APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1987, p. 2388–2393
0099-2240/87/102388-06$02.00/0

Vol. 53, No. 10

# Transport and Phosphorylation of Disaccharides by the Ruminal Bacterium *Streptococcus bovis*

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Received 12 May 1987/Accepted 8 July 1987

**Toluene-treated cells of *Streptococcus bovis* JB1 phosphorylated cellobiose, glucose, maltose, and sucrose by the phosphoenolpyruvate-dependent phosphotransferase system. Glucose phosphorylation was constitutive, while all three disaccharide systems were inducible. Competition experiments indicated that separate phosphotransferase systems (enzymes II) existed for glucose, maltose, and sucrose. [$^{14}$C]maltose transport was inhibited by excess (10 mM) glucose and to a lesser extent by sucrose (90 and 46%, respectively). [$^{14}$C]glucose and [$^{14}$C]sucrose transports were not inhibited by an excess of maltose. Since [$^{14}$C]maltose phosphorylation in triethanolamine buffer was increased 160-fold as the concentration of $P_i$ was increased from 0 to 100 mM, a maltose phosphorylase ($K_m$ for $P_i$, 9.5 mM) was present, and this activity was inducible. Maltose was also hydrolyzed by an inducible maltase. Glucose 1-phosphate arising from the maltose phosphorylase was metabolized by a constitutive phosphoglucomutase that was specific for α-glucose 1-phosphate ($K_m$, 0.8 mM). Only sucrose-grown cells possessed sucrose hydrolase activity ($K_m$, 3.1 mM), and this activity was much lower than the sucrose phosphotransferase system and sucrose-phosphate hydrolase activities.**

*Streptococcus bovis* is a common ruminal bacterium, but it is rarely present in high numbers unless large amounts of starch or high-quality forages are fed. When these feedstuffs are present, *S. bovis* grows faster and lactate production increases. Increased numbers of *S. bovis* are often associated with low ruminal pH and dysfunction of the rumen (12, 30). Even though numbers are lower in hay-fed ruminants, *S. bovis* can utilize cellobiose and short-chain water-soluble cellodextrins that are released by the cellulolytic ruminal bacteria (11, 27).

Previous work indicated that *S. bovis* had higher affinities for sucrose and maltose than for glucose or cellubiose (29) and that glucose and sucrose were used in preference to maltose and cellobiose (28). Since the preferred substrates caused an almost immediate inhibition of nonpreferred substrate utilization, it appeared that the phosphoenolpyruvate-dependent phosphotransferase system (PEP-PTS) might regulate sugar transport. Recently, toluene-treated cells of *S. bovis* JB1 were found to have PEP-PTS activity for glucose and 2-deoxyglucose (2-DG) (22). Since this organism can also metabolize cellobiose, maltose, and sucrose, the following series of experiments examined how these disaccharides were phosphorylated and transported into the cell.

(This work was done by S. A. Martin in partial satisfaction of the requirements for the Ph.D. degree from Cornell University, Ithaca, N.Y.)

## MATERIALS AND METHODS

**Organism and growth conditions.** The JB1 strain of *S. bovis* was used (28), and previous work has shown that this strain is characteristic of the species (31). Basal medium contained 292 mg of $K_2HPO_4$, 292 mg of $KH_2PO_4$, 480 mg of $(NH_4)_2SO_4$, 480 mg of NaCl, 100 mg of $MgSO_4 \cdot 7H_2O$, 64 mg of $CaCl_2 \cdot 2H_2O$, 4,000 mg of $Na_2CO_3$, 600 mg of cysteine hydrochloride, 1,000 mg of Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 1 mg of resazurin, 500 mg of yeast extract (Difco Laboratories, Detroit, Mich.), 28.3 mmol of acetic acid, 8.1 mmol of propionic acid, 3.4 mmol of butyric acid, and 1.0 mmol each of valeric, isovaleric, isobutyric, and 2-methylbutyric acids per liter (pH 6.7). Sugars were prepared as separate anaerobic solutions (20% [wt/vol]) under $N_2$ and added (usually 6 g/liter) to the basal medium after autoclaving. Incubations were performed anaerobically at 39°C in batch culture.

**Selection of glucose PTS-deficient mutant *S. bovis* JB1-SA.** Glucose-grown cells of *S. bovis* JB1 were inoculated into basal medium (see above) that contained 11 mM maltose and 0.5 mM 2-DG and incubated at 39°C for 3 days as previously described (38). Since maltose utilization is catabolite repressed by glucose (28), cells that took up the nonmetabolizable glucose analog, 2-DG, were unable to grow. However, mutants that could not transport 2-DG could grow because maltose metabolism was not repressed. The spontaneous mutants were picked from an agar plate that contained maltose and 2-DG and were repeatedly transferred in basal medium containing the same substrates.

**Toluene-treated cells.** Cells in logarithmic growth (optical density at 600 nm, approximately 1.0) were harvested (40 ml) by centrifugation (10,000 × *g*, 10 min, 4°C) and washed once with either 100 mM sodium-potassium phosphate buffer (pH 7.2) plus 5 mM $MgCl_2$ or 50 mM triethanolamine buffer (pH 7.5) plus 5 mM $MgCl_2$. Cells were suspended in 10 ml of buffer and stored on ice. One milliliter of cell suspension was treated with 30 μl of a toluene-ethanol mixture (1:9 [vol/vol]) as previously described (16, 22).

**Cell extracts.** Cells from 400 ml of culture were harvested as above and washed in either phosphate buffer or 50 mM histidine buffer (pH 7.2) plus 5 mM $MgCl_2$. The cell pellet was sonicated for 30 min (Branson model 200 Sonifier; microtip; 30% duty cycle; 0°C), unbroken cells were removed by centrifugation (37,000 × *g*, 20 min, 0°C), and the cell extract was stored on ice.

**PTS assays.** PEP-dependent sugar phosphorylation was initially measured with radiolabeled sugars, and this activity

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was compared to that observed with ATP (8, 22). The reaction mixture (1.0 ml) contained either 100 mM sodium-potassium phosphate buffer (pH 7.2) or 50 mM triethanolamine buffer (pH 7.5), 5 mM $MgCl_2$, 1 mM dithiothreitol, 10 mM PEP (Sigma Chemical Co., St. Louis, Mo.) or 10 mM ATP (Sigma), and 100 μl of toluene-treated cells. The reaction was started by the addition of 1 mM sugar that contained 48 μM D-[U-$^{14}$C]glucose, 0.40 μM [U-$^{14}$C]maltose, or 0.36 μM [U-$^{14}$C]sucrose. After incubation at 39°C for 30 min, the phosphorylated product was precipitated with 10 ml of a $BaBr_2$ solution (30 mM in 90% [vol/vol] ethanol, 20 min, 0°C). The precipitate was then collected on 0.45-μm-pore membrane filters (Millipore Corp., Bedford Mass.) and rinsed with 80% (vol/vol) ethanol. The filters were air dried and counted in a Packard Tri-Carb B2450 scintillation counter. Endogenous phosphorylation was estimated from controls lacking PEP or ATP.

Since radiolabeled cellobiose was not commercially available, the method of Kornberg and Reeves was also used to measure PEP-dependent transport (16). Both methods gave similar results for glucose, sucrose, and maltose. Each reaction mixture (1.0 ml) contained 100 mM sodium-potassium phosphate buffer (pH 7.2), 5 mM $MgCl_2$, 10 mM PEP, 2 U of L-lactate dehydrogenase (EC 1.1.1.27; Sigma), 0.2 mM β-NADH (Sigma), and 100 μl of toluene-treated cells. The reaction was initiated by the addition of 5 mM sugar, and the decrease in $A_{340}$ (Gilford spectrophotometer model 260 with cuvettes of 1-cm light path) was monitored for approximately 5 min. Endogenous phosphorylation or NADH oxidase activity was estimated from controls lacking PEP. All incubations and experiments were performed in triplicate, and variance is indicated by the standard deviation.

**Maltose uptake experiments.** The effect of several inhibitors on [U-$^{14}$C]maltose uptake was studied with intact cells of *S. bovis* JB1-SA. Cells were harvested (40 ml) anaerobically and washed once with $O_2$-free triethanolamine buffer plus 5 mM $MgCl_2$. Cells were then suspended in 10 ml of buffer, and 100-μl samples were added to a 1.0-ml reaction mixture that contained buffer, $MgCl_2$, and inhibitors at the following final concentrations: carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), 20 μM; *N*,*N'*-dicyclohexylcarbodiimide (DCCD), 20 μM; chlorhexidine diacetate, 0.20 mM. All inhibitors were purchased from Sigma. Since CCCP and DCCD were prepared in 2% (vol/vol) ethanol, control incubations containing the same concentration of ethanol were used. The reaction mixture was preincubated for 3 min at room temperature, and the reaction was started by the addition of 1 mM maltose that contained 0.40 μM [U-$^{14}$C]maltose. After incubation at 39°C for 12 min, the reaction was stopped by placing the reaction tubes in an ice bath and adding 5 ml of ice-cold triethanolamine buffer. The cells were collected by filtration through a 0.45-μm-pore membrane filter (Millipore) and rinsed with 3 ml of buffer. The filters were air dried and counted as described above. All incubations and experiments were performed in triplicate in a Coy anaerobic glove box (Coy Laboratory Products, Ann Arbor, Mich.).

**Disaccharide phosphate hydrolase.** Since cellobiose, maltose, and sucrose phosphate are not commercially available, an assay based on the endogenous production of disaccharide phosphate by the PTS was used (34). Disaccharide hydrolysis was estimated from the formation of glucose 6-phosphate and subsequent $NADP^+$-linked glucose 6-phosphate dehydrogenase activity. The reaction mixture (1.0 ml) contained either sodium-potassium phosphate buffer or triethanolamine buffer, 5 mM $MgCl_2$, 10 mM PEP or 10 mM ATP, 4 mM sugar, 0.8 mM $NADP^+$, 6.4 U of glucose 6-phosphate dehydrogenase (EC 1.1.1.49; Sigma), and 100 μl of toluene-treated cells. Control incubations without PEP or ATP were used to measure endogenous phosphorylation.

TABLE 1. Effect of the sugar supporting growth on specific activity of PEP-dependent sugar phosphorylation by toluene-treated cells of *S. bovis* JB1[a]

| Growth substrate | Sugar phosphorylated (nmol of NADH oxidized/mg of protein per min)[b] | | | |
|---|---|---|---|---|
| | Glucose | Maltose | Sucrose | Cellobiose |
| Glucose | 98 ± 9.3 | 9 ± 4.0 | 14 ± 3.1 | 16 ± 0.1 |
| Maltose | 105 ± 8.8 | 87 ± 16.4 | 29 ± 2.2 | 32 ± 9.0 |
| Sucrose | 108 ± 14.7 | 10 ± 4.0 | 111 ± 9.7 | 8 ± 5.7 |
| Cellobiose | 189 ± 19.5 | 4 ± 1.0 | 17 ± 0.8 | 181 ± 13.6 |

[a] Assayed by the lactate dehydrogenase-coupled method (16).
[b] Corrected for endogenous phosphorylation (<10%).

**Maltose phosphorylase assay.** Maltose phosphorylase activity was determined by a spectrophotometric method that assayed glucose 6-phosphate formation in the absence of PEP, and the reaction mixture (1.0 ml) contained 50 mM triethanolamine buffer (pH 7.5), 5 mM $MgCl_2$, 10 mM ATP, 4 mM maltose, 0.8 mM $NADP^+$ (Sigma), 6.4 U of glucose 6-phosphate dehydrogenase, 0 to 100 mM $P_i$, and 100 μl of toluene-treated cells.

**Phosphoglucomutase assay.** Phosphoglucomutase activity was measured spectrophotometrically by monitoring the conversion of α-D-glucose 1-phosphate to glucose 6-phosphate (4, 13). The reaction mixture (1.0 ml) contained 50 mM histidine buffer (pH 7.2), 5 mM $MgCl_2$, 0.8 mM $NADP^+$, 6.4 U of glucose 6-phosphate dehydrogenase, 1 mM α-D-glucose 1-phosphate (Sigma), and 100 μl of cell extract. No activity was observed when 1 mM β-D-glucose 1-phosphate was used as the substrate.

**Sucrose hydrolase assay.** The hydrolysis of sucrose to glucose and fructose was determined by an enzyme assay (34). The 1-ml reaction mixture contained 100 mM sodium-potassium phosphate buffer (pH 7.2), 5 mM $MgCl_2$, 10 mM ATP, 0.2 mM $NADP^+$, 6.4 U of glucose 6-phosphate dehydrogenase, 2 U of hexokinase (EC 2.7.1.1; Sigma), 2 U of phosphoglucose isomerase (EC 5.3.1.9; Sigma), 4 mM sucrose, and 100 μl of cell extract.

**Protein determination.** Protein from 0.2 N NaOH-hydrolyzed cells (100°C, 15 min) or cell extracts was determined by the method of Lowry et al. (18) or Bradford (5) and compared with a bovine serum albumin standard.

**Radioisotopes.** [U-$^{14}$C]maltose (501 μCi/μmol; 1 μCi = 37 kBq) and [U-$^{14}$C]sucrose (552 μCi/μmol) were obtained from Amersham Corp., Arlington Heights, Ill. D-[U-$^{14}$C]glucose (4.2 μCi/μmol) was purchased from New England Nuclear Corp., Boston, Mass.

## RESULTS

**PEP- and ATP-dependent phosphorylation.** When toluene-treated cells of *S. bovis* JB1 were examined for PEP-PTS activity by the enzymatic method of Kornberg and Reeves (16), PEP-dependent phosphorylation of glucose was observed for cells grown on each of the four sugars (Table 1). Glucose activity was unaffected by the sugar used for growth, but all three disaccharide systems were low unless the same sugar supported growth. Maltose-grown cells also had some activity for sucrose and cellobiose, but PEP-

TABLE 2. Effect of the sugar supporting growth on specific activity of ATP-dependent (kinase) sugar phosphorylation by toluene-treated cells of *S. bovis* JB1

| Growth substrate | Sugar phosphorylated (nmol/mg of protein per min)[a] | | |
|---|---|---|---|
| | Glucose | Maltose | Sucrose |
| Glucose | 126 ± 15.0 | 1 ± 0.3 | Nil[b] |
| Maltose | 190 ± 18.9 | 88 ± 1.9 | Nil |
| Sucrose | 204 ± 11.3 | 1 ± 0.2 | 4 ± 0.6 |

[a] Includes total (labeled and unlabeled) sugar phosphorylated. Corrected for endogenous phosphorylation (<10%).
[b] Nil, Experimental value did not exceed control value.

dependent phosphorylation for these sugars was approximately one-third the maltose rate.

Previous work showed that toluene-treated cells of *S. bovis* JB1 had significant ATP-dependent glucose phosphorylation or glucokinase activity (22). [$^{14}$C]glucose was phosphorylated by ATP, even when the cells were grown on maltose or sucrose (Table 2). Glucose- and sucrose-grown cells were unable to utilize ATP to phosphorylate either maltose or sucrose, but maltose-grown cells were able to phosphorylate maltose as well as glucose. Low rates of sucrose phosphorylation were observed, even if the cells were grown on sucrose.

**Competition experiments.** To ascertain whether glucose, sucrose, and maltose entered the cells by the same or different components of the PTS, competition experiments were performed (Table 3). Radiolabeled substrates were incubated alone or with a large excess (10 mM) of unlabeled sugar. Since maltose and sucrose contained <0.26% glucose, effects of glucose contamination should have been negligible. The lack of contamination was likewise illustrated by the inability of cold maltose or sucrose to inhibit [$^{14}$C]glucose phosphorylation. Unlabeled glucose inhibited the phosphorylation of [$^{14}$C]glucose by 89%, an amount similar to the theoretical dilution (91%). Unlabeled glucose also inhibited [$^{14}$C]maltose, but it did not affect [$^{14}$C]sucrose phosphorylation. Maltose caused substantial (73%) inhibition of [$^{14}$C]maltose phosphorylation, but it did not decrease the phosphorylation of [$^{14}$C]glucose or [$^{14}$C]sucrose. [$^{14}$C]maltose and [$^{14}$C]sucrose phosphorylations were inhibited by sucrose (46 and 88%, respectively), but the phosphorylation of [$^{14}$C]glucose was unaffected.

**Maltose transport by *S. bovis* JB1-SA.** Since high rates of maltose phosphorylation (maltose-grown cells) were observed in the presence of ATP as well as PEP (Tables 1 and 2), we decided to examine maltose phosphorylation with a glucose PTS-deficient mutant. Both the mutant and wild-type strains had similar growth rates (2.18 and 1.73 $h^{-1}$, respectively) and maltose PTS activity (116 and 87 nmol of NADH oxidized /mg of protein per min, respectively) when they were grown on maltose. However, glucose phosphorylation by the mutant cells (15 nmol of NADH oxidized/mg of protein per min) was only 15% of the activity of the parent strain (105 nmol of NADH oxidized/mg of protein per min). Since the mutant had little glucose PTS activity, it was unlikely that much of the maltose was hydrolyzed extracellularly and subsequently transported by the glucose PTS. To determine if maltose could be transported by a proton motive force-mediated mechanism as well as the PTS, we measured the effect of chlorhexidine diacetate, the proton-conducting ionophore CCCP, and the ATPase inhibitor DCCD on [$^{14}$C]maltose uptake by intact cells of *S. bovis* JB1-SA. All three compounds inhibited [$^{14}$C]maltose uptake, but chlorhexidine caused the greatest amount of inhibition (Table 4).

TABLE 3. Effect of unlabeled sugars on PEP-dependent phosphorylation of $^{14}$C-labeled sugars by *S. bovis* JB1[a]

| Radiolabeled sugar[b] | % Inhibition by unlabeled sugars | | |
|---|---|---|---|
| | Glucose | Maltose | Sucrose |
| D-[U-$^{14}$C]glucose | 89 | 3 | 5 |
| [U-$^{14}$C]maltose | 90 | 73 | 46 |
| [U-$^{14}$C]sucrose | 3 | 0 | 88 |

[a] Each reaction mixture contained 50 mM triethanolamine buffer (pH 7.5), 5 mM $MgCl_2$, 10 mM PEP, 0 or 10 mM unlabeled competing sugars, 1 mM unlabeled sugar that contained $^{14}$C-labeled sugars (glucose, 48 μM; maltose, 0.40 μM; sucrose, 0.36 μM), and 100 μl of toluene-treated cells.
[b] Cells grown on the same but unlabeled sugar.

TABLE 4. Effect of CCCP, DCCD, and chlorhexidine diacetate on [$^{14}$C]maltose uptake in intact cells of *S. bovis* JB1-SA grown on 2-DG and maltose[a]

| Inhibitor (concn) | % Inhibition |
|---|---|
| None | 0 |
| CCCP (20 μM) | 28 |
| DCCD (20 μM) | 42 |
| Chlorhexidine diacetate (0.2 mM) | 85 |

[a] Specific activity of [$^{14}$C]maltose uptake in cells treated with only 2% ethanol was 6.5 nmol/mg of protein per min.

**Disaccharide phosphate hydrolysis.** Since significant PEP-dependent phosphorylation was observed for maltose, sucrose, and cellobiose (Table 1), it seemed likely that a hydrolase was present to metabolize resulting disaccharide phosphates:

$$\text{disaccharide} + \text{PEP} \xrightarrow{\text{PTS}} \text{disaccharide-P} + \text{pyruvate}$$

$$\text{disaccharide-P} \xrightarrow{\text{hydrolase}} \text{hexose} + \text{glucose 6-P}$$

$$\text{hexose} + \text{ATP} \xrightarrow{\text{glucokinase}} \text{glucose 6-P}$$

When toluene-treated cells were incubated with disaccharide and PEP, glucose 6-phosphate resulting from the hydrolysis of disaccharide phosphate was detected for all three disaccharides (Table 5). The disaccharide phosphate hydrolase activity was particularly high for sucrose. If ATP was substituted for PEP, little disaccharide phosphate and, hence, glucose 6-phosphate were produced from cellobiose or sucrose. However, equivalent activity was observed for maltose regardless of the phosphoryl donor. From these observations it was not clear whether a hydrolase specific for maltose phosphate was present. The ATP-dependent activity (Tables 2 and 5) suggested that some maltose was converted to glucose monomers (maltase activity), which were then phosphorylated by a glucokinase.

**Maltose phosphorylase, phosphoglucomutase, and maltase.** Initial rates of $P_i$-dependent maltose phosphorylation were

TABLE 5. Effect of growth sugar on specific activity of disaccharide phosphate hydrolase in toluene-treated cells of *S. bovis* JB1

| Growth and enzyme substrate | Sp act (nmol of glucose phosphorylated/mg of protein per min) | |
|---|---|---|
| | PEP | ATP |
| Cellobiose | 25 | ND[a] |
| Maltose | 91 | 113 |
| Sucrose | 207 | ND |

[a] ND, Not detected.

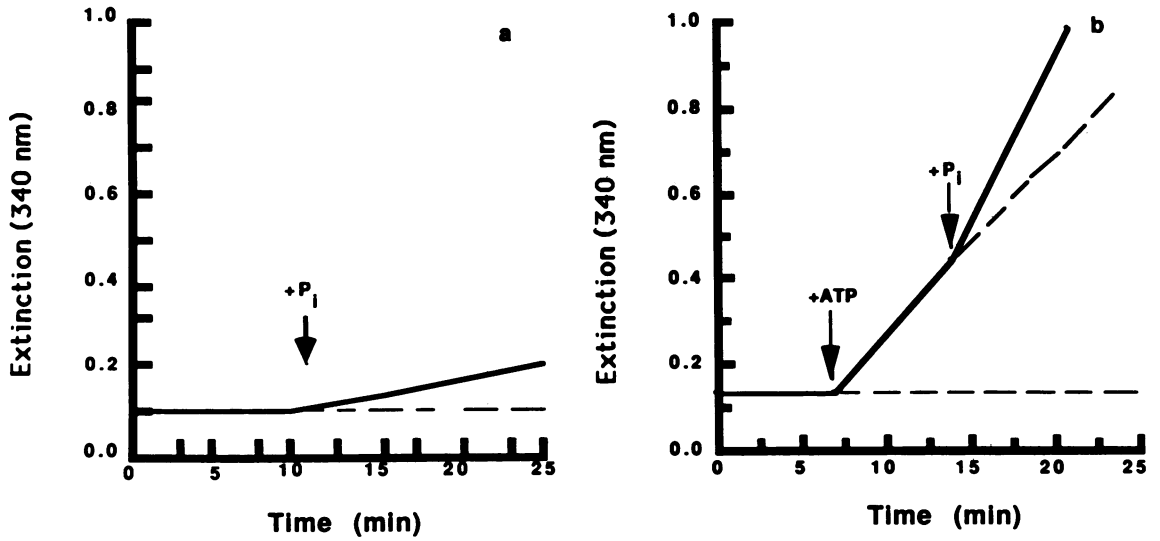


FIG. 1. Effect of 100 mM $P_i$ (a) or 10 mM ATP plus 100 mM $P_i$ (b) on rate of $NADP^+$ reduction (extinction at 340 nm) in toluene-treated cells of *S. bovis* JB1 incubated with maltose.

measured by a spectrophotometric method (Fig. 1a). When 100 mM $P_i$ was added to a reaction mixture that contained maltose, $NADP^+$, glucose 6-phosphate dehydrogenase, and toluene-treated cells, the rate of $NADP^+$ reduction (extinction at 340 nm) increased. Since $P_i$ served as the phosphoryl donor (no ATP or PEP present), it seemed likely that a phosphorylase was present. Because phosphate had no effect if the cells were grown on glucose, sucrose, or cellobiose (data not shown), it seemed that the phosphorylase activity was inducible and specific for maltose. Maltose phosphorylase yields glucose 1-phosphate rather than glucose 6-phosphate (3)

$$\text{maltose} + P_i \xrightarrow{\text{phosphorylase}} \text{glucose 1-P} + \text{D-glucose}$$

and since ATP was not present to allow phosphorylation by the glucokinase, phosphoglucomutase must have been present in the cells

$$\text{glucose 1-P} \xrightarrow{\text{mutase}} \text{glucose 6-P}$$

Maltose-grown cells as well as cells grown on glucose, sucrose, or cellobiose had phosphoglucomutase activities that were nearly the same (449 nmol of glucose phosphorylated/mg of protein per min), and it appeared that this enzyme was constitutive. The enzyme was specific for α-glucose 1-phosphate ($K_m$, 0.83 mM; $V_{max}$, 794 nmol of $NADP^+$ reduced/mg of protein per min), and no activity was measured when β-glucose 1-phosphate served as the substrate.

Rates of glucose 6-phosphate production and $NADP^+$ reduction were increased if ATP was added to the toluene-treated cells (Fig. 1b), and this meant that the cells contained a maltase which would yield free glucose

$$\text{maltose} \xrightarrow{\text{maltase}} \text{glucose} + \text{glucose}$$

$$2\ \text{glucose} + 2\ \text{ATP} \xrightarrow{\text{glucokinase}} 2\ \text{glucose 6-P}$$

However, additional $P_i$ caused a further increase in the rate of glucose 6-phosphate formation, even if ATP was already present. The apparent $K_m$ for $P_i$, as determined from Lineweaver-Burk plots, was approximately 9.5 mM, and the $V_{max}$ was 159 nmol of glucose phosphate/mg of protein per min.

**Sucrose hydrolysis.** Cell extracts of *S. bovis* JB1 only hydrolyzed sucrose when sucrose was the growth substrate, and this activity was not very high (21 nmol of $NADP^+$ reduced/mg of protein per min). The apparent $K_m$ for sucrose hydrolysis was approximately 3.1 mM, with a $V_{max}$ of 30.3 nmol of $NADP^+$ reduced/mg of protein per min. The low activity of sucrose hydrolysis was consistent with the low rates of ATP-dependent phosphorylation observed with toluene-treated preparations of sucrose-grown cells (Table 2). The very high activity of sucrose phosphate hydrolase (Table 5) would compensate for the lack of sucrose hydrolase.

## DISCUSSION

The PEP-PTS is favorable to anaerobic bacteria because it allows the concomitant transport and phosphorylation of a sugar without expending additional ATP in a glucokinase reaction. The PTS also plays an important role in regulating the uptake of many PTS and non-PTS sugars (6, 26, 32). PEP-dependent phosphorylation of a sugar is routinely used to determine if a microorganism possesses PTS activity. In *S. bovis* JB1, PEP-dependent phosphorylation of glucose was constitutive while the phosphorylations of cellobiose, maltose, and sucrose were inducible (Table 1). *Escherichia coli* can transport β-glucosides such as cellobiose by the PEP-PTS (7, 26), but this activity to our knowledge had not previously been reported in streptococci. Disaccharide phosphate hydrolases, which would be needed to hydrolyze disaccharide phosphates arising from the PTS, were likewise detected (Table 5).

Since glucokinases and hexokinases are unable to phosphorylate maltose (3), the inducible ATP-dependent maltose phosphorylation observed with maltose (Table 2) must have been due to a maltase. Because a maltase was detected, maltose could have been hydrolyzed to glucose extracellularly and subsequently transported by the constitutive glucose PTS (Table 1). However, a glucose PTS-deficient mutant grew rapidly on maltose and was still able to use PEP to phosphorylate maltose. Therefore, much of the maltose must have been transported and phosphorylated by a maltose PTS. Toluene-treated cells of *Streptococcus mutans* were previously found to have an inducible maltose PTS (34).

Bailey reported that *S. bovis* possessed a sucrose phosphorylase (2), and Ayers detected a cellobiose phosphorylase in the cellulolytic ruminal bacteria *Ruminococcus flavefaciens* (1). We were unable to detect significant hydrolase or phosphorylase activity for sucrose or cellobiose in toluene-treated cells or cell extracts, but an inducible maltose phosphorylase and maltase were observed (Fig. 1; Table 2). Maltose phosphorylases in streptococci were previously described (17, 25). When *S. bovis* JB1 was grown on maltose at rapid dilution rates in chemostats (29), small amounts of free hexose accumulated in the cell-free medium. These results indicated that some maltose cleavage was occurring on the outside of the cell.

Proton and other ion gradients across a membrane generate an electrochemical potential or proton motive force that can be used to drive nutrient uptake (23, 24). Several reports suggested that many oral streptococci have proton motive force-driven sugar uptake mechanisms in addition to PEP-PTS activity (9, 14, 15, 21). Inhibition of uptake by uncouplers that dissipate the proton motive force were used as criteria for ion-driven active transport (9, 14, 15, 19). The metabolic inhibitors CCCP and DCCD inhibited [$^{14}$C]maltose uptake by intact cells of *S. bovis* JB1-SA (Table 4); however, in each case the inhibition was <50%. Since the inhibition was not great, the presence of ion-driven transport in *S. bovis* JB1 is at best ambiguous. Intracellular energy sources (ATP or PEP) may be expended to regenerate the membrane potential, and the availability of PEP for transport could be depleted (39).

Chlorhexidine, a compound previously cited as a "specific" PTS inhibitor (15, 19, 20), completely inhibited PEP-dependent maltose phosphorylation (data not shown) by toluene-treated cells and maltose uptake by intact cells of *S. bovis* JB1-SA (Table 4). Chlorhexidine also significantly (90%) inhibited the uptake of xylose, a non-PTS carbohydrate, by intact cells of *Selenomonas ruminantium* HD4 (unpublished results). Therefore, it is doubtful that chlorhexidine is solely specific for PTS-mediated transport mechanisms. The lack of specificity is likewise supported by the ability of chlorhexidine to inhibit membrane-bound ATPases and interfere with membrane permeability to specific ions (10, 33). Based on these observations, the conclusion that chlorhexidine is a specific PTS inhibitor (15, 19, 20) should be re-evaluated.

Earlier work with *S. bovis* JB1 showed that glucose and sucrose could inhibit cellobiose and maltose utilization (28). When maltose-grown cells were incubated with [$^{14}$C]maltose, a 10-fold excess of glucose caused a 90% inhibition of PEP-dependent [$^{14}$C]maltose phosphorylation (Table 3). If glucose-grown cells were incubated with [$^{14}$C]glucose, a 10-fold excess of maltose caused only a 3% inhibition of [$^{14}$C]glucose phosphorylation. Since a significant inhibition was not observed in the latter case, and since a glucose PTS-deficient mutant was still able to transport maltose, these sugars were probably transported and regulated by different components of the PTS.

Cold glucose had little (3%) effect on [$^{14}$C]sucrose phosphorylation and cold sucrose had little (5%) effect on [$^{14}$C]glucose phosphorylation (Table 3). This lack of competition indicated that each of these sugars was transported by a separate enzyme II. The interaction between maltose and sucrose was not as straightforward. Unlabeled maltose did not inhibit [$^{14}$C]sucrose phosphorylation, but unlabeled sucrose inhibited [$^{14}$C]maltose phosphorylation by 46%. Since reciprocal inhibitions were not observed, it is unlikely that both sugars were transported by the same enzyme II. The inhibition of [$^{14}$C]maltose phosphorylation by sucrose might be explained by sucrose acting as a competitive inhibitor of the maltose enzyme II (26).

*S. mutans* has a PEP-PTS for sucrose as well as extracellular glucosyl- and fructosyltransferases and invertases (34–37). Sucrose hydrolase activity was detected in sucrose-grown cells of *S. bovis* JB1 (Table 2), but the activity was much less than the specific activities of the sucrose PTS (Table 1) or sucrose-phosphate hydrolase (Table 5). Thus, the sucrose PTS appeared to be the predominant sucrose uptake mechanism.

Previous work with *Neisseria perflava* indicated that the phosphoglucomutase was specific for β-glucose 1-phosphate (4). However, the *S. bovis* JB1 phosphoglucomutase was specific for α-glucose 1-phosphate and did not react with β-glucose 1-phosphate. Since β-glucose 1-phosphate was not utilized, the maltose phosphorylase must have yielded α-glucose 1-phosphate rather than β-glucose 1-phosphate like other phosphorylases (4, 25). Lohmeier-Vogel et al. were also unable to detect any β-glucose 1-phosphate resulting from maltose phosphorylase activity in *Streptococcus lactis* (17).

Our experiments demonstrated that disaccharide PTS activity in *S. bovis* JB1 was inducible, while the PTS for glucose was constitutive. Competition experiments indicated that separate enzymes II appear to exist for glucose, maltose, and sucrose. Maltose was also catabolized by an inducible maltase and a phosphorylase (Table 2; Fig. 1). The physiology of these redundant maltose phosphorylation and transport mechanisms is not clear and should warrant further study. Sucrose hydrolase and phosphorylase activities were very low, which would indicate that sucrose was transported primarily by the PTS.

## ACKNOWLEDGMENTS

S.A.M. was supported by a graduate research fellowship provided by International Minerals Corp., Terre Haute, Ind. J.B.R. is a member of the U.S. Dairy Forage Research Center, Madison, Wis.

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