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Galactose Transport in *Salmonella typhimurium*

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We have studied the various systems by which galactose can be transported in *Salmonella typhimurium*, in particular the specific galactose permease (GP). Mutants that contain GP as the sole galactose transport system have been isolated, and starting from these mutants we have been able to select point mutants that lack GP. The *galP* mutation maps close to another mutation, which results in the constitutive synthesis of GP, but is not linked to *galR*. Growth of wild-type strains on galactose induces GP but not the β-methylgalactoside permease (MGP). Strains lacking GP are able to grow slowly on galactose, and MGP is induced; however, D-fucose is a much better inducer of MGP. Induction of GP or MGP is not prevented by a *pts* mutation, although this mutation changes the apparent K_m of MGP for galactose. *pts* mutations have no effect on GP. GP has a rather broad specificity: galactose, glucose, mannose, fucose, 2-deoxygalactose, and 2-deoxyglucose are substrates, but only galactose and fucose can induce this transport system.

Transport of galactose in *Escherichia coli* and *Salmonella typhimurium* can be catalyzed by a number of different systems (16). Two of these, methyl β-D-thiogalactoside (TMG) I and TMG II, are the transport systems for lactose and melibiose, respectively. Galactose is both a substrate of these translocators and an inducer under certain conditions (16). A third system, the β-methylgalactoside permease (MGP), has been described which is able to transport both galactose and β-methylgalactoside. It also involves the galactose-binding protein (3). At least four genes (*mglA*, *-B*, *-C*, and *-D*), different from the *gal* operon, are involved in the synthesis of MGP (12, 13, 15). D-Fucose is able to induce the synthesis of both MGP and the fourth galactose transport system, the galactose permease (GP). Galactose and glucose are the main substrates of this system (16, 17). A regulatory gene, *galR*, which is involved in the inducible expression of both GP and the *gal* operon (5), has been identified. Finally, it has been shown recently (14) that the membrane-bound, sugar-specific enzyme II of the phosphoenolpyruvate-sugar phosphotransferase system (PTS) is also able to catalyze the transport of galactose under special conditions.

The purpose of this paper is to examine some of the properties of GP in *S. typhimurium*, the least known of the galactose transport systems. To study this transport system, we have isolated strains that possess only GP. In addition, we have isolated strains that are specifically defective in GP or express it constitutively. Finally, we will describe some of the conditions under which induction of MGP and GP occurs. A preliminary report of this work has been published elsewhere (P. W. Postma, Abstr. FEBS Symp. Biochem. Membrane Transport, p. 33, 1976).

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1. The phenotypic characteristics of representative strains are described in Table 2.

Media and growth conditions. Cells were cultured at 37°C on a rotatory 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. For growth of bacteria, it was supplemented with 20 μg of tryptophan per ml and the required carbon source (0.2%). Media were solidified with Difco agar (1.5%, wt/vol). Eosin methylene blue plates were made using eosin methylene blue (Difco) with 1% sugar added after sterilization.

Chemicals. D-[U-^{14}C]galactose (50 μCi/0.14 mg), D-[1-^{14}C]mannose (50 μCi/0.085 mg), [^{14}C]methyl β-D-galactopyranoside (50 μCi/2.03 mg), and [^{14}C]-methyl β-D-thiogalactopyranoside (50 μCi/1.2 mg) were obtained from New England Nuclear Corp. D-[1-^{3}H]fucose (1.3 Ci/mmol) and D-[U-^{14}C]glucose (284 mCi/mmol) were purchased from Amersham. D-Fucose and 2-deoxygalactose were obtained from Sigma Chemical Co. Methyl β-D-galactopyranoside was purchased from Koch-Light Lab. Uridine 5'-diphosphate (UDP)galactose, UDPglucose, galactose 1-phosphate, phosphoglucomutase, glucose 6-phosphate dehydrogenase, and UDPglucose dehydrogenase were obtained from Boehringer. All other reagents were of the best grade commercially

TABLE 1. *Origin and genotype of Salmonella strains*[a]

Strain	Relevant genotype	Isolation procedure, parental strain	Source[b]
SB3507	*trpB223*		E. Balbinder
SB2138	*mem-1 trpB223*	DES	A
SB3586	*gal-113 mem-1 trpB223*	DES	B
SB3593	*gal-113 mem-1*FR16 *trpB223*	Mal$^+$ SB3586, DES	B
SB3632	*gal-113 mem-1*FR15 *trpB223*	Suc$^+$ SB3586, DES	B
SB3633	*gal-113 mtlC584 mem-1 trpB223*	Mtl$^+$ SB3586, DES	B
PP116	*galC1891 trzA-ptsHI41 trpB223*	DES, SB2309	B
PP117	*galR1892 trzA-ptsHI41 trpB223*	DES, SB2309	B
PP144	*galT1898 gal-113 mem-1 trpB223*	DES, SB3586	B
PP146	*gal-1900 gal-113 mem-1 trpB223*	DES, SB3586	B
PP148	*gal-1902 gal-113 mem-1 trpB223*	DES, SB3586	B
PP149	*galP1903 mem-1 trpB223*	DES, SB3586	B
PP150	*gal-1904 mem-1 trpB223*	DES, SB3586	B
PP151	*galP1905 mem-1 trpB223*	DES, SB3586	B
PP153	*galP1907 mem-1 trpB223*	DES, SB3586	B
PP161	*galT1898 gal-113 mem-1*FR24 *trpB223*	Man$^+$ PP144, DES	B
PP165	*galP1903 mem-1*FR27 *trpB223*	Man$^+$ PP149, DES	B
PP217	*ptsI405 galP1903 mem-1*FR27 *trpB223*	DES, PP165	B
PP224	*gal-113 mem-1 trpB223*	P22 (SB3586) × PP149	B
PP230	*ptsI405 galC1891 mem-1*FR27 *trpB223*	P22 (PP116) × PP217	B
PP267	*cysA20 galP1903 mem-1*FR27 *trpB223*	P22 (*cysA20*) × PP217	B
PP269	*trzA-ptsHIΔ41 galP1903 mem-1*FR27 *trpB223*	P22 (SB2309) × PP267	B
PP271	*trzA-ptsHIcrrΔ49 galP1903 mem-1*FR27 *trpB223*	P22 (SB2950) × PP267	B
PP289	*galP1903 mem$^+$ trpB223*	P22 (SB3507) × PP149	B
PP291	*galP1905 mem$^+$ trpB223*	P22 (SB3507) × PP151	B
SB3676	F'*lacO^{c}15*/*mem-1 trpB223*	SB2138 × E7084I1	B
SB2309	*trzA-ptsHIΔ41 trpB223*		J. C. Cordaro
SB2950	*trzA-ptsHIcrrΔ49 trpB223*		J. C. Cordaro
	cysA20		P. E. Hartman
E. coli			
E7084	F'*lacO^{c}15*/*lac-proAB thi*		W. Reznikoff
E7084I1	F'*lacO^{c}15*/*lac-proAB thi ilv*		B

[a] Genetic nomenclature according to B. Bachmann et al. (1). *galP* and *galC* are proposed for mutations leading to a defective galactose permease (GP) and constitutive expression of GP, respectively. FR is used to designate "general" reversions of *mem-1*, resulting in the complete parental phenotype. DES, diethylsulfate; Mal, maltose; Suc, succinate; Mtl, mannitol; Man, mannose.

[b] A, P. W. Postma, J. C. Cordaro, and S. Roseman, manuscript in preparation; B, this study.

TABLE 2. *Phenotypes of strains containing galP, mem, and pts defects*[a]

Genotype	Phenotype[b]			
	Glc	Gal	Mtl	Mal
trpB223	+	+	+	+
trpB223 mem	±	−	−	−
trpB223 mem gal$^+$	±	+	−	−
trpB223 mem galP	±	−	−	−
trpB223 mem$^+$ galP	+	±	+	+
trpB223 mem$^+$ galP ptsHI del	−	±	−	−
trpB223 mem$^+$ galP ptsHI-crr del	−	±	−	+
trpB223 mem$^+$ galP^c ptsI	+	+	−	−

[a] Abbreviations: Glc, Glucose; Gal, galactose; Mtl, mannitol; Mal, maltose; *del*, deletion.

[b] Growth was monitored on chemically defined media and fermentation was tested on eosin methylene blue medium. +, Growth and fermentation after 48 h of incubation at 37°C; −, no growth and fermentation under these conditions.

available. All sugars used in this study were of the D-configuration.

Preparation of cell-free extracts. Cells were grown overnight in 1,000 ml of medium A containing 0.2% carbon source plus required supplements. Cells were centrifuged for 20 min at 6,000 × *g* at 4°C, washed twice with 0.9% NaCl, and resuspended in approximately 10 ml of 25 mM potassium phosphate buffer, pH 7.0, containing 0.5 mM ethylenediaminetetraacetate and 0.5 mM dithiothreitol. After sonication for 2.5 min on a Branson Sonifier, intact cells and debris were removed by centrifugation for 20 min at 12,000 × *g*. The resulting cell-free extract was centrifuged at 200,000 × *g* for 60 min at 4°C. The clear supernatant contained the enzymes involved in galactose metabolism (see below).

Enzyme assays. Galactokinase (EC 2.7.1.6), galactose 1-phosphate uridyl transferase (EC 2.7.7.12), and UDPglucose 4-epimerase (EC 5.1.3.2) were determined in the 200,000 × *g* supernatant. For galac-

tokinase the reaction mixture contained, in a final volume of 0.1 ml: 10 mM adenosine 5'-triphosphate, 5 mM $MgCl_2$, 2.5 mM dithiothreitol, 12.5 mM KF, 50 mM potassium phosphate buffer, pH 7.5, 10 mM [^{14}C]galactose (specific activity, 24 cpm/nmol), and varying amounts of the supernatant. After incubation at 37°C for 30 min, the reaction was stopped by rapid cooling in ice and diluted with two consecutive 2-ml volumes of distilled water. The galactose 1-phosphate formed was determined quantitatively by ion-exchange chromatography on Dowex AG 1-X2 (9). Specific activity is expressed as nanomoles of galactose 1-phosphate formed per minute per milligram of protein at 37°C. Galactose 1-phosphate uridyl transferase was determined essentially as described in reference 11. The reaction mixture contained, in a final volume of 0.55 ml: 100 mM glycine, pH 8.7, 10 mM mercaptoethanol, 0.4 mM UDPglucose, 0.4 mM galactose 1-phosphate, 0.4 mM nicotinamide adenine dinucleotide phosphate, 0.8 mM $MgCl_2$, 0.4 U of phosphoglucomutase (EC 2.7.5.1), 21 U of glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and varying amounts of supernatant. The change in optical density at 340 nm never exceeded 0.008 to 0.02/min. The specific activity is expressed as nanomoles of reduced nicotinamide adenine dinucleotide phosphate formed per minute per milligram of protein at 20°C. UDPglucose 4-epimerase was determined according to Darrow and Rodstrom (6). The reaction mixture contained, in a final volume of 0.6 ml: 100 mM glycylglycine, pH 8.9, 1 mM nicotinamide adenine dinucleotide, 0.16 mM UDPgalactose, 9 mU of UDPglucose dehydrogenase (EC 1.1.1.22), and supernatant such that the change in absorbancy at 340 nm never exceeded 0.05/min. The supernatant was diluted in 50 mM tris(hydroxymethyl)aminomethane-maleate, pH 6.5, plus 0.1% serum albumin. The specific activity is expressed as nanomoles of reduced nicotinamide adenine dinucleotide formed per minute per milligram of protein at 20°C. All enzyme activities are proportional to the amount of supernatant added.

Transport studies. Bacterial cultures (500 ml) were harvested in the midexponential phase of growth (optical density at 600 nm, 0.45 to 0.55) by centrifugation at 4°C for 20 min at 6,000 × *g*, washed twice with medium A minus carbon source and supplements, and resuspended in 5 ml of the same medium and stored at 0°C. For transport, cells (final concentration, 1 to 6 mg [dry weight]/ml) were incubated in medium A and aerated for 3 min. At zero time the ^{14}C-labeled compound was added. Samples (0.1 ml) were removed at the times indicated, diluted in 10 ml of medium A at room temperature, and rapidly filtered through glass-fiber filters (Whatman GF/F). Filters were counted in a liquid scintillation counter in a mixture containing 15 ml of toluene and Triton X-100 (2:1, vol/vol) and, per liter, 5.55 g of 2,5-diphenyloxazole (PPO) and 125 mg of 2,2'-*p*-phenylene bis(4-methyl-5-phenyloxazole) (dimethyl POPOP). Background radioactivity on filters was determined in a similar manner in the absence of cells. 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 (final volume, 1.6 ml). Substrates were added at the concentrations indicated. The oxidation velocity is expressed as microatoms of oxygen consumed per minute per milligram (dry weight) at 25°C.

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

Generation times. Cells were pregrown on 0.2% lactate and diluted 25-fold in medium A containing the required carbon source. Growth was followed by measuring the optical density at 600 nm at 37°C.

Genetic methods. Mutants were selected, after diethylsulfate mutagenesis, by standard penicillin enrichment techniques. Revertants were selected on plates with a filter disk saturated with diethylsulfate. Preparation of P22 transducing lysates and transduction with phage P22 was performed as described in reference 2.

RESULTS

Galactose permease. Until now no compounds have been found that are transported exclusively by GP. Consequently, direct study of this system as well as the isolation of mutants defective in GP is complicated. However, a point mutant of *S. typhimurium*, *mem-1*, has been isolated recently (J. C. Cordaro, P. W. Postma, and S. Roseman, Fed. Proc. 33:1326, 1974) which lacks a large number of membrane-bound proteins, including many transport systems. This mutant, SB2138, is unable to grow on galactose, the generation time being more than 10 h on 0.2% galactose, whereas the parent doubles every 50 min. Figure 1 shows that the mutant is unable to accumulate TMG, β-methylgalactoside, or galactose, from which we conclude that TMG II, MGP, and GP are absent in this strain. Under no condition is melibiose, galactose, or D-fucose able to induce any of these systems, in contrast to results with the parent.

Two classes of revertants can be isolated on galactose from this point mutant. The first class consists of "general" revertants, which upon selection on one carbon source regain the ability to grow on all carbon sources that were unable to support growth of *mem-1*, and represent true *mem*$^+$ revertants. The second class comprises "specific" revertants, which regain the ability to grow on galactose only and probably represent mutations in a gene different from *mem-1*. We have isolated a number of specific Gal$^+$ revertants. Figure 2 shows the transport properties of one of these revertants, SB3586. Growth of this revertant on galactose or lactate plus D-fucose induces the transport of galactose but not of β-methylgalactoside. In addition, it could be shown that the revertant does not grow on melibiose and is unable to

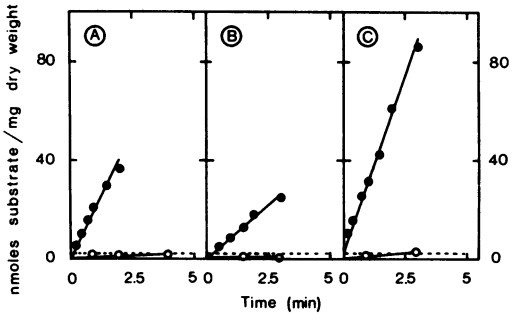

FIG. 1. *Activity of various galactose transport systems. Cells were grown as described in the text in a medium containing 0.2% lactate plus 0.2% melibiose (A) or 0.5 mM D-fucose (B, C). (A) TMG II was assayed with 1 mM [^{14}C]TMG (specific activity, 250 cpm/nmol). (B) MGP was assayed with 1 mM β-[^{14}C]methyl-galactoside (specific activity 540 cpm/nmol). (C) GP was assayed using 1 mM [^{14}C]galactose (specific activity 188 cpm/nmol). Symbols: ●, SB3507; ○, SB2138. The dotted line represents equilibration.*

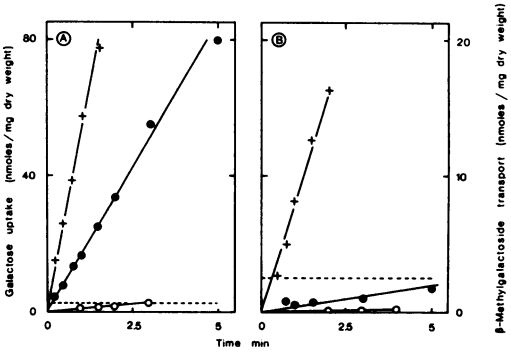

FIG. 2. *GP and MGP activity in SB3586. Cells were grown in a medium containing either 0.2% galactose or 0.2% lactate plus 1 mM D-fucose. (A) Transport of 1 mM [^{14}C]galactose (specific activity, 188 cpm/nmol). (B) Transport of 1 mM β-[^{14}C]methyl-galactoside (specific activity, 540 cpm/nmol). Symbols: +, SB3507; ○, SB2138; ●, SB3586. The dotted line represents equilibration.*

accumulate TMG (data not shown). Since both TMG I (*Salmonella* contains no *lac* operon) and enzyme II of the PTS are also absent (14), it is concluded that this revertant contains only GP, all other galactose transport systems still being defective. The seven specific Gal$^+$ revertants studied behave similarly. The rate of galactose transport varies between 6 and 8 nmol of galactose taken up/min per mg (dry weight) at 20°C, whereas β-methylgalactoside is not even equilibrated in 5 min.

A more detailed description of the defects caused by the *mem-1* mutation will be given in a separate paper (P. W. Postma, J. C. Cordaro, and S. Roseman, manuscript in preparation). It is sufficient to say here that *mem-1* affects, apart from many other processes, the primary step in galactose metabolism, its transport across the membrane. When an episome carrying a constitutive *lac* operon is introduced in SB2138, the resulting strain F'*lacO*^{c}/*mem-1*, can grow again on galactose since TMG I is able to catalyze galactose transport and is active in a *mem-1* background. We conclude that induction of all enzymes involved in galactose metabolism is normal in *mem-1* mutant strains.

Isolation of *galP* mutants. The above-described strain, SB3586, contains GP as the sole galactose transport system, and so it can be used to isolate mutants defective in GP. This is important since only one mutant lacking GP has been described in *E. coli* by Buttin (4). It was anticipated, however, that a large majority of Gal$^-$ mutants would be defective in one or more of the enzymes involved in galactose metabolism after the translocation step. For this reason the following selection procedure was devised. As described above, introduction of an *E. coli* episome carrying a constitutive *lac* operon into SB3586 leads to a second galactose transport system. The two classes of Gal$^-$ mutants of SB3586, due to a mutation in either GP or one of the enzymes involved in galactose metabolism, can be discriminated by replica-plating putative mutants onto the F'*lacO*^{c} strain. Mutants defective in one of the galactose-metabolizing enzymes are still unable to grow on galactose, whereas *galP* mutants now regain the ability to grow on galactose. Out of 250 Gal$^-$ mutants of SB3586, only four independently isolated mutants turned out to be defective in galactose transport after growth on lactate in the presence of either D-fucose (Table 3) or 0.2% galactose. Up to 10 mM D-fucose was unable to induce galactose transport. A few other mutants had rates of transport between 25 and 50% of the rate in the parent, SB3586. A

TABLE 3. *Transport of galactose in galP mutants*

Strain	Galactose transport[a]
SB3586	9.5
PP149	0
PP151	0.1
PP153	0.4
PP150	0.3
PP146	2.4
PP148	2.6

[a] Transport activities (0.5 mM [^{14}C]galactose) are expressed as nanomoles of galactose taken up/min per milligram (dry weight) at 20°C.

number of other Gal⁻ mutants were shown to be defective in one of the enzymes involved in galactose metabolism. Two mutants, PP149, defective in GP, and PP144, lacking galactose 1-phosphate uridyl transferase, were studied in more detail.

Mutants having *galP* constitutively. The localization of the *galP* gene(s) on the *E. coli* or *S. typhimurium* chromosome is unknown (see below). It is not linked to the *gal* operon (5). Mutants of *E. coli* that express GP and the enzymes of the *gal* operon constitutively at the same time are known, however. They are called *galR* (5) and map close to *lys* (1). Mutations in *galR* have also been established in *S. typhimurium* strains in which the soluble proteins of the PTS, enzyme I and HPr, are deleted (17). Because of this deletion, cells cannot grow on glucose (Glc), but Glc⁺ strains can be isolated that are *galR*. Glucose is a substrate but not an inducer of GP. When we repeated this procedure, the majority of the Glc⁺ mutants turned out to be *galR*, but in addition a mutant was found that expresses GP constitutively but still has to be induced for the *gal* operon (Table 4). MGP is still inducible. The mutation is different from *galR* and may represent an operator-constitutive mutation. If so, this operator is different from *galO*.

Characterization of *galP* mutants. One of the problems in studying inducible enzyme systems concerns the capacity of the inducer to reach its target site. For instance, D-fucose or galactose is unable to induce galactokinase in *mem-1*, presumably because all fucose and galactose transport systems are defective in this strain. On the other hand, galactokinase can be induced in a strain containing in a *mem-1* background, an altered GP (SB3586), or an episome coding for a constitutive TMG I. Similar difficulties are encountered with strain PP149, a presumptive *galP* mutant. Table 5 shows that none of the enzymes involved in galactose metabolism is induced by D-fucose in this strain. In contrast, in another Gal⁻ strain, PP144, derived from SB3586, synthesis of galactokinase and UDPgalactose 4-epimerase is normal, but no galactose 1-phosphate uridyl transferase is synthesized. To show that the inability of PP149 to grow on galactose is in fact due to a defect in galactose transport, and not due to a mutation in the galactose operon, we have transduced PP149 to *mem*⁺ with phage P22, resulting in PP289, or we have selected *mem*⁺ revertants by isolating general revertants of PP149 on succinate or mannose (PP165). Table 5 shows that in

TABLE 5. *Induction of galactose-metabolizing enzymes by* D-*fucose*

Strain[a]	Genotype	Galactokinase[b]	Galactose 1-phosphate uridyl transferase[c]	UDP-glucose-4-epimerase[d]
SB3507	Parent	600	57	109
SB2138	*mem-1*	30	3	20
PP149	*mem-1 galP1903*	10	2	38
PP165	*mem-1*FR27 *galP1903*	590	48	138
PP144	*mem-1 gal-113 galT1898*	550	0.4	165

[a] Cells were grown on lactate plus 0.5 mM D-fucose.
[b] Nanomoles of galactose 1-phosphate formed per minute per milligram of protein at 37°C.
[c] Nanomoles of NADPH formed per minute per milligram of protein at 20°C.
[d] Nanomoles of NADH formed per minute per milligram of protein at 20°C.

TABLE 4. *Induction of GP, MGP, and galactokinase*

Strain	Carbon source	Transport[a]			Galactokinase[b]
		Galactose	Fucose	β-Methyl-galactoside	
SB3507	Lactate	1	1	0.4	30
	Lactate + fucose	30	18	9.5	400
	Galactose	28	15	0	NT[c]
SB2309	Lactate	2	NT	0.1	20
	Lactate + fucose	24	NT	2	340
	Galactose	NT	NT	0	NT
PP116	Lactate	15	20	0.5	10
	Lactate + fucose	22	NT	4	300
	Galactose	NT	NT	0.2	NT

[a] Transport rates using 0.5 mM [^{14}C]galactose (specific activity 40 cpm/nmol), 1 mM [^{3}H]fucose (specific activity, 62 cpm/nmol), or 0.5 mM β-[^{14}C]methylgalactoside (specific activity, 366 cpm/nmol) are expressed as nanomoles of substrate taken up/minute per milligram (dry weight) at 20°C.
[b] Galactokinase activity is expressed in nanomoles of galactose phosphorylated/minute per milligram of protein at 37°C.
[c] NT, Not tested.

the resulting *mem*$^+$ *galP* strain PP165, all three enzymes can be induced. D-Fucose can enter now via MGP (see below), and the strain again grows on galactose, although slowly. However, as will be shown in more detail below, galactose is not a very good inducer in strains lacking GP, producing only 25% of the MGP activity reached with D-fucose.

Three of the earlier described *galP* mutants, PP149, PP151, and PP153, behave in this way. Transduction to *mem*$^+$ yields strains that again grow on galactose. The fourth one, PP150, could not be transduced to *mem*$^+$ by P22. Neither was it possible to revert it to *mem*$^+$ by diethylsulfate mutagenesis. The unknown mutation seems to prevent reversion to *mem*$^+$. The fact that PP150 could be reverted with a frequency of 10^{-7} to 10^{-8} on galactose with diethylsulfate to the parental Gal$^+$ phenotype shows that it is a point mutation and furthermore that it is still sensitive to the mutagen.

Table 6 summarizes the transport data obtained with the various mutants derived from SB3586. The third and fourth column shows which of the transport systems, MGP or GP, is present.

Transport studies. The set of mutants described above allowed us to determine the specificities of the various galactose transport systems. We have studied GP in detail.

Figure 3 shows that D-fucose is a substrate of GP. Both SB3586, containing GP after growth in the presence of D-fucose, and PP116, possessing GP constitutively, transport [^{3}H]fucose. Repeated incubation of the same fucose solution with new cell preparations of PP116 shows that no minor contamination is responsible for the uptake of labeled material. The apparent K_m of GP for D-fucose is rather high, being 2.2 mM in strain PP116, compared to 50 μM for galactose in the same strain. This is compatible with the observation that only high concentrations of D-fucose can inhibit galactose and glucose transport via GP. As has been shown previously (16), D-fucose is a substrate of MGP in *E. coli*. Similar results have been obtained with *Salmonella*. Strain PP165, containing only MGP after growth on lactate plus fucose, transports [^{3}H]fucose in addition to galactose and β-methylgalactoside (Table 6). The K_m of MGP for fucose has been determined to be 1.4 mM, close to the value found for GP.

In addition to the compounds tested by Rotman et al. (16), we have tested a number of galactose analogues in order to determine the specificity of GP. It was found that 2-deoxygalactose and 2-deoxyglucose were efficient competitors of galactose oxidation and galactose

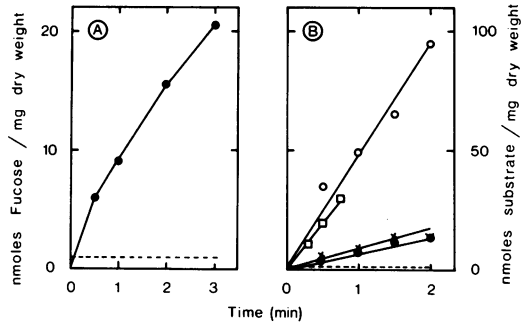

FIG. 3. *Specificity of GP. Cells (SB3507 or PP116) were grown in a medium containing 0.2% galactose. (A) Transport of 0.5 mM [^{3}H]fucose (specific activity, 62 cpm/nmol) by SB3507. (B) Transport of 0.5 mM [^{14}C]galactose (specific activity, 140 cpm/nmol) and 0.5 mM [^{14}C]mannose (specific activity, 60 cpm/nmol) by PP116. Symbols: ○, 0.5 mM galactose; ×, 0.5 mM galactose plus 6 mM 2-deoxygalactose; ●, 0.5 mM galactose plus 6 mM 2-deoxyglucose; □, 0.5 mM mannose. The dotted line represents equilibration.*

TABLE 6. *Transport in mutants defective in GP or MGP*

Strain	Carbon source	Presence of:		Transport[a]		
		GP	MGP	Galactose	Fucose	β-Methylgalactoside
SB3507	Galactose	+	−	25	NT[b]	0.3
	Lactate + fucose	+	+	35	20	10
SB2138	Lactate + fucose	−	−	0.5	0	0
SB3586	Lactate + fucose	+	−	15	4.5	0.5
PP149	Lactate + fucose	−	−	0	0	0
PP165	Lactate + fucose	−	+	4	3.7	5.5
PP144	Lactate + fucose	+	−	5	NT	0
PP161	Lactate + fucose	+	+	17.5	NT	9.5

[a] Transport rate with 1 mM [^{14}C]galactose, 1 mM [^{3}H]fucose, or 0.5 mM β-[^{14}C]-methylgalactoside is expressed as nanomoles of substrate taken up/minute per milligram (dry weight) at 20°C. For specific activities of labeled compounds, see footnote *a*, Table 4.

[b] NT, Not tested.

uptake via GP in either SB3507 or PP116 (Table 7, Fig. 3). As will be shown elsewhere (G. M. van Thienen, P. W. Postma, and K. van Dam, manuscript in preparation), addition of high concentrations of 2-deoxygalactose and 2-deoxyglucose to anaerobic cell suspensions caused an influx of H^+ into cells possessing GP, suggesting that those compounds are not only bound by GP but are actually transported. 6-Deoxyglucose, 6-*O*-methylgalactoside, and methyl α-glucoside had no effect on galactose transport in 10-fold excesses. Introduction of charged or bulky groups into galactose, such as galactosamine, *N*-acetylgalactosamine, or galacturonic acid, resulted in compounds that were noninhibitory when tested in 20-fold excess compared with galactose alone. It had been shown before that glucose is a substrate of GP (16, 17). Mannose is also transported in PP116 (Fig. 3), whereas neither glucose nor mannose are transported in the parental strain, SB2309, since it contains a *pts* deletion. GP shows little (if any) activity towards methyl α-glucoside.

We have also investigated GP in strain SB3586, a Gal^+ revertant of *mem-1*. Whereas GP has the same specificity as described above, the K_m for galactose is altered compared to the K_m of "wild-type" GP in SB3507 and PP116.

TABLE 7. *Inhibition of galactose oxidation via GP*[a]

Inhibitor	Galactose oxidation
No addition	0.21
0.6 mM 2-deoxygalactose	0.10
6 mM 2-deoxygalactose	0.015
6 mM 2-deoxyglucose	<0.01
6 mM 6-deoxyglucose	0.21
8 mM D-fucose	0.17

[a] SB3507 was grown on 0.2% galactose. Oxidation rate with 0.3 mM galactose is expressed as microatoms of oxygen taken up/minute per milligram (dry weight) at 25°C.

TABLE 8. *K_m of GP for galactose*[a]

Strain	K_m (mM)
SB3507	0.045
PP116	0.05
SB3586	0.30
SB3593	0.30
SB3632	0.35
SB3633	0.25

[a] Strains were grown on 0.2% galactose except for PP116, which was grown on either 0.2% galactose (for galactose oxidation) or 0.2% lactate (for [^{14}C]-galactose uptake). The K_m was determined by following either galactose oxidation (SB3507) or [^{14}C]galactose uptake (other strains), or both (PP116).

Table 8 shows that the K_m for galactose in SB3586 is 0.3 mM. This may be due to an altered GP protein since this strain was selected as a Gal^+ revertant of the membrane mutant *mem-1*. Transduction of SB3586 to mem^+ does not change the K_m of GP for galactose; this parameter is also not affected by a mutation that introduces a "specific" mannitol reversion, allowing the strain to grow also on mannitol (SB3633, Table 8). It should be emphasized that GP is the only galactose transport system present in those mem^+ strains when grown on galactose, although MGP could function again after transduction to mem^+; as will be shown below, growth on galactose induces only GP and not MGP in *Salmonella*. When grown on galactose, β-methylgalactoside transport is absent in strains SB3593, SB3632, and SB3633, amounting to less than 0.5 nmol of β-methylgalactoside transported/min per mg (dry weight) at 20°C. The apparent K_m of GP for D-fucose in SB3586 is higher than 5 mM, compared to 1.5 mM in the parental strain, SB3507, grown on galactose.

Induction studies. Since a number of potential transport systems for galactose exist, it is of interest to determine which one is induced by growth on galactose. Table 6 shows that growth of the wild-type strain, SB3507, on 0.2% galactose results in the induction of only GP, as evidenced by the presence of galactose transport but the absence of β-methylgalactoside transport. Transport of the latter substrate is induced only after growth in the presence of D-fucose. These results are somewhat different from those obtained with *E. coli*, in which growth on galactose does induce MGP (19).

Although MGP is not induced by galactose in the wild-type strain (carrying an intact GP), results with strain PP165, which lacks GP due to a mutation, suggest that in the absence of GP, MGP can be induced and is able to support, albeit slowly, growth on galactose. Generation times of these strains are given in Table 9. These results are paralleled by β-methylgalactoside transport studies in the mem^+ *galP* strains PP165, PP217, PP289, and PP291. Galactose is now able to induce β-methylgalactoside transport, but D-fucose is much more effective (Table 10).

Effect of *ptsI* mutation on galactose transport. It has been well documented that mutations in *S. typhimurium* and *E. coli*, which lead to the loss of the general proteins of the PTS, HPr and enzyme I, affect the growth of such mutants on many non-PTS sugars such as lactose, melibiose, maltose, and glycerol (18). Galactose is an exception to this rule. It has been shown that the induction of GP and the *gal*

TABLE 9. *Growth of galP mutants on galactose*

Strain	Genotype	Generation time[a] (min)
SB3507	Parent	60
SB2138	*mem-1*	>600
SB3586	*mem-1 gal-113*	100
PP149	*mem-1 galP1903*	>600
PP165	*mem-1* FR27 *galP1903*	190
PP217	*mem-1* FR27 *galP1903 ptsI405*	180
PP269	*mem-1* FR27 *galP1903 trzA-ptsHIΔ41*	130
PP271	*mem-1* FR27 *galP1903 trzA-ptsHIcrrΔ49*	145
PP230	*mem-1* FR27 *galC1891 ptsI405*	64

[a] Generation time was determined on 1% galactose.

TABLE 10. *Induction of MGP*[a]

Strain	Carbon source	Transport		
		Galactose	β-Methylgalactoside	Glucose
PP289	Lactate + galactose	3.5	4.5	
	Lactate + fucose	16	15.5	
PP291	Lactate + galactose	2.0	1	
	Lactate + fucose	11	12.5	
PP217	Lactate	0.8	0.1	0.4
	Lactate + fucose	5.5	3.4	4.0

[a] Strains were grown on lactate plus 0.2% galactose or 0.5 mM D-fucose. Rate of transport with 0.5 mM [^{14}C]galactose (specific activity, 65 cpm/nmol), 0.5 mM β-[^{14}C]methylgalactoside (specific activity, 417 cpm/nmol), or 1 mM [^{14}C]glucose (specific activity, 120 cpm/nmol) is expressed in nanomoles/minute per milligram (dry weight) at 20°C.

operon is normal in *ptsHI* deletions (17; Table 4). We have examined further the effect of *ptsI* on MGP and GP.

Strain PP165, which contains MGP but is defective in GP, grows on galactose with a generation time of 190 min (Table 9). Introduction of a *ptsI* mutation into PP165, yielding PP217, a *ptsI galP* strain, does not alter the generation time on galactose, 180 min. That the slow growth of PP165 and PP217 on galactose is not due to other unknown mutations in its background that affect galactose metabolism is shown by the observation that introduction of a constitutive GP into PP217 lowers the generation time on galactose to 64 min. This strain, PP230, was selected by crossing PP217 on glucose plates with phage P22 grown on PP116 and selecting for Glc$^+$ colonies. Mutants having a defective PTS can grow on glucose when GP is expressed constitutively (17). All colonies tested oxidized glucose after growth on lactate, showing that GP is constitutive. The parent strain, PP217, does not oxidize glucose unless induced with D-fucose. Similarly, transport of galactose, glucose, and β-methylgalactoside is induced when PP217, containing only MGP, is grown on lactate in the presence of D-fucose (Table 10). It can be concluded that glucose is a substrate of MGP but not an inducer since PP217 does not grow on glucose.

Induction of GP in strains carrying a *pts* deletion is normal, as shown in Table 4.

From the previous results it can be concluded that induction of MGP is possible in a *ptsI* background. PP217 does not grow on melibiose, maltose, or glycerol, showing that this *ptsI* mutation is indeed tight enough to prevent growth on these carbon sources (18). Although *ptsI* mutations do not inhibit growth of strain PP217 on galactose, preliminary experiments do show another effect. When comparing the apparent K_m of PP165 and PP217 for galactose, a value of approximately 1 μM was found with PP165, as measured by galactose oxidation, a value close to that found for MGP in *E. coli*. In contrast, the apparent K_m for galactose in strain PP217 was of the order of 2 mM. Transduction of PP217 to *pts*$^+$ restored the low K_m of MGP for galactose, showing that the change was not due to another (unknown) mutation in its background. It has been suggested that *ptsI* mutations exert their action through the inhibition of non-PTS sugar transport, thus lowering the endogenous inducer concentration (14a, 17). The finding that a *ptsI* mutation increases the K_m of the transport system for its substrate may explain the observations that *pts* mutants do not grow on a number of non-PTS carbon sources. As we have mentioned above, the K_m of GP for galactose is the same, about 50 μM, in both the wild-type strain (SB3507) and PP116, a strain that lacks enzyme I and HPr. Evidently, GP is not influenced by a *pts* mutation.

Genetics. As stated earlier, all isolated *galP* mutations are point mutations. Reversion on galactose occurs spontaneously with a frequency of 10^{-7} to 10^{-8}. Similarly, transduction of PP149 (*mem-1 galP*) with PP22 grown on SB3586 (*mem-1 galP*$^+$) yields Gal$^+$ transductants, which again transport galactose but not β-methylgalactoside. One of these strains, PP224, transports galactose, for instance, at a rate of 4 nmol/min per mg (dry weight) at 20°C, whereas β-methylgalactoside is not even equilibrated.

Mapping of the *galP* mutation has been impossible up to now since the Gal$^-$ phenotype is expressed only in a *mem-1* background. Reversion to *mem*$^+$ results in a cell that regains MGP and grows on galactose (Table 2). Consequently, transduction and Hfr crosses have to be done in the *mem-1* background, but in that case the transduced *galP*$^+$ gene cannot be ex-

pressed. It has been impossible to transduce PP217 to Glc$^+$ with P22 grown on PP117 (*galR ptsHI41*), suggesting that *galP* does not map closely to *galR*.

It can be established, however, that the *galP1903* mutation of PP149 is cotransducible with the *galC1891* mutation, which leads to the constitutive expression of GP in PP116. Using PP217 (*galP1903 ptsI*), which is unable to grow on glucose due to *ptsI*, transduction of this strain with P22 grown on PP116 yielded Glc$^+$ colonies. The percentage of cotransduction cannot be determined since only cells obtaining *galP*$^+$ together with *galC1891* grow on glucose. Plating in the presence or absence of D-fucose led to the same results. Eight recombinants were tested to determine whether GP was indeed synthesized constitutively. After growth on lactate, all eight recombinants were able to oxidize glucose at a rate of 0.16 to 0.19 μatoms of oxygen consumed/min per mg (dry weight) at 25°C, whereas the parent, PP217, is unable to do so (<5 natoms of oxygen consumed/min per mg [dry weight] at 25°C). Similar results were obtained with galactose transport. Transduction to *pts*$^+$ (which would also result in glucose oxidation) has been excluded since PP116 is a *ptsHI* deletion.

DISCUSSION

Galactose transport in *S. typhimurium* can be catalyzed by at least four different transport systems. By either using mutants that specifically lack one or more of these transport systems or employing different substrates, it has been possible to distinguish between these systems (16). With the exception of GP, the other galactose transport systems TMG I, TMG II, and MGP have been characterized genetically. GP synthesis is under the control of *galR*. Mutations in *galR* lead to the constitutive expression of both GP and the *gal* operon. Apart from the *E. coli* mutant 20SOK$^-$, described first by Buttin (4) and also characterized by Ganesan and Rotman (7), no mutants have been described that are specifically defective in GP. In the present paper we describe the isolation of a number of *galP* mutants. In addition, mutants were found that express GP constitutively but are still inducible for the *gal* operon. We have pointed out that we are unable at the moment to genetically map *galP* due to the interference of the other galactose transport systems, but *galP* is not linked to *galR*. From genetic crosses it could be concluded, however, that the mutation leading to a defective GP is closely linked to the mutation that results in the constitutive GP. These results suggest that the GP has its own operator which is closely linked to the *galP* gene. The *gal* operon probably has a very complex control system, consisting of two different operators, only one of which is regulated by *galR* (8). *galR* mutations probably represent mutations in which the galactose repressor, coded for by *galR*, is unable to act on both *galO* and *galC*, the operator belonging to *galP*, whereas a mutation in *galC* may result in the constitutive synthesis of only GP.

We have shown that GP is the only galactose transport system induced in *Salmonella* by growth on galactose. Different results have been obtained in *E. coli* in which growth on galactose also induces the β-methylgalactoside permease, as shown by Wilson (19). Mutants lacking GP can grow, although slowly, on galactose, suggesting that under certain conditions MGP can be induced by galactose. Transport studies, however, show that the rate of transport is rather low in these strains if galactose is the inducer. Using D-fucose as an inducer, much higher rates are obtained, approaching those of the *galP*$^+$ strains. It may be that galactose (or some metabolic product) is a poor inducer of MGP in *Salmonella*.

The properties of GP and MGP seem rather similar in *Salmonella* and *E. coli* if one considers both induction by D-fucose and substrate specificity. The apparent K_m of GP for galactose is somewhat lower in *Salmonella*, 50 μM compared to 140 μM (16), or 170 μM (19) in *E. coli*. The K_m of MGP for galactose is also very low in *Salmonella*, as is the case in *E. coli*, being less than 1 μM. Table 10 shows that glucose is a substrate of MGP. Glucose is unable, however, to induce the synthesis of MGP (or GP), since mutants containing *pts* deletions do not grow on glucose. It should be possible, however, to isolate *mglD* mutants by the procedure used to isolate *galR* and *galC1891*.

A number of substrates and substrate analogues that can be recognized by GP are now known. These include galactose, glucose, mannose, and the non-metabolizable compounds 2-deoxyglucose, 2-deoxygalactose, and fucose (6-deoxygalactose). Only galactose and fucose are inducers of this transport system. With the exception of 2-deoxygalactose and fucose, these sugars are also substrates of enzyme II-A/II-B of the PTS. This striking similarity between two systems, considered to be completely different, could be accidental. On the other hand, there may be a relationship between both transport systems, in line with the idea of a possible evolution of group translocation systems such as the PTS to active transport systems such as GP. It has been shown before that enzyme II of the PTS is able to catalyze the facilitated diffusion of galactose in the absence

of concomitant phosphorylation by phosphoenolpyruvate (14). The argument is somewhat weakened by the observation (Postma, unpublished observations) that in contrast to glucose, mannose, and 2-deoxyglucose neither 2-deoxygalactose nor fucose in 10-fold excess inhibits phosphoenolpyruvate-dependent phosphorylation of galactose by the PTS.

The effect of *pts* mutations on induction and functioning of both GP and MGP has been investigated in some preliminary experiments. No effect of a mutation leading to a defect in enzyme I and HPr has been found on either induction of GP or the apparent K_m of this transport system for its substrate, galactose. In both parent and *pts* mutant the K_m is about 50 μM. Different results have been obtained with MGP. The apparent K_m of MGP for galactose, as measured by galactose oxidation, is very low in a strain having an intact PTS, being 1 μM or less. This K_m increases considerably if a *ptsI* mutation is present in the same strain, resulting in a defective enzyme I. In that case the K_m is about 2 mM. Transduction of the *ptsI* mutant to *pts*$^+$ lowers the K_m again to about 1 μM. Although this observation does not explain what is happening in molecular terms, it may explain why *ptsI* mutants do not grow on many non-PTS compounds. If the K_m of a transport system is increased 1,000 to 10,000-fold, such a system seemingly does not function any longer since the concentration of the carbon source used during many growth studies is of low millimolarity.

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