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New Phosphoglucose Isomerase Mutants of *Escherichia coli*

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The mutants used to show that phosphoglucose isomerase, and glucose itself, are not essential components of *Escherichia coli* had not been characterized genetically, other than by mapping. We now describe two new *pgi* mutants, one amber and the other a Mu-phage insertion, presumably both complete inactivation mutations. The new mutations do not give a phenotype markedly different from those described earlier. However, they might be preferred for certain physiological studies, and we have prepared for this a new double mutant, strain DF214, with a Mu insertion in *pgi* and a deletion in *zwf* (glucose 6-phosphate dehydrogenase).

Mutants lacking phosphoglucose isomerase (*pgi*) are known in *Escherichia coli* and *Salmonella typhimurium*. They have been used to clarify pathways of glucose metabolism and, particularly is association with a glucose 6-phosphate dehydrogenase mutation (*zwf*), have been used in several physiological studies of the effect of inability to form or degrade glucose 6-phosphate (e.g., deoxyribonucleic acid glucosylation, hexose-phosphate transport, glycogen and lipopolysaccharide synthesis, catabolite repression, etc.). Such mutants are not conditional lethals: a *pgi*$^-$*zwf*$^-$strain has been reported to not grow at all on glucose, but to grow well on other carbon sources whose metabolism is not via glucose 6-phosphate, and the general finding has been that glucose 6-phosphate is simply not an essential metabolite in these microorganisms (reviewed in ref. 6).

A caveat to that conclusion has been that the few mutants reported to date have been genetically uncharacterized, except for map position. It is not known whether *pgi* is the structural gene, and even if it were the mutations might be missense and specify a product "leakier" in vivo than apparent from in vitro assay (typically 2 to 5% wild-type levels). Thus, although the level of glucose 6-phosphate or certain products derived from it (such as glycogen) are certainly very low or undetectable in the appropriate growth conditions, it is conceivable that a very small amount of this metabolite is essential and made even by the mutant enzyme.

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To answer these questions it would be useful to have complete inactivation mutation of *pgi*. The purpose of this paper is to report two likely such mutants, both found accidentally, one amber and the other a Mu-phage insertion. The properties of the new strains differ little from the ones described earlier, and the conclusion about phosphoglucose isomerase probably not being essential still applies.

MATERIALS AND METHODS

Bacterial strains. Strain RT534 carries allele *pfkA9* in a ϕ80-sensitive background (*pfkA9 pps pyrF trp str*, ref. 8). Strain RT559 is a revertant of RT534 selected on mannitol (8) and lacks phosphoglucose isomerase (this paper). The new mutation (*pgi-6*) was shown to be at the known *pgi* locus (2) by a transduction using as recipient strain AB1932 (*arg metA*); selection was for Met$^+$ on glycerol plates with arginine, and 80 recombinants gave the distribution 2 *arg*$^+$ *pgi*$^-$, 20 *arg*$^+$ *pgi*$^+$, 16 *arg*$^-$ *pgi*$^-$, 42 *arg*$^-$ *pgi*$^+$, i.e., *arg-metA-pgi-6*. RT808 (*pfkA9 pgi-6 pps str supF*$^+$) and RT809 (*pfkA9 pgi-6 pps str supF*) were derived from RT559 in two steps. First, a trp_{amber} mutation was put in by P1 transduction (PyrF$^+$ selection and scoring for trp_{amber} with ϕ80*psupF*), and then P1 transduction from trp$^+$ *supF* with scoring for *sup*$^+$ and *sup*$^-$ with a T4 amber mutant. Strain K10 is wild type (HfrC *tonA22 lambda*, ref. 1). Strain DF2000 (HfrC *lambda pgi-2 tonA22 zwf-2*) was described earlier (3).

The original *pgi*-Mu insertion strains appeared in selections by one of us (W.S.R.) for Mu-phage-induced *lac* mutations in strain R$^+$P$^+$W205, which is a recombinant between strains CA77 and XW205 (D. Mitchell, W. Reznikoff, and J. Beckwith, J. Mol. Biol., in press) carrying both a *lac* deletion (X74) and a *trp-lac* fusion caused by *tonB* mutation. Mu phage was applied to lawns of this strain, and after overnight

incubation samples from the zones of killing were grown in LB broth and spread on lactose-MacConkey plates. Apparent *lac*⁻ colonies were picked and characterized. A minority proved not to grow on glucose either, but to grow on fructose, glycerol, or succinate and to contain β-galactosidase. The three such isolates tested (e.g., strain "3C2") were found by assay to lack phosphoglucose isomerase. The genetic position was shown by phage P1 transduction into strain AB1932 (as with strain RT559 above). The mutation co-transduced with *metA*, but only when a Mu-lysogen was used as recipient, in which case the linkage was four *pgi*⁻ out of 67 *met*⁺. These results accord with the strains carrying Mu insertions in *pgi*, since one would expect zygotic induction in a nonlysogenic recipient, and Mu insertion would also decrease linkage.

Strain "3C2" was used to make strain DF214 (*pgi-Mu zwf*Δ) as follows. Strain DF213 [F⁻ *eda-1* (*edd-zwf*Δ1) *his metA str Mu*] is a *pgi*⁺ *metA* derivative of a strain (DF1671DZ1, ref. 4), carrying a *zwf* deletion, made by transduction to growth on glucose and scoring for *met* using phage P1 prepared on strain AB1932; the resulting strain, DF212, was then lysogenized with wild-type phage Mu to facilitate transduction of the *pgi*-Mu insertion mutation. Strain DF214 is from strain DF213 and contains the *pgi*-Mu insertion from strain "3C2" (see above), introduced by Met⁺ transduction and scoring.

Assay of glucose content. The strains were grown in medium 63 supplemented with 1% casein hydrolysate (Difco), 2 μg of thiamine-hydrochloride per ml, 20 μg of L-tryptophan per ml and 20 μg of uracil per ml from a 1:100 dilution, harvested in logarithmic phase, and washed once in medium 63. Cell pellets corresponding to 50 mg (dry weight) were suspended in 1 ml of 1 N H_2SO_4 in screw-capped tubes and heated 3 h in a boiling bath. Cell debris was removed by centrifugation, the pellets were washed twice with 1 ml of water, and the supernatant fluids were combined. Glucose isolation was according to Spiro (7). The 3-ml yellow hydrolysates were passed through a mixed ion exchange column (3-ml Dowex AG 50W-X4, 200 to 400 mesh [H^+] over 3-ml Dowex AG1-X2, 200 to 400 mesh [formate]), the glucose was eluted with water, and the total 20-ml effluent was concentrated at 40 C in a rotary evaporator. The dried hydrolysate was transferred in 2 ml of water and neutralized with NaOH, and glucose was assayed on portions. We used glucose oxidase (Glucostat, Worthington Biochemical Corp., Freehold, N.J.), with a final assay volume of 0.5 ml and incubation at 37 C for 45 min. The quantities of hydrolysate used (up to 0.3 ml) did not inhibit the assay. In a control where 1 μmol of glucose was added to a pellet of strain DF214 before hydrolysis, 88% was recovered.

RESULTS AND DISCUSSION

The amber *pgi* mutant was found during a study of phosphofructokinase (8). We were screening revertants of *pfkA* mutants for one whose revertant phenotype was sensitive to nonsense suppression. Although the aim was to find such reversion at *pfkA*, the only such revertant phenotypes proved to be caused by the *pgi* mutation. Thus, revertants of a strain carrying *pfkA9* (RT534, Table 1) were selected on mannitol and screened by cross-streaking on this medium with *ϕ80psupF* and *ϕ80psupF*⁺. One revertant, strain RT559, was apparently sensitive to the suppressor. Strain RT559 was unusual in another way; although an apparent revertant on mannose as well an mannitol, it did not appear as a revertant on glucose (Table 1). Enzyme assay showed strain RT559 still lacked phosphofructokinase, and a transduction showed the strain still carried *pfkA*⁻ in normal linkage (data not shown). The mannitol-glucose growth difference suggested that the new lesion might affect phosphoglucose isomerate (Fig. 1), and this suggestion was confirmed by enzyme assay (Table 1) and by mapping (Materials and Methods). (Two other mannitol revertants of

TABLE 1. *Growth, phosphoglucose isomerase, and glucose content*[a]

Strain	Growth (colony size, mm)					Phosphoglucose isomerase activity	Glucose content (mg/g [dry wt])
	Glycerol	Glucose	Mannitol	Mannose	Arabinose		
RT534 (*pfkA9*)	1.3	0.6	0.3	0.3	1.3	1.51	10.2
RT559 (*pfkA9 pgi-6*)	1.4	0.6	0.8	1.2	1.8	<0.05	0.007
RT808 (*pfkA9 pgi-6*)	1.2	0.6	0.9	1.0	1.9	<0.05	0.014
RT809 (*pfkA9, pgi-6, supF*)	1.0	1.0	0.3	0.4	2.0	0.67	3.3
K10 (wild type)	1.5	2.1	2.3	1.6	2.2	1.63	8.6
DF2000 (*pgi-2, zwf-2*)	1.0	0.0	1.4	0.6	1.9	<0.05	0.033
DF213 (*zwf* Δ)	2.0	2.6	0.8	2.8	1.5	1.77	8.4
DF214 (*zwf* Δ, *pgi*-Mu)	1.3	0.0	1.4	1.9	1.4	<0.05	0.015

[a] For plating the strains were grown in broth and diluted so as to give approximately 100 colonies per plate, and colony size was estimated after 48 h at 37 C. Phosphoglucose isomerase was assayed as earlier (5), using extracts prepared from cells grown in broth supplemented with 0.4% fructose. Glucose content was determined on hydrolsates of cells grown in casein hydrolysate (see Materials and Methods). Strains are described in Materials and Methods.

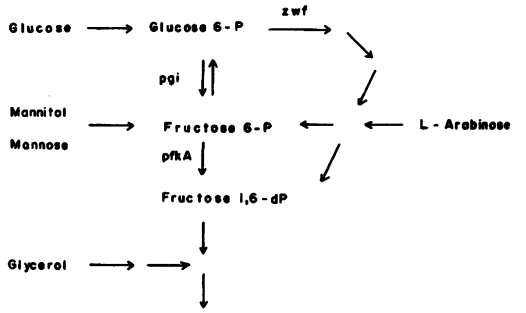

FIG. 1. *Pathways of glucose metabolism. Gene symbols are pgi, phosphoglucose isomerase; pfkA, phosphofructokinase; and zwf, glucose 6-phosphate dehydrogenase.*

strain RT534 also proved to be *pgi* mutants, but neither was suppressible by *ϕ80psupF*.)

Although the *ϕ80psupF* test for suppressibility of the *pgi* mutation of strain RT559 was clear, because of the instability of such lysogens we constructed isogenic strains by P1 transduction: strains RT808 (*pfkA9 pgi-6 supF*$^+$) and RT809 (*pfkA9 pgi-6 supF*). The first strain had the same growth phenotype as RT559, and the second strain was suppressed, resembling the *pgi*$^+$ strain, RT534, and having 40% its level of phosphoglucose isomerase activity (Table 1). Accordingly, *pgi-6* is an amber mutation.

The strains carrying *pgi-6* in Table 1 also carry *pfkA9*, so they did not show the typical *pgi* mutant growth phenotype. But the *pfkA*$^+$ *pgi-6* recombinants obtained in the mapping did not differ obviously from the *pgi-2* mutant described earlier (not shown).

We did one experiment to compare the heat lability of phosphoglucose isomerase from the suppressed strain with the wild-type enzyme (strains RT809 and RT534, respectively) and found no difference. We therefore cannot say that *pgi* is the structural gene for the enzyme, although that remains the most likely possibility.

The *pgi*-Mu insertion strains arose as apparent *lac* mutants in a culture treated with phage Mu (see Materials and Methods). We did not further characterize the growth of the original strains, since they arose in a *tonB* background. But we constructed a new strain, DF214, carrying *pgi*-Mu as well as deletion of *zwf* (glucose 6-phosphate dehydrogenase). Table 1 shows the phenotype of this strain, its *pgi*$^+$ parent, DF213, the earlier double mutant, strain DF2000, and the wild type, strain K10. The new double mutant blocked in both pathways of glucose 6-phosphate utilization did not differ markedly in growth from the old one.

It was shown earlier that *pgi* mutants, grown without glucose in the medium, contained little if any glucose. For example, DF40 (*pgi-2*) contained 2% or less the parental content of glucose after acid hydrolysis of whole cells grown in gluconate (5). In an attempt to put a new upper limit on this value we have refined the assay and done a similar experiment with the new strains (Table 1). The glucose content of the *pgi*$^+$ strains was approximately 1% by weight. In the *pgi* mutants the values were very low, ranging from 0.1 to 0.4% the parental level. These values are within blank range and we cannot say that the new strains are actually less "leaky" than the old; to a first approximation, glucose was undetectable. Nonetheless, for physiological studies the new strain, DF214, might be preferred and is available from the Coli Genetic Stock Center (B. Bachmann, Department of Microbiology, Yale University School of Medicine).

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