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Regulation of β-galactoside phosphate accumulation in *Streptococcus pyogenes* by an expulsion mechanism

(transport/exit/inducer expulsion/methyl β-D-thiogalactopyranoside phosphate)

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ABSTRACT *Streptococcus pyogenes* pregrown on lactose took up glucose, lactose, or methyl β-D-thiogalactopyranoside (MeSGal or TMG) by a phospho*enol*pyruvate-dependent phosphotransferase system. MeSGal accumulated in the cell as MeSGal-phosphate (MeSGal*P*). Three effects were noted when various sugars were added to MeSGal preloaded cells: (*i*) no decrease in intracellular MeSGal*P* concentration after addition of fructose, sucrose, *o*-nitrophenyl-β-D-galactoside, glycerol, 6-deoxyglucose, α-methyl D-glucoside, 2-deoxygalactose, glucose 1-phosphate, or glucose 6-phosphate; (*ii*) slow loss of preaccumulated MeSGal*P* evoked by lactose, 2-deoxy-D-glucose, or unlabeled MeSGal; and (*iii*) a short lag followed by extremely rapid expulsion of intracellular MeSGal*P* elicited by glucose or mannose and a slower expulsion elicited by glucosamine. The expelled compound was free MeSGal, indicating the involvement of dephosphorylation in the expulsion mechanism. Deoxyglucose inhibited the expulsion evoked by mannose, and prepoisoning of cells with fluoride or arsenate prevented the glucose-dependent expulsion. The expulsion is due to activation of an expulsion mechanism rather than to turnover of MeSGal*P* and leak of internal MeSGal with concomitant inhibition of MeSGal influx. The results suggest the need for phosphotransferase-dependent translocation of a preferential sugar or accumulation of the sugar catabolite for expulsion activation. The significance of the expulsion mechanism in synthesis regulation of enzymes involved in carbohydrate utilization is proposed.

The involvement of a phospho*enol*pyruvate (PEP)-dependent phosphotransferase system (PTS) for galactose, lactose, or methyl β-thiogalactopyranoside (MeSGal; also seen as TMG) translocation in certain streptococci has been established (1–8). In contrast, the transport of galactose or β-galactosides in *Escherichia coli* is energized by an ATP or proton motive force (9). Several early studies with *E. coli* have shown that glucose or α-methyl D-glucoside (α-MeGlc) inhibits uptake of β-galactosides despite the fact that they are taken up by a distinct system, PEP-dependent PTS (10–12). This inhibition by α-MeGlc was energy independent and occurred in energy-poisoned cells (11).

Recent studies have indicated that the PTS dominates and mediates the regulation of non-PTS sugar permeases as well as adenylate cyclase activity (for review, see ref. 13). As a result, a coordinate control mechanism was proposed (13) to explain the simultaneous inhibition of non-PTS sugar permeases and adenylate cyclase in *E. coli* by a sugar substrate of the PTS. However, this PTS-mediated repression of galactose and β-galactoside utilization in *E. coli*, by inducer exclusion and decrease in cyclic AMP levels, is probably not valid for Gram-positive bacteria which utilize these substrates by PEP-dependent PTS (1–8, 14). Also, the synthesis of inducible and catabolite enzymes in Gram-positive bacteria appears to be regulated without the apparent involvement of cyclic AMP (5, 15). A distinct mechanism controlling the uptake of nutrients and presumably depending on the accumulated levels of sugar phosphates recently has been proposed for Gram-positive bacteria (16).

The present communication describes an expulsion process of intracellular MeSGal*P* from *Streptococcus pyogenes* by metabolizable substrates of the glucose-PTS. This process may be instrumental for selecting a preferred PTS-sugar from a mixture during growth in Gram-positive bacteria.

MATERIALS AND METHODS

Organism and Media. *S. pyogenes*, type 12, was grown at 37°C without shaking in medium containing (g/liter): NaCl, 5; tryptone, 15; proteose peptone, 5; yeast extract, 2; and lactose, 5. Growth was measured by optical density at 660 nm.

Transport Studies. Cells in midlogarithmic growth were centrifuged, washed twice, and resuspended in 0.05 M Na phosphate, pH 7.0/0.01 M $MgCl_2$ (transport buffer). Uptake studies were performed at 37°C with cell suspensions (1.5–2.0 mg dry weight per ml) containing 1% peptone unless indicated otherwise. The following radioactive (specific activity, 1 μCi/μmol; 1 Ci = 3.7×10^{10} becquerels) sugars were used at the concentrations shown: [*methyl*-^{14}C]MeSGal (New England Nuclear), 0.5 mM; [D-*glucose*-1-^{14}C]lactose (Amersham), 0.1 mM; D-[*U*-^{14}C]glucose (Amersham), 0.15 mM; and 2-deoxy-D-[1-^{3}H]glucose (5 μCi/μmol; Amersham), 0.15 mM. Uptake was terminated by collecting cells (100–200 μg, dry weight) on Millipore filters (0.45 μm; 25 mm diameter). Filters were then washed twice with 3 ml of warm (37°C) 0.85% NaCl and dried before assay in a Packard Tri-Carb liquid scintillation spectrometer using a toluene scintillator. In exit studies, cells were preloaded (4–7.5 min) with [^{14}C]MeSGal and the expulsion of [^{14}C]MeSGal*P* was initiated by injection (5–10 μl) of the compounds tested into the cell suspension (0.5–1.0 ml).

PEP-Dependent PTS Assay. The assay mixture (100 μl) contained the following (in μmol): mercaptoethanol, 0.5; sodium phosphate at pH 7.3, 2.5; sodium fluoride, 1; PEP, 1.5; $MgCl_2$, 0.5; 2-deoxy-D-[^{3}H]glucose ([^{3}H]dGlc), 0.1, or [^{14}C]-MeSGal 0.75; and 50–65 μl of cells decryptified by freezing and thawing. The reaction mixture was incubated at 37°C (15–30 min) and then chilled in an ice bath. Aliquots (25 μl) were applied to DEAE-paper (DE-81, 25 mm, Whatman) discs, the discs were dried for 30 min at room temperature and washed with 50 ml of water, and the radioactivity of the retained MeSGal*P* was determined as above.

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Abbreviations: PEP, phospho*enol*pyruvate; PTS, phosphotransferase system; MeSGal, methyl β-D-thiogalactoside (TMG); α-MeGlc, α-methyl D-glucoside; dGlc, 2-deoxy-D-glucose.

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RESULTS

Evidence has been obtained that lactose-grown *S. pyogenes* takes up lactose and MeSGal via an inducible lactose-PTS. The initial rate of MeSGal uptake was inhibited (about 80%) by preincubation (10 min) with 20 mM NaF. Inhibition of lactose uptake under similar conditions was 90%. Inhibition by 0.5 mM 2,4-dinitrophenol or 10 μM carbonylcyanide *m*-chlorophenylhydrazone was slight (10–20%). The initial rates of lactose and MeSGal uptakes were slightly increased (10–20%) in the presence of 0.1 mM *N,N*-dicyclohexylcarbodiimide. Cells that had been incubated with [^{14}C]MeSGal were extracted with 5% butanol and the extracts subjected to paper chromatography (Whatman no. 2) (1) and radioautography. MeSGal accumulated in the cells as MeSGal*P* (R_F = 0.03) because after treatment with alkaline phosphatase (45 min at 37°C in 25 mM Tris, pH 8.5/2.5 mM $MgCl_2$) it regained the same R_F as MeSGal (R_F = 0.66). MeSGal phosphorylation by decryptified cells was dependent on PEP (20.5 nmol of MeSGal*P* per mg of protein, 100%); negligible phosphorylation (6.6%) was observed when ATP replaced PEP. Glucose 6-phosphate or 2-phosphoglycerate replaced PEP for MeSGal*P* formation in the absence of NaF (85.7% and 78.5%, respectively), but in its presence only slight phosphorylation (7.6% and 15.7%, respectively) was detected.

S. pyogenes pregrown on lactose has an inducible phospho-β-D-galactoside galactohydrolase which was demonstrated with decryptified cells by the hydrolysis of *o*-nitrophenyl-β-D-galactoside 6-phosphate. Hydrolysis of *o*-nitrophenyl-β-D-galactoside by these cells was negligible but did occur with intact cells. Neither hydrolysis of *o*-nitrophenylgalactoside by intact cells nor of *o*-nitrophenylgalactoside phosphate by decryptified cells was detected with pregrown cultures in glucose (unpublished data). Similarly, the uptake of MeSGal by cells pregrown on glucose or mannose was only 1–4% of that by cells grown on lactose. These results provide further support for the inducibility and role of lactose-PTS and phospho-β-D-galactoside galactohydrolase in the uptake and utilization of lactose by *S. pyogenes*. A PEP-dependent PTS is also involved in the uptake of glucose and dGlc (data not shown).

Expulsion of Preaccumulated MeSGal*P*. Glucose caused a rapid loss of radioactivity from MeSGal preloaded cells (Fig. 1*A*); after 1 min, only 5–10% of the initial β-galactoside remained in the cell. This expulsion exhibited first-order kinetics and the rate constant—$k = \ln 2/T_{1/2} = 2.77$ min^{-1}—was independent of the initial concentration of MeSGal*P* or glucose. However, the initiation of MeSGal*P* expulsion was related to glucose concentration; 20 mM glucose caused an instantaneous expulsion, but a 10-sec lag was observed with 5 mM glucose. Galactose grown cells preloaded with MeSGal also lost their labeled content rapidly when challenged with glucose. A similar displacement of radioactivity was observed when 5 mM mannose or glucosamine replaced glucose; however, the lag was longer and the expulsion rate was slower: 20 and 60 sec, and k = 1.13 and 0.38 min^{-1} for mannose and glucosamine, respectively. The exit rate constant was much lower when the MeSGal*P* expulsion was induced by lactose, dGlc, or unlabeled MeSGal: 0.063, 0.033, and 0.025 min^{-1}, respectively (Fig. 1*B*). The slow exit or rapid expulsion of MeSGal*P* evoked by dGlc or glucose, respectively, was also observed when peptone was omitted from the uptake system.

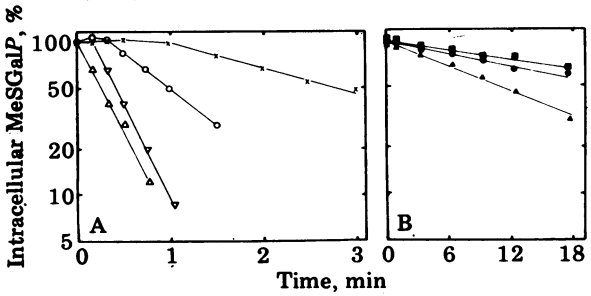

FIG. 1. Effect of various sugars on the intracellular pool of MeSGal*P*. Expulsion was initiated by rapid injection of the following sugars to suspensions of preloaded cells: (*A*) 20 mM glucose (△); 5 mM glucose (▽); 5 mM mannose (○); 5 mM glucosamine (×): (*B*) 5 mM dGlc (●); 5 mM MeSGal (■); 5 mM lactose (▲). The concentration of MeSGal*P* at 100% corresponded to 11.7, 13.1, 16.9, and 16.7 nmol/mg dry weight for cells exposed to glucose, mannose, glucosamine, and lactose, respectively, and 25.5 nmol/mg for cells exposed to dGlc or MeSGal.

No expulsion of MeSGal*P* was observed upon addition (5 mM) of 6-deoxyglucose, α-MeGlc, 2-deoxygalactose, glucose 1- or 6-phosphate, *o*-nitrophenylgalactoside, glycerol, fructose, or sucrose (data not shown).

The expulsion elicited by glucose, mannose, or glucosamine was temporary and its duration was dependent upon the concentration (1–5 mM) of carbohydrate added. (See, in Fig. 3, the onset of MeSGal reaccumulation after expulsion.) The eventual termination of expulsion and onset of MeSGal reaccumulation was presumably due to utilization and exhaustion of these carbohydrates. In contrast, the displacement of preaccumulated MeSGal by dGlc (1 mM) was apparently permanent because reaccumulation was not observed (data not shown). Uptake of MeSGal was also temporarily prevented by glucose at 1–4 mM; the duration of this inhibition was directly proportional to glucose concentration. When dGlc (2 mM) was used, this inhibition was permanent over the duration (20 min) of these experiments (data not shown). Similar inhibition as well as exhaustion of glucose at the end of the temporary inhibition period had been reported with *S. lactis* (17).

Does Glucose Evoke Expulsion by Inhibiting MeSGal Uptake or by Activating an Expulsion Mechanism? At steady-state concentrations of α-MeGlc accumulated by *E. coli*, a turnover of the intracellular hexose phosphate occurs. This process involves dephosphorylation and leakage of internal, free hexose concomitant with uptake of external substrate molecules (18, 19). If a similar turnover also applies to the MeSGal*P* pool in *S. pyogenes* then the apparent expulsion, induced by glucose, may be due to inhibition by glucose of MeSGal influx rather than to an activation of an expulsion mechanism. The results in Fig. 2 distinguish between these possibilities. No significant efflux was observed from MeSGal preloaded cells after they were collected and then resuspended and diluted in transport buffer devoid of external [^{14}C]MeSGal. Preloaded cells were also diluted in buffer containing 5 mM dGlc (inhibitor of MeSGal uptake) or MeSGal to ensure a unidirectional efflux of [^{14}C]MeSGal by eliminating recapture of escaped molecules. The efflux rate constants obtained with dGlc and MeSGal—0.027 and 0.02 min^{-1}, respectively—are similar to those obtained previously (Fig. 1*B*). When these cells were similarly exposed to glucose (Fig. 2), rapid expulsion was evident (k = 2.59 min^{-1}). Exposure of these cells to glucose 30 sec after addition of NaF did not result in rapid expulsion (data not shown). These results indicate that even if some turnover of MeSGal*P* did occur (i.e., see Fig. 2, dGlc or MeSGal), it would be at approximately 1% of the rate of the rapid expulsion evoked by glucose. Thus, the expulsion of MeSGal*P* by glucose is due directly to activation of an expulsion mechanism rather than indirectly to rapid turnover of MeSGal*P* and leak of internal MeSGal concomitant with inhibition by glucose of MeSGal influx.

Dephosphorylation of MeSGal*P* During Expulsion. Cells preloaded with [^{14}C]MeSGal and exposed to glucose contained

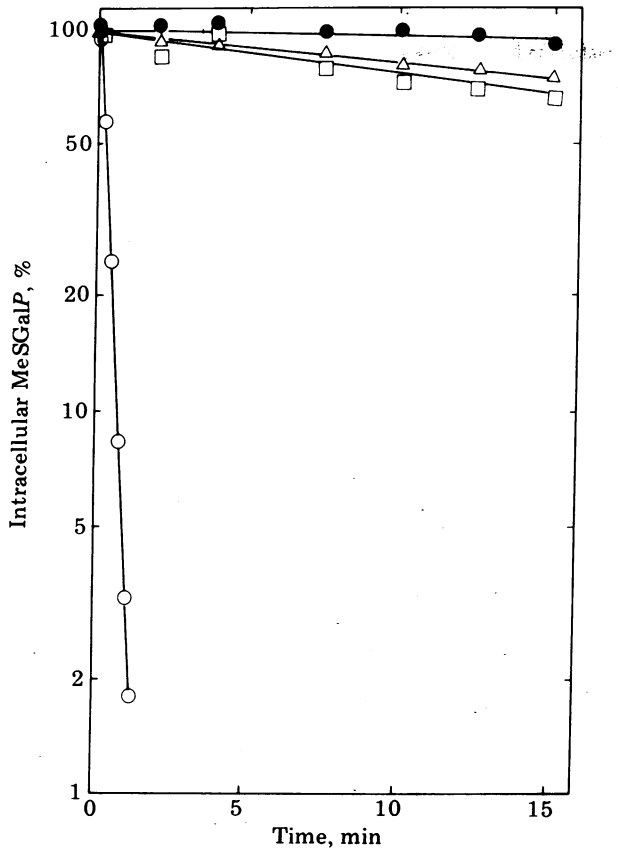

FIG. 2. Expulsion of MeSGal*P* after removal of [^{14}C]MeSGal from the medium. Cells (15 ml) were loaded with [^{14}C]MeSGal, external [^{14}C]MeSGal was removed by centrifugation, and cells were resuspended in 15 ml of transport buffer. Exit was induced by dilution (1:10) of cells in warm (37°C) transport buffer in the absence (●) or presence of 5 mM glucose (○), dGlc (□), or MeSGal (△). The concentration of MeSGal*P* at 100% varied from 17.2 to 19.7 nmol/mg dry weight.

a negligible amount of MeSGal*P* (2.3%); the bulk of the label (91.7%) was recovered extracellularly as MeSGal (Table 1). In contrast, cells not exposed to glucose contained a 24-fold higher amount (54.9%) of MeSGal*P* and the extracellular amount of MeSGal was one-seventh (12.8%). Qualitative verification of MeSGal and MeSGal*P* was obtained by chromatography and autoradiography. These results indicate that glucose does not displace MeSGal*P* but rather activates a dephosphorylation mechanism which precedes efflux of MeSGal from the cells. The small amount of MeSGal*P* in the medium may be due to an unspecific release during preparation of cell suspensions.

Table 1. *In vivo* dephosphorylation of MeSGal*P* by glucose

Material	cpm/20 μl		
	Total	MeSGal*P*	MeSGal
		With glucose	
Medium		650 (5.5%)	10,795 (91.7%)
	11,770 (100%)		
Cells		270 (2.3%)	55 (0.5%)
		Without glucose	
Medium		1,790 (17.1%)	1,335 (12.8%)
	10,465 (100%)		
Cells		5,750 (54.9%)	1,590 (15.2%)

Lactose grown cells preloaded with [^{14}C]MeSGal were centrifuged, cell pellets were resuspended in 1:10 dilution of warm transport buffer, and glucose (2 mM) was added as indicated. After 2-min incubation (37°C), cell suspensions were filtered, the extracellular filtrate was lyophilized, and the intracellular solutes were extracted with 5% butanol. Extracted cells were removed by centrifugation and the supernatant was lyophilized. Lyophilized preparations were dissolved in 0.25 ml of water and aliquots (20 μl) were assayed for MeSGal*P* retained on washed DE-81 paper discs. Free MeSGal was calculated by subtracting the MeSGal*P* radioactivity from the total counts in duplicate unwashed discs. After treatment with alkaline phosphatase, only about 0.5% of the total radioactivity could be detected on washed discs.

Effect of Fluoride and Arsenate on the Expulsion of MeSGal*P*. Glucose and dGlc uptakes by lactose pregrown *S. pyogenes* were extremely rapid, and maximum intracellular levels (about 14 nmol/mg dry weight) were reached within 15–30 sec. Because 5 mM NaF inhibits about 90% of glucose or dGlc uptake, it was possible to determine if the uptake or mere presence of extracellular glucose was obligatory for MeSGal*P* expulsion. When MeSGal preloaded cells were exposed to NaF before glucose addition, no MeSGal release was observed (Fig. 3). However, a rapid loss of MeSGal was observed when glucose was added 15–30 sec before NaF, thereby permitting cells to accumulate glucose. Thus, extracellular glucose does not elicit expulsion; instead, the uptake of glucose precedes dephosphorylation of MeSGal*P* and expulsion of free MeSGal. The possibility that NaF prevents the expulsion process by inhibition of MeSGal*P* dephosphorylation should also be considered.

Poisoning by arsenate reduces appreciably the amount of PEP and depresses the maximal extent of α-MeGlc accumulation attainable in *E. coli* and *Salmonella typhimurium* (20, 21). Treatment of *S. pyogenes* with arsenate (5 min, 37°C) also resulted in glucose uptake 4% of that in unpoisoned cells. However, *Salmonella typhimurium* preloaded with α-[^{14}C]MeGlc and poisoned with arsenate does exhibit vectorial transphosphorylation, resulting in the exit of intracellular label in the presence of extracellular α-[^{12}C]MeGlc (21). In contrast, glucose or mannose failed to evoke the characteristic rapid expulsion of MeSGal*P* in arsenate-poisoned *S. pyogenes*, and no significant difference was noted between the expulsion exerted by these hexoses and by dGlc (Fig. 4). The inability of

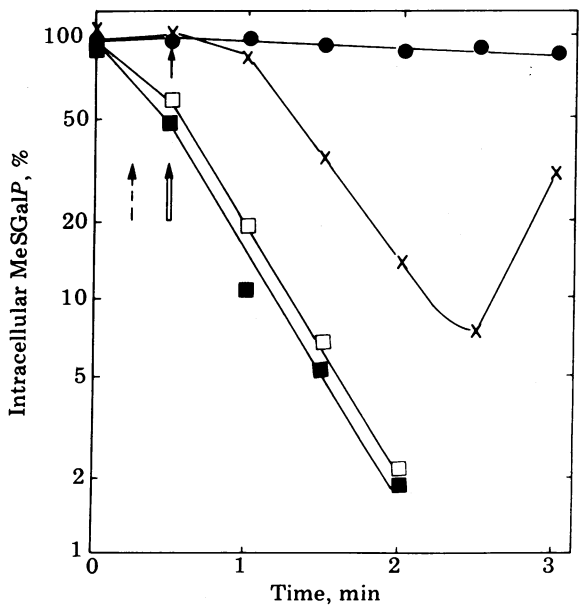

FIG. 3. Effect of NaF on the expulsion of MeSGal*P* by glucose. The following were added to preloaded cultures at the indicated times: x, control (1 mM glucose at 30 sec, ↑); ●, 15 mM NaF at zero time and 5 mM glucose at 30 sec (↑); ■, 5mM glucose at zero time and 15 mM NaF at 30 sec (open arrow); □, 5 mM glucose at zero time and 15 mM NaF at 15 sec (↑). The concentration of MeSGal*P* at 100% varied from 10.6 to 11.5 nmol/mg dry weight.

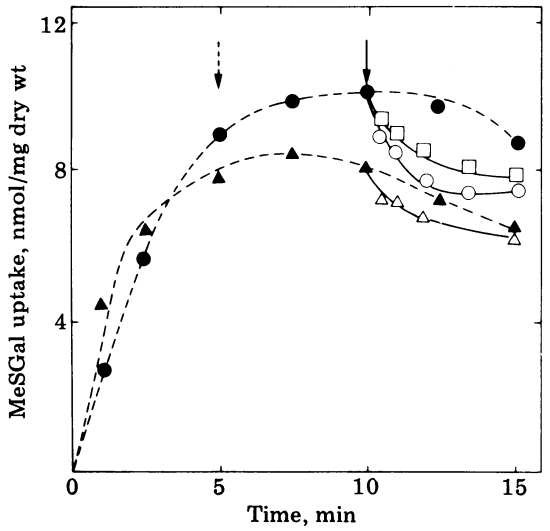

FIG. 4. Effect of arsenate on expulsion of MeSGal*P* by glucose, mannose, or dGlc. Cells in 0.05 M sodium maleate buffer, pH 7.0/0.01 M $MgCl_2$ were loaded (5 min) with MeSGal; then 5 mM arsenate was added (dashed arrow). At 10 min (solid arrow), aliquots were transferred to test tubes containing 5 mM glucose (○), dGlc (□), or mannose (△). Solid symbols and broken lines indicate control cultures treated with arsenate but without additions.

glucose to elicit expulsion of MeSGal*P* in the presence of NaF or arsenate distinguishes this expulsion mechanism from the vectorial transphosphorylation reaction (21).

Effect of dGlc on the Expulsion of MeSGal*P* by Mannose. PEP-dependent phosphorylation of dGlc by decryptified *S. pyogenes* was severely inhibited (91–93%) by 50 mM glucose, mannose, or glucosamine; only 50% inhibition of dGlc phosphorylation was obtained with α-MeGlc. These results suggest that *S. pyogenes* may have a constitutive and common PEP-dependent PTS for mannose and dGlc, presumably similar to the mannose-PTS in *E. coli* (22) and streptococci (23–25). This and the expulsion of MeSGal*P* evoked by mannose offered another approach to distinguish whether extracellular glucose or mannose or their translocation and preaccumulation were responsible for expulsion. The rapid expulsion of MeSGal*P* by extracellular mannose was prevented by the simultaneous addition of a 2-fold higher concentration of dGlc (Fig. 5) which also competitively inhibits mannose translocation (24). A similar effect was not observed when glucose replaced mannose, presumably because of an alternate translocating mechanism for glucose without dGlc (22). These findings, together with the inhibition of expulsion by NaF and arsenate, indicate that translocation and preaccumulation of the extracellular preferential carbohydrates or their high-energy metabolic products are required to initiate expulsion.

DISCUSSION

The transport of glucose (23–25), lactose, or galactose (or MeSGal) (1–8) by the corresponding PEP-dependent PTS and the preferential uptake of glucose (or dGlc) over MeSGal (17) in streptococci have been described. Our data accentuate and extend these findings. They demonstrate that glucose also evokes dephosphorylation and expulsion of MeSGal*P* preaccumulated in *S. pyogenes*. Earlier findings by Egan and Morse (26) also indicated qualitatively the displacement of preaccumulated lactose, by glucose, in *Staphylococcus aureus*.

Several lines of evidence suggest that this expulsion phenomenon is not caused by the previously characterized vectorial sugar phosphate:sugar transphosphorylation reaction (21, 27) which catalyzes an exchange group translocation.

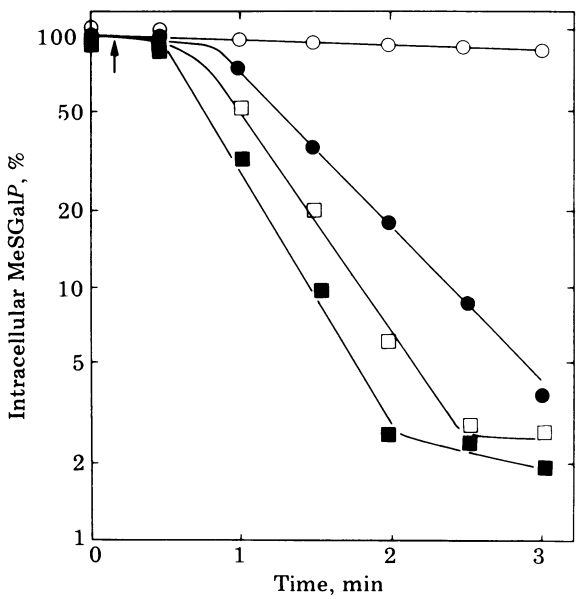

FIG. 5. Effect of dGlc on the expulsion of MeSGal*P* induced by mannose or glucose. Arrow indicates time (0.16 min) of addition of unlabeled compounds to MeSGal-preloaded cell suspensions: ■, 5 mM glucose; □, 5 mM glucose and 10 mM dGlc; ●, 5 mM mannose; ○, 5 mM mannose and 10 mM dGlc. The concentration of MeSGal*P* at 100% varied from 14.6 to 16.3 nmol/mg dry weight.

(*i*) The release of preaccumulated isopropyl-β-thiogalactoside from *Staphylococcus aureus* occurs with substrates of the lactose-PTS, and "several metabolizable carbon sources including glucose and glycerol were equally effective" (21). In contrast, the lactose-PTS substrates were much less effective than glucose, mannose, or glucosamine in promoting expulsion in *S. pyogenes*.

(*ii*) Rapid expulsion apparently depends on the ability of *S. pyogenes* to metabolize the displacing sugar. In contrast, the exchange group translocation also occurs with nonmetabolizable sugars.

(*iii*) The exchange group translocation depends on the activity of the membrane enzyme II complex and not on PEP or the soluble energy-coupling PTS proteins (21, 27). This reaction occurs in butanol- and urea-extracted membranes, in the presence of NaF or with arsenate-poisoned cells (21). In contrast, arsenate or NaF drastically inhibits expulsion in *S. pyogenes*.

(*iv*) If the expulsion of MeSGal*P* by glucose is due to exchange group translocation, then a similar phosphoryl exchange should also be expected in the reverse reaction—i.e., between glucose 6-phosphate and MeSGal as the phosphoryl donor and acceptor, respectively. However, glucose 6-phosphate failed to phosphorylate MeSGal when NaF was present during the *in vitro* phosphorylation utilizing decryptified *S. pyogenes*.

(*v*) The rate of the exchange group translocation is slower than the PEP-dependent translocation (21, 27). In contrast, the rate of MeSGal*P* expulsion by glucose was much faster than the uptake of MeSGal. Only 1.5–2.0 min was needed to displace about 95% of the MeSGal*P* previously accumulated after 7–10 min.

(*vi*) An initial lag preceded the displacement of MeSGal*P* from *S. pyogenes*. This lag is presumably due to accumulation of an effective concentration of the displacing sugar or a metabolic product thereof. A lag is not expected and was not reported in the exchange translocation reaction (21, 27).

The failure of dGlc to evoke rapid expulsion of MeSGal*P*, the inhibition by dGlc of the expulsion evoked by mannose, the lag that precedes the expulsion, and the inhibition of this expulsion

by arsenate or NaF all suggest that the expulsion process is energy dependent and requires a PTS-mediated translocation of the utilizable extracellular sugars or an accumulation of a common metabolic product thereof. The requirement for translocation of extracellular glucose is further indicated by the inability of lactose to elicit rapid expulsion although it is hydrolyzed intracellularly to glucose and galactose phosphate. The prerequisite sugar translocation further distinguishes this sequential expulsion mechanism from exchange translocation in which both substrates bind simultaneously to the enzyme II complex (28). Although our arguments suggest that this rapid expulsion process is not due to the previously characterized exchange group translocation, its involvement with it cannot be disregarded. Other studies with mutants and membrane preparations are needed to explore this possibility.

The almost instantaneous expulsion of MeSGal*P* after addition of glucose eliminates the possibility of induction and biosynthesis of an expulsion mechanism; apparently, it implies an activation of a preexisting dephosphorylation and efflux system. Dephosphorylation of MeSGal*P* by periplasmic phosphatase (29, 30) requires its prior exit across the cytoplasmic membrane. Instead, it is tempting to suggest that MeSGal efflux follows dephosphorylation of MeSGal*P* by a cytoplasmic or membranous phosphatase (31) or sugar-phosphate phosphatase (14, 32, 33). The rapid expulsion of MeSGal*P* and its low intracellular concentration at the end of the short expulsion period make it unlikely that diffusion is responsible for this expulsion. Instead, these findings may be compatible with MeSGal efflux facilitated by a carrier-mediated permease or PTS protein.

The displacement of intracellular MeSGal*P* may be regarded as inducer expulsion because of its structural homology to the lactose-PTS inducer, galactose 6-phosphate (34). The expulsion of intracellular inducer is distinct from inducer exclusion which is also exerted by a more preferable sugar (glucose) but results in prevention of uptake of extracellular inducer. The physiological significance of inducer expulsion may be similar to that of inducer exclusion—i.e., regulation of enzyme synthesis involved in the catabolism of less-advantageous sugars by modulation of intracellular inducer concentration. However, the eventual regulation of enzyme synthesis by the exclusion mechanism is indirect and relatively slow because the intracellular inducer concentration decreases slowly by dilution during growth. In contrast, the expulsion mechanism has the physiological advantage of instantaneous and direct modulation of the intracellular inducer concentration. Thus, the expulsion mechanism would be important when gratuitous or slowly metabolizable inducers accumulate intracellularly. In these cases, inducer expulsion has the advantage over the exclusion mechanism in that it permits cells to make an instantaneous effective arrest of enzyme induction and biosynthesis while choosing a more advantageous carbon and energy source. The expulsion mechanism is of special significance in cases such as lactose metabolism in which the effective inducer (galactose 6-phosphate) is evolved intracellularly after external lactose translocation and intracellular hydrolysis of lactose phosphate. Finally, this expulsion may protect the cells from the deleterious effects of accumulated hexose phosphates (24).

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