Regulation of the lactose phosphotransferase system of *Streptococcus bovis* by glucose: independence of inducer exclusion and expulsion mechanisms

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*Streptococcus bovis* had a diauxic pattern of glucose and lactose utilization, and both of these sugars were transported by the sugar phosphotransferase system (PTS). Lactose catabolism was inducible, and *S. bovis* used the tagatose pathway to ferment lactose. Since a mutant that was deficient in glucose PTS activity transported lactose as fast as the wild-type, it appeared that *S. bovis* has separate enzyme IIs for glucose and lactose. The nonmetabolizable glucose analogue 2-deoxyglucose (2-DG) was a noncompetitive inhibitor of methyl β-o-thiogalactopyranoside (TMG) transport, and cells that were provided with either glucose or 2-DG were unable to transport TMG or lactose. Because the glucose-PTS-deficient mutant could ferment glucose, but could not exclude TMG, it appeared that enzyme IIc rather than glucose catabolism per se was the critical feature of inducer exclusion. Cells that had accumulated TMG as TMG 6-phosphate expelled free TMG when glucose was added, but 2-DG was unable to cause TMG expulsion. The glucose-PTS-deficient mutant could still expel TMG in the presence of exogenous glucose. Membrane vesicles also exhibited glucose-dependent TMG exclusion and TMG expulsion. Membrane vesicles that were electroporated with phosphoenolpyruvate (PEP) and HPr retained TMG for more than 3 min, but vesicles that were electroporated with PEP plus HPr and fructose 1,6-diphosphate (FDP) (or glyceralate 2-phosphate) lost their ability to retain TMG. Because FDP was able to trigger the ATP-dependent phosphorylation of HPr, it appeared that inducer expulsion was mediated by an FDP-activated protein kinase. This conclusion was further supported by the observation that mutant forms of HPr differed in their ability to facilitate inducer expulsion. S46DHPr, a mutant HPr with aspartate substituted for serine at position 46, promoted TMG expulsion from membrane vesicles in the absence of FDP better than wild-type HPr or S46AHPr, a mutant form with alanine substituted for serine at position 46. Based on these results, it appeared that glucose catabolism was needed for inducer expulsion, but not inducer exclusion.

**Keywords:** phosphotransferase system, lactose transport, *Streptococcus bovis*, rumen, inducer exclusion and expulsion

**INTRODUCTION**

*Streptococcus bovis* is an opportunistic bacterium that outgrows other ruminal bacteria when there is an abundance of fermentable carbohydrate (Hungate et al.,
The overgrowth of S. bovis results in an accumulation of lactic acid in the rumen, a decrease in ruminal pH, acute indigestion, and even death of the animal (Slyter, 1976). S. bovis can be a significant inhabitant of the human colon (Darjee & Gibb, 1994). Increased faecal counts of S. bovis have been correlated with colon cancer (McMahon et al., 1991), and S. bovis-mediated septicaemia has been used as an index of gut wall deterioration (Reynolds et al., 1983).

The overgrowth of S. bovis in the rumen can be explained by its capacity to ferment starch (Slyter, 1976; Russell & Robinson, 1984), but the proliferation of S. bovis in the human colon has yet to be explained. It should be noted that all S. bovis strains grow rapidly on lactose (Hardie, 1986), and both colon cancer and lactose intolerance are late-onset human diseases (Reynolds et al., 1983). Gilbert & Hall (1987) reported that S. bovis strain HA3 had β-galactosidase activity and a lac operon similar to those of Escherichia coli, but many lactic acid bacteria use the phosphoenolpyruvate-phosphotransferase system (PEP-PTS) to transport and phosphorylate lactose (Reizer, 1989; Postma et al., 1993).

The lactose PTs of low-G+C Gram-positive bacteria are regulated by glucose-mediated inducer exclusion and inducer expulsion (Reizer & Panos, 1980; Thompson & Saier, 1981; Reizer et al., 1983; Reizer & Peterkošky, 1987; Reizer, 1989). In Streptococcus pyogenes (Reizer & Panos, 1980; Reizer et al., 1983) and Lactococcus lactis (Thompson & Saier, 1981), glucose prevents the uptake of the nonmetabolizable lactose analogue methyl β-D-thiogalactopyranoside (TMG), and the addition of glucose causes TMG expulsion. Inducer expulsion in L. lactis has been shown to be caused by a glucose-dependent increase in fructose 1,6-diphosphate (FDP) and a subsequent cascade of phosphorylation and dephosphorylation (Ye et al., 1994). When intracellular FDP concentrations increase, an ATP-dependent protein kinase is allosterically activated, and this protein kinase then phosphorylates HPr, a non-sugar-specific component of the PTS. The phosphorylation of HPr at serine 46 is thought to activate a sugar phosphate phosphatase that dephosphorylates TMG 6-phosphate (Ye et al., 1994). The dephosphorylation of TMG 6-phosphate then facilitates TMG expulsion.

Preliminary experiments indicated that at least 20 strains of S. bovis have a PTS for lactose transport (D. B. Kearns, G. M. Cook & J. B. Russell, unpublished results). The experiments described here were designed to delineate mechanisms of lactose utilization and transport in S. bovis. Because S. bovis has two systems of glucose transport, a PTS (Martin & Russell, 1987) and a facilitated diffusion mechanism (Russell, 1990; Cook & Russell, 1994), we felt that this bacterium might provide additional information on the mechanisms of glucose-dependent inducer exclusion and expulsion.

METHODS

Materials. All chemicals were analytical reagent grade. [14C]Methyl β-D-thiogalactopyranoside ([14C]TMG) was obtained from Amersham.

Cell growth. Streptococcus bovis JB1 was grown anaerobically at 39 °C in basal medium containing (per litre): 292 mg K2HPO4, 292 mg KH2PO4, 480 mg (NH4)2SO4, 480 mg NaCl, 100 mg MgSO4.7H2O, 64 mg CaCl2.2H2O, 600 mg cysteine hydrochloride, 1 g Trypticase (BBL Microbiology Systems) and 0.5 g yeast extract. Carbon sources and glucose analogues were all prepared anoxically and added from separately sterilized stock solutions to the desired final concentration. A glucose-PTS-deficient mutant (JB1D200) was isolated as previously described (Cook & Russell, 1994). The medium was adjusted to pH 6.7 and the final pH was never less than 6.5. The incubation temperature was 39 °C. Growth was monitored by the increase in turbidity (1 cm cuvette, 600 nm). The relationship between optical density and cell protein was 160 mg protein 1-1 per OD600 unit.

Toluene-treated cells. Cells (10 ml) were harvested during exponential growth (OD600 approximately 1.0) by centrifugation (10000 g, 10 min, 4 °C) and washed twice in 50 mM Tris/HCl (pH 7.0) buffer containing 5 mM MgCl2 and 2 mM dithiothreitol. Cells were then resuspended in 2 ml of the same buffer and stored on ice. The cell suspension (500 μl) was treated with 15 μl tolune/ethanol (1:9, v/v) as previously described (Kornberg & Reeves, 1972; Martin & Russell, 1986).

PTS and glucokinase activity. PTS and glucokinase activities were determined in toluene-treated cells at 39 °C as previously described (Martin & Russell, 1986). Specific activities were determined under first-order conditions (activity versus protein concentration was linear). All assays were performed in duplicate with appropriate controls. The replicates differed by less than 5%. Where available, commercial enzymes were used as a positive control.

β-Galactosidase and β-D-phosphogalactoside galactohydrolase (P-β-galactosidase) assays. To test for the presence of β-galactosidase (EC 3.2.1.23) in growing cells, cultures samples (1.0 ml) were removed, toluene-treated (as above), and assayed for the release of o-nitrophenol from o-nitrophenyl β-D-galactoside (ONPG) in an assay mix (500 μl) containing 50 mM Tris/HCl (pH 7.0) buffer, 5 mM MgCl2 and 2 mM dithiothreitol. Incubation was at 39 °C for 5 min and the reaction was stopped with 500 μl Na2CO3. After 10 min, the assay tubes were centrifuged at 8000 g for 5 min at room temperature, and the A420 of the supernatant was read. One A420 unit was equivalent to 0.52 μmol o-nitrophenol and was proportional to the quantity of cells in the assay mixture. The assay for P-β-galactosidase measured the release of o-nitrophenol from ONPG 6-phosphate under the same conditions.

Preparation of membrane vesicles. Membrane vesicles of S. bovis were prepared as previously described (Russell et al., 1988). These vesicles possessed only 22% of the cellular HPr activity, but they retained substantial activities of the membrane-associated HPr(Ser) kinase and HPr(Ser-P) phosphatase.

Transport of radioactively labelled sugars. Cells were harvested by centrifugation (10000 g, 5 min, 4 °C), washed twice in anaerobic buffer (50 mM Tris/HCl, pH 7.0, containing 5 mM MgCl2 and 2 mM dithiothreitol, under N2), and incubated (approximately 80 mg protein in 200 μl) anaerobically with [14C]TMG (final concentration 60 μM). Transport was terminated by the addition of 2 ml ice-cold 0.1 M LiCl to the
reaction mixture and rapid filtration through 0.45 μm pore size cellulose nitrate membrane filters. Filters were washed with 2 ml 0.1 M LiCl, dried for 20 min at 120 °C, and radioactivity was determined by liquid scintillation counting. The transport kinetics were first order (activity versus protein was linear).

**Preparation of TMG-6-phosphate-loaded cells.** The preloading buffer (200 μl) contained 50 mM Tris/HCl buffer (pH 7.0), 5 mM dithiothreitol, 5 mM MgCl₂, [³⁵S]TMG (final concentration 60 μM), and 5–10 mg S. bovis protein (cells or vesicles). After incubation for 10 min at 39 °C, after which time the maximal accumulation of TMG-6-phosphate had been achieved, the cells were used in expulsion studies as described in Results. The radioactive material in the cells and vesicles was shown to consist of [³⁵S]TMG-6-phosphate as determined by ion-exchange chromatography (5 × 0.5 cm column size) according to Kundig & Roseman (1971).

**Electroporation of HPr and metabolites into membrane vesicles of S. bovis.** Bacillus subtilis HPr (50 μM) or one of the mutant proteins S46AHPr or S46DHPr (also 50 μM) (Reizer *et al.*, 1989) were added to a gene pulser cuvette (Bio-Rad) containing 100 μl S. bovis vesicles (10 mg protein). The mixture was then electroporated twice at 0 °C and 700 V (resistance 200 Ω; capacitance 25 μF) for 1.5 ms. The mixture was left on ice for 20 min before the electroporated vesicles were used for TMG uptake as described above.

**Assay of ATP-dependent phosphorylation of HPr by a protein kinase in membrane vesicles of S. bovis.** The assay mixture for HPr(Ser) phosphorylation (50 μl final volume) contained 50 mM Tris/HCl buffer (pH 7.0), 5 mM dithiothreitol, 5 mM MgCl₂, 1.0 mM [γ-³²P]ATP (ICN Pharmaceuticals; 500–2000 c.p.m./pmol⁻¹), 50 μM HPr and 5 mg vesicle protein. Various sugars and sugar metabolites were also present as indicated in Results. The assay mixture was electroporated as indicated and incubated for 30 min at 39 °C before the reaction was terminated by the addition of SDS quench buffer. Proteins were separated by SDS-gel electrophoresis (Laemmli, 1970; Reizer *et al.*, 1988); the gels were stained for proteins with Coomassie Blue R in 25% methanol, 10% acetic acid (v/v) (1 h at 55 °C), and then destained with the same solvent (4 h at 55 °C), before drying under reduced pressure. Radioactivity in the dry gels was determined by autoradiography.

**Other analyses.** Glucose was analysed by an enzymic method using hexokinase and glucose-6-phosphate dehydrogenase (Bergmeyer & Klotzsch, 1965). Lactose was assayed by converting lactose to glucose using β-galactosidase followed by enzymic determination of glucose. Protein from NaOH-hydrolysed cells (0.2 M NaOH, 100 °C, 15 min) was assayed by the Lowry method. Galactokinase and galactose-6-phosphate isomerase were assayed as described by Crow *et al.* (1983).

**RESULTS**

**Glucose and lactose transport by S. bovis**

*S. bovis* JB1 grew rapidly on glucose or lactose, but the maximal specific growth rate was always lower with lactose than with glucose (Table 1). Cells grown on either glucose or lactose had high rates of PEP-dependent glucose phosphorylation, but lactose PTS activity was very low unless lactose was the energy source. Cells which were successively transferred on glucose plus 2-DG lost most of their glucose PTS activity (JB1²DG), but were still able to grow rapidly on glucose or lactose (Table 1). The

### Table 1. Glucose and lactose utilization by *S. bovis*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth substrate</th>
<th>Growth rate (h⁻¹)</th>
<th>Glucose PTS*</th>
<th>Lactose PTS*</th>
<th>β-Galactosidase activity*</th>
<th>P-β-Galactosidase activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>JB1</td>
<td>Glucose</td>
<td>1.85</td>
<td>996 ± 63.0</td>
<td>100 ± 3.0</td>
<td>80 ± 6.0</td>
<td>120 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td>0.85</td>
<td>933 ± 40.0</td>
<td>415 ± 29.0</td>
<td>153 ± 7.1</td>
<td>908 ± 65.0</td>
</tr>
<tr>
<td>JB1²DG</td>
<td>Glucose</td>
<td>1.75</td>
<td>333 ± 5.0</td>
<td>200 ± 4.0</td>
<td>150 ± 3.8</td>
<td>200 ± 6.4</td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td>0.80</td>
<td>160 ± 3.0</td>
<td>403 ± 41.0</td>
<td>80 ± 2.2</td>
<td>1004 ± 80.0</td>
</tr>
</tbody>
</table>

* All activities are expressed as nmol substrate converted to product per min per mg protein by toluene-treated cells at 39 °C. Results are the means of duplicates, ± range.
wild-type strain and strain JB12DG had approximately the same lactose PTS activity when lactose was the energy source for growth. Toluene-treated cells that had been grown on lactose hydrolysed ONPG, but the rate of ONPG hydrolysis was more than 20-fold less than the rate of lactose phosphorylation via the PTS (Table 1). The rate of ONPG 6-phosphate hydrolysis was 50-fold greater than that of ONPG, and the cells also had an inducible galactose-6-phosphate isomerase [335-71 nmol min⁻¹ (mg protein)⁻¹]. Little, if any, galactokinase activity was detected [< 20 nmol min⁻¹ (mg protein)⁻¹]. Lactose transport could not be driven by an artificial membrane potential (ΔΨ) or pH gradient (ΔpH), and the protonophore 3,3',4',5-tetrachlorosalicylanide (TCS) and the sodium/proton antiporter monensin had no effect on the rate of lactose fermentation by washed cells (data not shown).

Inducer exclusion and expulsion in intact S. bovis cells

When S. bovis cells that had been pre-grown on glucose were provided with a combination of glucose and lactose, a diauxic growth pattern was observed (Fig. 1). Glucose was used preferentially to lactose and no lactose utilization was noted until the glucose was depleted from the growth medium. Cells that had been pre-grown on lactose exhibited a short lag phase when transferred to medium containing lactose (Fig. 2). Glucose addition to cultures growing on lactose immediately inhibited lactose uptake and lactose uptake did not continue until glucose was completely utilized (Fig. 2).

Washed cells of S. bovis JB1 grown on lactose transported [¹⁴C]TMG or [¹⁴C]lactose at a rapid rate (data not shown). No [¹⁴C]TMG or [¹⁴C]lactose uptake was noted if the cells were grown on glucose. Rapid transport of [¹⁴C]glucose or 2-[¹⁴C]DG was observed whether the cells were grown on glucose or lactose (data not shown). The non-metabolizable sugars 2-DG and TMG were competitive inhibitors of glucose and lactose uptake, respectively (data not shown). When glucose or 2-DG was added to lactose-grown cells prior to the addition of [¹⁴C]TMG, [¹⁴C]TMG uptake was completely blocked (Fig. 3a). The Lineweaver–Burk plot of [¹⁴C]TMG uptake in the presence of unlabelled 2-DG indicated that 2-DG was a noncompetitive inhibitor of TMG transport (data not shown).
Regulation of the lactose PTS of *S. bovis* by glucose

The PTS-deficient mutant strain JB1<sup>2DG</sup> (defective in enzyme I<sup>10IC</sup>) grew rapidly on glucose, but it had very low rates of PEP-dependent glucose or 2-DG phosphorylation (Table 1). When JB1<sup>2DG</sup> was pre-incubated with 2-DG prior to <sup>14</sup>C]TMG addition, there was no effect on <sup>14</sup>C]TMG uptake (Fig. 3b). If glucose was added, <sup>14</sup>C]TMG was still transported, but after 2 min the rate of <sup>14</sup>C]TMG accumulation decreased. These results were consistent with the idea that glucose catabolism causes <sup>14</sup>C]TMG expulsion, but not exclusion.

Wild-type cells incubated with <sup>14</sup>C]TMG retained <sup>14</sup>C]TMG as the phosphate ester, TMG 6-phosphate, for more than 10 min as determined by ion-exchange chromatography. The addition of glucose to TMG-6-phosphate-loaded cells caused immediate efflux of free TMG (not TMG 6-phosphate), but the addition of 2-DG had no effect (Fig. 4a). Glucose addition also caused expulsion of <sup>14</sup>C]TMG from TMG-6-phosphate-loaded cells of JB1<sup>2DG</sup> (Fig. 4b), but the rate of expulsion was slow compared with that in wild-type cells. 2-DG had no effect on TMG expulsion from JB1<sup>2DG</sup>.

Membrane vesicles were prepared from lactose-grown cells of wild-type JB1 and tested for their ability to accumulate <sup>14</sup>C]TMG. When membrane vesicles were incubated with <sup>14</sup>C]TMG, no uptake was observed, even after 3 min incubation. However, if the membrane vesicles were first electroporated with 20 mM PEP, rapid rates of <sup>14</sup>C]TMG uptake were noted (Fig. 5a). Virtually all of the intravesicular TMG was present as TMG 6-phosphate. Electroporation of the membrane vesicles with ATP did not promote <sup>14</sup>C]TMG uptake, and additional HPr did not increase the rate of PEP-dependent TMG uptake (Fig. 5a). Membrane vesicles that were electroporated with PEP and incubated with glucose or 2-DG (extra-vesicularly) prior to <sup>14</sup>C]TMG addition lost their ability to take up <sup>14</sup>C]TMG (Fig. 5b).

Membrane vesicles accumulated <sup>14</sup>C]TMG as TMG 6-phosphate, which was retained for more than 3 min, but rapid TMG efflux was observed if glucose was added.
extravesicularly (Fig. 6a). 2-DG was unable to trigger TMG efflux from membrane vesicles. Membrane vesicles that were electroporated with PEP and FDP were unable to retain TMG 6-phosphate, particularly if additional HPr was also added intravesicularly (Fig. 6b). Intravesicular glycerate 2-phosphate could replace FDP in decreasing TMG 6-phosphate retention, but other glycolytic intermediates were less effective (Table 2).

**Demonstration of vesicular HPr phosphorylation**

When HPr, FDP (20 mM) and 1 mM [$\gamma$-$^{32}$P]ATP were electroporated into the membrane vesicles, followed by incubation at 39 °C for 30 min, there was an increased rate of [$\gamma$-$^{32}$P]ATP-dependent phosphorylation of HPr as determined by autoradiography. Gels (SDS-PAGE) stained for proteins with Coomassie Blue R confirmed that the radioactive spots were indeed HPr (data not shown). Strong phosphorylation of HPr was also observed when glycerate 2-phosphate or glucose was added to the electroporation buffer. Little if any [$\gamma$-$^{32}$P]ATP-dependent phosphorylation of HPr could be detected if other glycolytic intermediates were added (data not shown). Very little phosphorylation was observed when [$\gamma$-$^{32}$P]ATP was electroporated into membrane vesicles without HPr in the presence of glucose. Similarly, when both [$\gamma$-$^{32}$P]ATP and HPr were electroporated into the vesicles, but glucose or a metabolite of glucose was absent, very little phosphorylation was observed. These latter observations supported the idea that additional intravesicular HPr as well as a metabolite of glucose were needed for strong ATP-dependent phosphorylation of HPr.

**Regulation of TMG efflux by serine-46 in HPr**

To demonstrate the possibility that HPr plays a direct role in the regulation of the lactose PTS of *S. bovis* by glucose,

![Image](image-url)

**Table 2. Regulation of [$^{14}$C]TMG expulsion from *S. bovis* membrane vesicles**

Membrane vesicles were electroporated in the presence of 20 mM PEP plus 50 μM HPr and the compound listed in the table at a final concentration of 20 mM. TMG 6-phosphate remaining in the membrane vesicles was measured after 1 min. All values reported are the means of duplicates, ± range.

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>TMG 6-phosphate [nmol (mg protein)$^{-1}$]</th>
<th>TMG 6-phosphate retained (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no metabolite addition)</td>
<td>4.35 ± 0.65</td>
<td>100</td>
</tr>
<tr>
<td>Glycerate 2-phosphate</td>
<td>0.78 ± 0.22</td>
<td>17.9</td>
</tr>
<tr>
<td>Fructose 1,6-diphosphate (FDP)</td>
<td>0.97 ± 0.16</td>
<td>22.2</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.20 ± 0.23</td>
<td>27.6</td>
</tr>
<tr>
<td>Fructose 6-phosphate</td>
<td>1.71 ± 0.36</td>
<td>39.3</td>
</tr>
<tr>
<td>Fructose 1-phosphate</td>
<td>1.82 ± 0.21</td>
<td>41.8</td>
</tr>
<tr>
<td>Glycerate 3-phosphate</td>
<td>2.68 ± 0.16</td>
<td>61.6</td>
</tr>
<tr>
<td>Glucose 6-phosphate</td>
<td>3.00 ± 0.27</td>
<td>68.9</td>
</tr>
<tr>
<td>Gluconate 6-phosphate</td>
<td>3.32 ± 0.19</td>
<td>76.3</td>
</tr>
<tr>
<td>Galactose 6-phosphate</td>
<td>4.01 ± 0.10</td>
<td>92.1</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>4.00 ± 0.25</td>
<td>92.0</td>
</tr>
</tbody>
</table>
site-specific mutant forms of HPr modified in the seryl 46 residue were tested for their ability to cause TMG efflux in membrane vesicles. When S46DHPr, which is conformationally similar to serine-phosphorylated HPr (Reizer et al., 1989), was substituted for wild-type HPr in the electroporation step, the vesicles lost their ability to retain TMG 6-phosphate, even if FDP or another glycolytic intermediate was not present (Fig. 7). S46AHPPr, an HPr derivative with a neutral alanine residue at position 46, also caused TMG expulsion, but to a lesser extent than S46DHPr.

**DISCUSSION**

Many bacteria are able to utilize some energy sources preferentially, and sequential patterns of energy source utilization allow bacteria to minimize protein synthesis and maximize their growth rates (Magasanik, 1961). In *E. coli*, glucose is the preferred energy source, and the ability of glucose to inhibit other utilisations was originally called the 'glucose effect' (Monod, 1947). In the 1960s, Magasanik (1961) coined the term catabolite repression, and later work indicated that glucose, via cyclic AMP and a catabolite activator protein, regulated the transcription of the lactose operon (Peterkofsky & Gazdar, 1974). McGinnis & Paigen (1969) noted that *E. coli* also had a short-term mechanism of regulating lactose utilization and introduced the term 'catabolite inhibition'. Subsequent work indicated that this latter effect was due to an inhibition of lactose transport by glucose (McGinnis & Paigen, 1973).

Saier & Roseman (1976) demonstrated that the glucose PTS of *E. coli* could inhibit the lac permease, and later work indicated that inducer exclusion was mediated by a direct interaction of enzyme II<sup>A</sup> with the permease (Saier, 1985, 1989). In some Gram-positive bacteria, the glucose PTS regulates other PTS systems (Reizer & Panos, 1980; Thompson & Saier, 1981; Reizer et al., 1983, 1984) and causes inducer expulsion as well as inducer exclusion. A process analogous to inducer expulsion has been reported in Gram-negative bacteria (Haguenauer & Kepes, 1971, 1972; Winkler, 1991). In fermentative, low-G+C Gram-positive bacteria, inducer expulsion involves the action of a sugar phosphate phosphatase that dephosphorylates the sugar prior to its being 'expelled' from the cell (London et al., 1985; Reizer et al., 1983, 1985; Sutrina et al., 1988; Thompson & Chassy, 1983a, b).

Inducer expulsion was first demonstrated in *Streptococcus pyogenes* (Reizer & Panos, 1980), and under these conditions the phosphate carrier protein, HPr, was phosphorylated at seryl residue 46 (Ser 46) as well as its catalytic domain (Deutscher & Saier, 1983; Reizer et al., 1983). The phosphorylation of HPr at Ser 46 was mediated by an ATP-dependent protein kinase (Deutscher & Saier, 1983). Because the protein kinase seemed to be allosterically activated by a glucose-dependent increase in FDP, it appeared that HPr was controlling inducer expulsion (Reizer et al., 1984). This conclusion was supported by the observation that a mutant form of HPr having a negatively charged aspartyl residue rather than serine at position 46 (S46DHPr) triggered the expulsion of the non-metabolizable lactose analogue TMG from membrane vesicles of *Lactococcus lactis*, even if FDP was not present (Ye et al., 1994). Another mutant of HPr having a neutral alanine residue (S46AHPPr) was inactive or less effective at triggering expulsion.

In *S. bovis*, lactose was transported by a PEP-dependent mechanism, and there was no evidence that this sugar was taken up by active transport. Because toluene-treated cells could hydrolyse ONPG 6-phosphate, but not ONPG, at a rapid rate, it appeared that the low β-galactosidase activity observed might be little more than an artifact. When lactose 6-phosphate is converted to glucose and galactose 6-phosphate, the latter is usually fermented by the tagatose pathway (Crow et al., 1983). Because lactose 6-phosphate hydrolase and galactose 6-phosphate isomerase, but not galactokinase activity, could be detected, it is likely that *S. bovis* also uses the tagatose pathway for lactose fermentation.

Because *S. bovis* had a diauxic pattern of glucose and lactose utilization, and glucose was a noncompetitive inhibitor of TMG uptake, it appeared that the lactose PTS must be regulated by inducer exclusion. The non-metabolizable glucose analogue 2-DG was able to mediate exclusion to the same extent as glucose. The glucose-PTS-deficient mutant *S. bovis* JBl<sup>1486</sup> also fermented glucose, but in this case neither glucose nor 2-DG could exclude TMG. Based on these results, it appeared that enzyme II<sup>C</sup>, rather than glucose catabolism per se, was the critical feature of inducer exclusion. In *E. coli*, enzyme II<sup>C</sup> has a higher affinity for phospho-HPr than enzyme II<sup>M</sup>, and competition for phospho-HPr allows for the preferential utilization of glucose to the exclusion of mannitol (Saier, 1985). Dills & Seno (1983) reported that a glucose PTS mutant of *Streptococcus mutans* lost its ability to exclude hexitols, but the effect of glucose catabolism was not studied.
Glucose, but not 2-DG, was able to promote the expulsion of TMG from S. bovis cells and membrane vesicles, and this result indicated that glucose catabolism was an obligate feature of inducer expulsion. This conclusion is supported by the observation that membrane vesicles could not expel TMG until they were electroporated with FDP, an intermediate in the Embden–Meyerhof pathway of glucose catabolism. The role of FDP in inducer expulsion was further reinforced by the demonstration that FDP was needed to promote the ATP-dependent phosphorylation of HPr in membrane vesicles, and also by the observation that HPr modifications at serine 46 affected both the rate and extent of TMG expulsion from membrane vesicles. Because S46DHPr, an HPr derivative conformationally similar to serine-phosphorylated HPr, was more effective than S46AHPr at causing TMG efflux, it appears that ATP-dependent phosphorylation of Ser-46 by the observation that HPr modifications at serine 46 was more effective than S46AHPr at causing TMG efflux, while ATP had the direct role in inducer expulsion. This conclusion is likewise supported by the finding that the membrane-bound sugar phosphate phosphatase of S. bovis was more effective than S46AHPr at causing TMG efflux, while ATP had the direct role in inducer expulsion. This mutant lost its ability to exclude TMG, but it retained its capacity to metabolize glucose, produce FDP, and catalyse the ATP-dependent phosphorylation of HPr. Because JBT2DG was able to dephosphorylate TMG 6-phosphate, TMG uptake was only a transient process when glucose was present.

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Proprietary or brand names are necessary to report factually on others that may be suitable.

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