JOURNAL OF BACTERIOLOGY, Apr. 1983, p. 177–184
0021-9193/83/040177-08$02.00/0

Vol. 154, No. 1

Chromosomal Location of the Gene Encoding Phosphoribosylpyrophosphate Synthetase in *Escherichia coli*

BJARNE HOVE-JENSEN

Enzyme Division, Institute of Biological Chemistry B, DK-1307 Copenhagen K, Denmark

Received 17 September 1982/Accepted 24 January 1983

A mutant of *Escherichia coli* with a partially defective phosphoribosylpyrophosphate synthetase (ribosephosphate pyrophosphokinase) has been characterized genetically. The genetic lesion causing the altered phosphoribosylpyrophosphate synthetase, *prs*, was mapped at 26 min on the linkage map by conjugation. Transductional analysis of the *prs* region established the gene order as *purB-fadR-dadR-tre-pth-prs-hemA-trp*. Two additional mutations were identified in the mutant: one in *gsk*, the gene encoding guanosine kinase, and one in *lon*, conferring a mucoid colony morphology. The contribution of each mutation to the phenotype of the mutant has been evaluated.

Phosphoribosylpyrophosphate (PRPP) synthetase (ribosephosphate pyrophosphokinase; ATP:D-ribose-5-phosphate pyrophosphotransferase, EC 2.7.6.1) catalyzes the formation of PRPP from ATP and ribose-5-phosphate. PRPP is an important intermediate in cellular metabolism, being a component in the biosynthesis of purine and pyrimidine nucleotides, of the coenzymes NAD^+ and $NADP^+$, and of the amino acids histidine and tryptophan. In nucleotide and coenzyme synthesis, PRPP is consumed by both the de novo pathways and by auxiliary pathways, i.e., enzymatic reactions utilizing exogenously added or endogenously formed adenine, hypoxanthine, xanthine, guanine, uracil, and nicotinic acid for nucleotide synthesis. Thus, a total of 10 enzymes compete for PRPP as a substrate in *Escherichia coli* and *Salmonella typhimurium*. The PRPP synthetase reaction therefore constitutes the branch point of a highly branched pathway and is subjected to metabolic regulation (24, 32). Among the many compounds formed from PRPP, ADP has been shown to be the most potent inhibitor of PRPP synthetase from *S. typhimurium* (6, 30). In addition, it has been shown that a pyrimidine nucleotide is involved in the repression of the synthesis of the enzyme (22). Otherwise, the genetics of the synthesis of PRPP synthetase and its regulation have not been elucidated. The isolation of a mutant of *E. coli* with defective PRPP synthetase has been reported from this laboratory (12). The mutant was isolated from a purine-requiring strain defective in purine nucleoside phosphorylase and adenine phosphoribosyltransferase (i.e., genotype *purE deoD apt*). This strain cannot utilize nucleosides as purine sources. Mutants able to grow with guanosine as the sole purine source were selected. The properties of one of the mutants (strain SØ1172) include, besides the ability to use guanosine (or adenosine or inosine) as the purine source, an increased K_m for ribose-5-phosphate and ATP of PRPP synthetase. The K_m values of the mutant enzyme were 240 μM and 1 mM for ribose-5-phosphate and ATP, respectively, with comparative values of 45 and 60 μM for the wild-type enzyme. The specific activity of PRPP synthetase of the mutant was double that of the parent. Furthermore, the regulation of the purine de novo pathway was altered in the mutant, as evidenced by the reduced accumulation of aminoimidazoleriboside, the substrate of the enzyme encoded by *purE*, in the mutant during purine starvation. The genetic lesion responsible for the altered PRPP synthetase has been designated *prs* (12). The present work describes the genetic characterization of strain SØ1172.

MATERIALS AND METHODS

Bacterial strains. The strains used and their genotypes are listed in Table 1. All the strains are derivatives of *E. coli* K-12. Strain SØ003 and its derivatives were found to harbor an amber suppressor. This suppressor mapped between *trp* and *hemA* (data not shown) and was assumed to be *supF*. Strain SØ003 was usually used as a donor in removing nutritional requirements by transduction. Isolation of strains with insertions of Tn*10* (a transposable element coding for tetracycline resistance [Tc^r]) was performed as described previously (16). A random pool of approximately 3×10^4 independent Tn*10* insertions was generated and used for the preparation of a P1 lysate. Strain HO276 *hemA* was infected with the P1 (Tn*10* pool) lysate, and Hem^+ Tc^r recombinants were selected. Nomenclature for insertions of transposable elements follow the rules of Chumley et al. (4). The

TABLE 1. Bacterial strains[a]

Strain	Sex	Genotype	Parent/derivation/source
SØ003	F^-	*rpsL relA supF metB*	*E. coli* K-12 derivative of strain 58-161F^+
SØ446	F^-	*purE deoD apt*[b]	P. Nygaard (15) from SØ003
SØ1172	F^-	*lon gsk*[c] *prs*[b,d]	(12) from SØ446
HO47	F^-	*lacY*[b,d]	(12) from SØ446
HO52	F^-	*lon gsk prs lacY pyrF*[b,d]	(12) from SØ1172
HO54	F^-	*lon gsk prs proC*[b,d]	SØ1172 was made *lacY*[e] and then *lac*$^+$ *proC* with P1(AT3134)
HO72	F^-	*gsk prs*[b,d]	HO54 with P1(SØ003), Pro^+
HO74	F^-	*lon*[b,d]	HO47, transduction to Lac^+, $ProC^-$ and Pro^+, Lon^- with P1(SØ1172)
HO82	F^-	*pyrF lacY*[b,d]	(12) from HO47
HO86	HfrH	*pyrF thi deoD gyrA lacY*	AT2242 was made *deoD*[f], then *gyrA* (10) and *lacY*
HO88	HfrH	*pyrF thi purE deoD apt gyrA*	HO86 was made F^- phenocopy (20) and conjugated with HO112, selecting *lac*$^+$ *gyrA*
HO112	HfrH	*rpsL relA supF purE deoD apt*	SØ446 was conjugated with AT2242 for 3 h, selecting Met^+, Sm^r and screening Hfr donor potency (20)
HO190	F^-	*gsk prs pyrF*[b,d]	Lon^+ of HO52, as HO72 via *proC*
HO193	F^-	*lon gsk prs proC thr*[b,d]	HO54 was made *deoB* and then *deoB*$^+$ *deoD thr* with P1(H913)
HO226	F^-	*fadR prs hemA his tyrA thi rpsL*	RS3059 with P1(SØ1172), Pur^+
HO276	Hfr	*metB tre hemA*	RS3039 with P1(CB962), Pur^+
HO278	F^-	*purB dadR pth*	JK268 with P1(AA7852), Trp^+
HO279	F^-	*purB dadR pth trpE trpA zch-2410*::Tn*10*	HO278 with P1(*trpE trpA zch-2410*::Tn*10*), Tc^r
HO286	F^-	*lacY pyrF srl*::Tn*10 recA*[b,d]	HO82 with P1(NK5304), Tc^r
HO287	F^-	*gsk prs pyrF recA srl*::Tn*10*[b,d]	HO190 as HO286
HO299	F^-	*fadR prs hemA his tyrA thi rpsL trpE trpA zch-2410*::Tn*10*	HO226 with P1(*trpE trpA zch-2410*::Tn*10*), Tc^r
HO300	F^-	*dadR tre pth trpE trpA zch-2410*::Tn*10*	HO279 with P1(CB962), Pur^+
HO349	F^-	*dadR tre pth trpE trpA*	HO300 was made Trp^+, $PyrF^-$, Tc^s and then Pyr^+, Trp^- with P1(JK268)
HO359	F^-	*gsk prs trpE trpA*[b,d]	HO190 with P1(JK268), Pyr^+
HO360	F^-	*fadR*::Tn*10 dadR tre pth hemA trpE trpA*	HO349 with P1(RS3039), Tc^r
HO402	Hfr	*metB zcg-2402*::Tn*10*	HO276, Tn*10* insertion near *prs*
HO410	Hfr	*metB tre zch-2410*::Tn*10*	HO276, Tn*10* insertion near *prs*
AA7852	F^-	*pth*[g]	J. Menninger (19)
AT2242	HfrH	*pyrF thi*	A. L. Taylor, CGSC[h]
AT3134	F^-	*proC*[g]	CGSC
CB962	F^-	*tre*[g]	(2)
H913	F^-	*thr*[g]	deHaan, CGSC
JK268	F^-	*purB dadR trpE trpA*	Identical to JK266, Hadar et al. (9), CGSC
KLF25/KL181	F′	F125 *pyrD*$^+$ *trp*$^+$/*thi pyrD his trp recA rpsL*	K. B. Low (17), CGSC
NK5304	Hfr	*srl*::Tn*10 recA*[g]	N. Kleckner
RS3039	Hfr	*purB fadR13*::Tn*10 hemA metB*	R. Simons (27)
RS3059	F^-	*purB fadR hemA his tyrA thi rpsL*	R. Simons (27)

[a] Gene symbols are from Bachmann and Low (1).
[b] Also harbors *rpsL relA supF metB*.
[c] This mutation results in a functional gene product with altered enzymatic properties.
[d] Also harbors *purE deoD apt*.
[e] Mutations in *lacY* were selected as described (28).
[f] The *deoD* strains were constructed as follows. Low-thymine-requiring derivatives (*thy deoB*) were prepared (3, 29). Then, *deoB* was exchanged with *deoD* with P1(SØ446), and *thy* was removed by transduction.
[g] Relevant genotype.
[h] Obtained through Coli Genetic Stock Center (CGSC).

insertion *zcg-2402*::Tn*10* mapped at 26 min on the linkage map (1); *zch-2410*::Tn*10* mapped at 27 min.

Genetic procedures. Conditions for cell growth were as described previously (12). Uninterrupted conjugation, transduction with P1 *vir*, and episome transfer were performed as described by Miller (20). Recombinants from conjugations, recombinants from transductions, and sexductants were always reisolated at least once before use. Hem$^+$ transductants were selected on succinate minimal plates lacking δ-aminolevulinic acid, and *hemA* was scored on L-broth plates; *fadR* was scored on plates with decanoic acid as the carbon source (27); *dadR* was scored on plates with D-tryptophan serving as tryptophan source (9); *tre* was scored on MacConkey or eosin-methylene blue indicator plates supplemented with 1% trehalose (2); *pth*, a temperature-sensitive marker, was scored at 42°C (19). Tc^r was selected and screened on L-broth plates containing tetracycline (5 μg/ml). Excretion of aminoimidazoleriboside was assayed as described previously (12).

Assay of PRPP synthetase. One unit of enzyme activity was defined as the amount catalyzing the formation of 1 nmol of product per min at 37°C. Protein was determined by the method of Lowry et al. (18) with bovine serum albumin as the standard. PRPP synthetase was assayed by the method of Jensen et al. (13). To discriminate between wild-type and mutant PRPP synthetase in genetic experiments, a modified assay was employed. Recombinants were grown for 24 h at either 37 or 30°C in 0.5 ml of minimal medium with glucose limitation (0.08%). These cultures were treated with 5 μl of toluene-ethanol (1:1, vol/vol) followed by vortexing for 1 s and incubation at 37°C for 30 min. Cells (10 μl) were added to 40 μl of assay mixture to give the following final concentrations as compared with those of the assay of Jensen et al. (13), which are given in parentheses: 50 mM potassium phosphate buffer, pH 7.5 (50 mM); 20 mM NaF (20 mM); 3 mM $MnCl_2$ (3 mM); 0.5 mM ribose-5-phosphate (5 mM); 150 μM ATP (3 mM). The assay contained 15 nCi of [γ-^{32}P]ATP. Assays were performed at 37°C for 100 min; 25 μl of 0.33 M HCOOH was added, and the assay mixtures were cooled on ice. Then, 15 μl was applied on polyethyleneimine cellulose-coated thin-layer plates (Baker-flex), dried with cold air, and developed in 0.85 M potassium phosphate, pH 3.4. The plates were dried and exposed to X-ray film (Curix, Agfa) overnight.

Chemicals. All chemicals were obtained from commercial sources, except that 2',3'-dideoxyadenosine was obtained from Drug Research and Development, National Cancer Institute, Bethesda, Md.

RESULTS

The PRPP synthetase-defective strain (SØ1172) displays a complex phenotype (12). The properties useful for genetic characterization include the ability of the mutant to use guanosine as the sole purine source, increased K_m values for the substrates of PRPP synthetase, and a reduced excretion of aminoimidazoleriboside during purine starvation.

Effect of 2',3'-dideoxyadenosine. Among a number of purine and pyrimidine analogs and histidine and tryptophan analogs tested, only 2',3'-dideoxyadenosine was found to have a selective effect on the growth of the mutant strain. The effect of this analog was shown to be due to both the *lon* and the *prs* mutations. Apparently the double mutant SØ1172 (*prs lon*) is more sensitive than either HO72 (*prs*) or HO74 (*lon*). The last two strains seem to be equally sensitive (Table 2). However, the fact that *lon* strains form mucoid colonies allows discrimination between *prs lon*$^+$ and *prs*$^+$ *lon*.

TABLE 2. Effect of 2',3'-dideoxyadenosine on *prs* and *lon* strains

Strain	Genotype[a]		Growth response to 2',3'-dideoxyadenosine at a concn (μM)[b] of:		
	lon	*prs*	0	10	50
SØ446	+	+	+	+	+
HO74	−	+	+	+	−
HO72	+	−	+	+	−
SØ1172	−	−	+	−	−

[a] All strains were *purE deoD apt*.

[b] Growth was scored on minimal agar plates after 24 h at 37°C. Plates contained glucose methionine, thiamine, hypoxanthine, and analog as indicated. Symbols: +, growth; −, lack of growth.

Screening assay of PRPP synthetase. To make possible the screening of recombinants for the PRPP synthetase phenotype (i.e., increased K_m for ribose-5-phosphate and ATP) independently of other phenotypes, a simple screening assay was developed. This assay was based on the kinetic properties of the mutant enzyme. Thus, the ATP concentration was far below the K_m value for ATP of the mutant enzyme. The activity of the wild-type enzyme, when determined by the screening assay, was 50% of the activity obtained when assayed under optimal conditions, whereas the residual activity of the mutant enzyme was only 1% of the activity obtained under optimal conditions. Figure 1 shows the result obtained by submitting 11 strains to the screening assay. The procedure allows an easy discrimination between mutant and wild-type PRPP synthetase.

Mapping by the gradient of transmission. Figure 2 shows the results of analysis of recombinants from two gradient of transmission experiments (5). The following conclusions can be drawn: (i) the *prs* locus, responsible for the altered PRPP synthetase, is located at 26 min; (ii) the locus responsible for the guanosine growth phenotype (tentatively designated GR) is located far away from *prs* at 12 min; (iii) the *lon* locus is found, as expected, at 10 min; (iv) PRPP synthetase, aminoimidazoleriboside excretion,

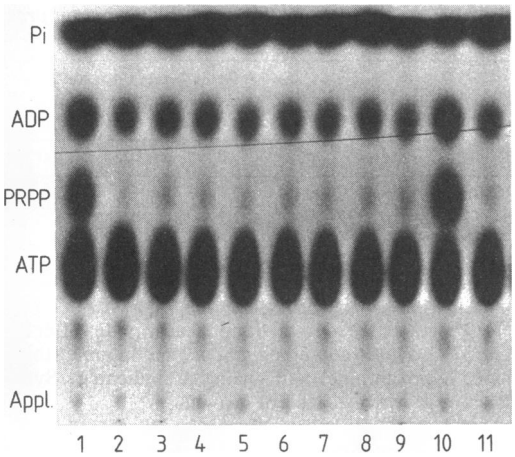

FIG. 1. Screening assay of PRPP synthetase. Cells were grown and PRPP synthetase was assayed as described in the text. The strains assayed were: lane 1, RS3059 *hemA prs*$^+$; lane 2, SØ1172 *hemA*$^+$ *prs*; lanes 3 through 11, *hem*$^+$ recombinants obtained by P1 transduction with SØ1172 as the donor and RS3059 as the recipient.

and 2',3'-dideoxyadenosine phenotypic traits are all displayed by the *prs* mutation (a 100% coincidence of the three phenotypes was found).

Transductional mapping of *prs*. The mapping data presented above suggest that the *prs* locus is located in the 25- to 27-min region of the linkage map. Therefore, attempts were made to map further the *prs* locus in the *purB-trp* region. Results of two four-factor crosses are shown in Table 3. The data indicate the gene order *purB-fadR-prs-hemA-trp*. Cotransduction of *purB* with *prs* was 4.5%, and cotransduction of *purB* with *hemA* was 2.8%. However, among the 10 *purB*$^+$ *hemA*$^+$ transductants, 9 inherited the *prs* mutant allele from the donor, showing linkage of *prs* with *hemA*, which would not be expected if the gene order were *prs-purB-hemA*. The cotransduction frequency of *purB* with *fadR* was 58%, giving the gene order above. Analogous rationalization with *hemA*$^+$ selection gives the same gene order. The cotransduction of *hemA* with *prs* was 82%. When *trp*$^+$ was the selected marker, *prs* was located as described above between *fadR* and *hemA* (Table 3).

To map further the *prs* locus relative to other genetic markers in the *fadR-hemA* region, the two crosses shown in Table 4 were carried out. By using a rationale similar to that above, it can be concluded that in the *hemA*$^+$ selection all the unselected markers are on the *fadR* side of *hemA*. In the Tn*10* selection all the unselected markers, except *dadR*, are on the *hemA* side of *fadR*. There was a slight discrepancy with respect to the location of the *dadR* marker. The two crosses in Table 4 placed *dadR* on both sides of *fadR*. With spontaneous *fadR* mutations (*fadR1* or *fadR12* [27]) in the donor and with strain JK268 *purB dadR* as the recipient, the gene order repeatedly was found to be *purB-fadR-dadR* (see Fig. 3 for cotransduction frequencies), whereas with *fadR13*::Tn*10*, the gene order was *purB-dadR-fadR*. This seems to suggest that a small inversion occurred in the *fadR13*::Tn*10* strain. Taking all results into consideration, the gene order of the markers is *purB-fadR-dadR-tre-pth-prs-hemA-trp*. A map of the *purB-trp* region of the linkage map is shown in Fig. 3.

Attempts were made to include the markers *rimC*, *gdh*, and *nirC* (1) on the map. Apparently,

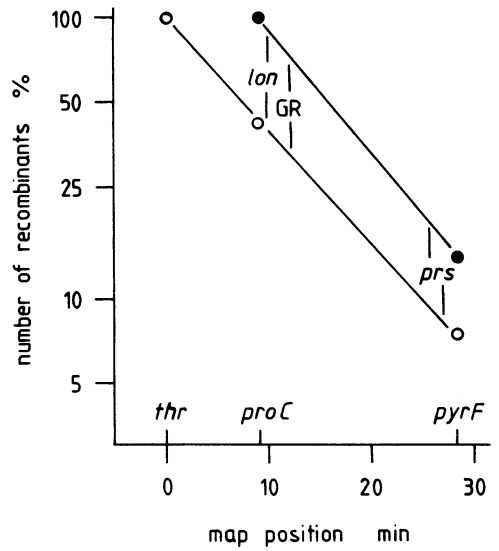

FIG. 2. Localization of *prs*, the GR locus, and *lon* by the gradient of transmission. GR designates the guanosine growth phenotype; GR$^+$ indicates the ability to use guanosine as the sole purine source, GR$^-$ indicates inability to use guanosine. Uninterrupted conjugations were performed for 2 h as described in the text. The donor strain was HO88 HfrH *purE deoD apt thi pyrF* GR$^-$. The selection plates contained hypoxanthine and guanosine as the purine sources. The presence of *prs* was scored by the screening assay of PRPP synthetase, resistance or sensitivity to 2',3'-dideoxyadenosine, and by excretion of aminoimidazoleriboside. GR was scored on plates with guanosine as the sole purine source; *lon* was scored by colony morphology on minimal plates (8). Symbols: ○, The recipient was HO54 F$^-$ *purE deoD apt proC lon* GR$^+$ *prs rpsL*; the selected phenotype was Pro$^+$, Sm^r; 351 recombinants were examined. ●, The recipient was HO193, a *thr* derivative of HO54; the selected phenotype was Thr$^+$, Sm^r; 345 recombinants were examined. The positions of *thr*, *proC*, and *pyrF* were taken from Bachmann and Low (1).

TABLE 3. Mapping of *prs* in the *purB-trp* region by transduction[a]

Donor	Recipient	Selected marker (no. scored)	Unselected markers	
			Genotype	No. of recombinants
SØ1172 *prs*	RS3059 *purB fadR hemA*	*purB*$^+$ (359)	*fadR prs*$^{+b}$ *hemA*	148
			fadR$^+$ *prs*$^+$ *hemA*	194
			fadR$^+$ *prs hemA*	6
			fadR$^+$ *prs hemA*$^+$	9
			fadR prs hemA	1
			fadR prs hemA$^+$	0
			fadR$^+$ *prs*$^+$ *hemA*$^+$	1
			fadR prs$^+$ *hemA*$^+$	0
		hemA^{+c} (116)	*fadR prs*$^{+b}$	20
			fadR prs	78
			fadR$^+$ *prs*	17
			fadR$^+$ *prs*$^+$	1
RS3039 *fadR*::Tn*10 hemA*	HO359 *trp prs*	*trp*$^{+d}$ (352)	*prs*^{e} *hemA*$^+$	240
			prs hemA	15
			prs$^+$ *hemA*	92
			prs$^+$ *hemA*$^+$	5

[a] Transductions were performed as described in the text.
[b] *prs* was scored by assay of PRPP synthetase.
[c] Three recombinants inherited the donor *purB* allele.
[d] Three recombinants inherited the donor *fadR* allele.
[e] *prs* was scored on plates with 2',3'-dideoxyadenosine.

none of these markers mapped within the *purB-trp* region since none showed linkage with *zcg-2402*::Tn*10* (joint transduction frequencies less than 0.5%).

As mentioned previously, the mutant had a twofold increase in PRPP synthetase activity. This property was found to be linked to the K_m defect: all *prs*$^+$ recombinants assayed under optimal conditions showed normal activity (40 U/mg of protein), and all *prs* recombinants had increased activity (90 U/mg of protein).

Analysis of *prs* merodiploid strains. The kinetic properties of the mutant PRPP synthetase presented here and elsewhere (12) indicate that the *prs* locus is the gene encoding PRPP synthetase. To further assess this aspect, the F125 episome, covering the *purB-trp* region, was transferred into *prs*$^+$ *recA* and *prs recA* strains (HO286 and HO287, respectively). A gene dosage effect was observed in the *prs*$^+$/F*prs*$^+$ strain since the total PRPP synthetase activity was 68 U/mg of protein in the merodiploid as compared with 40 U/mg of protein in the *prs*$^+$ haploid strain. This indicates that the gene encoding PRPP synthetase resides on the F125 episome. In the *prs*/F*prs*$^+$ strain, the PRPP synthetase activity showed kinetic properties different from those observed for both *prs* and *prs*$^+$ strains; the K_m value for ATP of the enzyme from the merodiploid strain was lower than that from the *prs* strain but higher than that from the *prs*$^+$ strain. Given the knowledge that PRPP synthetase is multimeric (26), the results presented above suggest that *prs* is the structural gene for PRPP synthetase.

Other properties of strain SØ1172. The data presented in Fig. 2 suggest that the guanosine growth property and the K_m defect of PRPP synthetase were caused by two independent mutations. The guanosine growth locus mapped at 12 min, indicating that the *gsk* gene, encoding guanosine kinase, might be affected. This was confirmed by further genetic analysis (data not shown). In accordance with these data, the guanosine kinase of strain SØ1172 has been shown to have altered thermal and kinetic properties in vitro (P. Nygaard, personal communication).

The growth properties of strains harboring either *gsk* or *prs* were analyzed. The data indicate that growth on guanosine is mainly caused by the *gsk* mutation and that growth is somewhat improved by introduction of *prs* in addition to *gsk*. Apparently there may be a selective advantage of the *gsk prs* double mutant over the *gsk* single mutant (data not shown).

Yet a third genetic lesion in strain SØ1172 was identified within the *lon* gene (Fig. 2). Analysis of a *lon*$^+$ derivative (HO72) of the mutant as well as a *lon* derivative (HO74) of the parent revealed no effect of *lon* on PRPP or purine nucleotide metabolism as examined by analysis of PRPP

TABLE 4. Mapping of *prs* in the *fadR-hemA* region by transduction[a]

Donor	Recipient	Selected marker (no. scored)	Unselected markers						No.
			fadR	*dadR*	*tre*	*pth*	*prs*	*hemA*	
HO300 *dadR tre pth*	HO299 *fadR prs hemA*	*hem*$^+$ (368)	−	+	+	+	−[b]		57
			−	+	+	+	+		118
			−	+	+	−	+		99
			−	+	−	−	+		39
			−	−	−	−	+		9
			+	−	−	−	+		37
			−	+	+	−	−		5
			−	+	−	+	+		2
			−	−	+	−	+		1
			+	−	+	−	−		1
HO360 *fadR*::Tn*10* *dadR tre pth hemA*	HO359 *prs*	Tn*10* (1,065)		+	+	+	−[c]	+	128
				−	+	+	−	+	383
				+	−	+	−	+	22
				−	−	+	−	+	163
				+	−	−	−	+	1
				−	−	−	−	+	23
				+	−	−	+	+	32
				−	−	−	+	+	6
				+	−	−	+	−	243
				−	−	−	+	−	38
				+	+	−	−	+	1
				−	+	−	−	+	4
				+	+	−	+	+	3
				+	+	−	+	−	1
				+	+	+	+	+	2
				+	+	+	+	−	1
				+	+	+	−	−	2
				−	+	+	−	−	2
				+	−	+	+	+	1
				−	−	+	+	+	1
				+	−	+	+	−	4
				−	−	+	+	−	1
				−	−	+	−	−	2
				−	−	−	−	−	1

[a] Transductions were performed as described in the text.
[b] *prs* was scored by assay of PRPP synthetase.
[c] *prs* was scored on plates with 2',3'-dideoxyadenosine.

synthetase, PRPP, and nucleotide pools (data not shown).

DISCUSSION

This report presents, for the first time, a detailed genetic characterization of a mutant of a microorganism with altered PRPP synthetase. The strain was isolated as a mutant with improved utilization of guanosine during a study of guanosine utilization for nucleotide synthesis in *E. coli* (12). Both the phenotype and the genotype of the characterized mutant, strain SØ1172, were complex. At least two mutations were necessary for optimal growth on guanosine. One of the mutations affected a new gene (*prs*), resulting in the synthesis of a PRPP synthetase with altered K_m, suggesting a mutation within the structural gene for this enzyme. The second mutation mapped in the *gsk* gene. It is not completely understood why such a *gsk prs* double mutant appeared by the selection procedure employed (12). However, a number of other mutants obtained in the same selection were double mutants, namely *gsk purF*, *gsk purG*, or *gsk purI* (unpublished results). Similarly, mutants with secondary blocks in the purine de novo pathway have been selected from a *purE deoD* strain of *S. typhimurium* (7).

The growth of the *prs* mutant was inhibited by the deoxyribonucleoside analog 2',3'-dideoxyadenosine. The biochemical basis of the effect of the analog is not resolved. In vitro, there was no effect of the compound on PRPP synthetase activity (data not shown), suggesting that the compound has to be metabolized or that the target of the compound is not PRPP synthetase itself. In cells of higher organisms, the synthesis of PRPP has frequently been reported to be

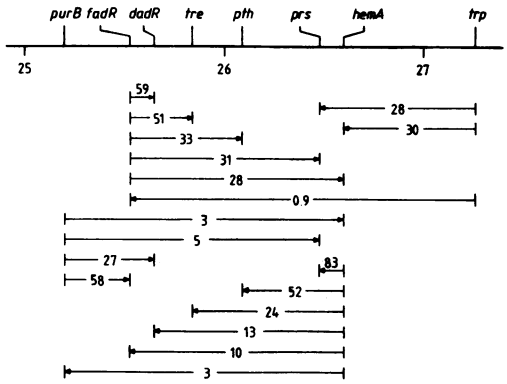

FIG. 3. A map of the *purB-trp* region of the *E. coli* chromosome. Values are percentages of joint transduction based on data from Tables 3 and 4. Arrowheads point to the unselected markers.

inhibited by nucleoside analogs such as 2',3'-dideoxyadenosine, 2',5'-dideoxyadenosine (31), or 5'-deoxyadenosine (11).

The *prs* locus was mapped by a series of four- and six-factor crosses. Since in many crosses the Prs phenotype was scored by assay of PRPP synthetase, such crosses were preferred over two- and three-factor crosses, thereby reducing the number of recombinants that had to be assayed. Transductional analysis revealed a location of the *prs* gene very close to *hemA*, and the detailed map of the *prs* region (Fig. 3) included several markers not previously located accurately. Since mutants with deficiencies in PRPP synthetase have been isolated in *S. typhimurium* (see below), it is of interest to note that the *prs* gene is flanked by genes known to be located on a chromosomal fragment with inverted gene orders between *E. coli* and *S. typhimurium* (1, 21, 25). These genes are *dadR*, *tre*, and *hemA* (2, 33, 34). Therefore, *prs* must also be on this fragment, and if a homologous locus is present in *S. typhimurium*, it should be located near *hemA*.

A PRPP synthetase mutant of *S. typhimurium* has been isolated by the same approach used for the *E. coli prs* mutant described here (14). The mutation was located at 7 map units on the *S. typhimurium* linkage map. However, more recent experiments have located the lesion approximately 2 map units clockwise of *pyrF* (B. Hove-Jensen, unpublished results), which is at 33 map units. This map position therefore seems to be analogous to the *prs* locus in *E. coli* and is in agreement with the prediction made above, i.e., the gene encoding PRPP synthetase in *S. typhimurium* is located on the inverted chromosomal segment near *hemA*.

Interpretation of the results is slightly complicated by the results obtained with another mutant of *S. typhimurium*. It was isolated by screening temperature-sensitive mutants for lack of PRPP synthetase activity in vitro (23). This mutation was mapped at 47 map units. The molecular defect of the latter mutant remains to be established. It is probably not the structural gene, as PRPP synthetase consists of only a single species of subunit (26). Alternatively, it may be a regulatory gene, or the gene product may be involved in post-translational modification of PRPP synthetase, although at present there is no evidence for such a modification of PRPP synthetase.

ACKNOWLEDGMENTS

I am grateful to B. Bachmann, R. Simons, M. C. Pascal, and C. F. Beck for kindly supplying bacterial strains and bacteriophage; to R. Kelln for carefully reading the manuscript; to P. Nygaard for valuable discussions; and to J. Steno Christensen and C. Jespersen for excellent technical assistance.

This work was supported by the Danish Natural Science Research Council.

LITERATURE CITED

1. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44**:1–56.
2. **Becerra de Lares, L., J. Ratouchniak, and F. Casse.** 1977. Chromosomal location of gene governing the trehalose utilization in *Escherichia coli* K12. Mol. Gen. Genet. **152**:105–108.
3. **Breitman, T. R., and R. M. Bradford.** 1964. The induction of thymidine phosphorylase and excretion of deoxyribose during thymine starvation. Biochem. Biophys. Res. Commun. **17**:786–791.
4. **Chumley, F., R. Menzel, and J. R. Roth.** 1979. Hfr formation directed by Tn*10*. Genetics **91**:639–655.
5. **de Haan, P. G., W. P. M. Hoekstra, C. Verhoef, and H. S. Felix.** 1969. Recombination in *Escherichia coli*. III. Mapping by the gradient of transmission. Mutat. Res. **8**:505–512.
6. **Gibson, K. J., K. R. Schubert, and R. L. Switzer.** 1982. Binding of the substrates and the allosteric inhibitor adenosine 5'-diphosphate to phosphoribosylpyrophosphate synthetase from *Salmonella typhimurium*. J. Biol. Chem. **257**:2391–2396.
7. **Gots, J. S., C. E. Benson, B. Jochimsen, and R. K. Koduri.** 1977. Microbial models and regulatory elements in the control of purine metabolism, p. 23–41. *In* Purine and pyrimidine metabolism: CIBA Foundation Symposium vol. 48. Elsevier, N.V., Uitg. Mij., Amsterdam.
8. **Gottesman, S., and D. Zipser.** 1978. Deg phenotype of *Escherichia coli lon* mutants. J. Bacteriol. **133**:844–851.
9. **Hadar, R., A. Slonim, and J. Kuhn.** 1976. Role of D-tryptophan oxidase in D-tryptophan utilization by *Escherichia coli*. J. Bacteriol. **125**:1096–1104.
10. **Hane, M. W., and T. H. Wood.** 1969. *Escherichia coli* K-12 mutants resistant to nalidixic acid: genetic mapping and dominance studies. J. Bacteriol. **99**:238–241.
11. **Henderson, J. F.** 1978. Effects of nucleoside analogs on purine metabolism. Pharmacol. Ther. Part A Chemother. Toxicol. Metab. Inhibitors. **2**:751–769.
12. **Hove-Jensen, B., and P. Nygaard.** 1982. Phosphoribosylpyrophosphate synthetase of *Escherichia coli*. Identification of a mutant enzyme. Eur. J. Biochem. **126**:327–332.
13. **Jensen, K. F., U. Houlberg, and P. Nygaard.** 1979. Thin-layer chromatographic methods to isolate ^{32}P-labeled 5-phosphoribosyl-α-1-pyrophosphate (PRPP): determination of cellular PRPP pools and assay of PRPP synthetase activity. Anal. Biochem. **98**:254–263.
14. **Jochimsen, B., B. Garber, and J. S. Gots.** 1980. Phosphori-

bosylpyrophosphate synthetase mutant in *Salmonella typhimurium*. Adv. Exp. Med. Biol. **122B**:131–136.
15. **Jochimsen, B., P. Nygaard, and T. Vestergaard.** 1975. Location on the chromosome of *Escherichia coli* of genes governing purine metabolism. Mol. Gen. Genet. **143**:85–91.
16. **Kleckner, N., D. F. Barker, D. G. Ross, and D. Botstein.** 1978. Properties of the translocatable tetracycline-resistance element Tn*10* in *Escherichia coli* and bacteriophage lambda. Genetics **90**:427–461.
17. **Low, K. B.** 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. **36**:587–607.
18. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
19. **Menninger, J. R., C. Walker, P. F. Tan, and A. G. Atherly.** 1973. Studies on the metabolic role of peptidyl-tRNA hydrolase. Mol. Gen. Genet. **121**:307–324.
20. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Mojica-A, T.** 1975. Transduction by phage P1CM*clr-100* in *Salmonella typhimurium*. Mol. Gen. Genet. **138**:113–126.
22. **Olszowy, J., and R. L. Switzer.** 1972. Specific repression of phosphoribosylpyrophosphate synthetase by uridine compounds in *Salmonella typhimurium*. J. Bacteriol. **110**:450–451.
23. **Pandey, N. K., and R. L. Switzer.** 1982. Mutant strains of *Salmonella typhimurium* with defective phosphoribosylpyrophosphate synthetase activity. J. Gen. Microbiol. **128**:1863–1871.
24. **Sadler, W. C., and R. L. Switzer.** 1977. Regulation of *Salmonella* phosphoribosylpyrophosphate synthetase activity *in vivo*. Deductions from pool measurement. J. Biol. Chem. **252**:8504–8511.
25. **Sanderson, K. E., and P. F. Hartman.** 1978. Linkage map of *Salmonella typhimurium*, edition V. Microbiol. Rev. **42**:471–519.
26. **Schubert, K. R., R. L. Switzer, and E. Shelton.** 1975. Studies of the quaternary structure and the chemical properties of phosphoribosylpyrophosphate synthetase from *Salmonella typhimurium*. J. Biol. Chem. **250**:7492–7500.
27. **Simons, R. W., P. A. Egan, H. T. Chute, and W. D. Nunn.** 1980. Regulation of fatty acid degradation in *Escherichia coli*: isolation and characterization of strains bearing insertion and temperature-sensitive mutations in gene *fadR*. J. Bacteriol. **142**:621–632.
28. **Smith, T. F., and J. R. Sadler.** 1971. The nature of lactose operator constitutive mutations. J. Mol. Biol. **59**:273–305.
29. **Stacey, K. A., and E. Simson.** 1965. Improved method for the isolation of thymine-requiring mutants of *Escherichia coli*. J. Bacteriol. **90**:554–555.
30. **Switzer, R. L., and D. C. Sogin.** 1973. Regulation and mechanism of phosphoribosylpyrophosphate synthetase. V. Inhibition by end products and regulation by adenosine diphosphate. J. Biol. Chem. **248**:1063–1073.
31. **Tyrsted, G., and A. C. Sartorelli.** 1968. Inhibition of the synthesis of 5-phosphoribosyl-1-pyrophosphate by 3'-deoxyadenosine and structurally related nucleoside analogs. Biochim. Biophys. Acta **155**:619–622.
32. **White, M. N., J. Olszowy, and R. L. Switzer.** 1971. Regulation and mechanism of phosphoribosylpyrophosphate synthetase: repression by end products. J. Bacteriol. **108**:122–131.
33. **Wild, J., and T. Klopotowski.** 1981. D-Amino acid dehydrogenase of *Escherichia coli* K12: positive selection of mutants defective in enzyme activity and localization of the structural gene. Mol. Gen. Genet. **181**:373–378.
34. **Wild, J., W. Walczak, K. Krajewska-Grynkiewicz, and T. Klopotowski.** 1974. D-Amino acid dehydrogenase: the enzyme of the first step in D-histidine and D-methionine racemization in *Salmonella typhimurium*. Mol. Gen. Genet. **128**:131–146.