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3-Deoxy-3-Fluoro-D-Glucose-Resistant *Salmonella typhimurium* Mutants Defective in the Phosphoenolpyruvate:Glycose Phosphotransferase System[1]

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Three classes of phosphotransferase system mutants in *Salmonella typhimurium* were selected through their resistance to 3-deoxy-3-fluoro-D-glucose (DFG). Strains with mutations in the *ptsH* (HPr) and/or *ptsI* (enzyme I) genes were selected on medium containing lactate plus DFG. Strains with mutations in *ptsH* but not *ptsI* were selected on medium containing fructose plus DFG. Clones isolated from fructose plus DFG semisolid plates and selected for ability to swarm were mutant in either *ptsH* or *ptsG*. Mutants of the latter class were defective in enzyme IIB', a membrane component of the glucose transport system. Some pleiotropic properties of one representative *ptsG* mutant are described.

The metabolism of the glucose analogue 3-deoxy-3-fluoro-D-glucose (DFG) (6) has been studied in *Saccharomyces cerevisiae* (18; R. J. Miles and S. J. Pirt, Biochem. J. 144:10p, 1969) and in *Pseudomonas fluorescens* (17). The only product of DFG metabolism detected was 3-deoxy-3-fluoro-D-gluconic acid. Miles and Pirt (11) found that frozen and thawed cells of *Escherichia coli* converted DFG to 3-deoxy-3-fluoro-D-glucose-6-phosphate via catalysis by the phosphoenolpyruvate:sugar phosphotransferase transport system (PTS) (9). DFG at 0.1 to 10 mM severely inhibited the uptake and utilization of lactose, fructose, glycerol, succinate, acetate, and pyruvate by intact *E. coli* cells. Lactose utilization was found to be prevented due to the inhibition by DFG or its phosphate ester of the synthesis of β-galactosidase and galactoside permease (11).

DFG-resistant mutants of *E. coli* have been characterized as enzyme II mutants of the PTS by virtue of their inability to form 3-deoxy-3-fluoro-D-glucose-6-phosphate and by growth studies (11) or by growth and transport studies (7). Enzyme II activity of the PTS was not assayed directly in membrane preparations. In this paper we report the isolation and characterization of *Salmonella typhimurium* DFG-resistant mutants and show these to be deficient in various components of the PTS. The enzymatic composition of the PTS has been reviewed (8, 12) and is presented in summary form in the accompanying paper (2). We show below that selection of mutants resistant to DFG during growth on D-fructose as the sole source of carbon allows the isolation of *ptsH* (i.e., HPr^-) and enzyme II^- mutants. Growth on DFG plus lactate yields *ptsH* and *ptsI* (i.e., HPr^- and enzyme I^-) mutants. One enzyme II mutant has been characterized as defective in the glucose transport system mediated by membrane component enzyme IIB'. Some pleiotropic effects of this genetic lesion are summarized.

MATERIALS AND METHODS

Bacterial strains and mutant isolation. *S. typhimurium* LT-2 strains are described below and listed in Table 1. Mutants resistant to DFG were isolated from strain SB3507 (*trpB223 pts*$^+$) by direct plating of nitrous acid-mutagenized cultures on minimal medium agar plates containing either 0.2% lactate or 0.2% fructose as sole carbon source and 20 μg of L-tryptophan per ml. Crystals of DFG were applied to each plate, the plates were incubated for 3 days at 37°C, and single colonies appearing in the inhibition zones were picked to analogous media lacking DFG. After 2 days of incubation, these plates were replica plated onto eosin-methylene blue (EMB) mannitol and EMB fructose plates. Presumptive fermentation-negative mutants were placed through single-colony isolation from the EMB mannitol plates. Strains SB3799 through SB3802 were isolated from the lactate selection, and strains SB3769 and SB3798 were isolated from the fructose selection. Another series of mutants (strains SB3730 through SB3734) was isolated as spontaneous mutants by stabbing individual colonies of the *pts*$^+$ parent (strain

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TABLE 1. *Salmonella typhimurium LT-2 strains, genotypes, and isolation methods*

Strain	Genotype	Isolation[a]	Reference
SB2676	*ptsH28 pts-224*	Revertant on mannitol of *ptsH28* (overproduces HPr-fructose)	J. C. Cordaro (unpublished data)
SB3507	*trpB223 pts*$^+$		This paper
SB3730	*trpB223 ptsG217*	DFG-fructose swarm	This paper
SB3731	*trpB223 ptsG218*	DFG-fructose swarm	This paper
SB3732	*trpB223 ptsG219*	DFG-fructose swarm	This paper
SB3733	*trpB223 ptsG220*	DFG-fructose swarm	This paper
SB3734	*trpB223 ptsG221*	DFG-fructose swarm	This paper
SB3735	*trpB223 ptsG222*	DFG-fructose swarm	This paper
SB3736	*trpB223 ptsG223*	DFG-fructose swarm	This paper
SB3737	*trpB223 ptsH196*	DFG-fructose swarm	This paper; 2
SB3769	*trpB223 ptsH197*	DFG-fructose	This paper; 2
SB3770	*trpB223 ptsG217 ptsI184*	Fosfomycin resistance in SB3730	This paper; 2
SB3771	*trpB223 ptsG217 cysA20*	Transduction of *cysA20* into SB3730	This paper
SB3774	*trpB223 ptsG217 ptsH196*	Transduction from SB3737 into SB3771	This paper
SB3798	*trpB223 ptsH198*	DFG-fructose	This paper; 2
SB3799	*trpB223 ptsI199*	DFG-lactate	This paper; 2
SB3800	*trpB223 ptsH192*	DFG-lactate	This paper; 2
SB3801	*trpB223 ptsI193*	DFG-lactate	This paper; 2
SB3802	*trpB223 ptsI194*	DFG-lactate	This paper; 2

[a] Methods are described in text.

SB3507) into minimal medium swarm plates. These semisolid plates contained 1 mM fructose as sole carbon source, 20 μg of L-tryptophan per ml, 0.05 mg of 2,3,5-triphenyltetrazolium chloride per ml, 0.6 mM DFG, and 0.25% Ionagar (Oxoid). After 2 days of incubation at 37°C, bacteria were picked from the clearly delineated circumferences of independent swarms on the tetrazolium-containing medium, and the bacteria were streaked out for single-colony isolation. Phenotypes of presumptive mutants were tested on fermentation media by replica plating. The three strains, SB3735 through SB3737, were selected from among 30 spontaneously mutant colonies growing near DFG crystals on fructose plates. These three strains grew and swarmed freely when stabbed into fructose-DFG semisolid plates of the composition noted above; the remaining 27 colonies failed to grow in the semisolid plates.

Media and genetic tests. The preparation of growth and indicator media, the propagation of phage lysates, techniques for transduction and reversion analyses, and nitrous acid mutagenesis were performed as previously described (1-3).

DFG. DFG was synthesized according to the procedure of Foster et al. (6).

Construction of double-mutant strains. Strain SB3770 (*ptsG217 ptsI184 trpB223*) was isolated as a spontaneous mutant resistant to 40 μg of fosfomycin per ml in strain SB3730 (*ptsG217 trpB223*) by plating with 0.2% lactate as sole carbon source (2). Strain SB3774 (*ptsG217 ptsH196 trpB223*) was constructed in two steps by transduction of *cysA20* into strain SB3730 (3), resulting in strain SB3771 (*ptsG217 cysA20 trpB223*). Phage propagated on strain SB3737 (*ptsH196 trpB223*) was then used as donor, and *cysA*$^+$ recombinants were selected on minimal lactate plates containing L-tryptophan. Recombinant clones were scored for content of the *ptsH196* marker, jointly transduced with *cysA*$^+$ (cf. reference 3).

Sugar transport. Bacteria were cultured to mid-exponential growth phase in 500 ml of minimal medium containing 0.2% carbon source and 20 μg of L-tryptophan per ml. The bacteria were harvested, washed twice in cold minimal medium minus carbon source and supplements (MO), and concentrated 100-fold by centrifugation at 4°C for 10 min at 23,000 × *g*. Samples of 1.25 ml were added to 1.25 ml of MO for transport measurements. A stop-flow apparatus (15) was used to rapidly mix these diluted cells at room temperature with 2.5 ml of MO containing 0.5 mM labeled sugar: D-[U-^{14}C]glucose (specific activity, 3.6 × 10^5 cpm/μmol) or D-[U-^{14}C]mannose (specific activity, 2.4 × 10^5 cpm/μmol). Samples of 0.1 ml were removed at intervals and diluted into 10 ml of MO at room temperature. The mixtures were filtered rapidly through Reeve Angel glass-fiber filters (984 H) premoistened with MO and mounted on a manifold (Millipore Corp.). The time from mixing of the cell suspension with the labeled sugar to dilution of each sample was recorded automatically, representing the time for uptake. The time between dilution and complete filtration of each sample was less than 5 s. The filters were dried and then counted in a toluene-based scintillation fluid containing Triton X-100. Dry-weight measurements were made for each culture after desiccation of a sample at room temperature for 2.5 days. Transport data are expressed as micromoles of sugar taken up per gram (dry weight) of cells at 23°C.

Cell extracts. Bacterial cultures grown in tryptophan-supplemented minimal medium with 0.2% lactate as sole carbon source (unless otherwise specified) were incubated in a New Brunswick gyratory shaker at 37°C and harvested when the bacteria were in late exponential growth phase (i.e., usually at about 0.8 to 1.0 units of optical density at 590 nm as measured in a Beckman spectrophotometer). After sedimentation in a GSA rotor in a Sorvall refrigerated centrifuge at 16,000 × *g* for 20 min, the

bacteria were resuspended and washed in two-thirds of the original volume of a 0.9% NaCl solution and resedimented. A second wash preceded resuspension in 5 ml of 0.01 M tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.5 (all buffers contained 1 mM ethylenediaminetetraacetic acid and 0.1 mM dithiothreitol). Cell suspensions were passed through an Aminco French pressure cell at 20 to 25,000 lb/in^2, and the debris was sedimented in a Sorvall centrifuge at 17,000 × g for 10 min at 4°C. The supernatant was centrifuged for 2 h at 220,000 × g (Ti50 rotor) in a Beckman L-2 preparative ultracentrifuge to sediment membrane fragments. The clear supernatant was recentrifuged for an additional 2 h to eliminate membrane fragments more completely from the soluble fraction. The pellet fractions were resuspended with a Teflon pestle homogenizer in the 0.01 M Tris buffer (total volume of 10 ml) and recentrifuged at 220,000 × g. The washed pellet was resuspended in 1 ml of 0.01 M Tris buffer. Extracts prepared in this manner could be kept at 4°C for 3 to 4 days without significant loss of activity; however, all assays reported here were performed within 24 h after preparation. The supernatant fraction contained the soluble components of the PTS (i.e., HPr, factor III, and enzyme I), whereas the pellet contained the membrane-bound proteins (enzyme IIA, enzyme IIB, and enzyme IIB') (8, 12).

PTS assays. HPr was assayed by two methods. The first assay procedure measured HPr as a substrate of enzyme I (E. Bruce Waygood, personal communication), and the second measured phospho-HPr as a substrate of enzyme II (9). The former assay was used to standardize HPr preparations in terms of molar concentration based on the observation (9) that fully phosphorylated HPr contains 1 mol of phosphate per mol of protein, and for each mole of HPr phosphorylated, 1 mol of pyruvate is produced. The pyruvate was measured directly by coupling to lactate dehydrogenase and observing oxidation of reduced dihydronicotinamide adenine dinucleotide. The incubation mixture contained the following components in a final volume of 1.0 ml: 10 μmol of phosphoenolpyruvate, 5 μmol of $MgCl_2$, 12.5 μmol of KF, 0.25 μmol of dithiothreitol or dithioerythritol, 0.15 μmol of dihydronicotinamide adenine dinucleotide, 15 μg of lactate dehydrogenase (chicken heart; Schwarz/Mann Corp.), 0.4 U of enzyme I, and 50 μmol of potassium phosphate buffer, pH 7.5.

A second assay procedure for the amount of HPr utilized a labeled-sugar assay (9). The incubation mixtures contained the following components in a final volume of 1.0 ml: 1 μmol of phosphoenolpyruvate, 0.5 μmol of $MgCl_2$, 1 μmol of methyl-α-[^{14}C]glucoside (specific activity, 200,000 cpm/μmol), 1.25 μmol of KF, 0.25 μmol of dithiothreitol, 0.35 to 0.5 U of enzyme II (membranes from strain SB2950 [= *cysK ptsPHIcrrΔ42 trpB223*] served as a source of enzyme II; see reference 2), more than 2 U of enzyme I, and 50 μmol of potassium phosphate buffer, pH 7.5. A unit of enzyme I and/or enzyme II is that amount of enzyme that generates 1 μmol of methyl-α-glucoside-6-phosphate in 30 min using 10 mM methyl-α-glucoside and 25 μM HPr under the standard assay conditions. The HPr assays were incubated at 37°C for 30 min, at which time the reaction was stopped by dilution with water in an ice bath. The diluted assay mixtures were immediately placed on Dowex (AG1, X2, 50 to 100 mesh; Bio-Rad) ion-exchange columns (3- to 5-ml bed volume) and washed with 3 volumes (10 ml each) of water to remove the free sugar. The sugar-[^{14}C]phosphate was eluted from the columns by washing with two 3-ml volumes of 1 M LiCl and collected in scintillation vials to which 6 ml of scintillation fluid (preblended 3a70b; Research Products International Corp.) was added. The amount of ^{14}C material present was determined by using a Packard Tri-Carb liquid scintillation spectrometer.

The assay conditions for enzyme I activity were the same as those used for the labeled sugar assay for HPr except that each 0.1-ml assay mixture contained 0.03 μmol of HPr, more than 2 U of enzyme II (an excess), and no added enzyme I. The assay relies upon the immediate utilization by the excess of enzyme II of the phospho-HPr produced by the addition of an unknown quantity of enzyme I.

The enzyme IIA/IIB assay was essentially the same as the labeled HPr assay except that to maintain 0.03 μmol of HPr (a saturating quantity when fully phosphorylated), more than 2 U of enzyme I (an excess) was used per 0.1 ml of incubation mixture.

Assay conditions for the enzyme IIB' of the PTS were the same as those for enzyme IIA/IIB with the exception that an excess of partially purified factor III was added to the incubation mixture. The increase in rate of phosphorylation of sugar upon the addition of factor III was used as a measure of enzyme IIB' activity.

In the assay for factor III activity, partially purified enzyme IIB' (8) was added to an incubation mixture containing components of the radioactive assay for HPr. Sugar phosphorylation in the presence of enzyme IIB' was used to measure the quantity of factor III. Enzyme IIB' was preincubated with 33 mM dithiothreitol and 1.7 mg/ml of phosphotidylglycerol-Triton X-100 (5:1) mixed micelles (9) for 5 min at 24°C before the addition of the other assay components (8).

All reactions were carried out under conditions such that the observed rate was linear with regard to time of incubation and to concentration of the protein to be assayed. The protein content of extracts was determined by a biuret procedure, using bovine serum albumin as the standard (10).

RESULTS

Mutant phenotypes and genetic tests. Four DFG-resistant strains (SB3737, 3769, 3798, and 3800) exhibited the fermentation phenotype of *ptsH* mutants defective in HPr activity; namely, they were negative for fermentation on glucose, mannose, and mannitol EMB plates but positive on fructose and galactose EMB plates. The strains came both from DFG-fructose and DFG-lactate selections. All four mutations were shown to lie within the *ptsH* gene by transduction tests involving deletion mapping (see Fig. 2 in reference 2).

Three DFG-resistant strains (SB3799, 3801, and 3802) exhibited the fermentation phenotype of *ptsI* mutants; namely, they were negative on glucose, mannose, mannitol, and fructose EMB plates but positive on galactose EMB plates. All three strains came from the DFG-lactate selection. All three mutations were shown to lie within the *ptsI* gene by transduction tests involving deletion mapping (see Fig. 2 in reference 2).

The seven *ptsH* and *ptsI* strains were examined for reversion by exposure to diethyl sulfate and to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine on minimal mannitol plates. Reversion was induced in all strains, indicating base-substitution mutations, except for strain SB3802, which failed to revert. In transduction tests, the *ptsI194* mutation in strain SB3802 mapped as a short multisite mutation in the proximal portion of the *ptsI* gene (see Fig. 2 in reference 2).

Seven DFG-resistant strains selected for swarming ability in DFG-fructose selections exhibited a fermentation phenotype not seen with previously isolated *Salmonella pts* mutants (2). Fermentation tests on EMB sugar plates showed strains SB3730 through SB3736 to be negative on mannose, weakly positive on glucose, and strongly positive on fructose, mannitol, and galactose. Table 2 summarizes some swarming patterns, growth properties, and fermentation patterns of the *pts*$^+$ parent (strain SB3507) and five of these mutants. Growth on and fermentation of fructose were essentially unchanged, but utilization of mannose and glucose was severely impaired. Two strains (SB3732 and SB3733) show lessened chemotaxis on fructose-containing plates, but the significance of this accessory defect has not been assessed by thorough genetic testing. This new class of *Salmonella pts* mutants is designated *ptsG* for reasons given below. None of the *ptsG* mutations is cotransducible with *cysA*, as are all known *ptsH* and *ptsI* mutations (2, 3).

Sugar transport. Transport studies with [^{14}C]glucose (Fig. 1) and [^{14}C]mannose (Fig. 2) showed that the three *ptsG* mutants tested were deficient in the uptake of these two sugars relative to the *pts*$^+$ parental strain, SB3507. The transport defect could account for the slower growth rates on glucose and on mannose noted for these three mutant strains. In separate experiments with labeled methyl-α-glucoside, an apparent K_m of about 0.5 mM and a V_{max} of 4 μmol/g (dry weight) per min were

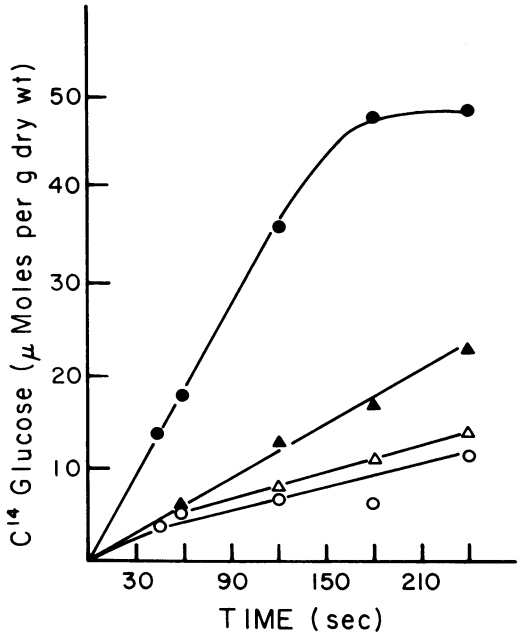

FIG. 1. *Glucose transport in DFG-resistant strains. The following strains were exposed to* D-[^{14}C]*glucose as described in Materials and Methods:* (●) *SB3507 = pts*$^+$*; ptsG mutant strains SB3730* (○), *SB3731* (△), *and SB3732* (▲).

TABLE 2. *Swarming, fermentation, and growth properties of some ptsG mutants and their pts*$^+$ *parent*

Strain	Relevant genotype	Swarm diam[a] (mm)	Doubling times[b] (min)			Fermentation[c]		
			Mannose	Glucose	Fructose	Mannose	Glucose	Fructose
SB3507	*pts*$^+$	28	72	60	72	+	+	+
SB3730	*ptsG*	25	168	180	84	−	±	+
SB3731	*ptsG*	20	168	180	81	−	±	+
SB3732	*ptsG*	5	132	172	78	−	±	+
SB3733	*ptsG*	3	108	120	78	−	±	+
SB3734	*ptsG*	20	108	196	78	−	±	+

[a] Measured on minimal A plates with 1 mM D-fructose as sole carbon source and 0.6 mM DFG, and solidified with 0.25% Ionagar (Oxoid).

[b] Doubling times of bacteria growing at 37°C in liquid minimal A medium with 0.2% of the sole carbon sources indicated.

[c] Fermentation patterns when streaked on EMB indicator plates containing 1% (wt/vol) of the sugar indicated and incubated for 18 h at 37°C. Symbols: +, Green sheen; ±, dark center but no sheen; −, pale-pink colonies.

determined for glucose-grown strain SB3730 (data not shown); the comparable values for the *pts*$^+$ parental strain SB3507 were about 0.2 mM and 100 μmol (J. Stock, personal communication).

In vitro PTS assays. The data in Table 3 on extracts of lactate-grown bacteria document the specific HPr deficiency in strains SB3737, 3769, 3798, and 3800 and the specific enzyme I deficiency in strains SB3799, 3801, and 3802. In contrast, strain SB3730 contained essentially normal levels of all soluble PTS components

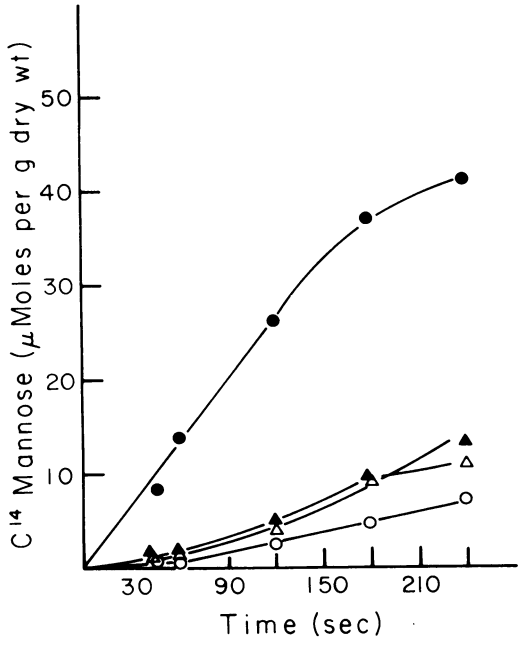

FIG. 2. *Mannose transport in DFG-resistant strains. The following strains were exposed to* D-[^{14}C]*mannose as described in Materials and Methods:* (●) *SB3507 = pts*$^+$*; ptsG mutant strains SB3730* (○), *SB3731* (△), *and SB3732* (▲).

(factor III levels were quite sensitive to culture conditions), but lacked appreciable membrane-bound enzyme IIB′ activity and was also low in membrane-bound enzyme IIA/IIB activity.

In enteric bacteria, a fructose-induced HPr activity can substitute for HPr (14, 15), explaining our ability to select *ptsH* (HPr$^-$) mutants on DFG-fructose medium. Also, there are separate enzymes II for D-fructose and D-glucose (4, 5, 12). To ascertain whether strain SB3730 was defective in all membrane-bound PTS transport proteins, we were interested in the enzyme IIA/IIB profile of strain SB3730 carrying the *ptsG* mutation in an assay system in which the transport activities were substituted or supplemented by fructose-induced activities. Strain SB3730 (*ptsG*) and strain SB3737 (*ptsH*) were grown either in 0.2% lactate or 0.2% fructose minimal medium and assayed with [^{14}C]fructose as substrate, using HPr from similarly grown bacteria of strain SB2676. Enzyme IIA/IIB activity in membranes from lactate-grown strain SB3730 was low in the presence of either source of HPr (Table 4). On the other hand, fructose-grown strain SB3730 contained normal enzyme IIA/IIB activity when supplemented with HPr from fructose-grown bacteria. These results agree with the ability of strain SB3730 to grow well on fructose. They suggest that the lowered activity of the enzyme IIA/IIB complex noted in our assays of lactate-grown bacteria is not due to a general membrane defect lowering all sugar transport activities. Therefore, the primary lesion in strain SB3730 appears to be in its defect of enzyme IIB′. Our data also suggest that fructose-induced enzyme II/fructose HPr activity is not active with DFG as substrate since HPr mutants may be selected as DFG resistant on fructose, a condition that allows the inducion of the fructose-specific activities.

Double *pts* mutants. We observed that *ptsG*

TABLE 3. *Relative specific activities of PTS components of DFG-resistant strains*[a]

Strain	Relevant genotype	Relative sp act[b]				
		HPr	Enzyme I	Factor III	Enzyme IIA/IIB	Enzyme IIB′
SB3507	*pts*$^+$	1.0	1.0	1.0	1.0	1.0
SB3730	*ptsG217*	1.25	0.81	0.50	0.12	0.09
SB3737	*ptsH196*	0.03	1.01	1.70	1.60	0.95
SB3769	*ptsH197*	0.03	0.89	1.00	1.66	0.65
SB3798	*ptsH198*	0.03	0.48	0.58	1.73	0.31
SB3799	*ptsI199*	0.83	0.01	0.50	0.80	1.44
SB3800	*ptsH192*	0.30	0.78	0.83	1.13	0.98
SB3801	*ptsI193*	2.48	0.03	1.90	3.8	2.03
SB3802	*ptsIΔ194*	1.36	0.001	0.50	2.46	1.99

[a] Methyl-α-[^{14}C]glucoside (specific activity, 1.7 × 10^5 cpm/μmol) was used as substrate.

[b] For strain SB3507, the following specific activities were set as unity (micromoles of sugar phosphate formed in 30 min at 37°C per milligram of protein): HPr, 0.6; enzyme I, 6.6; factor III, 0.12; enzyme IIA/IIB complex, 1.5; and enzyme IIB′, 1.23.

mutants exhibit reduced chemotaxis toward glucose but that chemotaxis was restored in *ptsG-ptsH* or *ptsG-ptsI* double mutants (T. Melton et al., in preparation). Therefore, we examined the phenotypes of a relatively isogenic set of these strains (Table 5) and assayed levels of PTS components (Table 6). There may have been a higher level of enzyme IIA/IIB activity in the double mutants (last two lines of Table 4) compared with SB3730, but it remained significantly lower than the activity observed in *pts*$^+$ (top line, Table 4).

DISCUSSION

Our studies have demonstrated that DFG can be effectively used to select mutants defective either in some of the soluble components of the PTS system (HPr, enzyme I) or in a membrane-bound component (enzyme IIB'). The three classes of mutants can be distinguished from one another phenotypically, genetically (2), and by enzyme analyses (Table 3). All seven enzyme IIB' (*ptsG*) mutants isolated appear to be phenotypically similar to one another on fermentation plates, the five measured have similar growth patterns (Table 2), and the three measured carry similar transport defects (Fig. 1 and 2). Only one of the *ptsG* mutants has been characterized in detail enzymologically (Tables 3, 4, and 6), and genetic tests are underway to examine for homology. Preliminary data of Zlata Hartman (personal communication) indicate that the genetic lesion in *ptsG* strain SB3730 is not located at a position on the *Salmonella* chromosome homologous to that found for the lesion in the DFG-resistant membrane mutant isolated in *E. coli* by Kornberg

TABLE 4. *Specific activities of D-fructose enzyme IIA/IIB activity using HPr from lactate- and fructose-grown bacteria in the enzyme II assay*

Strain	Relevant genotype	Sp act			
		Lactate-grown cells[a]		Fructose-grown cells	
		Lactate[b]	Fructose	Lactate	Fructose
SB3507	*pts*$^+$	1.6	1.0	1.5	1.2
SB3730	*ptsG217*	0.45	0.22	0.75	1.5
SB3737	*ptsH196*	1.9	0.83	1.7	1.4

[a] The three bacterial strains were grown at 37°C in minimal A medium with either 0.2% lactate or 0.2% D-fructose as sole carbon source. Enzyme IIA/IIB activity was measured with D-[^{14}C]fructose (specific activity, 1.2×10^5 cpm/μmol) as substrate. Specific activities are given as micromoles of sugar phosphate formed in 30 min at 37°C per milligram of protein.

[b] "Lactate" refers to HPr isolated and partially purified from strain SB2676 grown on minimal medium with 0.2% lactate as sole carbon source, and "fructose" refers to a similar preparation from the same strain grown on 0.2% D-fructose. The HPr preparations were kindly supplied by E. Bruce Waygood.

TABLE 5. *Fermentation patterns on EMB plates of strains SB3730, SB3770, and SB3774*[a]

Strain	Relevant genotype	D-Mannose	D-Glucose	D-Fructose	D-Galactose
SB3507	*pts*$^+$	+	+	+	+
SB3730	*ptsG217*	−	±	+	+
SB3770	*ptsG217 ptsI184*	−	−	−	+
SB3774	*ptsG217 ptsH196*	−	−	+	+

[a] EMB plates containing 1% (wt/vol) of the specified sugar were incubated for 18 h at 37°C and scored: +, green sheen; ±, dark center but no sheen; −, pale-pink colonies.

TABLE 6. *Relative specific activities of PTS components of strains carrying ptsG217*[a]

Strain	Relevant genotype	Relative sp act[b]				
		HPR	Enzyme I	Factor III	Enzyme IIA/IIB	Enzyme IIB'
SB3507	*pts*$^+$	1.0	1.0	1.0	1.0	1.0
SB3730	*ptsG217*	1.5	0.8	0.5	0.12	0.09
SB3770	*ptsG217 ptsI184*	1.7	0.01	1.0	0.23	<0.01
SB3774	*ptsG217 ptsH196*	0.13	0.8	0.58	0.20	<0.01

[a] Lactate-grown bacteria were assayed with methyl-α-[^{14}C]glucoside (specific activity, 1.7 × cpm/μmol) as substrate.

[b] The specific activities (micromoles of sugar phosphate formed in 30 min at 37°C per milligram of protein) for strain SB3507 were: HPr, 0.32; enzyme I, 6.6; factor III, 0.12; enzyme IIA/IIB, 1.5; and enzyme IIB', 1.23.

and Smith (7) or the *ptsG* mutants of Curtis and Epstein (4); rather, the mutation in strain SB3730 appears to map in the general chromosome region analogous to the *ptsM* ("*mpt*") region in *E. coli* (4). A number of mutants have been described with properties overlapping those of our *ptsG* mutants (cf. references 12, 14). Further genetic and biochemical analyses are required to define their relationships.

The enzyme IIB′ mutant SB3730 also shows decreased activity of the membrane-bound enzyme IIA/IIB complex in extracts of lactate-grown bacteria (Table 3) and decreased chemotaxis toward glucose (Melton et al., in preparation). A deficit in fructose enzyme II activity (4, 5, 13) is not found in membranes of fructose-grown bacteria supplemented with fructose-induced HPr (Table 4); therefore, the *ptsG* mutant differs from a *Salmonella mem* mutant with a more generalized membrane transport defect (Cordaro et al., in preparation). Other experiments demonstrate that the effect on chemotaxis toward glucose of the *ptsG* mutant is relieved in *pts* double-mutant strains and by the addition of exogenous glucose and cyclic 3′,5′-adenosine monophosphate (Melton et al., in preparation). Saier et al. (13) have reported the inducibility by glucose of enzyme II^{Glu} activity in a cyclic 3,′5-adenosine monophosphate phosphodiesteraseless mutant of *E. coli*. Because glucose enzyme IIA/IIB activity but not glucose enzyme IIB′ activity can be partially restored under altered growth conditions in our *ptsG* strain SB3730, we assume that the primary defect in the *ptsG* strain is, in fact, a deficiency in enzyme IIB′ activity and that the other effects are secondary pleiotropic manifestations of this primary lesion.

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