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Biochemistry

ATP-dependent phosphorylation of serine-46 in the phosphocarrier protein HPr regulates lactose/H^+ symport in *Lactobacillus brevis*

(lactose transport/facilitated diffusion/protein kinase/protein phosphorylation/membrane vesicles)

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ABSTRACT *Lactobacillus brevis* takes up lactose and the nonmetabolizable lactose analogue thiomethyl β-galactoside (TMG) by a permease-catalyzed lactose/H^+ symport mechanism. Earlier studies have shown that TMG, previously accumulated in *L. brevis* cells, rapidly effluxes from the cells upon addition of glucose, and that glucose inhibits further uptake of TMG. We have developed a vesicular system to analyze this regulatory mechanism and have used electroporation to shock proteins and membrane-impermeant metabolites into the vesicles. Uptake of TMG was dependent on an energy source, effectively provided by intravesicular ATP or extravesicular arginine. TMG uptake into these vesicles was not inhibited, and preaccumulated TMG did not efflux from them upon addition of glucose. Intravesicular but not extravesicular wild-type phosphocarrier protein HPr of *Bacillus subtilis* restored regulation. Glucose could be replaced by intravesicular (but not extravesicular) fructose 1,6-bisphosphate, gluconate 6-phosphate, or 2-phosphoglycerate, but not by other phosphorylated metabolites, in agreement with the allosteric activating effects of these compounds on HPr(Ser) kinase measured *in vitro*. Intravesicular serine-46 → alanine mutant HPr could not promote regulation of lactose permease activity when electroporated into the vesicles with or without glucose or the various phosphorylated metabolites, but the serine-46 → aspartate mutant HPr promoted regulation, even in the absence of glucose or a metabolite. HPr(Ser-*P*) appears to convert the lactose/H^+ symporter into a sugar uniporter. These results establish that HPr serine phosphorylation by the ATP-dependent metabolite-activated HPr kinase regulates lactose permease activity in *L. brevis*. A direct allosteric mechanism is proposed.

The phospho*enol*pyruvate:sugar phosphotransferase system (PTS) catalyzes the concomitant uptake and phosphorylation of its numerous sugar substrates. This process requires the sequential phosphorylation of four proteins or protein domains—enzyme I, the phosphocarrier protein HPr, and the sugar-specific enzymes IIA and IIB—before sugar phosphorylation and transport can occur. In addition, the PTS functions in a variety of regulatory capacities (1, 2).

In early studies, it was shown that addition of a rapidly metabolizable sugar such as glucose to streptococcal cells resulted in inhibition of the uptake of other sugars (inducer exclusion) as well as rapid efflux of preaccumulated sugars or sugar metabolites (inducer efflux) (3, 4). *In vivo* experiments (5) provided evidence that the simultaneous accumulation of cytoplasmic ATP and glycolytic intermediates was required for rapid inducer efflux. Further, phosphorylation of a small protein was detected following exposure of cells to inorganic [^{32}P]phosphate under the conditions which promoted efflux of thiomethyl β-galactoside (TMG). These *in vivo* studies led to the discovery of an ATP-dependent metabolite-activated HPr kinase and an HPr(serine phosphate) [HPr(Ser-*P*)] phosphatase that reversibly phosphorylate HPr (6). This kinase was shown to phosphorylate serine-46 in HPr, and it and the HPr(Ser-*P*) phosphatase were shown to be present in partial association with the cytoplasmic membrane in a wide variety of low-G+C Gram-positive bacteria (7).

Lactobacillus brevis has been shown to possess a lactose/H^+ symport permease that exhibits the glucose-promoted phenomenon of inducer efflux (8). This organism possesses HPr and the kinase/phosphatase system that reversibly phosphorylates serine-46 in this protein, but enzyme I and the various sugar-specific enzyme II complexes of the PTS are apparently lacking (9). The function of HPr and its reversible phosphorylation in *L. brevis* might be supposed to be regulation of non-PTS carbohydrate transport, but no direct evidence for this postulate has been forthcoming.

In this paper we describe the development and use of a vesicular system that allowed us to test the postulate that HPr plays a direct role in the regulation of the *L. brevis* lactose permease. We show that both inducer exclusion and inducer efflux are dependent on intravesicular HPr and a metabolic intermediate such as fructose 1,6-bisphosphate (Fru-P_2) or gluconate 6-phosphate. The results provide compelling evidence regarding the mechanism of non-PTS transport regulation in Gram-positive bacteria.

MATERIALS AND METHODS

Organisms, Growth, and Vesicle Preparations. Growth conditions and the bacterial strain used, *L. brevis* strain ATCC367, were described previously (8). Cells were grown for 18 hr at 30°C in the presence of 25 mM galactose, harvested, washed, and used directly for transport experiments or for membrane vesicle preparation following the method described by Kaback for *Escherichia coli* (10) with the following modifications. In the spheroplast formation step, lysozyme (2 mg/ml) in 10 mM potassium EDTA (pH 7.0) was incubated with cells for 180 min at room temperature. The final membrane fractions were resuspended in 50 mM Tris/maleate buffer, pH 7.0, to a concentration of 50 mg of vesicle protein per ml and stored at −70°C. These vesicles were observed by phase-contrast microscopy to have lost the rod-shaped appearance of intact cells and exhibited a spherical morphology. They were shown to possess only 12% of the cellular β-galactosidase specific activity and about 5% of the total cellular HPr.

Uptake Measurements. Cells or vesicles were suspended in 50 mM Tris/maleate buffer (pH 7.0) at a cell density of 0.5 mg of dry weight per ml or a vesicle density of 10 mg of protein per ml. Various additions as specified in *Results* or as

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Abbreviations: PTS, phospho*enol*pyruvate:sugar phosphotransferase system; TMG, thiomethyl β-galactoside; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

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described below were made to a 100-μl cell or vesicle suspension incubated at 30°C. [^{14}C]Methyl β-thiogalactopyranoside ([^{14}C]TMG) (New England Nuclear) was added to a final concentration of 60 μM (58 mCi/mmol; 1 Ci = 37 GBq) at zero time except where otherwise noted. The energy source (arginine, 20 mM) was usually added at t = 0 min, while glucose or other potential external inhibitors were added at t = −5 min. Samples of 10–20 μl were removed at appropriate intervals, filtered on 25-mm membrane filters (0.45 μm pore size; Millipore), and washed with cold 50 mM Tris/maleate buffer (pH 7.0). Washed filters with cells or vesicles were then transferred to vials containing 5 ml of scintillation fluid for determination of radioactivity. All data reported are the means of three or four experiments.

Preparation of [^{14}C]TMG-Loaded Vesicles for Efflux Studies. The preloading medium (2 ml) contained 50 mM Tris/maleate buffer (pH 7.0), 0.20 mM [^{14}C]TMG (0.5 mCi/mmol), and 20 mg of *L. brevis* vesicle protein. After an incubation period of 10 min at 30°C, after which time the maximal accumulation of [^{14}C]TMG had been achieved, the suspension was chilled rapidly to 0°C. When appropriate, vesicles were then subjected to electroporation with designated compounds present. The vesicles were collected by centrifugation (12,000 × g, 1 min), the supernatant fluid was removed by aspiration, and excess liquid was removed with cotton buds. The [^{14}C]TMG-loaded vesicle pellet was resuspended in 1 ml of ice-cold 50 mM Tris/maleate buffer (pH 7.0) by rapid mixing with a Vortex stirrer. Of this suspension, 100 μl (equivalent to 2 mg of vesicle protein) was used for TMG efflux studies. Aliquots (20 μl) were periodically removed for determination of intravesicular TMG as indicated above.

Counterflow Studies with *L. brevis* Vesicles. Counterflow studies were conducted essentially as described previously (8) except that vesicles rather than intact cells were used. Briefly, two sets of *L. brevis* vesicle preparations (10 mg of vesicle protein per ml) were electroporated with or without HPr and incubated in the presence of 20 mM L-arginine plus nonradioactive TMG (5 mM, final concentration) for 10 min at 30°C. In the case of [S46D]HPr, the order was reversed, so that TMG preloading preceded electroporation. Glucose (20 mM) in buffer was then added to one set, while only buffer was added to the other set, and vesicles were incubated for an additional 10 min. The vesicle suspensions were diluted 20-fold with the same solutions (containing either 20 mM L-arginine plus 20 mM glucose or 20 mM L-arginine alone) except that 60 μM [^{14}C]TMG (specific radioactivity of 58 mCi/mmol) was added. Samples were taken immediately after dilution and at suitable time intervals thereafter.

Electroporation of HPr and Metabolites into Vesicles. Preliminary experiments revealed that anionic metabolites as well as HPr and its various mutant derivatives readily entered *L. brevis* vesicles during electroporation under standard conditions. *Bacillus subtilis* HPr (usually at 100 μM) or one of the mutant proteins [S46A]HPr or [S46D]HPr (also at 100 μM) (11) was added to a Gene Pulser cuvette (Bio-Rad) containing 50–100 μl of *L. brevis* vesicles. The mixture was then electroporated twice at 0°C and 700 V (resistance of 200 Ω; capacitance of 25 μF) for 1.5 msec. It was left on ice for at least 10 min but not more than 30 min before the electroporated vesicles were used for TMG uptake studies.

Enzyme Assays. The standard assay mixture for HPr(Ser) phosphorylation (50 μl final volume) contained 50 mM Tris/maleate buffer (pH 7.0), 2 mM dithiothreitol, 2 mM $MgCl_2$, 0.2 mM [γ-^{32}P]ATP (ICN; 500–2000 cpm/pmol), 100 μM HPr, and vesicles at 10–20 mg of protein per ml. Various sugars and sugar metabolites were also present as specified. The assay mixture was electroporated and incubated at 37°C for 30 min before the reaction was terminated by addition of SDS "quench buffer." Proteins were separated by SDS gel electrophoresis (11–13); the gels were stained for proteins with Coomassie blue R in 25% methanol, 10% acetic acid (vol/vol) (1 hr at 55°C); and the gels were destained with the same solvent (4 hr at 55°C) before drying under reduced pressure. Radioactivity in the dry gels was determined by autoradiography.

Chemicals were purchased from Sigma and were of the highest purity available. β-Galactosidase activity (14), PTS enzyme activities (9, 11), and protein concentrations (15) were determined by published procedures.

RESULTS

Inducer Exclusion and Efflux in Intact *L. brevis* Cells. With arginine as an energy source, TMG, a lactose analogue and substrate of the lactose/H$^+$ symporter of *L. brevis*, was rapidly accumulated against a concentration gradient in intact cells as reported previously (ref. 8; data not shown). When glucose was present during the uptake experiment, accumulation was largely blocked, and the radioactive substrate merely reached a level comparable to the equilibration level. When TMG was preaccumulated in the cytoplasm with arginine used as the energy source and glucose was subsequently added, rapid efflux was observed until the equilibra-

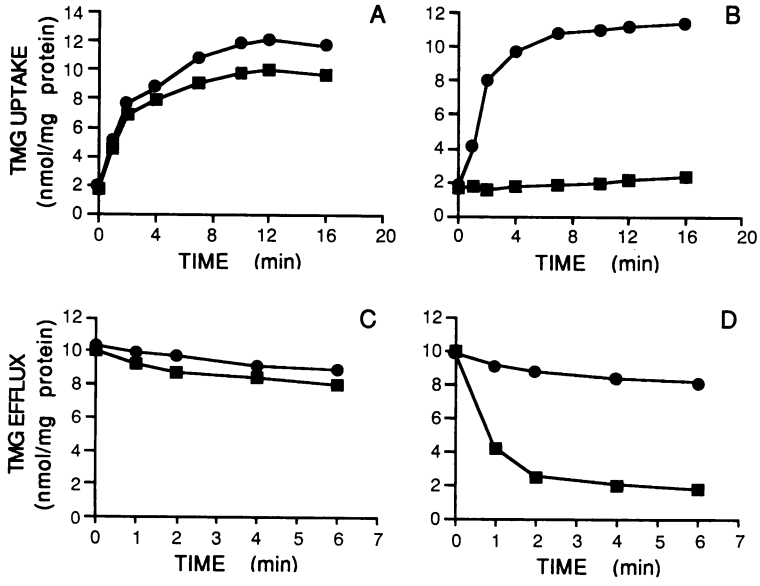

FIG. 1. Time courses for the uptake (*A* and *B*) or efflux (*C* and *D*) of [^{14}C]TMG by membrane vesicles of galactose-grown *L. brevis* cells with L-arginine as an energy source. Glucose was either present at a concentration of 20 mM (■) or absent (●). In *A* and *C*, vesicles were subjected to electroporation in the absence of HPr. In *B* and *D*, *B. subtilis* HPr (100 μM) was present during electroporation.

tion level was approached. The results are consistent with earlier conclusions (8) suggesting that glucose, metabolized in vesicles as in intact cells to gluconate 6-*P*, converts the carrier from a H^+ symporter to an energy-uncoupled uniporter (see also below).

Proline uptake, like that for TMG, appeared to be dependent on the protonmotive force (pmf). Uptake in the presence of arginine was inhibited 63% by 20 μM carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). Glucose did not inhibit proline uptake, and in fact it exerted a slight stimulatory effect (data not shown).

Regulation of TMG Accumulation in *L. brevis* Membrane Vesicles. Vesicles prepared from *L. brevis* cells as described under *Materials and Methods* exhibited different behavior (Fig. 1 *A* and *C*). Although TMG was accumulated in the presence of arginine to an extent comparable to that observed with intact cells, glucose was ineffective in regulating this activity. However, when purified wild-type HPr from *B. subtilis* was electroporated into the vesicles, regulation was restored. Glucose then inhibited uptake (Fig. 1*B*) and stimulated efflux (Fig. 1*D*).

The results of experiments designed to reveal the requirements for TMG accumulation and its regulation in *L. brevis* vesicles are summarized in Table 1. In the absence of arginine, no accumulation was observed. With arginine present, TMG was accumulated against a concentration gradient, but less than 20% inhibition was observed when glucose, Fru-P_2, or HPr was added to the extravesicular fluid. By contrast, when HPr was electroporated into the vesicles, glucose strongly inhibited uptake. When Fru-P_2 was electroporated into the vesicles together with HPr, no accumulation of TMG was observed.

Table 1. Regulation of [^{14}C]TMG uptake into *L. brevis* vesicles: Effects of intravesicular versus extravesicular substances

Vesicle treatment	Extravesicular substance*				TMG uptake, nmol/mg†
	Arg	Glc	Fru-P_2	HPr	
Electroporated alone	+	−	−	−	11.2 ± 1.1
	+	+	−	−	9.6 ± 1.2
	+	−	+	−	10.9 ± 0.9
	+	−	−	+	10.2 ± 1.4
	+	+	+	−	9.5 ± 1.8
	+	+	−	+	9.4 ± 1.6
	+	−	+	+	10.1 ± 1.2
	+	+	+	+	9.0 ± 2.1
	−	−	−	−	1.3 ± 0.2
Electroporated with HPr	+	−	−	−	11.7 ± 1.3
	+	+	−	−	2.8 ± 0.7
	+	−	+	−	11.2 ± 1.4
	+	−	−	+	11.7 ± 1.5
	+	+	+	−	2.8 ± 0.3
	+	+	−	+	3.2 ± 0.8
	+	−	+	+	10.6 ± 1.4
	+	+	+	+	2.6 ± 1.1
	−	−	−	−	1.1 ± 0.5
Electroporated with HPr and Fru-P_2	+	+	−	−	1.3 ± 0.2
	+	−	−	−	1.4 ± 0.3
	−	−	−	−	1.2 ± 0.2

*Arginine (Arg), glucose (Glc), and Fru-P_2 were all added to a final concentration of 20 mM. HPr was added to a concentration of 100 μM.

†Values (expressed in nmol/mg of protein at 15 min) of three experiments were averaged and are reported ± SD.

TMG accumulation into arginine-energized vesicles was blocked by addition of a 20 μM concentration of the protonophore CCCP. Similarly, proline uptake into arginine-energized vesicles was strongly inhibited by CCCP, but glucose did not inhibit proline uptake, regardless of whether or not HPr had been electroporated into the vesicles (data not shown). These observations suggest that the inhibitory effect of glucose on TMG uptake is exerted at the level of the lactose permease rather than on the energy source for TMG accumulation.

Metabolites That Influence Intravesicular TMG Accumulation. Table 2 provides quantitative information regarding the

Table 2. Regulation of [^{14}C]TMG uptake into *L. brevis* vesicles: Dependency on the concentrations of ATP, HPr, Fru-P_2, gluconate 6-*P*, and other metabolites

Vesicle treatment	Arg*	TMG uptake, nmol/mg†
Electroporated alone	−	1.2 ± 0.3
Electroporated with ATP (2.5 mM)	−	11.3 ± 1.2
Electroporated with HPr (100 μM) plus ATP at		
2.5 mM	−	10.6 ± 1.5
0.25 mM	−	7.4 ± 1.7
0.025 mM	−	2.9 ± 0.7
2.5 mM (+ extravesicular 20 mM glucose)	−	1.7 ± 0.1
Electroporated with Fru-P_2 (5 mM) plus HPr at		
0 μM	+	10.3 ± 1.5
10 μM	+	8.9 ± 1.1
30 μM	+	7.2 ± 1.3
100 μM	+	1.3 ± 0.2
Electroporated with HPr (100 μM) plus Fru-P_2 at		
0 mM	+	10.2 ± 1.4
0.02 mM	+	9.2 ± 2.1
0.2 mM	+	5.8 ± 1.8
2 mM	+	2.2 ± 0.5
Electroporated with HPr (100 μM) plus gluconate 6-*P* at		
0 mM	+	10.0 ± 1.7
0.02 mM	+	7.8 ± 1.5
0.2 mM	+	6.3 ± 1.2
2 mM	+	2.1 ± 0.3
Electroporated with HPr (100 μM) plus the following compound (20 mM):		
Fructose 1,6-P_2	+	1.1 ± 0.3
Gluconate 6-*P*	+	1.4 ± 0.2
Glycerate 2-*P*	+	2.3 ± 0.5
Glucose 6-*P*	+	8.7 ± 1.0
Fructose 1-*P*	+	10.5 ± 1.3
Fructose 6-*P*	+	4.3 ± 0.8
Glucose 1-*P*	+	10.3 ± 1.1
Glucose 1,6-P_2	+	9.7 ± 2.1
Phospho*enol*pyruvate	+	11.1 ± 1.4
Pyruvate	+	10.6 ± 1.2
3-*P*-glycerate	+	9.1 ± 2.1
Lactate	+	11.2 ± 1.0
Gluconate	+	10.3 ± 1.4
Ribulose 5-*P*	+	10.2 ± 1.3
Ribose 5-*P*	+	9.6 ± 1.1

*Arginine (Arg), when present, was added to a concentration of 20 mM.

†Values (expressed in nmol/mg of protein at 15 min) of three experiments were averaged and are reported ± SD.

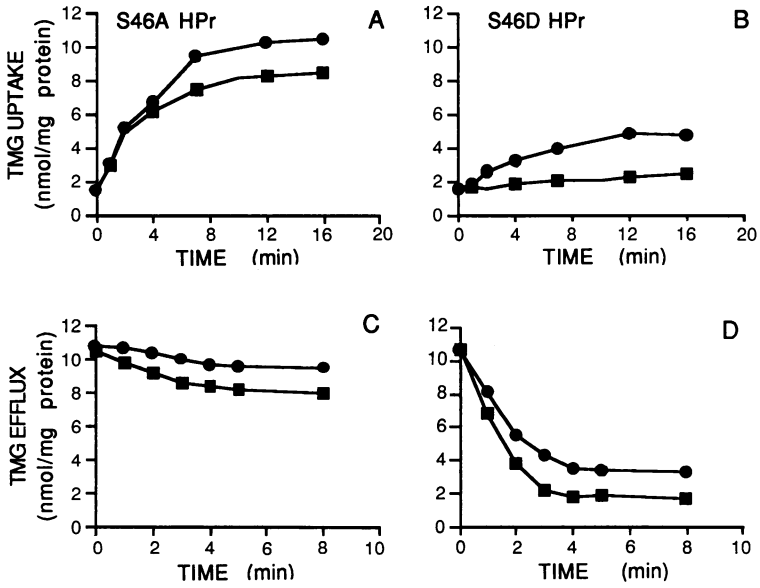

FIG. 2. Effects of S46A (*A* and *C*) and S46D (*B* and *D*) mutant HPr proteins (each at 100 μM) on TMG uptake into (*A* and *B*) or efflux from (*C* and *D*) *L. brevis* vesicles with (■) or without (●) 20 mM glucose.

metabolite and protein concentrations required to observe both uptake and inhibition of uptake of TMG into *L. brevis* vesicles. ATP (2.5 mM) could replace arginine as an energy source for the accumulation of TMG, provided that it was electroporated into the vesicles. A concentration of 250 μM ATP was sufficient to provide half-maximal stimulation of TMG accumulation. In the presence of excess ATP, glucose blocked uptake. The concentration of HPr required during electroporation for half-maximal inhibition was about 50 μM, while the concentrations for Fru-P_2 and gluconate 6-*P* were about 200 μM and 500 μM, respectively. These apparent K_i values are slightly lower than those reported for allosteric activation of the streptococcal kinase *in vitro* (13).

Dependency of Regulation on Serine-46 in HPr. Fig. 2 portrays the results of experiments in which intravesicular wild-type *B. subtilis* HPr was replaced with mutant forms of HPr in which the target serine residue of ATP-dependent kinase-catalyzed phosphorylation was replaced with a neutral alanine residue (S46A; Fig. 2 *A* and *C*) or with the negatively charged aspartate residue (S46D; Fig. 2 *B* and *D*). In the former case TMG uptake occurred to the extent observed with the wild-type HPr, but an insignificant degree of inhibition by glucose was observed (Fig. 2*A*), and glucose could not promote TMG efflux (Fig. 2*C*). By contrast, in the latter case, uptake of TMG was reduced to a low level, even when glucose was not present (Fig. 2*B*). Moreover, TMG-preloaded vesicles rapidly lost the radioactive sugar, even in the absence of glucose (Fig. 2*D*). These results are consistent with the observation that the S46D mutation renders HPr conformationally similar to serine-phosphorylated HPr, as has been revealed by multidimensional NMR studies (16). [S46D]- (but not [S46A]-) HPr also behaves like serine-phosphorylated HPr in PTS enzyme assays *in vitro* (11).

Direct Demonstration of Vesicular HPr Phosphorylation Under Conditions of TMG Uptake Inhibition. Fig. 3 shows the results of an experiment in which the phosphorylation of wild-type HPr was studied. *B. subtilis* HPr was electroporated into the vesicles together with [^{32}P]ATP. When [^{32}P]ATP was electroporated into the vesicles without HPr in the absence or presence of glucose or a glucose metabolite, very little phosphorylation was observed (Fig. 3, lane 1, and data not shown). Similarly, when both [^{32}P]ATP and HPr were electroporated into the vesicles, but glucose or a metabolite of glucose was absent, very little phosphorylation was observed (lane 6). However, when in addition to HPr and [^{32}P]ATP, Fru-P_2, 2-phosphoglycerate, or gluconate 6-*P* (each at 20 mM) was electroporated into the vesicles, or when glucose was added to the extravesicular fluid, phosphorylation of HPr was observed (lanes 2–5). When either [S46A]- or [S46D]HPr instead of wild-type HPr had been electroporated into the vesicles, the results were comparable to those shown in lane 6 (data not shown). Control experiments showed that wild-type HPr was not appreciably phosphorylated when it was only present extravesicularly, even when Fru-P_2 was present (data not shown). These results establish that intravesicular wild-type HPr is phosphorylated under the conditions which promote inhibition of TMG accumulation.

Evidence That HPr(Ser-*P*) Promotes Lactose/Lactose Counterflow. The results summarized in Fig. 4 provide evidence consistent with the conclusion of Romano *et al.* (8) that glucose in intact cells [and thus HPr(Ser-*P*) in cells and vesicles] converts the lactose/H^+ symporter into a lactose uniporter. In all experiments shown, vesicles were preloaded with nonradioactive TMG in the presence or absence of glucose and then diluted 20-fold into buffer containing [^{14}C]TMG with or without glucose. In the experiments in which values are represented by circles, HPr had been electroporated into the vesicles. In all cases [^{14}C]TMG was rapidly accumulated, but only in the presence of intravesicular HPr and exogenously added glucose was uptake followed by rapid efflux. This behavior is characteristic of counterflow and establishes that the permease is functional for TMG exchange under these conditions, even though it does not catalyze accumulation of TMG.

Kinetic Analysis of Glucose-Promoted Inhibition of TMG Uptake. The vesicle system was utilized to measure the

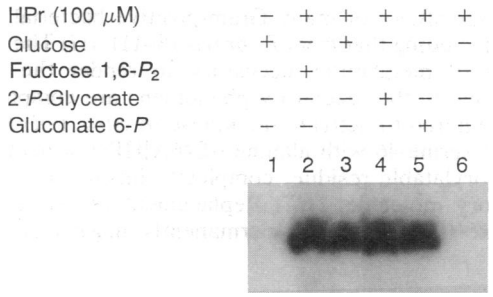

FIG. 3. Phosphorylation of intravesicular HPr by [^{32}P]ATP. Vesicles were electroporated in the presence of [^{32}P]ATP, with or without HPr and various metabolites as indicated. The concentration of HPr added prior to electroporation was 100 μM, while that of the metabolites was 20 mM.

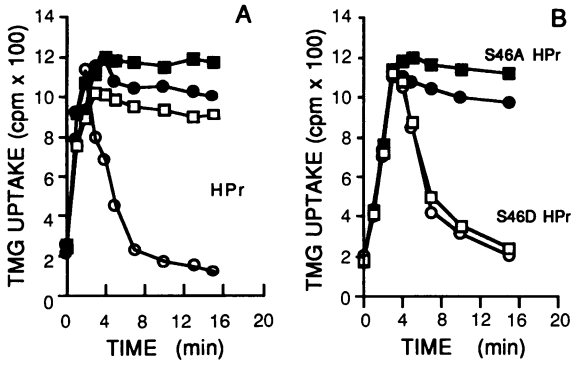

FIG. 4. TMG counterflow in the presence of arginine plus glucose, and in the presence or absence of intravesicular HPr. The experiment was conducted with vesicles preloaded with a high concentration of nonradioactive TMG in the absence (squares) or presence (circles) of 20 mM glucose. (*A*) Solid symbols, vesicles without HPr; open symbols, 100 μM wild-type HPr was electroporated into the vesicles. (*B*) Vesicles were electroporated with [S46A]HPr (closed symbols) or [S46D]HPr (open symbols) before initiation of the counterflow experiment.

kinetic parameters for TMG uptake under conditions of glucose-promoted inhibition. HPr (50 μM) was electroporated into the vesicles which were used for TMG uptake measurements with and without glucose. A double-reciprocal (Lineweaver–Burk) plot revealed that glucose decreased the maximal velocity of uptake (V_{max}) without altering the K_m (0.5 mM; data not shown).

DISCUSSION

The experiments reported in this communication provide virtually unequivocal evidence for the postulate that phosphorylation of serine-46 in HPr regulates the *L. brevis* lactose/H^+ symporter. The evidence is as follows: (*i*) Uptake of lactose is dependent on an energy source (arginine as an ATP-generating system or intravesicular ATP) but not on HPr or a metabolite of glucose (e.g., Fru-P_2 or gluconate 6-*P*). (*ii*) Vesicles that have been prepared by osmotic shock and are largely depleted of the small phosphocarrier protein of the PTS, HPr, are insensitive to inhibition of TMG uptake and stimulation of TMG efflux by glucose. (*iii*) Electroporation of HPr into the vesicles (but not extravesicular HPr) restores sensitivity of the lactose permease to glucose-promoted regulation. (*iv*) The time courses for TMG uptake and efflux, and the degrees of inhibition and stimulation by glucose, are comparable in the *in vivo* and reconstituted vesicular systems. (*v*) Glucose can be replaced by a few intravesicular (but not extravesicular) metabolites of glucose such as Fru-P_2, gluconate 6-*P*, and 2-phosphoglycerate but not by various other metabolites. This specificity corresponds to the activation specificity of many Gram-positive bacterial HPr kinases, including that from *L. brevis* (6–11). (*vi*) The concentrations of metabolites necessary to render the vesicles responsive to the regulatory phenomena are comparable to those required to activate the kinase *in vitro*. (*vii*) Replacement of serine-46 with alanine ([S46A]HPr), a neutral nonphosphorylatable residue, completely inactivates HPr as a regulatory molecule. (*viii*) Replacement of serine-46 with aspartate ([S46D]HPr), a permanently negatively charged residue resembling phosphorylated serine with regard to its charge, gives rise to an inhibited permease, in agreement with conformational and functional properties of the mutant protein as described previously (11, 16). (*ix*) Regulation appears to convert the vesicular transporter from a TMG/H^+ symporter to a TMG uniporter, capable of catalyzing rapid counterflow, in agreement with *in vivo* results (8). (*x*) Regulation depresses the maximal velocity of TMG uptake without altering the K_m when TMG uptake (rather than exchange or counterflow) is studied. (*xi*) Finally, proline uptake, which is also energized by the protonmotive force, is not regulated by a comparable mechanism.

These results, taken together, argue strongly for a direct allosteric mechanism whereby HPr(Ser-*P*) binds to the lactose/H^+ symporter of *L. brevis*. It is noteworthy that a comparable regulatory process occurs in *E. coli*, except that the inhibitory species is the free form of the glucose-specific enzyme IIA of the PTS (1, 17). In this case, the enzyme I-catalyzed phosphorylation of enzyme IIA^{glc} on histidine prevents binding to the target permeases and consequent inhibition of inducer uptake (2). Further biochemical and molecular genetic studies will be required to elucidate the mechanistic fine details of the process whereby HPr(Ser-*P*) uncouples lactose from H^+ translocation in the lactose permease of *L. brevis*.

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