JOURNAL OF BACTERIOLOGY, Feb. 1978, p. 607-613
0021-9193/78/0133-0607$02.00/0

Vol. 133, No. 2
Printed in U.S.A.

2-Deoxygalactose, a Specific Substrate of the *Salmonella typhimurium* Galactose Permease: Its Use for the Isolation of *galP* Mutants

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Received for publication 13 September 1977

2-Deoxygalactose is a specific substrate of the galactose permease. The apparent K_m is about 500 μM, compared to 45 μM for galactose, whereas the maximal rate of uptake is one-half to one-third of that of galactose. None of the other galactose transport systems, including methyl β-D-thiogalactosides I and II, the β-methylgalactoside permease, and both arabinose systems, is able to catalyze transport of 2-deoxygalactose to a significant extent. 2-Deoxygalactose can also be used to isolate mutants defective in galactose permease, since it is bacteriostatic. Colonies that grow with lactate, malate, or succinate as a carbon source in the presence of 0.5 to 2 mM 2-deoxygalactose were found to be mostly *galP* mutants, lacking galactose permease. Spontaneous 2-deoxygalactose-resistant strains arose with a frequency of about 2×10^{-6}. *galP* mutants have also been derived from *pts* deletion mutants that require galactose permease for growth on glucose. Revertants have been obtained that have acquired the parental phenotype.

Growth of *Salmonella typhimurium* on galactose leads to the induction of a single galactose transport system, the specific galactose permease (GP, 12). Transport of galactose across the membrane of *S. typhimurium* and *Escherichia coli* can be catalyzed, however, by a multiplicity of transport systems (7, 12, 15). The majority of these transport systems have other natural substrates. For instance, methyl β-D-thiogalactoside I and II both transport galactose but are coded for by genes in the *lac* and *mel* operons, respectively, and are involved in the metabolism of lactose and melibiose. A third system, the methyl β-galactoside permease (MGP) can be induced by galactose, but only when the GP is defective (12). Other galactose-transporting systems are those for arabinose (2, 10) and part of the phosphotransferase system (PTS, 11).

The study of the major galactose transport system in *Salmonella*, the GP, has been hampered by the lack of a specific substrate. In addition, it has been impossible until recently to select mutants defective in GP. Using a special mutant of *Salmonella* that lacks all galactose transport systems except GP, we succeeded in the isolation of *galP* mutants, but the selection method used could be applied only to certain strains (12). It was found also, using these and other strains, that 2-deoxygalactose inhibited galactose transport via GP and induced the uptake of protons in anaerobic cells of *Salmonella*, suggesting that 2-deoxygalactose is a substrate of GP (12, 18). Since no labeled 2-deoxygalactose was available at that time, direct transport studies were impossible.

We now report that 2-deoxygalactose is a specific substrate of the GP. In addition, it will be shown that 2-deoxygalactose can be used to isolate mutants defective in the GP.

METHODS

Bacterial strains. The strains used in this study are listed in Table 1.

Growth of cells. Cells were grown 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; and supplemented with 20 μg of tryptophan per ml and a carbon source (0.2%). Growth of PP267 required in addition 20 μg of cysteine per ml.

Chemicals. [^{14}C]methyl β-D-thiogalactopyranoside (14.4 mCi/mmol), [^{14}C]methyl β-D-galactopyranoside (0.05 mCi per 0.25 mg), and D-[*U*-^{14}C]galactose (0.05 mCi per 0.027 mg) were obtained from New England Nuclear Corp., Boston, Mass. D-[*U*-^{14}C]glucose (284 mCi/mmol) was purchased from The Radiochemical Centre, Amersham, England. 2-Deoxy-D-[1-^{3}H]galactose (20 Ci/mmol, Amersham) was a gift from J. van Steveninck, University of Leiden. Methyl β-D-galactopyranoside was purchased from Koch-Light Lab. D-Fucose, 2-deoxygalactose, and methyl β-D-thiogalactopyranoside were obtained from Sigma Chemical Co., St. Louis, Mo. All sugars used in this study were

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

Strain	Relevant genotype	Isolation procedure, parental strain	Source[b]
SB3507	*trpB223*		E. Balbinder
PP267	*cysA20 galP1903 mem-1*FR27 *trpB223*		(12)
PP418	*galP283 trpB223*	2DG res, SB3507	A
PP508	*F'lacO^c 15/galP283 trpB223*	E7084I1 × PP418	A
SB2637	*galR106 Δ(trzA-ptsHI)41 trpB223*		P. E. Hartman
PP354	*galP1914 galR106 Δ(trzA-ptsHI)41 trpB223*	2DG res, SB2637	A
PP355	*galP1915 galR106 Δ(trzA-ptsHI)41 trpB223*	2DG res, SB2637	A
PP356	*galP1916 galR106 Δ(trzA-ptsHI)41 trpB223*	2DG res, SB2637	A
SB2634	*galR103 Δ(trzA-ptsHIcrr)49 trpB223*		P. E. Hartman
PP362	*galP272 galR103 Δ(trzA-ptsHIcrr)49 trpB223*	2DG res, SB2634	A
PP364	*galP274 galR103 Δ(trzA-ptsHIcrr)49 trpB223*	2DG res, SB2634	A
PP438	*galP274*R1 *galR103 Δ(trzA-ptsHIcrr)49 trpB223*	Glc$^+$ PP364	A
PP116	*galC1891 Δ(trzA-ptsHI)41 trpB223*		(12)
PP415	*galP280 galC1891 Δ(trzA-ptsHI) 41 trpB223*	2DG res, PP116	A
SB2950	*Δ(trzA-ptsHIcrr)49 trpB223*		P. E. Hartman
SB1690	*ptsI40 trpB223*		P. E. Hartman
SB2226	*ptsH38 trpB223*		P. E. Hartman
	galK9		K. Sanderson
E. coli			
E7084I1	*F'lacO^c 15/lac-proAB thi ilv*		(12)

[a] Genetic nomenclature according to K. E. Sanderson (17). *galP* and *galC* are mutations leading to a defective GP and constitutive expression of GP, respectively. R is used to designate revertants of *galP* that acquire the parental phenotype. 2DG res, Resistant to 2-deoxygalactose; Glc, glucose.

[b] A, This study.

of D-configuration, unless mentioned otherwise.

Preparation of cell-free extracts and enzyme assays. Cell-free extracts were prepared exactly as described (12). Galactokinase (EC 2.7.1.6) was determined in the 200,000 × *g* supernatant (12). The reaction mixture contained, in a final volume of 0.1 ml: 10 mM ATP, 5 mM $MgCl_2$, 2.5 mM dithiothreitol, 12.5 mM KF, 50 mM potassium phosphate buffer (pH 7.5), 5 mM [^{14}C]galactose (specific activity, 322 cpm/nmol) or 5 mM [^{3}H]2-deoxygalactose (specific activity, 228 cpm/nmol), and varying amounts of supernatant. Phosphoenolpyruvate (PEP)-dependent phosphorylation of galactose and 2-deoxygalactose was measured as follows. The reaction mixture (0.1 ml) contained 10 mM PEP, 5 mM $MgCl_2$, 2.5 mM dithiothreitol, 12.5 mM KF, 50 mM potassium phosphate buffer (pH 7.5), 5 or 10 mM labeled sugar, and varying amounts of the 200,000 × *g* supernatant of strains SB1690 and SB2226 as a source of HPr and enzyme I, respectively. As a source of the membrane-bound, sugar-specific enzymes II, the membrane fraction obtained after centrifugation at 200,000 × *g* was used. The phosphate esters formed during the galactokinase and phosphotransferase (PTS) assays were determined by ion-exchange chromatography (8). The specific activity is expressed as nanomoles of substrate phosphorylated per minute per milligram of protein at 37°C. All enzyme activities were proportional to the amount of protein added.

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

Protein. Protein was determined by the method of Lowry et al. (9), using bovine serum albumin as a standard.

Isolation of mutants. Mutants resistant to 2-deoxygalactose were isolated by spreading about 10^8 cells on agar plates containing 0.4% DL-lactate as a carbon source and supplemented with 1.5 mM 2-deoxygalactose. 2-Deoxygalactose-resistant colonies appeared spontaneously after incubation for 48 to 72 h at 37°C. PP508 (F'*lacO^{c}15/galP283 trpB223*) was obtained from a cross of PP418 (*galP283 trpB223*) with *E. coli* E7084I1 on medium A agar plates containing 0.2% lactose supplemented with 20 μg of tryptophan per ml.

RESULTS

2-Deoxygalactose, a specific substrate of GP. Figure 1 shows that growth of the wild-type strain of *S. typhimurium*, SB3507, on galactose resulted in the induction of transport of both galactose and 2-deoxygalactose. Uninduced cells, grown on DL-lactate, transported both compounds at very low rates. The following lines of evidence show that transport is not due to a minor contamination present in the labeled 2-deoxygalactose. (i) The labeled compound was 98% pure as measured by paper chromatography (6). (ii) Cells that lacked GP but possessed an active MGP did not accumulate 2-deoxygalactose (Table 3). This excludes galactose as a possible contaminant, since the K_m of MGP for galactose is about 1 μM. (iii) At low 2-deoxygalactose concentrations (50 to 200 μM), more than 10% of the added label was accumulated within 4 min; even higher uptake was achieved with longer incubations. (iv) Repeated incubations of

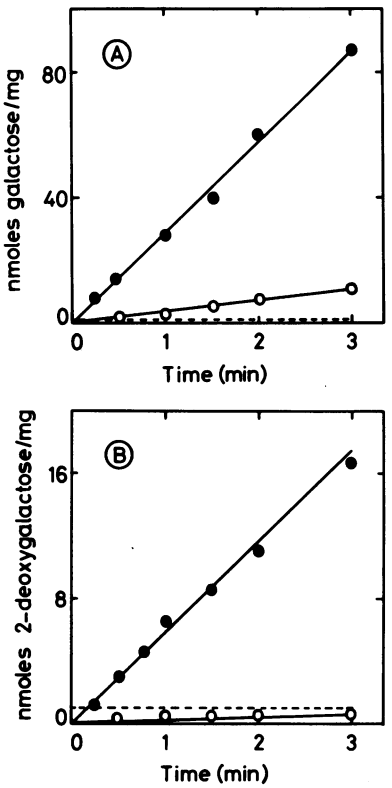

FIG. 1. *Transport of galactose and 2-deoxygalactose. SB3507 was grown in a medium containing 0.4% DL-lactate (○) or 0.2% galactose (●). (A) Transport of 0.5 mM [^{14}C]galactose (specific activity, 90 cpm/nmol). (B) Transport of 0.5 mM [^{3}H]2-deoxygalactose (specific activity, 402 cpm/nmol). The dotted line represents equilibration. The amount of uptake is expressed as nanomoles of substrate taken up per milligram (dry weight) at 20°C. Note the difference in the scales on the ordinates.*

the same 2-deoxygalactose solution with fresh cells always resulted in uptake of label.

Strains that express the GP constitutively (*galR* and *galC*) transported 2-deoxygalactose (and galactose) after growth on DL-lactate (Tables 2 and 6). Table 2 also shows that mutants lacking GP, such as PP267, did not accumulate 2-deoxygalactose when grown on lactate plus D-fucose, but still transported galactose. In this case, galactose transport occurred via MGP, which is induced by D-fucose. Induction of MGP is also shown by the transport of methyl β-galactoside (20 nmol/min per mg [dry weight] at 0.5 mM methyl β-galactoside).

Since we have shown previously (12) that cells of *Salmonella* grown on galactose synthesize only the GP of all the known galactose transport systems, these results demonstrated that 2-deoxygalactose is a substrate of GP. To show that 2-deoxygalactose is not transported by any of the other galactose transport systems, we induced each of these systems one at a time. A strain defective in the GP (PP418, see below) was used to avoid problems with inducers such as D-fucose. Table 3 shows the activities of the various transport systems with the specific substrates and 2-deoxygalactose. Methyl β-D-thiogalactoside II was induced by growth on melibiose and MGP by growth on lactate plus D-fucose. In both cases, transport of galactose was induced in addition to that of the specific substrates methyl β-D-thiogalactoside and methyl β-galactoside. 2-Deoxygalactose transport was negligible. To induce the arabinose transport systems, PP418 was grown on lactate in the presence of 0.2% arabinose, since the wild-type strain, SB3507, and all strains derived from it (including PP418) cannot grow on L-arabinose alone. No 2-deoxygalactose transport was detected. Methyl β-D-thiogalactoside I was tested by introducing into *Salmonella* (which lacks the *lac* operon) an episome carrying a constitutive *lac* operon. Again, no transport of 2-deoxygalactose was observed (Table 3, strain PP508). Finally, involvement of the PTS (13) was excluded by the data presented in Fig. 1. Cells grown on lactate contain, in addition to the general proteins enzyme I and HPr, constitutive levels of the sugar-specific membrane-bound enzymes II of the PTS (13), but do not transport 2-deoxygalactose.

Figure 2 shows the rate of 2-deoxygalactose uptake in the wild-type strain at various 2-deoxygalactose concentrations. An apparent K_m of about 500 μM can be calculated. Under the same conditions, the K_m for galactose is 45 μM (12). The maximal rate of uptake of 2-deoxygalactose

TABLE 2. *Transport of 2-deoxygalactose in galC and galP strains*

Strain	Relevant genotype	Carbon source	Transport[a]	
			2-deoxygalactose	galactose
SB3507	Parent	Lactate	0.3	1
		Lactate + fucose	5	30
PP116	Δ*ptsHI41 galC1891*	Lactate	2.8	15
		Galactose	4	22
PP267	*galP1903*	Lactate	<0.1	0.5
		Lactate + fucose	<0.1	16

[a] Transport rates using 0.5 mM [^{3}H]2-deoxygalactose (specific activity, 402 cpm/nmol) and 0.5 mM [^{14}C]galactose (specific activity, 65 cpm/nmol) are expressed as nanomoles of substrate taken up per minute per milligram (dry weight) at 20°C.

TABLE 3. *Transport of 2-deoxygalactose in strains induced for different galactose transport systems*

Strain	Carbon source	Transport[a]			
		2-Deoxygalactose	Galactose	Methyl β-galactoside	Methyl β-thiogalactoside
PP418	Lactate	<0.1	0.5	0.5	0.5
	Lactate + fucose	<0.05	9	5	NT
	Melibiose	<0.05	12	NT	5
	Lactate + L-arabinose[b]	<0.05	2	NT	NT
PP508	Lactose[c]	<0.05	3	0	5

[a] Transport rates using 0.5 mM [^{3}H]2-deoxygalactose (specific activity, 365 cpm/nmol), 0.5 mM [^{14}C]galactose (specific activity, 90 cpm/nmol), 0.5 mM β-[^{14}C]methylgalactoside (specific activity, 365 cpm/nmol), and 0.44 mM [^{14}C]methylthiogalactoside (specific activity, 510 cpm/nmol) are expressed as nanomoles of substrate taken up per minute per milligram (dry weight) at 20°C. NT, Not tested.

[b] Cells were grown on 0.4% DL-lactate + 0.2% arabinose.

[c] Since PP508 can grow only on lactose when the cells possess the episome, this carbon source was used to ensure that all cells contain the lactose transport system.

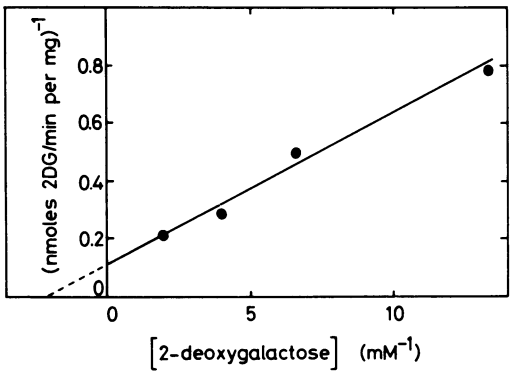

FIG. 2. *Lineweaver-Burk plot of 2-deoxygalactose transport. SB3507 was grown in a medium containing 0.2% galactose. The rate of uptake is expressed as nanomoles of substrate taken up per minute per milligram (dry weight) at 20°C. 2DG, 2-Deoxygalactose.*

is one-half to one-third of that of galactose. We have shown previously that 2-deoxygalactose inhibits both uptake and oxidation of galactose when GP is the sole galactose transport system (12). 2-Deoxygalactose had no effect on galactose or methyl β-galactoside transport via MGP. The rate of methyl β-galactoside transport (0.5 mM methyl β-galactoside) in strain PP267 was 20 nmol/min per mg (dry weight) in the absence or presence of 2.5 mM 2-deoxygalactose. Similarly, the uptake of 2-deoxygalactose was not inhibited by methyl β-galactoside in cells grown either on lactate plus D-fucose (parent strain, SB3507) or on galactose (strain PP267) (data not shown).

We have tried to investigate whether 2-deoxygalactose is an inducer of the GP. Since 2-deoxygalactose is toxic to *Salmonella* (1; see below), induction was studied in a *galK* strain that is resistant. Unfortunately, the GP activity in noninduced (lactate-grown) cells is rather high. Nevertheless, 2-deoxygalactose induced GP about 2.5-fold, even more than D-fucose (Table 4).

Metabolism of 2-deoxygalactose. Table 5 shows that 2-deoxygalactose was converted to (presumably) 2-deoxygalactose 1-phosphate by galactokinase, present in the high-speed supernatant of broken wild-type cells grown on galactose. The reaction was completely dependent on ATP and Mg^{2+} ions. The supernatant of broken cells grown on lactate did not catalyze the ATP-dependent phosphorylation of 2-deoxygalactose. The rate of 2-deoxygalactose phosphorylation in galactose-grown cells was low, about 5% of that with galactose as a substrate. We have not investigated whether 2-deoxygalactose phosphate is metabolized any further, as has been found in yeast by Jaspers and van Steveninck (6). It has been suggested by Floyd (as quoted in ref. 1) that 2-deoxyglucose phosphate is the toxic compound derived from 2-deoxygalactose.

It has been shown earlier that galactose can be phosphorylated to galactose 6-phosphate at the expense of PEP by the PEP:sugar PTS (W. Kundig, F. Dodyk-Kundig, B. E. Anderson, and S. Roseman, Fed. Proc., **24**:658, 1965). Table 5 shows that the membrane fraction of a *pts* deletion mutant, SB2950 (lacking HPr and enzyme I), catalyzed the PEP-dependent phosphorylation of 2-deoxygalactose when HPr and enzyme I were added. The reaction was completely dependent on PEP, enzyme I, and HPr. ATP had no effect. Using 10 mM 2-deoxygalactose, the phosphorylation rate was 22 nmol/min per mg of protein, about 50% of the rate with which membranes of the wild-type strain, SB3507, catalyze the PEP-dependent phosphorylation of galactose (11). It should be remembered, however, that *pts* deletion mutants have elevated levels

of enzyme II (4). Since the apparent K_m of enzyme II-A/II-B for 2-deoxygalactose is probably high (>10 mM, as is the case with galactose [11]), transport via the PTS under the conditions normally used (2-deoxygalactose concentration, <1 mM) is not important, as is also clear from Fig. 1.

The observation that *ptsHI* deletion mutants are still sensitive towards 2-deoxygalactose excludes 2-deoxygalactose 6-phosphate as the toxic compound.

Isolation of *galP* mutants. Alper and Ames (1) have isolated mutants with deletions in the *gal* operon region by selecting mutants resistant to 2-deoxygalactose. Since we have shown that 2-deoxygalactose is a specific substrate of the GP, it seemed reasonable to expect that another class of 2-deoxygalactose-resistant mutants would lack GP (*galP*). 2-Deoxygalactose-resistant strains of SB3507 appeared at a frequency of 2×10^{-6} when cells were plated on DL-lactate in the presence of 2-deoxygalactose (0.5 to 2 mM). The spontaneously arising colonies were tested for growth on galactose, since it was reasoned that *galP* mutants would still be able to grow on galactose due to the MGP, which is induced by galactose in the absence of GP (12). Mutants defective in the *gal* operon would not grow on galactose. Transport studies with one such *galP* mutant, PP418, are shown in Table 3. It was found that most 2-deoxygalactose-resistant strains derived from the parent strain, SB3507, grew normally on galactose (>90%) when lactate was used as a carbon source during the selection procedure. Similar results were obtained with succinate (94%, 47/50) and L-malate (92%, 46/50). When glycerol was used as a carbon source instead, 50% of the 2-deoxygalactose-resistant colonies were unable to grow on galactose (25/50), indicating that in this case *gal* operon mutations were appearing at a frequency of 10^{-6}, close to the value reported by Alper and Ames (1).

Since the phenotype of *galP* and *galP*$^+$ strains is in most cases identical, except for their sensitivity towards 2-deoxygalactose, isolation of revertants is difficult. For this reason, we selected for *galP* mutations in a *ptsHI* deletion mutant containing also a *galR* or a *galC* mutation. *ptsHI* deletion mutants do not grow on glucose (Glc), but when the GP is expressed constitutively (due to a *galR* or *galC* mutation), these strains regain growth on glucose, since glucose is a substrate of GP (12, 16). 2-Deoxygalactose-resistant strains were isolated from *galR* Δ*ptsHI* (SB2634, SB2637) and *galC* Δ*ptsHI* (PP116) mutants. Loss of the GP now results in strains that grow somewhat slower on galactose but are unable to grow on glucose. Several of these Glc$^-$ strains have been analyzed for transport of galactose, glucose, and 2-deoxygalactose, and the results with some mutant strains are shown in Table 6. All strains tested were shown to be deficient in GP. Since these strains can still grow on galactose, the *gal* operon can be expressed normally. This rules out a mutation in *galR* that prevents induction of both GP and the *gal* operon even in the presence of inducer. Since the transport studies shown in Table 6 were performed with lactate-grown cells, it should be shown that the *galR* mutation was still present. Table 7 shows that galactokinase was still synthesized constitutively in the *galP galR* mutants. Finally,

TABLE 4. *Induction of GP by 2-deoxygalactose*

Strain	Carbon source	Transport of 2-deoxygalactose[a]
galK9	Lactate	4
galK9	Lactate + 1 mM D-fucose	6.6
galK9	Lactate + 1 mM 2-deoxygalactose	9.4

[a] Transport rate using 0.5 mM [^{3}H]2-deoxygalactose (specific activity, 402 cpm/nmol) is expressed as nanomoles of substrate taken up per minute per milligram (dry weight) at 20°C.

TABLE 5. *Phosphorylation of 2-deoxygalactose*

Strain	Carbon source	Enzyme assay	Phosphorylation of 2-deoxygalactose[a]
SB3507	Galactose	Galactokinase, complete system	19
		Galactokinase, −ATP	<0.1
PP116	Lactate	Galactokinase, complete system	<1.0
SB2950	Lactate	Enzyme II-A/II-B, complete system	22
		Enzyme II-A/II-B, −PEP	<0.1
		Enzyme II-A/II-B, ATP instead of PEP	<1.0

[a] Rate of phosphorylation of [^{3}H]2-deoxygalactose (specific activity, 228 cpm/nmol) is expressed as nanomoles of 2-deoxygalactose phosphorylated per minute per milligram of protein at 37°C. In the case of galactokinase, specific activity is expressed per milligram of supernatant protein; in the case of enzyme II-A/II-B, per milligram of membrane protein.

growth of *galP galR* strains on lactate plus D-fucose led to uptake of galactose, glucose, and methyl β-galactoside, all substrates of MGP, but not to uptake of 2-deoxygalactose (data not shown). From these *galP galR* Δ*ptsHI* strains, *galP*$^+$ revertants could be isolated by selection for growth on glucose. The reversion frequency was dependent on the particular *galP* mutant studied, and varied between 10^{-7} and 2×10^{-8}. The properties of one such revertant, PP438, are shown in Table 6. Transport of galactose, glucose, and 2-deoxygalactose was almost similar to that of the original *galP*$^+$ strain, SB2634. Although, in principle, Glc$^+$ strains could arise due to the constitutive expression of the MGP (*mglD*; ref. 15), we have not found such strains among the revertants tested for transport. Selection should be easy, since *mglD* mutants would be resistant to 2-deoxygalactose, whereas *galP*$^+$ strains would be sensitive.

As we have mentioned earlier, the ratio between *galP* and *gal* operon mutants, selected on the basis of their resistance to 2-deoxygalactose, depends on the carbon source used. Furthermore, it is not possible to isolate 2-deoxygalactose-resistant strains by using any carbon source. A wild-type *galP*$^+$ strain was resistant to 2-deoxygalactose on a minimal A medium with various sugars as the carbon source, such as glucose, galactose, or mannose. This was probably due to exclusion of 2-deoxygalactose by direct competition for the transport system or due to catabolite repression. On the other hand, a *galP*$^+$ strain was sensitive when mannitol, fructose, melibiose, maltose (partly), glycerol, or the tricarboxylic acid cycle intermediates mentioned above were used. The strain was not sensitive to 2-deoxygalactose on nutrient agar plates.

TABLE 6. *Mutants defective in GP*

Strain[a]	Relevant genotype	Transport[b]		
		Galactose	Glucose	2-Deoxygalactose
SB3507		30	NT	5
PP418	*galP283*	9	NT	<0.05
SB2637	*galR106*	33	22	NT
PP354	*galR106 galP1914*	<0.1	<0.1	<0.1
PP355	*galR106 galP1915*	<0.1	<0.1	<0.1
PP356	*galR106 galP1916*	<0.1	<0.1	<0.1
SB2634	*galR103*	32	20	4.5
PP362	*galR103 galP272*	<0.1	<0.1	<0.1
PP364	*galR103 galP274*	<0.1	<0.1	<0.1
PP438	*galR103 galP274R1*	30	17	4
PP116	*galC1891*	15	NT	4
PP415	*galC1891 galP280*	0.7	NT	<0.1

[a] Cells were grown on 0.4% DL-lactate, except for SB3507 and PP418, which were grown on 0.4% DL-lactate + fucose.

[b] Transport rates using 0.5 mM [^{14}C]galactose (specific activity, 90 cpm/nmol), 0.5 mM [^{14}C]glucose (specific activity, 84 cpm/nmol), and 0.5 mM [^{3}H]2-deoxygalactose (specific activity, 402 cpm/nmol) are expressed as nanomoles of substrate taken up per minute per milligram (dry weight) at 20°C. NT, Not tested.

TABLE 7. *Activity of galactokinase in galR strains*

Strain	Galactokinase[a]
SB2637	370
PP355	220
PP356	325

[a] Strains were grown on 0.4% DL-lactate. Galactokinase activity is expressed in nanomoles of galactose phosphorylated per minute per milligram of protein at 37°C.

DISCUSSION

Galactose transport in *S. typhimurium* can be catalyzed by a number of different transport systems. Only one, the "specific" GP, is induced when cells are grown on galactose (12). Two factors have hampered a thorough investigation of this transport system: (i) no specific substrate is known for GP; and (ii) it has been impossible to isolate mutants deficient in GP. Only one such *galP* mutant has been found in *E. coli* (3) and has been characterized by Rotman and coworkers (5, 15).

In this paper, we report that 2-deoxygalactose is a specific substrate of the GP. In addition, this compound can be used to isolate mutants that lack specifically GP.

We have shown previously that 2-deoxygalactose inhibits galactose uptake and oxidation in galactose-grown cells (12). Similar to galactose, 2-deoxyglucose, and D-fucose, 2-deoxygalactose induces uptake of H^+ when the sugar is added to anaerobic wild-type cells that contain the galactose transport system (18). These results suggested that 2-deoxygalactose is a substrate of GP. We have here extended these studies using labeled 2-deoxygalactose. Transport studies with cells in which one or more of the known galactose transport systems was induced indicate that none of these transport systems except GP is able to transport 2-deoxygalactose (Table 3). Recently, it has come to our attention that similar results have been obtained in *E. coli* (P. J. F. Henderson, personal communication). Although a large number of substrates for GP of *S. typhimurium* is known by now, each of these, except 2-deoxygalactose, is a substrate of at least one other transport system. Glucose is a substrate of the MGP and two different PTS (13). 2-Deoxyglucose and mannose (and fructose) are also transported by the PTS (13). D-Fucose is a substrate of MGP. It is clear that the "specific" GP has a rather broad specificity. Interestingly, it has all known substrates in common with the

enzyme II-A/II-B of the PTS. This can be shown easily with glucose, 2-deoxyglucose, mannose, fructose, and galactose. The data of Table 5 show furthermore that 2-deoxygalactose can be phosphorylated in vitro by the PTS. Finally, we have shown that D-fucose, which is unable to act as phosphate acceptor due to the absence of the hydroxyl group at the 6 position, partly inhibits galactose phosphorylation by the PTS (P. W. Postma, unpublished results). We have speculated elsewhere on the possible relation between both systems (P. W. Postma and G. M. van Thienen, *Proceedings of the International Symposium on Mechanism of Proton and Calcium Pumps,* in press).

Alper and Ames (1) have used the sensitivity of *S. typhimurium* towards 2-deoxygalactose as a means to isolate deletions extending through the *gal* operon. Our finding that 2-deoxygalactose is a specific substrate of GP suggested a selection method for the isolation of *galP* mutants. Since, in the absence of GP, galactose induces MGP (12), thus enabling *galP* mutants to grow on galactose, mutants have been isolated that are resistant to 2-deoxygalactose but are still able to grow on galactose. The latter property indicates that the *gal* operon is still intact. Although we have reported earlier the isolation of *galP* mutants (12), the procedure described could only be applied to certain strains that contained the GP as the sole galactose transport system. The present method can be used with almost any strain. The mutations are most likely not in the regulatory gene, *galR*, since the *gal* operon is still inducible. We have been unable to map the *galP* mutation up until now. It has been suggested by Kornberg that *galP* in *E. coli* is cotransducible with *fda* and thus is close to *galR* (7). We have found, however, that *galP* is cotransducible with *galC*, a mutation resulting in a constitutive GP, but does not seem to be linked to *galR* (as measured by transduction with phage P22).

ACKNOWLEDGMENTS

We thank J. van Steveninck for the gift of labeled 2-deoxygalactose.

This work was supported by grants from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.) under the auspices of the Netherlands Foundation for Chemical Research (S.O.N.).

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