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Permease-Specific Mutations in *Salmonella typhimurium* and *Escherichia coli* That Release the Glycerol, Maltose, Melibiose, and Lactose Transport Systems from Regulation by the Phosphoenolpyruvate:Sugar Phosphotransferase System

MILTON H. SAIER, JR.,* HILIARY STRAUD, LYNN S. MASSMAN, JOHN J. JUDICE, MICHAEL J. NEWMAN, AND BRIGITTE U. FEUCHT

Department of Biology, John Muir College, University of California at San Diego, La Jolla, California 92093

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Several carbohydrate permease systems in *Salmonella typhimurium* and *Escherichia coli* are sensitive to regulation by the phosphoenolpyruvate:sugar phosphotransferase system. Mutant *Salmonella* strains were isolated in which individual transport systems had been rendered insensitive to regulation by sugar substrates of the phosphotransferase system. In one such strain, glycerol uptake was insensitive to regulation; in another, the maltose transport system was resistant to inhibition; and in a third, the regulatory mutation specifically rendered the melibiose permease insensitive to regulation. An analogous mutation in *E. coli* abolished inhibition of the transport of β-galactosides via the lactose permease system. The mutations were mapped near the genes which code for the affected transport proteins. The regulatory mutations rendered utilization of the particular carbohydrates resistant to inhibition and synthesis of the corresponding catabolic enzymes partially insensitive to repressive control by sugar substrates of the phosphotransferase system. Studies of repression of β-galactosidase synthesis in *E. coli* were conducted with both lactose and isopropyl β-thiogalactoside as exogenous sources of inducer. Employing high concentrations of isopropyl β-thiogalactoside, repression of β-galactosidase synthesis was not altered by the lactose-specific transport regulation-resistant mutation. By contrast, the more severe repression observed with lactose as the exogenous source of inducer was partially abolished by this regulatory mutation. The results support the conclusions that several transport systems, including the lactose permease system, are subject to allosteric regulation and that inhibition of inducer uptake is a primary cause of the repression of catabolic enzyme synthesis.

The phosphoenolpyruvate:sugar phosphotransferase system (PTS), found in a variety of bacterial genera, catalyzes the following reactions (10, 11, 17, 18, 29, 30):

$$\text{Phosphoenolpyruvate} + \text{HPr} \xrightarrow{\text{enzyme I}} \text{HPr} \sim \text{P} + \text{pyruvate}$$

$$\text{HPr} \sim \text{P} + \text{sugar} \xrightarrow[\text{complex}]{\text{enzyme II}} \text{sugar} - \text{P} + \text{HPr}$$

Enzyme I and HPr are necessary for the phosphorylation of all sugar substrates of the PTS, whereas the enzyme II complexes are sugar specific. In addition to its role in the phosphorylation of sugars, the PTS appears to catalyze the transport of its sugar substrates across the cytoplasmic membrane. In recent communications we showed that this same enzyme system in gram-negative bacteria regulates the utilization of certain carbohydrates that are not substrates of the system (21, 24, 25). These carbohydrates include glycerol, maltose, and melibiose in *Salmonella typhimurium* and the same sugars as well as lactose in *Escherichia coli*. Regulation of the utilization of these carbon sources reflected, in part, the sensitivities of the corresponding catabolic enzyme systems to repression of enzyme synthesis, and we have suggested that repression of enzyme synthesis in *S. typhimurium* was due, in part, to inhibition of the activities of the corresponding permease proteins (5, 19-23; M. H. Saier, Jr., and E. G. Moczydlowski, *In* B. P. Rosen, ed., *Bacterial Transport*, in press), i.e., to the phenomenon of inducer exclusion (1, 9, 13, 17, 33).

In the present communication this suggestion is substantiated. Mutants are described in which single transport systems have become resistant to regulation. The mutational defects were shown to map in or near the genes that code for the transport proteins. The same mutations ren-

dered induction of the corresponding catabolic enzyme systems and utilization of the corresponding carbohydrates less sensitive to regulation. These observations allow us to draw certain conclusions regarding the nature of the regulatory system. Accounts of some of these results have appeared in preliminary form (22, 23; M. H. Saier, Jr., H. Stroud, and J. Judice, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, P209, p. 179).

MATERIALS AND METHODS

Materials. Nonradioactive sugars were obtained from Calbiochem with the exception of melibiose, which was from Sigma Chemical Co. Grade A maltose (Calbiochem) was used for transport and induction studies, whereas grade B maltose was used for fermentation studies and growth experiments. [1-^{14}C]glycerol and ^{14}C-labeled substrates of the lactose permease were purchased from New England Nuclear Corp., or the Amersham/Searle Corp. [1-^{14}C]maltose was from Calatomic, and [^{3}H]melibiitol was synthesized by reduction of melibiose with [^{3}H]sodium borohydride, followed by purification of the radioactive product by paper chromatography employing butanol-pyridine-water (10:3:3) as the solvent system (5).

Growth media. The complex medium employed was doubly concentrated nutrient broth (Difco). The minimal salts medium was a modified medium 63 (24). This was supplemented with a carbon source (autoclaved separately) at 0.5% for growth in liquid medium. Solid minimal medium consisted of medium 63, 1.5% agar (Difco), 0.2% of a carbon source, and 0.1% methyl α-glucoside when present. Fermentation was estimated on eosin-methylene blue fermentation agar plates (Baltimore Biological Laboratory) supplemented with a carbon source at 1% and methyl α-glucoside at 0.1% where indicated.

Isolation of mutant bacterial strains. *Salmonella* strains used in the present study (Table 1) were derived from *S. typhimurium* strain LT-2. Strains SB1476 (*ptsI17*) and SB1796 (*ptsI17 crrA1*) have been described (22). SB2878, SB2880, and LJ14 were derived from SB1476 as follows. Three drops of a nutrient broth culture of SB1476 cells were spread on a minimal agar plate containing glycerol and methyl α-glucoside. This strain could not grow on these plates because methyl α-glucoside prevented induction of the glycerol catabolic enzymes and inhibited glycerol uptake (22, 23, 25). Mutation was induced with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Mutant clones capable of glycerol utilization in the presence of methyl α-glucoside appeared after 2 to 3 days at 37°C. They were purified and tested for their ability to utilize and ferment a variety of carbon sources. Mutants were selected that exhibited the same phenotype as the parental strain except with respect to glycerol utilization.

Two types of glycerol-specific mutants were isolated by this procedure. One type (Table 1, strains SB2878 and SB2880) grew on glycerol and glycerol plus methyl α-glucoside at the same rate and fermented glycerol poorly, as did the parental strain. The other mutant type (Table 1, strain LJ14) fermented glycerol more

TABLE 1. *Bacterial strains*

Strain	Genotype	Parent	Defect
S. typhimurium			
SB1476	*ptsI17*	LT-2	Enzyme I (leaky)
SB1796	*ptsI17 crrA1*	SB1476	Enzyme III^{glc}
SB2878	*ptsI17 glp-260*	SB1476	Regulation-resistant glycerol permease
SB2880	*ptsI17 glp-261*	SB1476	Regulation-resistant glycerol permease
LJ14	*ptsI17 glp-252*	SB1476	Up promoter?
LJ20	*ptsI17 glp-252 glpK255*	LJ14	Glycerokinase
LJ68	*glp-261*	SB2880	*ptsI*$^{+}$ Revertant of SB2880
LJ64	*ptsI17 mal-507*	SB1476	Regulation-resistant maltose permease
SB1667	*malQ62*	LT-2	Amylomaltase
SB1669	*malB64*	LT-2	Maltose permease
LJ28	*ptsI17 glp-252 malQ504*	LJ14	Amylomaltase
LJ30	*ptsI17 glp-252 malB506*	LJ14	Maltose permease
LJ65	*mal-507*	SB1669 × φLJ64	*mal*$^{+}$ transductant
LJ56	*ptsI17 mel-304*	SB1476	Regulation-resistant melibiose permease
LJ3	*melA301*	LT2	α-Galactosidase
LJ5	*melB303*	LT2	Melibiose permease
LJ61	*mel-304*	LJ3 × φLJ56	*mel*$^{+}$ transductant
E. coli			
LJ143 (1101)	Hfr *thi ptsH315*	1100	HPr
LJ140	Hfr *thi ptsH315 crrA157*	LJ143	Enzyme III^{glc}
LJ141	Hfr *thi ptsH315 lac-3004*		Regulation-resistant lactose permease
LJ180 (MP27)	F^{-} *thi rpsL Δpro-lac*		Deleted for the *lac* and *pro* genes
LJ178	F^{-} *thi rpsL*	LJ143 × LJ180	*lac*$^{+}$ *pro*$^{+}$ conjugant
LJ179	F^{-} *thi rpsL lac-3004*	LJ141 × LJ180	*lac*$^{+}$ *pro*$^{+}$ conjugant

efficiently than did the parental strain but showed noticeable inhibition of glycerol utilization by methyl α-glucoside. This second mutant type was found to take up [^{14}C]glycerol and to excrete negatively charged radioactive metabolites derived from glycerol at rates that were about threefold greater than those observed with the parental strain when either DL-lactate or glycerol served as the sole carbon source for growth. These mutants resemble the presumed "up promoter" mutants isolated in *E. coli* by Berman-Kurtz et al. (4). An analogous procedure was used for the isolation of strain LJ64 except that the minimal medium employed contained 0.2% maltose instead of glycerol.

Although SB1476 (*ptsI17*) cells do not utilize glycerol or maltose in the presence of methyl α-glucoside at 37°C, they can utilize melibiose under the same conditions. The utilization of melibiose in the presence of methyl α-glucoside could be prevented by shifting the growth temperature to 42°C, and, thus, mutants resistant to inhibition of melibiose utilization by methyl α-glucoside (i.e., strain LJ61 in Table 1) could be isolated as described above except that selection would be performed at 42°C. Temperature-dependent melibiose utilization is due to temperature sensitivity of the melibiose permease system and will be the subject of a separate communication.

The isolation of a strain that fermented glycerol efficiently (strain LJ14 in Table 1) greatly facilitated the isolation of glycerokinase-negative mutants used for genetic mapping. LJ14 Cells were grown for 12 generations in nutrient broth in the presence of 2-aminopurine (0.5 mg/ml), centrifuged from the medium, washed twice with sterile medium 63, and resuspended in 1 volume of sterile medium 63. A portion (0.1 ml) of this cell suspension was incubated at 37°C for 48 h without shaking in 3 ml of medium 63 containing 0.2% glycerol and 200 U of penicillin G. Subsequently, the cells were diluted and spread on eosin-methylene blue–glycerol fermentation plates. Clones incapable of glycerol fermentation were selected after growth at 37°C for 48 h. A similar procedure was employed to isolate strains incapable of maltose or melibiose utilization, the only difference being that the carbon source employed during penicillin screening and in the fermentation agar was maltose and melibiose, respectively.

RESULTS

Isolation and growth properties of *Salmonella* mutants. *S. typhimurium* strain SB1476 (*ptsI17*), a leaky enzyme I mutant, could utilize glycerol, maltose, or melibiose in the absence of methyl α-glucoside, but not in its presence (24, 25). We previously attributed the methyl α-glucoside-dependent growth stasis of this strain, in part, to repression of the synthesis of the requisite catabolic enzyme systems. We also described the isolation of a mutation (*crrA1*) that allowed utilization of all three of these carbohydrates in the presence of methyl α-glucoside (21, 22).

Sugar-specific mutations were isolated in strain SB1476 (*ptsI17*), each of which permitted utilization of a single carbohydrate. The *glp-260* mutation allowed strain SB1476 to utilize glycerol (but not maltose or melibiose) in the presence of methyl α-glucoside, whereas the *mal-507* and *mel-304* mutations specifically allowed utilization of maltose and melibiose, respectively. This effect of the mutations could be demonstrated both in liquid and on solid minimal media, and the mutations also allowed the strains to ferment the corresponding sugars in the presence of methyl α-glucoside when both substrate and inhibiting glucoside were present in the eosin-methylene blue agar plates.

Repression of catabolic enzyme synthesis in *Salmonella* mutants. Table 2 summarizes the effects of the sugar-specific regulatory mutations on the rates of sugar utilization under conditions that reflect the induction of the catabolic enzymes responsible for the utilization of glycerol, maltose, and melibiose. Methyl α-glucoside strongly repressed synthesis of all of these catabolic enzyme systems in strain SB1476 (*ptsI17*). The *glp-260* mutation desensitized the glycerol catabolic enzyme system to repression by methyl α-glucoside, and the *mal-507* mutation specifically diminished sensitivity of the maltose enzymes to repression by methyl α-glucoside, whereas the *mel-304* mutation specifically abolished sensitivity of the melibiose catabolic enzyme system to repression. These mutations also decreased the sensitivity of the different catabolic enzymes to repression by another sugar substrate of the PTS, mannitol, showing that the effect was not specific to methyl α-glucoside. Growth inhibition, therefore, correlated with the sensitivity of the synthesis of the individual catabolic enzyme systems to repression by sugar substrates of the PTS.

Inhibition of permease activities in the *Salmonella* mutants. The glycerol, maltose, and melibiose transport systems are subject to strong inhibition by sugar substrates of the PTS in strains carrying the *ptsI17* mutation (21, 23). The natural substrates of these transport systems are rapidly metabolized after entering the cell. However, inhibition was demonstrable in *malQ* (amylomaltase$^-$) and *melA* (α-galactosidase$^-$) mutants that could not catabolize maltose and melibiose, respectively (reference 23 and Table 4). Moreover, inhibition of the uptake of a non-metabolizable substrate of the melibiose transport system, methyl β-thiogalactoside, has been demonstrated (23). These results showed that transport activity, and not a subsequent metabolic reaction, was the site of regulation by the PTS.

In Table 3, the effects of the sugar-specific regulatory mutations on permease function are

TABLE 2. *Effects of mutations on repression of enzyme synthesis by methyl α-glucoside in S. typhimurium strain SB1476 (pts I17)*[a]

Strain	Genotype	Carbon source and inducer (0.2%)	Repression by 2 mM methyl α-glucoside (%)
SB1476	*ptsI17*	Glycerol	91
SB1476	*ptsI17*	Maltose	83
SB1476	*ptsI17*	Melibiose	68
SB2878	*ptsI17 glp-260*	Glycerol	39
SB2878	*ptsI17 glp-260*	Maltose	71
SB2878	*ptsI17 glp-260*	Melibiose	79
LJ64	*ptsI17 mal-507*	Glycerol	92
LJ64	*ptsI17 mal-507*	Maltose	24
LJ64	*ptsI17 mal-507*	Melibiose	86
LJ56	*ptsI17 mel-304*	Glycerol	92
LJ56	*ptsI17 mel-304*	Maltose	76
LJ56	*ptsI17 mel-304*	Melibiose	4

[a] Strains were grown overnight in medium 63 containing 0.5% DL-lactate to the stationary phase of growth. Portions of these cell suspensions (0.5 ml) were used to inoculate 25 ml of media of the same composition in 125-ml Erlenmeyer flasks. Flasks were rotated at 250 rpm for 2 h at 37°C (for subsequent induction of the glycerol or maltose catabolic enzyme systems) or at 30°C (for subsequent induction of the melibiose catabolic enzyme system). With the cells growing exponentially 2 h later, the carbon source indicated above was added to a concentration of 0.2%. To one of two flasks containing a particular strain and carbon source, methyl α-glucoside was simultaneously added to a final concentration of 2 mM. No methyl α-glucoside was added to the other flask. Cells were allowed to grow for 3 h at 37°C (for induction of the glycerol or maltose degradative enzymes) or for 4 h at 30°C (for induction of the melibiose enzyme system). Subsequently, the cells were harvested during exponential growth, washed three times with medium 63, and resuspended in medium 63 to a cell density of 0.2 mg of dry cells per ml. Uptake of [^{14}C]-glycerol, [^{14}C]maltose, or [^{3}H]melibiitol was measured in cells grown in the presence of glycerol, maltose, or melibiose, respectively, as well as cells grown in the presence of these carbon sources and methyl α-glucoside. Values reported indicate the percent to which uptake rates were depressed by inclusion of methyl α-glucoside in the growth medium. These values reflect the extent to which methyl α-glucoside depressed the induced synthesis of the corresponding catabolic enzyme system (25). Uptake rates were measured at 28°C in the presence of 200 μM [^{14}C]glycerol (specific activity, 50,000 cpm/μmol), 80 μM [^{14}C]maltose (specific activity, 130,000 cpm/μmol), or 200 μM [^{3}H]melibiitol (specific activity, 140,000 cpm/μmol). For melibiitol uptake, sodium chloride was added to the medium to a final concentration of 10 mM. Nonrepressed rates of uptake by the various strains were between 25 and 40 μmol of glycerol per min, 1.8 and 2.6 μmol of maltose per min, and 2.4 and 4.6 μmol of melibiitol per min.

shown. In these experiments, the cells were grown in minimal media with the inducer of the permease to be studied as the sole source of carbon, and the washed cells were tested for transport activities in the presence and absence of an inhibitory concentration of a sugar substrate of the PTS. Methyl α-glucoside strongly inhibited uptake of radioactive glycerol, maltose, and melibiitol into SB1476 (*ptsI17*) cells (Table 3). The *glp-260* mutation rendered glycerol uptake insensitive to inhibition by the glucose analog but did not alter sensitivity of the melibiose or maltose transport systems to inhibition. By contrast, the *mal-507* and *mel-304* mutations specifically rendered maltose and melibiose uptake insensitive to inhibition by methyl α-glucoside, respectively. In parallel experiments it was shown that these same mutations also rendered the corresponding transport systems insensitive to inhibition by mannitol (data not shown). These results showed that the sugar-specific regulatory mutations determined the sensitivities of the individual transport systems to regulation by sugar substrates of the PTS.

To study the effect of the sugar-specific regulatory mutations on carbohydrate uptake into cells containing normal concentrations of enzyme I, mutants were constructed that differed from strain LT-2 only with respect to these regulatory mutations (see above and Table 1).

TABLE 3. *Effect of mutations on inhibition of solute uptake by methyl α-glucoside in S. typhimurium strain SB1476 (ptsI17)*[a]

Strain	Genotype	Radioactive substrate	Inhibition of uptake rate by methyl α-glucoside (%)
SB1476	*ptsI17*	Glycerol	93
SB1476	*ptsI17*	Maltose	87
SB1476	*ptsI17*	Melibiitol	89
SB2878	*ptsI17 glp-260*	Glycerol	7
SB2878	*ptsI17 glp-260*	Maltose	90
SB2878	*ptsI17 glp-260*	Melibiitol	84
LJ64	*ptsI17 mal-507*	Glycerol	94
LJ64	*ptsI17 mal-507*	Maltose	0
LJ64	*ptsI17 mal-507*	Melibiitol	77
LJ56	*ptsI17 mel-304*	Glycerol	80
LJ56	*ptsI17 mel-304*	Maltose	85
LJ56	*ptsI17 mel-304*	Melibiitol	4

[a] Cells were grown overnight in medium 63 containing 0.5% glycerol or maltose (at 37°C), or melibiose (at 30°C). Portions of these stationary-phase cultures (1 ml) were used to inoculate 25 ml of the same media, and growth was allowed to proceed for 3 h at the same temperatures. Subsequently, the cells growing exponentially with glycerol or maltose as the carbon source were harvested. Cells utilizing melibiose were incubated for an additional hour at 42°C before harvesting. The cells were washed three times with medium 63 and resuspended in the same medium to a cell density of 0.2 mg of dry cells per ml. Rates of uptake of the radioactive substrates indicated below were measured at 28°C employing cells induced for the corresponding catabolic enzyme systems. Uptake rates were determined both in the presence and absence of 0.5 mM methyl α-glucoside. Percent inhibition of the uptake rate by methyl α-glucoside is indicated above. Experimental conditions, concentrations of radioactive substrates, and uninhibited rates of incorporation of radioactive compounds into cellular material were the same as those given in the footnote to Table 2.

This became possible when the map positions of these mutations became known (see below). The bacterial strains were grown as described in the footnote to Table 4 so that synthesis of the catabolic enzyme system for glycerol, maltose, or melibiose as well as the glucose enzyme II was induced (20). Under these conditions, uptake of radioactive glycerol, maltose, or melibiitol was strongly inhibited, both in strain LT-2 and in mutants which could not catabolize the substrates (Table 4). The sugar-specific regulatory mutations desensitized the transport systems to inhibition by methyl α-glucoside (Table 4). The glucose enzyme II was normally inducible in these strains, eliminating the possibility that loss of sensitivity to inhibition was due to abnormal induction.

Genetic mapping of sugar-specific regulatory mutations in *Salmonella*. Evidence for the map positions of the genes that code for the glycerol, maltose, and melibiose transport proteins has been published (26, 31). To map the sugar-specific regulatory mutations, appropriate mutants were isolated. Three *glpK* (glycerokinase-negative) mutants were isolated in strain LJ14 (*ptsI17 glp-252*) as described above (i.e., LJ20 in Table 1). By employing phage P22 *int-4* as carrier (6), these mutations were shown to be about 25% cotransducible with *metB87*, but <5% cotransducible with *metF95*. The probable map order in *S. typhimurium* is therefore *glpK-metB-metF* as in *E. coli* (31).

The *glp-260* and *glp-252* mutations were found to be closely linked to the *glpK255* mutation as follows. Phage P22 *int-4* were grown on strains SB1476, SB2878, and LJ14, and these phage preparations were used to transduce the *glpK*$^+$ gene into strain LJ20 (6). Six transductants capable of growth on glycerol minimal medium were isolated from each cross and were characterized with respect to their growth and fermentation properties on solid media. The three donor strains could be distinguished on the basis of these properties. The identity of the transductants was confirmed by measuring rates of [^{14}C]glycerol uptake in the presence and absence of methyl α-glucoside as described in Table 3. When SB1476 was employed as the donor strain, the six transductants exhibited growth behavior typical of strain SB1476, and glycerol uptake was strongly inhibited by methyl α-glucoside. When the phage were grown on SB2878, the transductants exhibited the growth properties of this strain, and glycerol uptake was resistant to inhibition by methyl α-glucoside. Finally, when LJ14 was employed as donor, all transductants exhibited the growth, fermentation and uptake properties of strain LJ14. These results establish that the *glpK255*, *glp-252*, and *glp-260* mutations are closely linked on the *Salmonella* chromosome.

To map the *mal-507* mutation, mutants defective for either amylomaltase (*malQ* in the *malA* region) or the maltose permease (the *malB* region) were isolated in a strain bearing the *ptsI17* mutation. The former strains lacked detectable amylomaltase activity in vitro but were fully inducible for maltose transport activity, whereas the latter contained amylomaltase activity but could not transport maltose (unpublished data). Phage were grown on strains SB1476 and LJ64, and with strain LJ30 as recipient, maltose-positive transductants were isolated. When the donor was SB1476 six out of six transductants could utilize maltose in the absence, but not in the presence of methyl α-glucoside, and maltose uptake was subject to strong inhibition by methyl α-glucoside. By contrast, when strain LJ64 was employed as donor, the phenotype of the transductants was that of strain LJ64.

The assignment of the *mal-507* mutation to the *malB* region (8) was confirmed with maltose-negative strains that had been mapped by conjugation (27). The *malA202* and *malA203* mutations mapped at about 112 min on the *Salmonella* chromosome, whereas the *malB205* and *malB206* mutations mapped at about 130 min (27). These strains were transduced to maltose utilization with phage grown on strain LJ64. The transductants were purified and grown as described in Table 4 and tested for inhibition of maltose uptake by methyl α-glucoside. Transductants from crosses with *malA* mutants, but not those from crosses with *malB* mutants, were sensitive to inhibition by methyl α-glucoside. It was therefore concluded that the *mal-507* mutation maps within, or very near, the *malB* region.

The genes that code for the melibiose permease protein and α-galactosidase appear to comprise an operon in *E. coli* and *S. typhimurium* (12, 28). We isolated mutant *S. typhimurium* strains in which the melibiose transport system (but not other transport systems) was rendered resistant to inhibition by methyl α-glucoside and mannitol (strain LJ56, Tables 1 and 3). Strains LJ3 and LJ5, which lacked α-galactosidase and melibiose transport activities, respectively, were used to map this mutation. Phage grown on strain LJ56 were used to transduce LJ3 and LJ5 to melibiose utilization. The transductants were then grown as described in Table 4 and tested for [^{3}H]melibiitol uptake, both in the presence and absence of methyl α-glucoside. Six transductants tested (three from each melibiose-negative strain) were resistant to

TABLE 4. *Effects of mutations on inhibition of solute uptake by methyl α-glucoside in S. typhimurium strains containing normal activities of the enzymes of the phosphotransferase system*[a]

Strain	Genotype	Radioactive substrate	Inhibition of uptake rate by methyl α-glucoside (%)
LT-2		[^{14}C]glycerol	96
LJ68	*glp-261*	[^{14}C]glycerol	9
LT2		[^{14}C]maltose	82
SB1667	*malQ62*	[^{14}C]maltose	80
LJ65	*mal-507*	[^{14}C]maltose	27
LT-2		[^{3}H]melibiitol	87
LJ3	*melA301*	[^{3}H]melibiitol	81
LJ61	*mel-304*	[^{3}H]melibiitol	12

[a] Cells were grown as described in Table 3 except that the inoculum size was reduced by one-half and 0.5% DL-lactate was present in the growth medium. After growth for 3 h in the presence of the inducing carbohydrate (glycerol, maltose, or melibiose), glucose (0.2%) was added to induce the glucose enzyme II (20). Cells were permitted to grow for 2 h more before harvesting. In the case of melibiose-induced cells, growth was continued at 30°C for 1 h after glucose addition, but the temperature was brought to 42°C for the remaining 1 h before harvesting. Subsequently, cells were prepared, and uptake rates were measured in the presence and absence of 0.5 mM methyl α-glucoside as described in the footnote to Table 3. Numerical values represent percent inhibition of the rate of uptake of the radioactive substrate by methyl α-glucoside.

inhibition of melibiitol uptake by methyl α-glucoside. Therefore, it appeared that the *mel-304* mutation mapped within or near the *mel* operon.

Effects of regulatory mutations in the glycerol regulon on the regulation of glycerol uptake by the PTS in *E. coli*. Berman et al. have reported that mutations that render synthesis of glycerol kinase constitutive and that render this enzyme resistant to feedback inhibition by fructose 1,6-diphosphate allow tight enzyme I mutants of *E. coli* to utilize glycerol (2, 3). Consequently, the effects of these mutations on the regulation of glycerol uptake in a wild-type genetic background was examined. The strains employed (obtained from E. C. C. Lin, Harvard Medical School) were strains 7 (*glpR*) and 43 (*glpR glpK*^{i}) (2). When these strains were grown in nutrient broth and harvested during exponential growth, glycerol uptake was not sensitive to inhibition by methyl α-glucoside. By contrast, when the same cells were grown in nutrient broth and glucose (0.5%) was added to the exponentially dividing culture 2 h before the cells were harvested, glycerol uptake in both cell types exhibited strong sensitivity to inhibition by methyl α-glucoside. By employing our standard assay conditions (see footnote to Table 3), half-maximal inhibition was observed at 10 to 20 μM methyl α-glucoside for both strains. Glycerol uptake was inhibited more than 70% by concentrations of methyl α-glucoside in excess of 100 μM. These results showed that the *glpR* and *glpK*^{i} mutations do not abolish regulation of glycerol uptake by the PTS when the cells are grown under sensitizing conditions (20).

Isolation and growth properties of lactose-specific, regulation-resistant mutants in *E. coli*. *ptsH* (HPr^{-}) and *ptsI* (enzyme I^{-}) mutants of *E. coli* grow poorly with lactose as the sole source of exogenous carbon (7, 16, 25, 32). Derivatives of an *E. coli ptsH* mutant capable of growth on lactose were isolated as described above. The same procedure was also used to isolate glycerol-, maltose-, and melibiose-specific, regulation-resistant mutants as well as general *crrA* mutants in this genetic background. The latter strains had the growth and fermentation properties noted above for the *Salmonella* mutants. The fermentation properties of pertinent bacterial strains (Table 1) are summarized in Table 5. Whereas the *crrA157* mutation in the *ptsH315* genetic background (7) allowed utilization of several non-PTS sugars, the *lac-3004* mutation specifically enhanced the rate of lactose utilization both in the presence and absence of methyl α-glucoside. It did not permit fermentation at the wild-type rate. Inclusion of cyclic AMP in the fermentation medium enhanced the fermentation response of strain LJ141, suggesting that the *lac-3004* mutation did not render transcription of the lactose operon independent of cyclic AMP control (16). The fermentation responses recorded in Table 5 for strain LJ141 were the same as those observed for three other independently isolated lactose-specific mutants (*lac-3005–lac-3007*), all derived from strain LJ143.

Mapping of the *lac-3004* mutation. Since the *lac-3004* mutation was introduced into an Hfr strain (7), the approximate location of the mutation on the bacterial chromosome could be estimated by conjugational analysis (31). When strain LJ141 or LJ143 was crossed with a female strain (LJ180), deleted for the *lac pro* region of the chromosome, conjugants capable of lactose utilization in the absence of proline were isolated. Ten conjugants were tested for sensitivity to repression of β-galactosidase synthesis by glucose with lactose as inducer (see below). The results of these studies showed that all conjugants from the LJ180 × LJ143 cross were fully

sensitive to glucose repression, whereas conjugants from the LJ180 × LJ141 cross were partially resistant to glucose repression. These results suggested that the *lac-3004* mutation maps within or near the *lac* operon.

Effect of the *lac-3004* mutation on repression of β-galactosidase synthesis. Preliminary induction studies showed that strain LJ143 could be induced for β-galactosidase synthesis when isopropyl β-thiogalactoside was added to a growing-cell suspension (16), but that lactose was not an effective inducer in the presence of methyl α-glucoside. Introduction of the *lac-3004* mutation allowed induction by exogenous lactose, even in the presence of methyl α-glucoside. To quantitate the effect of the *lac-3004* mutation on the rates of catabolic enzyme synthesis in a normal genetic background, the repression of β-galactosidase synthesis by various sugars was examined employing strains LJ178 and LJ179. These two strains are wild type for the *pts* genes and differ genetically only with respect to the *lac-3004* mutation. By employing a saturating concentration of isopropyl β-thiogalactoside as the inducer, the rates of β-galactosidase synthesis and the extent of glucose repression were the same in the two strains (Table 6). Comparable repression was observed when the cells were grown in either lactate or glucose minimal medium. Lactose was a less effective exogenous source of inducer than was isopropyl β-thiogalactoside, but in the absence of a repressing sugar, the rates of β-galactosidase synthesis were the same in the two strains (Table 6). With lactose as the inducer, however, glucose was far more effective in repressing β-galactosidase synthesis in strain LJ178 than it had been when isopropyl β-thiogalactoside was the inducer, and this enhanced sensitivity to repression was overcome by the *lac-3004* mutation. The degree of glucose repression in strain LJ179 was about the same when either lactose or isopropyl β-thiogalactoside served as the exogenous source of inducer. In a similar fashion, the *lac-3004* muta-

TABLE 5. *Fermentation of sugars by E. coli strains*[a]

Strain	Genotype	Fermentation response on medium containing:					
		Mannitol	Lactose	Lactose + cyclic AMP	Lactose + methyl α-glucoside	Melibiose + methyl α-glucoside	Maltose + methyl α-glucoside
1100	Wild type	++	++	++	++	++	++
1101	*ptsH315*	−	±	++	−	−	−
LJ140	*ptsH315 crrA157*	−	++	++	++	++	++
LJ141	*ptsH315 lac-3004*	−	+	++	+	−	−

[a] Fermentation responses were recorded as follows: ++, strong fermentation; + or ±, less fermentation; −, no fermentation.

TABLE 6. *Repression of β-galactosidase synthesis by sugar substrates of the phosphotransferase system*[a]

Carbon source for growth	Inducer and repressing sugar added	Time of induction (min)	o-Nitrophenyl β-galactoside (mmol/min per g [dry wt]) hydrolyzed by strain:	
			LJ178 (wild type)	LJ179 (*lac-3004*)
Lactate	IPTG	30	19.2	19.8
Lactate	IPTG + glucose	60	5.8	5.9
Glucose	IPTG	60	4.2	5.7
Lactate	Lactose	60	8.2	7.6
Lactate	Lactose + αMG	60	3.6	6.0
Lactate	Lactose + glucose	120	<0.1	1.7
Glucose	Lactose	120	<0.1	1.2
Lactate + glucose	Lactose	120	<0.1	0.7
Lactate	Lactose + mannitol	120	0.2	2.0

[a] Cells (LJ178 or LJ179 as indicated) were grown in medium 63 containing 1.0% DL-lactate, 0.5% sugar, or both as indicated in column 1. With the cells growing exponentially, inducer (isopropyl β-thiogalactoside, [IPTG, 0.5 mM]) or lactose (10 mM) was added alone or together with a repressing sugar as indicated in column 2. After the induction period indicated in column 3, cells were harvested, washed three times with medium 63, and resuspended for determination of β-galactosidase activity in toluenized cells (20). Values are in millimoles of *O*-nitrophenyl β-galactoside hydrolyzed per minute per gram (dry weight) of cells at 37°C.

tion partially relieved the severe repression caused by addition of mannitol or methyl α-glucoside to the cell suspension, suggesting that this effect was not specific for glucose, but was general for sugar substrates of the phosphotransferase system. The results are consistent with the conclusion that the *lac-3004* mutation specifically abolished the phenomenon of PTS-mediated inducer exclusion (1, 9, 14–16).

Effects of the *lac-3004* mutation on PTS-mediated inhibition of β-galactoside transport. If the suggestion is correct that the *lac-3004* mutation abolished the phenomenon of inducer exclusion, it should be possible to demonstrate that this mutation rendered the activity of the lactose permease insensitive to inhibition by sugar substrates of the PTS. This fact was established employing a hydrolyzable transport substrate of the lactose permease, *o*-nitrophenyl β-galactoside (Fig. 1A), and a non-metabolizable lactose analog, methyl β-thiogalactoside (Fig. 1B). In both cases, the *lac-3004* mutation reduced the regulatory effects of glucose and methyl α-glucoside so that these sugars exerted only weak inhibitory effects on galactoside transport.

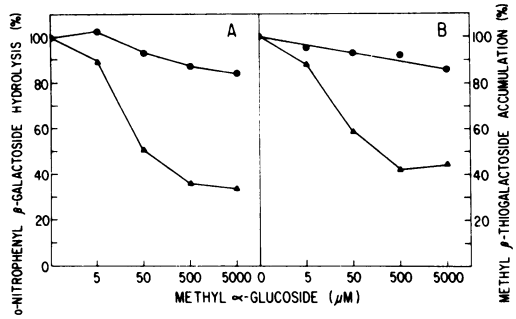

FIG. 1. *Effect of the lac-3004 mutation on the regulation of β-galactoside transport by methyl α-glucoside. (A) LJ178 (▲) and LJ179 (●) cells were grown at 37°C in medium 63 supplemented with 5 μg of thiamine per ml and 0.5% lactose. With the cells in the exponential growth phase, glucose (0.2%) was added, and 45 min later the cells were harvested, washed three times with medium 63, and resuspended in salts medium 63 for the in vivo hydrolysis experiment, conducted as described previously (20). Hydrolysis rates were not corrected for non-permease-mediated hydrolysis, which amounted to about 15% of the total. (B) Cells were grown in medium 63 supplemented with 1% DL-lactate, 5 μg of thiamine per ml, and 0.5 mM isopropyl β-thiogalactoside. Glucose was added to the growing culture 45 min before the cells were harvested. The washed cells were resuspended to a density of 1.6 mg of dry weight per ml, methyl α-glucoside was added to the concentration indicated on the abscissa, and the cell suspensions were equilibrated at 37°C with constant aeration before initiation of the transport experiment by addition of methyl-β-[^{14}C]thiogalactoside (final concentration, 0.13 mM). Portions (100 μl) were periodically removed, diluted, and filtered, and the filters were washed three times before determination of intracellular radioactivity as described previously (20).*

DISCUSSION

Reports from several laboratories have demonstrated that sugar substrates of the glucose permease (i.e., the glucose PTS) inhibit the activities of the lactose, maltose, glycerol, and melibiose permeases in *E. coli* and *S. typhimurium* (for a recent review see Saier and Moczydlowski, *in* B. P. Rosen [ed.], *Bacterial Transport*, in press). Moreover, we have shown that the activity of adenylate cyclase is subject to coordinate regulation by the PTS (5, 19, 20). To characterize the regulatory system, both biochemical and genetic approaches have been taken. We have shown that mutants deficient for (but not completely lacking) either enzyme I or HPr of the PTS (the general energy-coupling proteins of this system [17]) were hypersensitive to regulation. This result implicated enzyme I and HPr in the regulatory process. In addition, we isolated three classes of mutants in which resistance to regulation was observed.

One class of regulation-resistant mutants was defective for the enzyme II complexes of the PTS. In these mutants the glycerol, maltose, and melibiose transport systems were insensitive to inhibition by the sugars for which the defective enzyme II complexes were specific (23). These transport systems remained sensitive to inhibition by sugar substrates of other enzyme II complexes that remained functional. Therefore, it appeared that the enzyme II complex was an essential component of the regulatory system. Recently, extensive induction studies have corroborated this conclusion and extended it to a variety of wild-type and mutant *E. coli* strains (20).

A second type of mutation (the *crrA* mutation) rendered all transport systems resistant to regulation by all sugar substrates of the PTS. These mutations were found to map adjacent to the *pts* operon and resulted in loss of a sugar-specific protein involved in the phosphorylation of glucose and methyl α-glucoside. The nature of the *crrA* mutation has not been clearly established.

The present report describes a third class of mutants in which individual transport systems were insensitive to inhibition by the PTS. Each of these mutations rendered a specific transport system resistant to regulation by all sugar substrates of the PTS but did not alter the sensitivity of other transport systems to inhibition. We showed that these mutations also rendered in-

duction of enzyme synthesis and utilization of the corresponding carbohydrates resistant to PTS-mediated regulation, substantiating the suggestion that repression of enzyme synthesis in *S. typhimurium* is primarily due to inducer exclusion (14, 15). Genetic mapping of these sugar-specific regulatory mutations showed that they were localized near or within the genes that code for the individual transport proteins. The results suggest that the transport proteins are normally subject to allosteric regulation and that the mutations altered the regulatory properties of the transport systems without altering transport function; i.e., a regulatory and not a catalytic component of the transport system was the mutational target. Although a mechanism for transport regulation has been proposed (17, 18; Saier and Mocyzdlowski, *in* B. P. Rosen [ed.], *Bacterial Transport*, in press), the precise molecular details of this regulatory mechanism have yet to be established. Fine-structure genetic analyses of the lactose-specific regulatory mutants (presently in progress) should provide evidence concerning the allosteric nature of the lactose permease.

In addition to the mutant classes described above, Berman and co-workers described three classes of glycerol-specific mutations in *E. coli* that permitted a *ptsI* mutant to utilize glycerol. None of these mutants corresponded to the mutants described in this report. The three types of mutants isolated by these workers were (i) *glpR* (glycerol constitutive [3]), (ii) *glpK*^{i} (mutants in which glycerokinase was resistant to feedback inhibition by fructose 1,6-diphosphate [2]), and (iii) presumed up promoter mutants of the *glpK* operon (4). In view of the data reported here and the discovery of inducer-dependent desensitization to PTS-mediated regulation (unpublished data), it seems reasonable that each of the mutations isolated by Berman et al. did not abolish PTS-mediated regulation, but desensitized the cells to this phenomenon by virtue of the generation of enhanced cellular inducer concentrations. Indeed, the data reported in this communication, showing that glycerol uptake in each of these mutant types was sensitive to inhibition by methyl α-glucoside under strongly sensitizing conditions (i.e., when the cells were grown in the presence of glucose) tend to substantiate this conclusion. Mutations evidently can influence the regulation of transport either by a primary mechanism (as in the case of the regulatory mutations described here) or secondarily by altering the degree of desensitization (as appears to be the case in the mutants described by Berman et al. [2–4] and by Wang et al. [32]). The presence of a regulatory mechanism superimposed on a preexisting regulatory process complicates biochemical and genetic analyses and leads to the possibility that any of a variety of genetic lesions can manifest themselves in a single growth phenotype.

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