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Mechanism of Adenine Toxicity in *Escherichia coli*

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The mechanism of adenine toxicity in an *hpt gpt* strain of *Escherichia coli* that is extremely sensitive to adenine inhibition was investigated. Adenine-resistant derivatives had secondary mutations in adeninephosphoribosyltransferase or the *purR* repressor. Growth studies with various purine salvage pathway mutants and the ability of guanosine to prevent adenine toxicity indicated that adenine exerts its toxic effects by depleting guanine nucleotide pools. In the presence of adenine, ATP pools increased twofold in wild-type cells and stabilized after 5 min. In contrast, ATP pools continued to rise in *hpt gpt* cells up to 25 min and increased sevenfold after adenine addition. *hpt gpt* cells were shown to have higher levels of adeninephosphoribosyltransferase than did wild-type cells. In response to adenine addition, GTP pools dropped three- to fourfold in all strains tested. Although GTP levels returned to near normal values in wild-type cells after 35 min, no restoration of GTP pools was observed in the *hpt gpt* strain during this period. Measurements of guanine pools before and after the addition of adenine indicated that guaninephosphoribosyltransferese plays an important role in maintaining GTP pools by converting free guanine to GMP during guanine nucleotide depletion.

The growth of enteric bacteria is inhibited by high concentrations of adenine (9). Brooke and Magasanik (2) found that the bacteriostatic action of adenine could be reversed by thiamine. Subsequent studies by Moyed (17) and Newell and Tucker (18) indicated that the inhibitory effect of adenine or adenosine resulted from an inhibition of de novo purine biosynthesis and therefore the synthesis of a thiamine precursor (Fig. 1). Similar results were obtained by Dalal et al. (5).

Inhibition of de novo purine biosynthesis and the resulting depletion of thiamine precursors cannot be the sole explanation for the inhibitory effect of adenine on growth. Shive and Roberts (20) observed adenine toxicity in *Escherichia coli*, and this toxicity could be reversed by adenosine, inosine, xanthine, or guanine. Histidine has also been reported to reverse adenine toxicity in *Salmonella* (9). None of these reversing agents increased the rate of de novo purine biosynthesis in the presence of adenine. Additional studies indicated that adenine could exert its toxic effects by inhibiting H_2-folate metabolism (4, 8, 20, 21). Dalal et al. (5) isolated a series of *Salmonella* mutants that were sensitive to adenine, and this sensitivity could be prevented by the addition of compounds that were involved in folic acid synthesis or were products of one-carbon transfer reactions requiring folic acid. Further studies indicated that AMP inhibited the conversion of GTP to pteridine, the first step in folic acid biosynthesis (4).

In the process of studying the relationship between purine salvage and de novo pathways in *E. coli*, we isolated an *hpt gpt* mutant that was extremely sensitive to inhibition by adenine (16). In this paper we describe the effect of adenine on the growth characteristics and nucleotide pools of various adenine-sensitive and adenine-resistant mutants. The results indicate that adenine exerts its toxic effects by depleting guanine nucleotide pools.

MATERIALS AND METHODS

Strains. Table 1 lists the strains of *E. coli* K-12 used. X761 *pur*$^+$ was the parent organism for all derivatives.

Media. The minimal salts medium of Vogel and Bonner (VB) (22) was supplemented with glucose (0.5%, wt/vol). For ^{32}P nucleotide analysis, cells were grown in a low-phosphate medium (13). Amino acids were added as needed to a final concentration of 40 μg/ml. Purines were added as described in the text. Adenine-sensitive strains were always grown in purine-free medium unless otherwise noted.

Chemicals. [^{3}H]adenine (22 Ci/mol), [^{14}C]glycine (118 mCi/mmol), and $^{32}P_i$ were purchased from Amersham Corp. (Arlington Heights, Ill.).

Growth curves. Cells were grown overnight in VB medium without purines. Five milliliters of overnight culture was centrifuged, washed once in 10 mM $MgSO_4$, and resuspended in 5 ml of 10 mM $MgSO_4$. Fresh VB medium containing various additions was inoculated with 0.1 ml of the overnight culture and

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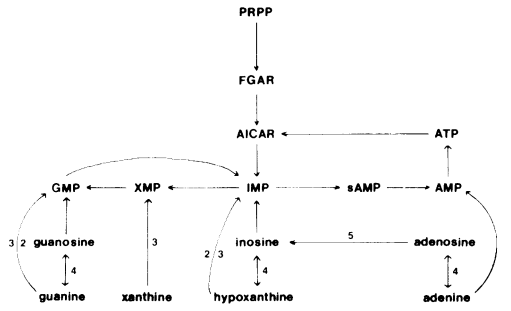

FIG. 1. Purine salvage pathway in *E. coli*. (1) APRT; (2) HPRT; (3) GPRT; (4) purine nucleoside phosphorylase; (5) adenosine deaminase.

incubated at 37°C with shaking. Growth was monitored at various times by measuring the absorbance at an optical density of 540 nm (OD_{540}) in a Bausch & Lomb Spectronic 20.

PRTase assays. Late-log-phase cells were harvested, washed twice in 10 mM $MgSO_4$, and sonicated for 45 s at 0°C in a Heat Systems-Ultrasonics, Inc., sonicator (model W185F). Extracts were clarified by centrifugation, and protein content was determined by the Bio-Rad protein-binding assay (1). Purine phosphoribosyltransferase (PRTase) assays were performed as described previously (10).

^{32}P analysis of nucleotide pools. Nucleotide pools were analyzed by the method of Jensen et al. (14). To measure total pools in the absence of adenine, carrier-free $^{32}P_i$ was added to an exponentially growing culture to a specific activity of 40 to 60 Ci/mol. Cultures were shaken at 37°C, and when the OD_{436} reached 0.7 to 0.8, 0.5 ml of culture was removed and pipetted directly into 0.1 ml of cold 2 M formic acid. After a 30- to 60-min incubation on ice, extracts were centrifuged in a Beckman microfuge for 1 min. The supernatants were used for chromatographic analysis either the same day or after storage overnight at −70°C. Nucleotide pool measurements in adenine-treated cultures were performed essentially as described above, except that ^{32}P was added at an OD_{436} of 0.05 to 0.1 and adenine (100 μg/ml) was added at an OD_{436} of about 0.4. Samples were processed at various times before and after the addition of adenine.

^{32}P-labeled nucleotides were separated on polyethyleneimine-impregnated cellulose thin-layer plates. Thirty microliters of extract was mixed with 10 μl of marker solution (nucleotide triphosphates; 4 mg/ml each) and applied to the plates. The plates were dried under air, washed for 10 min in methanol, dried, and chromatographed in 0.85 M KH_2PO_4 (pH 3.4). The chromatograms were dried, the nucleotide markers were visualized with UV light, the ATP and GTP spots were cut out, and the radioactivity was determined by liquid scintillation counting.

Intracellular guanine pool measurements. Seven hundred microliters of [^{14}C]glycine (118 mCi/mmol) was added to an exponentially growing 35-ml culture of an appropriate *E. coli* strain. Twelve-milliliter samples were removed just before and 15 min after the addition of adenine. Cells were centrifuged and resuspended in 1 ml of 10 mM $MgSO_4$. After centrifugation in the microfuge, the supernatant was carefully removed and the pellet was suspended in 100 μl of 0.5 M sodium formate to extract purine pools under conditions which minimize nucleotide degradation. After incubation on ice for 40 min, the samples were centrifuged and the supernatant was frozen at −70°C. Samples were spotted with 5 μl of marker guanine (4 mg/ml) on cellulose thin-layer plates and chromatographed in 1 M ammonium acetate. Guanine spots were visualized under UV light and cut out, and the radioactivity was determined as described above. The amount of free guanine is given as counts per minute in guanine per OD_{540} unit.

RESULTS

An *hpt gpt* mutant is adenine sensitive. In the process of isolating *E. coli* strains having mutations in various purine salvage enzymes, we

TABLE 1. Bacterial strains

E. coli strain	Genotype	Source or prep
X761 *pur*$^+$	F$^-$ *argA his ileu metA* or *-B thi trp ara gal lacZ mtl xyl tonA tsx str proA leu* T5^r	*pur*$^+$ derivative of X761 (from J. Wall [12])
JW2 *pur*$^+$	Same as X761 *pur*$^+$ except *gpt pro*$^+$	*pur*$^+$ derivative of JW2 (from J. Wall [12])
TL471	Derived from X761 *pur*$^+$, *gpt hpt leu*$^+$	P1 transduction
TL471 S-1	Derived from TL471, *purR*	See text
TL471 FA3	Derived from TL471, *apt*	See text
TL505-6	Derived from X761 *pur*$^+$, *hpt pro*$^+$ *leu*$^+$ *ara*$^+$	Conjugation using *hpt Hfr* H
TL540	Derived from X761 *pur*$^+$, *deoD pro*$^+$ *leu*$^+$ *ara*$^+$	Conjugation using *pup Hfr* H
TL541	Derived from JW2 *pur*$^+$, *deoD gpt leu*$^+$ *ara*$^+$	Conjugation using *pup Hfr* H
TL601	*his*$^+$ derivative of TL471	P1 transduction

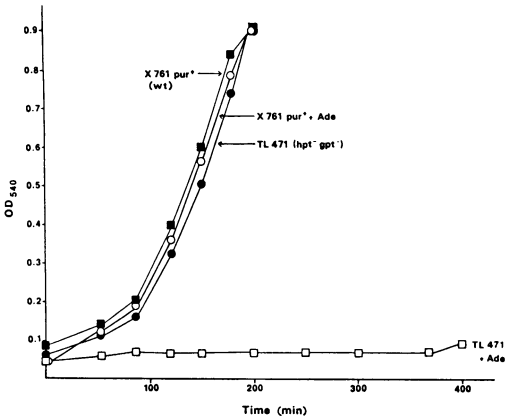

FIG. 2. Growth of TL471 in the presence or absence of adenine. An overnight culture of TL471 and X761 *pur*$^+$ grown in VB medium was inoculated into fresh VB medium with or without adenine (100 μg/ml). Cells were shaken at 37°C, and at various times the absorbancy at OD_{540} was determined. X761 *pur*$^+$, no adenine (■); X761 *pur*$^+$ plus adenine (100 μg/ml) (○); TL471, no adenine (●); TL 471 plus adenine (100 μg/ml) (□).

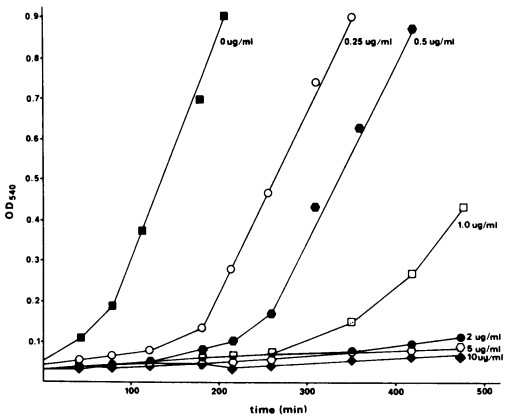

FIG. 3. Effect of different adenine concentrations on the growth of TL-471. TL471 was inoculated into VB medium containing various concentrations of adenine. Cells were shaken at 37°C, and OD_{540} readings were taken at various times. No adenine (■); 0.25 μg/ml (○); 0.5 μg/ml (●); 1 μg/ml (□); 2 μg/ml (●); 5 μg/ml (○); 10 μg/ml (◆).

constructed an *hpt* derivative of JW2 *pur*$^+$(*gpt*) by P1 transduction. This strain (TL471) was unable to grow in minimal medium plus adenine even 18 h after inoculation. Figure 2 compares the effect of adenine (100 μg/ml) on the growth of TL471 (*hpt gpt*) and X761 *pur*$^+$(Wt). In the absence of adenine, both strains grew at identical rates. However, in the presence of adenine, the growth of the parental strain X761 *pur*$^+$ was only slightly inhibited, whereas the growth of TL471 was inhibited even after several hours.

To test the effect of various adenine concentrations on the growth of TL-471, a fresh overnight culture grown in the absence of purines was inoculated into VB medium containing different concentrations of adenine. Growth was monitored at various times by measuring the increase in turbidity at OD_{540}. The growth of TL471 was inhibited by adenine at concentrations as low as 0.25 μg/ml (lowest concentration tested) (Fig. 3). This inhibition was manifested as an increase in the lag period with increasing adenine concentration.

Growth of adenine-resistant mutants. We have previously described the selection for *purR* and *apt* derivatives of TL471 (16). The growth of two of these derivatives, TL471 S-1 (*hpt gpt purR*) and TL471 FA3 (*hpt gpt apt*), was measured in VB medium containing 100 μg of adenine per ml (Fig. 4). As expected, both strains were able to grow in the presence of adenine, but the *purR* derivative exhibited a considerable growth lag and a reduced growth rate. These results indicated that transcription of de novo purine genes alleviated the toxic effects of adenine in an *hpt gpt* strain. It seems unlikely that all of the de novo purine enzymes are involved in this process because even in a a *purR* strain the first enzyme in the pathway, phosphoribosyl pyrophosphate amidotransferase, and therefore de novo purine biosynthesis as a whole, is inhibited by feedback inhibition.

Adenine sensitivity of *gpt* and *hpt* strains. To determine the relationship between adenine toxicity and specific purine salvage enzymes, the growth characteristics of a series of guanine and hypoxanthine PRTase mutants were studied

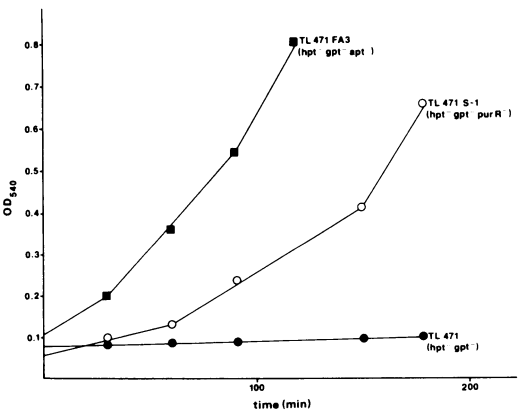

FIG. 4. Growth of adenine-resistant derivatives of TL471. Growth conditions are as described previously in the presence of adenine (100 μg/ml). TL471 (●); TL471 FA3 (■); TL471 S-1 (○).

in the presence of adenine (100 μg/ml). All of the purine PRTase mutants were sensitive to adenine to some degree. This sensitivity was manifested as an extended lag phase of about 45 and 90 min for *hpt* and *gpt* mutants, respectively. Mutants deficient in hypoxanthine PRTase (HPRT) or guanine PRTase (GPRT) alone, however, were not nearly as sensitive to the toxic effects of adenine as was the *hpt gpt* double mutant.

GPRT plays an important role in adenine toxicity. In *E. coli*, adenine can be converted to IMP via adenosine, inosine, and hypoxanthine (see Fig. 1). HPRT might play a crucial role in preventing adenine toxicity by allowing the conversion of excess adenine to IMP and GMP via the salvage pathway. However, growth studies (Fig. 5) indicated that *gpt* cells were even more sensitive to adenine than were *hpt*$^-$ cells. To confirm that GPRT plays an important role in alleviating adenine toxicity, the growth of *pup* and *pup gpt* strains was measured in the presence of adenine. In these strains, adenine could be converted to IMP only via AMP and the de novo purine pathway (see Fig. 1). Although a *pup gpt*$^+$ cell grew poorly in the presence of adenine, a *pup gpt* cell was extremely sensitive to adenine inhibition (Fig. 5). GPRT therefore played an important role in minimizing the toxic effects of adenine.

Guanosine prevents adenine toxicity. On the basis of the above-described experiments, we proposed that, in the presence of adenine, an *hpt gpt* strain may be depleted of guanine nucleotides. The evidence in support of this model includes: (i) the involvement of the de novo purine pathway (*purR* mutants are adenine resistant) and (ii) the role played by GPRT in alleviating the effects of adenine.

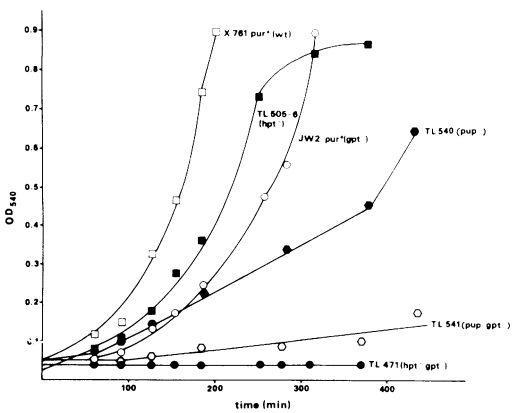

FIG. 5. Growth of purine PRTase and *pup* mutants in the presence of adenine. Various purine PRTase and *pup* mutants were inoculated into VB medium containing adenine (100 μg/ml). Growth was monitored as described previously. X761 *pur*$^+$ (□); TL505-6 (■); JW2 *pur*$^+$ (○); TL471 (●); TL540 (◐); TL541 (◯).

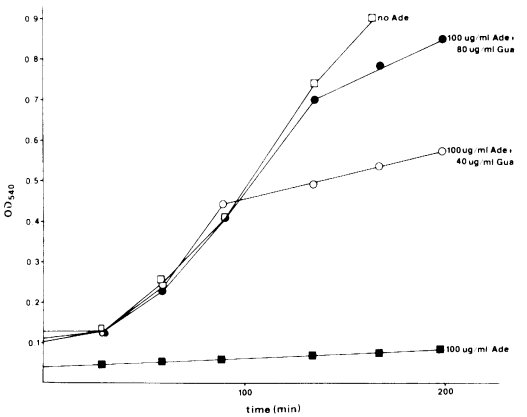

FIG. 6. Guanosine prevents adenine toxicity in TL471. TL471 was grown in VB medium containing adenine (100 μg/ml) with and without guanosine. A culture with no additions was included as a positive control. Growth was monitored as described previously. No adenine (□); adenine (100 μg/ml) (■); adenine (100 μg/ml) plus guanosine (40 μg/ml) (○); adenine (100 μg/ml) plus guanosine (80 μg/ml) (●).

To test this proposal, an overnight culture of TL471 was inoculated into VB medium containing adenine (100 μg/ml) with or without guanosine. In a cell deficient in GPRT, guanosine can be converted to GMP by guanosine kinase (6). Guanosine, at 80 μg/ml, prevented adenine toxicity in an *hpt gpt* cell (Fig. 6).

***his*$^+$ derivatives of TL471 are adenine resistant.** The conversion of AMP to IMP via the de novo purine pathway is inhibited by histidine (6). Since TL-471 requires histidine for growth, it is possible that the inhibition of this pathway contributes to guanine nucleotide starvation. A *his*$^+$ derivative (TL601) of TL471 was constructed by P1 transduction and grown in the presence or absence of adenine and histidine. Adenine toxicity was partially relieved in TL601 in the absence of histidine (Fig. 7). However, in the presence of histidine, adenine toxicity was more pronounced. These results confirm that the conversion of AMP to IMP via the de novo purine pathway is important in alleviating adenine toxicity.

Effect of adenine on nucleotide pools. To determine the effect of exogenous adenine on nucleotide pools in wild-type, *hpt*, *gpt*, and *hpt gpt* strains, overnight cultures were inoculated in VB medium. After a short period of growth, ^{32}P was added as described in Materials and Methods. Adenine (100 μg/ml) was added to each culture when the OD_{436} reached 0.3 to 0.4, and 0.5-ml samples were removed and processed for

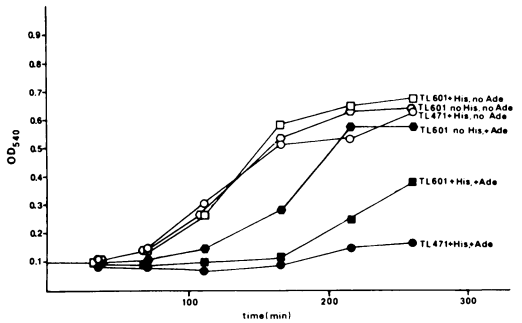

FIG. 7. Growth of TL601 in the presence of adenine. TL601 is a *his*$^+$ transductant of TL471. The growth of TL601 and TL471 (*his*) was compared in the presence and absence of adenine (100 μg/ml). Growth was monitored as previously detailed. Histidine concentration was 20 μg/ml.

nucleotide pool analysis at various times relative to the addition of adenine. The growth of an unlabeled control culture was also monitored. Upon addition of adenine, ATP pools increased in all four strains (Fig. 8A). However, whereas wild-type pools increased by twofold and stabilized after 5 min, ATP pools continued to rise in *hpt gpt* cells up to 25 min after adenine addition and increased sevenfold. *Gpt* and *hpt* strains exhibited intermediate levels of ATP.

Analysis of GTP pools (Fig. 8B) confirmed that guanine nucleotide starvation occurred in response to adenine. GTP pools dropped three- to fourfold in all four strains after the addition of adenine but returned to near-normal values in wild-type cells after 35 min. HPRT-deficient cells recovered more slowly, and GTP levels in cells lacking GPRT increased only slightly by 35 min after the addition of adenine. GTP pools in *hpt gpt* cells remained low, even after 35 min.

The relative effect of adenine on the purine PRTase mutants was most easily seen by measuring the change in ATP to GTP ratios. The ATP/GTP ratio (Fig. 8C) increased in all four strains in response to adenine; however, in the *hpt gpt* mutant the ratio increased 20-fold and remained elevated after 35 min, whereas in wild-type cells the ratio increased only fivefold. The relative order of the four strains with regard to ATP/GTP values was the same as that noted previously in growth inhibition studies.

Guanine pools drop in *gpt*$^+$ cells in the presence of adenine. Adenine lowers GTP pools in *E. coli*, and the ability to grow in the presence of adenine is related to the cell's ability to restore normal GTP levels. GPRT has been shown to play a crucial role in this process. GPRT probably restores guanine nucleotide pools by converting free guanine in the cell to GMP. To

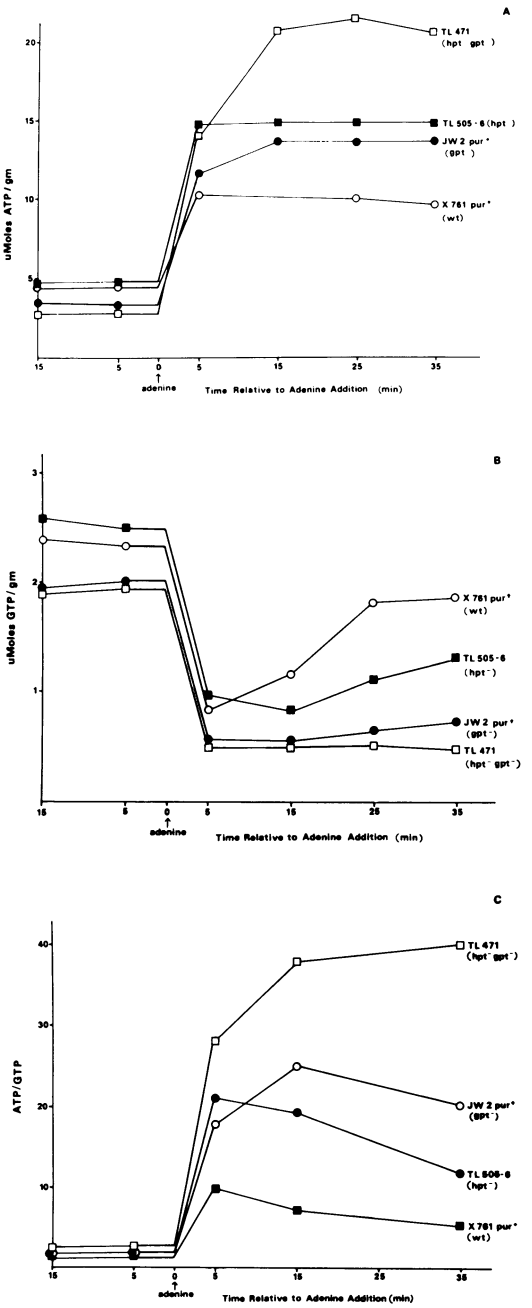

FIG. 8. Effect of adenine on ATP and GTP pools. Exponentially growing cells were labeled with ^{32}P for several generations. At an OD_{436} of 0.4, adenine (100 μg/ml) was added to each culture. Samples of 0.5 ml were removed at various times before and after the addition of adenine, and ATP and GTP pools were measured by polyethyleneimine thin-layer chromatography as described in Materials and Methods. Nucleotide pools measurements were done in duplicate and repeated at least twice. (A) Effect of adenine on ATP pools. X761 *pur*$^+$ (○); JW2 *pur*$^+$ (●); TL505-6 (■); TL471 (□). (B) GTP pools. (C) ATP/GTP ratios.

verify this prediction, total purine pools in wild-type and *hpt gpt* cells were labeled with [^{14}C]glycine for several generations. Samples were removed before and after the addition of adenine (100 μg/ml) and analyzed for radioactivity in guanine pools by thin-layer chromatography as described in Materials and Methods. Guanine pools decreased by 50% in the presence of adenine in *gpt*$^+$ cells, whereas no decrease was seen in the *hpt gpt* derivative (Table 2). GPRT therefore acted to restore guanine nucleotide pools by converting free guanine to GMP.

APRT levels in wild-type and *hpt gpt* cells. The increase in ATP pools observed after the addition of adenine to the medium was twofold lower in wild-type cells than in the *hpt gpt* derivative. The elevated ATP levels in this latter strain may have repressed the synthesis of de novo purine enzymes and prevented the conversion of AMP to GMP.

Adenosine PRTase (APRT) is the major enzyme responsible for the uptake and conversion of adenine to AMP in *E. coli* (11). Higher levels of APRT might therefore result in higher AMP and ATP pools. To determine whether APRT levels are altered in the presence of adenine, APRT activity in extracts from wild-type and *hpt gpt* cells was determined before and after the addition of adenine as described above. These experiments indicated that *hpt gpt* cells had 65% higher APRT activity than did wild-type cells in the absence of adenine. Upon the addition of adenine, APRT activity declined in both strains but still remained higher in the adenine-sensitive strain.

DISCUSSION

While studying purine biosynthesis in *E. coli*, we isolated an *hpt gpt* double mutant that was extremely sensitive to adenine-mediated growth inhibition. In the presence of adenine (100 μg/ml), the growth of wild-type cells was slightly inhibited. *hpt gpt* cells, however, had a lag of at least 3 h in the presence of adenine. Concentrations of adenine as low as 0.25 μg/ml (lowest concentration tested) inhibited the growth of the *hpt gpt* strain. This adenine concentration was 20 to 30 times lower than the amount of purine required to support the growth of a purine auxotroph. At adenine concentrations below 2 μg/ml, growth resumed after a lag period which was directly related to adenine concentration. This lag probably represents a period of detoxification or the time required to restore an essential metabolite depleted in the presence of adenine.

TABLE 2. Effect of adenine on guanine pools

Strain	cPM in guanine/OD_{540} units[a]		% Decrease
	Pre-adenine	Post-adenine	
X761 *pur*$^+$ (Wt)	10,437 ± 1,296	4,782 ± 267	54.2
TL471 (*hpt gpt*)	8,999 ± 667	9,098 ± 117	

[a] Samples were removed before and 15 min after the addition of adenine (100 μg/ml), and the number of counts in guanine was determined as described in Materials and Methods. Values represent the average and standard deviation of two separate experiments.

We have previously reported that *apt* and *purR* derivatives of TL471 are resistant to adenine toxicity (16). Cells deficient in APRT are able to grow in VB medium containing adenine, confirming that the conversion of adenine directly to AMP is required for adenine to exert its toxic effects. *purR* derivatives of TL471 are also able to grow in the presence of adenine, but these cells exhibit a considerable growth lag compared with *apt* cells. This lag may be due to the effect of histidine on nucleotide conversion. The involvement of the *purR* repressor in adenine toxicity suggests that de novo purine biosynthesis plays some role in the process. A likely step is the conversion of AMP to IMP via the de novo pathway. Growth studies with a *his*$^+$ derivative of TL471 confirm that this pathway plays an important role in preventing adenine toxicity. In addition, the conversion of amidoimadole carboxamide ribotide to IMP is catalyzed to enzymes that are regulated by the *purR* gene product (6).

Sensitivity to adenine was also observed in *gpt* and *hpt* strains of *E. coli*. This sensitivity was again manifested as an extended lag period before the onset of exponential growth. *gpt* cells were more sensitive to adenine inhibition than *hpt* cells, but neither strain alone was inhibited to the same extent as the *hpt gpt* double mutant.

In wild-type cells, adenine can be converted to IMP via AMP and the de novo purine pathway and also by conversion to adenosine, inosine, hypoxanthine, and IMP. The final step is catalyzed principally by HPRT, but GPRT can carry out this reaction at a reduced rate (3, 7). GPRT and HPRT may play crucial roles in preventing adenine toxicity by converting hypoxanthine to IMP and GMP. The importance of GPRT in minimizing adenine toxicity was confirmed in growth studies with *pup* and *pup gpt* cells. In *pup* cells, adenine cannot be converted to hypoxanthine. The increased sensitivity of *pup gpt* cells to adenine indicates that GPRT plays an important role in minimizing adenine toxicity and that this role does not involve the conversion of excess adenine-derived hypoxanthine to IMP.

Studies in which guanosine prevented adenine toxicity in the *hpt gpt* strain are consistent with a

model of adenine toxicity in which adenine inhibits growth by lowering guanine nucleotide pools. Biochemical analysis of ATP and GTP pools in various purine PRTase mutants before and after the addition of adenine to the media supports this model. Upon addition of adenine, ATP pools rose rapidly in all strains, but the greatest increase was seen in the *hpt gpt* strain. A concurrent drop in GTP pools was seen in all of the strains tested. The eventual recovery of GTP pools in wild-type and *hpt* cells indicates that these strains are able to restore GTP pools rapidly, probably by converting free guanine to GMP. GTP pools remain low in *gpt* and *hpt gpt* strains for at least 35 min, underscoring the importance of GPRT. Measurements of guanine pools before and after adenine addition in wild-type and *hpt gpt* cells are consistent with the idea that GPRT acts to convert free guanine to GMP in response to guanine nucleotide starvation.

The large increase in ATP pools seen in *hpt gpt* cells is probably a result of a 65% increase in APRT activity compared with wild-type cells. APRT is synthesized constitutively in *E. coli* (15), and the increase in APRT activity observed in *hpt gpt* cells in the absence of purines may be a result of the lowered ATP pools in this strain under these conditions (R. A. Levine and M. W. Taylor, unpublished data).

The elucidation of the mechanism of adenine toxicity has led to a better understanding of purine biosynthesis and the mechanism by which *E. coli* is able to maintain levels of purine nucleotides that are compatible with balanced growth. Adenine exerts its toxic effects by a mechanism that involves: (i) the de novo purine pathway, (ii) the conversion of ATP to AICAR, and (iii) GPRT. In the presence of adenine, ATP pools rise dramatically, resulting in guanine nucleotide depletion. ATP acts as a corepressor to inhibit de novo purine biosynthesis and the conversion of excess ATP to IMP. As a result of this repression, IMP synthesis is inhibited and guanine nucleotide levels fall. Wild-type cells are able to restore GTP levels by three mechanisms. First, ATP pools increase only two- to threefold in wild-type cells due in part to the lower activity of APRT in these cells. These levels of ATP may not totally repress all of the enzymes of the de novo purine pathway and thus allow the conversion of excess ATP to GMP. Second, wild-type cells are able to convert adenine to IMP via adenosine, inosine, and hypoxanthine. The final step requires a functional HPRT, and this explains the adenine sensitivity exhibited by *hpt* strains. Finally, GPRT plays a major role in maintaining GTP pools by converting free guanine to GMP during guanine nucleotide depletion.

TL471 is especially sensitive to adenine toxicity because the conversion of ATP to AICAR is inhibited by histidine present in the medium. Guanine nucleotide depletion, however, cannot be completely explained by this "histidine effect." The growth of a *his*$^+$ derivative of TL471 is still inhibited by adenine to some extent. In addition, independent experiments indicate that GPRT and *pur* transcription are involved in overcoming adenine toxicity.

The mechanism of adenine toxicity described above may help to explain the adenine-mediated depletion of folic acid derivatives reported by various workers (4, 8, 20, 21). Folic acid derivatives are synthesized from guanine nucleotides in *E. coli* (19). Under certain conditions, the adenine-mediated reduction in GTP pools might reduce the concentration of folate coenzymes to levels that are rate limiting for growth.

It must be emphasized that there is an essential difference between adenine toxicity as described previously and the work reported here. Adenine-mediated thiamine or folic acid depletion has previously been assayed as an inhibition of the overnight growth of a culture in the presence of adenine. Adenine does not substantially inhibit the growth of these adenine-sensitive strains, however, when an inoculum greater than 5×10^5 cells/ml is used (Levine and Taylor, unpublished data). Inhibition of growth by adenine in the experiments reported in this paper occurs with inocula 100 to 200 times larger. An *hpt gpt* mutant is therefore more sensitive to adenine at high cell densities than are wild-type or adenine-sensitive mutants isolated by other workers.

Although the ability of folic acid precursors to prevent adenine toxicity in the *hpt gpt* strains has not been tested, it seems unlikely that folic acid depletion is the main mechanism of growth inhibition in this strain. Dalal et al. (5) found that thiamine and methionine, both products of one-carbon transfer reactions requiring folic acid derivatives, could minimize the effects of adenine on adenine-sensitive cells. *hpt gpt* cells are extremely sensitive to adenine in the presence of both compounds, which are routinely added to VB medium. In addition, a 75% drop in GTP pools is likely to have more immediate effects on cell growth, including inhibition of RNA, DNA, and protein synthesis.

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