r*=resistant

[b] +=wildtype regulatory properties; –=*purR* mutant

mine the relationship between 6MP resistance and the *purR* phenotype. Ninety five 6MP resistant and 50 adenine resistant derivatives of these strains were replica plated onto the corresponding selective medium to which they had not previously been exposed (1 mM 6MP or melibiose+200 μg/ml adenine at 42° C). The results are presented in Table 5. Eighty-seven percent of the 6MP resistant cells were also able to grow at 42° C on melibiose in the presence of 200 μg/ml adenine whereas only 42% of the adenine resistant colonies were also resistant to 6MP. These results are consistant with the hypothesis that 6MP selects directly for *purR* mutants and that cells that grow at 42° C on melibiose in the presence of high concentrations of adenine are not all *purR* mutants. To verify these conclusions we assayed extracts from 6MP sensitive and resistant derivatives of adenine cells grown overnight under repressed conditions and tested their ability to synthesize FGAR. The results in Table 6 confirm that whereas adenine resistant 6MP resistant cells are predominantly *purR* mutants, adenine resistant 6MP sensitive cells are not. This latter class of adenine resistant cells are probably *purE* operator mutants.

These results make it difficult to explain the high frequency of *hpt*$^-$, *purR*$^-$ double mutants that are found among 6MP resistant derivatives of CSH 26. It seems unlikely that *hpt*$^-$ mutants are in some way selected for in *purR*$^-$ cells because *purR* mutants that have never been exposed to 6MP have wildtype levels of HPRT (data not shown). 6MP should not be able to select for *purR* mutants in cells deficient in HPRT activity because 6MP is dependent on HPRT for its transport into the cell. To confirm this we incubated exponentially growing cells of the different phosphoribosyl transferase mutants with ^{14}C-6MP at 37° C. Aliquots were removed at various times and collected on Millipore filters as described in Materials and Methods. The results shown in Fig. 3 indicate that 6MP is taken up very poorly if at all in HPRT deficient cells.

These results suggest that 6MP initially selects for HPRT mutants and that *purR* mutants are selected for in a *hpt*$^-$ background. To test this a *hpt*$^-$, *purR*$^+$ strain was constructed using a 6MP resistant derivative of Hfr H 259 to transfer the *hpt* mutant phenotype to a streptomycin resistant derivative of CSH 26 without a concurrent transfer of *purR*. Under these conditions all of the conjugants had wildtype GPRT activity. Conjugants having no HPRT activity were tested for the *purR*$^-$ phenotype using the FGAR in vitro synthesizing system. After many generations of growth in minimal media with or without purines we saw no evidence of the *purR* mutant phenotype (data not shown). If *purR* mutants had a definite growth advantage in this strain we would have expected to see an increase in the amount of FGAR synthesized in vitro under repressed conditions.

A Hpt$^-$*-Gpt*$^-$ *Mutant is Adenine Sensitive*

We next investigated the possibility that purR mutants are selected for in *hpt*$^-$, *gpt*$^-$ cells. A P1 *vir* lysate grown on Sϕ 606 was used to transduce JW2pur$^+$(gpt$^-$) to leucine prototrophy. Twenty-four isolates were assayed for HPRT activity and one, TL 471, was found to be deficient in the enzyme. In order to test for the *purR* mutant phenotype TL 471 was inoculated into VB medium containing 200 μg/ml adenine. To our surprise there was no evidence of growth after 18 h. We reinoculated fresh media with the strain and observed growth only after 48 h.

The relative effects of adenine on TL 471 and the strains from which it was derived was determined by monitoring the growth of X761 pur$^+$, JW2 pur$^+$ (gpt$^-$), and TL 471 (*hpt*$^-$, *gpt*$^-$) in the presence or absence of 100 μg/ml adenine. The results presented in Fig. 4 indicate that whereas all strains are inhibited by adenine to some extent, only the *hpt*$^-$, *gpt*$^-$ double mutant is unable to grow in its presence.

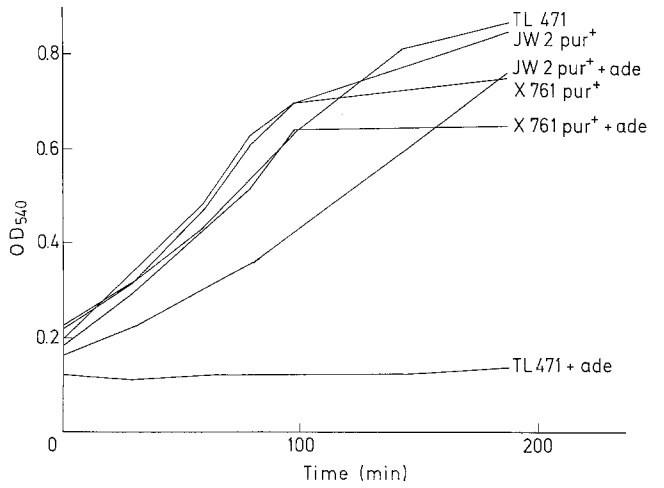

Fig. 4. Growth of X761 pur$^+$ Derivatives in the Presence or Absence of 100 µg/ml Adenine. A fresh overnight culture of each strain was inoculated into VB medium with or without 100 µg/ml adenine and shaken at 37° C. At various times the tubes were removed and the OD_{540} determined in a Bausch and Lomb Spectronic 20

Table 7. Analysis of adenine resistant derivatives of TL 471

Strain	APRT activity (% wt)	Repressed FGAR (nmoles/min/mg)
JW2 pur$^+$	100	0.07
TL 471[a]	100	0.68
TL 471 S-1[b]	100	2.93
TL 471 S-2	100	2.61
TL 471 L-1	<0.1	0.04
TL 471 L-2	<0.1	0.10

[a] TL 471 was grown in VB medium containing 200 µg/ml adenine. Growth was observed only after 48 h

[b] All other TL 471 derivatives were isolated as members of the large (L) or small (S) size class of adenine resistant mutants

Selection for PurR Mutants

Benson and Gots (1976) have previously shown that *purA* mutants are adenine sensitive in the absence of vitamin B1 and that *purR* mutants present in the population may have a selective advantage. A similar situation appears to exist with *hpt*$^-$, *gpt*$^-$ double mutants in the presence of adenine. Approximately 10^7 TL 471 cells grown overnight in the absence of purines were spread on minimal plates containing 200 µg/ml adenine. After 48 h two size classes of adenine resistant colonies were seen. Colonies representative of both size classes were reisolated and assayed for the *purR* phenotype as were the TL 471 mutants able to grow after 48 h in liquid medium in the presence of adenine. We had previously determined that fluoroadenine resistant derivatives of TL 471 were also resistant to adenine and so we also assayed the extracts for APRT activity. The results are presented in Table 7. The large size class of adenine resistant mutants are deficient in APRT. The smaller size class mutants however are *purR* mutants as indicated by the presence of the FGAR synthesizing enzymes in extracts from cells grown in the presence of adenine. Likewise, extracts from TL 471 mutants able to grow in liquid culture containing adenine were able to synthesize an intermediate level of FGAR in vitro. This intermediate level is consistant with the finding that not all adenine resistant derivatives of TL 471 are *purR* mutants.

Discussion

This study describes the isolation of an *E. coli hpt*$^-$ *gpt*$^-$ double mutant that is extremely sensitive to adenine. Our original observations indicated that a 6MP resistant derivative of CSH 26 was deficient in HPRT and in the regulation of de novo purine synthesis. This relationship was confirmed by the isolation of several independent 6MP resistant CSH 26 mutants that were both *hpt*$^-$ and *purR*$^-$. The apparent indirect relationship between HPRT activity and the *purR*$^-$ phenotype in these mutants suggests that HPRT might act as an bifunctional protein or that HPRT and the *purR* repressor are transcribed as part of a single unit. However, these possibilities were eliminated when the restoration of wildtype HPRT activity failed to alter the *purR* phenotype.

Although resistance to 6MP has generally been attributed to a deficiency in HPRT our results using a F factor carrying a fused *purE-lac* operon indicated that 6MP could select for *purR*$^-$ or *hpt*$^-$ mutants independently. We have seen no evidence to suggest that HPRT mutants are selected for in *purR*$^-$ strains. The mechanism by which 6MP selects for *purR* mutants is not well understood. We suggest that at high concentrations 6MP is able to repress the de novo pathway, thus shutting off the cells supply of purines. If the analog is not itself utilized efficiently as a purine source the cells will be starved for purines and grow slowly. *PurR* mutants would however have a fully functional de novo pathway and thus be able to satisfy the cells purine requirements. Consistent with this model is the finding that the addition of a small amount of adenine to plates containing 6MP reduces the inhibitory effect of the analog (data not shown).

It is unlikely that 6MP is able to select for *purR* mutants in *hpt*$^-$ cells because the analog is taken up very poorly in the absence of HPRT. 6MP is not lethal to wildtype *E. coli* and a substantial amount of background growth is seen after 48 hrs on 1 mM 6MP plates. Although at this concentration 6MP will enter cells by passive diffusion a cell lacking HPRT should accumulate considerably less 6MP than wildtype strains. Under these conditions selection for 6MP resistant mutants should be minimized.

Analysis of a *hpt*$^-$ strain that had never been directly exposed to 6MP indicated that *purR* mutants are not selected for in the presence or absence of adenine in cells deficient in HPRT alone. However, in a *gpt*$^-$ background such as CSH 26, an HPRT deficient cell is extremely sensitive to the toxic effects of adenine. Concentrations of adenine as low as 0.25 µg/ml have been shown to alter the growth of this strain (manuscript in preparation).

Two size classes of adenine resistant derivatives of TL 471 have been isolated on plates. The large size class mutants are deficient in APRT. The conversion of adenine directly to AMP is apparently a prerequisite for adenine sensitivity in this strain. The small size class mutants are *purR* mutants. In addition, *purR* mutants have been found to make up a substantial fraction of the population of cells able to grow in liquid medium containing adenine.

We are presently studying the mechanism of adenine toxicity in this strain and the way in which *purR*$^-$ and *apt*$^-$ mutants are able to protect TL 471 from these effects. Our preliminary results indicate that in the presence of adenine these cells are being starved for guanine nucleotides. A complete report of these findings will be presented in a future publication.

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