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Lactose Uptake Driven by Galactose Efflux in *Streptococcus thermophilus*: Evidence for a Galactose-Lactose Antiporter†

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Galactose-nonfermenting (Gal⁻) *Streptococcus thermophilus* TS2 releases galactose into the extracellular medium when grown in medium containing excess lactose. Starved and de-energized Gal⁻ cells, however, could be loaded with galactose to levels approximately equal to the extracellular concentration (0 to 50 mM). When loaded cells were separated from the medium and resuspended in fresh broth containing 5 mM lactose, galactose efflux occurred. De-energized, galactose-loaded cells, resuspended in buffer or medium, accumulated [^{14}C]lactose at a greater rate and to significantly higher intracellular concentrations than unloaded cells. Uptake of lactose by loaded cells was inhibited more than that by unloaded cells in the presence of extracellular galactose, indicating that a galactose gradient was involved in the exchange system. When de-energized, galactose-loaded cells were resuspended in carbohydrate-free medium at pH 6.7, a proton motive force (Δp) of 86 to 90 mV was formed, whereas de-energized, nonloaded cells maintained a Δp of about 56 mV. However, uptake of lactose by loaded cells occurred when the proton motive force was abolished by the addition of an uncoupler or in the presence of a proton-translocating ATPase inhibitor. These results support the hypothesis that galactose efflux in Gal⁻ *S. thermophilus* is electrogenic and that the exchange reaction (lactose uptake and galactose efflux) probably occurs via an antiporter system.

Lactose metabolism by *Streptococcus thermophilus* occurs in a manner unlike that of most other lactic acid bacteria. This organism lacks a lactose phosphoenolpyruvate-dependent phosphotransferase system and instead accumulates and hydrolyzes lactose via an ATP-energized proton motive force (Δp)-mediated system and β-galactosidase, respectively (11, 18, 22). Another unusual feature of lactose metabolism by *S. thermophilus* is that most strains are unable to metabolize either free extracellular galactose or the galactose generated intracellularly as a result of lactose hydrolysis (9, 16, 20, 21). The galactose is subsequently released into the extracellular environment via an undefined efflux process.

Since growth on lactose and lactose uptake occur at rapid rates (11), and because galactose efflux and lactose uptake occur at equimolar rates (9, 22), the downhill efflux of galactose appears to serve as a potential driving force for energy-requiring reactions. Otto et al. (17), for example, reported that the downhill release of lactate, coupled to $n > 1$ protons, could generate a proton motive force (PMF) in glycolyzing cells of *Lactococcus lactis* subsp. *cremoris*, thus sparing the cell of ATP normally required to expel protons. Driessen et al. (4) also reported that uptake of arginine by *L. lactis* subsp. *lactis* was driven by efflux of the metabolic product, ornithine, via an electroneutral antiporter.

In this report we show that galactose efflux in *S. thermophilus* TS2 is coupled to uptake of lactose. Although efflux could generate a PMF, exchange of intracellular galactose for extracellular lactose occurred in cells in the absence of a PMF, indicating the presence of a more direct, antiporter-like system.

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MATERIALS AND METHODS

Organisms and growth conditions. *S. thermophilus* TS2 was used for all experiments. This strain is unable to ferment free galactose and excretes galactose into the extracellular environment when grown in medium containing excess lactose (21, 22). The organism was routinely propagated at 42°C in Elliker broth (6) containing 0.5% lactose.

Uptake assays. Cells were grown to mid-log phase and harvested by centrifugation (10,000 × *g*) for 15 min. Pellets were washed twice with 100 mM citrate-phosphate buffer (pH 6.8), resuspended in the same buffer to an optical density (625 nm) of approximately 0.8 to 1.0, and incubated at 42°C for 1 h to deplete cells of energy reserves. Cells were loaded with galactose by incubating cell suspensions with galactose (0 to 50 mM) for 30 min at 42°C. Unloaded cells were also incubated for 30 min at 42°C. Cells were centrifuged as before, the supernatant was discarded, and the sides of the tubes were carefully dried with cotton swabs. Loaded or unloaded cells were resuspended in buffer containing radiolabeled sugars, and the reaction mixtures were incubated at 42°C with gentle orbital shaking (50 rpm). At various times, 1.0-ml samples were removed and cells were separated from the medium by centrifugation through silicone oil as described previously (10). The radioactivity in the supernatant and in the cell pellets was determined by scintillation counting in a Beckman LS 3801 (Beckman Instruments, Fullerton, Calif.). Intracellular concentrations of accumulated sugars were calculated based on a cell volume of 2.34 μl/mg (dry weight) of cells (15). For dual label experiments, the radioactivity in the samples was determined from ^{3}H and ^{14}C quench curves. Initial uptake rates were determined from duplicate samples taken at 0.5 and 1.0 min of incubation.

In some experiments, ethanolic solutions of the protonophore carbonyl cyanide *m*-chlorophenylhydrazone and the ATPase inhibitor dicyclohexylcarbodiimide, or an equivalent amount of ethanol (as a control), were added to the reaction mixtures prior to the addition of labeled sugar.

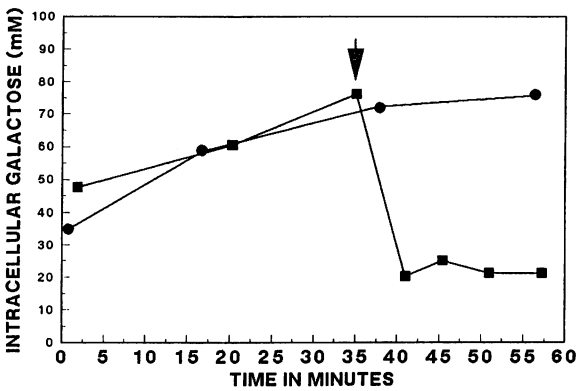

FIG. 1. Galactose uptake and efflux in Gal⁻ *S. thermophilus* TS2. Elliker broth-lactose-grown cells (mid-log phase) were washed and resuspended in 0.1 M citrate-phosphate buffer (pH 6.8) and incubated at 42°C. After 60 min of incubation, the cells were divided into two portions, 50 mM [^{14}C]galactose (0.1 μCi/μmol) was added to both tubes, and uptake was measured (● and ■). After 33 min (arrow), one tube of cells was centrifuged at 10,000 × *g* for 5 min. The supernatant was removed, and the sides of the tube were dried carefully with cotton swabs. The cell pellet was then resuspended in buffer containing 5.0 mM lactose (■), and the remaining radioactivity in the cells was determined by liquid scintillation counting.

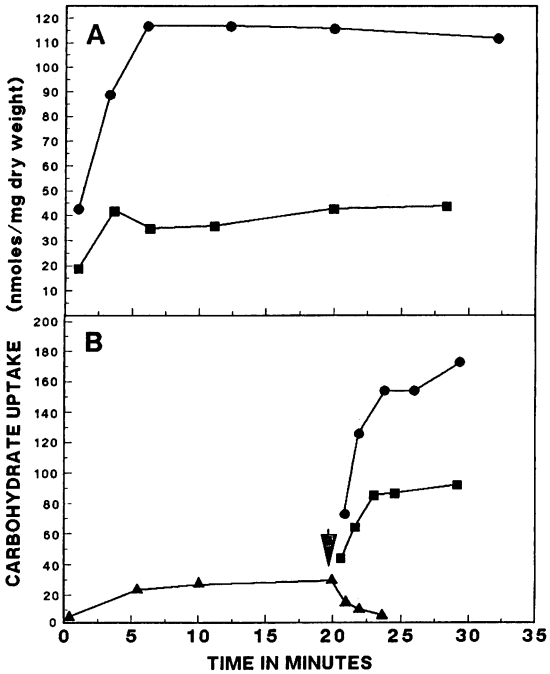

FIG. 2. Lactose uptake in galactose-loaded and unloaded cells. (A) Cells were loaded with 50 mM galactose (●), as described in the text, and resuspended in buffer containing 5.0 mM [^{14}C]lactose (0.08 μCi/μmol). A parallel portion of cells remained unloaded (■). (B) Cells were incubated with 50 mM [^{3}H]galactose (▲) for 20 min; as indicated by the arrow, the loaded cells (●) were then resuspended in buffer containing 5.0 mM [^{14}C]lactose. A parallel portion of cells remained unloaded (■).

PMF. The PMF (Δp) was determined by using isotope distribution methods, as described previously (12), with modifications. To measure both the membrane potential and ΔpH, log-phase cells were harvested and washed in fresh Elliker broth (pH 6.8) containing no added carbohydrate. Cells were resuspended in this medium (5.0 ml) and 50 mM galactose was added. After 30 min of incubation at 42°C, cells were centrifuged (10,000 × *g* for 10 min), the supernatant was removed, and the sides of the tubes were carefully dried with cotton swabs. The cells were resuspended in 5.0 ml of Elliker broth (pH 6.8, without carbohydrate) containing either 0.01 μM [^{3}H]tetraphenylphosphonium bromide (TPP; 10 mCi/μmol) or 0.03 mM [^{14}C]benzoic acid (7.3 mCi/mmol). Mixtures were incubated for 10 min, and 1.0-ml portions were centrifuged through silicone oil as before. Parallel samples were also treated with 5% butanol to correct for TPP binding. The average (n = 5) membrane potential and ΔpH values were calculated as described previously (12).

Materials. [^{3}H]TPP and [^{14}C]benzoic acid were obtained from New England Nuclear Corp., Boston, Mass. [^{14}C]lactose, [^{14}C]glucose, and [^{3}H]galactose were obtained from Amersham Corp., Arlington Heights, Ill., and [^{14}C]galactose was obtained from Sigma Chemical Co., St. Louis, Mo. All other chemicals were obtained from Sigma.

TABLE 1. Effect of extracellular galactose on lactose uptake rates in galactose-loaded and unloaded cells

Addition	Uptake rate (% of control)[a]	
	Unloaded cells	Loaded cells
None	100	100
5.0 mM galactose	96	67
50.0 mM galactose	56	7

[a] Initial uptake rates were 40 and 66 nmol of lactose per min per mg (dry weight) of cells for unloaded and loaded cells, respectively.

RESULTS

Galactose loading and efflux. Although *S. thermophilus* TS2 is phenotypically galactose negative (Gal⁻), it was shown previously that when this strain was energized with a fermentable carbohydrate it had the capacity to actively transport galactose (8). However, when starved cells were incubated in buffer or medium containing high concentrations of galactose, galactose could be accumulated to an intracellular concentration approximately equal to that in the medium (Fig. 1). When the galactose-loaded cells were separated from the galactose-containing medium and resuspended in medium containing 5.0 mM lactose, efflux of intracellular galactose occurred. Since this organism possesses little galactokinase activity and the intracellular galactose remains essentially free and unphosphorylated (8), greater depletion of galactose was expected. However, about 25 to 30% of the galactose remained associated with the cells.

Lactose uptake in galactose-loaded cells. Cells loaded with 50 mM galactose accumulated lactose more rapidly and to higher internal concentrations than nonloaded cells (Fig. 2A). When cells were loaded with 0.5 or 5.0 mM galactose, lactose uptake rates were reduced (data not shown). More direct evidence that galactose-lactose exchange was occurring was provided by dual label experiments in which cells were loaded with 50 mM [^{3}H]galactose prior to [^{14}C]lactose addition (Fig. 2B). Uptake of [^{14}C]glucose, which has low affinity for the galactose carrier (8), was the same in both galactose-loaded cells and unloaded cells (data not shown),

TABLE 2. Formation of a PMF (Δp) during galactose efflux

Trial	Loaded cells			Unloaded cells		
	Δψ (mV)	59 ΔpH (mV)	Δp (mV)	Δψ (mV)	59 ΔpH (mV)	Δp (mV)
1	70.4	19.5	89.9	50.4	11.6	62.0
2	68.1	17.7	85.8	35.5	4.0	39.4
3	70.2	14.7	84.9	56.6	11.5	68.1

indicating that galactose efflux could not drive glucose uptake.

Uptake of lactose by galactose-loaded cells was inhibited in the presence of extracellular galactose (Table 1). However, the effect was due only in part to competitive inhibition by galactose for lactose binding, since greater inhibition of lactose uptake occurred in loaded cells as compared with unloaded cells. Therefore, inhibition must also have been caused by inhibition of galactose efflux in the galactose-loaded cells.

PMF in galactose-effluxing cells. When galactose-loaded cells were resuspended in galactose-free Elliker medium, a PMF about 50% greater than in unloaded cells was formed (Table 2). It was interesting to note that the Δp values for de-energized cells of *S. thermophilus* TS2 were considerably lower (particularly in trial 2) than those observed for resting cells of lactococci (12). Both the membrane potential (Δψ) and ΔpH were greater in the loaded cells. However, the uptake of lactose by galactose-loaded cells was not affected by the addition of the protonophore carbonyl cyanide *m*-chlorophenylhydrazone, the proton-translocating ATPase inhibitor dicyclohexylcarbodiimide, or the metabolic poison NaF (Table 3), indicating that exchange activity was independent of the Δp.

DISCUSSION

There have been several recent descriptions of novel antiporter systems occurring in anaerobic bacteria. These include the arginine-ornithine system in *Lactococcus lactis* subsp. *lactis* (4), the oxalate-formate system in *Oxalobacter formigenes* (1), the phosphate-hexose 6-phosphate system in *L. lactis* subsp. *lactis* (14), and the agmatine-putrescine system in *Enterococcus faecalis* (5). Maloney (13) has termed these systems precursor-product antiporters since the uptake of a substrate or precursor is driven by efflux of one of the metabolic end products. As Maloney has suggested (13), these novel antiporters may be physiologically important for energy conservation in lactic acid bacteria. Electrogenic exchange of lactate for malate, for example, may result in energy production by *Leuconostoc* sp. and other lactic acid bacteria (3). In contrast, the electroneutral exchange of ornithine for arginine provides cells with an inexpensive route for transporting an energy-yielding nutrient (4).

TABLE 3. Effect of uncouplers and metabolic poisons on lactose uptake in galactose-loaded and unloaded cells

Treatment[a]	Rate of uptake (nmol of lactose/min/mg [dry wt] of cells)	% of control
Loaded	65.5	100
Unloaded	36.0	54
Loaded + CCCP (25 μM)	66.6	101
Loaded + DCCD (25 μM)	72.8	111
Loaded + NaF (2.5 mM)	61.7	94

[a] CCCP, Carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, dicyclohexylcarbodiimide.

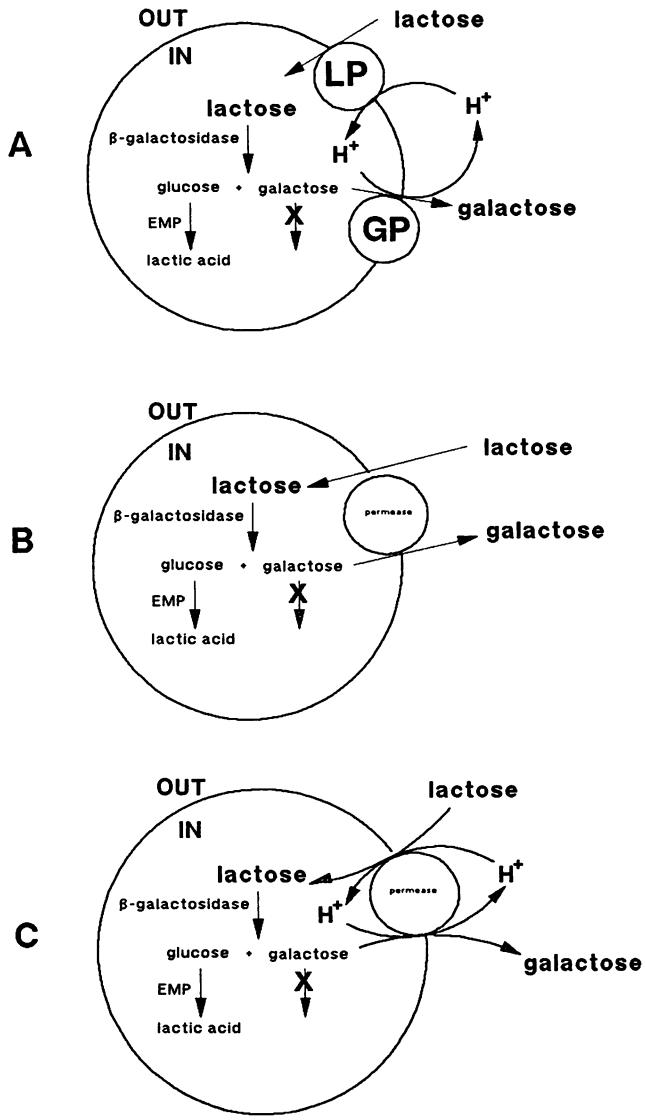

FIG. 3. Proposed models for galactose-driven lactose uptake in Gal⁻ *S. thermophilus*.

S. thermophilus achieves greater growth yields on lactose compared with glucose, even though only the glucose moiety of lactose is metabolized (data not shown). This suggested the possibility that galactose efflux may provide cells with an alternative source of energy or may conserve energy normally required to perform active transport. The results from this investigation support the hypothesis that galactose efflux drives uphill transport of lactose.

Several models can be envisaged to account for the counterflow-like galactose-lactose exchange system described in this paper (Fig. 3). In one of these possible models (Fig. 3A), galactose efflux and lactose uptake are indirectly coupled via a proton circuit, in a system analogous to those previously described in *Escherichia coli* (2, 7). In this scenario, a galactose efflux-generated PMF would be used to

drive a separate PMF-mediated uptake system for lactose. Lactose uptake has been suggested previously to occur via proton symport and a lactose permease (18). That galactose-fermenting mutants of *S. thermophilus* could be derived from wild-type Gal$^-$ strains (21), and that the former transported galactose at a significantly greater rate than the Gal$^-$ parental strains, would support the presence of distinct galactose and lactose permeases. Although genetic evidence for the presence of a galactose permease has not yet been reported, genes coding for other Leloir pathway enzymes have been identified (19).

Alternatively, a lactose-galactose antiporter may serve directly as the means of heterologous exchange (Fig. 3B). In this system, downhill galactose efflux would drive uptake of lactose. This model would imply that galactose and lactose uptake systems occur via a common carrier, as suggested by Poolman et al. (18). The relatively high affinity of galactose for the lactose carrier (8, 11, 18) would support this model. That lactose uptake in loaded cells occurred even when the PMF was dissipated by the addition of protonophores provides additional evidence that a PMF is not essential for exchange to occur. Also, galactose efflux did not increase the glucose uptake rate, although uptake of glucose may be mediated not by a PMF, but rather by a glucose phosphotransferase system (18). It appears, then, that uptake of lactose and efflux of galactose in *S. thermophilus* may be mediated by an antiporter-exchange type of system.

A third model (Fig. 3C), therefore, may account for the results observed in these experiments. Galactose-lactose exchange may occur via a common galactoside antiporter, but both sugar uptake and efflux may occur in symport with a proton. This would explain why a membrane potential is generated by galactose efflux and also why electroneutral exchange could occur in the absence of a proton gradient. Although the apparent stoichiometry of lactose-galactose exchange was considerably greater than 1:1 (Fig. 2B), intracellular hydrolysis of lactose and subsequent release of galactose would have provided an additional driving force for lactose uptake.

Finally, in light of efforts in progress to develop stable galactose-fermenting strains of *S. thermophilus*, it should be recognized that there may be metabolic or bioenergetic consequences of such a shift in metabolism. Most lactose-grown, galactose-fermenting strains initially excrete galactose into the medium, in part because galactokinase activity remains low. However, another explanation for this observation may be that the galactose efflux system in *S. thermophilus* competes successfully against the enzyme (i.e., galactokinase) necessary for galactose catabolism.

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