Mutation in the Phosphoribosylpyrophosphate Synthetase Gene (prs) That Results in Simultaneous Requirements for Purine and Pyrimidine Nucleosides, Nicotinamide Nucleotide, Histidine, and Tryptophan in Escherichia coli

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A mutant of Escherichia coli harboring a temperature-labile phosphoribosylpyrophosphate (PRPP) synthetase was characterized. Despite the lack of a detectable PRPP pool or PRPP synthetase activity at 40°C, the strain was fully viable at this temperature as long as guanosine, uridine, histidine, tryptophan, and nicotinamide mononucleotide were added to the growth medium. Viability of the strain was dependent upon mutations in genes of the nucleoside salvage pathways that improved the utilization of exogenous nucleosides. The properties of the strain are those expected of a PRPP-less strain and suggest that PRPP synthetase is dispensable for E. coli.

The metabolite 5-phospho-D-ribosyl-α-1-pyrophosphate (PRPP) is a biosynthetic precursor of purine and pyrimidine nucleotides, the pyridine nucleotide coenzyme NAD, and the amino acids histidine and tryptophan (8). In nucleotide synthesis, PRPP is used in the de novo pathways as well as in the auxiliary pathways by which bases are converted to the nucleotides. Thus, in Escherichia coli 10 enzymes utilize PRPP as a substrate (Fig. 1A). The synthesis of PRPP is catalyzed by PRPP synthetase (ATP:D-ribosyl-5-phosphate pyrophosphotransferase, EC 2.7.6.1) as follows: ribose 5-phosphate + ATP → PRPP + AMP. This enzyme, encoded by the prs gene (4–6), is believed to be essential for the growth of all organisms. The ultimate products of some of the PRPP pathways, i.e., purine and pyrimidine nucleotides, are impermeable to cells and therefore cannot be fed exogenously. Instead, in E. coli wild-type cells the nucleotides and nucleosides are catabolized rapidly to the nucleobases, and the nucleobases in turn are converted intracellularly to the nucleotides by consumption of PRPP (Fig. 1B) (3, 12, 14). Moreover, E. coli cells are impermeable to PRPP. In the present work, I describe conditions under which PRPP is apparently dispensable. These conditions were achieved by using a strain with a temperature-sensitive mutation within the prs gene and by mutational manipulation of the nucleoside salvage pathways, so that the ribonucleotides were directly phosphorylated to ribonucleotides rather than degraded to nucleobases. As a consequence of these mutations, the strain had a simultaneous requirement for purine, pyrimidine, and pyridine compounds as well as for histidine and tryptophan.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The E. coli K-12 strains used are shown in Table 1. Standard genetic techniques were used (4). The udp, deoD, and gsk alleles were manipulated in a hemA prs− host strain which was then transduced to hem− with a bacteriophage P1 lysate grown on HOS41 (prs-2) (5), and isogenic hem− prs-2 and hem− prs+ strains were obtained. The markers hemA and prs are very closely linked (4). The manipulation of the markers was performed in a hemA prs+ strain, rather than in a prs-2 strain, to avoid eventual counterselection of prs-2. Strains harboring prs-2 were maintained at 25°C. All udp strains contained the udp::Tn5 allele originally present in strain AM427 (A. Mironov, unpublished data), whereas all deoD strains contained the allele from strain S0446 (4). The gsk-3 allele was selected as described previously (7). The strains also contained metB relA spoF supF lamB rpsL and were derived from the defined wild-type strain S0003 (4). Growth curves were obtained by culturing the cells in the phosphate-buffered AB minimal salt medium of Clark and Maaløe (1). Glucose (0.2%) was used as the carbon source. Methionine (40 mg/liter) and thiamine (0.5 mg/liter) were added to all growth media. When indicated, the following compounds were added: guanosine (30 mg/liter), uridine (20 mg/liter), histidine (40 mg/liter), tryptophan (40 mg/liter), and nicotinamide mononucleotide (NMN; 33 mg/liter). Growth was recorded as absorbance in an Eppendorf photometer at 436 nm. An A436 of 1 (1-cm light path) corresponds to approximately $3 \times 10^8$ cells per ml.

Determination of PRPP and ribonucleoside triphosphate pools. Cells were grown in Tris-buffered minimal medium containing 0.3 mM P₄ (9). After at least six generations of exponential growth, carrier-free $^{32}$P₄ (Forsøgsanlaeg Rissø, Denmark) was added to a specific radioactivity of 1.1 to 4.8 TBq/mol. After approximately three generations of growth, samples were removed, and the PRPP and ribonucleoside triphosphates were extracted and separated by two-dimensional thin-layer chromatography on polyethyleneimine-imregnated cellulose plastic sheets (9). After the radioactive spots were identified by autoradiography, they were cut out, and the radioactivity was determined by liquid scintillation counting.

Assay of PRPP synthetase activity. Cells were grown exponentially in AB medium. At an A436 of 1, the cells were cooled, harvested, and washed in unsupplemented minimal medium. After an 80-fold concentration in 50 mM potassium phosphate buffer (pH 7.5), the cells were homogenized by sonication, and the debris was removed by centrifugation. The assay was conducted at 20°C by mixing 10 μl of prewarmed cell extract and 90 μl of prewarmed substrate mixture to give the following final concentrations: 50 mM...
potassium phosphate–50 mM glycine–KOH buffer, pH 9.5; 20 mM NaF; 5 mM ribose 5-phosphate; 3 mM \(^{32}P\)ATP (55 GBq/mol); 8 mM MgCl\(_2\). Samples of 10 \(\mu\)l were removed at intervals (1, 5, 10, and 20 min), mixed with 5 \(\mu\)l of 1.0 M HCOOH, and applied to polyethyleneimine-cellulose thin-layer chromatographic plates. After drying, the chromatograms were developed in 0.85 M KH\(_2\)PO\(_4\) (adjusted to pH 3.4 with 0.85 M H\(_3\)PO\(_4\)). After autoradiography the PRPP spots were cut out, and the radioactivity was determined by liquid scintillation counting (9). The protein content was determined with bovine serum albumin as the standard (11).

**RESULTS**

**Rationale for establishing presumptive PRPP-less conditions.** Because of the various pathways that utilize PRPP, a PRPP-less strain becomes phenotypically Pur~ Pyr~ His~ Trp~ Nad~ (Fig. 1A) and needs to be supplied with exogenous sources of purine, pyrimidine, and pyridine nucleotides together with histidine and tryptophan. Purine and pyrimidine compounds are most conveniently offered as the ribonucleosides. However, complex pathways exist for the metabolism of ribonucleosides (Fig. 1B). Wild-type strains degrade the nucleosides to the bases, catalyzed by nucleoside phosphorylases (3). The base moieties in turn may be converted to nucleotides by consumption of PRPP. Alternatively, the ribonucleosides can be phosphorylated directly to the ribonucleotides by ribonucleoside kinases. Phosphorylation (i.e., degradation) is much more predominant than phosphorylation, and under PRPP-less conditions the nucleosides are almost exclusively converted to bases (Fig. 1B) (3, 12, 14). Mutational inactivation of uridine phosphorylase (upd) and purine nucleoside phosphorylase (deoD) prevent the degradation of the nucleosides, and the kinase-catalyzed reactions are the sole functioning pathways of nucleoside metabolism. Uridine and guanosine, therefore, are converted to UMP and GMP exclusively via phosphorylation by uridine kinase (adk) and guanosine kinase (gsk), respectively; i.e., purine and pyrimidine nucleotides may be synthesized by PRPP-independent pathways (Fig. 1B and C) (12, 14). In the case of purine nucleosides, a deoD mutation is not sufficient for efficient utilization of guanosine. Thus, pur deoD strains cannot use purine ribonucleosides as the sole purine source (7, 10, 14), and it might be expected that a PRPP-less (Pur~) deoD strain could not be supplemented with a proper purine source. For this reason the gsk-3 allele was introduced (strain HO608 and its derivatives). Strains harboring this allele contain a threefold-increased specific activity of guanosine kinase (unpublished results). The effect of this mutation, therefore, is to improve the utilization of guanosine.

NMM was chosen as source of NAD. NMN is taken up intact by the cells and deaminated to nicotinic acid mononucleotide and then NAD (2).

**FIG. 1. Rationale for establishing PRPP-less conditions.** (A) Pathways that require PRPP as a precursor. Solid arrows indicate de novo pathways leading to purine (Pur), pyrimidine (Pyr), and NAD nucleotides and to the amino acids histidine (His) and tryptophan (Trp). Broken arrows indicate the consumption of PRPP by the auxiliary pathways which by exogenously added or endogenously formed bases are converted to the nucleotides. (B) General scheme for salvage and interconversion of purine and pyrimidine nucleotides, nucleosides, and bases in wild-type strains. Enzymatic reactions are indicated by numbers as follows: 1, guanosine kinase or uridine kinase; 2, purine nucleoside phosphorylase or uridine phosphorylase; 3, phosphoribosyltransferases. Heavy arrows indicate the predominant pathways. (C) In phosphorylase-deficient strains, the nucleosides can be phosphorylated (12, 14). The metabolic pathways of pyridine compounds are slightly different, in that they do not usually involve nucleosides. Instead exogenous NMN is taken up intact and converted to nicotinic acid mononucleotide and then NAD (2).

**Growth properties of the prs-2 strain.** Analysis of strain HO609 showed that guanosine, uridine, histidine, tryptophan, and NMN had to be added to the growth medium to support growth at 37°C and higher temperatures. Figure 2 shows the response obtained when the prs-2 strain and an isogenic prs~ strain were grown at 40°C in a medium containing all five components and shifted to media lacking one of the compounds. Removal of either one of the nucleosides or one of the amino acids caused a rapid cessation of growth of the prs-2 strain, whereas removal of NMN caused a slow cessation, typical of deprivation of a vitamin (Fig. 2A). Growth of the prs~ strain did not change, except for a small stimulation in the absence of uridine (Fig. 2B). The prs-2 strain may be regarded as an auxotrophic strain, requiring the simultaneous addition of guanosine, uridine, histidine, tryptophan, and NMN (Fig. 2A). At lower temperatures the response was different: at 30°C the prs-2 strain did not require NMN or tryptophan for growth, whereas at 20°C it did not require NMN, tryptophan, or histidine (data not shown). The behavior of strain HO609 described above is exactly that expected of a strain in which the synthesis of PRPP is limited in a temperature-dependent way.

**PRPP pool and PRPP synthetase activity in the prs-2 strain.** To determine whether the growth response obtained for the

<table>
<thead>
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<th>TABLE 1. Bacterial strains</th>
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<tr>
<td>Strain</td>
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<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>HO608</td>
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<tr>
<td>HO609</td>
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<tr>
<td>HO634</td>
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<tr>
<td>HO635</td>
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<td>HO636</td>
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<tr>
<td>HO637</td>
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<td>HO638</td>
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<td>HO639</td>
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* Gene designations: prs, PRPP synthetase; upd, uridine phosphorylase; deoD, purine nucleoside phosphorylase; gsk, guanosine kinase.
prs-2 strain was, indeed, caused by limitations in PRPP, the PRPP and ribonucleoside triphosphate pools were determined in HO609 (prs-2) and HO608 (prs+) grown at various temperatures with the nucleosides, the amino acids, and NMN present (Table 2). At 20°C, the pool sizes of the prs-2 strain were close to those of the wild-type strain, except that the PRPP pool was reduced to 50%. However, this reduction in the PRPP pool did not result in slower growth; the two strains grew at identical rates. At 30°C, the PRPP pool of the prs-2 strain was greatly reduced, whereas the ribonucleoside triphosphate pool sizes, except that of ATP, were nearly normal as compared with HO608 (prs+) at 30°C. Finally, at 40°C the prs-2 strain contained no detectable PRPP. Under these conditions, the purine ribonucleoside triphosphate pools were somewhat reduced, whereas the pyrimidine ribonucleoside triphosphate pools were much less abnormal. Despite the lack of a detectable PRPP pool, the prs-2 strain showed exponential growth, although at a reduced rate compared with the otherwise isogenic prs+ strain.

The lack of PRPP in the prs-2 strain was caused by nonfunctioning of PRPP synthetase (Table 2). HO609 (prs-2) contained no assayable PRPP synthetase activity in extracts of cells grown at 40°C. The PRPP synthetase activity in HO609 grown at 30°C was 15% of the activity in the prs+ strain, whereas the PRPP pool in the prs-2 strain was only a few percent of the PRPP pool in the wild-type strain. Also, when cells were grown at 20°C the PRPP synthetase activity of the prs-2 strain was reduced as compared with the prs+ strain.

**TABLE 2.** PRPP and ribonucleoside triphosphate pool sizes and PRPP synthetase activity in prs-2 and prs+ strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth temp (°C)</th>
<th>Doubling time (min)</th>
<th>Pool size (nmol/mg dry weight)</th>
<th>PRPP synthetase activity (nkat/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PRPP</td>
<td>ATP</td>
</tr>
<tr>
<td>HO609 prs-2</td>
<td>20</td>
<td>189 (204)</td>
<td>0.23</td>
<td>3.00</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>78 (72)</td>
<td>0.03</td>
<td>2.47</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>109 (73)</td>
<td>&lt;0.01</td>
<td>1.31</td>
</tr>
<tr>
<td>HO608 prs+</td>
<td>20</td>
<td>189 (228)</td>
<td>0.50</td>
<td>4.02</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>67 (67)</td>
<td>0.81</td>
<td>4.40</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>60 (52)</td>
<td>0.56</td>
<td>4.19</td>
</tr>
</tbody>
</table>

* Cells were grown with glucose as the carbon source and with guanosine, uridine, histidine, tryptophan, and NMN present.
* Values obtained in medium with 0.3 mM F. In parenthesis are the values obtained in AB medium (64 mM F).

Effect of the mutations in udg, deoD, and gsk. prs-2 and prs+ strains that lacked the various mutant alleles were constructed (Table 1). Figure 3A shows the behavior of these prs-2 strains grown at 25°C and shifted to 40°C with all the required components present. From the data given in Table 2, it is expected that the prs-2 cells exhaust their PRPP pool after the shift in the temperature. Strain HO609 continued exponential growth with a stimulation in growth rate upon the shift. The deo+ strain (HO637) displayed a quite different growth curve. This strain stopped growing after two to three generations of exponential growth. At this time, all of the added guanosine had been incorporated into nucleic acids or converted to purine bases, which cannot be converted to nucleotides if there is no PRPP available. In addition lack of PRPP prevents de novo purine synthesis. The guanosine salvage pathways of strain HO637 compared with those of strain HO609 are illustrated in Fig. 4A. In contrast, the gsk+ derivative (HO635) immediately ceased exponential growth upon the shift, and the culture became stationary after about 12 h of incubation. The reason for the lack of growth of HO635 at 40°C is that guanosine is not properly salvaged, due to inefficiency of the wild-type guanosine kinase, and there is no PRPP available for de novo purine nucleotide synthesis. The guanosine salvage pathways of strain HO635 are given in Fig. 4A. The kinetics of the cessation of growth of strain HO635 indicates dilution of some vital cellular component. This may very likely be PRPP synthetase (and thence PRPP) by assuming that the enzyme once formed at 25°C is stable, even at 40°C, and that enzyme formed

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**FIG. 2.** Growth responses of prs-2 and prs+ strains to shifts in medium composition. Cells were grown exponentially for at least five generations at 40°C in AB medium supplemented with guanosine, uridine, histidine, tryptophan, and NMN. At an A460 of 0.8, the cells were cooled rapidly, harvested, washed three times, and suspended in cold unsupplemented medium. The cells were then inoculated at time zero (arrow) to A460 of approximately 0.1 in fresh medium with the same composition as before or in medium in which one of the supplements was omitted. (A) HO609 (prs-2); (B) HO608 (prs+). Abbreviations: Con, control medium containing all five compounds; -Guo, medium lacking guanosine; -Urd, medium lacking uridine; -His, medium lacking histidine; -Trp, medium lacking tryptophan; -NMN, medium lacking NMN.
postshift is inactive. In fact, after an upshift in temperature, the specific activity of PRPP synthetase decreased in a manner reciprocal to the growth curve of strain HO635 (data not shown).

Figure 3B shows the results of similar growth experiments with the prs' strains that were otherwise isogenic with those used for Fig. 3A. These prs' strains did not require any of the added compounds, and there was no cessation of growth beyond the shift in the temperature of strain HO608, HO634, or HO636. The udp' strains (HO639 [prs-2]) in Fig. 3A, HO638 [prs'] in Fig. 3B) slowed growth at 25°C even before reaching the cell density of the temperature shift, indicating rapid phosphorolysis of uridine to uracil and ribose 1-phosphate (Fig. 4B). The reason for this behavior is at present unknown. However, it seems to be partly due to the presence of the gsk-3 allele, which in some way causes inhibition of the pyrimidine de novo synthesis when guanosine is present and uridine absent (data not shown).

**DISCUSSION**

The pool and enzyme analyses described above show that an organism under certain conditions may be completely deficient in detectable PRPP and yet fully viable. This deficiency created, at the restrictive temperature, a multiple requirement for guanosine, uridine, NMN, histidine, and tryptophan in the strain containing the prs-2 allele.

The results of analysis of the growth of this strain indicate that there is a hierarchy in the utilization of PRPP by the various pathways. Thus, removal of the nucleosides is lethal to the prs-2 strain at all temperatures. Removal of the amino acids is much less severe, and removal of NMN is even less severe. This effect, likely, is a composite of the efficiency with which PRPP is used by the pathways and of the quantitative demand of PRPP for the various PRPP end products. It has been calculated that purine and pyrimidine nucleotide synthesis each consume 30 to 40% of the PRPP synthesized, histidine and tryptophan each consume 10 to 15% whereas only 1 to 2% is used for NAD synthesis (8). The results furthermore confirm the accumulated information about PRPP as precursor in the purine, pyrimidine, and pyridine nucleotide pathways and in histidine and tryptophan biosynthesis. In addition, the fact that strain HO609 is viable only when supplied with the relevant compounds demonstrate that no PRPP-utilizing pathways have been overlooked.

**FIG. 4.** Ribonucleoside metabolism in strains with wild-type alleles of deoD, gsk, or udp. The pathways operating under PRPP-less conditions are given. (A) Pathways for conversion of guanine-containing compounds of strains HO609, HO637 (deo'), and HO635 (gsk'). Heavy arrows indicate the predominant pathways. The broken arrow indicate wild-type guanosine kinase, which functions very poorly. (B) Pathways for conversion of uracil-containing compounds of strains HO609 and HO639 (udp'). Heavy arrows indicate the predominant pathways; the light arrow indicates the less predominant uridine kinase reaction in the udp' strain.
The results presented in Fig. 3A show that the presence of the gsk-3 allele is indispensable for establishing the phenotype of HO609, i.e., growth at 40°C in the presence of guanosine, uridine, histidine, tryptophan, and NMN. The mutant deoD allele is semidispensable; its presence improves the growth properties of the strain. The strains harboring the deoD allele only inefficiently utilize guanosine, but this inefficiency can be overcome by increasing the guanosine concentration. The mutant udp allele was also necessary to achieve optimal growth of theprs-2 strain. The udp allele was also required by theprs+ strain, so that the impact of udp on prs-2 could not be directly assessed.

The results obtained by analysis of strain HO609 at the restrictive temperature are those expected for a strain that is completely defective in PRPP synthesis; (i) no detectable PRPP pool or PRPP synthetase activity; (ii) requirement for purine, pyrimidine, and pyridine compounds and for histidine and tryptophan; and (iii) impaired growth when the nucleosides can be cleaved to the nucleobases. These results very strongly suggest that PRPP, and therefore PRPP synthetase activity, is dispensable forE. coli. Proof of this hypothesis, however, depends on the isolation of a true null mutation in theprs gene, i.e., a nonsense, an insertion, or a deletion mutation. An implication of the results is that prs mutants may be obtained by screening mutants for simultaneous requirements for purine, pyrimidine, and pyridine compounds as well as for histidine and tryptophan in adeoD udp gsk-3 genetic background.

The experiments described in the present work are based on a detailed knowledge of the nucleoside salvage pathways as they occur in E. coli. The rationale, i.e., the exchange of nucleoside phosphorylase reactions by nucleoside kinase reactions, may be valid in other organisms as well. However, in organisms that do not possess ribonucleoside kinases, it would not be possible to supply nucleotides without the intermediate formation of nucleobases, and thus PRPP-less conditions may not be established. Whereas pyrimidine ribonucleoside kinases are widely distributed among microorganisms (12), purine ribonucleoside kinases have been discovered only inE. coli and Salmonella typhimurium (14).

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LITERATURE CITED