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Phosphorylation of D-Glucose in *Escherichia coli* Mutants Defective in Glucosephosphotransferase, Mannosephosphotransferase, and Glucokinase

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Genetic studies show that *Escherichia coli* has three enzymes capable of phosphorylating glucose: soluble adenosine 5'-triphosphate-dependent glucokinase, which plays only a minor role in glucose metabolism; an enzyme II, called glucosephosphotransferase, with high specificity for the D-glucose configuration; and another enzyme II, called mannosephosphotransferase, with broader specificity. The former enzyme II is active on glucose and methyl-α-glucopyranoside, whereas the latter is active on D-glucose, D-mannose, 2-deoxy-D-glucose, D-glucosamine, and D-mannosamine. Mutations leading to loss of glucosephosphotransferase activity and designated by the symbol *gpt* are between the *purB* and *pyrC* markers in a locus previously called *cat*. The locus of mutations to loss of mannosephosphotransferase, *mpt*, is between the *eda* and *fadD* genes. Mutations to loss of glucokinase, *glk*, are between the *ptsI* and *dsd* genes.

Many species of bacteria have a unique sugar-phosphorylating system in which phosphoenolpyruvate (PEP) acts as phosphate donor in a multi-step reaction (18, 35). A typical reaction scheme for this PEP-dependent phosphotransferase system (PTS) in *Escherichia coli* is (35):

$$\text{PEP} + \text{HPr} \xrightleftharpoons{\text{enzyme I, } Mg^{2+}} \text{HPr-P} + \text{pyruvate} \quad \text{(i)}$$

$$\text{HPr-P} + \text{II}_a \xrightarrow{Mg^{2+}} \text{II}_a\text{-P} + \text{HPr} \quad \text{(ii)}$$

$$\text{II}_a\text{-P} + \text{sugar} \xrightarrow{\text{II}_b,\ Mg^{2+}} \text{sugar-P} + \text{II}_a \quad \text{(iii)}$$

Enzyme I and Hpr are soluble proteins common to all phosphorylation reactions catalyzed by the PTS, whereas II_a and II_b are protein components of phosphohistidinoprotein-hexose phosphotransferases (EC 2.7.1.69) that are membrane bound and sugar specific (18, 25, 26, 35). In some reaction sequences, a soluble, sugar-specific protein, called factor III, takes the place of II_a in the above scheme (35). The phosphorylation of glucose in *E. coli* appears to proceed by the PTS pathway, since an enzyme II for glucose has been described (23-26) and mutants defective in enzyme I or Hpr grow poorly or not at all on glucose (40). However, mutants unable to grow on glucose because they are unable to phosphorylate glucose, but which can phosphorylate and grow on other PTS-metabolized sugars, have not been described to date. This fact suggests that *E. coli* has multiple enzymes capable of phosphorylating glucose. One known pathway is via the constitutive soluble adenosine 5'-triphosphate-dependent glucokinase (EC 2.7.1.2), but the available evidence suggests that this enzyme does not play an important role in glucose catabolism (11).

In this paper we report genetic studies showing the presence of two constitutively synthesized glucose-phosphorylating enzymes II in *E. coli*. One of these, glucosephosphotransferase (GPT), is quite specific for sugars with the D-glucose configuration. The other, mannosephosphotransferase (MPT), phosphorylates glucose and mannose, as well as their derivatives altered at the C2 position. Mutants lacking both GPT and MPT grow very slowly on glucose. If in addition the mutants lack glucokinase activity, they cannot grow on glucose. The isolation and genetic characterization of mutations affecting these enzyme activities are described below. Preliminary reports of some of this work have been presented (6; S. Curtis and W. Epstein, Fed. Proc. **30**:1123, 1971).

MATERIALS AND METHODS

Bacterial strains. The principal strains used, all *E. coli* K-12, are listed in Table 1. Three new genetic symbols are used: *gpt*, a gene for GPT activity; *mpt*, a gene for MPT activity; and *glk*, a gene for glucokinase activity. In a preliminary report (6), *gptA* and *ptB* were used for genes now designated *gpt* and *mpt*, respectively. Mutants bearing *glk* and *mpt* mutations

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were isolated by the authors. The *gpt* mutations *gpt-1* and *gpt-2*, were identified in strains isolated by others. The *cat-1* mutation in strain LA12 (42) is listed here as *gpt-1* in view of evidence presented below that this mutation affects GPT activity. An *E. coli* chromosome map showing the genetic loci and the origins of transfer of several Hfr strains used in this research is shown in Fig. 1. Genetic symbols other than *gpt*, *mpt*, and *glk* are those described by Taylor and Trotter (41).

Culture media and growth of bacteria. Minimal medium consisted of: sodium phosphate-buffered medium K10 (7); thiamine-hydrochloride, (1 mg/liter); a carbon source (2 to 10 g/liter); and, if required, amino acids (25 mg/liter) and adenine or uracil (50 mg/liter). One percent Casamino Acids medium contained 10 g of Casamino Acids (Difco) per liter of K10 medium. ML medium (7) was used for growth of cells for transductions and for matings. Cultures were usually grown in a New Brunswick gyratory incubator at 37 C.

Solid media contained 15 g of agar per liter. MacConkey agar base (Difco) indicator plates contained 10 g of glucose or mannose per liter. Mannitol-sorbitol tetrazolium indicator medium was prepared as described previously (8). BCIG indicator medium was prepared as described by Tyler et al. (42) and consisted of 10 g of glucose, 2 g of lactose, 15 g of agar, and 20 mg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside per liter of K10 medium.

Genetic methods. Matings, P1*kc*-mediated transduction, and penicillin selection of mutants were performed by standard techniques (7). The multiplicity of infection in transductions was 0.1 to 0.3.

Unless otherwise specified, *gpt*$^+$ and *mpt*$^+$ recombinants were selected on minimal medium plates containing 250 mg of glucose per liter. *fadD*$^+$ recombinants were selected on minimal medium containing 1 g of oleic acid per liter (31), *ptsI*$^+$ recombinants were selected on mannitol (10 g/liter), *eda*$^+$ recombinants were selected on sodium gluconate (10 g/liter) minimal medium (34), and *dsd*$^+$ recombinants were selected on *d*-serine (0.5 g/liter) minimal medium. The *mpt* and *gpt* mutations were scored on indicator plates as indicated in Table 2.

Preparation of the gpt glk mutant ZSC103. A mating was performed between strains W1895D1 (*gpt-2*) and X9172a (F$^-$ *trp purC lac strA*) and *purC*$^+$ *trp*$^+$ *strA* recombinants selected on glycerol minimal medium containing 400 mg of streptomycin sulfate per liter. Recombinants were scored on glucose-MacConkey indicator plates for the presence of the *gpt* mutation. One of the *gpt* recombinants in which the defect was confirmed by assay of methyl-α-D-glucopyranoside (α-MG) phosphorylation in extracts was mated with strain CHEp40 (HfrK116 *thi ptsI40*), and *ptsI strA* recombinants obtained by plating the mated cells on mannitol-sorbitol tetrazolium medium containing streptomycin (8). A *ptsI* recombinant was transduced to *ptsI*$^+$ with a P1*kc* lysate of the *glk* mutant ZSC17. Transductants appearing slightly lighter in color on glucose-MacConkey indicator medium than the parental *gpt* strain were assayed for glucokinase and GPT activity. Strain ZSC103 was one

TABLE 1. *Bacterial strains*

Strain[a]	Mating type	Pertinent genotype	Source or derivation
AB259	Hfr Hayes	*thi*	R. B. Helling (1)
AB259 6-1	Hfr Hayes	*thi purB*	R. B. Helling (1)
CAC-1	Hfr Hayes	*thi gpt-2*	Transductant of AB259 6-1
CAC-2	Hfr Hayes	*thi gpt-2 mpt-3*	UV[b] mutant of CAC-1
CAC-3	Hfr Hayes	*thi mpt-3*	Transductant of CAC-2
EM3003	F$^-$	*dsd purF aroC argH strA*	E. M. McFall
FF7046	F$^-$	*ptsI211 strA*	UV mutant; derived from FF8005 (8)
K63-C	F$^-$	*fadD his gal strA*	From K63 (31)
LA12	F$^-$	*gpt-1*	W. F. Loomis, Jr. (28)
RP102	F$^-$	*aroD arg pro strA*	J. Adler
RP864-C	F$^-$	*eda pps strA*	From RP864 of J. Adler
W1895	Hfr Cavalli	*metB*	C. F. Fox
W1895D1	Hfr Cavalli	*metB gpt-2*	D. P. Kessler (20)
X7014-L	F$^-$	*purB pyrC*	B. Tyler (42)
X7014-4	F$^-$	*purB gpt-1*	B. Tyler (42)
ZSC7	F$^-$	*ptsI211 glk-7 strA*	UV mutant of FF7046
ZSC17	F$^-$	*glk-7 strA*	Transductant of ZSC7
ZSC13	F$^-$	*glk-3 strA*	Analogous to ZSC17
ZSC103	F$^-$	*gpt-2 glk-7 strA*	See text
ZSC112 (103a)	F$^-$	*gpt-2 mpt-1 glk-7 strA*	UV mutant of ZSC103
ZSC113	F$^-$	*gpt-2 mpt-2 glk-7 strA*	UV mutant of ZSC103
ZSC114 (103agl)	F$^-$	*mpt-1 glk-7 strA*	Transductant of ZSC112
ZSC160	F$^-$	*ptsI gal strA*	From ZSC13 by matings
ZSC161	F$^-$	*glk-3 gal strA*	Transductant of ZSC160
ZSC162	F$^-$	*gal strA*	Transductant of ZSC160

[a] The designations in parentheses are those used for these strains in earlier reports (2, 6).
[b] UV, Ultraviolet light.

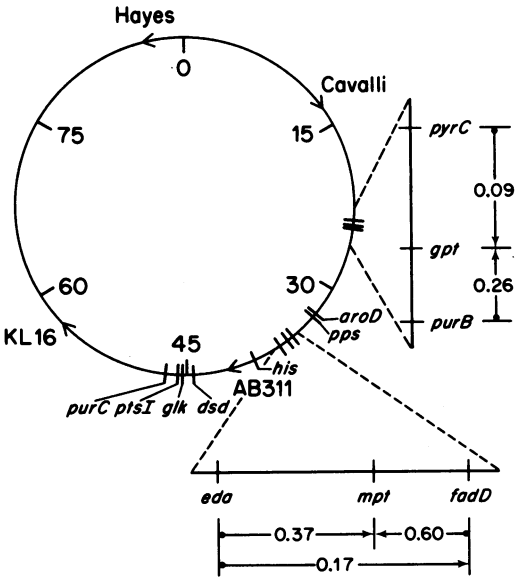

FIG. 1. *Representation of the circular chromosome of E. coli according to Taylor and Trotter (41), showing origins and direction of transfer of Hfr strains and positions of loci used in this work. The linkage data for the gpt and mpt loci are from Tables 5 and 7; arrows point from selected marker to scored, unselected marker. Data in Table 6 provide the basis for locating glk between ptsI and dsd.*

of the transductants found to lack both GPT and glucokinase activity.

Isolation of the gpt mpt glk mutants ZSC112 and ZSC113, and the mpt glk mutant ZSC114. A culture of ZSC103 was mutagenized by irradiation with ultraviolet light and subjected to penicillin selection in 1% glucose minimal medium. The penicillin treated culture was plated on glucose-MacConkey indicator medium. Colonies that were lighter in color than strain ZSC103 and which could grow in mannitol, but not in liquid 1% glucose minimal medium, were assayed for MPT activity. Several of these mutants were found to have very low rates of PEP-dependent glucose phosphorylation in sonic extracts. Two independently isolated *gpt mpt glk* mutants of this type are strains ZSC112 and ZSC113.

To isolate a *mpt glk* mutant, strain ZSC112 was transduced with a lysate of a wild strain and transductants able to grow on 0.025% glucose minimal medium were selected. The *gpt*$^+$ *mpt* and the *gpt mpt*$^+$ transductants produced could be readily distinguished on glucose-MacConkey and mannose-MacConkey indicator medium (Table 2). Strain ZSC114 is one of the transductants that, when assayed, was found to have normal GPT activity but lacked MPT activity.

Isolation of gpt and mpt mutants of AB259 6-1 (Hfr Hayes purB). Strain AB259 6-1 was transduced to *purB*$^+$ with a lysate of strain W1895D1. *purB*$^+$ *gpt-2* transductants produced were identified on indicator plates and assayed for GPT activity. Strain CAC-1 is one of these *gpt-2* transductants. The *gpt mpt* mutant CAC-2 was isolated from strain CAC-1 by the method described above for isolating strain ZSC112 (*gpt mpt glk*) from strain ZSC103. The *mpt* mutant CAC-3 was isolated as a *gpt*$^+$ transductant of strain CAC-2.

Isolation of glk mutants. It had been observed that all *pts* mutants isolated in strain FF8005 (F$^-$ *proC*$^-$ *strA*, see reference 8) grew slowly on glucose (W. Epstein, unpublished data). It was surmised that such growth was dependent on glucokinase. Strain FF7046, derived from strain FF8005 and carrying the *ptsI211* mutation, was mutagenized with ultraviolet light and subjected to penicillin selection in K10 medium containing 10 g of glucose per liter and 1 mM adenosine-3',5'-monophosphate. Strain FF7046 grows faster on glucose in the presence of the nucleotide. Survivors of the penicillin selection were plated on glucose-MacConkey medium. Mutants that were even paler than the parental *ptsI* strain on these plates and that could not grow on 0.1% glucose with or without cyclic adenosine-3',5'-monophosphate but could grow on galactose were tested for glucokinase activity. Three independent mutants defective in glucokinase were isolated in this way. Two of them, ZSC3 and ZSC7, lack detectable activity in the assay (less than 1% of the wild-type levels of 55 to 60 μmol/g per min), whereas the third, ZSC4, has slight activity of 1.1 μmol/g per min. The *glk* mutants ZSC13 and ZSC17 were obtained by transduction of strains ZSC3 and ZSC7, respectively, to *ptsI*$^+$.

Enzyme assays. The preparation of cell extracts for enzyme assays was performed at 0 to 5 C unless otherwise specified.

For assays of GPT to MPT activity, a strain was grown in 1% Casamino Acids medium to a density of 4 × 10^8 cells per ml. The cells were harvested by centrifugation, washed with 50 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.6), and suspended at 10^{10} cells per ml in the same buffer. The cells were then disrupted by sonic oscillation with a Branson Sonifier to produce a crude sonic extract. Assays contained, in a total volume of 200 μl: KF, 10

TABLE 2. *Appearance of gpt and mpt mutants on indicator media*[a]

Genotype	Appearance on:		
	Glucose-MacConkey	Mannose-MacConkey	BCIG
Wild	Red	Red	White
gpt	Light red	Red	Blue
mpt	Red	Very light pink	White
gpt mpt	Very light pink	White	Blue
gpt glk mpt	White	White	Blue

[a] Certain *gpt* mutant strains such as LA12 are not correctly scored on the MacConkey plates because they have PEP-dependent glucose phosphorylating activity that is greater than that normally found in most *gpt* mutant strains.

mM; $MgCl_2$, 0.05 mM; PEP, 12.5 mM; radioactive sugar substrate (specific activity 1,200 to 1,800 counts/min per nmol), 0.5 mM; enzyme I plus HPr extract, 50 μl; sonic extract, 50 μl; and Tris-hydrochloride (pH 7.6), 50 mM. In control assays, 50 μl of Tris-hydrochloride was substituted for the sonic extract. The reaction was initiated by addition of the sonic extract to a tube containing all of the other components of the assay. After incubation at 28 C for 20 min, the reaction was terminated by addition of 50 μl of 2 mM ethylenediaminineteetraacetic acid to the reaction mixture. Phosphorlyated sugar produced was measured by applying 50 μl of the reaction mixture to a strip (2 by 4 cm) of diethylaminoethyl paper (Whatman, DE81), which was dried and then washed extensively with deionized water (38). The strip was placed in 10 ml of scintillation fluid (33), and the radioactivity on the strip was counted in a Packard liquid scintillation spectrometer. Assays contained saturating amounts of enzyme I and HPr, such that enzyme activity was proportional to the amount of sonic extract present in the assay. One unit of GPT or MPT activity produces 1 μmol of sugar phosphate per min at 28 C.

To prepare extracts containing enzyme I and HPr, cells were grown in 50 to 100 liters of Casamino Acids medium in a New Brunswick Fermacell fermentor to a density of 10^9 cells per ml. Extracts were initially prepared from strain W1895D1; later when strain ZSC112 had been isolated it was used. The cells were harvested with a Sharples centrifuge, washed with 1 liter of TM buffer (Tris-hydrochloride, [pH 7.6], 10 mM; β-mercaptoethanol, 10 mM), and suspended in the buffer to 1 g of cells (wet weight) per 4 ml. The suspension was subjected to sonic oscillation and then centrifuged at 12,000 $\times$ *g* for 30 min. Nucleic acids were precipitated from the supernatant fluid with streptomycin sulfate (12 g/liter). The extract was then fractionated with $(NH_4)_2SO_4$. The proteins precipitating between 35 and 80% saturation were dialyzed against several changes of TM buffer, and the protein extract was then centrifuged at 100,000 $\times$ *g* for 2.5 h in a Beckman L2-65 ultracentrifuge to remove the membrane-bound enzyme II. To remove any GPT or MPT activity not eliminated by the centrifugation, the extract was chromatographed on a Bio-Gel P-300 polyacrylamide resin column (2.5 by 20 cm) with TM buffer. The void volume containing remaining membrane fragments in the extract was discarded. Fractions of eluant containing proteins significantly retarded by the column were pooled, and the proteins were precipitated with 80% $(NH_4)_2SO_4$ and then dialyzed against TM buffer. The dialyzed enzyme I plus HPr extract had a protein concentration of 25 to 30 mg/ml. Extracts were stored at -20 C with no appreciable loss of activity in 1 year.

For glucokinase assays, bacteria were grown overnight to stationary phase in 1% Casamino Acids medium. The cells were harvested by centrifugation, washed with 0.15 M NaCl, and suspended at 1/80 of the culture volume in buffer containing 50 mM Tris-hydrochloride (pH 7.6) and 10 mM $MgCl_2$. The cells were disrupted by sonic oscillation, and the sonic extract centrifuged at 27,000 $\times$ *g* in a Sorvall centrifuge. The supernatant fluid obtained was assayed for glucokinase at 25 C by the spectrophotometric method in which phosphorylation of glucose is coupled to the nicotinamide adenine dinucleotide phosphate-linked oxidation of glucose-6-phosphate by glucose-6-phosphate dehydrogenase (12).

Other methods. Uptake of α-MG was assayed in cells suspended at a density of 10^9 cells per ml in phosphate buffer identical to K10 medium except that it lacked $(NH_4)_2SO_4$, potassium salts were replaced with the corresponding sodium salts, and 40 μg of chloramphenicol per ml was added. After preincubation for 15 min at 30 C, [^{14}C]α-MG was added to a final concentration of 25 μM. Ten minutes later 0.2 ml of the assay mixture was filtered on a membrane filter (Millipore Corp., type HA), and the filter was then washed with 10 ml of cold phosphate buffer and dried. The filter was placed in 10 ml of scintillation fluid and counted in a liquid scintillation spectrometer. As a control assay, uptake of [^{14}C]α-MG in the presence of 50 mM nonradioactive α-MG was measured. Uptake activity is expressed as micro-moles of α-MG accumulated per gram of cell protein. The accumulation at 10 min represents the plateau level of uptake.

Protein concentrations were measured by the Folin-phenol method of Lowry et al. (29), with bovine serum albumin as the standard.

Chemicals. D-[U-^{14}C]glucose and [U-^{14}C]α-MG were purchased from the Calbiochem Co. Radioactive sugars were chromatographed on Dowex 1-formate to remove anionic radioactive contaminants. D-Glucose was obtained from the J. T. Baker Chemical Co.; D-mannose, containing no more than 0.02% glucose, was from the Calbiochem Co.; α-MG and D-fructose were from Pfanstiehl Laboratories, Inc.; and trisodium PEP, adenosine-5'-triphosphate, nicotinamide adenine dinucleotide phosphate, D-glucose-6-phosphate, D-glucosamine, and D-galactose were from Sigma Chemical Co.

RESULTS

Initial evidence for two enzymes II for glucose. The properties of a mutant strain, W1895D1, first indicated that there were two enzymes II in *E. coli* capable of phosphorylating glucose. This strain, isolated by Kessler and Rickenberg (20) and found by Schaefler (36) to be defective in transport of α-MG, was shown by Fox and Wilson (10) to lack PEP-dependent phosphorylation of α-MG in crude sonic extracts. In view of earlier evidence that α-MG and glucose are transported and phosphorylated by the same system (23, 24, 40), it was concluded that strain W1895D1 lacks an enzyme II for glucose. However, the strain does grow on glucose, although at a somewhat slower rate than its parent strain W1895, and PEP-dependent glucose phosphorylation in crude sonic extracts of W1895D1 is approximately 60% of that of W1895 (Table 3). This activity is due to a membrane-bound activity, since 91% of the

activity is in the pellet fraction obtained after high-speed centrifugation of a crude extract of strain W1895D1 (Table 4). Thus PEP-dependent phosphorylation of glucose by strain W1895D1 is not due to a soluble enzyme such as glucokinase. Further evidence for the presence of two enzymes II active on glucose came from measurements of inhibition of glucose phosphorylation by α-MG (Fig. 2). The analogue is quite a good inhibitor of approximately 40% of the activity in two wild-type strains, but the remaining 60% of the activity is resistant. None of the activity in strain W1895D1 is inhibited by α-MG.

In view of these results, we postulated the existence of two enzymes II for glucose with different substrate specificities, a hypothesis confirmed by the genetic work presented below. The activity absent in strain W1895D1 is GPT and is active on glucose and α-MG; a second enzyme II activity, MPT, although it is active on glucose, is the principal enzyme II active on mannose and appears to be identical with the mannose enzyme II described by Kundig and Roseman (26).

Mapping of the gpt gene. The locus of the *gpt* gene was estimated from the results of matings between strain W1895D1 (Hfr Cavalli; Fig. 1) and various F⁻ strains. In these matings, various markers were selected, and the frequency of inheritance of the *gpt* mutation in the recombinants was determined by scoring them on indicator plates (Table 2). The results indicated that *gpt* was transferred by W1895D1 to a recipient strain relatively late. When *ptsI*⁺ recombinants were selected, 8 of 78 recombinants were *gpt* mutants. Of 66 *his*⁺ recombinants, 10 were *gpt* mutants; of 78 *trp*⁺ recombinants, 26 were also *gpt*. These results suggested that the *gpt* gene was located between the *trp* locus and the origin of W1895D1 was near *purE*.

Precise mapping of the *gpt* mutation was facilitated by our identification of the *cat* mutation in strain LA12 as a *gpt* mutation. We suspected this from the fact that LA12 grows more slowly on glucose than its parent (28), as is

TABLE 4. *Glucose phosphorylation in membrane and soluble fractions of W1895 and W1895D1*[a]

Strain	% Glucose phosphorylation	
	Membranes	Soluble fraction
W1895	94	5
W1895D1	91	3

[a] A crude sonic extract of each strain was centrifuged at 100,000 × *g* for 2 h. PEP-dependent glucose phosphorylation activity was measured in the soluble and membrane fractions obtained. The percentage of glucose phosphorylation was calculated as [glucose phosphorylation in fraction/glucose phosphorylation in sonic extract] × 100.

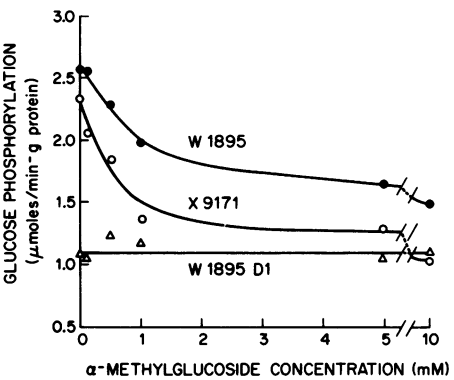

FIG. 2. *Crude sonic extracts of three different strains were tested for glucose phosphorylation in the presence of* α-MG concentrations ranging up to 10 mM. X9171 is a strain unrelated to W1895 and is wild type for all three glucose phosphorylating enzymes.

TABLE 3. *Phosphorylation of glucose and α-MG in wild-type and mutant strains*[a]

Strain	Genotype	α-MG uptake (μmol/g of protein)	α-MG phosphorylation (μmol/min per g of protein)	Glucose phosphorylation (μmol/min per g of protein)	Glucokinase activity (μmol/min per g of protein)
W1895	Wild type	14.2	1.8, 1.9	4.5, 4.6	52
W1895D1	*gpt-2*	0.04	0.13, 0.15	2.4, 2.5	53
LA12	*gpt-1*	—	0.12	—	—
CW101	*gpt-1*	—	0.04	—	—
ZSC103	*gpt-2 glk*	—	0.10	2.0	<0.5
ZSC112	*gpt-2 mpt-1 glk*	—	0.04	1.1	<0.5
ZSC113	*gpt-2 mpt-2 glk*	—	0.01	0.84	<0.5

[a] α-MG uptake in whole cells, α-MG and glucose phosphorylation in crude sonic extracts, and glucokinase in the supernatants of sonic extracts after centrifugation were measured as described in the text. Each value is the result of a separate determination. A dash indicates measurement was not performed.

the case for strain W1895D1. LA12 is defective in phosphorylation of α-MG (Table 3), and this defect is due to the *cat* mutation since *purB*$^+$ *pyrC*$^+$ *cat* transductants of X7014-L, such as strain CW101, are also defective in phosphorylation of α-MG (Table 3).

Transductional crosses (Table 5) showed that *gpt-2* is between *purB* and *pyrC*, and is closely linked to the *cat* mutation (called *gpt-1* here). The linkage data for *gpt-2* with the two flanking markers are similar to the data of Tyler et al. (42) for *cat* presented for comparison in the last column of the table. The *gpt-2* mutation is inherited with very high frequency (83%) when both *pyrC*$^+$ and *purB*$^+$ are selected, a much higher frequency of inheritance of the donor *gpt-2* mutation than when only one of these markers is selected. The last two crosses in the table give a measure of the recombination frequency between the *gpt-1* and *gpt-2* mutations. In each cross, *purB*$^+$ recombinants were selected and scored for *gpt*$^+$, which would be obtained by recombination between the two *gpt* mutations. In neither cross were any *gpt*$^+$ recombinants obtained. Since *gpt* and *purB* are approximately 25% linked, approximately 50 recombinants in each cross would have received the donor *gpt* region. In one of the crosses, which is not known since the order of *gpt-1* and *gpt-2* is not known, a double crossover would have resulted in *gpt*$^+$ recombinants. Since none was seen, it can be inferred that recombination between these two is infrequent, probably less than 2%.

Isolation of glk mutants and mapping of the glk gene for glucokinase. Although earlier work suggested that the soluble glucokinase of *E. coli* did not play an important role in glucose catabolism (11), it was not known whether the presence of this enzyme would complicate the identification of mutants defective in *mpt*. Thus, a strain lacking glucokinase as well as GPT activity was initially used in the isolation of *mpt* mutants. Three glucokinase mutants were isolated from a strain lacking enzyme I activity. Strain ZSC17 was then used in the preparation of a *gpt glk* mutant.

To facilitate preparation of a *gpt glk* mutant, the *glk* mutation was mapped. It was thought that the *glk* gene might be located near the *ptsI* gene, since both genes have a role in glucose metabolism. Matings were performed between the Hfr strain CHE9, which injects the *ptsI* region early, and three independently isolated *glk* mutants, and *ptsI*$^+$ recombinants were selected. A recombinant from each of the three matings was assayed and found to have normal glucokinase activity. This result indicated that *glk* is located near *ptsI*. Transductions in which *ptsI*$^+$ transductants of *ptsI glk* were selected showed that *ptsI* and *glk* are closely linked (Table 6). From the results of further transductional analysis, it was determined that the donor *glk* marker is inherited when both the *dsd* and *ptsI* markers of the donor are selected (Table 6). The *glk* marker of the donor is not inherited when both the *ptsI* and *purC* markers of the donor are selected in a transduction. From these results we conclude that the *glk* gene is approximately 70% linked to *ptsI* and is located between the *ptsI* and *dsd* genes. The gene order determined is *purC-ptsI-glk-dsd*.

TABLE 6. *Mapping of glk by transductional crosses*

P1*kc* donor[a]	Recipient	Recombinant class tested	Incidence of donor *glk* marker
Wild	*ptsI glk*	*ptsI*$^+$	8/11[b]
ptsI glk	*purC*	*purC*$^+$ *ptsI*	0/8
ptsI glk	*dsd*	*dsd*$^+$ *ptsI*	10/10

[a] The donor in the second cross was ZSC3; in the third cross it was a transductant of ZSC3 retaining the parental *glk* mutation but carrying the temperature-sensitive ptsI106R mutation.

[b] Results are composite for three crosses utilizing three different recipients (ZSC3, ZSC4, and ZSC7) with this genotype.

TABLE 5. *Mapping of gpt by transductional crosses*

P1*kc* donor[a]	Recipient[b]	Selected marker	Scored genotype	Incidence	Incidence with *gpt-1* as donor[c]
gpt-2	*purB pyrC*	*purB*$^+$	*gpt*	36/163 (26%)	59/218 (27%)
gpt-2	*purB pyrC*	*pyrC*$^+$	*gpt*	18/204 (9%)	50/225 (22%)
gpt-2	*purB pyrC*	*purB*$^+$ *pyrC*$^+$	*gpt*	30/36 (83%)	16/18 (88%)
gpt-2	*purB gpt-1*	*purB*$^+$	*gpt*$^+$	0/181	
gpt-1	*purB gpt-2*	*purB*$^+$	*gpt*$^+$	0/208	

[a] The *gpt-2* donor was strain W1895D1; the *gpt-1* donor was strain LA12G, a derivative of strain LA12.

[b] The recipient in the first three crosses was strain X7014-L. The *purB gpt-1* recipient was strain X7014-4. The recipient in the last cross was a *pyrC*$^+$ *gpt-2* transductant of strain X7014-L.

[c] These data, presented here for comparison, are those reported for *cat* by Tyler et al. (42).

Isolation of mpt mutants. Mutant strain ZSC103, defective in GPT and glucokinase activities, was mutagenized and subjected to penicillin selection in glucose medium. Survivors that were glucose negative on indicator plates were tested, and two found to have markedly reduced glucose phosphorylation in sonic extracts were used in further work. These two strains, ZSC112 and ZSC113, have glucose phosphorylation rates approximately 20% of that of the wild-type strain (Table 3), but we believe that this residual activity does not contribute significantly to glucose phosphorylation in vivo since the mutants do not grow on glucose. This residual activity is not enriched in membrane preparations over that of crude extracts, suggesting that it is not due to an enzyme II (unpublished data). Later we found that *mpt* mutants are readily identified even in strains with normal glucokinase activity. Several *mpt* mutants were then isolated from strain CAC-1 by using the methods used to obtain strains ZSC112 and ZSC113. These mutants were used in some of the mapping described below.

Mapping the mpt locus. Recombinants able to grow on minimal glucose plates were selected in matings of strain ZSC112 with different Hfr strains, and the recombinants were scored for genotype on indicator plates (Table 2). When an Hfr Hayes strain was the donor, virtually all of such recombinants were *gpt*$^+$ *mpt*, whereas crosses with strain KL16 or AB311 yielded mainly *mpt*$^+$ *gpt* recombinants. This result suggested that *mpt* was located between the origin of transfer of strain AB311 and *gpt*, but not very close to the latter. In transductional crosses, *mpt* is linked 37% with *eda* and 60% with *fadD* (Table 7). No linkage was observed with the *pps* and *aroD* markers that are in this region of the *E. coli* chromosome. Since linkage of *mpt* to either *eda* or *fadD* is greater than linkage of *eda* to *fadD* (17%), *mpt* should lie between these two. This location is supported by a three-factor cross (Table 7). An *fadD* donor was used to transduce an *eda mpt* recipient to *eda*$^+$. Of 39 transductants receiving the donor *fadD* marker, 38 also received the donor *mpt*$^+$ marker, whereas only 1 retained the recipient *mpt* marker. The order *eda-mpt-fadD* allows the former class to be obtained by a double crossover; the latter class requires a less frequent quadruple crossover.

Roles of GPT and MPT in sugar metabolism. The mutations under study here affect growth rates on three sugars: glucose, mannose, and glucosamine (Table 8). The roles of GPT and MPT are seen most clearly in the comparison of a strain having only one of these activities, with the corresponding mutant lacking both. Thus, GPT activity alone (compare ZSC114 with ZSC112, and CAC-3 with CAC-2) allows wild-type growth rate on glucose, but increases growth rates on the other sugars only modestly in the ZSC strains and not all in those derived from strain AB259. MPT activity alone (compare ZSC103 with ZSC112, and CAC-1 with CAC-2) permits growth on mannose and glucosamine at over 60% of the wild-type rate, while bringing glucose growth rates to approximately 40% of wild-type. These results show that GPT is of primary importance for growth on glucose, since it alone is sufficient to achieve wild-type growth rates on this sugar, whereas

TABLE 7. *Mapping of mpt by transductional crosses*[a]

P1*kc* donor	Recipient	Selected marker	Genotype scored	Incidence[b]
mpt	*aroD*	*aroD*$^+$	*mpt*	0/78 (0)
mpt	*pps eda*	*pps*$^+$	*mpt*	0/143 (0)
mpt	*pps eda*	*eda*$^+$	*mpt*	54/136 (37)
mpt	*fadD*	*fadD*$^+$	*mpt*	134/224 (60)
fadD	*pps eda*	*eda*$^+$	*fadD*	23/140 (17)
fadD	*mpt eda*	*eda*$^+$	*mpt*$^+$ *fadD*	38/223 (17)
			mpt$^+$ *fadD*$^+$	86/223 (39)
			mpt fadD	1/223 (0.5)

[a] The *mpt-3* allele was used in all crosses.
[b] Values in parentheses are percentages.

TABLE 8. *Growth of gpt and mpt mutants on sugars*[a]

Strain	Pertinent genotype	Activity present			Doubling time (min)					
		GPT	MPT	GLK[b]	Glucose	Mannose	Glucosamine	Fructose	Mannitol	Glycerol
ZSC13	*glk*	+	+	−	77	94	109	69	67	89
ZSC103	*gpt glk*	−	+	−	198	166	166	80	90	83
ZSC114	*mpt glk*	+	−	−	75	286	1,300	83	90	90
ZSC112	*gpt mpt glk*	−	−	−	>2,000	1150	>2,000	80	92	87
AB259	Wild	+	+	+	63	121	126	78	70	94
CAC-1	*gpt*	−	+	+	132	150	160	92	85	104
CAC-3	*mpt*	+	−	+	63	950	750	80	72	92
CAC-2	*gpt mpt*	−	−	+	295	770	730	103	77	103

[a] Cultures were grown in K10 minimal medium (at 37 C) containing 2 g of the indicated carbon source per liter.
[b] Glucokinase.

MPT alone allows only 40% of the normal growth rate. MPT, on the other hand, is the chief enzyme needed for rapid metabolism of mannose and glucoseamine, although both MPT and GPT are need to achieve wild-type growth rates on these sugars.

Glucokinase plays only a minor role in growth on the sugars. In strains lacking only glucokinase, no effect on growth rates for any of the sugars listed in Table 8 can be detected, such strains growing just as fast as the isogenic strain having glucokinase activity (data not shown). The role of glucokinase can be seen in strains lacking both GPT and MPT. In such a strain (CAC-2), there is slow growth on glucose and very slow growth on mannose and glucosamine. When glucokinase activity is also lost, as in strain ZSC112, there is no longer detectable growth on glucose or glucosamine, while slow growth on mannose persists. This result agrees with the known substrate specificity of glucokinase; active on glucose and glucosamine but not on mannose (3). Slow growth on mannose may be due to the soluble kinase active on mannose and fructose (37).

Glucokinase makes its presence felt even in the presence of GPT and MPT when strains utilize glucose generated inside the cell by hydrolysis of lactose. In *gal* mutants, growth on lactose is dependent on metabolism of glucose liberated inside the cell by the action of β-galactosidase. Growth on lactose is 20% slower in a *gal glk-3* strain than in the otherwise isogenic *gal glk*$^+$ strain (Table 9). Growth rates of the two strains on glucose and glycerol are comparable. This result suggests that the rate at which GPT and MPT phosphorylate glucose presented inside the cell is lower than when glucose is presented to these enzymes from the outside.

None of the mutations studied here has significant effects on utilization of other metabolizable sugars. Growth rates of the mutants on fructose and mannitol, both metabolized via the PTS (27, 39), and glycerol are shown in Table 8. In other experiments (data not shown), the triple mutant strain ZSC112 was found to have wild-type growth rates on xylose, sodium gluconate, galactose, and glucose-6-phosphate.

TABLE 9. *Role of glucokinase in growth on lactose*[a]

Strain	Pertinent	Doubling time (min)		
		Glucose	Glycerol	Lactose[b]
ZSC161	*glk-3 gal*	73	91	94, 101
ZSC162	*glk*$^+$ *gal*	70	92	78, 79

[a] Measurements were made as described in Table 8.
[b] Data for two separate experiments are shown.

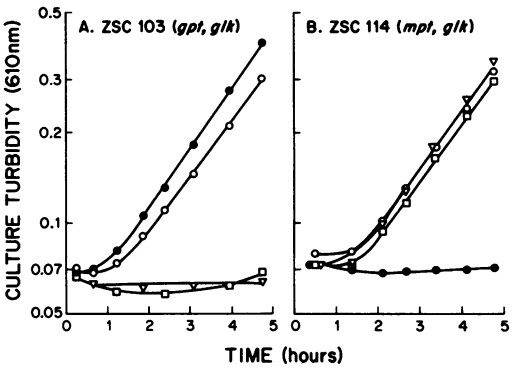

FIG. 3. *Inhibitory effect of non-metabolizable sugar analogues on adaptation to glycerol. Strains ZSC103 (A) and ZSC114 (B) were grown in minimal medium containing 15 mM* DL-*lactate to mid-logarithmic phase, washed, and suspended at approximately 8 × 10*^{7} *cells per ml in minimal medium containing glycerol (2 g/liter) and other additions (at 2 g/liter) as follows:* ○, *no addition;* ●, *α-MG;* □, *2-deoxyglucose;* △, *mannosamine. The tubes (18 by 150 mm) were shaken at 37 C, and culture turbidity was read at 610 nm in a Bausch & Lomb Spectronic colorimeter.*

Growth experiments can reveal the specificity of GPT and MPT for phosphorylation of sugar analogues that can not be metabolized to produce energy. The nometabolizable analogue α-MG, 2-deoxy-D-glucose, and mannosamine produce only a modest reduction of 20 to 40% in the growth rate of most strains of *E. coli* when added to cultures growing on glycerol. However, these analogues severely inhibit growth adaptation of *E. coli* when transferred from one carbon source, such as lactate, to glycerol or lactose. α-MG severely inhibits growth adaptation to glycerol of strains that have normal GPT activity, whereas 2-deoxyglucose and mannosamine inhibit strains that have MPT activity (Fig. 3). These effects are very specific, since 2-deoxyglucose and mannosamine have no detectable effect on strains lacking MPT and α-MG has no effect on strains lacking GPT. These results confirm that α-MG is a substrate for GPT and indicate that 2-deoxyglucose and mannosamine are substrates for MPT (2).

Preliminary experiments suggest that both catabolite repression and alterations in inducer metabolism mediate inhibition by the analogues. Both of these effects must be overcome to allow prompt adaptation to growth on the new carbon source. Catabolite repression alone does not account for the long lags upon adaptation to a new carbon source, since concentrations of cyclic adenosine monophosphate as high as 10 mM did not appreciably shorten the

growth lags. This concentration of cyclic adenosine monophosphate reverses both transient and catabolite repression (32). The roles of inducer transport and metabolism were tested in adaptation to lactose. Lactose enters cells via the inducible lactose transport system (19) but induces only after conversion by β-galactosidase to lactose isomers that are inducers (4, 17). Both the transport and conversion steps can be bypassed by isopropyl-β-D-thiogalactoside, which at a 10^{-3} M concentration readily enters cells and directly induces the lactose operon. We found that isopropyl-β-D-thiogalactoside alone had no effect, but when both isopropyl-β-D-thiogalactoside and cyclic adenosine monophosphate were present cells pregrown on sodium lactate began to grow on lactose in the presence of α-MG after a lag of 3 h. The lag in the absence of α-MG was 2 h, whereas with α-MG present but without both isopropyl-β-D-thiogalactoside and cyclic adenosine monophosphate the lag was over 8 h.

DISCUSSION

Our studies show that *E. coli* has three distinct enzymes capable of phosphorylating glucose at rates sufficient to allow growth on this sugar: the soluble adenosine-3',5'-triphosphate-dependent glucokinase, a highly glucose-specific enzyme II (GPT), and an enzyme II active on glucose and several other sugars (MPT). These are appropriate names since the former enzyme II is of major importance in permitting rapid growth on glucose, whereas the latter is necessary for rapid growth on mannose. These two enzymes appear to be identical to the glucose and mannose enzymes II, respectively, of Kundig and Roseman (26), since the former phosphorylates α-MG as does GPT, whereas the latter is active on *N*-acetylmannosamine which is a substrate for MPT (unpublished data).

The genes affecting these three activities are scattered over one quadrant of the chromosome of *E. coli* (Fig. 1). The *glk* gene is quite near the structural gene for enzyme I of the PTS, *ptsI*, but these two do not form a continguous cluster of genes affecting sugar metabolism. Recent mapping data (13, 21) for the gene for deoxyribonucleic acid ligase, *lig*, combined with the data presented here indicate that *lig* lies between *ptsI* and *glk*.

A major purpose of our studies has been the characterization of mutants defective in GPT and/or MPT activity to provide genetic evidence for the roles of these enzymes in sugar metabolism. The recognition that α-MG and glucose share a system for uptake in *E. coli* (14, 16) predates the discovery of the PTS. Subsequently, a number of mutants defective in enzymes II were isolated without an appreciation of the nature of the primary function affected by the mutation. The lesion in strain W1895D1 was identified by Fox and Wilson (10) some years after the strain was isolated. The discovery that strain LA12 is defective in GPT was made in the course of work reported here. Dietz and Heppel (5) isolated a mutant resistant to 2-deoxyglucose inhibition of adaptation to glycerol and observed the mutant did not accumulate 2-deoxy-glucose. It is probable that the mutant was defective in MPT, since such strains are not inhibited by 2-deoxyglucose (Fig. 3) and do not take up this compound (2).

Kornberg and Smith (22) have isolated *umg* mutants defective in uptake of α-MG. These are probably identical to our *gpt* mutants. The *umg* locus is near min 24.5 on the map and is co-transduced with *purB*. Co-transduction of *umg* with *pyrC* was not observed. Recently, Kornberg and Jones-Mortimer (FEBS Lett., in press) provided good evidence that the *ptsX* locus, first identified by its role in fructose uptake (9), is probably identical to our *mpt*. The *ptsX* locus is 53% co-transduced with *eda*, and *ptsX* mutants, like *mpt* mutants, grow very slowly on glucoseamine and mannose.

The marked inhibitory effect of analogues exploited by several groups of workers (5, 20, 22) is a useful method of selecting *gpt* and *mpt* mutants. We tested a number of compounds and found that there is good inhibition of adaptation to lactose as well as to glycerol. In our strains the selection is not absolute, since the parental strains usually begin to grow in the presence of the analogue after approximately 8 h. By performing two cycles of selection, an initial one from lactate to glycerol and a second from glycerol to lactose, we obtained over 100-fold enrichments for *gpt* or *mpt* mutants depending on the analogue present during the selection.

Analogues appear to inhibit adaptation to growth on new carbon sources by two effects: catabolite repression and by interfering with the accumulation of inducer in the cell. Analogues such as α-MG are known to produce rather severe transient repression and to interfere with transport of β-galactoside into the cell (30). Another point of action of the analogues is suggested by the observation that the inhibitory effect is particularly marked in adaptation to lactose or to glycerol. These two carbon sources are not inducers of their respective pathways; the true inducer in each case is a product of the

first enzyme of the pathway (4, 15, 17). Any inhibition of the first enzyme, β-galactosidase, and glycerol kinase, respectively, will inhibit induction. Since it seems hardly likely that all of the different analogues would inhibit these enzymes, we suggest that these effects are mediated by the phosphorylated derivatives of the analogues, known to accumulate in the cell (5, 35). The inhibition could be direct or could be indirect if the analogues deranged glycolysis so as to lead to an increase in cell fructose-1,6-diphosphate, the latter a known potent inhibitor of glycerol kinase (43).

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