Regulation of Sorbitol Metabolism by Glucose in *Clostridium pasteurianum*: a Role for Inducer Exclusion

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Cells of *Clostridium pasteurianum* grown on glucose lacked enzyme systems specific for sorbitol metabolism: the phosphoenolpyruvate (PEP)-dependent phosphotransferase (PTS) and sorbitol-6-phosphate dehydrogenase. These activities were induced by growth on sorbitol, but were partially repressed by glucose. During growth on excess glucose and sorbitol, the sorbitol PTS was absent until the growth rate declined at the onset of stationary phase. Synthesis of at least two PTS proteins, one soluble and one membrane-bound, was repressed in exponentially growing cells. In contrast to the PTS, sorbitol-6-phosphate dehydrogenase was present during the exponential phase.

Glucose and methyl α -glucoside inhibited the activity of the sorbitol PTS in permeabilized cells, and this inhibition was competitive at the level of PEP. This implies that in intact cells sorbitol is excluded by glucose, at least in part due to competition between the glucose and sorbitol phosphotransferases for a common pool of phosphate and energy. Exclusion of sorbitol may be an important factor in the repression of sorbitol metabolism by glucose.

INTRODUCTION

In bacteria, many enzyme systems involved in carbohydrate metabolism are inducible. Induction of such enzyme systems is often prevented in the presence of a second carbon source which can be readily utilized by the cells. In *Escherichia coli*, glucose is metabolized in preference to other sugars (Magasanik, 1970). This was first referred to as the 'glucose effect', but is now generally known as catabolite repression. At least two factors contribute to the glucose preference (Dills *et al.*, 1980; Postma & Lengeler, 1985). First, in a phenomenon termed inducer exclusion, glucose inhibits the entry of other sugars into the cell (by several distinct mechanisms) and thus prevents induction of the appropriate enzyme system. Second, the activity of adenylate cyclase is inhibited, leading to a lowered intracellular concentration of cAMP; since cAMP, in association with the cAMP binding protein (CAP), acts as a positive effector of many catabolic operons, this also results in inhibition of enzyme synthesis.

Catabolite repression is also observed in Gram-positive bacteria such as bacilli and clostridia, but the mechanisms involved are not known. However, these organisms in general appear to lack cAMP (Botsford, 1981; Setlow & Sacks, 1983), and so the mechanism of regulation of gene expression may be expected to be different from that in *E. coli*. We are interested in the regulation of gene expression in clostridia, and have studied growth of *Clostridium pasteurianum* on media containing two carbon sources (Mitchell *et al.*, 1987). In most cases co-metabolism of the two growth substrates was observed. The only instance of strong repression of utilization of a substrate was found between glucose and sorbitol. When cells were pregrown on glucose and then exposed to both substrates, no appreciable removal of sorbitol from the medium occurred

Abbreviations: PEP, phosphoenolpyruvate; PTS, phosphotransferase system; RCM, Reinforced Clostridial Medium.

until the glucose was almost exhausted. Prior exposure of the cells to sorbitol, however, protected against the repressive effects of glucose. We now present the results of a more detailed investigation of the mechanisms by which glucose inhibits sorbitol metabolism in *C. pasteurianum*. Some of the data have appeared in a preliminary form (Mitchell *et al.*, 1985).

METHODS

Organism and growth of cells. *Clostridium pasteurianum* NCIB 9486 was maintained as a spore suspension in distilled water at 4 °C. Samples (0.5 ml) of the spore suspension were treated at 80 °C for 10 min, inoculated into 20 ml Reinforced Clostridial Medium (RCM; Oxoid), and allowed to grow at 37 °C for up to 24 h. Vegetative culture (8 ml) was transferred to 500 ml defined salts and vitamins medium, and growth was continued at 37 °C for a further 18–24 h, by which time the cells were in early stationary phase. The defined medium, modified from Mackey & Morris (1971) contained (l^{-1}): NH_4Cl , 3.0 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $NaCl$, 0.1 g; $CaCl_2 \cdot 2H_2O$, 0.01 g; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.01 g; $MnCl_2$, 0.015 g; $FeNaEDTA$, 0.275 g; d-biotin, 0.12 mg; *p*-aminobenzoic acid, 2 mg; carbon source, 20 g. The pH of the medium was adjusted to 7.4 with NaOH and, after autoclaving, potassium phosphate buffer (pH 7) was added to a concentration of 50 mM. Growth vessels were sealed using Suba-seals, and the headspace rendered free of oxygen by evacuation and flushing with oxygen-free nitrogen. For experiments investigating induction of sorbitol phosphotransferase activity (Fig. 1), 5 l cultures were grown in an aspirator with a lower port. The medium was autoclaved and allowed to cool under an atmosphere of oxygen-free nitrogen, and inoculated with 40 ml of initial RCM culture. Samples were withdrawn through the port using oxygen-free nitrogen to apply positive pressure above the culture.

Treatment of cells. Cells for use in experiments were prepared using previously described techniques (Booth & Morris, 1975; Mitchell & Booth, 1984) with minor modifications. Harvesting was done by centrifugation (10000g, 10 min) at 4 °C. Cells were washed with 50 mM-potassium phosphate buffer, pH 7, and resuspended in the same buffer. Buffers were made anaerobic by autoclaving immediately before use and allowing to cool under oxygen-free nitrogen. When cells were to be made permeable to low molecular mass solutes, the buffer contained 0.1% (w/v) L-cysteine hydrochloride. Cells were permeabilized by treatment with 1% (v/v) toluene; the mixture was vortexed for 20 s and incubated at 37 °C for 20 min. Whole and permeabilized cells were stored on ice, for a maximum period of 3 h, before use. Cell density was estimated from the relationship $A_{600} = 1.0$, equivalent to 0.25 mg dry wt ml^{-1} .

Preparation of cell-free extracts. Cells were suspended in 50 mM-potassium phosphate buffer, pH 7, at a concentration of 4 ml buffer (g wet wt cells) $^{-1}$. Cells were broken and extracts prepared as described by Mitchell & Booth (1984). The protein concentration of extracts was estimated by a microbiuret method (Zamenhof, 1957) using bovine serum albumin as standard.

Assay of carbohydrate uptake and phosphorylation. For assay of carbohydrate uptake into whole cells, 1 ml cell suspension was removed from the storage vessel using a gas-tight syringe and transferred to a tube which had been previously sparged with oxygen-free nitrogen; the suspension was then kept anaerobic by directing a stream of nitrogen over the surface. After pre-equilibration at 37 °C, a small volume of an anaerobic solution of radiolabelled carbohydrate was added to give a final concentration of 0.1 mM, sufficient to saturate the appropriate phosphotransferase system (PTS). Samples (0.14–0.20 ml) were removed at the times indicated, filtered using Whatman GF/F filter discs, washed with 5 ml potassium phosphate buffer, pH 7, and dried under a heat lamp. The dried filters were then placed in polypropylene minivials (BDH Scintran); 4 ml Scintillation cocktail O (BDH Scintran) was added, and radioactivity determined using a Packard Tricarb model 3320 liquid scintillation spectrometer.

For phosphorylation assays, toluene-treated cells were supplemented with 5 mM- $MgCl_2$ and phosphoenolpyruvate (PEP) at the concentrations indicated for individual experiments. The experiments were done anaerobically as described above, and radiolabelled substrate was added routinely after a 3 min preincubation period at 37 °C. Samples were removed and carbohydrate phosphate precipitated in 2 ml of a solution of 1% (w/v) $BaBr_2$ in 80% (v/v) ethanol. The precipitate was trapped by filtration and washed with 5 ml 80% ethanol. Filters were dried and radioactivity was determined as described above. Phosphorylation activity in extracts was measured as described by Mitchell & Booth (1984). All assay mixtures contained 50 mM-potassium phosphate buffer, pH 7, 5 mM- $MgCl_2$, 2 mM-dithiothreitol, 12 mM-NaF and PEP at the indicated concentrations.

Assay of sorbitol-6-phosphate dehydrogenase activity. The assay mixture for the measurement of sorbitol-6-phosphate dehydrogenase activity in cell-free extracts contained, in a total volume of 1.0 ml: 50 mM-Tris/HCl, pH 7.5, 5 mM- $MgCl_2$, 1 mM-dithiothreitol and 1 mM-NAD $^{+}$. After 3 min preincubation at 37 °C, sorbitol 6-phosphate was added to a final concentration of 1 mM and the rate of formation of NADH was followed as the absorbance at 340 nm. Extracts prepared in potassium phosphate buffer were dialysed into Tris/HCl buffer before dehydrogenase assays were done. Activity was proportional to extract concentration within the range studied.

Assay of carbohydrate concentration in culture supernatants. Culture samples (1.0 ml) were centrifuged in an Eppendorf centrifuge, and the supernatant was removed for assays. Glucose concentration was measured using Sigma assay kit no. 510, while sorbitol concentration was determined using a sorbitol assay kit obtained from Boehringer-Mannheim.

Materials. Growth media were purchased from Oxoid. PEP [tri(cyclohexylammonium) salt], DL-dithiothreitol, D-biotin, *p*-aminobenzoic acid, and D-sorbitol 6-phosphate (barium salt) were from Sigma and β -NAD⁺ was from BDH. All other chemicals were of the highest purity commercially available. D-[U-¹⁴C]Sorbitol, D-[U-¹⁴C]glucose and methyl(α -D-[U-¹⁴C]gluco)pyranoside were purchased from Amersham.

RESULTS

In *C. pasteurianum*, sorbitol is accumulated by a PEP-dependent PTS (Booth & Morris, 1982). The resultant intracellular sorbitol 6-phosphate is then oxidized by an NAD-dependent dehydrogenase to form fructose 6-phosphate (B. Mackey, personal communication). The two reactions thus represent the sorbitol-specific metabolism of the organism. These activities were measured in cells and extracts after overnight growth on glucose or sorbitol, or in the presence of both carbon sources. As shown in Table 1, PTS and dehydrogenase activities were found after growth on sorbitol, but were absent from cells grown on glucose. When both substrates were provided for growth, intermediate levels of the activities were observed. Thus it appears that the required enzymes are induced in the presence of sorbitol, and at least partially repressed by glucose. The rates of glucose and methyl α -glucoside uptake in cells grown on glucose plus sorbitol are also shown in Table 1. These sugars are accumulated at a rate comparable to the uptake of sorbitol.

In a previous report (Mitchell *et al.*, 1987) it was demonstrated that cells growing exponentially in the presence of glucose and sorbitol lacked sorbitol PTS activity. The time-course of induction of sorbitol PTS activity was therefore examined (Fig. 1). Glucose prevented significant removal of sorbitol from the medium and exponentially growing cells had extremely low sorbitol PTS activity. As the cells entered stationary phase, however, the sorbitol PTS was induced up to 25-fold despite a considerable residual concentration of glucose in the medium. The possibility that repression of sorbitol metabolism required a minimum glucose concentration greater than the residual concentration was eliminated by the demonstration of an identical induction curve when the starting glucose concentration was as low as 0.5% (w/v) (not shown). In addition, 0.18% glucose prevented utilization of sorbitol by exponentially growing cells (Mitchell *et al.*, 1987). Thus, some other factor must be responsible for induction or derepression of sorbitol PTS activity late in growth. In contrast to the PTS, sorbitol 6-phosphate dehydrogenase was present throughout the growth period over which samples were taken. Therefore the synthesis of the dehydrogenase enzyme and the PTS did not appear to be regulated coordinately.

Although they have not been completely characterized, the glucose and sorbitol phosphotransferases of *C. pasteurianum* have been shown to comprise both soluble and membrane-bound components (Mitchell & Booth, 1984; Mitchell *et al.*, 1985). When cells were grown on glucose, membranes were completely inactive in assays of sorbitol phosphorylation. The soluble fraction supported a low rate of sorbitol phosphorylation in the presence of membranes prepared from sorbitol-grown cells, but the specific activity was less than 10% of that found in a sorbitol soluble fraction (data not shown). The results indicated that growth on sorbitol induced the synthesis of at least two sorbitol-specific proteins (one soluble and one membrane-bound), perhaps analogous to III^{gut} and Enzyme II^{gut} of *Salmonella typhimurium* (Grenier *et al.*, 1985). Exponential cells from a culture such as that in Fig. 1 gave rise to extracts which behaved exactly as glucose extracts, showing a multiple defect in synthesis of sorbitol PTS components.

One effective mechanism of regulation of inducible operons in enteric bacteria is exclusion of the inducer molecule from the cell, generally observed as inhibition of uptake of the substrate of the enzyme system by the inhibiting sugar (Saier & Roseman, 1976; Saier & Moczydlowski, 1978). The effect of glucose on uptake of sorbitol was therefore examined in intact cells and in cells permeabilized with toluene; in the latter, sorbitol phosphorylation was used as a measure of sorbitol PTS activity. These experiments were done using cells grown in the presence of both

Table 1. *Metabolic activities in C. pasteurianum*

Growth and treatment of cells, and enzyme assays, were as described in Methods. All measurements of solute uptake into whole cells were done after preincubation of cells at the experimental temperature (37 °C) for 13 min, under which conditions maximum rates of uptake via the respective phosphotransferase systems were achieved (see Fig. 3). Phosphorylation assays were done using permeabilized cells; the PEP concentration in all assays was 0.25 mM. The values in the Table are means $\pm$ SD (number of determinations in parentheses). ND, Not determined.

Growth substrate	Sorbitol PTS activity		Glucose PTS activity			Sorbitol-6-phosphate dehydrogenase activity* [μ mol NADH min ⁻¹ (mg protein) ⁻¹]
	Uptake [nmol min ⁻¹ (mg dry wt cells) ⁻¹]	Phosphorylation [nmol min ⁻¹ (mg dry wt cells) ⁻¹]	Glucose uptake	Methyl α -glucoside		
				Uptake [nmol min ⁻¹ (mg dry wt cells) ⁻¹]	Phosphorylation [nmol min ⁻¹ (mg dry wt cells) ⁻¹]	
Sorbitol	21.5 $\pm$ 3.4 (3)	15.0 $\pm$ 2.0 (3)	ND	ND	ND	1.41 $\pm$ 0.16 (8)
Glucose + sorbitol	3.79 $\pm$ 1.17 (16)	3.89 $\pm$ 1.79 (6)	4.22 $\pm$ 0.75 (6)	3.72 $\pm$ 0.93 (10)	2.21 $\pm$ 0.77 (4)	0.27 $\pm$ 0.09 (6)
Glucose	0.07 $\pm$ 0.03 (3)	0.04†	ND	ND	ND	0‡

* Assays done using crude cell-free extracts. † Mean of duplicate experiments. ‡ Three experiments.

Table 2. *Inhibition of sorbitol phosphorylation by methyl α -glucoside in cell-free extracts of C. pasteurianum*

A. In the presence of concentrations of both sorbitol and glucose soluble extracts which saturated the respective membranes for phosphorylation of sorbitol and methyl α -glucoside. Assay mixtures (1.0 ml) contained 4.1 mg sorbitol soluble extract, 0.25 mg sorbitol membranes, 1.6 mg glucose soluble extract, 0.2 mg glucose membranes, 0.25 mM-PEP and 0.1 mM [¹⁴C]sorbitol. B. In the presence of sub-saturating concentrations of the sorbitol soluble extract. Assay mixtures (1.0 ml) contained 2.08 mg sorbitol soluble extract and 1.48 mg glucose soluble extract; other additions were as in A. All assays were done as described in Methods. Assay mixtures were preincubated for 3 min at 37 °C before addition of the substrate. Where methyl α -glucoside was present, it was added at the beginning of the preincubation period.

PEP concn (mM)	Rate of sorbitol phosphorylation (nmol min ⁻¹)	Methyl α -glucoside		Percentage inhibition by methyl α -glucoside
		—	+	
A. 0.01	0.10	0.01	0.01	90.0
0.05	0.43	0.01	0.01	97.7
0.10	0.48	0.06	0.06	87.5
0.20	0.51	0.45	0.45	11.8
0.50	0.55	0.55	0.55	0
B. 0.10	0.34	0.31	0.31	8.8

Fig. 1. Growth (○) of *C. pasteurianum* in defined medium containing glucose (■) and sorbitol (▲). Culture conditions and assays of PTS and dehydrogenase activity and of carbohydrate concentrations were as described in Methods. Cell density for experiments measuring sorbitol PTS activity (□) was in the range 3.0–4.0 mg dry wt ml⁻¹. Protein concentrations in experiments measuring sorbitol-6-phosphate dehydrogenase activity (●) were in the range 4.0–35.0 μg ml⁻¹, and activity was proportional to the extract concentration in each case.

Fig. 2. Effect of glucose and methyl α -glucoside on sorbitol uptake by whole cells of *C. pasteurianum*. Cells were grown in medium containing both glucose and sorbitol at a concentration of 2% (w/v). Assays of sorbitol uptake were done as described in Methods. Sorbitol concentration in all experiments was 0.1 mM. (a) Effect of glucose. Preincubation time (before substrate addition) and additions for individual experiments were as follows: ○, 3 min, no addition; ●, 13 min, no addition; □, 3 min, 0.1 mM-glucose added at start of preincubation; ■, 13 min, 0.1 mM-glucose added 3 min before substrate; ▲, 13 min, 0.1 mM-glucose added simultaneously with substrate. Cell density in experiments was 2.52 mg dry wt ml⁻¹. (b) Effect of methyl α -glucoside. Preincubation time and additions for individual experiments were as follows: ○, 3 min, no addition; ●, 13 min, no addition; □, 3 min, 0.1 mM-methyl α -glucoside added at start of preincubation; ■, 13 min, 0.1 mM-methyl α -glucoside added 3 min before substrate; ▲, 13 min, methyl α -glucoside added simultaneously with substrate. Cell density in experiments was 1.64 mg dry wt ml⁻¹.

Fig. 3. Competition between glucose and sorbitol phosphotransferase systems for PEP. Cells were grown on medium containing both glucose and sorbitol at a concentration of 2% (w/v). Sorbitol phosphorylation assays were done as described in Methods. Cell density in experiments was 2.20 mg dry wt ml⁻¹, and sorbitol concentration was 0.1 mM. Phosphorylation rates were measured as nmol min⁻¹ (mg dry wt cells)⁻¹. ○, control experiments (no methyl α-glucoside present); ●, 0.1 mM-methyl α-glucoside present.

glucose and sorbitol to ensure that both sugars would be taken up by the cells at a reasonable rate. As shown in Fig. 2(a) the effect of glucose on sorbitol uptake by whole cells was dependent on the time of preincubation at 37 °C. With a minimum preincubation time of 3 min, the rate of sorbitol uptake was low, and was significantly stimulated in the presence of glucose. These results suggest that the rate of sorbitol uptake by resting cells is limited by the intracellular concentration of PEP, which would be expected to increase after preincubation with glucose. The control rate of sorbitol uptake itself increased gradually with increasing preincubation time up to 13 min, at which time the rates in the presence and absence of glucose were equal. The lack of stimulation or inhibition by glucose is not due to partial depletion of the hexose during preincubation since simultaneous addition of glucose and sorbitol gave a rate identical to the control. Rather, the implication is that PEP accumulates in the cell during the preincubation period. This conclusion is strengthened by the fact that the rate of methyl α-glucoside uptake via the glucose PTS is also stimulated, and that the increase in rate is prevented by the glycolysis inhibitor iodoacetate (data not shown).

The non-metabolizable glucose analogue methyl α-glucoside strongly inhibited sorbitol uptake by whole cells when added before the substrate, irrespective of the preincubation time (Fig. 2b). Also, when methyl α-glucoside and sorbitol were added simultaneously after 13 min preincubation, sorbitol uptake was inhibited although not apparently during the first 30 s. Thus, when PEP cannot be generated from the hexose, the sorbitol PTS is strongly inhibited and this may be due in part to depletion of intracellular PEP as a result of methyl α-glucoside uptake.

Both glucose and methyl α-glucoside inhibited sorbitol phosphorylation, and to a similar extent, in experiments involving permeabilized cells to which PEP was added (data not shown). In this case, fluctuations in PEP concentration are avoided and the differences between the effects of the two hexoses observed in Fig. 2 would not be expected. Sorbitol had no effect on either the uptake or phosphorylation of methyl α-glucoside in our experiments.

Since methyl α-glucoside is not a competitive inhibitor of sorbitol phosphorylation at the level of Enzyme II for sorbitol (data not shown), inhibition of sorbitol uptake may be dependent on the glucose PTS and require transport and phosphorylation of the inhibitor (glucose or methyl α-glucoside), i.e. either hexose phosphate is the regulator or inhibition is a consequence of hexose transport itself. Glucose 6-phosphate significantly inhibited sorbitol phosphorylation by permeabilized cells only when added at concentrations of 1 mM and above, thus effectively excluding it as an inhibitor in our experiments (data not shown).

The glucose and sorbitol phosphotransferase systems share a common energy source, PEP, and inhibition of the sorbitol system by hexose may be the result of competition for this phosphate donor. The inhibition of sorbitol phosphorylation by methyl α -glucoside as a function of PEP concentration was investigated using permeabilized cells. Inhibition was maximal when the PEP concentration was low and limiting for the phosphorylation rate. When PEP was present at saturating concentrations, methyl α -glucoside had little effect. Kinetic analysis showed that the inhibition was competitive at the level of PEP (Fig. 3). It is therefore clear that the two phosphotransferases compete for a common supply of PEP, and the intracellular concentration of PEP will thus be of critical importance in determining the sensitivity of the sorbitol PTS to glucose. The competition is probably a major factor in the regulation of sorbitol utilization by glucose in *C. pasteurianum*.

We attempted to demonstrate inhibition of sorbitol phosphorylation by methyl α -glucoside using cell-free extracts. When extracts were prepared by standard techniques (see Methods) the glucoside had no effect on the sorbitol PTS. However, glucose PTS activity in extracts of *C. pasteurianum* is not limited by the concentration of PEP down to 25 μ M (Mitchell & Booth, 1984) and the same is true for the sorbitol PTS (data not shown). In order to see an effect of methyl α -glucoside it is necessary to generate conditions in which PEP does limit the phosphorylation rate. This can be achieved by using sufficient soluble extract to saturate membranes in the PTS assay. When soluble extracts from both glucose- and sorbitol-grown cells were present at saturating levels, methyl α -glucoside did inhibit sorbitol phosphorylation, and once again the degree of inhibition varied with PEP concentration (Table 2). Comparison of the effect of methyl α -glucoside in the presence of different amounts of sorbitol soluble extract shows that significant inhibition occurs only when the rate of sorbitol phosphorylation is limited by the PEP concentration. The results cannot be explained by competition between the two phosphotransferases for a common, rate-limiting PTS protein.

DISCUSSION

We have examined the inhibition of sorbitol metabolism by glucose in the obligate anaerobe *C. pasteurianum*. At least three proteins or enzymes specific to sorbitol metabolism were induced in the presence of the substrate: two components of the PTS (one soluble and one membrane-bound) and the enzyme sorbitol-6-phosphate dehydrogenase. Although the sorbitol PTS has previously been reported to be constitutive, activity in glucose-grown cells was extremely low (Booth & Morris, 1982). We also found that soluble and membrane components of the sorbitol PTS were absent, or present at extremely low levels in glucose-grown cells.

One mechanism by which glucose prevents metabolism of sorbitol is by exclusion of the hexitol from the cell. Both carbon sources are accumulated via a PTS mechanism (Booth & Morris, 1982), and the transport systems compete for a common phosphate and energy supply in the form of PEP (Fig. 3). The PTS of *C. pasteurianum* has not been fully characterized, and it is therefore not possible to identify the level at which competition between the glucose and sorbitol systems occurs. Similar interaction between phosphotransferases has been found in other bacteria. In *E. coli* and *S. typhimurium* competition between Enzyme II complexes for the common phosphoryl donor phospho-HPr has been identified as a physiologically significant mechanism for inhibition of uptake of carbohydrates (Dills *et al.*, 1980; Scholte & Postma, 1981). In addition, Dills & Seno (1983) have shown that in the Gram-positive bacterium *Streptococcus mutans*, uptake of hexitols is inhibited by glucose and 2-deoxyglucose by a mechanism which is consistent with competition for phospho-HPr. The exclusion of hexitols from the cell resulted in repression of hexitol metabolic systems.

The physiological significance of the competition mechanism can be seen by comparison of the effect of glucose on cells which are induced or uninduced for sorbitol metabolism (Saier, 1985). The strongest inhibition will be exhibited when the Enzyme II for glucose is induced while that for sorbitol is not, since the sorbitol PTS will be totally starved of PEP. When the sorbitol Enzyme II is induced and glucose Enzyme II is present at reduced levels, inhibition will be less severe. The extent of inhibition of sorbitol utilization by growing cells is reduced when

the cells have been previously exposed to the hexitol (Mitchell *et al.*, 1987). In addition, the energy status of the cell, reflected in the intracellular concentration of PEP, will be important in determining the effect of glucose on sorbitol uptake. Our experiments using resting cell suspensions failed to show inhibition of sorbitol uptake by glucose, apparently because the cells contained sufficient PEP to support sorbitol uptake in the presence of glucose (Fig. 2). Booth & Morris (1982) reported inhibition of sorbitol uptake by glucose in resting whole cells of *C. pasteurianum*, although glucose was present in a 100-fold concentration excess and may have inhibited sorbitol binding at the cell exterior. Inhibition of the sorbitol PTS by glucose 6-phosphate is not relevant in experiments involving permeabilized cells. However in intact cells an effect of hexose phosphate, which may be present in millimolar concentrations, cannot yet be excluded.

In the presence of both glucose and sorbitol in the growth medium, glucose prevented induction of the sorbitol PTS until late in the growth period (Fig. 1), but as the culture entered stationary phase, glucose repression was bypassed and the sorbitol PTS was expressed although the residual glucose concentration was still very high. The reason for induction or derepression of the sorbitol PTS by cells entering stationary phase, even in the presence of excess glucose, is not clear. Booth & Morris (1982) similarly reported that, at the onset of sporulation, the activity of a number of transport systems increased. For the PTS sugars glucose and fructose, this occurred irrespective of whether the substrate was present in the growth medium. Induction of the sorbitol PTS was dependent on sorbitol, as shown by the lack of activity in a glucose-only culture (Table 1).

As for the sorbitol PTS, sorbitol 6-phosphate dehydrogenase was induced by sorbitol but was not present in cells grown on any other carbon source tested. The effects of glucose on expression of the dehydrogenase and the sorbitol PTS were, however, quite different (Fig. 1). Further studies are necessary in order to examine the organization of the genes of the sorbitol regulon in *C. pasteurianum* and to provide a more complete understanding of the mechanisms involved in regulation of their expression.

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REFERENCES

- BOOTH, I. R. & MORRIS, J. G. (1975). Proton-motive force in the obligately anaerobic bacterium *Clostridium pasteurianum*: a role in galactose and gluconate uptake. *FEBS Letters* **59**, 153-157.
- BOOTH, I. R. & MORRIS, J. G. (1982). Carbohydrate transport in *Clostridium pasteurianum*. *Bioscience Reports* **2**, 47-53.
- BOTSFORD, J. L. (1981). Cyclic nucleotides in prokaryotes. *Microbiological Reviews* **45**, 620-642.
- DILLS, S. S. & SENO, S. (1983). Regulation of hexitol catabolism in *Streptococcus mutans*. *Journal of Bacteriology* **153**, 861-866.
- DILLS, S. S., APPERSON, A., SCHMIDT, M. R. & SAIER, M. H., JR (1980). Carbohydrate transport in bacteria. *Microbiological Reviews* **44**, 385-418.
- GRENIER, F. C., HAYWARD, I., NOVOTNY, M. J., LEONARD, J. E. & SAIER, M. H., JR (1985). Identification of the phosphocarrier protein enzyme $\text{III}^{\text{P}}_{\text{Hsc}}$: essential component of the glucitol phosphotransferase system in *Salmonella typhimurium*. *Journal of Bacteriology* **161**, 1017-1022.
- MACKEY, B. M. & MORRIS, J. G. (1971). Ultrastructural changes during sporulation of *Clostridium pasteurianum*. *Journal of General Microbiology* **66**, 1-13.
- MAGASANIK, B. (1970). Glucose effects: inducer exclusion and repression. In *The Lactose Operon*, pp. 189-219. Edited by J. R. Beckwith & D. Zipser. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.
- MITCHELL, W. J. & BOOTH, I. R. (1984). Characterization of the *Clostridium pasteurianum* phosphotransferase system. *Journal of General Microbiology* **130**, 2193-2200.
- MITCHELL, W. J., ROOHI, M. S. & MORRISON, D. (1985). Regulation of sorbitol metabolism in *Clostridium pasteurianum*. *Biochemical Society Transactions* **13**, 936-937.
- MITCHELL, W. J., ROOHI, M. S., MOSELY, M. J. & BOOTH, I. R. (1987). Regulation of carbohydrate utilization in *Clostridium pasteurianum*. *Journal of General Microbiology* **133**, 31-36.
- POSTMA, P. W. & LENGELER, J. W. (1985). Phosphoenolpyruvate: carbohydrate phosphotransferase system of bacteria. *Microbiological Reviews* **49**, 232-269.
- SAIER, M. H., JR (1985). *Mechanisms and Regulation of Carbohydrate Transport in Bacteria*, pp. 110-113. Orlando: Academic Press.

- SAIER, M. H., JR & MOCZYDLOWSKI, E. G. (1978). The regulation of carbohydrate transport in *Escherichia coli* and *Salmonella typhimurium*. In *Bacterial Transport*, pp. 103–125. Edited by B. P. Rosen. New York: Marcel Dekker.
- SAIER, M. H., JR & ROSEMAN, S. (1976). Sugar transport. Inducer exclusion and regulation of the melibiose, maltose, glycerol, and lactose transport systems by the phosphoenolpyruvate:sugar phosphotransferase system. *Journal of Biological Chemistry* **251**, 6606–6615.
- SCHOLTE, B. J. & POSTMA, P. W. (1981). Competition between two pathways for sugar uptake by the phosphoenolpyruvate-dependent sugar phosphotransferase system in *Salmonella typhimurium*. *European Journal of Biochemistry* **114**, 51–58.
- SETLOW, P. & SACKS, L. E. (1983). Cyclic AMP is not detectable in *Clostridium perfringens*. *Canadian Journal of Microbiology* **29**, 1228–1230.
- ZAMENHOF, S. (1957). Preparation and assay of deoxyribonucleic acid from animal tissue. *Methods in Enzymology* **3**, 696–704.