JOURNAL OF BACTERIOLOGY, Dec. 1987, p. 5589–5596
0021-9193/87/125589-08$02.00/0

Vol. 169, No. 12

Regulation of β-Galactoside Transport and Accumulation in Heterofermentative Lactic Acid Bacteria

ANTONIO H. ROMANO,[1]* GINO BRINO,[1] ALAN PETERKOFSKY,[2] AND JONATHAN REIZER[2]

Department of Molecular and Cell Biology, The University of Connecticut, Storrs, Connecticut 06268,[1] and Laboratory of Biochemical Genetics, National Heart, Lung, and Blood Institute, Bethesda, Maryland 20892[2]

Received 25 June 1987/Accepted 16 September 1987

Galactose-grown cells of the heterofermentative lactic acid bacteria *Lactobacillus brevis* and *Lactobacillus buchneri* transported methyl-β-D-thiogalactopyranoside (TMG) by an active transport mechanism and accumulated intracellular free TMG when provided with an exogenous source of energy, such as arginine. The intracellular concentration of TMG resultant under these conditions was approximately 20-fold higher than that in the medium. In contrast, the provision of energy by metabolism of glucose, gluconate, or glucosamine promoted a rapid but transient uptake of TMG followed by efflux that established a low cellular concentration of the galactoside, i.e., only two- to fourfold higher than that in the medium. Furthermore, the addition of glucose to cells preloaded with TMG in the presence of arginine elicited a rapid efflux of the intracellular galactoside. The extent of cellular TMG displacement and the duration of the transient effect of glucose on TMG transport were related to the initial concentration of glucose in the medium. Exhaustion of glucose from the medium restored uptake and accumulation of TMG, providing arginine was available for ATP generation. The nonmetabolizable sugar 2-deoxyglucose elicited efflux of TMG from preloaded cells of *L. buchneri* but not from those of *L. brevis*. Phosphorylation of this glucose analog was catalyzed by cell extracts of *L. buchneri* but not by those of *L. brevis*. Iodoacetate, at a concentration that inhibits growth and ATP production from glucose, did not prevent efflux of cellular TMG elicited by glucose. The results suggested that a phosphorylated metabolite(s) at or above the level of glyceraldehyde-3-phosphate was required to evoke displacement of intracellular TMG from the cells. Counterflow experiments suggested that glucose converted the active uptake of TMG in *L. brevis* to a facilitated diffusion mechanism that allowed equilibration of TMG between the extra- and intracellular milieux. The means by which glucose metabolites elicited this vectorial regulation is not known, but similarities to the inducer expulsion that has been described for homofermentative *Streptococcus* and *Lactobacillus* species suggested the involvement of HPr, a protein that functions as a phosphocarrier protein in the phosphotransferase system, as well as a presumptive regulator of sugar transport. Indeed, complementation assays with extracts of a *Staphylococcus aureus ptsH* mutant revealed the presence of HPr in *L. brevis*, although this lactobacillus lacked a functional phosphoenolpyruvate-dependent phosphotransferase system for glucose, 2-deoxyglucose, or TMG.

A number of bacterial mechanisms for the regulation of sugar transport and accumulation have been described previously (for reviews, see references 6, 13, 19, and 22). These mechanisms include those that are operative at the level of transcription of genetic determinants encoding specific transport systems (induction and repression), those that are operative at the level of activity of specific permeases (inducer exclusion), and those that operate by regulating the intracellular pool of a preaccumulated substrate (inducer expulsion). Both inducer exclusion and inducer expulsion have consequences at the transcriptional level, as these terms imply, since they allow mechanisms whereby a preferred carbohydrate, such as glucose, can limit the intracellular concentration of a less-preferred substrate that might otherwise induce the synthesis of permeases and enzymes for catabolic pathways that would be superfluous and, therefore, disadvantageous.

These regulatory mechanisms have been most thoroughly elucidated for organisms that transport a number of sugars by the phosphoenolpyruvate (PEP):sugar phosphotransferase system (PTS). Specific regulatory roles have been ascribed to protein components of the PTS, and studies on these control mechanisms have served to emphasize the importance of the PTS in metabolic regulation beyond its primary function in sugar transport and phosphorylation. For example, in *Escherichia coli* and *Salmonella typhimurium*, the degree of phosphorylation of III^{Glc}, which is affected by the presence or absence of glucose, regulates the activity of the lactose permease (10, 11, 13) and has been assigned a role in regulation of the intracellular level of cyclic AMP (13), which in turn is involved in the expression of a number of inducible operons (12). Thus, the PTS plays a central role in inducer exclusion, induction, and repression. Inducer expulsion in *Streptococcus* species is manifested as follows (14, 16, 17, 24). When *Streptococcus pyogenes* or *Streptococcus lactis* cells induced for lactose transport are allowed to take up the nonmetabolizable analog methyl-β-D-thiogalactopyranoside (TMG), they accumulate intracellular TMG-phosphate (TMG-P) through the operation of the PEP: lactose PTS. The addition of glucose to the preloaded cells elicits rapid depletion of intracellular TMG-P. In a two-step process, glucose uptake first triggers the activity of a phosphatase which hydrolyzes the phosphate ester bond of TMG-P, and then free TMG exits from the cell. Expulsion of the intracellularly formed TMG requires ATP and metabolites derived from glucose, and it appears to correlate with the action of a protein kinase which catalyzes ATP-dependent phosphorylation of HPr at a seryl residue (5, 14, 15, 16). The exit of TMG from the cell is presumably facilitated by enzyme II^{Lac} (20).

* Corresponding author.

Much less is known of the mechanisms regulating sugar transport and accumulation in organisms that do not transport sugars by the PTS. The present study of representative heterofermentative lactic acid bacteria was undertaken because they show important differences from streptococci and other homofermentative lactic acid bacteria in the means by which they transport and metabolize sugars; heterofermentative species metabolize sugars by the pentose phosphoketolase pathway (4) and lack a PEP:glucose PTS (21). We show here that *Lactobacillus brevis* and *Lactobacillus buchneri* also lacks a PTS for TMG transport. Furthermore, although accumulation of this galactoside depended upon provision of metabolic energy, the metabolism of glucose satisfied this requirement only transiently. Instead, glucose metabolism provoked an efflux of preaccumulated TMG from these lactobacilli in a mode that was similar in overall manifestation to the inducer expulsion in streptococci. Moreover, the presence of HPr in *L. brevis* was demonstrated, where it may play a regulatory role in the apparent absence of a function in group translocation.

MATERIALS AND METHODS

Organisms and growth conditions. The following organisms were used: *L. bacillus brevis* ATCC 367, *L. buchneri* ATCC 4005, *Lactobacillus casei* ATCC 393, *Staphylococcus aureus* S710A (*ptsI*), and *S. aureus* S797A (*ptsH*). The latter two strains are mutants lacking enzyme I and HPr, respectively (7). Cells were grown for 18 h at 30°C in a complex medium containing (grams per liter): tryptone, 15; protease peptone, 5; yeast extract, 2 (all from Difco Laboratories, Detroit, Mich.); and NaCl, 5. Sugar (galactose unless specified otherwise) was sterilized separately and added to a final concentration of 25 mM. Cells were harvested by centrifugation at 12,000 × *g* for 15 min, washed, and suspended in specified buffer solutions for uptake measurements or for cell extract preparation.

Uptake measurements. Cells were suspended in 0.05 M Na-potassium phosphate buffer (pH 7.2) containing 0.2 g of $MgSO_4 \cdot 7H_2O$ per liter at a cell density of 0.56 mg/ml (a reading of 220 Klett units at 540 nm). Various additions as specified in Results and Discussion were made to a 4.4-ml cell suspension incubated at 30°C in a shaking water bath to a final volume of 5.0 ml. [*methyl*-^{14}C]β-D-thiogalactopyranoside ([^{14}C]TMG) (DuPont New England Nuclear Products, Boston, Mass.) was added to a final concentration of 0.25 mM (0.5 mCi/mmol) at zero time. Samples of 0.5 ml were taken at appropriate intervals, filtered on 25-mm membrane filters (0.45-μm pore size; Millipore Corp., Bedford, Mass.), and washed with cold buffer. Filters with cells thereon were transferred to vials containing 10 ml of scintillation fluid (2) for determination of radioactivity.

For determination of intracellular pools of free or phosphorylated TMG, cells that were incubated with [^{14}C]TMG, sampled, filtered, and washed as above were extracted with 3 ml of boiling water for 15 min. After filtration to remove cells and debris, the filtrates (0.5 ml) were applied to columns (0.5 by 4 cm) containing AG1-X2 anion exchange resin in the formate form (analytical grade, 100/200 mesh, Bio-Rad Laboratories, Richmond, Calif.). Free TMG was eluted directly into scintillation vials containing 10 ml of scintillation fluid with six 0.5-ml portions of water; TMG-P was subsequently eluted with six 0.5-ml portions of 0.5 M ammonium formate in 0.2 M formic acid.

Preparation of cell extracts. Six liters of culture was harvested by centrifugation, washed, and suspended in 3 ml of 0.02 M Tris maleate buffer (pH 7.0) containing 1 mM dithiothreitol and 0.1 mM phenylmethylsulfonyl fluoride. The suspensions were subjected to four cycles of sonication (2 to 3 min each) at maximum power (Braunsonic 1510, B. Braun Instruments, Burlingame, Calif.) in the presence of glass powder (5-μm particle size, 30% [vol/vol]) while immersed in a dry ice-acetone bath. Extracts were centrifuged at 12,000 × *g* for 15 min to remove unbroken cells and cell debris. Protein content was determined by the method of Lowry et al. (9).

Assay of sugar phosphorylation by cell extracts. The standard assay mixture (0.1 ml final volume) contained the following: phosphate buffer (pH 7.4), 40 mM; $MgCl_2$, 5 mM; NaF, 25 mM; PEP or ATP, 10 mM; [^{14}C]TMG or 2-deoxy-D-[U-^{14}C]glucose ([^{14}C]2-DOG) (ICN Pharmaceuticals Inc., Irvine, Calif.), 1 mM (0.5 mCi/mmol); and cell extract containing 0.2 to 0.7 mg of protein. Reaction mixtures were incubated in a 37°C water bath for 30 min. Reactions were stopped by addition of 1 ml of ice-cold water. Phosphorylated sugar was determined essentially as described by Kundig and Roseman (8). The diluted reaction mixtures were transferred to columns (Bio-Rad Econocolumns; 0.8 by 4 cm) containing 1.4 ml of Bio-Rad AG1-X2 anion exchange resin (analytical grade, 100/200 mesh, chloride form). Excess ^{14}C-sugar was eluted from the columns with 15 ml of distilled water. Phosphorylated ^{14}C-sugars were subsequently eluted with 3 ml of 1 N LiCl into scintillation vials. The vials were then completely filled with scintillation fluid, and radioactivity was determined in a model 1217 Rackbeta liquid scintillation counter (LKB Instruments, Inc., Gaithersburg, Md.). Counting efficiency of ^{14}C in the presence of 3 ml of 1 N LiCl was 80%.

RESULTS AND DISCUSSION

Properties of β-galactoside transport system of *L. brevis* and *L. buchneri*. A comparison of the characteristics of a representative homofermentative (*L. casei*) versus a heterofermentative (*L. brevis*) lactic acid bacterium with regard to their respective TMG uptake systems is shown in Fig. 1. *L. casei* rapidly accumulated TMG in the absence of an added energy source. This is because starved cells of this species, as well as the streptococci, have an intracellular store of PEP, which is available to drive the uptake of TMG via the PEP:lactose PTS (3, 25). The addition of an exogenous energy source (L-arginine) had no significant effect on TMG accumulation. Moreover, as expected in the operation of a PEP-driven PTS, the proton-conducting ionophore carbonyl cyanide *m*-chlorophenylhydrazone had no inhibitory effect on TMG accumulation. In contrast, starved *L. brevis* cells did not accumulate TMG to a significant extent in the absence of an exogenous energy source (Fig. 1B). The addition of L-arginine, which generates ATP by the arginine deiminase pathway (1), led to the accumulation of TMG to a level approximately 20 times higher than that of the external medium (based on an intracellular water volume of 2 μl/mg [dry wt] of cells). TMG accumulation in *L. brevis* was abolished by carbonyl cyanide *m*-chlorophenylhydrazone, indicating that TMG accumulation in this organism was energized by a proton motive force, generated by hydrolysis of ATP derived from the metabolism of L-arginine.

Further evidence that TMG uptake in *L. brevis* takes place by active transport rather than by the PTS was provided by ion-exchange chromatography of aqueous extracts of *L. casei* and *L. brevis* cells that had been allowed to take up [^{14}C]TMG. All the radioactivity appeared in the nonanionic

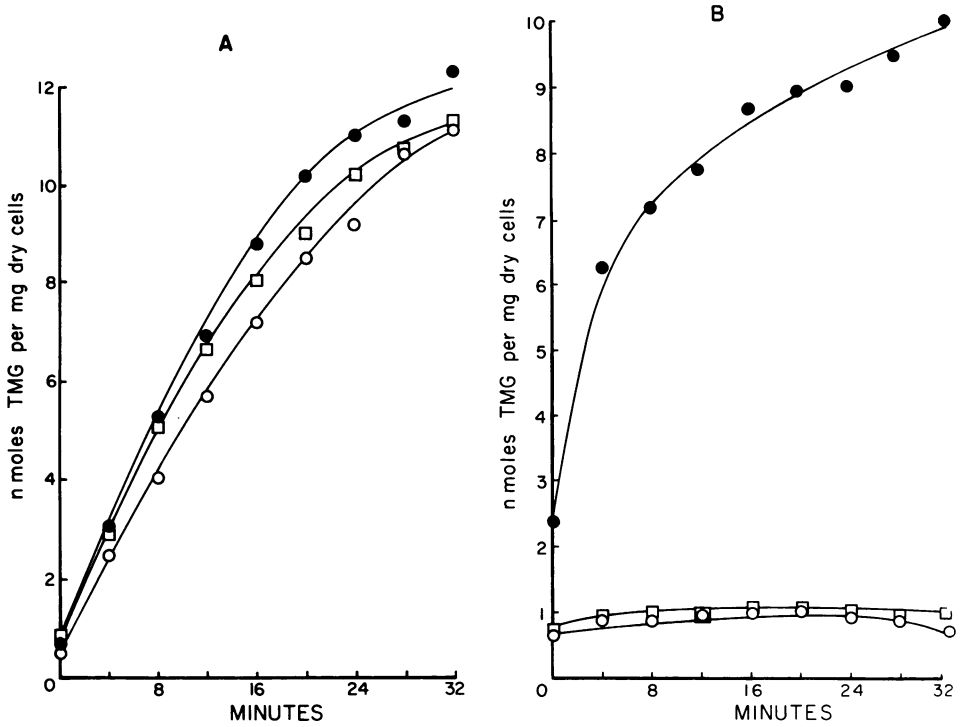

FIG. 1. Uptake of [^{14}C]TMG by *L. casei* (A) and *L. brevis* (B). Galactose-grown cells were incubated with 0.25 mM [^{14}C]TMG (0.5 mCi/mmol added at zero time) and the following additions: ○, none; ●, 20 mM L-arginine at −30 s; □, 20 mM L-arginine at −30 s and 0.05 mM carbonyl cyanide *m*-chlorophenylhydrazone at −1 min.

free sugar fraction for *L. brevis*, whereas the preponderance of radioactivity (84%) appeared in the phosphorylated sugar fraction for *L. casei*. The identity of the radioactive substance in the *L. brevis* cell extracts was confirmed by thin-layer chromatography on cellulose. A single radioactive spot which comigrated with authentic TMG was detected (results not shown).

Results (not shown) obtained with *L. buchneri*, another heterofermentative species, were the same as those described above for *L. brevis*.

The absence of PTS activity with respect to TMG and to the D-glucose analog 2-DOG was further shown in cell extracts of *L. brevis* and *L. buchneri* (Table 1). There was no PEP-dependent phosphorylation of 2-DOG or TMG by extracts of *L. brevis* or *L. buchneri*, although both these sugars were phosphorylated in the presence of PEP by the *L. casei* extract. The *L. buchneri* extract showed a high ATP-dependent phosphorylation activity with 2-DOG, a feature that is not common among procaryotes. The significance of this observation will be referred to later in this paper.

Effect of glucose on TMG accumulation. The transport systems of *L. brevis* and *L. buchneri* for both glucose and TMG are completely different from the PTS for these sugars found in the homofermentative *Streptococcus pyogenes*, *Streptococcus lactis*, and *L. casei* (Fig. 1); however, the addition of glucose to cell suspensions of *L. brevis* or *L. buchneri* that had been allowed to accumulate TMG provoked rapid efflux of TMG, a response similar in its overall manifestation to that previously shown in the homofermentative species (3, 14, 16, 17, 24). The effects of glucose on TMG uptake and accumulation in *L. brevis* and *L. casei* are compared in Fig. 2. In *L. casei* (Fig. 2A), glucose addition before commencement of TMG uptake prevented TMG accumulation; glucose addition to cells preloaded with TMG-P provoked a rapid expulsion of TMG, as previously reported for this organism and for streptococci (3, 14, 16, 17, 24). In *L. brevis* (Fig. 2B), the addition of glucose 1 min before [^{14}C]TMG addition, in the presence of L-arginine, caused no decrease in the initial rate of TMG uptake; in fact, in many experiments the initial rate was increased somewhat. However, after 4 min, there was a rapid decrease in intracellular TMG. Glucose alone (without L-arginine) also brought about an initial uptake and accumulation of TMG, followed by a rapid exit. The addition of glucose (at 16 min) to cells that had accumulated TMG to a steady-state level in

TABLE 1. PEP- or ATP-dependent phosphorylation of 2-DOG and TMG by cell extracts

Extract[a] and phosphate donor added	Sugar phosphate formed[b]	
	2-DOG-P	TMG-P
L. casei		
None	3.9	2.7
PEP	49.6	32.4
ATP	9.9	3.4
L. brevis		
None	1.8	0.9
PEP	1.9	0.9
ATP	1.3	0.9
L. buchneri		
None	1.9	0.6
PEP	1.9	0.7
ATP	76.3	0.8

[a] Protein present in assay reaction: *L. casei*, 0.62 mg; *L. brevis*, 0.53 mg; *L. buchneri*, 0.61 mg.

[b] Shown in nanomoles per milligram of protein per 30 min.

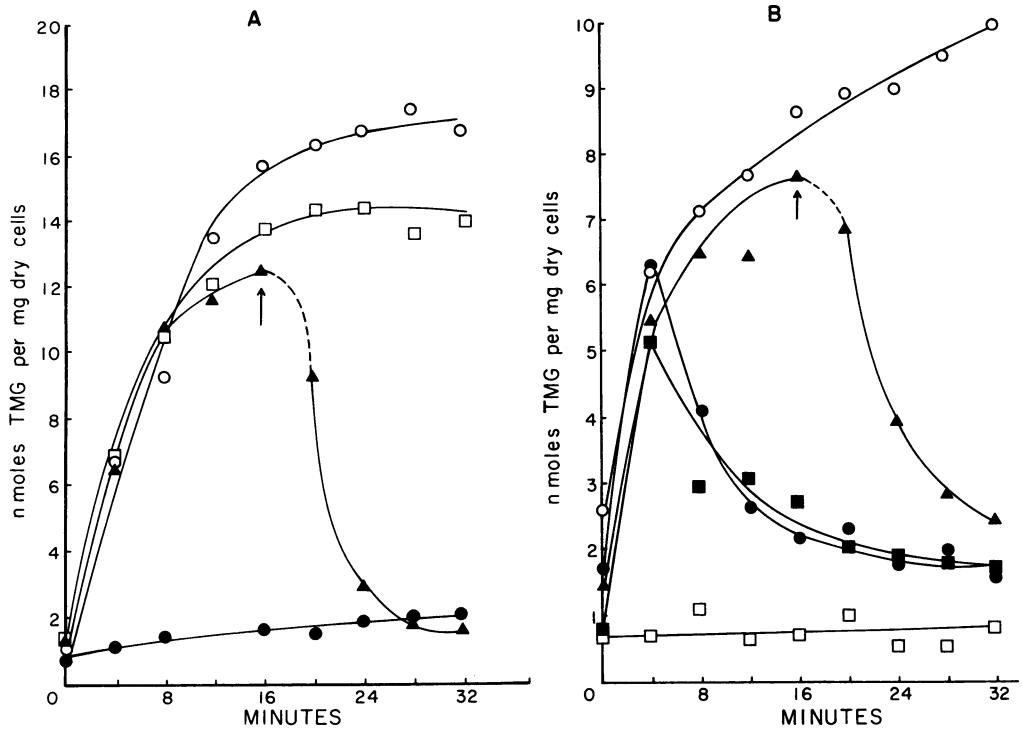

FIG. 2. Effect of glucose on [^{14}C]TMG uptake and accumulation in *L. casei* (A) and *L. brevis* (B). Galactose-grown cells were incubated with 0.25 mM [^{14}C]TMG (0.5 mCi/mmol added at zero time) and the following additions: □, none; ○, 20 mM L-arginine at −30 s; ●, 20 mM L-arginine at −30 s and 10 mM glucose at −1 min; ■, 10 mM glucose at −1 min; ▲, 10 mM L-arginine at −30 s and 10 mM glucose at 16 min (time of glucose addition indicated by arrow).

the presence of L-arginine also provoked a rapid efflux of intracellular TMG.

The fact that glucose did not decrease the initial rate of TMG uptake appears to rule out the possibility of simple competitive inhibition or exchange of intracellular TMG for glucose via a common carrier. Rather, a reasonable interpretation of these results is that the initial metabolism of glucose generates ATP that can energize active transport of TMG (as does L-arginine), but its continued metabolism results in the accumulation of metabolite(s) that can negatively affect the active uptake or maintenance of an intracellular concentration of TMG above that of the suspending medium.

Glucose was as active at 1 mM as it was at 10 mM. Lower concentrations (0.35 mM) were effective, but when added 1 min before the start of TMG uptake, there was a reversal of the glucose effect after 16 min, probably due to the depletion of glucose and its metabolites.

There was a time lag in the expulsive effect of glucose when added at 1 min before commencement of TMG uptake (Fig. 2). Similarly, when glucose at concentrations of 0.35 to 1 mM was added after 16 min of TMG accumulation, there appeared to be an initial increase in TMG uptake, followed by rapid exit (data not shown). This was confirmed by sampling at closer time intervals (Fig. 3). This delay in the manifestation of the expulsive effect elicited by glucose, whether added before commencement of TMG uptake or after achievement of a steady-state concentration of intracellular TMG, was probably due to the time required to accumulate some critical intracellular concentration of metabolite(s) derived from glucose, which triggers TMG efflux. This interpretation is supported by the observation that preincubation of cells with 10 mM glucose (a higher concentration was used to preclude exhaustion of glucose during preincubation)–20 mM L-arginine 10 min before [^{14}C]TMG addition prevented significant accumulation of TMG (Fig. 3).

Effect of other sugars. Figure 4 shows the effect of a

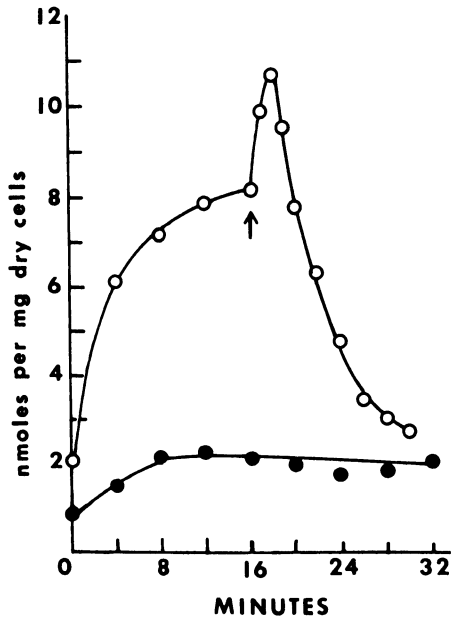

FIG. 3. Time course of glucose effect on [^{14}C]TMG accumulation. Galactose-grown cells were incubated with 20 mM L-arginine–0.25 mM [^{14}C]TMG (0.5 mCi/mmol); 1 mM glucose was added at 16 min (○), or 10 mM glucose was added at −10 min (●).

number of other hexoses on TMG accumulation. Fructose, mannose, and 2-DOG at 20 mM were without effect on galactose-grown *L. brevis*, whereas D-glucosamine and D-gluconate, which are constitutively utilized by this organism, had effects similar to that of glucose. Galactose prevented any uptake of TMG, because of its action as a competitive inhibitor. When *L. brevis* was grown on 25 mM fructose–25 mM galactose to induce systems for fructose utilization as well as TMG uptake, 20 mM fructose also caused TMG efflux, but the effect was only one-third as great as that of 1 mM glucose, with respect to both rate and extent of TMG efflux. Lower concentrations had no significant effect (results not shown).

L. buchneri behaved similarly to *L. brevis* with respect to the effect of glucose on TMG accumulation, but there was one important difference: 2-DOG was as effective as glucose in provoking TMG efflux in *L. buchneri* (results not shown). It has been shown already that the cell extract of *L. buchneri* catalyzed an ATP-dependent phosphorylation of 2-DOG, whereas the *L. brevis* extract did not (Table 1). These results suggest that 2-DOG or glucose must be phosphorylated to promote the efflux of intracellular TMG. Moreover, since 2-DOG is not metabolized beyond the 2-deoxy-D-glucose-6-phosphate stage (2-DOG is toxic to the growth of *L. buchneri*), it can be presumed that hexose phosphates are among the metabolites that are effective in triggering TMG efflux. This conclusion is supported by the lack of effect of iodoacetate on the efflux of TMG elicited by glucose (see below).

Effect of iodoacetate. The addition of glucose to *L. brevis* cells would be expected to have two effects: (i) the generation of phosphorylated metabolic intermediates and (ii) the generation of ATP and a resultant enhancement of the transmembrane proton motive force. Iodoacetate, an inhibitor of glyceraldehyde-3-phosphate dehydrogenase, should prevent the generation of ATP and enhancement of the transmembrane proton motive force by glucose but should not prevent the generation of phosphorylated intermediates at or above the level of glyceraldehyde-3-phosphate, as long as another source of ATP (L-arginine) was present to initiate metabolism. The effect of this inhibitor was determined. In control experiments it was found that 25 mM sodium iodoacetate inhibited growth of *L. brevis* and prevented glucose-energized active transport of alpha-aminoisobutyric acid, an amino acid analog whose active uptake by *L. brevis* is dependent upon a proton motive force generated by glycolytic ATP, as has been shown for *Streptococcus pyogenes* (18); glucose-energized alpha-aminoisobutyric acid accumulation is abolished by carbonyl cyanide *m*-chlorophenylhydrazone or iodoacetate in both organisms. Yet, 25 mM iodoacetate had no significant inhibitory effect on the ability of glucose to provoke the efflux of TMG from *L. brevis*, whether glucose was added before the start of TMG uptake or at the steady state of TMG accumulation (Fig. 5).

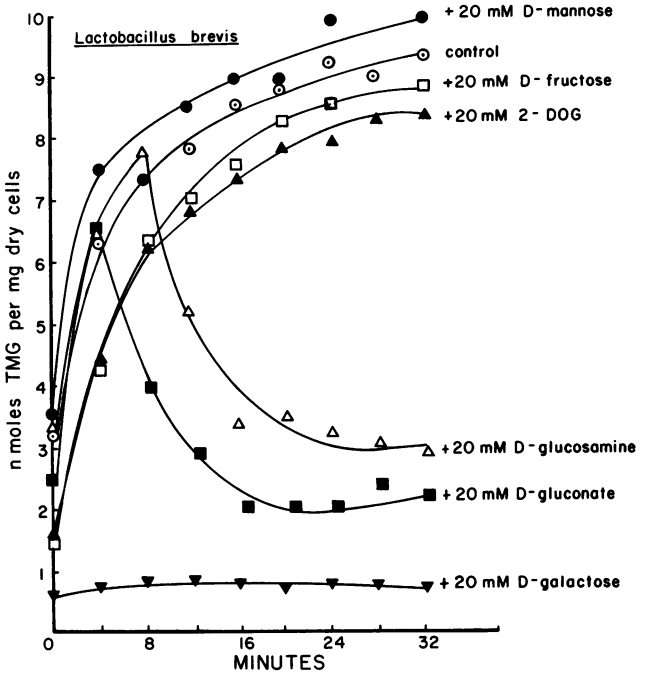

FIG. 4. Effect of various hexoses on TMG accumulation by *L. brevis*. Galactose-grown cells were incubated with 0.25 mM [^{14}C]TMG (0.5 mCi/mmol added at zero time) and 20 mM L-arginine (added at −30 s). Hexose additions were made at −1 min).

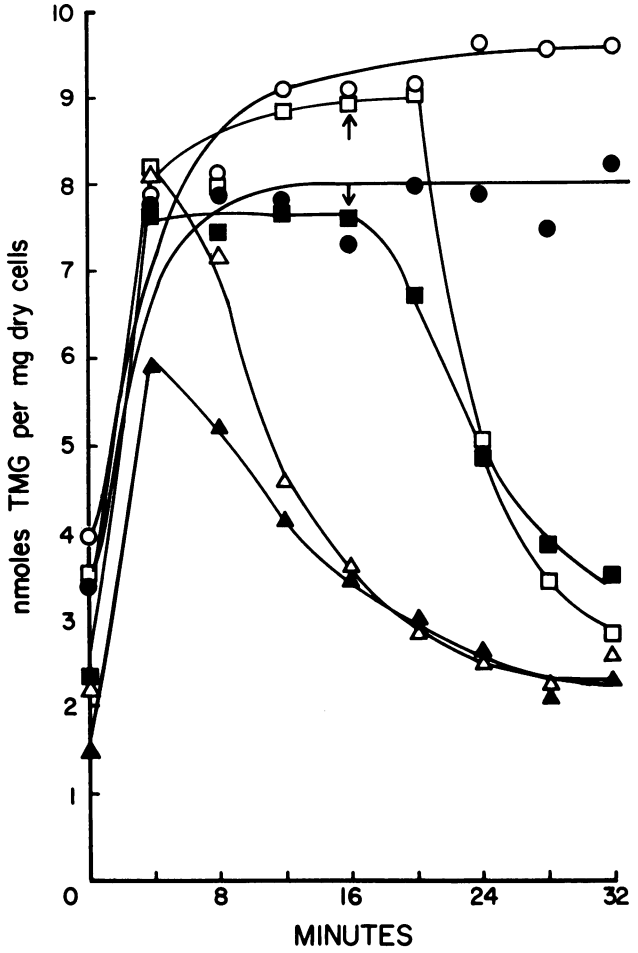

FIG. 5. Effect of iodoacetate on glucose-induced efflux of TMG in *L. brevis*. Galactose-grown cells were incubated with 20 mM L-arginine (added at −30 sec) and 0.25 mM [^{14}C]TMG (0.5 mCi/mol added at zero time). Further additions were as follows: ○, none; ●, 25 mM sodium iodoacetate; △, 10 mM glucose added at −1 min in the absence of sodium iodoacetate; ▲, 10 mM glucose added at −1 min in the presence of 25 mM sodium iodoacetate; □, 10 mM glucose added at 16 min (arrow) in the absence of sodium iodoacetate; ■, 10 mM glucose added at 16 min (arrow) in the presence of 25 mM sodium iodoacetate. All additions of iodoacetate were made 5 min before the [^{14}C]TMG addition.

Two conclusions seem justified by these results. First, glucose metabolites at or above the level of glyceraldehyde-3-phosphate can trigger the efflux of accumulated TMG from *L. brevis*. Second, glucose-induced efflux of TMG is not due to an increase of the transmembrane proton motive force above some threshold level which dissipates the concentration gradient of TMG, since glucose still exerted its effect in the presence of iodoacetate, where an increase in the proton motive force due to glucose metabolism was precluded. It is also unlikely that glucose exerted its effect by decreasing the proton motive force, since the ability of *L. brevis* to concentrate alpha-aminoisobutyric acid was increased by glucose. The possibility that the glucose effect was due to increased ΔpH as a result of acid production was also considered. However, nigericin, which collapses ΔpH by promoting transmembrane H^+-K^+ exchange, did not interfere with the glucose-elicited efflux of TMG from preloaded cells at a concentration (2 μg/ml) that inhibited the growth of *L. brevis* in the complex (low K^+) medium used in this study (results not shown). Finally, the effectiveness of 2-DOG in promoting TMG efflux in *L. buchneri* argues against the involvement of proton motive force components, since these would not be generated from this nonmetabolizable analog.

Nature of glucose effect on β-galactoside carrier system. A possibility to be considered was that metabolites derived from glucose triggered some as yet unknown series of intracellular events that resulted in the conversion of the β-galactoside active transport system to a facilitated diffusion system, so that preaccumulated TMG flowed out of the cell down its concentration gradient. Such a possibility was investigated by pretreating cells with glucose in the presence of L-arginine, then examining whether these cells showed the characteristics of facilitated diffusion, *i.e.*, nonconcentrative carrier-mediated uptake of TMG, and influx counterflow (countertransport) (23). The experiment (Fig. 6) was carried out as follows. Two pairs of *L. brevis* cell suspensions (10 mg [dry wt] per ml) were incubated in the presence of 20 mM glucose plus 20 mM L-arginine (experimental) or 20 mM L-arginine only (control) for 10 min at 30°C. Nonradioactive TMG (5 mM final concentration) was added to one tube of each pair to preload both experimental cells, pretreated with glucose, and control cells, while leaving one tube from each pair unloaded. After an additional 10-min incubation, all four cell suspensions were diluted 20-fold in the continued presence of either glucose plus L-arginine (20 mM each) or L-arginine alone, with suitable additions of [^{14}C]TMG such that the final concentration and specific radioactivity of isotopic TMG was 0.25 mM and 0.5 mCi/mmol, respectively, in all cases. Sampling at 1-min intervals was begun immediately after dilution. Control cells (treated with L-arginine alone) showed a typical concentrative uptake of [^{14}C]TMG with an intracellular concentration maintained at a level several-fold higher than that of the external medium, whether or not the cells had been preloaded with nonradioactive TMG (Fig. 6). In contrast, only a low level of [^{14}C]TMG accumulation was obtained by cells pretreated with glucose and arginine but not preloaded with nonradioactive TMG. Glucose-plus-arginine-treated cells that had been preloaded with nonradioactive TMG showed the typical characteristics of influx counterflow—an initial rapid influx and apparent transient accumulation of radioactive TMG (occasioned by the temporary saturation of the carrier in the outward direction by nonradioactive TMG), followed by a gradual decrease to the same low equilibrium level as that of glucose-treated cells that had not been preloaded with nonradioactive TMG. These results are consistent with the notion that the metabolism of glucose by *L. brevis* resulted in the conversion of the β-galactoside transport system from an active transport mechanism to a facilitated diffusion system, although the possibility that a separate facilitator was involved cannot be excluded at present. Interestingly, expulsion of intracellularly formed free TMG in *Streptococcus pyogenes* also appears to be catalyzed by a facilitated diffusion mechanism which promotes equilibration of the sugar between the intracellular and extracellular milieux (S. Sutrina, J. Reizer, and M. H. Saier, Jr., submitted for publication).

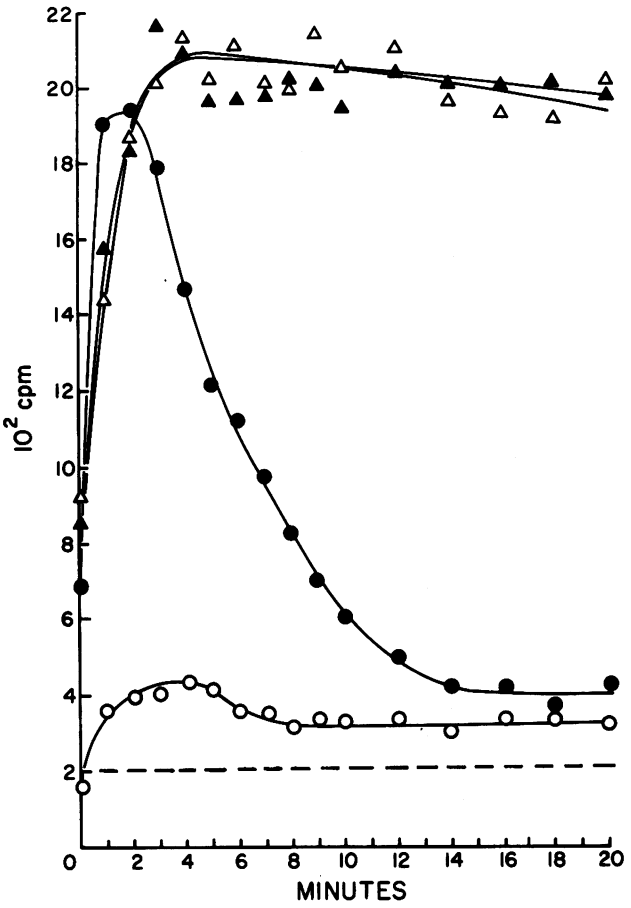

FIG. 6. Influx counterflow in *L. brevis* treated with glucose. Galactose-grown cells were treated as follows: ●, 20 mM L-arginine plus 20 mM glucose for 10 min, then preloaded with 5 mM TMG for 10 min; ○, 20 mM L-arginine plus 20 mM glucose for 10 min, not preloaded with TMG; ▲, 20 mM L-arginine for 10 min, then preloaded with 5 mM TMG for 10 min; △, 20 mM L-arginine for 10 min, not preloaded with TMG. At zero time, all cell suspensions were diluted to a final concentration of 0.25 mM [^{14}C]TMG (0.5 mCi/mmol) in the presence of 20 mM L-arginine plus 20 mM glucose (●, ○) or 20 mM L-arginine (▲, △). ----, Intracellular [^{14}C]TMG concentration equal to that of the external medium.

Details of the mechanism by which metabolites derived from glucose trigger this ultimate effect on galactoside transport and accumulation are the subject of continued investigation. Reizer et al. (14, 15, 19) have shown that for the homofermentative *Streptococcus pyogenes*, metabolites derived from glucose activate an ATP-dependent protein kinase which phosphorylates HPr at a seryl residue. This HPr kinase of *Streptococcus pyogenes* has been demonstrated both in vivo and in vitro and has been partially

TABLE 2. Complementation assays for HPr and enzyme I showing PEP-dependent phosphorylation of TMG by cell extracts

Assay and extract[a]	Phosphate donor added	[^{14}C]TMG-P (nmol/30 min)
HPr		
S. aureus ptsH	None	0.31
	PEP	0.34
	ATP	0.35
L. brevis	None	0.16
	PEP	0.19
	ATP	0.19
S. aureus ptsH + *L. brevis*	None	0.27
	PEP	12.50
	ATP	0.41
S. aureus ptsH + purified HPr[b]	None	0.25
	PEP	13.14
	ATP	1.40
Enzyme I		
S. aureus ptsI	None	0.25
	PEP	0.40
	ATP	0.19
S. aureus ptsI + *L. brevis*	None	0.23
	PEP	0.28
	ATP	0.24
S. aureus ptsI + purified enzyme I[c]	None	0.36
	PEP	3.77
	ATP	0.51

[a] Protein present in assay reaction: *S. aureus ptsH*, 0.35 mg; *S. aureus ptsI*, 0.30 mg; *L. brevis*, 0.19 mg.
[b] From *Streptococcus pyogenes* (2.5 μg).
[c] From *Streptococcus faecalis* (2.5 μg).

purified. The complete correspondence between conditions promoting its activity and conditions promoting inducer expulsion of TMG provides strong presumptive evidence that this ATP-dependent seryl phosphorylation of HPr can play a role in the regulation of sugar transport by exclusion or expulsion mechanisms (19). In view of the similarities in the overall manifestation of the glucose-induced efflux of TMG in the heterofermentative bacteria studied here in comparison with that of *Streptococcus pyogenes* (though their means of transporting both glucose and TMG are different), we considered the possibility that HPr might be present in these heterofermentative bacteria and serve a regulatory function in the absence of a transport function. Accordingly, the presence of HPr and enzyme I in cell extracts of *L. brevis* was investigated by PEP-dependent sugar phosphorylation assays in the presence of cell extracts of *S. aureus ptsH* and *ptsI* mutants that lack HPr and enzyme I, respectively (7). Results of these complementation assays with *L. brevis* extracts are shown in Table 2. It is clear that the *L. brevis* extract complemented *S. aureus ptsH*, whereas it did not complement *S. aureus ptsI*. These results confirm the absence of enzyme I in *L. brevis*, providing further evidence for the absence of a functional PTS, and demonstrate the presence of an HPr-like protein in this organism. This is the first report demonstrating the presence of HPr activity in an organism lacking the PTS and suggests that this protein may play a regulatory role without a transport function. We have made a preliminary report of an ATP-dependent HPr kinase in *L. brevis* (J. Reizer, A. Peterkofsky, and A. H. Romano, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, K-156, p. 228). Details of this continuing biochemical study will be the subject of a future publication.

ACKNOWLEDGMENT

This work was supported in part by a grant from The University of Connecticut Research Foundation.

LITERATURE CITED

1. **Abdelal, A. T.** 1979. Arginine catabolism in microorganisms. Annu. Rev. Microbiol. **33**:139–168.
2. **Bray, G. A.** 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem. **1**:279–285.
3. **Chassy, B. M., and J. Thompson.** 1983. Regulation of lactose-phosphoenolpyruvate-dependent phosphotransferase system and β-D-phosphogalactoside galactohydrolase activities in *Lactobacillus casei*. J. Bacteriol. **154**:1195–1203.
4. **DeMoss, R. D., R. C. Bard, and I. C. Gunsalus.** 1951. The mechanism of the heterolactic fermentation: a new route of ethanol formation. J. Bacteriol. **62**:499–511.
5. **Deutscher, J., and M. H. Saier, Jr.** 1983. ATP-dependent protein kinase-catalyzed phosphorylation of a seryl residue in HPr, a phosphocarrier protein of the phosphotransferase system in *Streptococcus pyogenes*. Proc. Natl. Acad. Sci. USA **80**:6790–6794.
6. **Dills, S. S., A. Apperson, M. R. Schmidt, and M. H. Saier, Jr.** 1980. Carbohydrate transport in bacteria. Microbiol. Rev. **246**:1407–1418.
7. **Hengstenberg, W., W. K. Penberthy, K. L. Hill, and M. L. Morse.** 1969. Phosphotransferase system of *Staphylococcus aureus*: its requirement for the accumulation and metabolism of galactosides. J. Bacteriol. **99**:383–388.
8. **Kundig, W., and S. Roseman.** 1971. Sugar transport. II. Characterization of constitutive membrane-bound Enzymes II of the *Escherichia coli* phosphotransferase system. J. Biol. Chem. **246**:1407–1418.
9. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
10. **Nelson, S. O., J. K. Wright, and P. Postma.** 1983. The mechanism of inducer exclusion. Direct interaction between purified III^{Glc} of the bacterial phosphoenolpyruvate: sugar phosphotransferase system and the lactose carrier of *Escherichia coli*. EMBO J. **2**:715–720.
11. **Osumi, T., and M. H. Saier, Jr.** 1982. Regulation of lactose permease activity by the phosphoenolpyruvate: sugar phosphotransferase system: evidence for direct binding of the glucose-specific Enzyme III to the lactose permease. Proc. Natl. Acad. Sci. USA **79**:1457–1461.
12. **Peterkofsky, A.** 1981. *Escherichia coli* adenylate cyclase as a sensor of sugar transport function. Adv. Cyclic Nucleotide Res. **14**:215–228.
13. **Postma, P. W., and J. W. Lengeler.** 1985. Phosphoenolpyruvate: carbohydrate phosphotransferase system of bacteria. Microbiol. Rev. **49**:232–269.
14. **Reizer, J., J. Deutscher, S. Sutrina, J. Thompson, and M. H. Saier, Jr.** 1985. Sugar accumulation in gram-positive bacteria: exclusion and expulsion mechanisms. Trends Biochem. Sci. **10**:32–35.
15. **Reizer, J., M. J. Novotny, W. Hengstenberg, and M. H. Saier, Jr.** 1984. Properties of ATP-dependent protein kinase from *Streptococcus pyogenes* that phosphorylates a seryl residue in HPr, a phosphocarrier protein of the phosphotransferase system. J. Bacteriol. **160**:333–340.
16. **Reizer, J., M. J. Novotny, C. Panos, and M. H. Saier, Jr.** 1983. Mechanism of inducer expulsion in *Streptococcus pyogenes*: a two-step process activated by ATP. J. Bacteriol. **156**:354–361.
17. **Reizer, J., and C. Panos.** 1980. Regulation of β-galactoside

phosphate accumulation in *Streptococcus pyogenes* by an expulsion mechanism. Proc. Natl. Acad. Sci. USA **77**:5497–5501.
18. **Reizer, J., and C. Panos.** 1982. Transport of α-aminoisobutyric acid by *Streptococcus pyogenes*. J. Bacteriol. **149**:211–220.
19. **Reizer, J., and A. Peterkofsky (ed.).** 1987. Regulatory mechanisms for sugar transport in gram-positive bacteria, p. 333–364. Ellis Horwood Ltd., Chichester, England.
20. **Reizer, J., and M. H. Saier, Jr.** 1983. Involvement of lactose enzyme II of the phosphotransferase system in rapid expulsion of free galactosides from *Streptococcus pyogenes*. J. Bacteriol. **156**:236–241.
21. **Romano, A. H., J. D. Trifone, and M. Brustolon.** 1979. Distribution of the phosphoenolpyruvate: glucose transport system in fermentative bacteria. J. Bacteriol. **139**:93–97.
22. **Saier, M. H., Jr.** 1985. Mechanisms and regulation of carbohydrate transport in bacteria. Academic Press, Inc., Orlando, Fla.
23. **Stein, W. D.** 1981. Concepts of mediated transport, p. 123–157. *In* S. L. Bonting and J. J. H. H. M. de Pont (ed.). Membrane transport. Elsevier/North-Holland Biomedical Press, Amsterdam.
24. **Thompson, J., and M. H. Saier, Jr.** 1981. Regulation of methyl-β-D-thiogalactopyranoside-6-phosphate accumulation in *Streptococcus lactis* by exclusion and expulsion mechanisms. J. Bacteriol. **146**:885–894.
25. **Thompson, J., and T. D. Thomas.** 1977. Phosphoenolpyruvate and 2-phosphoglycerate: endogenous energy source(s) for sugar accumulation by starved cells of *Streptococcus lactis*. J. Bacteriol. **130**:583–595.