Mechanisms of Sugar Transport in the Rumen Bacterium *Selenomonas ruminantium*

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(Received 16 July 1987; revised 25 September 1987)

Toluene-treated cells of *Selenomonas ruminantium* HD4 used phosphoenolpyruvate (PEP) to phosphorylate glucose and sucrose. Glucose activity was constitutive, while the phosphorylation of sucrose was inducible. Competition experiments indicated that separate phosphotransferase (PTS) enzymes II were present for glucose and sucrose, but it appeared that maltose was hydrolysed by an inducible extracellular maltase and then transported by the glucose PTS. *S. ruminantium* HD4 grew more slowly on maltose than glucose or sucrose and the specific activity of maltase was rate limiting. The maltase was competitively inhibited by glucose and sucrose. Xylose was not phosphorylated by PEP or ATP, and its uptake was inhibited by the protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), and by chlorhexidine diacetate. The absence of PEP-dependent phosphorylation and the effects of CCCP suggested that xylose was transported by an active transport mechanism.

INTRODUCTION

Selenomonas ruminantium is a Gram-negative anaerobe that was originally isolated from the rumen (Lessel & Breed, 1954). Latham *et al.* (1971) determined that up to 16% of the total bacterial counts in the rumen were *S. ruminantium*. Recent work showed that this species accounted for more than 20% of the isolates from the caecal contents of pigs (Robinson *et al.*, 1981). Many different carbohydrates are fermented by *S. ruminantium*, and it can grow under a variety of dietary conditions (Hungate, 1966). When it is grown in batch culture (high growth rate) with glucose, lactate is the predominant fermentation product (Hobson, 1965). Rapid growth and lactate production by *S. ruminantium* contributes to the acidity of rumen fluid, and it is more acid tolerant than many other rumen bacteria (Russell & Dombrowski, 1980). After hexose is depleted, the bacterium then utilizes the lactate as an energy and carbon source. In this secondary fermentation, acetate and propionate are the primary products (Scheifinger *et al.*, 1975; Russell & Baldwin, 1978). *S. ruminantium*, however, has a low affinity for lactate (Russell & Baldwin, 1979), and may not be a significant lactate utilizer in the rumen (Counotte *et al.*, 1981).

Previous studies showed that *S. ruminantium* had high affinities for glucose, maltose, sucrose and xylose (Russell & Baldwin, 1979), but glucose, sucrose and xylose were used in preference to maltose (Russell & Baldwin, 1978). Glucose and sucrose caused an inhibition of maltose utilization and it appeared that catabolite regulatory mechanisms controlled maltose utilization (Russell & Baldwin, 1978). Recent work (Martin & Russell, 1986) showed that toluene-treated cells of *S. ruminantium* HD4 possessed PTS activity for glucose and 2-DG, but no information

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Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; 2-DG, 2-deoxy-D-glucose; PNPG, *p*-nitrophenyl α -D-glucopyranoside; PEP, phosphoenolpyruvate; PTS, phosphotransferase system; p.m.f., protonmotive force.

was available on the transport of other sugars. Since transport is the first step in sugar metabolism, and since uptake is often a key characteristic determining the success of bacteria in natural environments (Matin & Veldkamp, 1978), we sought to examine how maltose, sucrose and xylose were translocated across the cell membrane.

METHODS

Organism and growth conditions. The HD4 strain of *Selenomonas ruminantium* was used (Bryant, 1956). Basal medium contained (l^{-1}) (adjusted to pH 6.7): K_2HPO_4 , 292 mg; KH_2PO_4 , 292 mg; $(NH_4)_2SO_4$, 480 mg; NaCl, 480 mg; $MgSO_4 \cdot 7H_2O$, 100 mg; $CaCl_2 \cdot 2H_2O$, 64 mg; Na_2CO_3 , 4 g; cysteine.HCl, 0.6 g; Trypticase (BBL), 1 g; resazurin, 1 mg; yeast extract, 0.5 g; acetic acid, 1.7 g; propionic acid, 0.6 g; butyric acid, 0.3 g; valeric, isovaleric, isobutyric and 2-methylbutyric acids, (0.1 g each). Sugars were prepared as separate anaerobic solutions (20%, w/v) under N_2 and added (6 g sugar l^{-1}) to the basal medium after autoclaving. Incubations were done anaerobically (under CO_2) at 39 °C in batch culture.

Spontaneous glucose-PTS-deficient mutants were selected for their resistance to the non-metabolizable glucose analogue 2-DG. Maltose- or sucrose-grown cells of *S. ruminantium* HD4 were inoculated into basal medium containing either 11 mM-maltose and 0.5 mM-2-DG or 11 mM-sucrose and 0.5 mM-2-DG and were incubated at 39 °C for 3–7 d. The spontaneous mutants (*S. ruminantium* HD4-S1 and HD4-S2) were isolated as previously described (Vadeboncoeur & Trahan, 1982; Martin & Russell, 1987); these cultures were repeatedly transferred in basal medium containing 2-DG.

Toluene-treated cells. Cells were harvested (40 ml) during exponential growth (optical density at 600 nm approximately 1.0; 138 mg cell protein l^{-1}) by centrifugation (10000 g, 10 min, 4 °C) and washed once with 100 mM-sodium/potassium phosphate buffer (50 mM-sodium phosphate, 50 mM-potassium phosphate, pH 7.2) containing 5 mM-MgCl₂. Cells were then suspended in 10 ml of buffer (553 µg cell protein ml^{-1}) and stored on ice. Cell suspension (1.0 ml) was treated with 30 µl of toluene/ethanol mixture (1:9, v/v) as previously described (Kornberg & Reeves, 1972; Martin & Russell, 1986).

Sonicated cell extracts. Cells were harvested (400 ml) and washed as described above. The cells were suspended in 4 ml of buffer and sonicated for 2 min (Branson model 200 Sonifier; microtip, 30% duty cycle, 4 °C). Unbroken cells were removed by centrifugation (37000 g, 20 min, 4 °C), and the cell extract was stored on ice.

Cell-free extracts and membrane preparation. Maltose-grown cells from 400 ml of culture were harvested and washed once in buffer containing 1 mM-dithiothreitol. The cells were suspended in 4 ml of buffer and broken in a French pressure cell (140 MPa, 4 °C; SLM Instruments). The cell extract was then centrifuged at low speed (5000 g, 10 min, 4 °C) to remove any unbroken cells, and the resulting supernatant was centrifuged at high speed (200000 g, 2 h, 4 °C) to sediment membrane fragments. The pellet (membrane fraction) was resuspended in 4 ml of buffer. Both the membrane and cytoplasmic fractions were stored at –20 °C overnight. No significant loss of enzyme activity was observed under these storage conditions.

Phosphorylation assays. PEP-dependent sugar phosphorylation was measured with radiolabelled sugars and this activity was compared to that observed with ATP (Gachelin, 1970; Vadeboncoeur *et al.*, 1983; Martin & Russell, 1986). The reaction mixture (1.0 ml) contained 100 mM-sodium/potassium phosphate buffer (pH 7.2), 5 mM-MgCl₂, 1 mM-dithiothreitol, 10 mM-PEP or 10 mM-ATP, and 100 µl of toluene-treated cells (final concentration 48 µg protein ml^{-1}). The reaction was started by the addition of 1 mM of sugar that contained 0.2 µCi (7.4 kBq), D-[U-¹⁴C]glucose, [U-¹⁴C]maltose, [U-¹⁴C]sucrose or D-[U-¹⁴C]xylose. After incubation at 39 °C for 30 min, the phosphorylated product was precipitated with 10 ml BaBr₂ solution (30 mM in 90%, v/v, ethanol; 20 min, 0 °C). The precipitates were then collected on 0.45 µm pore size membrane filters (Millipore) and rinsed with 80% (v/v) ethanol. The filters were air-dried and counted in a Packard Tri-Carb B2450 scintillation counter. The effect of Tris buffer on PTS activity in toluene-treated cells was also examined. Endogenous phosphorylation was estimated from controls lacking PEP or ATP, and these values were subtracted. All incubations and assays were done in triplicate, and variance is indicated by SD values.

Uptake by intact cells. ¹⁴C-labelled sugar uptake was examined in cells that were harvested (40 ml) anaerobically and washed once with O₂-free sodium/potassium phosphate buffer (100 mM, pH 7.2) plus 5 mM-MgCl₂. The cells (100 µl in 1 ml; 54.3 µg cell protein ml^{-1}) were preincubated in buffer or buffer plus inhibitor for 3 min, and the reaction was started by adding 1 mM sugar that contained 0.2 µCi (7.4 kBq) D-[U-¹⁴C]glucose, [U-¹⁴C]maltose, [U-¹⁴C]sucrose or D-[U-¹⁴C]xylose as above. 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 buffer. The cells were collected by filtration through 0.45 µm pore size membrane filters and rinsed with 3 ml of buffer. The filters were air-dried and counted as described above. The metabolic inhibitors CCCP (final concentration 20 µM) and chlorhexidine diacetate (final concentration 0.2 mM) were prepared in 2% (v/v) ethanol, and the final concentration of ethanol added to each reaction mixture was 0.1%. Control incubations contained the same final concentration (0.1%) of ethanol. All incubations and assays were done in triplicate in a Coy anaerobic (under CO_2) glove box.

Maltose hydrolase assay. The hydrolysis of maltose to glucose was determined by an enzyme-coupled assay. The reaction mixture (1.0 ml) contained 100 mM-sodium/potassium phosphate buffer (pH 7.2), 5 mM-MgCl₂, 10 mM-ATP, 4 mM-maltose, 0.8 mM-NADP⁺, 6.4 U glucose-6-phosphate dehydrogenase (EC 1.1.1.49), 2 U hexokinase (EC 2.7.1.1), and 100 µl of cell extract (52.2 µg protein ml⁻¹), cell-free extract (125 µg protein ml⁻¹) or membrane protein (245 µg ml⁻¹). Maltase (EC 3.2.1.20) activity was also measured continuously as the hydrolysis of PNPG at 400 nm. The reaction mixture (3.0 ml) contained 100 mM-potassium phosphate buffer (pH 7.0), 4 mM-PNPG and 100 µl of cell extract (final concentration of protein 175 µg ml⁻¹). Initial rates of PNPG hydrolysis were calculated using the molar extinction coefficient for *p*-nitrophenol (10718 M⁻¹ cm⁻¹, pH 7.15; Kesters-Hilderson *et al.*, 1982).

Sucrose hydrolase assay. Sucrose hydrolysis was determined by the enzymic method of St Martin & Wittenberger (1979). Each 1.0 ml reaction mixture contained 100 mM-sodium/potassium phosphate buffer (pH 7.2), 5 mM-MgCl₂, 10 mM-ATP, 0.2 mM-NADP⁺, 6.4 U glucose-6-phosphate dehydrogenase, 2 U hexokinase, 2 U phosphoglucose isomerase (EC 5.3.1.9), 4 mM-sucrose and 100 µl of cell extract (final concentration of protein 585 µg ml⁻¹). The inhibitory effect of 20 mM-Tris buffer on maltose and sucrose hydrolysis was also examined.

Identification of extracellular sugars. Maltose-grown cells (40 ml) were harvested anaerobically, washed once with O₂-free 100 mM-sodium/potassium phosphate buffer (pH 7.2) plus 5 mM-MgCl₂, and resuspended in 10 ml. Aliquots (100 µl; 54.3 µg ml⁻¹) were added to a 1.0 ml reaction mixture that contained the same buffer plus 5 mM-MgCl₂, 0.2 mM-chlorhexidine diacetate and 1 mM-maltose (0.2 µCi [U-¹⁴C]maltose). After incubation at 39 °C for 30 min, the cells were removed by centrifugation (10000 *g*, 5 min) and the supernatant was saved. All assays and incubations were done anaerobically under CO₂. Reaction samples (10 µl) were applied to a cellulose thin layer chromatogram, which was developed in a butyl alcohol/pyridine/water (6:4:3, by vol.) solvent system. Radioactive areas were qualitatively visualized by autoradiography and identified by co-chromatography with ¹⁴C-labelled glucose and maltose. *R_F* values (relative to the solvent front) were 0.52 for maltose and 0.64 for glucose.

Protein determination. Protein content was determined by the Lowry method or according to Bradford (1976). All samples were treated with 0.2 M-NaOH (100 °C, 15 min) and compared with a bovine serum albumin standard.

Chemicals. ATP, PEP, Tris, CCCP, chlorhexidine diacetate, PNPG, 2-DG, glucose, maltose, sucrose, xylose, dithiothreitol, BaBr₂, NADP⁺, glucose-6-phosphate dehydrogenase, hexokinase and phosphoglucose isomerase were from Sigma. All other chemicals were of the highest purity commercially available. [U-¹⁴C]Maltose, [U-¹⁴C]sucrose, and D-[U-¹⁴C]xylose were obtained from Amersham. D-[U-¹⁴C]Glucose was purchased from New England Nuclear.

RESULTS

Phosphorylation of sugars

PTS activity was examined in toluene-treated cells of *S. ruminantium* HD4 by measuring the PEP-dependent phosphorylation of radiolabelled glucose, maltose, sucrose and xylose (Table 1). Glucose phosphorylation was observed even if the cells were grown on other sugars, but the maltose and sucrose activities were low unless the organism was grown on the same sugar. Sucrose was also phosphorylated by maltose-grown cells, but at approximately one-third the rate for maltose. No PTS activity was detected for xylose. Cells grown on maltose or xylose had the lowest maximum specific growth rates.

ATP-dependent [¹⁴C]glucose phosphorylation in toluene-treated cells is characteristic of glucokinase or hexokinase activity (Romano *et al.*, 1979), and recent work showed that *S. ruminantium* HD4 had a glucokinase rather than a hexokinase (Martin & Russell, 1986). Glucokinase activity was observed if the cells were grown on sugars other than glucose (Table 2), but glucose- and xylose-grown cells were unable to hydrolyse maltose or sucrose and phosphorylate the resulting monomers. Maltose-grown cells incubated with maltose phosphorylated sugar at less than half the rate of glucose, and ATP-dependent phosphorylation of [¹⁴C]sucrose was less than one-tenth the glucose rate even if the cells were grown on sucrose. These results indicated that inducible hydrolases were present for maltose and sucrose. Phosphorylation of [¹⁴C]xylose by ATP was never significant.

Maltose and sucrose hydrolysis

Cell extracts from maltose-grown cells had significant levels of maltase activity (Table 3), but sucrose was only hydrolysed at approximately one-tenth of this rate. Addition of 20 mM-Tris buffer, a disaccharidase inhibitor (Dahlqvist, 1964; Groleau & Forsberg, 1981), decreased

Table 1. *Effect of sugars on growth rate and the specific activity of PEP-dependent sugar phosphorylation by toluene-treated cells of S. ruminantium HD4*

Growth substrate	μ^* (h ⁻¹)	Sugar phosphorylated†			
		Glucose	Maltose	Sucrose	Xylose
Glucose	0.66	104 ± 30	8 ± 0	7 ± 1	2 ± 0
Maltose	0.51	170 ± 24	73 ± 7	24 ± 4	3 ± 1
Sucrose	0.76	77 ± 21	10 ± 1	33 ± 9	2 ± 0
Xylose	0.53	49 ± 4	8 ± 2	8 ± 1	3 ± 1

* Maximum specific growth rate.

† nmol phosphorylated (mg protein)⁻¹ min⁻¹. The reaction mixture was incubated at 39 °C for 30 min.Table 2. *Effect of the sugar supporting growth on the specific activity of ATP-dependent (kinase) sugar phosphorylation by toluene-treated cells of S. ruminantium HD4*

Growth substrate	Sugar phosphorylated*			
	Glucose	Maltose	Sucrose	Xylose
Glucose	141 ± 23	5 ± 1	3 ± 0	1 ± 0
Maltose	138 ± 25	50 ± 8	6 ± 1	2 ± 1
Sucrose	156 ± 17	4 ± 1	12 ± 1	1 ± 0
Xylose	63 ± 2	2 ± 1	4 ± 1	2 ± 1

* nmol phosphorylated (mg protein)⁻¹ min⁻¹. The reaction mixture was incubated at 39 °C for 30 min.Table 3. *Effect of maltose, sucrose and Tris buffer on maltose and sucrose hydrolase activity in cell extracts from maltose- and sucrose-grown cells*

Growth substrate	Addition	Hydrolase activity*
Maltose	Maltose	488
	Sucrose	53
	Maltose + 20 mM-Tris†	86
Sucrose	Sucrose	59
	Maltose	7
	Sucrose + 20 mM-Tris†	35

* nmol hexose (mg protein)⁻¹ min⁻¹.

† Tris buffer was added to the reaction mixture before adding maltose or sucrose.

maltose hydrolysis by 82%. If the cell extract was divided into cytoplasmic and membrane fractions by high speed centrifugation, a greater proportion of the specific activity was found in the cytoplasmic fraction than the membrane fraction [223 and 108 nmol (mg protein)⁻¹ min⁻¹, respectively]. However, in terms of total activity, maltase was evenly distributed between the two fractions. The apparent K_m for maltose hydrolysis, as determined from Lineweaver-Burk plots, was 1.7 mM with a V_{max} of 658 nmol (mg protein)⁻¹ min⁻¹. The K_m for PNPG, a maltose analogue, was 2.5 mM and the V_{max} was 152 nmol (mg protein)⁻¹ min⁻¹. Glucose and sucrose were competitive inhibitors of PNPG hydrolysis with K_i of 8.7 mM for glucose and 17.4 mM for sucrose.

Cell extracts from sucrose-grown cells also hydrolysed sucrose and maltose, but these activities were low (Table 3) and consistent with the low rates of ATP-dependent phosphorylation (Table 2). Tris buffer inhibited sucrose hydrolysis by 41%. The apparent K_m for sucrose hydrolysis was 2.3 mM with a V_{max} of 78 nmol (mg protein)⁻¹ min⁻¹.

Table 4. Effect of unlabelled sugars and Tris buffer on the PEP-dependent phosphorylation of ^{14}C -labelled sugars by toluene-treated cells of *S. ruminantium* HD4

Reaction mixtures contained 100 mM-sodium/potassium phosphate buffer (pH 7.2), 5 mM-MgCl₂, 10 mM-PEP, 0 or 10 mM unlabelled competing sugars or 0.2 mM-Tris buffer, 1 mM sugar that contained 0.2 μCi ^{14}C -labelled sugars and 100 μl of toluene-treated cells (48 μg protein ml^{-1}). Competing sugars or Tris buffer were added to the reaction mixture before the radiolabelled substrates. The reaction mixture was incubated at 39 °C for 30 min.

Radiolabelled sugar*	Percentage inhibition of phosphorylation by:			
	Glucose	Maltose	Sucrose	Tris
Glucose	85	8	6	0
Maltose	68	79	29	49
Sucrose	35	18	77	0

* Cells grown on the same but unlabelled sugar.

Table 5. Effect of maltose or sucrose on the maximum specific growth rates and PTS specific activities for glucose and maltose in toluene-treated cells of *S. ruminantium* HD4, HD4-S1 or HD4-S2

Organism	Substrate	μ^* (h^{-1})	PTS specific activity†		
			Glucose	Maltose	Sucrose
<i>S. ruminantium</i> HD4	Maltose	0.51	170 $\pm$ 24	73 $\pm$ 7	ND
<i>S. ruminantium</i> HD4-S1	Maltose	0.21	34 $\pm$ 5	11 $\pm$ 3	ND
<i>S. ruminantium</i> HD4	Sucrose	0.76	77 $\pm$ 21	ND	33 $\pm$ 9
<i>S. ruminantium</i> HD4-S2	Sucrose	0.66	22 $\pm$ 2	ND	28 $\pm$ 6

ND, Not determined.

* Maximum specific growth rate.

† nmol phosphorylated (mg protein) $^{-1}$ min $^{-1}$. The reaction mixture was incubated at 39 °C for 30 min.

Competition experiments

To determine whether glucose, maltose and sucrose were transported by the same or different components of the PTS, a series of competition experiments was done (Table 4). In these experiments, a ^{14}C -labelled sugar was incubated with a large excess (10 mM) of unlabelled sugar. Maltose and sucrose contained less than 0.26% glucose, and unlabelled maltose or sucrose were unable to significantly inhibit [^{14}C]glucose phosphorylation. Unlabelled glucose inhibited the phosphorylation of [^{14}C]glucose by 85% and this inhibition was close to the theoretical dilution (91%). [^{14}C]Maltose and [^{14}C]sucrose phosphorylation was also inhibited by unlabelled glucose. Maltose strongly inhibited [^{14}C]maltose phosphorylation, but [^{14}C]sucrose phosphorylation was also inhibited by 18%. Phosphorylation of [^{14}C]sucrose or [^{14}C]maltose was inhibited by sucrose, but [^{14}C]glucose phosphorylation was unaffected. Tris buffer only inhibited the PEP-dependent phosphorylation of [^{14}C]maltose.

Phosphorylation by PTS mutants

Since maltose and sucrose were phosphorylated by ATP as well as PEP (Tables 1 and 2), we examined disaccharide phosphorylation with glucose-PTS-deficient mutants (Table 5). When *S. ruminantium* HD4 and HD4-S1 were grown on maltose, the growth rate of the mutant (HD4-S1) was decreased by 60% and PEP-dependent phosphorylation of glucose and maltose were both decreased by approximately 80%. *S. ruminantium* HD4-S2, another glucose-PTS-deficient mutant, and the wild-type had similar growth rates on sucrose and similar sucrose PTS activity even though glucose phosphorylation was reduced by 71%.

Extracellular hydrolysis of disaccharides

Since significant maltase activity was observed with membrane fractions, it seemed likely that maltose was hydrolysed to glucose extracellularly. When maltose-grown cells were incubated in the presence of [^{14}C]maltose and chlorhexidine diacetate, a compound that inhibited the uptake of all sugars by approximately 90% (data not shown), extracellular [^{14}C]glucose was detected by TLC. This qualitative analysis indicated that maltose was indeed hydrolysed extracellularly, and that the resulting glucose monomers were available for transport into the cell as glucose.

Xylose transport

The lack of PTS activity for xylose suggested that a p.m.f. could be involved in transport. Therefore, we measured the effect of the protonophore CCCP (20 μM) on [^{14}C]xylose uptake by intact cells. Uptake [44 nmol (mg protein) $^{-1}$ min $^{-1}$] was inhibited by 83%. This indicated that transmembrane proton gradients may be involved in the transport process. Chlorhexidine diacetate (0.2 mM) was also a strong inhibitor of [^{14}C]xylose uptake (95%).

DISCUSSION

Generally, anaerobic micro-organisms obtain low yields of ATP and the phosphoenolpyruvate:carbohydrate PTS is advantageous because transport and phosphorylation occur simultaneously and require less energy than active transport and kinase reactions (Postma & Lengeler, 1985). PEP-dependent phosphorylation of a sugar is commonly used to determine if a micro-organism possesses PTS activity. Because bacterial cells utilizing the PTS often contain ATP-dependent glucokinase activity, the non-metabolizable sugar 2-DG is often used to verify the presence of a glucose PTS. Most bacterial glucokinases are unable to phosphorylate 2-DG, but *S. ruminantium* possesses an ATP-dependent 2-DG kinase (Martin & Russell, 1986). The presence of this latter enzyme confounded the differentiation of ATP- and PEP-dependent mechanisms. However, the PEP-dependent activity was primarily associated with the membrane fraction and little PEP-dependent phosphorylation of glucose could be detected in cell-free extracts (Martin & Russell, 1986). These latter observations indicated that PEP-dependent phosphorylation of sugars was indicative of the PTS in this organism. In these experiments PEP-dependent phosphorylation of glucose was constitutive, while the phosphorylations of maltose and sucrose were inducible (Table 1). PTS-mediated transport of disaccharides (β -glucosides, lactose, sucrose) has been described in enteric bacteria (Postma & Lengeler, 1985), and an inducible maltose PTS has been observed in streptococci (St Martin & Wittenberger, 1979; Martin & Russell, 1987).

Even though PEP-dependent phosphorylation could be demonstrated when [^{14}C]maltose was the substrate, the presence of a specific maltose PTS in *S. ruminantium* HD4 was not assured. Maltose could have been hydrolysed by an inducible maltase (Tables 2 and 3) and the resulting glucose monomers subsequently transported by the constitutive glucose PTS. Evidence supporting this mechanism was obtained from several observations: (i) much of the maltase was associated with the membrane fraction, and the appearance of extracellular glucose indicated that at least some of this activity was extracellular; (ii) Tris buffer significantly inhibited maltase activity (Table 3), and this compound also inhibited PEP-dependent sugar phosphorylation when [^{14}C]maltose was provided (Table 4); (iii) unlabelled glucose inhibited PEP-dependent sugar phosphorylation resulting from [^{14}C]maltose by 68%, but unlabelled maltose (maltase not induced) only inhibited [^{14}C]glucose phosphorylation by 8% (Table 4); (iv) a glucose-PTS-deficient mutant grew much more slowly on maltose and PEP-dependent phosphorylation of maltose was decreased to the same extent as glucose (Table 5); (v) the maximum specific growth rate on maltose was 23% less than the rate on glucose (Table 1), PEP-dependent phosphorylation of maltose was 29% less than the rate of glucose phosphorylation (Table 1), and maltase activity appeared to be a rate-limiting step in maltose utilization. Since maltose was hydrolysed extracellularly by an inducible hydrolase, and since significant PEP-dependent maltose phosphorylation could not be demonstrated if the glucose PTS was lacking, it is unlikely that maltose was taken up by a separate and inducible maltose PTS.

Glucokinases and hexokinases are unable to phosphorylate disaccharides directly (Barman, 1969). The inducible ATP-dependent sucrose phosphorylation observed (Table 2) must therefore have been due to a sucrose hydrolase (Table 3). The rate of sucrose hydrolysis was much less than the activity observed for maltose, and Tris buffer was much less inhibitory (41%). Because a sucrose hydrolase was present, sucrose could have also been hydrolysed extracellularly and transported as hexose. However, a glucose-PTS-deficient mutant (*S. ruminantium* HD4-S2) grew rapidly on sucrose and was still able to phosphorylate sucrose (Table 5). Even though Tris buffer inhibited sucrose hydrolysis in cell extracts (Table 3), no inhibition of PEP-dependent sucrose phosphorylation was observed in toluene-treated cells (Table 4). These results indicated that little sucrose was hydrolysed extracellularly and subsequently transported by the glucose PTS.

Low rates of PEP-dependent sucrose phosphorylation by maltose-grown cells (Table 1) could be explained by maltase-mediated hydrolysis of sucrose (Table 3). Maltases from a variety of organisms have been shown to hydrolyse sucrose in addition to maltose (Barman, 1969). Because sucrose did not induce high levels of maltase activity (Table 3), PEP-dependent sucrose phosphorylation by sucrose-grown cells could not be explained by maltase-mediated hydrolysis of sucrose and subsequent transport by the glucose PTS.

Earlier work with *S. ruminantium* HD4 showed that maltose utilization is inhibited by the addition of glucose or sucrose (Russell & Baldwin, 1978). When maltose-grown cells were incubated with [^{14}C]maltose, a 10-fold excess of glucose caused a 68% inhibition of PEP-dependent [^{14}C]maltose phosphorylation, but a 10-fold excess of sucrose caused only a 29% inhibition of phosphorylation (Table 4). Since maltose was hydrolysed extracellularly, it seemed likely that glucose was regulating the maltase as well as competing for the glucose PTS. Neither glucose nor sucrose repressed maltase synthesis (data not shown), but both sugars competitively inhibited the hydrolysis of PNPG (a maltose analogue). The K_i for glucose (8.7 mM) was 50% of the K_i for sucrose (17.4 mM), and in toluene-treated cells, glucose inhibited [^{14}C]maltose phosphorylation more than sucrose did (Table 4). These results are consistent with the differences in K_i . While the inhibition constants were rather high it should be noted that the $V_{\max}$ for PNPG hydrolysis was approximately one-fifth that for maltose. Since K_i is inversely proportional to $V_{\max}$ (Segel, 1976), the K_i for maltose inhibition may be less.

Unlabelled glucose inhibited [^{14}C]sucrose phosphorylation to a greater extent than maltose did (35% versus 18%), but the presence of an extracellular maltase meant that glucose could have been responsible in either case (Table 4). Since unlabelled sucrose did not significantly inhibit [^{14}C]glucose phosphorylation, and since a glucose-PTS-deficient mutant was still able to phosphorylate sucrose (Table 5), it is unlikely that glucose and sucrose were transported by the same enzyme II. The presence of separate PTSs infers some specificity, but competition of different PTS sugars for non-sugar-specific components of the PTS has been noted (Postma & Lengeler, 1985; Saier, 1985). We were unable to detect an accumulation of sucrose phosphate. However, previous workers were only able to detect the sucrose phosphate intermediate in mutants of *Streptococcus mutans* which lacked sucrose phosphate hydrolase (St Martin & Wittenberger, 1979).

The lack of significant PEP-dependent phosphorylation of xylose (Table 1) indicated that an active transport mechanism was probably used. ATP-dependent phosphorylation was not detected either, but the absence may have been due to the equilibrium between xylose and xylulose. Xylose isomerase favours xylose formation by a factor of 6.25 (Barman, 1969). In general, pentoses are not transported by the PTS, but pentitol-specific PTSs have been observed in *Lactobacillus casei* (London & Chase, 1977, 1979; London & Hausman, 1982). It is well established that transmembrane gradients of protons or other ions can drive nutrient uptake. When intact cells of *S. ruminantium* HD4 were assayed for xylose uptake, significant inhibition (83%) was measured in the presence of CCCP. This indicated that proton gradients may be involved in the transport of xylose. Lam *et al.* (1980) reported that the transport of xylose into *Escherichia coli* was by a p.m.f.-driven mechanism.

CCCP also caused some inhibition of glucose, maltose and sucrose uptake by whole cells (data not shown), but these results alone are not conclusive proof for active transport. Membrane

Fig. 1. Probable mechanisms of soluble carbohydrate transport by *S. ruminantium*. PTS, phosphoenolpyruvate phosphotransferase system; AT, active transport.

vesicle studies will be necessary to determine if these sugars are also actively transported. Chlorhexidine, a compound previously reported to be a 'specific' PTS inhibitor (Keevil *et al.*, 1984; Marsh *et al.*, 1983, 1984), inhibited the uptake of all four sugars by approximately 90%. Since xylose did not appear to be taken up by a PTS, chlorhexidine is probably not a 'specific' PTS inhibitor. Chlorhexidine can also inhibit membrane-bound ATPases and affect membrane permeability (Harold *et al.*, 1969; Sissons & Midgley, 1981).

Probable mechanisms of sugar transport by *S. ruminantium* HD4 are shown in Fig. 1. Glucose and sucrose appeared to be phosphorylated by separate PTS components. Maltose was hydrolysed by an extracellular maltase and resulting glucose could then be transported by the constitutive glucose PTS. Since maltase activity was competitively inhibited by glucose and sucrose, glucose and sucrose can be used in preference to maltose. Some sucrase activity was detected, but this activity was much less than the maltase activity and approximately one-third the activity of the sucrose PTS. While small amounts of sucrose may also be hydrolysed extracellularly, most of the sucrose appears to be transported by a sucrose-specific PTS. Xylose was not phosphorylated by PEP, but its uptake was strongly inhibited by CCCP, a protonophore which interferes with active transport.

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 US Dairy Forage Research Center, Madison, Wis.

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