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Genetic Locus, Distant from *ptsM*, Affecting Enzyme IIA/IIB Function in *Escherichia coli* K-12

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Most strains of *Escherichia coli* K-12 are unable to use the enzyme IIA/IIB (enzyme II^{Man}) complex of the phosphoenolpyruvate:sugar phosphotransferase system (PTS) in anaerobic growth and therefore cannot utilize glucosamine anaerobically. Introduction into these strains of a *ptsG* mutation, which eliminates activity of the enzyme III^{Glc}/IIB' complex of the PTS, resulted in inability to grow anaerobically on glucose and mannose. Derivative strains able to grow anaerobically on glucosamine had mutations at a locus close to *man*, the gene coding for phosphomannose isomerase, and had higher enzyme IIA/IIB activities during anaerobic growth than did the parental strain. These results establish a locus affecting function of enzyme IIA/IIB that maps distant from *ptsM*, the probable structural gene for enzyme IIB.

In *Escherichia coli*, glucose, mannose, and glucosamine are among the sugars transported by the phosphoenolpyruvate:sugar phosphotransferase system (PTS) (11, 28, 32). Transport of glucose and mannose may occur by either of two distinct enzyme II complexes, the factor III^{Glc}/enzyme IIB' system or the factor IIA/enzyme IIB system (Fig. 1). The III^{Glc}/IIB' system is termed the "high-affinity" glucose PTS system (11, 28). It transports glucose, with high affinity, and mannose (11, 20, 28), and transports the glucose analog α-methyl-D-glucoside (αMG) well, but has a low affinity and V_{max} for 2-deoxyglucose (2-DOG) (11, 20, 28). The III^{Glc}/IIB' system is likely to play a key role in establishing glucose as a favored carbon source; mutations (*crr*) that reduce PTS regulation of catabolic genes for less favored carbon sources also lower the amount of factor III^{Glc}, and may lie in its structural gene (6, 10, 32, 34). The IIA/IIB system, termed the "low-affinity" glucose PTS system (11), or enzyme II^{Man} (32), acts, with decreasing affinity, on glucose, mannose, glucosamine, and fructose (32). This enzyme II complex has a much higher affinity for 2-DOG than for αMG. It is therefore possible to assay the III^{Glc}/IIB' system by αMG transport and the IIA/IIB system by 2-DOG transport (28).

Activity of the III^{Glc}/IIB' system is eliminated by mutation at *ptsG* (2), which probably codes for enzyme IIB' (6, 11). Mutations at *ptsM* (2), a locus distant from *ptsG*, eliminate activity of the IIA/IIB system, probably by affecting enzyme IIB; it is not known whether enzyme IIA is affected by *ptsM* mutations (6, 11). Growth experiments with quasi-isogenic wild-type, *ptsG* (*gpt*) mutant, *ptsM* (*mpt*) mutant, and *ptsG ptsM* double-mutant strains have clarified the physiological roles of the two gene products. Strains lacking the *ptsG* product grow at about half the wild-type rate on glucose and grow somewhat slowly on mannose, using the *ptsM* product (7). Strains lacking the *ptsM* product grow at wild-type rates on glucose and grow slowly on mannose, using the *ptsG* product (7). *ptsM* mutants are unable to grow on glucosamine (7, 16, 33).

We found that *ptsG* mutant derivatives of a number of *E. coli* strains were, unexpectedly, unable to grow on either glucose or mannose under anaerobic conditions unless an electron acceptor such as nitrate was provided. Strains in which a *ptsG* mutation had this effect were unable to utilize glucosamine anaerobically, suggesting that in these strains the IIA/IIB system was not functional under anaerobic conditions. Revertants of these *ptsG* strains selected for anaerobic growth on glucose included strains still lacking the III^{Glc}/IIB' system but able to transport glucose and mannose anaerobically via the IIA/IIB system as a result of mutations close to the gene for phosphomannose isomerase (*man*). Most of these revertants were able to use glucosamine anaerobically. The differing ability of *E. coli* K-12 strains to utilize glucosamine anaerobically was also found to be determined by genetic differences at a site close to *man*, a locus distant from *ptsM*.

MATERIALS AND METHODS

Bacterial strains. The strains of *E. coli* K-12 used are listed in Table 1, with genotypes and some details of derivation.

Media and growth conditions. Minimal medium 007 (5) was supplemented with the carbon source at 0.2% (unless otherwise stated), amino acids at 25 or 50

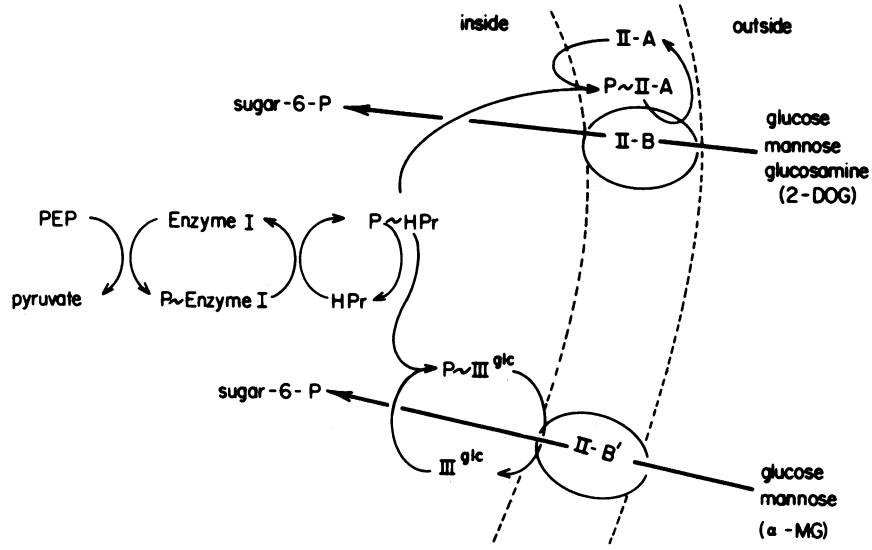

FIG. 1. *PTS routes for transport of glucose, mannose, and glucosamine, modified from Postma and Roseman (28). PEP, Phosphoenolpyruvate; enzyme I and Hpr, cytoplasmic proteins used in all PTS-mediated phosphorylations. Components of the enzyme II complexes, the phosphoryl carrier proteins, the membrane-bound factor IIA and cytoplasmic factor III^{Glc}, and the phosphoryl transfer enzymes, membrane-bound enzymes IIB and IIB', are described in the introduction. ptsM mutations eliminate activity of the IIA/IIB complex, and ptsG mutations eliminate activity of the III^{Glc}/IIB' complex (28).*

TABLE 1. *Strains of E. coli K-12 used*[a]

Strain	Sex	Genotype	Derivation, source, or reference
GMS343	F⁻	*aroD6 argE3 dgsA galK2 lacY1 man-4 mtl-1 rpsL700 tsx-29? supE44?*	CGSC (25); *dgsA* isolate from original mixed culture
GMS343-1	F⁻	*aroD6 argE3 galK2 lacY1 man-4 mtl-1 rpsL700 tsx-29? supE44?*	CGSC (25); *dgsA*⁺ isolate from original mixed culture of GMS343
K10	HfrC	*tonA22* λ	(1)
K12			CGSC 5073 (1)
RR246	F⁻	*dsdA galK his pps-1 ptsM (mpt-2) rpsL tyrA*	R. A. Roehl, Ph.D. thesis, University of Connecticut, Storrs, 1979
RR274	HfrC	*ptsG tonA22* λ	K10, EMS, maltose Blu (29)
RR313	HfrC	Δ(*dgsA-man*) *ptsG tonA22* λ	Spontaneous revertant of RR274 to anaerobic growth on glucose
RR345	HfrC	Δ(*dgsA-man*) *ptsG ptsM (mpt-2) tonA22* λ	2-DOG-resistant transductant of RR313, donor ZSC113L
RR370	HfrC	*ptsG tonA22* λ	Aerobic mannose-utilizing transductant of RR313, donor K10
RR371	HfrC	*dgsA ptsG tonA22* λ	Aerobic mannose-utilizing transductant of RR313, donor W3110(HMS)
RT500	F⁻	*aroD6 edd-1 galK his pfkA1 pps-2 pyrD rpsL*	(35)
W3110	F⁻		CGSC 4474 (1)
W3110(HMS)	F⁻	*dgsA*	D. G. Fraenkel
ZSC103L	F⁻	*ptsG (gpt-2) glk-7 rpsL*	W. Epstein; lactose-utilizing transductant of ZSC103 (7)
ZSC113L	F⁻	*ptsG (gpt-2) ptsM (mpt-2) glk-7 rpsL*	W. Epstein; lactose-utilizing transductant of ZSC113 (7)

[a] Gene designations are as previously used (2) except *dgsA*, this study. *rpsL* was formerly called *strA*. All known markers are included. Abbreviations: EMS, ethyl methane sulfonate mutagenesis; CGSC, strain obtained from B. Bachmann, *E. coli* Genetic Stock Center, Yale University.

μg/ml as required, and thiamine hydrochloride at 1 μg/ml. Sugars were the D form; amino acids were the L form. BTYEX 7 medium was minimal medium with 10 g of tryptone (Difco Laboratories, Detroit, Mich.) and 4 g of yeast extract (Difco) per liter. Solid medium had 1.5% agar (Difco). Solid medium for anaerobic growth contained 0.01% Casamino Acids (Difco) in addition to the carbon source. Anaerobic incubation

of agar plates was in GasPak anaerobic jars (BBL Microbiology Systems, Cockeysville, Md.). Anaerobic liquid cultures were in completely filled and tightly stoppered flasks. Media used for phage P1 propagation, assay, and transductions were those of Lennox (22) and Rosner (31).

Genetic procedures. Transductions were done with bacteriophage P1 (35) or with P1CM to simplify preparation of lysates (31). Conjugations were done as described by Miller (24). In genetic crosses, selection of *aroD*$^+$ was for growth on glycerol agar without phenylalanine, tyrosine, tryptophan, and shikimic acid; selection of *man*$^+$ was for growth with mannose as carbon source. Recombinants were purified on selective agar before scoring for unselected markers. Scoring of *ptsG* was by resistance to αMG (7) in fructose or glycerol minimal agar or by anaerobic growth on glucose. Transduction of *ptsM* mutations into *dgs* (deoxyglucose sensitive) strains was done by plating cells adsorbed with transducing phage to glycerol minimal agar and placing a filter paper disk saturated with 0.1 M 2-DOG onto the agar. Transductants appeared where diffusion had given the proper time for phenotypic expression and were purified on glycerol agar with 1 mM 2-DOG.

Enzyme assays. Phosphomannose isomerase was assayed by the method of Kang and Markovitz (15). Mannose 6-phosphate-dependent production of NADH was followed by absorption at 340 nm in a coupled system with extract and added phosphoglucose isomerase and glucose 6-phosphate dehydrogenase. Assay of wild-type and known deficient strains confirmed the procedure. Protein content of streptomycin-treated extracts was determined by UV absorption (9). Proline oxidase was assayed as described (8).

Transport assays. Aerobic cells were from 25-ml exponential cultures grown at 37°C in 250-ml flasks on a rotary shaking water bath at 300 cycles per min. Cells were centrifuged, washed, and suspended to an optical density at 580 nm of 0.5 in 007 buffer (minimal medium without carbon source), with 50 μg of chloramphenicol per ml. Cell suspensions were stored briefly on ice and then equilibrated at 32°C for 15 min in tubes agitated by a wrist-action shaker before transport assays were initiated by the addition of ^{14}C-labeled substrate. Anaerobic cells were grown at 37°C in completely filled 20-ml screw-cap tubes. Cells in exponential phase were centrifuged, washed in 007 buffer that had been made anaerobic by autoclaving and cooling while bubbled with nitrogen, and suspended in anaerobic buffer to which chloramphenicol had been added. Transport assays were done as for aerobic cells, with cell suspensions bubbled continuously with nitrogen if assays were to be under anaerobic conditions. ^{14}C-labeled αMG and 2-DOG were used at a specific activity of 0.2 mCi/mmol; glucose was used at 1.0 mCi/mmol. At intervals, samples of cells were filtered and washed with 10 ml of 32°C 007 buffer, and radioactivity was determined by scintillation counting.

Phosphotransferase assays. Phosphoenolpyruvate-dependent conversion of ^{14}C-labeled sugar to the sugar phosphate by cells made permeable by treatment with toluene was measured at 30°C by trapping the sugar phosphate on ion-exchange filter paper and determining radioactivity as described previously (30). Cells made permeable by treatment with benzene in ethanol (4) were assayed at 23°C for PTS activity for a number of sugars by measuring the sugar-dependent conversion of phosphoenolpyruvate to pyruvate, detected by following the decrease in absorbance due to NADH at 340 nm in a coupled system with lactic dehydrogenase as described previously (17, 18). Sugars were present in the reaction mixture at 5 mM. Cells made permeable with toluene showed higher NADH oxidase activities than cells treated with benzene, and could not be assayed by this method. Activities were expressed as nanomoles per minute per milligram (dry weight) of cells; the dry weight of cells was estimated from an optical density determination and a standard curve for dry weight versus optical density.

Chemicals. Uniformly ^{14}C-labeled αMG, D-glucose, and 2-DOG were from New England Nuclear Corp., Boston, Mass. Unlabeled αMG was from Mann Research Laboratories, and other biochemicals, including auxiliary enzymes for the phosphomannose isomerase and coupled PTS assays, were from Sigma Chemical Co., St. Louis, Mo.

RESULTS

Anaerobic growth of *ptsG* mutants. We observed that our strain RR274, a *ptsG* derivative of the wild-type *E. coli* K-12 strain K10, grew well aerobically on solid medium with glucose and mannose as carbon sources but was completely unable to grow anaerobically on agar with either sugar as carbon source unless nitrate was added as electron acceptor (Table 2). RR274 was able to grow anaerobically in glucose and mannose liquid medium, but at greatly reduced rates (Table 2). A well-characterized *ptsG* mutant in another genetic background, ZSC103L (7), was found to have the same growth properties.

Failure of *ptsG* strains to grow anaerobically on glucose and mannose was not due to an inability to produce acid fermentation products from these sugars. The *ptsG* strains produced enough acid from glucose and mannose on MacConkey indicator agar (7) to give a color only slightly less intense than that given by strain K10, whereas *ptsG ptsM* double mutants produced no acid in this test (data not shown).

The observation that *ptsG* mutant strains, which lack enzyme III^{Glc}/IIB′ activity, were unable to use glucose and mannose anaerobically suggested either that the alternate transport system for these sugars, enzyme II^{Man} (enzyme IIA/IIB, in part the *ptsM* product; Fig. 1), did not function under anaerobic conditions in wild-type cells or that a *ptsG* mutation somehow prevented anaerobic use of enzyme II^{Man}. Growth on glucosamine as a sole source of carbon provided a means for deciding between these possibilities, because enzyme II^{Man} func-

TABLE 2. *Aerobic and anaerobic growth of representative strains*

Strain	Relevant genotype			Growth on solid medium[a]								
				Aerobic				Anaerobic				
	ptsG	*ptsM*	*dgsA*[b]	Glu	Man	Glm	G6P	Glu	Glu + NO_3^-	Man	Glm	Glm + NO_3^-
K10	+	+	+	+ (65)	+ (75)	+ (415)	+ (55)	+ (95)	+	+ (145)	− (745)	+
RR274	−	+	+	+ (145)	+ (125)	+ (465)	+ (55)	− (330)	+	− (580)	− (640)	+
RR313	−	+	−	+ (80)	− (>3,000)	+ (390)	+ (60)	+ (135)	+	− (>3,000)	+ (220)	+
RR345	−	−	−	− (325)	− (>3,000)	− (905)	+ (80)	− (>3,000)	−	− (>3,000)	− (>3,000)	−
W3110(HMS)	+	+	−	+	+	+ (150)	+	+	+	+	+ (150)	+
RR370	−	+	+	+	+	+	+	−	ND	−	−	ND
RR371	−	+	−	+	+	+	+	+	ND	+	+	ND

[a] Growth at 27°C on minimal agar with 0.2% of the specified sugar is indicated by (+) strong growth or (−) very weak or no growth of an inoculum applied with the flat surface of a toothpick. Anaerobic agar contained 0.01% casein hydrolysate and 25 mM KNO_3 where indicated. *dgsA*$^+$ strains were negative on anaerobic glucosamine agar if inoculated from aerobic glucose agar, but an inoculum from aerobic complex agar sometimes grew appreciably. Scoring for growth was more reliable if strains were streaked for determination of colony diameters. Doubling times at 37°C in liquid medium are given in parentheses below the growth response. Aerobic liquid cultures were grown in a rotary shaking water bath and anaerobic cultures were grown in completely filled and stoppered tubes, both inoculated with washed cells from aerobic cultures with gluconate as carbon source. Abbreviations: Glu, glucose; Man, mannose; Glm, glucosamine; G6P, glucose 6-phosphate; ND, not done.

dgsA (deoxyglucose sensitive) is a genetic locus defined in this study. Strains with mutations at *dgsA* have elevated activities of the enzyme II^{Man} system and are typically very sensitive to inhibition by 2-DOG (Table 4).

tion is required for use of this compound (7). Strains K10, RR274 (*ptsG*), and RR313 (a spontaneous revertant of RR274 that was unable to grow anaerobically on glucose but still carried the *ptsG* lesion; described below) were compared for ability to grow on glucosamine anaerobically. The results (Table 2) indicated that enzyme II^{Man} was not used anaerobically by K10 or RR274 but could be used by the revertant, RR313. (The inability of RR313 to utilize mannose under any conditions is due to loss of a mannose catabolic enzyme, as described below.)

A survey of *E. coli* K-12 cultures revealed that most strains did not use enzyme II^{Man} anaerobically. Strains K-12 (Lederberg wild type, CGSC 5073), W3110 (CGSC 4474), X7187 (35), DF1651 (35), and CSH59 (24) shared this trait with the strains noted above, as tested by anaerobic phenotype on glucosamine agar for the first two strains and by anaerobic phenotype on glucose and mannose agar after introduction of a *ptsG* mutation for the remaining strains. However, our laboratory culture of strain W3110, obtained at Harvard Medical School and designated W3110(HMS), was able to use glucosamine anaerobically (Table 2), as were roughly half of isolated colonies of a culture of strain GMS343 (25) from the *E. coli* Genetic Stock Center.

Transport activity of enzyme III^{Glc}/IIB' and enzyme II^{Man} systems under anaerobic conditions. Strains K10, RR274 (*ptsG*), and the latter's anaerobic glucose-utilizing revertant RR313 were compared for aerobic and anaerobic transport of αMG, a substrate of the enzyme III^{Glc}/IIB' system, using cells grown aerobically and anaerobically. RR274 and RR313 were unable to transport αMG aerobically or anaerobically (data not shown), indicating that anaerobic use of glucose by RR313 did not involve restoration of transport by enzyme III^{Glc}/IIB'.

The ability of RR313 to use glucosamine anaerobically (Table 2) suggested that anaerobic glucose utilization by this strain occurred via enzyme II^{Man}. In this case, mannose should compete strongly with glucose for anaerobic transport by RR313 (11, 28), and this was found to occur. Whereas RR313 showed nearly wild-type anaerobic accumulation of radioactivity from [^{14}C]glucose, much greater than that by RR274, a 10-fold excess of unlabeled mannose depressed accumulation of radioactivity by RR313 to the low level seen for RR274 under the same conditions (Fig. 2). Strain K10, possessing a functional enzyme III^{Glc}/IIB' system, was resistant to inhibition of glucose transport by mannose (Fig. 2). That RR274 was able to transport glucose anaerobically at a low but significant level (Fig. 2) was in accord with liquid culture growth rates (Table 2).

Anaerobic transport via enzyme II^{Man} in anaerobically grown cells was assayed directly, using 2-DOG as a substrate (Fig. 3). Strain RR313 showed much greater initial rates of 2-DOG accumulation than did K10 and RR274, al-

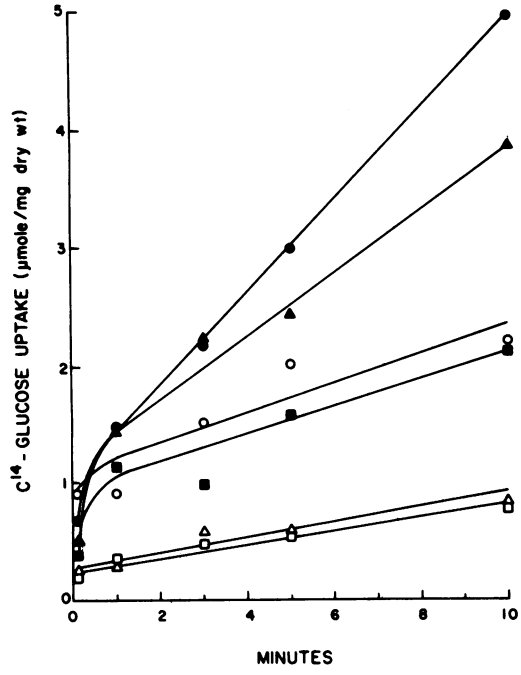

FIG. 2. *Effect of mannose on anaerobic glucose transport. Cells for assay were from cultures grown to late exponential phase anaerobically at 37°C in 0.4% gluconate minimal medium and then shifted to anaerobic 0.4% glucose medium at 37°C for 2 h before harvest. Cells were washed and suspended in anaerobic minimal medium without carbon source with 50 μg of chloramphenicol per ml and assayed under nitrogen for transport at 32°C.* D-*[^{14}C]glucose was used at 0.2 mM, and* D-*mannose, when added, was used at 2.0 mM. Symbols: ○, K10; □, RR274; △, RR313. Solid symbols, Transport with only glucose present; open symbols, transport of glucose in the presence of 10× mannose.*

though the latter strains, inferred from phenotype to be deficient in anaerobic enzyme II^{Man} function, showed appreciable transport activities. Moreover, no marked difference was seen between aerobic uptake and anaerobic uptake of 2-DOG by K10 and RR274. Aerobic transport by aerobically grown cells of both strains was found to be only slightly more rapid than anaerobic transport by anaerobically grown cells; aerobic transport by anaerobically grown cells and vice versa also gave results much like those shown in Fig. 3, RR313 always having more rapid uptake than K10 and RR274 (data not shown). Use of a variety of procedures to increase the completeness of anaerobiosis of growth cultures and cell suspensions for transport assay did not affect this result. The lack of significant accumulation of 2-DOG by a known *ptsM* mutant confirmed this as an assay for enzyme II^{Man} activity (Fig. 3, strain RR246; RR246 had wild-type ability to transport αMG [data not shown]). Anaerobic activities of the enzyme II^{Man} system as assayed by 2-DOG transport were too great to explain the anaerobic solid medium phenotypes of K10 and RR274, but may fit with the growth of these strains in anaerobic liquid culture (Table 2).

Phosphotransferase activity measured in permeable cells. Direct phosphotransferase assays of cells made permeable with toluene or benzene permit the use of metabolizable substrates and may provide more reliable measures of PTS activity than transport assays (17). Permeabilization procedures are also very rapid; the repeated washings required for transport assays might permit changes to occur in the cells. Phosphoenolpyruvate-dependent phosphorylation of 2-DOG by permeable cells from aerobic stationary cultures (Fig. 4) confirmed that RR313 had greater PTS activity for 2-DOG than did K10 and RR274, and showed that enzyme II^{Man} activity was depressed in stationary-phase *ptsG* cells. Transport studies with stationary-phase cells confirmed lower accumulation of 2-DOG by RR274 than by K10 (data not shown), unlike

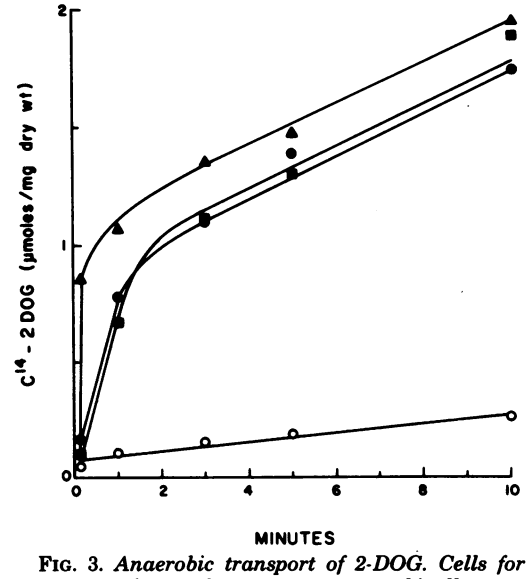

FIG. 3. *Anaerobic transport of 2-DOG. Cells for assay were from cultures grown anaerobically overnight at 37°C in 0.4% gluconate minimal medium and then shifted to fresh anaerobic gluconate medium and allowed to double several times before harvest. Cells were washed and suspended in anaerobic minimal medium without carbon source with 50 μg of chloramphenicol per ml and assayed under nitrogen at 32°C for transport of 2-DOG (^{14}C labeled), which was added to 0.2 mM. Symbols: ●, K10; ■, RR274; ▲, RR313; ○, RR246.*

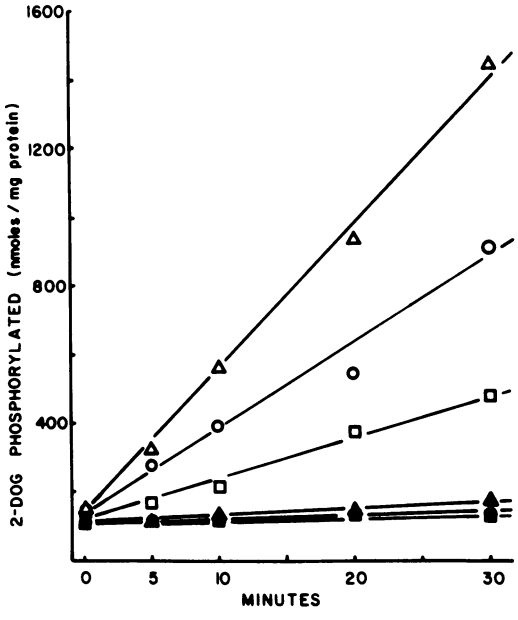

FIG. 4. *Phosphoenolpyruvate-dependent phosphorylation of 2-DOG by toluene-treated cells at 30°C. Cells were from fresh stationary cultures in 0.2% mannitol minimal medium grown aerobically at 37°C. 2-DOG (^{14}C labeled) was present in the reaction mixture at 0.8 mM. The procedure for toluene treatment was such that the activity of the enzyme III^{Glc}/IIB' system was not released (12); cells of the three strains showed essentially no phosphorylation of αMG. Symbols: ○, K10; □, RR274; △, RR313. Open symbols, Reaction mixtures with phosphoenolpyruvate added; closed symbols, reaction mixtures without phosphoenolpyruvate.*

the results with exponential-phase cells in Fig. 3.

Permeable-cell PTS assays of cells grown aerobically and anaerobically (Table 3) showed a markedly decreased activity for enzyme II^{Man} substrates in anaerobically grown cells of K10 and RR274 and a greatly increased activity for these substrates in anaerobically grown RR313. Strain RR313 showed low levels of all PTS activities in aerobic cells made permeable with benzene (Table 3). The assays reported in Table 3 were done under aerobic conditions. The effect of anaerobiosis on enzyme II^{Man} activities may then be at the level of synthesis rather than of activity, but the lack of correspondence between transport assays and permeabilized cell assays of PTS activity in anaerobic cells complicates this interpretation.

Revertants of a *ptsG* mutant able to grow anaerobically on glucose. Spontaneous revertants of RR274 able to grow on anaerobic glucose agar occurred at a frequency of 50 for every 10^8 cells plated. Revertants fell into four phenotypic classes (Table 4). Revertants of class I were sensitive to inhibition by αMG (Table 4) and were shown to have back mutations at *ptsG* (7). Revertants of the remaining classes remained resistant to αMG but showed increased sensitivity to inhibition by 2-DOG (Table 4). Class IV revertants were unable to utilize mannose anaerobically or aerobically due to deletion of the gene for a mannose catabolic enzyme, as shown below. Surprisingly, some or all revertants of classes II, III, and IV were defective in growth on proline as a carbon source (Table 4).

TABLE 3. *PTS activities of aerobically and anaerobically grown cells*

Strain	Genotype	PTS activity (nmol/min per mg, dry wt)[a]											
		Aerobic						Anaerobic					
		Mtl	2-DOG	Glm	Man	αMG	Glu	Mtl	2-DOG	Glm	Man	αMG	Glu
K10		48.4	59.5	23.1	55.8	29.4	48.5	4.7	1.4	<0.2	6.7	5.7	5.6
RR274	*ptsG*	43.3	15.2	10.7	20.6	3.0	9.4	4.1	0.7	<0.2	2.2	<0.2	<0.2
RR313	Δ(*dgsA-man*) *ptsG*	12.7[b]	18.4	13.9	18.4	10.5	6.9	5.7	6.0	12.4	23.4	3.0	15.6

[a] PTS activities at 23°C, using the coupled assay with lactic dehydrogenase and cells made permeable by treatment with benzene (see Materials and Methods). Aerobic cells were from shaking cultures grown in 0.2% mannitol minimal medium at 37°C, which had been in stationary phase for between 2 and 3 h. Cells from exponential aerobic cultures showed activities essentially identical to those for cells from stationary cultures. Anaerobic cells were from cultures grown in 0.2% mannitol medium in completely filled and stoppered flasks at 37°C and harvested at an optical density at 580 nm of 0.55, about half final yield. Abbreviations: Mtl, mannitol; Glm, glucosamine; Man, mannose; Glu, glucose.

[b] PTS activities of aerobically grown cells of RR313 and *dgs* strains of class III (Table 4) were low in assays with cells made permeable by treatment with benzene. Treatment with toluene, expected to give high activities for RR313 (Fig. 5), revealed extensive NADH oxidase activity in all these strains, which prevented PTS assay by the coupled reaction with lactic dehydrogenase. Anaerobic cells of a typical class III strain, made permeable with benzene, showed PTS activities comparable to those of RR313.

TABLE 4. *Phenotypes of glucose-fermenting revertants of RR274 and of other representative strains*[a]

Strain	No. of revertants[b]	Anaerobic growth[c]				Growth on:			Genotype
		Glu	Man	Glm	Gal	Glycerol + 2-DOG[d]	Glycerol + αMG[e]	Proline[f]	
K10		+	+	−	−	+	−	+	
RR274		−	−	−	−	+	+	+	*ptsG*
Class I revertants	8	+	+−	−	−	+	−	+	*ptsG*$^+$
Class II revertants	6	+	+−	−	++[g]	−	+	−	*dgsB ptsG*
Class III revertants	80	+	+	22+ 58+− 65−	15+[h]	−	+	−, +−, +	*dgsA ptsG*
Class IV revertants (including RR313)	4	+	−	+	+	−	+	−	Δ(*dgsA-man*) *ptsG*
RR345		−	−	−	+	+	+	−	Δ(*dgsA-man*) *ptsG ptsM*
RR370[i]		−	−	−	−	+	+	+	*ptsG*
RR371[i]		+	+	+	+	−	+	+	*dgsA ptsG*
W3110(HMS)		+	+	+	+	+	−	+	*dgsA*

[a] +, Strong growth; +−, weaker growth; − very weak or no growth. Streaking for isolated colonies made scoring more reliable.

[b] Ten independent cultures of RR274 were plated to anaerobic glucose agar, and about 10 colonies from each culture were purified. The strains listed represent at least 50 independent revertants.

[c] Anaerobic growth at 37°C on the indicated carbon source (glucose, mannose, glucosamine, galactose) with 0.01% casein hydrolysate. All strains grew on these sugars aerobically, except revertants of class IV, which could not utilize mannose, and RR345 (Table 2). All strains grew aerobically and anaerobically on fructose, glucose 6-phosphate, and mannitol.

[d] Aerobic growth at 37°C on glycerol agar with 1 mM 2-DOG.

[e] Aerobic growth at 37°C on glycerol agar with 0.2% αMG, inocula from lactate, or complex agar (7).

[f] Aerobic growth at 37°C with L-proline as carbon source. Revertants defective in proline utilization were impaired in aerobic growth on a number of carbon sources. RR313 grew nearly as well as RR274 on galactose, gluconate, glucose, glucose 6-phosphate, lactose, and maltose, and mannitol, but much less well on acetate, fructose, glycerol, lactate, proline, succinate, and xylose and on BTYEX 7 medium.

[g] Class II revertants had twofold greater colony diameters on anaerobic galactose agar than revertants of classes III and IV and were sensitive to inhibition by 2-deoxygalactose.

[h] The 15 class III strains able to use galactose anaerobically showed strong growth on anaerobic glucosamine plates; most of the remaining strains were weaker on anaerobic glucosamine agar.

[i] RR370 and RR371 are *man*$^+$ transductants of RR313 with strains K10 and W3110(HMS) as donors. Transductants with strains K-12 (CGSC 5073) and W3110 (CGSC 4474) as donors had phenotypes identical to that of RR370.

Also surprising were the growth phenotypes on galactose, a sugar not transported by the PTS and included as control. Whereas strains K10 and RR274 were unable to utilize galactose anaerobically, all revertants of classes II and IV and some of class III showed strong growth on anaerobic galactose agar (Table 4).

2-DOG is inhibitory to cells when transported by the enzyme II^{Man} system (7). The heightened 2-DOG sensitivity of RR313 and other anaerobic glucose revertants of RR274 (Table 4), and the ability of most of these strains to use glucosamine anaerobically (Table 4), suggested that mutations resulting in increased expression of enzyme II^{Man} were a common basis for anaerobic growth on glucose. A genetic demonstration that enzyme II^{Man} function was required for anaerobic growth on glucose was made for strain RR313. RR313 was readily transduced to 2-DOG resistance on aerobic glycerol agar with a bacteriophage P1 lysate prepared on a known *ptsM* mutant strain (ZSC113L [7]) but could not under the same conditions be transduced to resistance with a lysate made on a *ptsM*$^+$ strain. Purified 2-DOG-resistant transductants, verified as *ptsM* by genetic crosses, were unable to grow anaerobically or aerobically on glucose and glucosamine (strain RR345; Tables 2 and 4).

Genetic analysis of a class IV revertant. Strain RR313, a mannose nonutilizing revertant (class IV in Table 4), retained a *ptsG* mutation indistinguishable from that of its parent, as shown by transduction into a *pyrC* mutant strain (7). The inability of RR313 to transport αMG, noted above, made informational suppression of the *ptsG* mutation, or compensation for an altered enzyme IIB′ (the *ptsG* product) by a change in factor III^{Glc} (probable product of the

crr locus [32]), an unlikely explanation of anaerobic glucose utilization, and these possibilities were eliminated by genetic tests. Results presented in earlier sections indicated that RR313 used glucose anaerobically via enzyme II^{Man}, but transductional crosses showed no linkage of the reversion mutation to a marker (*edd*) cotransducing with *ptsM*. With the elimination of known PTS loci, the genetic designation *dgs* (deoxyglucose sensitive) was proposed for the mutations permitting anaerobic growth on glucose, to be used until a more specific designation might be warranted.

RR313 is an HfrC strain. Conjugational three-point crosses with multiply marked F^- strains gave an approximate location for the *dgs* locus of RR313 of *his-aroD-dgs-pyrD*, with *dgs* close to *aroD* (data not shown). The indicated map position for *dgs* was close to the position of the mannose utilization locus *man* (2, 23). This result and the mannose-negative phenotype of RR313 suggested that *dgs* mutations affected *man*. The *man* and *aroD* loci are about 1% cotransducing (14, 25; Table 5, cross 5), and the *dgs* mutation in RR313 showed a similar linkage to *aroD* (Table 5, cross 1). The product of the *man* locus, phosphomannose isomerase (23), was assayed in K10, RR274, and RR313; RR313 lacked activity (data not shown).

The *dgs* locus thus appeared to be a synonym of *man*. However, the known *man* mutant GMS343 was not sensitive to 2-DOG and was, in some isolates, unable to use glucosamine anaerobically while showing good aerobic growth on this sugar. That the *dgs* mutations in RR313 and other class IV revertants were likely to be deletions extending into the *man* locus was suggested by a total absence of reversion of these strains to mannose utilization, even after treatment with mutagens, and by the failure to find class IV revertants after ethyl methane sulfonate mutagenesis of RR274 (R. A. Roehl and R. T. Vinopal, unpublished data).

Our culture of the described *man* strain GMS343 contained both glucosamine-fermenting and -nonfermenting cells. These isolates made it possible to test for linkage between, or coincidence of the *man* and *dgs* loci. W3110(HMS), a strain naturally able to use glucosamine anaerobically (Table 2), was found to differ at the *dgs* locus from strains incapable of anaerobic growth on glucosamine. A bacteriophage P1 lysate prepared on W3110(HMS) and used to transduce a glucosamine-nonfermenting isolate of GMS343 to growth on mannose donated the ability to ferment glucosamine at about 40% (Table 5, cross 4), whereas a lysate prepared on K10 did not donate this trait (Table 5, cross 2; compare crosses 3 and 4). *dgs* was thus clearly distinct from *man*, consistent with RR313 being a deletion mutant. All of 16 transductants of RR313 to aerobic growth on mannose with W3110(HMS) as donor were able to use glucose, mannose, and glucosamine anaerobically, whereas all of 16 transductants with K10, K12, or W3110 as donors showed the phenotype of RR274 (compare transductants RR371 and RR370, Table 2). This confirmed RR313 as a deletion mutant and demonstrated that the anaerobic-negative phenotype resulting from the *ptsG* lesion in RR274 was dependent on the presence of the wild-type *dgs* allele. Transductants with W3110(HMS) as donor were able to use galactose anaerobically, whereas transductants with K10 as donor were not (compare RR371 and RR370, Table 4), confirming galactose fermentation as a component of the *dgs* phenotype.

The map order of the *aroD*, *dgs*, and *man* markers was deduced. If *dgs* is between *aroD* and *man*, then *dgs* point mutations should be about 12% linked by transduction to *aroD*, as

TABLE 5. *Mapping of the dgsA locus by transduction*

Donor	Recipient	Selected marker	No. of recombinants	Scoring[a]			
1. RR313 Δ(dgsA-man)	RT500 *aroD*	*aroD*$^+$	549	Man$^+$ 545		Man$^-$ 4	
2. K10	GMS343-1 *man*	*man*$^+$	21	GlmAn$^+$ 0		GlmAn$^-$ 21	
3. W3110(HMS) *dgsA*	GMS343 *dgsA man*	*man*$^+$	21	21		0	
4. W3110(HMS) *dgsA*	GMS343-1 *man*	*man*$^+$	21	8		13	
5. W3110(HMS) *dgsA*	GMS343-1 *aroD man*	*aroD*$^+$	489	Man$^+$ GlmAn$^+$ 0	Man$^+$ GlmAn$^-$ 5	Man$^-$ GlmAn$^+$ 0	Man$^-$ GlmAn$^-$ 484

[a] Scoring of *dgsA* was by growth on anaerobic glucosamine agar (GlmAn; *dgsA* strains are GlmAn$^+$) or, for the Δ(*dgsA*-man) mutation, by growth on aerobic mannose agar (Man). *dgsA* was also scored by sensitivity to 2-DOG or by growth on proline agar (Table 4); all methods gave the same results.

calculated by the method of Wu (36) from the observed linkage between *aroD* and *man* and between *dgs* and *man* (Table 5). Cross 5 in Table 5, potentially three point, showed no linkage between *aroD* and *dgs*. This result strongly implied that the linkage observed between *aroD* and the Δ(*dgs-man*) marker in RR313 was due to deletional shortening of the transducing particle and that the map order was *aroD-man-dgs*.

Genetic analysis of class II and class III revertants. Two typical members of each of classes II and III (Table 4) were analyzed. Revertants of both classes retained the *ptsG* mutation of RR274 by genetic tests. Transduction into RR313, a *dgs man* deletion strain, permitted rapid mapping of *dgs* mutations. Selection was for aerobic growth on mannose (repair of *man*), and scoring of recombinants was for traits of the donor revertant strain. The two class III revertants tested donated their revertant phenotype; every transductant of RR313 had all the characteristics of the class III revertants (Table 4), showing that class III revertants were altered at the *dgs* locus. However, the class II revertants donated the parental phenotype; every transductant of RR313 had the properties of RR274 (Table 4). The reversion mutation in class II strains was thus not close to *man*. The *dgs* locus close to *man* has been designated *dgsA*, and the unmapped locus at which class II revertants are altered has been designated *dgsB*. Although class II revertants were sensitive to inhibition by 2-DOG, none of them was able to utilize glucosamine under anaerobic conditions (Table 4). 2-DOG is likely to be a substrate for the galactose permease (27), and class II strains grew better than other revertant classes on anaerobic galactose agar (Table 4). *dgsB* revertants may use glucose anaerobically by a galactose permease rather than by enzyme II^{Man}.

Pleiotropic carbon source utilization phenotype of *dgs* mutant strains. All classes of glucose-fermenting revertants of RR274 other than back mutants at *ptsG* included strains that were slow or negative in growth on proline as carbon source. These revertants were also impaired in growth on a variety of other carbon sources (Table 4). Class IV strains were most dramatically affected, and their traits could have been due to deletion of a locus other than *dgsA* or *man*, although described strains with long deletions covering *man* do not show the pleiotropic phenotype of RR313 (14). Proline-utilizing derivatives of class II and III revertants included strains once again unable to utilize glucose anaerobically, making multisite mutations unlikely explanations for the pleiotropic phenotype of these revertants.

DISCUSSION

Strain RR313 and other *dgsA* derivatives of RR274 are evidently able to grow on glucose anaerobically by use of an enzyme II^{Man} system that is more active than in the wild-type, $dgsA^+$ strain: (i) RR313 gained the ability to grow anaerobically on glucosamine, a substrate of enzyme II^{Man} (Tables 2 and 4); (ii) mannose competed strongly with glucose for anaerobic transport by RR313 but not by K10 (Fig. 2); (iii) RR313 showed heightened sensitivity to 2-DOG (Table 4), a substrate of enzyme II^{Man}, and showed increased ability to transport this analog and to phosphorylate it with phosphoenolpyruvate as phosphoryl donor (Fig. 3 and 4); (iv) enzyme II^{Man} activities, low in anaerobically grown $dgsA^+$ cells, were increased dramatically in anaerobically grown cells of RR313 (Table 3); (v) introduction of a *ptsM* mutation into RR313, eliminating enzyme II^{Man} activity, completely prevented anaerobic (and aerobic) utilization of glucose (Tables 2 and 4).

It is not known what the *dgsA* gene specifies or how mutation at this locus affects enzyme II^{Man} activities. Although the *dgsA* mutation resulted in increased enzyme II^{Man} activity in anaerobically grown cells and permitted anaerobic growth on enzyme II^{Man} substrates, aerobically grown *dgsA* strains showed increased transport of 2-DOG and increased sensitivity to inhibition by it. The effect of the mutation is thus not limited to anaerobic growth. Cell levels of sugar-specific PTS components are affected by growth physiology (11, 13, 28) and might be affected nonspecifically by mutations altering growth. Although the enzyme II^{Man} system is often called the "constitutive" glucose PTS system to distinguish it from the glucose-inducible III^{Glc}/IIB′ system, levels of enzyme II^{Man} do vary with carbon source and growth rate (12, 28).

The enzyme II^{Man} complex has been resolved into a single, tightly membrane-bound phosphoryl transfer enzyme, enzyme IIB, and three less tightly bound phosphoryl carrier proteins, the "enzymes" IIA (11, 28). Individual enzymes IIA participate in the phosphorylation of different sugars, with some overlap in specificity: IIA^{Glc} is active with at least glucose, αMG, and mannose, IIA^{Fru} is active with at least fructose, and IIA^{Man} is active with at least mannose and *N*-acetylmannosamine (11, 28). It has not been reported which enzyme or enzymes IIA are active with glucosamine and 2-DOG. Enzyme IIB is probably the product of *ptsM* (6). The map locations of genes for the enzymes IIA are not known. A chromosomal location adjacent to *man* would be appropriate for at least the gene(s) coding for the enzyme(s) IIA active on

mannose; sugar-specific components of the PTS map adjacent to catabolic genes for fructose, sorbitol, mannitol, galactitol, and β-glucosides (32). However, *dgsA* strains had increased activities for all substrates of the enzyme II^{Man} complex tested (Table 3), and *dgsA* mutations, which include deletions, are evidently loss of function, more consistent with the gene for a regulatory protein than with the structural gene for an enzyme IIA.

The pleiotropic carbon source utilization phenotype of some revertants of RR274 (Table 4) may be the result of altered PTS regulation. The known involvement of the PTS system in regulation of catabolic gene expression (10, 32, 34) suggests that induction of catabolic enzymes may be affected in *dgs* strains with a pleiotropic carbon source utilization phenotype. The enzymes of proline degradation are not inducible in RR313 (Roehl and Vinopal, unpublished data). cAMP does not correct the inability of *dgs* strains to grow on proline or other restrictive carbon sources (Roehl and Vinopal, unpublished data), but neither does it correct the carbon source utilization deficiencies of some known PTS mutants (28). Heightened expression of enzyme II^{Man} in RR313 (Fig. 3 and 4) might lead to heightened PTS effects on catabolic gene expression, perhaps triggered by endogenous glucose or mannose. However, a *ptsM* mutant transductant of RR313 retained the pleiotropic phenotype (strain RR345, Table 4).

The ability of *dgsA* derivatives of K10 to grow anaerobically on galactose again raises the question of whether *dgsA* mutations affect only enzyme II^{Man} function or affect anaerobic growth in a more general way. PTS-mediated (aerobic) entry of galactose into cells without phosphorylation has been reported to occur by enzyme II^{Man} in *Salmonella* (26) and by enzyme III^{Glc}/IIB′ in *E. coli* (19). In each of these cases, PTS entry of galactose is manifested as slow growth on galactose of strains lacking the usual galactose transport systems (19, 26). K10, however, shows strong growth on galactose aerobically, and was one of the *E. coli* strains found to have vigorous galactose transport in the study reporting PTS entry of galactose in *E. coli* (19). PTS phosphorylation of galactose to galactose 6-phosphate in vitro by *E. coli* membranes has been shown (19, 26), and a mutant strain of *Salmonella* has been reported to phosphorylate galactose (presumably to galactose 6-phosphate) by the enzyme II^{Man} system (11). Although galactose catabolism via galactose 1-phosphate and the Leloir pathway has been amply demonstrated in enteric bacteria, an alternative route, via galactose 6-phosphate and the tagatose 6-phosphate pathway, as in certain gram-positive bacteria (3), seems possible; some components of a tagatose 6-phosphate pathway have been demonstrated in *E. coli* (21). However, galactose fermentation by one *dgsA* derivative of K10 is not prevented by *ptsM* (or *ptsG*) mutations (strain RR345, Table 4); hence a direct role of the PTS in anaerobic galactose utilization by this strain is unlikely. Strains carrying the $dgsA^+$ allele are not generally defective in anaerobic growth. They will grow anaerobically on glucose 6-phosphate, fructose, and mannitol, and if they have a functional enzyme III^{Glc}/IIB′ system will ferment glucose and mannose.

There is an effect, perhaps indirect, of *dgsA* on maltose catabolism, RR274 was isolated as a maltose Blu strain, identified by iodine staining as accumulating maltodextrin during aerobic growth on maltose (29). Maltose-grown RR313 is much less intensely blue staining with iodine vapor than is RR274. *dgsA* point mutant transductants of RR313 are also only faintly blue, whereas $dgsA^+$ transductants are intensely blue.

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