INFECTION AND IMMUNITY, Feb. 1984, p. 536–542
0019-9567/84/020536-07$02.00/0

Vol. 43, No. 2

Role of the Phosphoenolpyruvate-Dependent Glucose Phosphotransferase System of *Streptococcus mutans* GS5 in the Regulation of Lactose Uptake†

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Received 2 May 1983/Accepted 28 October 1983

When *Streptococcus mutans* GS5 was grown in equimolar (5 mM) amounts of glucose and lactose, a classical diauxic growth curve was obtained. Glucose was taken up during the first growth phase, followed by a 60-min lag, and then lactose was transported. Synthesis of lactose phosphotransferase system (PTS) enzymes was repressed until the complete exhaustion of glucose, indicative of an inducer exclusion mechanism of repression. The enzyme phospho-β-galactosidase, however, was found in small amounts even in the presence of glucose. Repression was not observed when GS5 was grown in equimolar amounts of fructose and lactose. Although fructose was taken up preferentially, synthesis of the lactose PTS occurred from the onset of growth in these sugars. It is proposed that a component of the glucose PTS may be a regulatory factor in lactose transport. Glucose PTS$^-$ mutants did not display diauxic growth in glucose-lactose mixtures and, in fact, transported the disaccharide preferentially.

The underlying mechanisms of regulation exerted by the phosphoenolpyruvate (PEP)-dependent glucose phosphotransferase system (glc-PTS) on potential substrates involve inhibition of transport as well as repression of gene expression for key metabolic enzymes. These mechanisms are termed inducer exclusion and catabolite repression, respectively, and have long been recognized as being the result of PTS-mediated uptake affecting the metabolism of non-PTS sugars (17). Saier has proposed a unifying hypothesis to explain these two distinct regulatory mechanisms (20). He proposes that key PTS proteins also function as regulators of adenylate cyclase and the non-PTS permeases by means of a phosphorylation-dephosphorylation mechanism. Central to this scheme is the enzyme III (EIII) of the high-affinity glc-PTS of enteric species which is suggested to be the regulatory protein linking PTS-mediated transport with regulation of transport of non-PTS sugars (20, 21).

More poorly understood, however, is the preferential utilization by enteric species of glucose over other PTS substrates, such as sorbitol (16) and fructose (9). Since all major sugars catabolized by *Streptococcus mutans* are translocated by PTS (7, 9), the physiological basis for the preferential utilization of one PTS substrate over another is relevant to our understanding of transport regulation in this species. A regulatory role for the glc-PTS of *S. mutans* has been proposed, but has been somewhat controversial. Hamilton and Lo (7) observed a diauxic effect when this species was grown in the presence of glucose and lactose, resulting from the apparent inhibition of the induction of the lactose (lac) PTS by glucose. However, this "glucose effect" was atypical since it was dependent on the glucose concentration. Slee and Tanzer (25) also demonstrated biphasic growth when *S. mutans* was grown in mixtures of glucose and sucrose. Recently, Dills and Seno (4) found that glucose repressed the utilization of sorbitol and mannitol by *S. mutans* 6715. They suggested inducer exclusion to be the primary mechanism of repression and demonstrated a classical diauxic growth curve to support this premise. However, other sugars (i.e., fructose and sucrose), besides glucose, also repressed hexitol uptake, thereby making the unique regulatory role of the glc-PTS less clear for this particular system.

In the present report, we describe the regulation of lactose uptake by the glc-PTS of *S. mutans* GS-5. A diauxic growth pattern is demonstrated which is independent of the glucose concentration and is apparently the result of the repressible synthesis of the lac-PTS. Biphasic growth is not exhibited by cultures growing in fructose plus lactose, which indicates that the constitutive fructose PTS is non-regulatory for transport of this dissacharide. glc-PTS$^-$ mutants, known to be deficient in EII^{glc}, do not repress lactose transport in the presence of glucose, clearly supporting a major regulatory role in sugar transport for the glc-PTS of this important cariogenic species.

(This work was presented in part at the 82nd Annual Meeting of the American Society for Microbiology, 7 to 12 March 1982, in Atlanta, Ga.)

MATERIALS AND METHODS

Cultures and cell growth. Cells of *S. mutans* GS5 were routinely maintained in a tryptone-yeast extract (TYE) broth (7). This medium was composed of 10 g of tryptone (Difco Laboratories, Detroit, Mich.), 5 g of yeast extract (Difco), and 3 g of dibasic potassium phosphate per liter. For maintenance, this medium was supplemented with 20 mM glucose.

Growth curves. Cells were initially grown in a defined medium (DM; 26) or TYE supplemented with 5 or 20 mM sugar. At midlogarithmic phase, cells (5 to 10% inocula) were transferred to fresh media supplemented with appropriate sugars at 5 mM concentrations. Incubation was carried out at 37°C in a candle jar, and growth was monitored with a Klett-Summerson photoelectric colorimeter, using a red filter no. 66 (Klett Manufacturing Co., Inc., New York, N.Y.), or at 600 nm with a Gilford 2600 spectrophotometer (Gilford Instruments, Inc., Oberlin, Ohio).

Sugar determinations in spent medium. Lactose was mea-

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† Florida Agricultural Experiment Station Journal Series no. 4687.

sured with the Boehringer Mannheim lactose/galactose kit and fructose was determined with the glucose/fructose kit (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). The glucose determination was by the glucose oxidase method, using the Sigma diagnostic kit (Sigma Chemical Co., St. Louis, Mo.).

Determinations of radiolabeled lactose uptake. For the measurement of uptake of radioactive lactose, cells were pregrown at 37°C in TYE plus 20 mM mannitol, harvested, and concentrated two times, and then 1 ml was transferred to Klett tubes containing TYE (10 ml) plus 5 mM glucose and 4.9 mM lactose, of which 0.2 mM was [^{14}C]lactose (0.97 μCi/μmol). Incubation was continued at 37°C. At specified times, absorbancy readings were taken, and 0.1-ml aliquots were removed for filtration through 0.2-μm membrane filters (Gelman Laboratory Filtration Products, Ann Arbor, Mich.). The filters were washed by passing several volumes of water through the system by suction and then transferred to 5 ml of scintillation fluor (Aquasol; New England Nuclear, Boston, Mass.) for counting in a Beckman LS8000 scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.).

Cell preparation for assays. For assay purposes, the cells were grown overnight at 37°C in TYE broth plus a 20 mM concentration of a given sugar. These cells were transferred to DM containing the sugar (5 mM) to be assayed. A 10% inoculum size was routinely used. Unless otherwise specified, cells were allowed to grow until late log or early stationary phase at 37°C in the DM, after which they were harvested by centrifugation at 12,000 × *g* for 10 min and washed once in 100 mM sodium phosphate (PB), pH 7.0, plus 5 mM $MgCl_2$. The centrifuge used for routine purposes was a Sorvall RC-5B (Dupont Instruments, Newton, Conn.).

In many of the assays outlined below, decryptified cells were used. The decryptification conditions were as follows: washed cells were resuspended in PB, pH 7.0, plus 5 mM $MgCl_2$ to one-tenth their original volume. A toluene-acetone mixture (1:3, vol/vol) was used to permeabilize the cells. The mixture was added in a ratio of 100 μl to each 1 ml of cell suspension, and decryptification was carried out by vigorously shaking on a Vortex mixer (Scientific Industries) for 1- to 2-min intervals interspersed with cooling in ice. The total duration of shaking was 3 to 5 min. This methodology was based on that used by Calmes (1) for *S. mutans*. If a further dilution was required, 100 mM PB plus 5 mM $MgCl_2$ was added after decryptification to achieve the desired cell concentration.

PTS and phospho-β-galactosidase assay. The assay for the determination of the lac-PTS utilized the lactose analog *o*-nitrophenyl-β-galactoside (ONPG; Sigma Chemical Co.). The standard reaction mixture contained 100 mM PB (pH 7.0), 5 mM $MgCl_2$, 8 mM sodium PEP (Sigma Chemical Co.), 8 mM ONPG, and decryptified cells (concentrations provided in footnotes to tables and figure legends). The total volume was 0.6 ml. This was incubated for 30 min at 37°C, after which the reaction was stopped by the addition of 1 ml of a 5% Na_2CO_3 solution (aqueous). The cells were centrifuged in a Sorvall table top centrifuge (Dupont Instruments), and the amount of *o*-nitrophenol (ONP) formed was determined by reading the absorbancy of the supernatant at 420 nm in a Gilford 2600 recording spectrophotometer. The amount of ONP was then determined from a standard curve, and the results were expressed in terms of picomoles of ONP per microgram (dry weight) of cells.

For the determination of phospho-β-galactosidase activity, the lactose-phosphate analog *o*-nitrophenyl-β-galactose-6-phosphate (ONPG-6-P; Sigma Chemical Co.) was used. Unless otherwise specified, the reaction mixture contained 100 mM PB (pH 7.0), 5 mM $MgCl_2$, 10 mM ONPG-6-P, and decryptified cells in a total volume of 0.5 ml. After 30 min at 37°C the reaction was terminated with 5% Na_2CO_3, the cells were removed by centrifugation, and the absorbancy of the supernatant was determined at 420 nm. The results were expressed as picomoles of ONP formed per microgram (dry weight) of cells. The assay for the lac-PTS was based on that described by Calmes (1), and that for the phospho-β-galactosidase was described by Calmes and Brown (2).

Determination of phosphorylation of radiolabeled sugars. For the determination of phosphorylation of radioactive sugars, the following conditions were used. In a total reaction volume of 200 μl, 60 mM PB (pH 7.0), 3.0 mM $MgCl_2$, 10 mM PEP, 10 mM sodium fluoride (Sigma Chemical Co.), and the specific isotopes (New England Nuclear) were reacted with 18 to 20 μg of decryptified cells. Cell dry weights were determined from a standard curve constructed by first measuring the absorbancy of suspensions at 600 nm before gravimetric measurements were made. After a 30-min incubation at 25°C, the reactions were stopped by diluting 100 to 200 μl of the reaction mixture 1:9 (vol/vol) in 1% "cold" sugar followed by rapid cooling in an ice bath. From this, the phosphorylated derivative was separated from the labeled substrate by filtration under vacuum through a DE-81 anion-exchange filter (Whatman, Clifton, N.J.) which had been prewashed with a 1% solution of underivatized sugar. The filters were washed one time with 1% sugar and four times with cold water. They were then counted in a Beckman LS8000 scintillation counter. Controls were run routinely, using reaction suspensions lacking PEP to determine non-PTS-associated uptake of label. These counts were never greater than 10% of the experimental samples. This assay is based on the methodology developed by Simoni et al. (24). The scintillation fluor used was Aquasol (5 ml).

Mutagenesis and mutant selection. A modification of the methanesulfonic acid ethyl ester (Sigma Chemical Co.) mutagenesis procedure developed by Shanmugam and Valentine (23) was used to mutagenize a culture of *S. mutans*. Cells were grown in DM plus 20 mM glucose. At early log phase (Klett = 34), 0.1 ml of methanesulfonic acid ethyl ester was added and the incubation of cells was continued for 60 min at 37°C. At the end of this time period, the cells were harvested at ambient temperture, washed once with sugar-free DM, and sonicated for 30 s. The volume was brought up to 10 ml with sugar-free medium, and the treated cells were incubated at 48°C for 40 min. The cells were pelleted at ambient temperature and resuspended in a small volume of TYE before sonicating for 30 s. The volume was brought up to 10 ml with TYE supplemented with 20 mM glucosamine, and the culture was incubated until growth resumed. At this time, streptozotocin was added to select for glc-PTS$^-$ mutants according to the procedure of Lengeler (15). Streptozotocin was added so that the final concentration of this glucose analog was 50 μg/ml, and incubation at 37°C proceeded for 5 h. The cells were harvested, washed once with sugar-free medium, and then allowed to grow overnight in TYE supplemented with 20 mM lactose. The cells were then transferred to fresh TYE supplemented with glucosamine, and at early log phase (Klett = 35) streptozotocin was added to yield a final concentration of 50 μg/ml. After 5 h at 37°C, the cells were harvested, washed once with sugar-free medium, and plated on TYE supplemented with 20 mM lactose plus 20 mM 2-deoxyglucose. After 48 h, the plates were replicated on TYE containing 20 mM glucosamine or 20 mM lactose. Colonies that grew on lactose but not

TABLE 1. lac-PTS and phospho-β-galactosidase activity in lactose- versus glucose-grown cells of *S. mutans* GS5[a]

Growth substrate	pmol of ONP produced/μg of cells		
	ONPG + PEP	ONPG − PEP	ONPG-6-P
Lactose	1.10	0.46	47.50
Glucose	0	0	3.40

[a] Cells were grown in DM plus 5 mM sugar to stationary phase, harvested, washed, and decryptified. Cells were resuspended in 1 ml of 100 mM PB, pH 7.0, and assayed as described in the text. Final cell concentrations were 52 to 81 μg/ml for these assays.

on glucosamine were patched onto lactose–2-deoxyglucose-containing TYE agar. These plates were finally replicated onto TYE plates containing either 20 mM glucosamine or 20 mM lactose. Colonies were chosen which grew only on lactose. These colonies were grown in TYE broth plus 20 mM mannitol, and the resultant cultures were stored in glycerol at −40°C.

Chemicals. Glucose, glucosamine, 2-deoxyglucose, and lactose were purchased from Sigma Chemical Co. Fructose and mannitol were obtained from Calbiochem-Behring (La-Jolla, Calif.). The purity of these products was determined by gas-liquid chromatography as described previously (6). The following isotopes were purchased from New England Nuclear: D-[*u*-^{14}C]fructose (359 mCi/mmol) and [*u*-^{14}C]lactose (0.97 mCi/mmol).

RESULTS

lac-PTS and phospho-β-galactosidase activities in lactose- and glucose-grown cells. The inducibility of the lac-PTS in *S. mutans* has been reported elsewhere (1, 2, 7). Table 1 demonstrates the inducible or repressible nature, or both, of the lac-PTS of strain GS-5. Cells grown in lactose show measurable levels of ONPG hydrolysis in the presence of PEP indicative of the lac-PTS, as well as ONPG-6-P hydrolysis which indicates an active phospho-β-galactosidase. The level of activity with ONPG as a PTS substrate, however, is low and likely reflects a weak affinity for this non-metabolizable analog.

As others also have demonstrated (1, 7), some activity can be detected when ONPG is assayed in the absence of PEP (Table 1). This result was highly variable in our hands, with the listed value representing the highest activity obtained for this control. Since sodium fluoride was not included in this particular assay, we could not distinguish between the presence of an endogenous PEP reserve, as has been shown in other studies of lactic microorganisms (3, 27), and the possible presence of a β-galactosidase able to react with ONPG. We believe the former is a better possibility because exogenous PEP stimulated ONP production approximately 2.5-fold, strongly indicating that a PTS was being measured in lactose-grown cells. To confirm these findings, we examined the inducibility of the lac-PTS, using the lactate dehydrogenase/NADH-linked assay of Kornberg and Reeves (12), and found that only lactose- and galactose-grown GS5 cells possessed the lac-PTS. Glucose- and mannitol-grown cells lacked this transport activity although they were able to transport glucose by a constitutive PTS (data not shown).

Whereas glucose-grown cells are repressed for the lac-PTS as shown by inactivity in the presence of ONPG plus PEP, we consistently measured small but significant levels of phospho-β-galactosidase in these cells (Table 1). This indicates a "leaky" control on the regulation of synthesis of this enzyme in *S. mutans*. A similar result for *Lactobacillus casei* has been reported by Chassy and Thompson (3).

Regulation of the lac-PTS by glucose. If noninduced cells are grown in a medium containing glucose and lactose, a biphasic growth pattern is observed (Fig. 1). The first phase of growth coincides with the uptake of glucose, whereas the second phase of growth coincides with the uptake of lactose. These two phases of growth are separated by a 60-min lag period. Glucose is completely exhausted from the medium

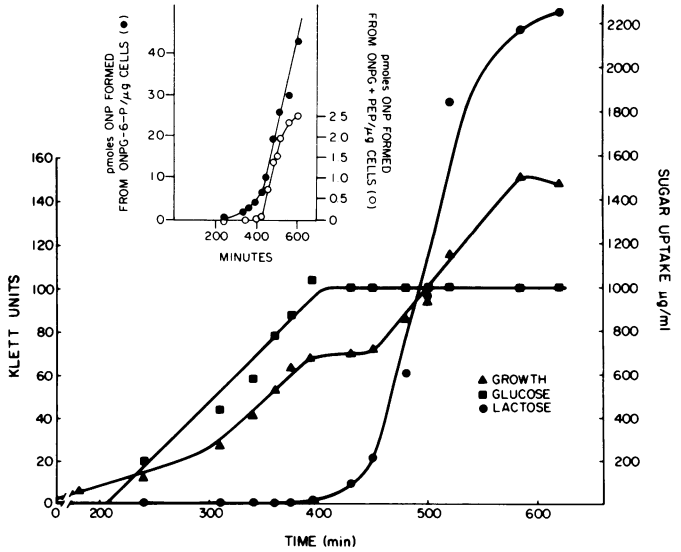

FIG. 1. Diauxic growth patterns by *S. mutans* GS5, using glucose and lactose substrates. Log-phase cells (10 ml) grown in DM plus 5 mM glucose were harvested, washed with sterile water, resuspended in 200 ml of DM plus 5 mM glucose and 5 mM lactose, and incubated at 37°C. At specified times, 10-ml aliquots were removed for optical density readings on the Klett colorimeter (▲). The cells were centrifuged and washed once with 10 ml of 100 mM PB (pH 7.0) plus 5 mM $MgCl_2$, and dry weights were determined. Cells then were resuspended in 1 ml of buffer and decryptified. Assays for the lac-PTS and phospho-β-galactosidase (inset) were done with ONPG + PEP (○) and ONPG-6-P (●), respectively. The volume of cells used for these assays was 50 μl. The spent medium from the original centrifugation was assayed for glucose (■) and lactose (●) as described in the text.

before lactose is taken up by the cells, and lactose uptake is preceded by a typical diauxic lag period. The lag period usually is indicative of the induction of enzymes required for uptake of the second sugar. This appears to be the case in these cells. The induction of the lac-PTS, as measured with the chromogenic analog ONPG, is concomitant with the second phase of growth and, furthermore, is not evident until the lag phase is complete (inset, Fig. 1). Its specific activity then increases coincident with the second phase of growth. A curve identical to that shown in Fig. 1 (inset) was generated when the lac-PTS was measured by using the Kornberg and Reeves assay (data not shown). As would be predicted from Table 1, phospho-β-galactosidase appears during the first growth phase in glucose, as its synthesis appears to precede the appearance of the lac-PTS. The possibility exists that one of its products, galactose-6-phosphate, is the inducer of the lac-PTS as has been proposed for *Staphylococcus aureus* (18).

Although glucose prevents induction of the lac-PTS in *S. mutans*, it does not appear to regulate lactose metabolism once the lactose-dissimilating enzymes are present. This can be seen from the data in Table 2, where lactose-grown cells were grown in a glucose-lactose mixture. Even though glucose uptake precedes lactose uptake upon growth initiation, once log-phase growth commences both are utilized in parallel. The slightly higher than expected concentration of lactose found in the medium most likely reflects carry-over from the inoculum.

One interesting point is the apparent expulsion of glucose from cells at 450 min (Table 2). This was a reproducible observation. Reizer and Panos (19) have shown that *Streptococcus pyogenes* exhibits a phenomenon they termed "inducer expulsion." When these cells are allowed to transport thiomethyl-β-D-galactopyranoside (TMG), thereby forming phosphorylated TMG, and are presented with a metabolizable sugar such as glucose, free TMG is expelled. However,

TABLE 2. Growth and sugar uptake by cells of *S. mutans* GS5 induced for lactose dissimilation in medium supplemented with lactose and glucose[a]

Time (min)	Klett units	Disappearance of sugar from medium (mM)[b]	
		Lactose	Glucose
0	7	0	0
180	15	0	1.0
240	22	0	1.4
300	38	1.2	1.9
345	52	0.5	1.5
370	66	1.8	2.0
390	83	2.5	2.5
430	115	3.9	3.6
450	137	4.8	1.7
540	175	6.5	3.3
575	185	6.6	4.1
595	186	ND[c]	ND

[a] Cells were grown in 10 ml of DM supplemented with 5 mM lactose to log phase. This culture was transferred to 100 ml of DM containing 5 mM lactose plus 5 mM glucose and incubated at 37°C in a candle jar. At specified times, 5-ml samples were removed to measure Klett units. Cells were removed from this sample by centrifugation, and the lactose and glucose concentrations in the spent medium were determined according to the text.

[b] The concentration determined at each point was subtracted from the starting concentration and this difference is the expressed result.

[c] ND, Not determined.

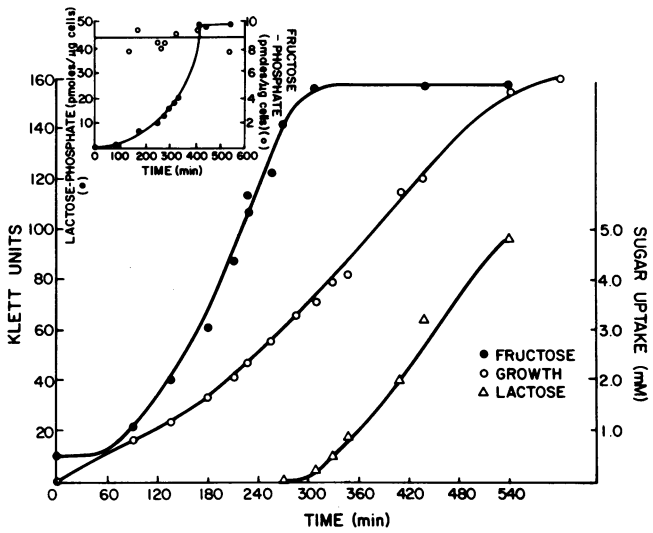

FIG. 2. Growth patterns displayed by *S. mutans* GS5 in fructose plus lactose. Log-phase cells, grown in DM plus 5.0 mM fructose (10 ml), were transferred directly to 90 ml of DM supplemented with 5 mM fructose plus 5 mM lactose and incubated at 37°C in a candle jar. At specified times aliquots were removed for optical density readings on the Klett colorimeter (○), and cells were removed by centrifugation. Spent-medium samples were assayed for fructose (●) and lactose (△) contents as described in the text. Cells were washed and dry weights were determined. To measure fructose PTS activity, washed cells were decryptified and suspended in 60 mM PB (pH 7.0)–3.0 mM $MgCl_2$–10 mM PEP–10 mM sodium fluoride–1.5 μM D-[*u*-^{14}C]fructose (359 μCi/μmol) to a final volume of 200 μl. The cell concentration was 18 to 20 μg/200 μl. The reaction proceeded for 30 min at 37°C and was stopped with excess cold fructose and filtered through a DE-81 filter. Labeled fructose phosphate (○) is expressed as picomoles per microgram of cells (inset). The lac-PTS was determined in a similar manner except the reaction mixture (200 μl) contained 210 μM [*u*-^{14}C]lactose (0.97 μCi/μmol) and the reaction was terminated in cold lactose. Labeled phosphorylated derivatives (●) are collectively expressed as picomoles per microgram of cells (inset). These are termed "lactose phosphate" for convenience but represent derivative compounds (e.g., galactose phosphate) as well.

in the case presented in Table 2 it is the metabolizable compound being excreted. A reasonable explanation may be that the free glucose, formed from the cleavage of lactose-phosphate to galactose-6-phosphate plus glucose, is being expelled in favor of the glucose-6-phosphate formed by the glc-PTS.

Lack of regulation of the lac-PTS by fructose. Figure 2 shows the results of growing noninduced cells in fructose and lactose. Growth does not appear to be biphasic, although a semilog plot of the data reveals two growth rates. The first rate is equal to a generation time of 82 min and is measurable during fructose uptake (0 to 310 min; Fig. 2); the second rate is equal to a generation time of 225 min and commences with lactose uptake (310 min; Fig. 2). Whereas lactose transport does not begin until fructose has been exhausted from the medium, a typical diauxic induction period is not evident. Indeed, lac-PTS induction occurs with the onset of growth (inset, Fig. 2). The fructose PTS does not vary in specific activity since it was maximized by prior growth in fructose. The conditions of the experiment caused variable carry-over of fructose with the cell inoculum, explaining the additional uptake of this sugar over lactose.

Loss of regulation of the lac-PTS by glc-PTS$^-$ mutants. To

verify the role of the glc-PTS in the regulation of lactose transport, glc-PTS$^-$ mutants were obtained and were compared with wild-type cells. These mutants have been found to lack EII^{glc} but possess normal levels of EI (Liberman and Bleiweis, manuscript in preparation). All cells were pregrown in TYE plus 20 mM mannitol, these conditions having been shown to be noninducive for the lac-PTS. Figure 3 presents the growth curves obtained when mutant strain 8B and the wild type were grown in glucose plus lactose. This mutant strain, and all others grown in a similar manner, exhibited a uniphasic growth pattern which was confirmed by plotting the data as a semilog graph (not shown). The mean generation time for 8B was calculated to be 72 min. As expected, wild-type cells exhibited biphasic growth separated by a short (20-min) lag period. Transformation of these data by semilog plots yielded two growth rates: 64 min followed by 120-min generation times.

Interestingly, the final cell density was the same for both the wild type and mutant strains. Recent studies (5, 8) have shown that *S. mutans* possesses an alternative glucose transport system which plays a significant physiological role under high glucose conditions. Thus, even glc-PTS$^-$ mutants would be expected to transport glucose. Strain 8B, furthermore, is slightly leaky for this genetic feature and possesses 13% of the normal glc-PTS activity (unpublished data).

Figure 4 demonstrates the unrepressed uptake of [^{14}C]lactose in the presence of 5 mM glucose by a tight glc-PTS$^-$ mutant (strain 3A) as compared with wild-type cells. Lactose uptake, after a short lag, was directly proportional to increasing cell density and proceeded at a rate of 40 cpm/Klett unit. Thus, even in the presence of glucose, the mutant transports lactose with little delay, indicating the absence of an inducer exclusion regulation of the lac-PTS. In the case of wild-type cells, however, lactose internalization is low (5.2 cpm/Klett unit) during the early stage of growth, and this repression is not relieved until the second phase of growth as would be predicted from Fig. 1. The second rate of lactose uptake is higher (19.4 cpm/Klett unit) and represents the major utilization of this sugar as a growth substrate. Growth curves (not shown) indicated uniphasic growth for mutant 3A (mean generation time, 109 min) and typical diauxic growth for the wild-type strain, with the lag occurring at 60 to 70 Klett units. The maximum growth obtained with the mutant was less than with the wild type. As measured in Klett units, the maximum absorbancy was 140 versus 170 for 3A and wild-type GS5, respectively. The cell densities achieved by the respective strains reflect the amount of glucose left in the spent media. In a separate experiment where glucose was measured, wild-type GS5 exhausted the glucose (5.0 mM) from the medium, whereas 2.2 mM remained after the cessation of growth of 3A. Restricted uptake of glucose by mutants may be explained by the observation of Hamilton and St. Martin (8) that the alternate glucose transport system is a low-affinity system.

DISCUSSION

The assignment of a regulatory role to the glc-PTS of *S. mutans* has been somewhat controversial. Hamilton and Lo (7) observed diauxie when strain Ingbritt was grown in an equimolar mixture of glucose and lactose. This effect, however, was leaky in that glucose-induced repression was concentration dependent. Thompson et al. (29) and Heller and Roschenthaler (10, 11) have argued in favor of a simple competition by the various PTS-linked permeases for the common PTS components (EI, HPr). Certain systems (e.g., glucose) would be more efficient than others (e.g., lactose), thereby causing an apparent inhibition of the less efficient transport system. The data presented here, however, support an argument in favor of a classical repression mechanism exerted by glucose on the synthesis of the lac-PTS of strain GS5 (Fig. 1). This regulatory role appears to be specific for glucose since fructose, although able to inhibit the transport of lactose, does not repress the synthesis of lac-PTS enzymes (Fig. 2).

Studies with glc-PTS$^-$ mutants support conclusions reached with the parent, wild-type strain. Such mutants do not exhibit biphasic growth in glucose-lactose mixtures (Fig. 3) and transport lactose actively even in the presence of equimolar glucose (Fig. 4), although glucose may enter the

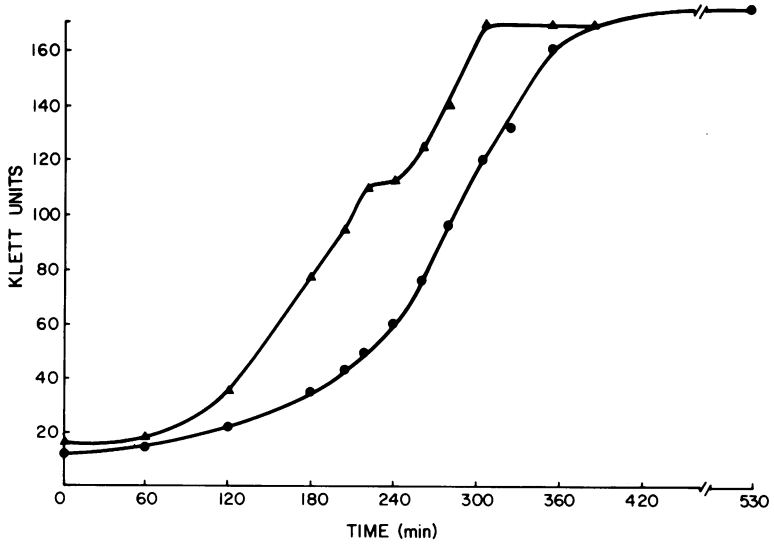

FIG. 3. Growth of *S. mutans* GS5 (wild type) and a glc-PTS$^-$ mutant in lactose plus glucose. Cells (wild type or glc-PTS$^-$ mutant strain 8B) were grown for 16 h in TYE plus 20 mM mannitol. Five percent inocula were used for growth in TYE supplemented with 5 mM glucose plus 5 mM lactose. Cultures were incubated at 37°C in a candle jar, and absorbancies (wild type, ▲; 8B, ●) were measured with a Klett colorimeter.

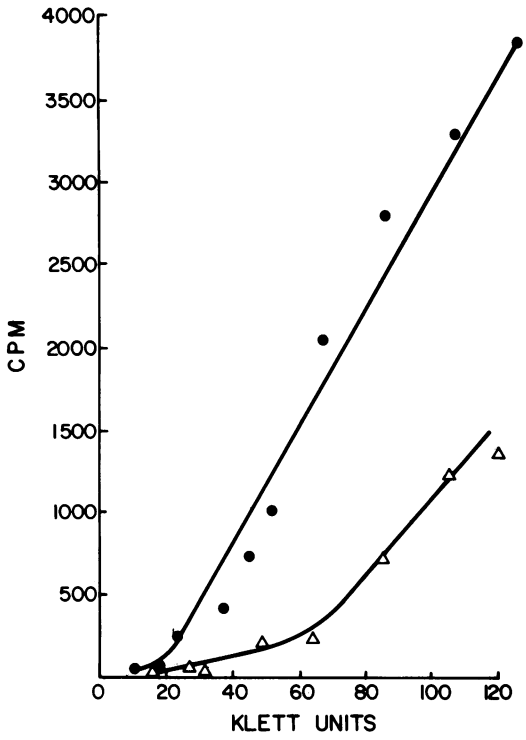

FIG. 4. Lactose uptake by *S. mutans* GS5 (wild type) and a glc-PTS$^-$ mutant as a function of cell density. Cells (wild type or glc-PTS$^-$ mutant strain 3A) were grown for 16 h in TYE plus 20 mM mannitol. After havesting, washing, and concentrating twofold, a 10% inoculum was used to initiate growth in 10 ml of TYE supplemented with 5 mM glucose plus 4.9 mM lactose (4.7 mM [^{12}C]lactose plus 0.2 mM [^{14}C]lactose [0.97 μCi/μmol]). Cultures were incubated at 37°C in a candle jar. At specified absorbancies as read on the Klett colorimeter, aliquots (0.1 ml) were removed for filtration to measure radioisotope uptake by cells (wild type, △; 3A, ●), expressed as counts per minute. Similar curves (not shown) were obtained when cells were harvested by centrifugation.

cell by an alternate route (8). Strain 3A is a "tight" mutant which fails to transport glucose and mannose by the glc-PTS but shows normal transport of fructose and mannitol by their respective PTS. This indicates that normal levels of EI and HPr are present in this clone, a characteristic recently confirmed by measuring cell-free extracts of 3A for the PEP-pyruvate exchange reaction (Liberman and Bleiweis, in preparation). Mutant strain 8B is leaky and still possesses 13% of the glc-PTS activity of the wild type. It too possesses normal levels of EI and HPr in cell-free extracts. When cell-free membranes of these mutants were prepared, strain 3A was found to lack the EII^{glc}, as determined by the glucose: glucose-6-phosphate transphosphorylation reaction, whereas strain 8B showed only 10% the activity of control (wild-type) membranes (Liberman and Bleiweis, in preparation). The major lesion in these mutants, therefore, appears to be the permease EII^{glc}. Since no information exists concerning the occurrence, molecular nature, or cellular localization of a putative EIII^{glc} in *S. mutans*, it is not presently possible to assign a regulatory role to any single molecular component of the glc-PTS. Such assignments await further investigations of the molecular biology of the streptococcal PTS along the lines taken by Kundig and Roseman (13, 14) in their classical studies of *Escherichia coli* or by Scholte et al. (22) with *Salmonella typhimurium*.

The similarity of the well-studied regulatory role of the *E. coli* glc-PTS to the *S. mutans* glc-PTS presently extends only to the observation that glucose is the preferred sugar in noninduced cells of both species. In *E. coli*, regulation is complex, involving distinct mechanisms (e.g., inducer exclusion versus catabolite repression) and distinct effector molecules such as cyclic AMP (21). Furthermore, regulation presumably involves the PTS functioning to control non-PTS-mediated transport (e.g., glucose versus lactose); thus, a role for EI and HPr can be ascribed. In *S. mutans*, regulation of one PTS over a second occurs. It should be noted here that, even though the focus of recent studies in *E. coli* has been on the PTS control over non-PTS-mediated transport, diauxie has been observed when cells are grown on two PTS substrates as in the cases of glucose plus fructose (9) and glucose plus sorbitol (16). Very little is known about this control mechanism, and its elucidation would probably aid in our understanding of regulation in *S. mutans*.

An attractive hypothesis has been put forth by Thompson et al. (29) to explain these apparent regulatory relationships. They propose that catabolite inhibition is responsible for the preferential utilization of glucose over other PTS sugars. That is, the affinity for HPr by the glucose-specific PTS proteins is greater than by the other sugar-specific PTS proteins (e.g., lactose). However, if this were the case a less stringent diauxie than that observed here (Fig. 1) would be expected. Indeed, Hamilton and Lo (7) demonstrated that diauxie in *S. mutans* Ingbritt was concentration dependent. That is, as the glucose concentration was lowered induction of the lac-PTS occurred. This would be an expected observation if competition between two PTS exhibiting differential affinities for a limited amount of HPr occurred, exactly as the catabolite inhibition theory proposes. However, the results presented in this report indicate that repression of synthesis rather than competitive inhibition is responsible since the lac-PTS is not induced until all of the glucose is exhausted. The necessity for the complete absence of glucose is supported by the definite lag period before lac-PTS activity is evident in a glucose-repressed culture. Furthermore, in cells preinduced for lactose, both glucose and lactose are utilized simultaneously (Table 2), suggesting that no such competition occurs.

Catabolite inhibition, however, may explain the results observed when fructose and lactose were present in the growth medium (Fig. 2). The lac-PTS was partially induced in the presence of fructose but did not function until fructose was exhausted; that is, lactose did not start disappearing from the medium until fructose was completely spent. However, since the lac-PTS already had been induced no diauxie occurred. These are exactly the conditions described by Thompson et al. (29) for the glucose preference over galactose in *S. lactis*, which they explained as being due to catabolite inhibition.

Recently, Thompson and Saier (28) and Chassy and Thompson (3) have reported an explusion mechanism in *Streptococcus lactis* and *L. casei* which results in the exclusion of TMG in the presence of glucose. Since TMG enters through the lac-PTS, these authors suggest that this mechanism may operate to allow the preferential utilization of glucose. Thompson and Saier (28) found evidence for a two-step process which involves a dephosphorylation of the lactose analog followed by the explusion of the underivatized form of this β-D-galactoside. If a similar mechanism is operating in *S. mutans* GS5, one would expect to detect free lactose accumulating in the medium when glucose was

present regardless of whether the cells are induced or noninduced. Table 2 clearly demonstrates that this is not the case when preinduced cells are used. One explanation for this may be that the phospho-β-galactosidase has a higher affinity for its substrate than does the putative phosphatase.

Finally, most investigators believe that regulation in streptococci is the result of inducer exclusion (4, 10, 11, 28); whether catabolite repression is also involved is unknown. Catabolite repression implies the presence of cyclic AMP, yet the evidence in favor of a role for cyclic AMP in gram-positive organisms is presently weak.

ACKNOWLEDGMENTS

We gratefully acknowledge Bruce M. Chassy of the National Institute of Dental Research (National Institutes of Health) and Keelnatham T. Shanmugam and Francis C. Davis of the University of Florida for advice during the course of these studies.

This work was supported by Public Health Service grant DE06051-01 from the National Institute of Dental Research, by grant BC-01440 from the Florida Agricultural Experiment Station, and by a President's Fellowship (to E.S.L.) from the American Society for Microbiology.

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