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Starvation-Induced Stimulation of Sugar Uptake in *Streptococcus mutans* Is Due to an Effect on the Activities of Preexisting Proteins of the Phosphotransferase System

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We examined the effects of sugar concentration in the medium on sugar uptake and phosphoenolpyruvate-dependent sugar phosphotransferase system (PTS) activities in *Streptococcus mutans* GS-5. Kinetic analyses of sucrose uptake in cells harvested under conditions of sucrose excess or sucrose limitation showed that increased uptake under the latter condition was almost completely due to an increase in the V_{max} of the high-affinity PTS. In a series of experiments in which cells growing under conditions of sucrose or glucose excess were shifted to a medium lacking sugar, starvation resulted in a stimulation of sugar uptake and a parallel increase in PTS activity. These starvation-induced increases in PTS-mediated uptake were not affected by the presence of either chloramphenicol or rifampin during the starvation period, indicating that neither protein nor RNA synthesis was necessary for the stimulation. In vivo labeling experiments with $^{32}P_i$ revealed that uptake stimulation during starvation was accompanied by a loss of acid-stable phosphate covalently bound to the phosphocarrier protein HPr of the PTS. We conclude, therefore, that stimulation of PTS-mediated uptake of sucrose and glucose during sugar limitation in *S. mutans* GS-5 is at least partially the result of increased activities of preexisting PTS proteins and that this may be due, at least in part, to dephosphorylation of a previously identified site in *S. mutans* HPr that can be phosphorylated by an ATP-dependent kinase.

Carbohydrate transport in anaerobic and facultatively anaerobic bacteria is often catalyzed by a phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) (5, 16, 19). In the aerotolerant anaerobe *Streptococcus mutans*, for example, glucose (20), sucrose (21, 22, 25), fructose and mannose (27), lactose (1), and mannitol (13) have all been shown to be taken up by a PTS. In addition, non-PTS modes of uptake for sucrose and glucose in *S. mutans*, possibly dependent on the proton motive force, have also been inferred (7, 10, 23, 24). The understanding of the mechanism of sugar transport and its regulation in *S. mutans* is important, especially for sucrose, since utilization of this disaccharide by *S. mutans* in the oral cavity is believed to be critical for its ability to initiate carious lesions in tooth enamel (9). For these reasons, we and others have been studying the biochemistry and regulation of the PTS in *S. mutans*, and we have very recently identified an ATP-dependent protein kinase in this organism which may be involved in regulation of carbohydrate uptake via the PTS (15).

Previous investigations have provided evidence that PTS-mediated phosphorylation of glucose (7) and sucrose (6, 23) in *S. mutans* is repressed under conditions of excess sugar availability and derepressed when sugar becomes limiting in the medium. In this report, we examined the effects of sugar concentration in the growth medium and starvation on sugar uptake and PTS activities in *S. mutans* GS-5. From our results, it is apparent that regulation of sucrose and glucose uptake by sugar availability is mediated, at least in part, by direct modulation of the activities of the PTS proteins involved rather than at the level of protein or RNA synthesis. We also present data that are consistent with the possible involvement in this regulation of an ATP-dependent kinase that phosphorylates the phosphocarrier protein HPr of the PTS in *S. mutans*.

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MATERIALS AND METHODS

Materials. [U-^{14}C]sucrose, [U-^{14}C]glucose, [4,5-^{3}H(N)]leucine, [5,6-^{3}H]uridine, and carrier-free $^{32}P_i$ were obtained from Dupont, NEN Research Products. All other chemicals were purchased from Sigma Chemical Co.

Bacterial strains and cell growth. *S. mutans* GS-5 (strain V843, serotype c) was provided by F. Macrina, and *S. mutans* NCTC 10449 (serotype c) was obtained from M. Stinson. These strains were maintained on TYE-MOPS agar plates, which consisted of tryptone (10 g/liter), yeast extract (5 g/liter), and agar (15 g/liter) (all from Difco Laboratories) containing, in addition, K_2HPO_4 (4 g/liter), KH_2PO_4 (1 g/liter), NaCl (2 g/liter), 0.1 mM $MgCl_2$, 0.1 M sodium morpholinepropanesulfonic acid (MOPS) (pH 7.2), and 20 mM sucrose. Cells were routinely transferred every week. For growth in liquid cultures, colonies were inoculated into 10 ml of TYE-MOPS broth, which had the same composition as above without the agar and were grown overnight at 37°C. This culture was then used to inoculate the final culture medium which consisted of TYE-MOPS broth containing either 5 or 50 mM sugar. Cells were grown at 37°C to either the midexponential phase (7 to 7.5 h; A_{550} of 0.35; final pH, 7.1) or to the early stationary phase (17 to 18 h; A_{550} of 1.1; final pH, 7.0). Cells were harvested at 4°C (15,000 × *g*, 10 min), washed twice with TDM buffer (50 mM Tris hydrochloride, 10 mM $MgCl_2$, 1 mM dithiothreitol [pH 7.5]), and then utilized for uptake experiments (see below).

For the starvation experiments, cells were grown to the midexponential phase on either 50 mM sucrose or glucose,

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harvested, washed twice with sterile TYE-MOPS broth without sugar, and suspended in the same medium. One half of the culture was used for uptake and PTS assays (see below) and was designated time zero. The other half was incubated at 37°C for various times and then harvested, washed, and assayed as above. In some experiments, chloramphenicol (final concentration, 35 μg/ml) or rifampin (final concentration, 50 μg/ml) was added at the beginning of the starvation period. Although these concentrations of these antibiotics have been shown to completely inhibit the growth of *S. mutans* (8; G. Jacobson, unpublished observations), we also directly determined their effects on protein and RNA synthesis, respectively. In these experiments midexponential-phase cells (100 ml) grown on 50 mM sucrose were prepared and suspended as above. To one 32-ml sample of the suspension was added 65 μCi of [^{3}H]leucine (60 Ci/mmol), and to a second 32-ml sample was added 65 μCi of [^{3}H]uridine (35 Ci/mmol). Each of these suspensions was split into thirds: to one was added 50 mM sucrose (unstarved control), to the second was added nothing (starved control), and to the third was added antibiotic as above (chloramphenicol to the [^{3}H]leucine-labeled cells and rifampin to the [^{3}H]uridine-labeled cells). The suspensions were then incubated for 2 h at 37°C, harvested, and washed twice with TDM buffer. To measure total protein synthesis, each [^{3}H]leucine-labeled pellet was suspended in 1 ml of 5% trichloroacetic acid; the samples were incubated at 90°C for 15 min, cooled to room temperature, and filtered through Whatman GF/A glass fiber filters to collect the precipitated protein. The filters were washed twice with 1 ml of 5% trichloroacetic acid and twice with 1 ml of 95% ethanol, air dried, and counted. Total RNA synthesis was estimated in the [^{3}H]uridine-labeled cells by suspension at 4°C of each cell pellet in 200 μl of buffer containing 50 mM glucose, 25 mM Tris hydrochloride (pH 8.0), and 10 mM EDTA and the addition of 400 μl of 0.2 M NaOH containing 1% sodium dodecyl sulfate, followed by 300 μl of 5 M potassium acetate (pH 4.8). Each sample was centrifuged (10 min, 10,000 × *g*), and 400 μl of the supernatant was extracted with 400 μl of phenol. To 300 μl of the aqueous phase was added 750 μl of ice-cold ethanol, and the samples were incubated overnight at −20°C and centrifuged at 4°C for 40 min at 10,000 × *g*. The pellets were suspended in H_2O and counted.

Assays. For uptake assays, *S. mutans* cells grown to the midexponential or stationary phase were harvested at 15,000 × *g* for 10 min at 4°C, washed twice with TDM buffer, suspended in TDM buffer at 4°C, and placed on ice for up to 2 h without detectable loss of uptake activity. Samples (5 ml) of the cell suspensions (0.4 to 0.8 mg [dry weight] of cells per ml) were used for uptake measurements. The concentration of cells for each experiment was determined by measuring the A_{550} of each suspension (an A_{550} of 1 corresponds to 0.25 mg [dry weight] of cells per ml). The cells were then prewarmed at 37°C for 5 min. Uptake was initiated by the addition of ^{14}C-labeled sugar followed by vigorous agitation. Samples (0.5 ml) were withdrawn every 15 s and filtered through a 0.45-μm nitrocellulose filter (type HAWP; Millipore Corp., Bedford, Mass.) which had been prewashed with TDM buffer. After two 5-ml washes with ice-cold TDM buffer, the filters were dried at 110°C for 30 to 40 min and then counted in a standard toluene–Triton X-100-based scintillation fluid.

For PTS assays, 1 ml of washed cells in TDM buffer (0.8 to 1.0 mg [dry weight] of cells per ml) was permeabilized by the addition of 10 μl of toluene followed by vigorous agitation for 45 s (7, 12). These cells were collected by centrifugation at 12,000 × *g*, washed twice with TDM buffer, and then treated with toluene as before. Unless otherwise indicated, PEP-dependent sugar phosphorylation was determined in mixtures (0.1 ml) containing 25 mM Tris hydrochloride (pH 8.0), 1 mM dithiothreitol, 5 mM $MgCl_2$, 10 mM KF, 0.01 M ^{14}C-labeled sugar (5 μCi/μmol), and the toluene-treated cells in the presence and absence of 1 mM PEP. Mixtures were incubated at 37°C for 15 to 20 min (when the reaction was still linear with time); then the reaction was stopped by the addition of 1 ml of ice-cold H_2O, and the solutions were filtered through DEAE-cellulose filter disks (Whatman, DE81) as described by Jacobson et al. (11). After three washes with 10-ml of ice-cold H_2O, the filters were air dried and counted in standard toluene-based scintillation fluid. PEP-dependent phosphorylation (PTS activity) was calculated as the difference between mixtures containing and lacking PEP in all cases.

In vivo ^{32}P labeling of *S. mutans*. *S. mutans* GS-5 was grown to the midexponential phase on low-phosphate tryptone-MOPS broth (TYE-MOPS broth lacking yeast extract, KH_2PO_4, and K_2HPO_4) containing 50 mM sucrose. The cells were harvested, washed with sterile growth medium, and finally suspended in 1 ml of low-phosphate tryptone-MOPS broth containing 50 mM sucrose. The final concentration of cells was 1.0 mg (dry weight) of cells per ml. To the suspension was added $^{32}P_i$ (final concentration, 0.25 mM [300 Ci/mol]), and the cells were incubated for 1 h at 37°C. The cells were then harvested (12,000 × *g*, 10 min, 4°C), washed free of any phosphate not incorporated, suspended in 10 ml of low-phosphate tryptone-MOPS broth lacking sugar, and incubated at 37°C. Samples (1 ml) were taken at various times, centrifuged at 12,000 × *g* at 4°C, washed with ice-cold TDM buffer, and suspended in 60 mM Tris hydrochloride (pH 6.8)–2% sodium dodecylsulfate–1% 2-mercaptoethanol–20 mM EDTA–10% glycerol–0.005% bromphenol blue. The mixture was boiled for 5 min, and then samples were applied to sodium dodecyl sulfate-polyacrylamide slab gels prepared by the method of Weber and Osborn (30). Gels were fixed in methanol-acetic acid-H_2O (5:1:5), dried, and exposed to Kodak X-Omat X-ray film at −70°C. Molecular weights of labeled bands were deduced from standards run in parallel and stained with Coomassie brilliant blue.

RESULTS

Kinetic characterization of sucrose uptake in *S. mutans* GS-5 grown under various conditions. Previous experiments by other workers (6, 21, 23, 25) have shown that sucrose PTS activity in *S. mutans* is low when cells are rapidly growing under sucrose-excess conditions, and that an increase in sucrose PTS activity occurs when cells are grown under sucrose-limited conditions or when the culture medium is depleted of sucrose. The initial rates of uptake of 25 μM sucrose by our laboratory strain of *S. mutans* GS-5 under sucrose-excess and sucrose-limited growth conditions are shown in Table 1. Uptake was lowest in cells grown with 50 mM sucrose and harvested at the midexponential phase (sucrose-excess conditions). In contrast, the initial uptake rate of sucrose in cells grown to the stationary phase on 5 mM sucrose (sucrose-depleted conditions) was nearly threefold greater. Cells grown to the midexponential phase on 5 mM sucrose or to the stationary phase on 50 mM sucrose showed an intermediate value. Thus, limitation of sucrose to *S. mutans* GS-5 either by lowering the sucrose concentration used for growth or by allowing the cells to reach the stationary phase resulted in significant increases in the

TABLE 1. Uptake of sucrose by *S. mutans* GS-5 at the midexponential and stationary phases[a]

Culture stage	Sucrose concn used for growth (mM)	Initial velocity[b]
Midexponential	5	10.0
Stationary	5	20.8
Midexponential	50	7.6
Stationary	50	12.0

[a] Uptake was measured at a [^{14}C]sucrose concentration of 25 μM as described in Materials and Methods.
[b] Expressed as nanomoles of sucrose taken up per minute per milligram (dry weight) of cells, measured 15 s after the addition of substrate.

sucrose uptake rate. Most likely, sucrose uptake in cells grown to the midexponential phase on 5 mM sucrose or to the stationary phase on 50 mM sucrose gave intermediate levels of sucrose uptake, since these conditions represent intermediate levels of sucrose limitation.

To determine whether a single transport system might be responsible for the stimulation in uptake that was seen in sucrose-limited cells, we determined the kinetic constants for sucrose uptake in cells grown under the conditions listed in Table 1 by using a concentration range of [^{14}C]sucrose of 10 μM to 1 mM. Kinetic data derived from least-squares analyses of Hanes plots (Table 2) revealed that under all growth conditions tested there appeared to be two sucrose uptake systems present. The K_m values for both the high and low affinity systems were not dramatically different in cells grown under sucrose-excess or sucrose-limited conditions. The most significant differences were seen in the V_{max} of the high affinity system, which increased over threefold in cells grown under sucrose-limited conditions (5 mM sucrose, stationary phase) relative to the V_{max} of sucrose-excess cells (50 mM sucrose, midexponential phase). In contrast, the V_{max} of the low affinity system did not change appreciably under all these conditions (Table 2). These changes in the maximal velocity of the high-affinity uptake system fully account for the stimulation of sucrose uptake activity measured at low sucrose concentrations that was seen under sucrose-limiting growth conditions (Table 1). As shown by others (23, 24), this system is a sucrose PTS.

Starvation-induced stimulation of sucrose uptake and PTS activities in *S. mutans* GS-5. The data presented above suggest that the sucrose PTS is utilized to transport sucrose under both sucrose-excess and sucrose-limited growth conditions, but that sucrose-limited conditions cause a marked increase in sucrose uptake at low substrate concentrations. The mechanism of this switch was investigated in a series of sucrose starvation experiments.

Sucrose uptake was measured in cells grown in excess sucrose (50 mM sucrose, midexponential phase) at 10 μM [^{14}C]sucrose. When the same cells were starved for 2 h at

TABLE 2. Kinetic constants for sucrose uptake under various growth conditions[a]

Sucrose concn (mM)	Phase	High affinity		Low affinity	
		K_m (μM)	V_{max}[b]	K_m (μM)	V_{max}[b]
50	Midexponential	70	23	232	100
50	Stationary	69	34	230	110
5	Midexponential	70	44	200	117
5	Stationary	50	72	200	134

[a] Calculated from least-squares analyses of Hanes plots.
[b] See footnote *b* of Table 1.

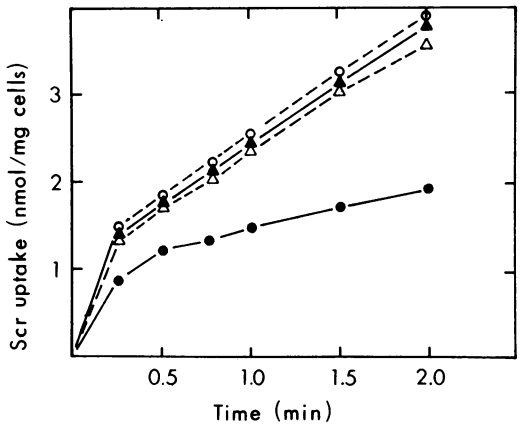

FIG. 1. Effect of sucrose starvation on sucrose uptake in *S. mutans* GS-5. Cells were grown to the midexponential phase on 50 mM sucrose and either assayed immediately or starved for 2 h before assay as described in Materials and Methods. The [^{14}C]sucrose concentration used for uptake was 10 μM. Symbols: (●) uptake by freshly harvested midexponential-phase cells, (○) uptake by sucrose-starved cells, (▲) uptake by sucrose-starved cells incubated with 35 μg of chloramphenicol per ml, (△) uptake by sucrose-starved cells incubated with 50 μg of rifampin per ml.

37°C and again assayed for sucrose uptake, significant increases in both initial and steady-state uptake rates for sucrose were seen (1.6-fold and 2.4-fold, respectively; Fig. 1). To test whether protein or RNA synthesis is required for this stimulation, identical starvation experiments were performed except that either chloramphenicol (35 μg/ml) or rifampin (50 μg/ml) was added during the starvation period. These antibiotics were found to be effective in inhibiting protein and RNA syntheses, respectively, occurring during the starvation period (Table 3). There was no significant effect of either inhibitor on the stimulatory effect of sucrose starvation (Fig. 1, Table 4). Moreover, PEP-dependent phosphorylation of sucrose in permeabilized cells increased about twofold upon sucrose starvation; this stimulatory effect also was not inhibited by the addition of either antibiotic (Table 4). Thus, the stimulation in sucrose PTS activity by starvation of *S. mutans* for sucrose is also not dependent on protein or RNA synthesis, suggesting that the components of the sucrose PTS are present under both rapid growth conditions and starvation, but that they are somehow regulated at the level of their activities by the availability of

TABLE 3. Effects of chloramphenicol and rifampin on protein and RNA syntheses during starvation of *S. mutans* GS-5

Culture[a]	cpm[b] in:	
	Protein	RNA
Unstarved (with 50 mM sucrose)	31,600 (100)	15,600 (100)
Starved (no sugar)	22,600 (71)	1,700 (11)
Starved plus chloramphenicol	5,300 (17)	
Starved plus rifampin		900 (6)

[a] Midexponential-phase cells were incubated for 2 h at 37°C under the indicated conditions. Protein and RNA syntheses occurring during this incubation period were determined as described in Materials and Methods.
[b] The specific molar amounts of [^{3}H]leucine and [^{3}H]uridine incorporated into protein and RNA respectively could not be determined since the medium used (TYE-MOPS) contains an unspecified amount of both compounds. The results therefore are expressed as counts per minute incorporated per 10-ml culture (see Materials and Methods) and are related to the unstarved control by the numbers in parentheses (percentage of control incorporation).

TABLE 4. Effects of starvation on sucrose uptake and PTS activities in *S. mutans* GS-5

Culture conditions	Uptake velocity[a]		PTS activity[b]
	Initial[c]	Steady state[d]	
Midexponential-phase cells[e]	4.0	0.48	0.52
Sucrose-starved cells[f]			
No addition	6.2	1.2	1.07
Plus chloramphenicol	6.1	1.1	1.05
Plus rifampin	6.1	1.1	1.06

[a] Uptake of 10 μM [^{14}C]sucrose expressed as nanomoles of sucrose taken up per minute per milligram (dry weight) of cells. Results are the means of four independent experiments.
[b] Expressed as nanomoles of sucrose phosphorylated per minute per milligram (dry weight) of cells measured in toluene-treated cells as described in Materials and Methods.
[c] Measured 15 s after the addition of substrate.
[d] Measured between 1 and 2 min after the addition of substrate.
[e] Grown on 50 mM sucrose.
[f] Cells were starved for 2 h at 37°C as described in Materials and Methods. Chloramphenicol (35 μg/ml) or rifampin (50 μg/ml) was added immediately before starvation.

sugar. A trivial explanation for these results, namely, that the increase in sucrose PTS activity upon starvation is due to increased intracellular PEP levels, can be excluded, since intracellular PEP was depleted before the assay by toluene treatment and washing and background PTS activities in the absence of PEP were ≤5% of those in its presence.

Thus, starvation induces increased sucrose uptake in *S. mutans* cells which can largely be accounted for by an increase in sucrose PTS activity that is independent of protein or RNA synthesis. Another *S. mutans* serotype c strain (NCTC 10449) showed nearly identical patterns of stimulation of sucrose uptake and PTS activities (data not shown), showing that these effects were not strain specific.

Effects of starvation on glucose uptake in *S. mutans* GS-5. Is the starvation-induced stimulation of sucrose uptake in *S. mutans* unique to this sugar, or do other sugars show this effect also? To answer this question, we carried out similar starvation experiments with glucose as the growth and uptake substrate. As with sucrose, cells grown to the midexponential phase on 50 mM glucose showed a severalfold lower uptake activity for glucose than did cells grown to the stationary phase on 5 mM glucose (data not shown). When midexponential-phase cells were harvested after growth on 50 mM glucose and subsequently starved for glucose for 2 h, again there was a significant increase in glucose uptake. Initial and steady-state uptake rates increased 2.3- and 2.9-fold respectively, and the addition of chloramphenicol or rifampin during the starvation period had little effect on the stimulation of glucose uptake (Fig. 2, Table 5). PEP-dependent phosphorylation of glucose was 2.4-fold greater in glucose-starved cells than in freshly harvested glucose-excess cells, and this stimulation was likewise insensitive to the addition of either antibiotic during the starvation period (Table 5). These results show that, like sucrose uptake, glucose uptake is stimulated in starved cells relative to freshly harvested cells and that this stimulation does not require either protein or RNA synthesis. Thus, sucrose and glucose uptake appear to be similarly regulated at the level of activity of one of the PTS components.

Correlation of starvation-induced uptake stimulation and in vivo phosphorylation of HPr. One target for regulation of PTS activity at the level of enzymatic activity could be the general PTS phosphocarrier protein HPr. It is now well

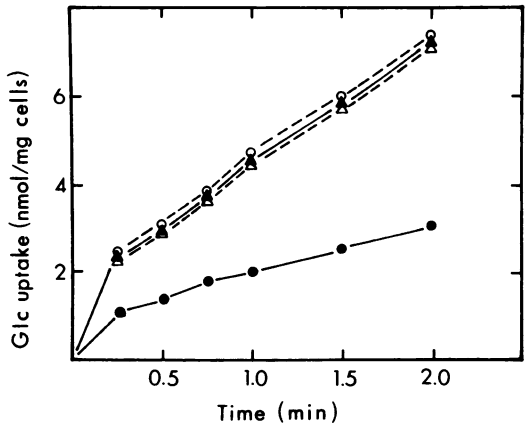

FIG. 2. Effect of glucose starvation on glucose uptake in *S. mutans* GS-5. Experiments were conducted as described in the legend to Fig. 1, except that cells were grown to the midexponential phase on 50 mM glucose and the uptake substrate was 10 μM [^{14}C]glucose. Symbols: (●) uptake by freshly harvested midexponential-phase cells, (○) uptake by glucose-starved cells, (▲) uptake by glucose-starved cells incubated with 35 μg of chloramphenicol per ml, (△) uptake by glucose-starved cells incubated with 50 μg of rifampin per ml.

documented that the HPrs of several gram-positive bacteria, including *S. mutans*, can be phosphorylated on a serine residue by an ATP-dependent protein kinase (2, 4, 15, 29), resulting in a decreased ability of HPr to be phosphorylated by PEP on a histidyl residue and inhibition of PEP-dependent phosphorylation of at least some sugars. In *S. mutans*, the ATP-dependent HPr kinase is regulated in crude extracts by glycolytic intermediates in a manner consistent with the energy needs of the cell under limiting and excess availability of sugars (15). To test whether ATP-dependent phosphorylation or phosphatase-catalyzed dephosphorylation of HPr might be responsible for the regulatory effects we observed, we labeled HPr with $^{32}P_i$ in vivo and tested the effects of sucrose starvation on the amount of incorporated acid-stable label. Sucrose-excess cells preloaded with $^{32}P_i$ contained a phosphorylated protein that corresponded to HPr on sodium dodecyl sulfate-polyacrylamide gels (Fig. 3, inset). Similar in vivo labelling of HPr was previously observed by Deutscher and Saier (4) in *Streptococcus pyogenes*. After prelabeling, the *S. mutans* cells were starved for sucrose as in previous experiments. Samples were taken at various time intervals, and the level of phosphorylation of HPr was determined after electrophoresis and autoradiography. The amount of label significantly decreased after 1 h, and by 2 h of starva-

TABLE 5. Effects of starvation on glucose uptake and PTS activities in *S. mutans* GS-5[a]

Culture conditions	Uptake velocity		PTS activity
	Initial	Steady-state	
Midexponential-phase cells[b]	4.4	1.12	0.32
Glucose-starved cells			
No addition	10.0	3.20	0.77
Plus chloramphenicol	9.8	3.10	0.75
Plus rifampin	9.8	3.10	0.76

[a] See footnotes *a*, *b*, *c*, *d*, and *f* of Table 4 except that glucose was used for uptake and PTS activities instead of sucrose.
[b] Grown on 50 mM glucose.

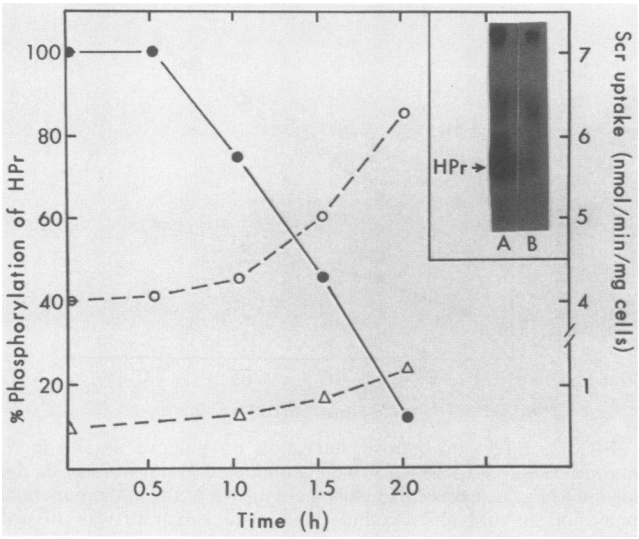

FIG. 3. Effect of sucrose starvation on the amount of acid-stable phosphorylation of HPr and rate of sucrose uptake in *S. mutans* GS-5. Cells were grown to the midexponential phase on tryptone-MOPS broth containing 50 mM sucrose but lacking the potassium phosphate supplements (see Materials and Methods). The cells were then preloaded with $^{32}P_i$, washed, and processed as described in Materials and Methods. Percent label in HPr (relative to time zero) was determined by densitometric scans of the autoradiograms. The inset shows the amount of label comigrating with HPr (apparent M_r at 17,000 in this gel system [14, 15]) at time zero (A) and after 2 h of starvation (B). Symbols: (●) percent phosphorylation of HPr relative to time zero; (○) initial and (△) steady-state rates of sucrose uptake (see footnote *a* of Table 4) measured at 10 μM [^{14}C]sucrose.

tion only 10% of the original label remained (Fig. 3). This decrease in the amount of phosphorylated HPr corresponded in time to the increase in sucrose uptake, measured at low sucrose concentrations, observed upon starvation (Fig. 3).

DISCUSSION

Previous work (6, 7, 23) had shown that PTS-mediated phosphorylation of sucrose and glucose in *S. mutans* appeared to be derepressed under conditions of sugar limitation or deprivation. The experiments presented in this report, however, show that at least part of this stimulation is due to direct modulation of the activity of PTS proteins, most probably that of HPr.

Kinetic analyses of sucrose uptake showed that uptake stimulation due to sugar limitation could be entirely accounted for by an increase in the V_{max} of the high-affinity PTS sucrose uptake system. The lower-affinity sucrose uptake system exhibited little change in either its K_m or V_{max} under all growth conditions tested. Therefore, the PTS appears to be the main, if not the sole, target of regulation of sucrose uptake by the availability of sugar. This phenomenon was further investigated in a series of starvation experiments. Starvation of cells after growth to the midexponential phase resulted in increased uptake activities for glucose and sucrose as well as parallel increases in glucose and sucrose PTS activities in whole cells. These increases were similar in magnitude to those observed for sucrose uptake in sucrose-limited cells relative to cells growing under sucrose-excess conditions. Neither chloramphenicol nor rifampin had any effect on the starvation-induced stimulations, showing that they must be due to a direct effect on the PTS uptake systems rather than to increased biosynthesis of PTS proteins. Results similar to these were obtained in another serotype c strain, *S. mutans* NCTC 10449, indicating that this type of regulation is not unique to strain GS-5.

It should be noted here that Slee and Tanzer (24) have suggested that *S. mutans* possesses at least three uptake systems for sucrose and that two of them, with kinetic constants similar to those shown in Table 2, are PTS dependent. Thus, if two different PTSs are involved in sucrose uptake in *S. mutans*, only the higher-affinity PTS appears to be regulated by the availability of sugar. We did not carry out our kinetic experiments at sucrose concentrations sufficiently high to detect the third uptake system reported by these workers (K_m of 3 mM [24]).

Recently, we identified ATP-dependent protein kinase activities in *S. mutans* (15). One target for kinase-mediated phosphorylation is HPr, the general, heat-stable phosphocarrier protein of the PTS. Based on experiments with *S. mutans* (15) and other streptococci (2–4, 17, 29), we postulated that in *S. mutans* ATP-dependent phosphorylation of HPr may have a regulatory function in vivo, such that phospho-(seryl)-HPr, the product of the kinase reaction, was a much poorer substrate for enzyme I of the PTS, resulting in lower PTS activities when HPr was in the phospho-(seryl) state (15). This hypothesis was consistent with the effects of various glycolytic intermediates on the HPr kinase activity in crude extracts. Intermediates that accumulate in cells grown with excess sugar activated the kinase, whereas intermediates present in sugar-depleted cells inhibited this activity (15). This system, therefore, provides one way in which PTS uptake could be regulated at the level of enzyme activity. If ATP-dependent phosphorylation of HPr were involved in the regulatory effects we observed, then one would predict that starvation of cells should lead to a dephosphorylation of phospho-(seryl)-HPr. Indeed, this is what we observed, and the time course of HPr dephosphorylation closely paralleled sugar uptake stimulation induced by starvation (Fig. 3). Although these results do not prove that the kinase-phosphatase system was responsible for these regulatory effects, they are fully consistent with this hypothesis. Further work will be necessary, however, to establish a direct involvement of ATP-dependent phosphorylation of HPr in this regulatory phenomenon.

Finally, what is the possible physiological significance, if any, of regulation by sugar availability of PTS-mediated sugar uptake in *S. mutans*? This question is especially pertinent since the maximum stimulation observed by sugar limitation under our culture conditions was approximately threefold. No doubt, the PTS in *S. mutans* has as one of its primary functions the scavenging of low concentrations of sugars from the medium, as has been suggested by others (6, 7, 10, 23, 24). Under conditions of sugar limitation, the PTS is an ATP-conserving uptake mechanism in fermentative organisms since only one ATP equivalent (in the form of PEP) is used to both transport and phosphorylate the sugar substrate. On the other hand, for sugars taken up by active transport systems (e.g., those dependent on the proton motive force) more than one ATP equivalent is required for transport and phosphorylation since these are two separate energy-requiring steps. Thus, it is clear why maximum PTS activities are of selective advantage to cells under conditions of severe sugar limitation. Moreover, even activation factors of two- to threefold are highly significant, since under these conditions sugar is most likely the limiting nutrient, and thus the growth rate of the cell would be directly proportional to the sugar uptake rate.

Our data also provide one possible explanation for direct inhibition of the PTS at high sugar concentrations. Under these conditions, streptococci, including *S. mutans*, accumulate relatively high steady-state concentrations of fructose 1,6-bisphosphate as well as other sugar phosphates (26, 31). Because intracellular sugar phosphate becomes inhibitory to many bacteria, including *S. mutans* (25), at high sugar concentrations the uptake and phosphorylation of sugars must be coordinated with the rate of sugar phosphate breakdown. It is possible, therefore, that if the V_{max} of the high-affinity system were not immediately lowered under conditions of sugar excess, the cell would be overwhelmed with inhibitory concentrations of sugar phosphate under these conditions, since the V_{max} of the high-affinity system under fully activated conditions approaches that of the lower-affinity system (Table 2).

Regulation of sucrose and glucose PTS activities in *S. mutans* Ingbritt (serotype c) as a function of sugar availability has been reported by others (6, 7), and this regulation was of the same order of magnitude that we report here for strain GS-5. In these previous reports, differences in activities were ascribed to repression or derepression of the PTS (implying an effect on gene expression). Our results, however, would indicate that this regulation is at least partially at the level of PTS activity itself rather than, or in addition to, being at the level of enzyme synthesis. More dramatic differences that have been observed in PTS activities due to the nature of the growth substrate (1, 7, 13, 21, 23, 25) or to various combinations of acidic pH, nitrogen limitation, and/or sugar limitation (18, 23, 28) could, of course, be due to regulation at the level of transcription or translation. Indeed, very recent reports (18, 28) suggest that this may be the case for at least some enzymes II, and possibly also for enzyme I, of the PTS. However, the type of mechanism we report here, based on direct modulation of the activity of a preexisting transport system, would seem to be the most ideally suited to respond to rapidly fluctuating levels of sugar in the oral cavity.

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