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Defective Enzyme II-B^{Glc} of the Phosphoenolpyruvate:Sugar Phosphotransferase System Leading to Uncoupling of Transport and Phosphorylation in *Salmonella typhimurium*

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Transport and phosphorylation of glucose via enzymes II-A/II-B and II-B^{Glc} of the phosphoenolpyruvate:sugar phosphotransferase system are tightly coupled in *Salmonella typhimurium*. Mutant strains (*pts*) that lack the phosphorylating proteins of this system, enzyme I and HPr, are unable to transport or to grow on glucose. From *ptsHI* deletion strains of *S. typhimurium*, mutants were isolated that regained growth on and transport of glucose. Several lines of evidence suggest that these Glc$^+$ mutants have an altered enzyme II-B^{Glc}, as follows. (i) Insertion of a *ptsG*::Tn*10* mutation (resulting in a defective II-B^{Glc}) abolished growth on and transport of glucose in these Glc$^+$ strains. Introduction of a *ptsM* mutation, on the other hand, which abolishes II-A/II-B activity, had no effect. (ii) Methyl α-glucoside transport and phosphorylation (specific for II-B^{Glc}) was lowered or absent in *ptsH*$^+$,*I*$^+$ transductants of these Glc$^+$ strains. Transport and phosphorylation of other phosphoenolpyrurate:sugar phosphotransferase system sugars were normal. (iii) Membranes isolated from these Glc$^+$ mutants were unable to catalyze transphosphorylation of methyl α-glucoside by glucose 6-phosphate, but transphosphorylation of mannose by glucose 6-phosphate was normal. (iv) The mutation was in the *ptsG* gene or closely linked to it. We conclude that the altered enzyme II-B^{Glc} has acquired the capacity to transport glucose in the absence of phosphoenolpyruvate:sugar phosphotransferase system-mediated phosphorylation. However, the affinity for glucose decreased at least 1,000-fold as compared to the wild-type strain. At the same time the mutated enzyme II-B^{Glc} lost the ability to catalyze the phosphorylation of its substrates via III^{Glc}.

The phosphoenolpyruvate (PEP):sugar phosphotransferase system (PTS) catalyzes the transport and concomitant phosphorylation of a large number of sugars in bacteria such as *Escherichia coli* and *Salmonella typhimurium* (14). Transfer of the phosphoryl group from PEP to sugar is catalyzed by two general proteins, enzyme I and HPr, and a number of sugar-specific complexes which are mainly localized in the cytoplasmic membrane (Fig. 1). Glucose transport in *S. typhimurium* is catalyzed by two different PTS complexes. One of these complexes, designated II-A/II-B or II^{Man}, is also responsible for the uptake and phosphorylation of mannose, fructose, and 2-deoxyglucose. The second complex, II-B^{Glc}/III^{Glc}, which consists of the membrane-bound II-B^{Glc} and the soluble factor III^{Glc}, is specific for glucose and the nonmetabolizable analogs methyl α-glucoside, β-D-thioglucose, and 5-thioglucose (14, 16, 21, 22).

Mutations in the sugar-specific enzymes II result in the inability of the cell to grow on a particular PTS sugar. In contrast, mutants which are defective in the general proteins enzyme I and HPr do not grow on any PTS sugar. It was therefore concluded that transport and phosphorylation catalyzed by the PTS are tightly coupled (14). In the absence of the general phosphoryl-transferring proteins, the enzymes II do not catalyze facilitated diffusion (15). In the case of glucose, in particular, this is very clear. *S. typhimurium* contains abundant glucokinase (15, 19), but *ptsHI* deletion mutants which lack HPr and enzyme I are unable to grow on glucose.

In this paper, we report the isolation of mutants of *S. typhimurium* in which transport and phosphorylation of glucose via enzyme II-B^{Glc} are uncoupled. The altered II-B^{Glc} complex is unable to catalyze PEP-dependent phosphoryl-

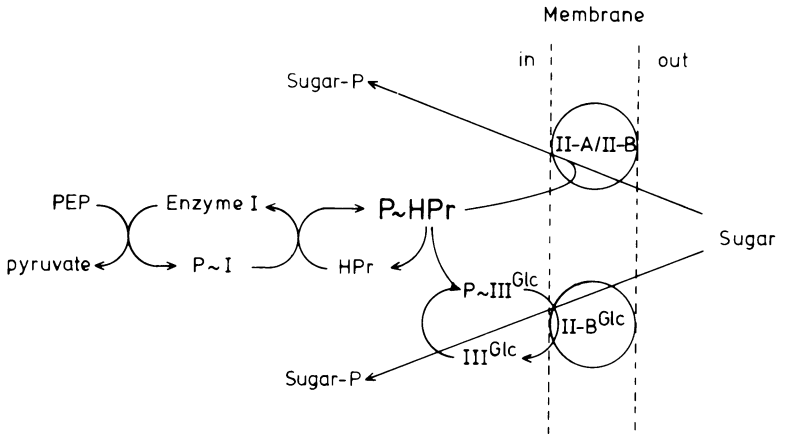

FIG. 1. *The PEP:sugar PTS. The phosphocarrier protein HPr is phosphorylated by enzyme I and is a substrate of either factor III^{Glc} or the II-A/II-B complex. P~III^{Glc} is a substrate of enzyme II-B^{Glc}. Abbreviations used: I, Enzyme I; II-A/II-B, the enzyme II^{Man} complex; II-B^{Glc}, enzyme II-B^{Glc}; P~, the high-energy phosphoryl derivative of the various PTS proteins.*

ation of methyl α-glucoside and glucose but allows facilitated diffusion of glucose in *ptsHI* deletion mutants.

MATERIALS AND METHODS

Chemicals. D-[*U*-^{14}C]glucose (284 mCi/mmol) and methyl(α-D[*U*-^{14}C]gluco)pyranoside (184 mCi/mmol) were purchased from the Radiochemical Centre, Amersham, England; [^{14}C]methyl β-galactoside (50 μCi/2.03 mg) and D-[*U*-^{14}C]mannose (50 mCi/mmol) were obtained from New England Nuclear Corp., Boston, Mass.

Bacterial strains. Table 1 lists the derivation of all bacterial strains used in this study.

Media and growth conditions. Cells were grown at 37°C on a rotary shaker in liquid medium A [containing, per liter of distilled water: $(NH_4)_2SO_4$, 1 g; K_2HPO_4, 10.5 g; KH_2PO_4, 4.5 g; $MgSO_4$, 0.1 g] supplemented with 20 μg of tryptophan per ml and a carbon source (0.2%), or on plates solidified with 1.5% agar (Difco Laboratories, Detroit, Mich.). Transductions involving Tn*10* were performed on nutrient agar plates (0.8% nutrient broth [Difco], 0.5% NaCl, and 1.5% agar) containing 20 μg of tetracycline per ml.

Preparation of cell-free extracts and enzyme assays. Cells were ruptured by passage through a French pressure cell, and cell-free extracts were prepared as described earlier (15). Membranes were prepared by centrifugation at 230,000 × *g* (Kontron TFT 65.13 rotor) for 90 min at 4°C. PEP:sugar phosphotransferase activity was determined by the ion-exchange method (9). As a source of enzyme I and HPr, the 230,000 × *g* supernatant of strain PP1120 was used; this lacks both II-A/II-B and II-B^{Glc} activity. As a source of III^{Glc} we used a partially purified fraction. Transphosphorylation catalyzed by enzyme II was measured at 37°C as described by Saier et al. (18), using the membrane fraction as a source of enzyme II.

Transport studies. Transport of labeled compounds was performed as described previously (12). The rate of transport is expressed as nanomoles of substrate taken up per minute per milligram (dry weight) at 20°C.

Oxygen consumption. O_2 consumption was measured with a Clark-type electrode in medium A and expressed as nanoatoms of oxygen consumed per minute per milligram (dry weight) at 25°C.

Phosphorylation in toluenized cells. Cells were toluenized, and PEP-dependent phosphorylation of sugar was measured as described elsewhere (21). Activity is expressed as nanomoles of NADH oxidized per minute per milligram (dry weight) at 20°C.

Protein. Protein was determined by the method of Lowry et al. (10), using bovine serum albumin as a standard.

Determination of generation times. The rate of growth of mutant strains on a given carbon source was determined as described elsewhere (12). Growth at 37°C was followed by measuring the increase in optical density at 600 nm.

Genetic methods. Preparation of P22(HT) transducing lysates and transduction with phage P22(HT) were performed as described by Ely et al. (4). Glc$^+$ revertants were isolated on minimal plates containing 0.2% glucose. Insertion of Tn*10* into the chromosome of *S. typhimurium* was performed as described by Davis et al. (3; see also reference 7), using a P22 lysate of strain NK337 (*hisC527 leu-414 supE* [P22 *c2*ts29 *12am*N1 *13am*H101 *int-3* Tn*10*]). Strain NK337 was a generous gift of J. Roth. The *ptsG415*::Tn*10* mutation was obtained by inserting Tn*10* into PP1108 (a strain lacking II-A/II-B due to the *mem-1* mutation [13]) and selecting for mutants that at the same time acquired resistance to tetracycline (Tet^r) and lost the capacity to grow on glucose. The *ptsM* mutations were isolated after diethylsulfate mutagenesis and penicillin selection as Glc$^-$ strains derived from strain SB3666, which lacks II-B^{Glc} (13). Tn*10* was inserted near *ptsM416* as described by Davis et al. (3). P22 was

TABLE 1. *Strains of S. typhimurium used in this study*

Strain	Relevant genotype[a]	Isolation procedure from parental strain[b]	Source[c]
PP801	*Δ(cysK-ptsHI)41 galP283 trpB223*		A
PP889	*Δ(cysK-ptsHI)41 galP283 crp-771 trpB223*		A
PP951	*pts-417 Δ(cysK-ptsHI)41 galP283 crp-771 trpB223*	Glc$^+$ PP889, spont.	B
PP952	*pts-418 Δ(cysK-ptsHI)41 galP283 crp-771 trpB223*	Glc$^+$ PP889, DES	B
PP995	*galP283 trpB223 crp-771*	PP889 × P22 (SB3507)	B
PP1031	*pts-417 galP283 crp-771 trpB223*	PP951 × P22 (SB3507)	B
PP989	*pts-418 galP283 crp-771 trpB223*	PP952 × P22 (SB3507)	B
PP1120	*ptsG415*::Tn*10 mem-1 mtlC579 trpB223*	Tn*10* insertion into PP1108	B
PP495	*ptsM420 ptsG204 trpB223*	Glc SB3666	B
PP497	*ptsM416 ptsG204 trpB223*	Glc SB3666	B
PP1125	*ptsM416 ptsG204 trpB223*, Tn*10* near *ptsM*		B
PP1133	*ptsM416 trpB223*	SB3507 × P22 (PP1125)	B
PP1162	*ptsG415*::Tn*10 Δ(cysK-ptsHI)41 trpB223*	PP801 × P22 (PP1120)	B
PP1135	*ptsG415*::Tn*10 pts-418 Δ(cysK-ptsHI)41 galP283 crp-771 trpB223*	PP952 × P22 (PP1120)	B
PP1138	*ptsM416 pts-418 Δ(cysK-ptsHI)41 galP283 crp-771 trpB223*	PP952 × P22 (PP1125)	B
PP1174	*ptsM416 pts-418 galP283 crp-771 trpB223*	PP1138 × P22 (SB3507)	B

[a] Genetic nomenclature according to Sanderson and Hartman (20).
[b] Glc, Glucose; spont., spontaneous; DES, diethylsulfate; P22, phage P22.
[c] A, Reference 14a. B, This study.

grown on the resulting strain and used to infect PP497, selecting for Tet^r Man$^-$ colonies. PP1133 was constructed by infecting strain SB3507 with P22, grown on PP1125, and selecting for Tet^r colonies that grew slowly on mannose.

RESULTS

Isolation of "uncoupled" enzyme II mutants. In an attempt to find mutant strains of *S. typhimurium* in which enzyme(s) II of the PTS was able to catalyze transport of glucose in the absence of PEP-dependent phosphorylation, cells of a *ΔptsHI galP* strain, PP889, were spread on agar plates containing 0.2% glucose. Colonies which regained growth on glucose were picked and purified. It had been shown earlier, however, that *ptsHI* deletion strains easily become Glc$^+$ due to a *galR* (17) or *galC* (12) mutation. Both mutations result in the constitutive synthesis of the galactose permease (GalP) which has affinity towards glucose. To eliminate this possibility, the starting strain, PP889, contained a *galP* mutation, resulting in a defective permease (12). Another possibility, constitutive synthesis of the methyl β-galactoside transport system (MglP), which also has affinity towards glucose, was examined by measuring transport of substrates of MglP in a number of Glc$^+$ mutants of strain PP889 grown under various conditions. Figure 2 shows the rates of methyl β-galactoside and glucose transport for a representative strain, PP952. Similar results were obtained with galactose transport. It is clear that MglP was still inducible because D-fucose, a gratuitous inducer of MglP, increased methyl β-galactoside transport (and glucose transport) at least 5- to 10-fold. Figure 2 shows also that growth on glucose did not result in the increase of activity of a glucose or methyl β-galactoside transport system or both. Similar results were obtained with six other independently isolated Glc$^+$ strains derived from PP889. Analogous Glc$^+$ strains were also isolated from a Δ(*ptsHI-crr*) *galP* mutant which lacks III^{Glc} in addition to enzyme I and HPr.

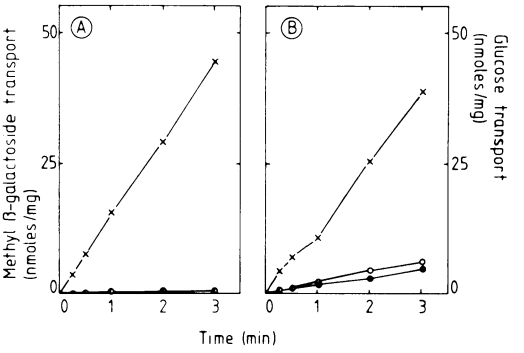

FIG. 2. *Transport of methyl β-galactoside and glucose in PP952. PP952 was grown in medium A containing as a carbon source 0.4% DL-lactate (●), 0.4% DL-lactate plus 1 mM D-fucose (×), or 0.2% glucose (○). Transport of (A) 0.5 mM [^{14}C]methyl β-galactoside (specific activity, 188 cpm/nmol) or (B) 0.5 mM [^{14}C]glucose (specific activity, 195 cpm/nmol) was measured as described in the text.*

Appearance of low-affinity glucose transport system in uncoupled mutants. Transport of glucose in PP952, as shown in Fig.

2, was measured at an extracellular glucose concentration of 0.5 mM, which is saturating for both glucose-specific enzymes II of the wild type under phosphorylating (i.e., intact PTS) conditions (14, 16). Under these conditions only a slight uptake of glucose was observed (Fig. 2B). However, the growth rate of PP952 and a similar strain, PP951, on 1% (56 mM) glucose was almost equal to that of the corresponding *pts*$^+$ strain (doubling times, 62 and 64 min, respectively, compared to 60 min in the parental *pts*$^+$ strain SB3507). For this reason we also studied glucose transport at higher concentrations.

Figure 3 shows that appreciable rates were indeed found in PP952 at 5 mM glucose. Under these conditions glucose transport was still negligible in PP889. Oxidation studies showed that PP952 contained a low-affinity glucose transport system which had an apparent K_m of 12 mM as measured by glucose oxidation (data not shown). In PP951 similar results were obtained, although with a slightly lower apparent K_m, 6.5 mM. The maximal velocities of glucose oxidation, 129 and 165 nanoatoms of oxygen consumed per min per mg (dry weight) in PP952 and PP951, respectively, were close to the value in *pts*$^+$ strains.

Uncoupling of glucose and methyl α-glucoside transport and phosphorylation. Figure 4 shows that whereas mannose (a II-A/II-B substrate) did not inhibit glucose transport in PP952, methyl α-glucoside (a II-B^{Glc}/III^{Glc} substrate) did. Conversely, PP952 showed some, although low, methyl α-glucoside transport at

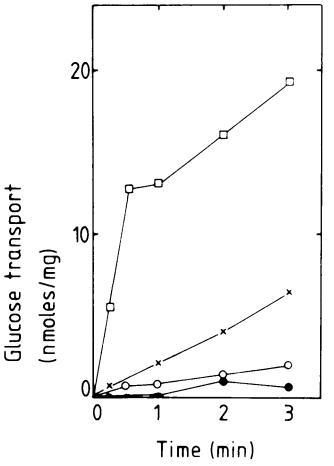

FIG. 3. *Glucose transport in ptsHI deletion strains. PP952 and PP889 were grown in medium A containing 0.4% DL-lactate, and transport of [^{14}C]-glucose (specific activity, 779 cpm/nmol) was measured.* (●) *PP889, 5 mM glucose;* (□) *PP952, 5 mM glucose;* (×) *PP952, 0.5 mM glucose;* (○) *PP952, 0.2 mM glucose.*

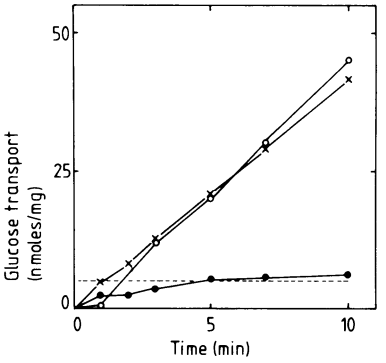

FIG. 4. *Effect of mannose and methyl α-glucoside on glucose transport. PP952 was grown in medium A containing 0.4% DL-lactate. Transport of 2.5 mM [^{14}C]glucose (specific activity, 181 cpm/nmol) was measured as described in the text.* (○) *No additions;* (×) *plus 20 mM mannose;* (●) *plus 20 mM methyl α-glucoside. Dotted line represents equilibration.*

high external concentrations which was partly inhibited by glucose but not by mannose (data not shown). These results suggested the possibility that II-B^{Glc} is involved in the transport of glucose in PP952. This conclusion was also confirmed by in vitro studies of PEP-dependent phosphorylation (Table 2). Thus, membranes of PP952 and PP951 were unable to catalyze III^{Glc}-dependent methyl α-glucoside phosphorylation but were able to catalyze mannose phosphorylation.

Our conclusion was further confirmed in *ptsH*$^+$*I*$^+$ transductants of the various Glc$^+$ PP889 derivatives. Figure 5 shows that P989, a *ptsH*$^+$*I*$^+$ transductant of PP952, had normal mannose transport as compared with the isogenic strain PP995 but did not transport methyl α-glucoside. Similarly, PP989 was also unable to catalyze PEP-dependent phosphorylation of methyl α-glucoside in contrast to the isogenic strain PP995 as measured in toluenized cells (data not shown). Similar results were obtained with the comparable strain PP1031 (*ptsH*$^+$*I*$^+$ PP951), although in that case some methyl α-glucoside phosphorylation was observed. Mannose and 2-deoxyglucose were phosphorylated in all three strains. These results strongly suggest that PP952 and similar strains have acquired a mutation in the II-B^{Glc} complex. The other component of the transport system, III^{Glc}, is present in normal amounts as determined by in vitro phosphorylation assays (data not shown).

Since it was found that the apparent K_m of glucose transport in strain PP951 was increased about 1,000-fold compared to the *ptsH*$^+$*I*$^+$ *ptsG*$^+$ strain, we also investigated phosphorylation of methyl α-glucoside and glucose at very high

TABLE 2. *II-B^{Glc} and II-A/II-B activity in various mutants*

Strain	Relevant genotype	PEP-dependent phosphorylation[a]	
		Methyl α-glucoside	Mannose
PP801		20	130
PP951	*pts-417*	0	150
PP952	*pts-418*	0	125

[a] Bacteria were grown in nutrient broth containing 0.2% glucose. Membranes were prepared as described in the text. PEP-dependent phosphorylation of 10 mM [^{14}C]mannose (specific activity, 93 cpm/nmol) was measured in the presence of added enzyme I and HPr, and phosphorylation of 0.5 mM [^{14}C]methyl α-glucoside (specific activity, 123 cpm/nmol) was measured in the presence of added enzyme I, HPr, and III^{Glc}. Activity is expressed as nanomoles of sugar phosphorylated per minute per milligram of membrane protein at 37°C.

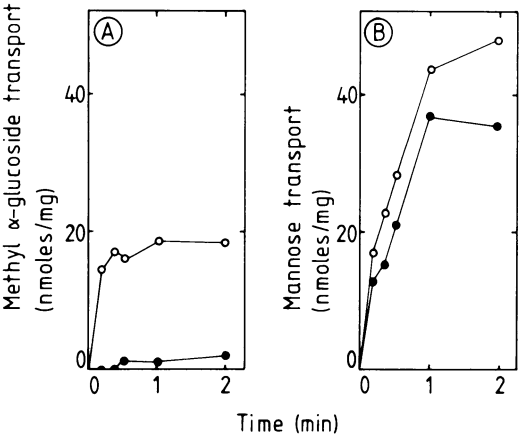

FIG. 5. *Methyl α-glucoside and mannose transport in a pts$^+$ transductant. PP989 and PP995 were grown in medium A containing 0.2% glucose. (A) Transport of 0.5 mM [^{14}C]methyl α-glucoside (specific activity, 211 cpm/nmol); (B) transport of 0.5 mM [^{14}C]mannose (specific activity, 43 cpm/nmol).* (○) *PP995;* (●) *PP989.*

concentrations. Phosphorylation of methyl α-glucoside was low or negligible even at concentrations as high as 40 mM. Glucose phosphorylation in strain PP989 via enzyme II-B^{Glc}, if any, will be obscured by the presence of II-A/II-B. This second pathway was eliminated in PP989 by introduction of a *ptsM* mutation in the *pts-418* background. Table 3 shows that whereas mannose and methyl α-glucoside phosphorylation in PP1174 was virtually zero, glucose was phosphorylated but only at very high concentrations. The complete absence of this PEP-dependent phosphorylation of glucose in *ptsG ptsM* double mutants such as PP495 proves that we are not dealing with an unknown bypass.

Transphosphorylation. We investigated whether the transphosphorylation reaction (18) can still be catalyzed by the altered enzyme II-B^{Glc} of strain PP952. Table 4 shows that membranes of PP952 and similar strains were unable to catalyze transphosphorylation from glucose 6-phosphate to methyl α-glucoside, but still catalyzed transphosphorylation from glucose 6-phosphate to mannose. The first reaction is dependent on II-B^{Glc}, whereas the second is catalyzed by II-A/II-B.

Effect of *ptsG*::Tn*10* and *ptsM* mutations. It was predicted that introduction of a second *ptsG* mutation (resulting in a defective II-B^{Glc}) would abolish glucose transport in PP952 and PP951, whereas a *ptsM* mutation (defective II-A/II-B) should have no effect. The *ptsG415* mutation used in this study results from a Tn*10* insertion and eliminates II-B^{Glc} activity completely (data not shown). The *ptsM416* mutation, which is localized close to a Tn*10* insertion

TABLE 3. *PEP-dependent phosphorylation in various enzyme II mutants*

Strain	Relevant genotype	PEP-dependent phosphorylation[a]				
		Glc			αMG (20 mM)	Man (5 mM)
		1 mM	5 mM	20 mM		
PP1174	*pts-418 ptsM*	6	20	36	5	1
PP495	*ptsG ptsM*	3	NT	4	1	4
PP1133	*ptsM*	70	NT	75	55	2

[a] Cells were grown in nutrient broth containing 0.2% glucose. Phosphorylation was measured in toluenized cells by using the indicated sugars and concentrations. Activity is expressed as nanomoles of NADH oxidized per minute per milligram (dry weight) at 20°C. Glc, Glucose; αMG, methyl α-glucoside; Man, mannose; NT, not tested.

TABLE 4. *Transphosphorylation*

Strain	Relevant genotype	Transphosphorylation[a]	
		Methyl α-glucoside	Mannose
PP801		0.90	1.4
PP952	*pts-418*	0.03	1.8
PP1135	*ptsG415*::Tn*10 pts-418*	<0.02	1.1
PP1162	*ptsG415*::Tn*10*	<0.02	1.2

[a] Cells were grown in nutrient broth containing 0.2% glucose. Preparation of membranes is described in the text. Transphosphorylation was measured with either 100 μM [^{14}C]methyl α-glucoside (specific activity, 383 cpm/nmol) or 100 μM [^{14}C]mannose (specific activity, 442 cpm/nmol) as acceptor and with 10 mM glucose 6-phosphate as a donor. Activity is expressed as nanomoles of sugar phosphorylated per minute per milligram of protein at 37°C.

(about 70% cotransduction), abolished mannose transport but not methyl α-glucoside transport (data not shown). Both mutations were introduced into PP952 and PP951 by selection for tetracycline resistance. All tetracycline-resistant transductants (15/15) from the cross of PP952 with phage P22 grown on the *ptsG*::Tn*10* strain PP1120 were unable to grow on glucose, whereas all transductants (35/35) derived from PP952 × phage P22 grown on the *ptsM* strain PP1125 were still able to grow on glucose. Transport studies also support the conclusion that glucose transport in PP951 is catalyzed by II-B^{Glc}. The *ptsG*::Tn*10*-containing strain PP1135, derived from PP952, lost the capacity to catalyze the transport of glucose in contrast to the *ptsM*-containing strain PP1138 (data not shown).

These results do not rigorously prove that the mutation which allows growth of PP952 on glucose necessarily is localized in the *ptsG* gene. Enzyme II-B^{Glc} could be composed of more than one subunit. *ptsG* might code for a protein that is involved in methyl α-glucoside phosphorylation, whereas a second gene might code for a protein which is responsible for transport per se (1, 5, 6). If those two genes are closely linked, introduction of the *ptsG*::Tn*10* mutation would at the same time eliminate also the *pts-418* mutation, which is responsible for the transport of glucose in Δ*ptsHI galP* strains. If the genes are not closely linked, it may be that the protein which contains the *pts-418* mutation is dependent for its activity on the protein that harbors the *ptsG*::Tn*10* mutation. The finding that PP1135 cannot be transduced to Glc$^+$ with P22 grown on a Δ*ptsHI ptsG*$^+$ strain (PP642) suggests that *pts-418* and *ptsG*::Tn*10* are closely linked. This conclusion is supported by preliminary mapping data which show that linkage between *ptsG*::Tn*10* and *purB* is more than 80% using Hfr K4. Similar results were obtained with *pts-418*.

DISCUSSION

ptsHI deletion mutants do not grow on glucose, nor do they transport glucose. It has been concluded that enzyme II-B^{Glc} is unable to catalyze glucose accumulation (or even equilibration) in the absence of the phosphoryl-transferring proteins enzyme I and HPr, due to a tight coupling between transport and PEP-dependent phosphorylation (15).

In this paper we describe mutants of *S. typhimurium* which have lost this tight coupling. Starting from *ptsHI* deletion mutants, we isolated strains that grow on glucose. A number of results indicate that this new capacity is due to a mutation in II-B^{Glc}. (i) Membranes of our uncoupled mutants lacked III^{Glc}-stimulated methyl α-glucoside phosphorylation but catalyzed normal mannose phosphorylation (Table 2). Similar results were obtained with transphosphorylation of methyl α-glucoside and mannose (Table 3). (ii) *ptsH*$^+$,*I*$^+$ transductants of our uncoupled mutants transported and phosphorylated mannose but not methyl α-glucoside (Fig. 5). (iii) The low-affinity glucose transport system which appeared in the Glc$^+$ *ptsHI* deletion strains was inhibited by methyl α-glucoside but not by mannose (Fig. 4). (iv) Introduction of a *ptsG*::Tn*10* mutation abolished this low-affinity glucose transport system. (v) The new *pts* mutations described in this paper were closely linked to the *ptsG* mutations described earlier.

From these results we conclude that the Glc$^+$ *ptsHI* deletion strains described in this paper have acquired an altered enzyme II-B^{Glc}. The complex is able to catalyze the transport of glucose in the absence of phosphorylation, in contrast to enzyme II-B^{Glc} in the wild-type strain. If the mutant strain contains enzyme I, HPr, and III^{Glc}, phosphorylation of methyl α-glucoside is not detectable; glucose, however, is phosphorylated although only at very high external concentrations. Transphosphorylation studies showed that the mutated II-B^{Glc} has also lost the capacity to transfer the phosphoryl group from glucose 6-phosphate to methyl α-glucoside. Although our mutants can catalyze glucose transport via II-B^{Glc} in the absence of PEP-dependent phosphorylation, the apparent affinity of the system for glucose is decreased at least 1,000-fold compared to the situation in wild-type *ptsH*$^+$*I*$^+$ strains. The apparent K_m values of glucose transport in the intact cells and glucose phosphorylation in toluenized cells and crude cell extracts of wild-type strains of *S. typhimurium* are in the micromolar range (14, 16), whereas in a *pts-418* background, which results in the altered II-B^{Glc}, an apparent K_m of 6.5 to 10 mM is found for transport. The much higher apparent K_m of methyl α-glucoside phosphorylation as compared to glucose in the wild-type strain, 60 μM (21), might be the reason why no methyl α-glucoside phosphorylation or transphosphorylation was detected in our mutant strain.

With respect to II-B^{Glc}, two different mutations have been described in *E. coli* and *S. typhimurium*. *ptsG* mutants lack methyl α-glucoside transport and phosphorylation (14). *tgl* mutants, on the other hand, have lowered methyl α-glucoside transport, but the rate of phosphorylation, as measured in extracts or toluenized cells, is equal to that in the wild type. It has been suggested by Gershanovitch and coworkers (1, 5) that II-B^{Glc} consists of two com-

ponents, one involved in transport, the other in phosphorylation.

The *pts-418* mutation which results in the uncoupled phenotype is clearly different from the *ptsG*::Tn*10* mutation (described in this paper) and *ptsG* mutations in *S. typhimurium* and *E. coli* described previously (2, 8, 11, 13, 16). In those cases, phosphorylation and transport of glucose, catalyzed by II-B^{Glc}, are completely absent, even at high external concentrations. The *pts-418* mutation is also different from the *tgl* mutation described by Gershanovitch and coworkers in *E. coli* (1, 5). *tgl* mutants are still able to phosphorylate methyl α-glucoside, provided it can reach its phosphorylation site inside the cell. Our mutants, in contrast, have lost the ability to phosphorylate II-B^{Glc}-specific substrates, even if the sugar can reach the cytoplasmic side of the membrane (toluenized cells, in vitro PTS assay). The transport of glucose in our Δ*ptsHI pts-418* strain is still saturable, in contrast to *tgl* strains (5), although the apparent K_m is increased 1,000-fold. The present lack of information on the structure of II-B^{Glc}, the nature of its active site(s), and the possibility of a phosphorylated intermediate do not allow us to conclude whether the specific defect in II-B^{Glc} is caused by an alteration of the active site for the sugar, the site with which phosphorylated III^{Glc} reacts, or a combination of both of these possibilities. Although the absence of the transphosphorylation reaction, which is independent of III^{Glc}, suggests that the binding site for sugar has been affected, it cannot be excluded at the moment that alterations in other parts of the II-B^{Glc} affect this site.

The striking property of the mutated enzyme II-B^{Glc} reported in this paper, namely, its capacity to catalyze the influx of glucose in intact cells in the absence of concomitant phosphorylation, suggests that enzyme II-B^{Glc} may act like a pore which is closed in wild-type cells unless phosphorylated III^{Glc} is present. In the latter case, transport of the sugar from outside to inside coupled to phosphorylation is possible. The mutation described here may alter II-B^{Glc} in such a way that the pore is open under all conditions. As a consequence, transport is always possible and is uncoupled from phosphorylation. We have not yet been able to determine whether this uncoupled II-B^{Glc} also facilitates the exit of glucose from the cell. Purification of wild-type and mutant II-B^{Glc} and reconstitution in liposomes might be a possible way to investigate the possibilities mentioned above.

Finally, we want to draw attention to mutants of *E. coli* with uncoupled lactose transport which have been described by Wilson and coworkers (23, 24) and which might be analogous to ours. In wild-type cells lactose is transported by a lactose-H^+ symport system, the M protein, in which the lactose and H^+ movements are obligatorily coupled. The driving force for lactose transport is the electrochemical proton gradient. In these mutants lactose and H^+ movement are uncoupled.

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