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Lack of Glucose Phosphotransferase Function in Phosphofructokinase Mutants of *Escherichia coli*

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Phosphofructokinase (*pfkA*) mutants of *Escherichia coli* are impaired in growth on all carbon sources entering glycolysis at or above the level of fructose 6-phosphate (nonpermissive carbon sources), but growth is particularly slow on sugars, such as glucose, which are normally transported and phosphorylated by the phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS). This is due to malfunction of the PTS in *pfkA* strains: *pfkA* mutants resemble PTS mutant strains in several ways. The residual slow growth of *pfkA* strains on glucose is dependent on low-affinity transport of free glucose and phosphorylation by glucokinase. Establishment by mutation of an active PEP-independent route for glucose entry greatly improves growth of *pfkA* strains on glucose. *pfkA* mutants growing on nonpermissive PTS sugars probably lack adequate PEP for PTS function and may thus be useful in physiological studies of the structurally intact but nonfunctioning PTS.

Mutants of *Escherichia coli* deficient in the major phosphofructokinase (*pfkA*) activity (D-fructose-6-phosphate, E.C. 2.7.1.11; the likely *pfkA* product [47]) are impaired in the use of carbon sources entering glycolysis at or above the level of fructose 6-phosphate (Fig. 1). These carbon sources are referred to as nonpermissive; carbon sources entering glycolysis below the level of fructose 6-phosphate, such as fructose, glycerol, succinate, are permissive for *pfkA* strains. Surprisingly, growth on nonpermissive carbon sources is not uniformly slowed; growth on sugars and sugar derivatives transported and phosphorylated by the phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS; 31, 32), for example growth on glucose, mannose and mannitol, is much more severely retarded by mutation at *pfkA* than is growth on sugars transported in other ways, for example galactose (52), lactose, and glucose 6-phosphate (26, 49).

It has been suggested that the particularly slow growth of *pfkA* mutants on PTS-transported sugars, such as glucose, is due to a lack of PEP for transport (18). the argument is stoichiometric. If hexose phosphate formed in *pfkA* strains is metabolized entirely through the hexose monophosphate (HMP) shunt (Fig. 1), one hexose phosphate would yield one triose phosphate (such as PEP) and three CO_2's (18). The PTS-mediated transport of glucose is written, glucose + PEP → glucose 6-phosphate + pyruvate (31, 32). Then, in metabolism of glucose by a *pfkA* mutant, one glucose would yield one pyruvate and three CO_2's. In the absence of a route for converting pyruvate to PEP under these conditions, there would be no triose phosphate for biosynthesis, or, as was suggested, if triose phosphate (or any precursor) is used for biosynthesis there would be insufficient PEP for the transport of glucose (18). Growth on a non-PTS carbon source, such as glucose 6-phosphate, would not be subject to this restriction. (By the same reasoning, it should not be possible for an organism to metabolize a PTS-transported sugar by the Entner-Doudoroff pathway, by which hexose phosphate → triose phosphate + pyruvate, and confirmation of this expectation is found in the fact that a glucose PTS is not present in a wide range of strictly aerobic bacteria [30], which normally use glucose by the Entner-Doudoroff route.)

In accord with the stoichiometric PEP limitation explanation of the *pfkA* phenotype is the finding that mutation to constitutive expression of the glyoxylate shunt, a mutation known to suppress the PEP synthetase (*pps*) mutant phenotype (Fig. 1), probably by establishing an alternate pathway for the conversion of pyruvate to PEP, also partially suppresses the *pfkA* phenotype on carbon sources transported by the PTS (49). However, mutational loss of the HMP shunt in a *pfkA* strain does not eliminate either growth on glucose 6-phosphate or glyoxylate shunt suppression on glucose, whereas loss of the second, minor, phosphofructokinase activity, by mutation at the *pfkB* locus (49), abolishes both growth on glucose 6-phosphate and glyoxylate shunt suppression on glucose in *pfkA* strains with a functional HMP shunt (48).

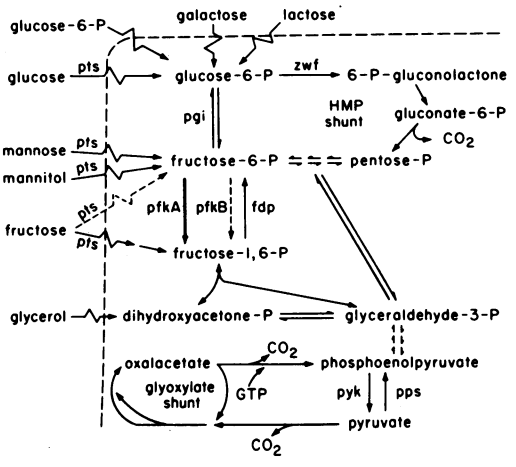

FIG. 1. *Central pathways of sugar metabolism in E. coli. Abbreviations: fdp, fructose diphosphatase; HMP, hexose monophosphate; pfk, phosphofructokinase; pgi, phosphoglucose isomerase; pps, PEP synthetase; pts, transport by PEP-dependent phosphotransferase system; pyk, pyruvate kinase; zwf, glucose 6-phosphate dehydrogenase. Fructose enters glycolysis mostly as fructose 1,6-diphosphate (11).*

It then appears that in a *pfkA* mutant strain metabolism of hexose phosphate is largely by glycolysis, through residual phosphofructokinase activity.

Whereas a stoichiometric inference of PEP shortage during growth on glucose and other PTS-transported sugars thus cannot be made, a PTS malfunction in *pfkA* strains caused by PEP limitation still remains an attractive hypothesis, as will be discussed. If a failure of PTS function is in fact the cause of the particularly slow growth of *pfkA* strains on glucose and other PTS sugars, then *pfkA* mutants should resemble *pts* mutants defective in enzyme I *(ptsI)* or in the histidine-containing phosphate carrier protein (*ptsH*) of the PTS (31, 32). Two specific predictions may be made. First, the establishment of a PEP-independent route for the entry of glucose into the cell, by mutation to constitutive expression of the galactose-specific galactose transport system (non-PTS, with an affinity for glucose [52, 53]), should suppress the *pfkA* phenotype on glucose just as it suppresses the *pts* mutant phenotype on glucose in *Salmonella typhimurium* (35, 42). Second, the residual slow growth of *pfkA* strains on glucose might be expected to depend on slow, non-PTS entry of free glucose into the cell followed by adenosine 5'-triphosphate-dependent phosphorylation, as has been found for residual growth on glucose of *pts* mutants of *Salmonella* (38) and of *E. coli* (7, 14, 45). We confirm these predictions here.

MATERIALS AND METHODS

Bacterial strains. The strains of *E. coli* K-12 used are listed in Table 1, with genotypes and details of derivation.

Media. Minimal medium 007 was used (4), supplemented with 1 μg of thiamine hydrochloride per ml, and the carbon source at 0.2%, unless otherwise stated. BTYEX 7 medium was minimal medium with 10 g of tryptone (Difco Laboratories, Detroit, Mich.) and 5 g of yeast extract (Difco) per liter. Casein hydrolysate medium was minimal medium with 10 g of Casamino Acids (Difco) per liter. Agar plates were of these media with 1.5% agar (Difco). Minimal top agar was minimal medium with 0.75% agar. Media used for phage P1 propagation and assay were those of Lennox (21). Nitrogen-free medium (20) was supplemented with D-serine at 8 mM as nitrogen source.

Genetic procedures. Preparation of phage P1 lysates, their use in transductions, mutagenesis with ethyl methane sulfonate, and penicillin counterselections were done as described (49).

thyA mutants were selected as forming colonies on glycerol plates with 10 μg of trimethoprim per ml and 50 μg of thymine per ml (24). A *galR*^{c} derivative of K10, strain RR3, was isolated using ethyl methane sulfonate mutagenesis and the selection of Buttin (3). The *galR*^{c} marker was moved into other strains by cotransduction with *thyA*. Thy$^+$ transductants were scored for the *galR* allele on tetrazolium overlay plates modified from Saedler et al. (34), by replacing membrane filters with filter paper circles (Shark skin, Schleicher and Schuell Co., Keene, N.H.) covered with 3 ml of minimal top agar. *galR* scoring was confirmed by galactokinase assay; *galR*$^+$ strains were induced more than 10-fold by addition of galactose to casein hydrolysate medium (from 0.02 to 0.3 μmol per min per mg of protein), whereas *galR*^{c} strains had high, constitutive activities. D-serine deaminase mutants (*dsdA*) were isolated following ethyl methane sulfonate mutagenesis and penicillin counterselection in nitrogen-free glycerol medium with 8 mM D-serine as nitrogen source (23). Because our strains are inhibited by contaminating L-serine (6), 25 μg of L-isoleucine per ml was added to all media containing D-serine. *ptsI* and *glk* mutations were introduced into *dsdA* strains by transductional repair to use of D-serine as nitrogen source. Because D-serine is toxic to *dsdA* strains (23) phenotypic expression of *dsdA*$^+$ was permitted by casting the transduction mixture in nitrogen-free minimal top agar on a nitrogen-free agar plate with lactate as carbon source and incubating overnight at room temperature before adding a layer of agar with D-serine. Purified *ptsI* transductants were identified as fructose negative and showed the typical *pts* phenotype. Purified *glk* transductants of *pfkA* strains were especially weak on glucose and were verified by glucokinase assay; *glk*$^+$ strains grown in casein hydrolysate medium had an activity of about 50 nmol per min per mg of protein and *glk*$^-$ strains, including DF52, had undetectable activity.

Enzyme assays. Galactokinase and glucokinase were assayed by a modification of the glycerol ki-

TABLE 1. *E. coli K-12 strains used*[a]

Strain	Sex	Genotype	Derivation, source, or reference
AM1	HfrC	*pfkA1, tonA22, lambda*	D. G. Fraenkel; from K10 (26)
DF51	HfrH	*lacI22, ptsI2, rel-1, thi-1, lambda*	D. G. Fraenkel; formerly MM-6 (14, 45)
DF52	HfrH	*glk, lacI22, ptsI2, rel-1, thi-1, lambda*	CGSC; formerly GN-2 (14, 45)
K10	HfrC	*tonA22, lambda*	D. G. Fraenkel (2)
RR25	HfrC	*galR^c, pfkA1, tonA22, lambda*	–[b]
RR30	HfrC	*pfkA1, tonA22, lambda*	as for RR25, but a *galR$^+$* transductant, by assay
RR45	HfrC	*galR^c, glk, pfkA1, tonA22, lambda*	–[c]
RR47	HfrC	*galR^c, pfkA1, tonA22, lambda*	as for RR45, but a *glk$^+$* transductant, by assay
RR53	HfrC	*glk, pfkA1, tonA22, lambda*	–[d]
RR57	HfrC	*pfkA1, tonA22, lambda*	as for RR53, but a *glk$^+$* transductant, by assay
RR65	HfrC	*pfkA1, ptsI2, tonA22, lambda*	–[e]
RR67	HfrC	*pfkA1, tonA22, lambda*	as for RR65, but a *ptsI$^+$* transductant

[a] Gene designations are according to Taylor and Trotter (46) except *glk*, glucokinase (7). All known markers are included. Phenotypic abbreviations use capitalized gene designations; Fruc is fructose utilization. Other abbreviations are EMS, ethyl methane sulfonate mutagenesis; pcs, penicillin counterselection; spon., spontaneous mutation; trans., transduction with phage P1 (followed by donor strain in parentheses). Thus, the second step in construction of RR45 would be read "a transductant to *dsdA$^+$* using P1 grown on DF52, identified as *glk* by assay and scored as able to grow on fructose." CGSC, Strain obtained through the courtesy of B. Bachmann, Coli Genetic Stock Center, Yale University, New Haven, Conn.

[b] From AM1 in two steps; first spon., selection for Thy$^-$[f]; then Thy$^+$ (GalR^c[assay]) trans. (RR3[f]).

[c] From RR25 in two steps; first EMS, pcs for Dsd$^-$[f]; then Dsd$^+$ (Glk[assay], Fruc$^+$) trans. (DF52).

[d] From AM1 in two steps; first EMS, pcs for Dsd$^-$[f]; then Dsd$^+$ (Glk [assay], Fruc$^+$) trans. (DF52).

[e] From AM1 in two steps, the first as in footnote [d], the second Dsd$^+$ (Fruc$^-$) trans. (DF51).

[f] See genetic procedures.

nase assay of Richey and Lin (29). Washed cells were sonicated in 0.05 M tris(hydroxymethyl)aminomethane (Tris) hydrochloride (pH 7.8) buffer with 5 mM $MgCl_2$ and 10 mM mercaptoethanol, and cell-free extracts were prepared by centrifugation for 20 min at 20,000 × *g*. The reaction mixture (41) contained 0.205 ml of the sonication buffer with 3.2 mM NaF, 0.02 ml of 18.75 mM adenosine 5′-triphosphate, 0.005 ml of diluted extract, and 0.02 ml of 6.25 mM D-[^{14}C]glucose or galactose (0.5 Ci/mol) (final volume, 0.25 ml). Reactions were carried out at 25 C. At 5, 10, and 15 min after addition of adenosine 5′-triphosphate a 0.05-ml aliquot was withdrawn, pipetted onto a diethylaminoethyl-ion exchange filter (Whatman DE81), and washed with 5 ml of 80% ethanol, and then three 10-ml volumes of H_2O. Filters, blotted dry, were placed into a vial for scintillation counting. Protein was assayed by the Folin method (22).

Chemicals. Glucose, glucose 6-phosphate, mannose, galactose, fucose (all D isomers), and D-serine were from Sigma Chemical Co., St. Louis, Mo. Uniformly ^{14}C-labeled glucose and galactose were from New England Nuclear Corp., Boston, Mass. Mannitol and fructose (D isomers) were from Fisher Scientific Co., Pittsburgh, Pa. Lactate was from Fisher or Sigma. Fisher sodium lactate, 70% syrup, contained a substance that was somewhat inhibitory to *pfkA* strains (49); the *ptsI* allele from DF52 eliminated inhibition. Sigma lactic acid, sodium salt, 60% syrup, was not inhibitory. Trimethoprim was the gift of R. E. Wolf, Jr.

RESULTS

Establishment of a PEP-independent route for entry and phosphorylation of glucose suppresses the *pfkA* phenotype on glucose. In their description of *pts* mutants of *S. typhimurium* Simoni et al. reported that induction of a transport system for galactose permitted growth of a *ptsI* mutant on glucose and referred to similar observations made with *E. coli* mutants (42). Saier et al. later found that mutation at *galR*, resulting in constitutive production of the galactose-degradative enzymes, and the galactose-specific galactose permease (34, 53), resulted in improved growth of *ptsI-ptsH* mutants of *Salmonella* on glucose (35). Suppression of the *pts* phenotype in these cases was presumably due to transport of free glucose by a galactose permease, for which glucose is a substrate but not an inducer, and adenosine 5′-triphosphate-dependent phosphorylation by glucokinase. Glucokinase-dependent glucose growth of an *E. coli* strain lacking both PTS enzymes II for glucose, following induction with galactose, has been described (1).

We tested for similar suppression of the *pfkA* phenotype in *E. coli*. Initial experiments showed that D-fucose, a nonmetabolizable inducer of the galactose system (42, 53), stimulated growth of *pfkA* mutants in glucose medium. For example, strain RR30 had a doubling time of 7.4 h in 0.2% glucose minimal medium, and the addition of D-fucose to 3.3 mM decreased this to 5.7 h. We then introduced a *galR^c* (constitutive) mutation into a *pfkA, thyA* strain by P1-mediated transductional repair of *thyA*, using a *galR^c* donor strain. (*thyA* and *galR* are ca. 50% linked by transduction [34].) The *galR^c* transductants, identified on tetrazolium overlay plates and verified by galactokinase assay, were faster growing than *galR$^+$* (inducible) transductants on glucose plates and in glucose minimal medium (Tables 2, 3, 4; cf. RR25 [*pfkA1, galR^c*] and the isogenic *galR$^+$* strain RR30). Addition of D-fucose to glucose

medium shortened the doubling time of *galR*$^+$ but not of *galR*^{c} transductants (data not given; it is not known if our strains have the β-methyl galactoside permease [53]). Introduction of *galR*^{c} also improved growth somewhat on mannose, which is probably also transported by the galactose permease (35; but with a lower affinity than for glucose, 33), but not on mannitol or on the non-PTS sugars glucose 6-phosphate and galactose (Tables 2, 3). Improved growth on lactose (Table 3) will be discussed.

If the *galR*^{c} mutation suppresses the *pfkA* phenotype on glucose by establishing a non-PTS route for entry of free glucose, then phosphorylation of glucose, and suppression, should be dependent on glucokinase (7, 14, 45). To test this, we introduced a glucokinase (*glk*) mutation into a D-serine deaminase (*dsdA*) mutant derivative of a *pfkA*, *galR*^{c} strain, using transductional repair of *dsdA*. (*glk* and *dsdA* are linked by P1 transduction [7].) The *glk* transductants (verified by assay) were in fact very slow growing on glucose (Table 3; cf. RR45 [*pfkA1*, *galR*^{c}, *glk*] and the isogenic *glk*$^+$ transductant RR47). Growth on mannose was not much affected by introduction of *glk* (Table 3), suggesting mannose transport and action of a mannokinase (38, 39) rather than use of contaminating glucose in the mannose.

Suppression of a *ptsI* or *ptsH* mutant on glucose by *galR*^{c} mutation would necessarily be by establishment of an alternate, non-PTS route for entry of glucose into metabolism (35). However, *galR*^{c} suppression of a *pfkA* mutant might be more complex. For example, if low PEP levels were limiting growth of *pfkA* strains on glucose, then PEP-independent glucose entry via the galactose permease would ease PEP restriction and might support extensive simultaneous functioning of the glucose

TABLE 2. *Colony sizes*[a]

Strain	Genotype		Carbon source (colony diam, mm)						
	pfkA	*galR*	Glucose	Mannose	Mannitol	Glycerol	Fructose	Galactose	Lactose
K10	+	+	2.4	2.2	2.0	1.5	1.6	2.2	2.3
RR30	−	+	0.5	0.4	0	1.7	2.0	0.8	0.6
RR25	−	c	1.2	0.8	0	2.0	1.9	0.7	0.9

[a] Minimal plates contained the carbon source at 0.2%. Cells from glycerol cultures were washed and diluted in buffer and spread to give 10 to 100 colonies per plate. After about 36 h at 37 C average colony diameters were obtained using a dissecting microscope with micrometer scale.

TABLE 3. *Doubling times in minimal media*[a]

Strain	Genotype			Carbon source (doubling time, h at 37 C)						
	pfkA	*galR*	*glk*	Glucose	Mannose	Mannitol	Glycerol	Glu-6-P	Galactose	Lactose
K10	+	+	+	1.1	1.5	1.2	1.3	1.0	1.3	1.0
RR30	−	+	+	8.1	6.9	>50	1.6	3.1	3.1	4.5
RR25	−	c	+	3.6	5.1	>50	1.6	3.1	3.2	3.0
RR47	−	c	+	6.9[b]	8.2	>50	1.4	ND[c]	ND	ND
RR45	−	c	−	17.0	9.2	>50	1.2	ND	ND	ND

[a] Minimal media contained the carbon source at 0.2%. Inocula were from fresh stationary cultures in 0.02% glycerol minimal medium. Absorbancy at 580 nm was determined periodically with a Perkin-Elmer spectrophotometer, model 139, for 50-ml cultures in 250-ml flasks on a New Brunswick gyratory shaking water bath and plotted as a semilogarithmic function of time. Abbreviation: Glu-6-P, Glucose 6-phosphate.

[b] RR45 and RR47 are transductants of a *dsdA* derivative of RR25 obtained by ethyl methane sulfonate mutagenesis. This derivative was slow growing in several media but retained the *galR*^{c} marker.

[c] ND, Not determined.

TABLE 4. *Effect of sugar concentration on colony size*[a]

Strain	Genotype		Glucose concn (colony diam, mm)						Mannose concn (colony diam, mm)					
	pfkA	*galR*	0.01%	0.05%	0.1%	0.5%	1.0%	2.0%	0.01%	0.05%	0.1%	0.5%	1.0%	2.0%
K10	+	+	0.6	1.4	1.7	1.9	1.7	1.5	ND[b]	ND	ND	ND	ND	ND
AM1	−	+	0	0.1	0.2	0.4	0.4	0.6	ND	ND	ND	ND	ND	ND
RR30	−	+	0	0.1	0.2	0.4	0.5	0.6	0.2	0.3	0.4	0.6	0.5	0.5
RR25	−	c	0.4	0.5	0.5	0.7	0.7	0.8	0.4	0.4	0.8	1.2	1.2	1.1

[a] Minimal plates contained sugar at the indicated concentration. Procedures as in Table 2. Growth on mannitol was not improved by increased concentration (Table 2).

[b] ND, Not determined.

PTS. That the PTS plays no essential role in glucose growth of a *pfkA*, *galR*^{c} strain was demonstrated by the introduction of a *ptsI* mutation into the *pfkA*, *galR*^{c}, *dsdA* strain by cotransduction with *dsdA*; growth in glucose medium was the same as that of an isogenic *pts*$^+$ transductant (data not given).

Residual growth of *pfkA* strains on glucose is dependent on glucose concentration and glucokinase. Mutants of *Salmonella* and *E. coli* defective in enzyme I or the histidine-containing phosphate carrier protein of the PTS grow better on glucose than on other PTS sugars, such as mannitol (9, 38). This is evidently due to entry of free glucose by low affinity transport systems and internal phosphorylation by glucokinase. Growth of *ptsI* mutants of *Salmonella* is faster at high glucose concentrations (42) and mutational loss of glucokinase activity sharply decreases growth rates on glucose in *pts* mutants of Salmonella (38) and of *E. coli* (7, 14, 45). *pfkA* mutants of *E. coli* also grow better on glucose than on mannitol (26, 49; Tables 2, 3). Dependence of the growth rate of a *pfkA* mutant, strain AMl, on glucose concentration is shown in Tables 4 and 5. The doubling time of AMl decreases threefold as glucose concentration increases from 0.02% to 2.5%, whereas the growth rate of wild-type *E. coli* is independent of glucose concentration in this range (40, 51; Table 5). A Lineweaver-Burk plot of the data in Table 5 (Fig. 2) suggests two routes for the entry of glucose into the *pfkA* mutant, one supporting half-maximal growth rate at 0.3 mM glucose, the other a lower affinity, higher V_{max} system with an apparent K_m of about 40 mM glucose (affinities estimated graphically). The use of each of these routes is dependent on glucokinase, as shown by the growth response of RR53, a *glk* derivative of AMl (Table 5, cf. RR53 and RR57, an isogenic *glk*$^+$ transductant; Fig. 2). The *glk* mutation in an otherwise wild-type strain does not affect growth rates on these concentrations of glucose (7, 14; and unpublished observations).

The finding that the residual slow growth of *pfkA* mutant strains on glucose is largely dependent on the action of glucokinase suggests nonphosphorylating routes of glucose entry. However, it would be possible that PEP spared by glucose metabolism through glucokinase would permit partial functioning of the PTS system in *pfkA* mutants, as discussed above. In this case, increasing concentrations of glucose might improve the "pump-priming" of the PTS and yield a Lineweaver-Burk plot of the general shape seen in Fig. 2. Loss of the PTS by mutation should then eliminate at least the low affinity transport system, as in the case of *glk* mutation. A *pfkA*, *ptsI* double mutant was constructed and compared in growth on various concentrations of glucose to an isogenic *ptsI*$^+$ strain (strains RR65 and RR67, Table 5; RR65, Fig. 2. A different pair of isogenic strains gave the same results, in a separate series of growth

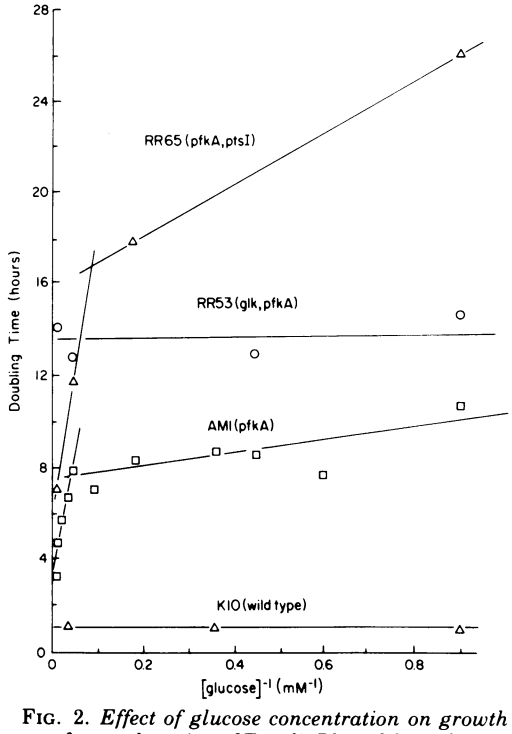

FIG. 2. *Effect of glucose concentration on growth rates of several strains of E. coli. Plotted from data in Table 5. Abbreviations: glk, glucokinase; ptsI, enzyme I of the PTS.*

TABLE 5. *Effect of glucose concentration on doubling time*[a]

Strain	Genotype			Glucose concn (doubling time, h at 37 C)										
	pfkA	*glk*	*ptsI*	0.02%	0.03%	0.04%	0.05%	0.1%	0.2%	0.4%	0.5%	1.0%	2.0%	2.5%
K10	+	+	+	1.0	–	–	1.1	–	–	–	1.1	–	–	–
AM1	–	+	+	10.6	7.7	8.5	8.7	8.3	7.1	7.9	6.8	5.8	4.7	3.3
RR57	–	+	+	7.5	–	6.4	–	–	7.7	5.3	–	–	3.8	–
RR53	–	–	+	14.7	–	13.4	10.1	–	15.5	13.4	–	–	14.0	–
RR67	–	+	+	8.7	–	–	–	7.0	–	6.2	–	–	4.4	–
RR65	–	+	–	26.0	–	–	–	17.8	–	11.7	–	–	6.9	–

[a] Minimal media contained glucose at the indicated concentration. Other procedures as in Table 3.

rate measurements.). Whereas the *ptsI* lesion does slow growth of a *pfkA* strain on glucose markedly, the growth response of the double mutant to changing glucose concentration remains biphasic, with the two transport components probably not changed; apparent K_m's for glucose in the double mutant are estimated as roughly 0.7 mM and 22 mM, respectively, from Fig. 2. The effect of *ptsI* mutation is then principally on V_{max}, and may thus be based on *pts*-increased sensitivity to glucose inhibition (31, 32, 36) of synthesis or activity of two unidentified non-PTS transport systems carrying glucose, rather than on direct elimination of a route for glucose entry.

Growth of *pfkA* strains on mannose is less concentration-dependent than growth on glucose (Table 4). Increasing mannitol concentration does not improve growth of *pfkA* strains on plates (data not shown), but this could be due simply to lack of a mannitol kinase.

Growth inhibition of *pfkA* strains by nonpermissive carbon sources. Growth of *pts* mutants on permissive carbon sources is typically inhibited by PTS sugars, due to interference with induction of catabolic enzymes (27, 31, 32, 37). *pfkA* mutants are also inhibited by some nonpermissive carbon sources, and this sensitivity resembles that of *pts* mutants (27) in that inhibition of *pfkA* strains by glucose may under some conditions be overcome by cyclic adenosine 3',5'-monophosphate (48). Of course, *pfkA* mutants, blocked in a major metabolic route, might accumulate phosphorylated intermediates, and such accumulation is known often to result in growth stasis (13, for accumulated glucose 6-phosphate; 10, for a number of other references). We have thus examined inhibition of *pfkA* mutants further.

pfkA strains are inhibited on succinate or casein hydrolysate medium by nonpermissive PTS sugars and not by galactose or lactose (unpublished observations), but this accords simply with the relative impairment of growth on these sugars as sole carbon source. Because slow growth of *pfkA* strains on glucose is dependent on glucokinase (Table 3; Fig. 2), *glk* mutation should directly or indirectly decrease production of phosphorylated intermediates from glucose. Then, if accumulation of an organic phosphate is the basis of glucose inhibition of *pfkA* strains, *glk* mutation should protect against it, whereas if glucose inhibition is like that seen for *pts* mutants *glk* mutation might even increase sensitivity to glucose by exacerbating PTS deficiency. A comparison of the sensitivities of the *pfkA* strain AM1 and a *glk* derivative of it to inhibition by PTS sugars (Table 6) supports the latter hypothesis. As with *pts* mutants (7, 31), the nonmetabolizable glucose analogues methyl-α-D-glucoside and 2-deoxyglucose also inhibit *pfkA* strains (unpublished data).

Surprisingly, glucose-resistant revertants of AM1 selected on complex medium include strains evidently mutated at *ptsI (H)*. These strains are also resistant to mannose and mannitol and are completely unable to grow on fructose, maltose, and lactose, as the result of a lesion mapping close to *dsdA*, by transduction (data not shown). *ptsI* derivatives of AM1 constructed by transduction are also protected against inhibition by PTS sugars (Table 6). However, the donor *ptsI* strain, DF51, is also insensitive to inhibition (Table 6) and might be a *crr* mutant (31, 32, 36, 37). (*ptsI [H]* mutants have been selected as revertants in a case of fructose toxicity involving accumulated phosphorylated intermediates [11].)

DISCUSSION

Whereas our studies support the idea that PTS malfunction is an important part of the phosphofructokinase mutant phenotype, the basis for the malfunction is not known. Cases of inhibition of the PTS by accumulated metabolic intermediates have been described (16, 17, 25; 8 discusses regulation of glucose PTS activity),

TABLE 6. *Inhibition by PTS-transported sugars*[a]

Strain	Genotype			Growth medium	Sugar tested (zone of inhibition, cm[b])		
	pfkA	*glk*	*ptsI*		Glucose	Mannose	Mannitol
K10	+	+	+	Succ	0	0	0
				CH7	0	0	0
AM1	−	+	+	Succ	2.2	1.7	3.8
				CH7	0	0	2.8
RR53	−	−	+	Succ	3.7	2.0	3.6
				CH7	2.8	0	2.9
RR65	−	+	−	Succ	0	0	0
				CH7	0	0	0
DF51	+	+	−	Succ	0	0	0
				CH7	0	0	0

[a] Cells from fresh stationary BTYEX7 cultures were washed in buffer, suspended in 2.5 ml of minimal top agar at ca. 5×10^7 cells/ml, and overlayered onto minimal plates with 0.2% sodium succinate or 1.0% Casamino Acids as carbon source. Filter paper disks (0.635 cm, Schleicher and Schuell, Inc., no. 740-E) were saturated with a 10% solution of the sugar tested and placed onto an overlayered plate. Incubation was at 37 C for 24 h. Abbreviations: Succ, succinate; CH, casein hydrolysate.

[b] Diameter of the zone of inhibition around paper disk is given; 0 indicates no visible inhibition at the time of inspection. Inhibition of AM1 by glucose and mannose was seen on CH7 media at shorter incubation times; data are comparative.

but in detail these examples do not support an explanation of the *pfkA* phenotype as involving deranged "fine control" of the PTS. Lack of adequate PEP for transport and biosynthesis in *pfkA* strains provided with nonpermissive PTS carbon sources was suggested by Kornberg and Smith on the basis of a logically appealing argument that is most likely incorrect (Introduction). However, for several reasons PEP limitation remains an attractive explanation for the particularly slow growth of *pfkA* strains on PTS sugars.

(i) Kornberg and Smith showed that *pfkA* cell suspensions are stimulated in the accumulation of label from radioactive glucose if preincubated with substances which can be converted to PEP or spare its use, such as pyruvate and malate (18). The stimulation by pyruvate was eliminated by introduction of a *pps* mutation (Fig. 1) in accord with conversion to PEP being required for the effect (18), although pyruvate has been reported to inhibit PTS function in *pps* strains (25).

(ii) Mutation to constitutive expression of the enzymes of the glyoxylate shunt increases growth rates of *pfkA* strains on PTS sugars, glucose, for example, supporting as rapid a growth rate as that on glucose 6-phosphate (49). An unregulated glyoxylate shunt would be likely to establish a route for conversion of pyruvate to PEP, and should at least decrease use of PEP for anaplerotic function (49).

(iii) If PEP limitation affects PTS function in *pfkA* strains, then a supplement of end products having PEP as a biosynthetic precursor might spare PEP removal and stimulate growth on PTS sugars. Growth of *pfkA* strains on mannose is stimulated by addition of aromatic amino acids and shikimic acid to the medium (49). However, addition of succinate or malate, which could halt removal of PEP for anaplerotic function, has no obvious effect (data not shown).

(iv) PEP insufficiently in *pfkA* strains could not be due simply to metabolism by the HMP shunt (Introduction). Still, if PEP is limiting in growth on PTS sugars, then any use of the HMP shunt would worsen PEP shortage. Loss of the shunt, for example, by mutation in glucose 6-phosphate dehydrogenase (*zwf*, Fig. 1), might then increase productive metabolism through residual phosphofructokinase and improve growth on PTS sugars. This is not ordinarily seen with glucose as carbon source (18, 49), but then growth of *pfkA* strains on glucose is largely dependent on non-PTS phosphorylation; on mannitol plates, however, *zwf* transductants of *pfkA* strains (49), verified by assay, always show slightly but distinctly stronger growth than zwf^+ transductants (unpublished observation). Similarly, mutation in phosphoglucose isomerase (*pgi*, Fig. 1) markedly improves mannitol growth of at least one *pfkA* mutant (50).

Why should PEP be limiting in *pfkA* mutants? Altered concentrations of PEP or its precursors in *pfkA* mutants could lead to excessive or uncoordinated activity of an enzyme acting on PEP (pyruvate kinase, for example) or on a precursor of PEP, and this could result in a selective effect on utilization of PTS sugars. Whereas lowered PEP concentration should not affect the PTS selectively, the affinity of the PTS for PEP being comparable to that of biosynthetic enzymes having PEP as substrate (5, 19, 44), failure of the PTS would quickly stop production of PEP. If a single unknown reaction were responsible for most PEP loss some partial revertants of *pfkA* strains might be altered in that activity. However, one set of 30 independent revertants selected on mannose did not include any of this class, all being assigned to other classes (48). We are continuing to look for such a revertant and are conducting direct measurements of cellular PEP pools following shift of *pfkA* strains to nonpermissive carbon sources.

It is of interest that growth of *pfkA* mutants on lactose, a non-PTS sugar, is improved by $galR^c$ mutation (Table 3). Lactose is split to glucose and galactose during metabolism, but improved use of galactose seems an unlikely basis for the effect because $galR^c$ does not improve growth on galactose (Table 3). Phosphorylation of endogenously generated glucose in *E. coli* may occur to a large extent by action of the PTS; loss of glucokinase results in only a 20% decrease in the growth rate on lactose of an *E. coli* mutant unable to use galactose (7). *pfkA* mutation does slow growth on lactose more than on galactose or glucose 6-phosphate (Table 3; 49). If PTS phosphorylation of endogenously generated glucose involves prior excretion (as in the case of fructose formed from sucrose in fructokinase mutants of *Aerobacter aerogenes* [15]) then establishment of a non-PTS route for glucose entry, as by $galR^c$ mutation, would improve lactose growth of a *pfkA* strain.

Mutation in galactokinase (*galK*) results in accumulation of endogenously produced galactose and induction of galactose permeases (53). The *pfkA* phenotype on glucose might be expected to be suppressed by *galK*, but *pfkA*,*galK* strains used in previous studies (47, 48) were not suppressed. Constitutivity of galactose permease in these strains has not been checked.

It is not clear what role the PTS plays in the residual slow growth of *pfkA* mutants on glu-

cose. The relevant data are presented in Fig. 2. Two transport systems for glucose (half-maximal growth rates at roughly 40 mM and 0.3 mM) are seen to function in *pfkA* strains. By contrast, the growth rate of wild-type *E. coli* on glucose is independent of glucose concentration except at very low concentrations, at which the apparent K_m for growth in about 1 μM (40, 51). The PTS shows a K_m for glucose of 0.4 mM in vitro (19; this value may be 10-fold lower when a cytoplasmic factor III participates [32]; low K_m values for glucose PTS transport by whole cells are reported in reference 1), hence the higher affinity route for glucose entry in *pfkA* strains might be the PTS. However, loss of glucokinase slows growth of *pfkA* strains at all glucose concentrations and results in essentially concentration-independent growth. This indicates that neither of the two transport systems can be an independently functioning PTS, and limits a possible role for the PTS to (i) carrying out group translocation of glucose by use of PEP made available by metabolism through glucokinase, or (ii) facilitating diffusion of glucose without phosphorylation (43), or both. Loss of enzyme I of the PTS does not eliminate either the higher or lower affinity glucose transport system, but instead results in decreased activity of each. One explanation of this would be that the synthesis or activity of the two unidentified transport systems carrying glucose is more sensitive to glucose inhibition in the *ptsI* strain (catabolite repression, inducer exclusion; 27, 31, 32, 36, 37), with the PTS playing no role in transport. The higher affinity system, for example, could be the galactose-specific galactose permease (53; cf. RR30 and RR25, Table 4; some *ptsI* mutants are impaired in growth on galactose [12]). However, arguments could also be made for residual enzyme I activity functioning with PEP spared by action of glucokinase or for an effect of the *ptsI* mutation on availability of enzymes II (1, 7) for facilitated diffusion of glucose (43). Glucose concentration responses of *pfkA* strains lacking one or both of the enzymes II for glucose (7) would help settle this question and are being carried out.

Whereas transport of a number of sugars by group translocation is the best understood function of the PTS, it is clearly not the only function. Mutants altered in components of the PTS are affected in the regulation of inducible enzyme synthesis, in transport of non-PTS substrates, in cyclic adenosine 3',5'-monophosphate metabolism, and in chemotaxis (1, 31, 32), thus it is likely that the PTS plays a central regulatory role (28, 36) not fully understood at present. Whereas mutant analysis of the PTS has been valuable in understanding it, the results have not all been easy to interpret, in part because mutational alteration in one component of a very complex system may not leave other components unaffected. *pfkA* mutants may permit study of a structurally intact but nonfunctioning PTS once the basis of PTS malfunction in these strains has been established.

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Addendum in Proof

A new pair of isogenic strains was constructed to eliminate the difficulty noted in footnote *b*, Table 3: RR95 (*galR*^{c}, *pfkA1*), and RR93 (*galR*^{c}, *glk*, *pfkA1*). Doubling times in minimal media (determined as in Table 3) were, for RR95, 2.9 h in glucose and 5.2 h in mannose; for RR93, 15.2 h in glucose and 7.4 h in mannose. Colony diameters (determined as in Table 2) were, for RR95, 0.35 mm on glucose, 0.35 mm on mannose; for RR93, less than 0.05 mm on glucose, and 0.20 on mannose.

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