JOURNAL OF BACTERIOLOGY, June 1996, p. 3557–3563 Vol. 178, No. 12
0021-9193/96/$04.00+0

Regulation of Sugar Uptake via the Phosphoenolpyruvate-Dependent Phosphotransferase Systems in *Bacillus subtilis* and *Lactococcus lactis* Is Mediated by ATP-Dependent Phosphorylation of Seryl Residue 46 in HPr

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Received 29 December 1995/Accepted 10 April 1996

By using both metabolizable and nonmetabolizable sugar substrates of the phosphoenolpyruvate-dependent phosphotransferase system (PTS), we show that PTS sugar uptake into intact cells and membrane vesicles of *Lactococcus lactis* and *Bacillus subtilis* is strongly inhibited by high concentrations of any of several metabolizable PTS sugars. Inhibition requires phosphorylation of seryl residue 46 in the phosphocarrier protein of the PTS, HPr, by the metabolite-activated, ATP-dependent protein kinase. Inhibition does not occur when wild-type HPr is replaced by the S46A mutant form of this protein either in vesicles of *L. lactis* or *B. subtilis* or in intact cells of *B. subtilis*. Nonmetabolizable PTS sugar analogs such as 2-deoxyglucose inhibit PTS sugar uptake by a distinct mechanism that is independent of HPr(ser-P) and probably involves cellular phosphoenolpyruvate depletion.

All low-G+C gram-positive bacteria that have been examined, including species of *Bacillus*, *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Lactococcus*, *Lactobacillus*, *Listeria*, and *Mycoplasma*, possess the phosphoenolpyruvate:sugar phosphotransferase system (PTS) as well as a metabolite-activated, ATP-dependent HPr(ser) kinase that phosphorylates the PTS phosphocarrier protein, HPr, on seryl residue 46 (16, 21, 22). The PTS catalyzes the concomitant uptake and phosphorylation of its sugar substrates, and sugar uptake via the PTS is regulated by multiple mechanisms that are well characterized from a physiological standpoint but poorly defined from a mechanistic standpoint (11, 14, 20, 23). In vitro experiments established that phosphorylation of seryl residue 46 in HPr strongly inhibits phosphoenolpyruvate-dependent sugar phosphorylation via the PTS (3, 4), thereby providing a potential mechanism for inhibition of sugar uptake via the PTS in vivo. However, the physiological significance of this observation was questioned when in vivo studies conducted with *Bacillus subtilis* and *Staphylococcus aureus* failed to reveal appreciable HPr(ser-P)-mediated inhibition of PTS and non-PTS sugar uptake under conditions that were thought to generate HPr(ser-P) in vivo (5, 6, 17, 18, 26). On the other hand, extensive studies with vesicles of *Lactococcus lactis*, *Lactobacillus brevis*, *Enterococcus faecalis*, *Streptococcus bovis*, and *Streptococcus pyogenes* provided evidence that inducer exclusion (inhibition of sugar uptake) and/or inducer expulsion (stimulated efflux of preaccumulated sugar) were mediated by HPr(ser) phosphorylation (1, 2, 25, 28–35). Moreover, Lodge and Jacobson (12) showed that intact cells of *Streptococcus mutans* exhibit elevated sugar uptake following carbon starvation, and this enhanced activity correlates with decreased ^{32}P labeling of an acid-stable HPr derivative and not with increased levels of the protein (see reference 9).

The negative results reported for HPr(ser) phosphorylation-mediated inhibition of PTS sugar uptake into *B. subtilis* and *S. aureus* cells might be explained as follows. The enzyme II complexes of the PTS that transport sugars using HPr(his-P) as the phosphoryl donor are known to bind HPr(his-P) with high affinity (18), and the degree of HPr(ser-P) accumulation observed in the experiments reported by Reizer et al. (17, 18), Sutrina et al. (26), and Deutscher et al. (5) might have been insufficient to deplete the HPr(his-P) concentration to a value that was appreciably lower than the K_m values of the PTS permeases for this phosphocarrier protein. In fact, in these studies, concentrations of the inhibitory sugar in excess of 1 mM were not used. Thus, appropriate conditions for demonstration of PTS sugar uptake inhibition by HPr(ser-P) might not have been found.

Accumulation of the natural metabolizable substrates of the lactose, glucose, mannose, and ribose permeases of *L. lactis* has been shown to be inhibited by HPr(ser) phosphorylation in vesicles (31). This fact suggested to us that, at least in these vesicle preparations, the PTS was being regulated. However, the possibility that inducer expulsion rather than inducer exclusion was responsible for the effects observed was not excluded (27). Further, the relevance of the vesicular system to PTS-mediated uptake in whole cells was not established.

Because of the uncertainties cited above, we initiated a study to determine if conditions could be found in which inhibition of PTS-mediated sugar uptake could be unequivocally shown to be due to HPr seryl phosphorylation. The work reported in this communication provides evidence that the gram-positive bacterial cell possesses an indirect feedback control mechanism for regulating the rate of PTS sugar uptake in response to the cytoplasmic concentrations of key carbohydrate metabolic intermediates.

MATERIALS AND METHODS

The basic methods used have been described in detail in our previous publications (27–35).

Organisms, growth conditions, and vesicle preparation. All bacterial strains (*L. lactis* ML3 and *B. subtilis* GM1221 [wild type] and GM1222 [*ptsH1*, encoding S46A HPr]) were grown at 37°C for 18 h in Luria-Bertani broth medium containing 25 mM galactose (for *L. lactis*) or fructose (for *B. subtilis* used for vesicle preparation) or 25 mM glucose, fructose, or mannitol (for *B. subtilis* used for

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TABLE 1. Lack of effect of growth substrate on inhibition of PTS sugar uptake in wild-type *B. subtilis* cells[a]

Sugar substrate during transport	Inhibitory sugar during transport	Sugar inducer during growth	Uninhibited uptake rate (nmol/min/mg [dry wt])	% Inhibition with inhibitory sugar concentration (mM) of:		
				0.5	5	50
Fructose	Glucose	Glucose	4.2	49 ± 6	88 ± 3	91 ± 2
Fructose	Glucose	Fructose	7.3	45 ± 10	75 ± 5	87 ± 4
Glucose	Fructose	Glucose	5.8	5.0 ± 0.6	18 ± 2	45 ± 12
Glucose	Fructose	Fructose	5.0	9.0 ± 1.0	25 ± 3	54 ± 8
Mannitol	Glucose	Glucose	3.4	25 ± 3	59 ± 8	78 ± 10
Mannitol	Glucose	Mannitol	4.6	17 ± 4	79 ± 12	82 ± 7
Glucose	Mannitol	Glucose	5.8	3.0 ± 0.6	16 ± 2	24 ± 8
Glucose	Mannitol	Mannitol	4.3	4.0 ± 0.06	19 ± 5	29 ± 4

[a] *B. subtilis* GM1221 was grown in the presence of the sugar inducer (glucose, fructose, or mannitol; 25 mM) in Luria broth medium and prepared for uptake measurements as outlined in Materials and Methods. The concentration of the sugar substrate was 100 μM, while that of the inhibitory sugar was as indicated above. Data of three experiments were averaged, and the results are reported ± standard deviations. Inhibition of [^{14}C]sugar uptake by 50 mM glucitol never exceeded 30% regardless of the growth substrate (data not shown). When GM1222 replaced GM1221 in experiments comparable to those reported above, inhibition was always markedly diminished, as illustrated in Fig. 5.

whole-cell uptake measurements) (0.2 liters for a typical preparation). Cells were grown to early stationary phase (an optical density at 600 nm of about 2.0) (31). Growth conditions were as described previously (31, 32), and these conditions differed from those used by other investigators studying the regulation of PTS sugar uptake (5, 6, 17, 18, 26). Cells were harvested, washed, and used directly for transport experiments or for preparation of membrane vesicles by the method described by Kaback (10) for *Escherichia coli* but with the modifications described by Ye et al. (31) or in the figure legends. *B. subtilis* vesicles were prepared as described previously for *L. lactis* (31) except that cells were incubated with 0.5 mg of lysozyme per ml at room temperature for 30 min before they were subjected to osmotic shock. Electroporation of HPr; its mutant derivative, S46A HPr; and anionic metabolites into *L. lactis* and *B. subtilis* vesicles was conducted as described previously (30, 31).

Uptake measurements. Cells or vesicles were suspended in 50 mM Tris-maleate buffer (pH 7.0) containing 20 mM $MgSO_4$ at a cell density of 0.5 to 1.0 mg (dry weight)/ml or a vesicle density of 2 mg of protein per ml. They were temperature equilibrated for 5 to 10 min at 37°C before initiation of the uptake experiment by addition of the radioactive substrate. Various additions, as specified in Results or as described below, were made to a 100-μl aliquot of cell or vesicle suspension incubated at 37°C.

For time-dependent sugar uptake measurements, [^{14}C]fructose, [^{14}C]glucose, [^{14}C]mannitol (all at a specific activity of 5.0 mCi/mmol), or [methyl-^{14}C]β-thiogalactopyranoside ([^{14}C]TMG; 68 mCi/mmol) was added to a final concentration of 1 mM at time zero except in the experiments reported in Table 1, for which the concentration was 100 μM. All radioisotopes were obtained from New England Nuclear Corp., Boston, Mass. Glucose or another inhibitory sugar (at a concentration of 25 mM unless otherwise indicated), when present, was added at $t = -5$ min. Samples of 20 to 30 μl were removed at appropriate intervals, filtered through 25-mm-diameter membrane filters (0.45-μm pore size; Millipore Corp., Bedford, Mass.), and washed three times with cold 50 mM Tris-maleate buffer (pH 7.0). Washed filters bearing the cells or vesicles were then transferred to vials containing 5 ml of scintillation fluid for determination of radioactivity. All data reported represent the means of three or four experiments.

For carbohydrate concentration-dependent uptake, [^{14}C]glucose (specific activity, 1.0 to 0.01 mCi/mmol), [^{14}C]fructose (specific activity, 5.0 to 0.05 mCi/mmol), or [^{14}C]mannitol (specific activity, 2.5 to 0.025 mCi/mmol) was added to a final concentration of 0.05 to 50 mM at time zero. The energy source used for measurement of sugar uptake in intact cells (5% Luria broth medium) was added to the buffer at $t = -1$ min. Samples were removed at $t = 1$ min and filtered as described above for determination of radioactivity. For measurement of the effects of enzyme II induction on inhibitory responses to exogenous sugars, cells were grown as described above in the presence of glucose, fructose, or mannitol, and [^{14}C]sugar (100 μm) uptake was measured in the presence of increasing concentrations of an inhibitory sugar. In other respects, the procedure was the same as described above.

Protein assays and reagents. Chemicals were purchased from Sigma Chemical Co., St. Louis, Mo., and were of the highest purity available. Protein concentrations and the protein contents of vesicles prepared from either *L. lactis* or *B. subtilis* were determined as described by Lowry et al. (13).

RESULTS

Inhibition of PTS sugar uptake by glucose in *L. lactis* cells. Figure 1 shows time courses for the effects of a high concentration of glucose (25 mM) on the uptake of two metabolizable PTS sugars, fructose and mannitol, as well as a nonmetabolizable lactose analog, TMG. It is apparent that uptake of all three sugars is strongly inhibited, even at the earliest time point (15 s). The fact that inhibition was observed at early time

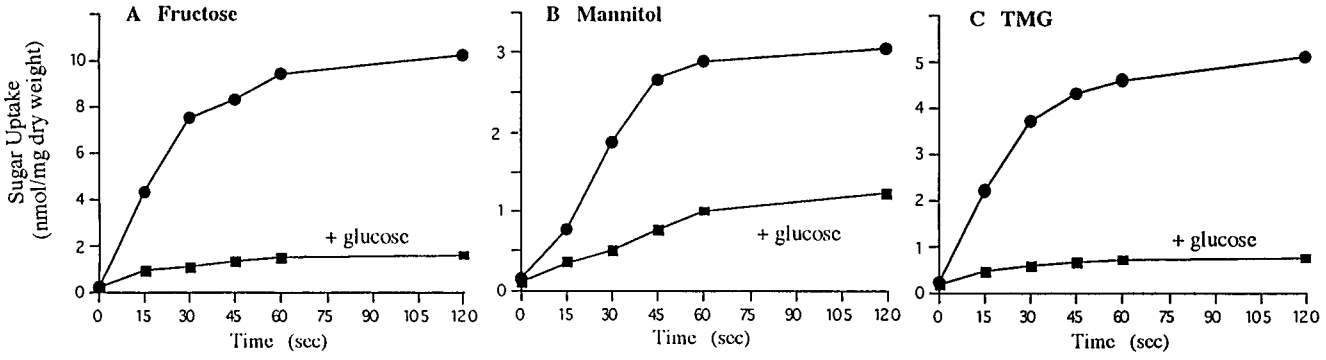

FIG. 1. Time courses for the uptake of [^{14}C]fructose (A), [^{14}C]mannitol (B), and [^{14}C]TMG (C) into *L. lactis* cells. The radioactive sugar was present at a concentration of 1 mM, and glucose was either present at a concentration of 25 mM (■) or absent (●). The experimental protocol was as described in Materials and Methods with cells grown in the presence of galactose as outlined previously (31).

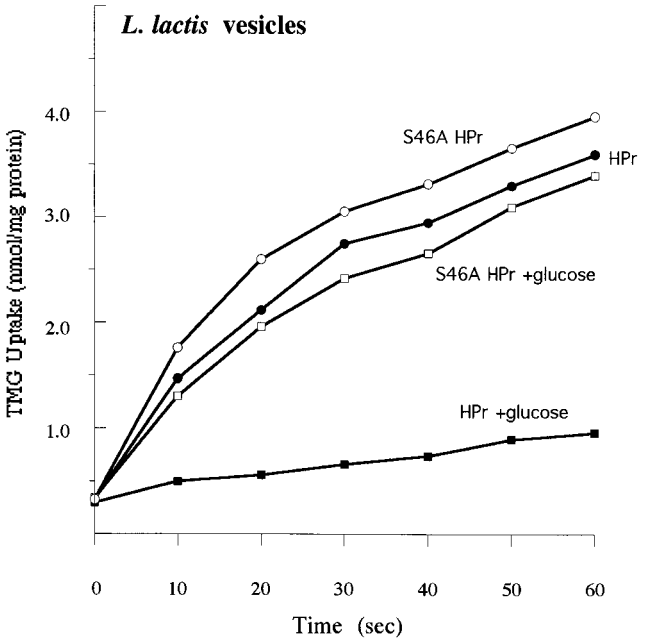

FIG. 2. Effects of wild-type *B. subtilis* HPr (50 μM) (closed symbols) or of mutant S46A HPr (50 μM) (open symbols) on TMG uptake into *L. lactis* vesicles with (squares) or without (circles) 25 mM glucose. The experimental protocol was as described in Materials and Methods with vesicles prepared from galactose-grown cells and HPr proteins introduced into the vesicles by electroporation as described by Ye et al. (31).

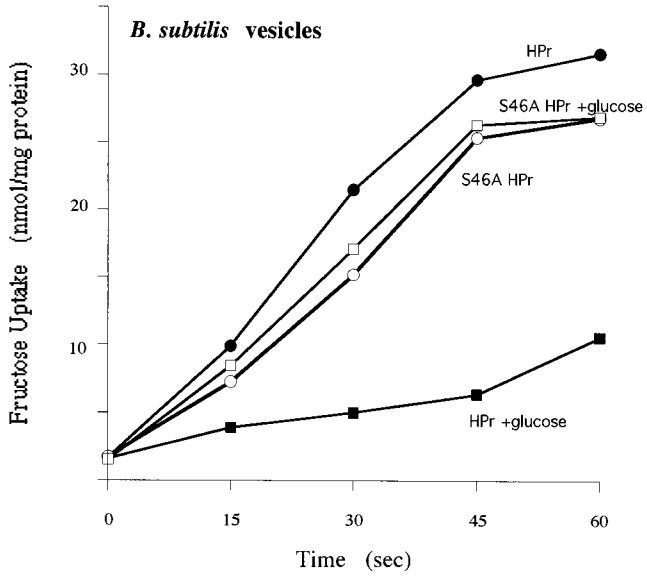

FIG. 3. Effects of wild-type HPr (50 μM) (closed symbols) or S46A mutant HPr (50 μM) (open symbols) on fructose uptake into vesicles prepared from fructose-grown *B. subtilis* GM1221 in the presence (squares) or absence (circles) of 25 mM glucose. The experimental protocol was as described in Materials and Methods.

points, when accumulation was minimal, argues in favor of an exclusion mechanism. This is particularly true because of the low affinity of the sugar-phosphate phosphatase II for its cytoplasmic substrates (see reference 35). The results, therefore, suggest, but do not prove, that sugar uptake via the PTS is a primary target of regulation.

Inhibition of TMG uptake in *L. lactis* vesicles. Figure 2 shows the rates of [^{14}C]TMG uptake into membrane vesicles of *L. lactis* under a variety of conditions. When wild-type HPr was introduced into the vesicles by electroporation, rapid uptake of TMG was observed, and this process was immediately and strongly inhibited when glucose was present, even at early time points. At $t = 10$ s, when minimal accumulation of the radioactive lactose analog had occurred, the inhibitory effect of glucose was almost as strong as it was at later time points. This fact clearly suggests that stimulation of TMG efflux by glucose was not solely responsible for the inhibitory effect of glucose on TMG accumulation. When S46A HPr was introduced into the vesicles by electroporation, the uptake rate was comparable to that observed in the presence of wild-type HPr, but uptake was subject to only weak inhibition by glucose. These results suggest that the inhibitory effect of glucose on PTS sugar accumulation in intact cells was due in considerable measure to inhibition of uptake and that seryl phosphorylation of HPr by the metabolite-activated HPr(ser) kinase was responsible for this inhibition.

Inhibition of fructose uptake by glucose in *B. subtilis* vesicles. In order to estimate the potential relevance of the results obtained with *L. lactis* vesicles (reported in Fig. 2) to *B. subtilis*, vesicles were made from the latter organism and an experiment comparable to the one whose results are shown in Fig. 2 was conducted. Because *B. subtilis* lacks the lactose-specific enzyme II complex of the PTS but possesses the fructose-specific system, fructose was selected as the radioactive substrate. Because *B. subtilis* apparently lacks the inducer expulsion phenomenon (5, 6, 28), this phenomenon could not account for any inhibition of sugar uptake that might be observed. For the experiment shown in Fig. 3, vesicles were prepared from cells grown in the presence of fructose in order to induce synthesis of the fructose-specific PTS enzyme II complex.

When wild-type *B. subtilis* HPr was introduced into the vesicles by electroporation, uptake of 1 mM fructose was strongly inhibited by the presence of 25 mM glucose. However, when the S46A mutant HPr was introduced into the same vesicles by electroporation, almost no inhibition by glucose was observed. These results strongly suggest that the inhibitory effect of glucose observed in the presence of wild-type HPr is due to seryl phosphorylation of HPr by the metabolite-activated protein kinase. Preincubation of the vesicles with a high concentration of glucose evidently generated sufficient fructose-1,6-diphosphate to activate the kinase to phosphorylate most of the vesicular HPr serine residue 46 (29–31).

Regulation of PTS sugar uptake in *B. subtilis* cells. A chromosomal mutant of *B. subtilis* is available in which the *ptsH* gene encoding wild-type HPr is replaced by the *ptsH1* gene encoding S46A HPr (5). The availability of this mutant strain (GM1222) and its isogenic parent (GM1221) allowed us to rigorously test the possibility that seryl phosphorylation of HPr is directly responsible for the inhibition of PTS sugar uptake by metabolizable PTS sugars in vivo. As shown in Fig. 4, the uptake of [^{14}C]fructose by these two isogenic strains was studied as a function of time in the presence and absence of excess glucose. It is apparent that glucose strongly inhibited fructose uptake in strain GM1221 but only weakly inhibited fructose uptake in strain GM1222. Since the only difference between strains GM1222 and GM1221 is the presence of the *ptsH1* mutation in the former strain, it must be concluded that the inhibitory effect of glucose in the latter strain is due to HPr(ser) phosphorylation by the kinase in response to metabolites generated during glucose catabolism, such as fructose-1,6-diphosphate.

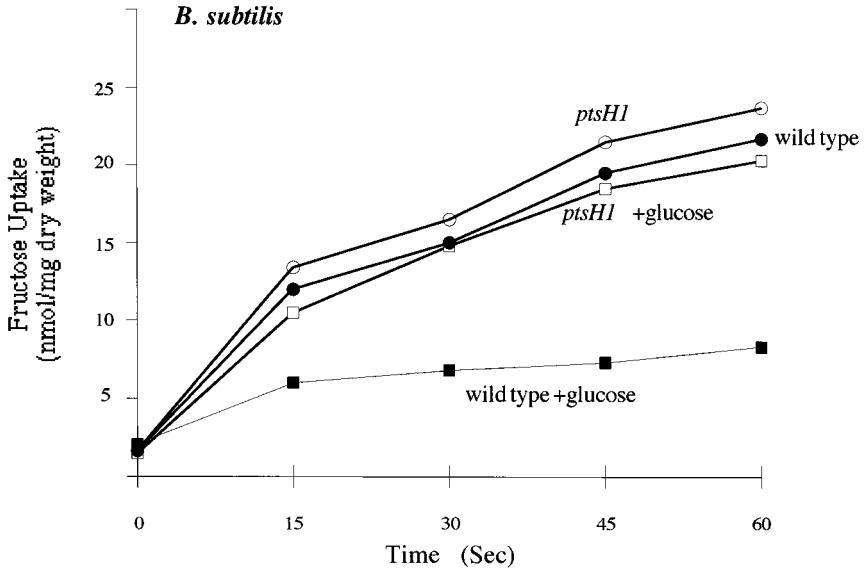

FIG. 4. Time courses for the uptake of [^{14}C]fructose into glucose-grown cells of wild-type *B. subtilis* (GM1221) (closed symbols) or of the S46A HPr mutant strain (GM1222) (open symbols). Glucose was either present at a concentration of 25 mM (squares) or absent (circles). The experimental protocol was as described in Materials and Methods.

All of the experiments described so far deal with the inhibition of PTS sugar uptake by glucose. Seryl phosphorylation might merely provide a mechanism that allows bacteria to construct a hierarchy of preferred PTS sugars, with glucose (presumably the preferred and most rapidly metabolized sugar) at the top of that hierarchy. Alternatively, seryl phosphorylation might also provide a feedback mechanism allowing the rapid metabolism of any PTS sugar, or saturation with a carbon source, to indirectly inhibit its own uptake. The experiment whose results are shown in Fig. 5 establishes the validity

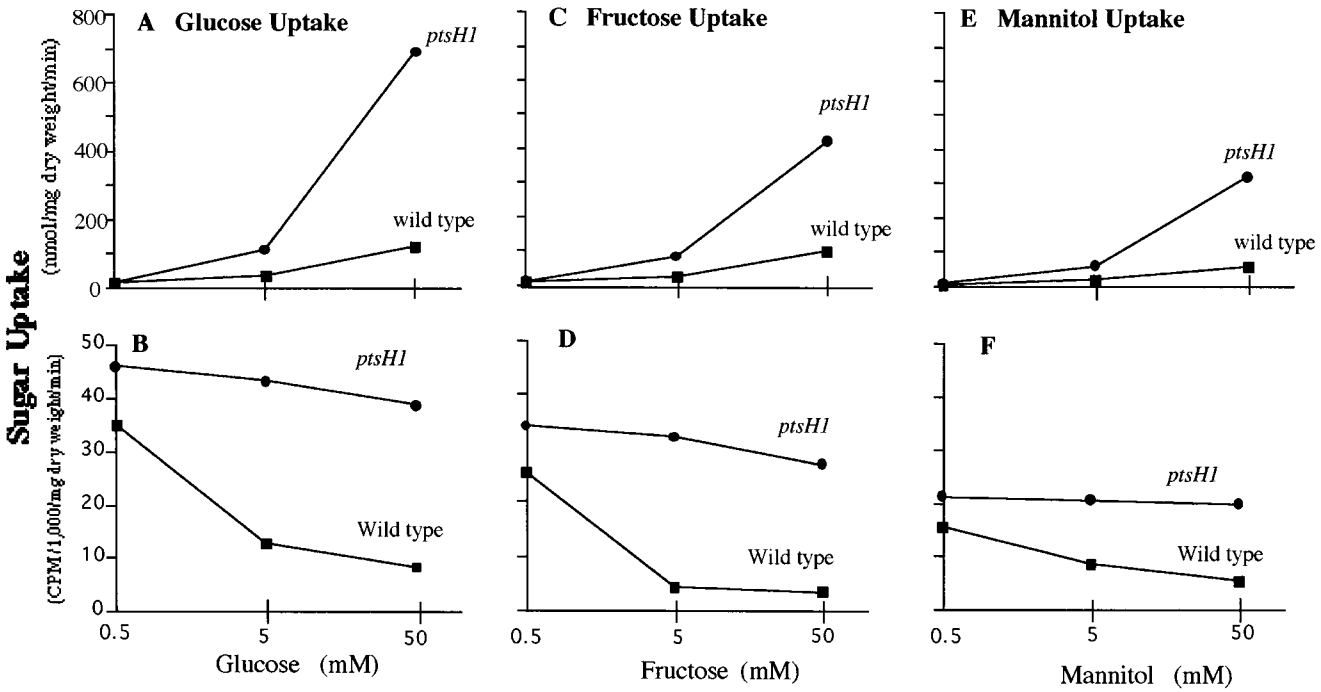

FIG. 5. Uptake of [^{14}C]glucose (A and B), [^{14}C]fructose (C and D), and [^{14}C]mannitol (E and F) by *B. subtilis* cells. (Uptake rates of the wild-type strain [GM1221] are always compared with those of the *ptsH1* mutant [GM1222].) In panels A, C, and E, sugar uptake is expressed in nanomoles per milligram of protein per minute, while in panels B, D, and F, sugar uptake is expressed in counts per minute. As the specific activity of the radioactive sugar was inversely related to the sugar concentration, the amount of radioactivity per volume of cell suspension was constant (see Materials and Methods). Sugar uptake (glucose-grown cells incubated at 37°C for 3 min with the indicated concentrations of radioactive sugar) was measured by rapid filtration as outlined in Materials and Methods. The data reported represent the averages of three experiments with triplicate values in agreement ±10%.

of the latter possibility. Thus, uptake rates for glucose, fructose, and mannitol were all diminished in *B. subtilis* GM1221 relative to those in strain GM1222 (bearing the *ptsH1* mutation) when the sugar concentration was high. These results clearly suggest that the metabolism of any one of several PTS sugars can inhibit its own uptake provided that the exogenous concentration of the sugar is high.

The experiments described above were conducted with cells harvested in the early stationary phase of growth. Under essentially the same growth and uptake conditions, the experiments whose results are shown in Fig. 4 and 5 were repeated with cells harvested during logarithmic-phase growth. In all cases, inhibition of radioactive-sugar uptake was observed as in stationary-phase wild-type cells, although the degree of inhibition was somewhat diminished (data not shown).

Synthesis of the glucose, fructose, and mannitol enzymes II of the PTS is known to be inducible by growth in the presence of glucose, fructose, and mannitol, respectively (15). Additionally, glucitol can be metabolized by an inducible non-PTS-mediated pathway (5). We therefore grew the *B. subtilis* strains in the presence of glucose, fructose, or mannitol to determine if induction of the enzyme II specific for either the inhibitory sugar or the sugar substrate used in the uptake experiment altered the inhibitory response. As revealed by the data in Table 1, glucose generally inhibited sugar uptake more strongly than did fructose and fructose inhibited sugar uptake more strongly than did mannitol. Glucitol was not strongly inhibitory, even at high concentrations, regardless of whether it was included in the growth medium (data not shown). In fact, no marked effect of the growth substrate was observed with any of the inhibitory sugars tested. Similar results were obtained when cells were harvested during logarithmic-phase growth. A plausible explanation for the surprising lack of effect of the growth substrate is provided in the Discussion.

Inhibition of sugar uptake by the nonmetabolizable PTS sugar 2-deoxyglucose. Activation of the HPr(ser) kinase is believed to be due to the generation of cytoplasmic metabolites such as fructose-1,6-diphosphate. Consequently, nonmetabolizable PTS sugar analogs, such as the glucose analog 2-deoxyglucose, should not inhibit by a mechanism that involves HPr(ser) phosphorylation. Nevertheless, such sugar analogs are known to inhibit both PTS and non-PTS sugar uptake in both gram-positive and gram-negative bacteria (see, for example, references 11, 14, 20, and 24). We examined the effect of 2-deoxyglucose on [^{14}C]fructose uptake into intact cells of *B. subtilis* GM1221 (wild type) and GM1222 (*ptsH1*). The results are presented in Fig. 6. As can be seen, fructose uptake in both strains was equally strongly inhibited by 2-deoxyglucose, showing that in contrast to inhibition by metabolizable sugars, the inhibitory response to this nonmetabolizable glucose analog could not be due to HPr(ser) phosphorylation.

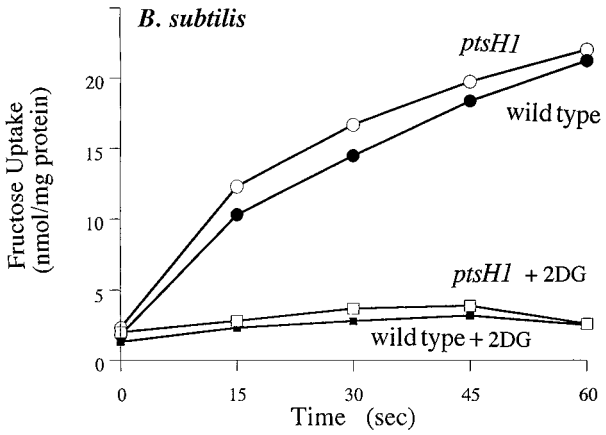

FIG. 6. Inhibition of [^{14}C]fructose uptake in strains GM1221 (wild type) (solid symbols) and GM1222 (*ptsH1*) (open symbols) in the presence (squares) or absence (circles) of 2-deoxyglucose (2DG; 25 mM). The fructose concentration was 100 μM. The experimental conditions were as described in Materials and Methods.

DISCUSSION

The results presented in this communication establish for the first time that seryl phosphorylation of the PTS phosphoryl carrier protein, HPr, in low-G+C gram-positive bacteria serves as a primary mechanism for the regulation of sugar uptake via the PTS. Since glucose inhibits sugar uptake more strongly than does fructose and fructose inhibits it more strongly than does mannitol or glucitol (Table 1 and data not shown), HPr(ser) phosphorylation provides a mechanism for creating a hierarchy of preferred sugars. However, the results lead to the conclusion that this regulatory mechanism not only provides a means for creating a hierarchy of preferred sugars, with the most rapidly metabolizable sugars at the top of the hierarchy, but also allows feedback inhibition of the uptake of each PTS sugar by its own metabolic products. Our quantitative data show that strong inhibition is observed only when the sugar concentration in the extracellular medium is high (>1 mM).

Regulation of PTS sugar uptake was demonstrated in two low-G+C gram-positive bacteria, *B. subtilis* and *L. lactis*. The demonstration of this regulatory mechanism in *B. subtilis* is particularly important, because this organism had previously been shown to lack the sugar-phosphate phosphatase which initiates the inducer expulsion phenomenon documented for *L. lactis* and certain other gram-positive bacteria (28, 31, 35). Since *B. subtilis* lacks the sugar-phosphate phosphatase that initiates inducer expulsion of preaccumulated cytoplasmic sugar phosphates and therefore does not exhibit expulsion of PTS-accumulated cytoplasmic sugar phosphates, the inhibitory effects on sugar uptake reported with this organism cannot be due to the inducer expulsion phenomenon. This fact strengthens the interpretation that the results reported for *L. lactis* in Fig. 1 and 2 are at least in part due to the direct inhibition of PTS sugar uptake. In other words, although *L. lactis* possesses the inducer expulsion-promoting, HPr(ser-P)-activated sugar-phosphate phosphatase, the apparent inducer exclusion observed is not likely to be explained solely by the stimulation of sugar phosphate hydrolysis and expulsion of the liberated sugar.

Some of the results reported in this communication were unexpected. Thus, the PTS permeases generally exhibit K_m values for their natural sugar substrates in the low-micromolar range, and yet strong inhibition of sugar uptake in *B. subtilis* required high (millimolar) sugar concentrations. Several metabolizable sugars were inhibitory, and inhibition was not appreciably dependent on the carbon source present in the growth medium (Table 1). Moreover, the high-efficiency carrier that was apparently responsible for sugar uptake in the millimolar concentration range exhibited low affinity for the sugar (K_m, about 20 mM) and was apparently sensitive to inhibition by HPr(ser-P) (see Fig. 5).

A low-affinity, high-efficiency carrier responsible for sugar uptake in the millimolar range has not previously been characterized for *B. subtilis*. However, a facilitated diffusion system meeting these specifications has been described for *S. bovis* (19). We have examined several low-G+C gram-positive bacteria, including *B. subtilis*, *E. faecalis*, *S. pyogenes*, and *L. lactis*,

and have found that each of these bacteria possesses such a transporter (36). Moreover, in these bacteria, the identified systems exhibit broad substrate specificity. The characterization of these transport systems and their regulation will be the subject of a separate communication.

The experiment described in Fig. 6 using the nonmetabolizable glucose analog 2-deoxyglucose revealed the occurrence in *B. subtilis* of a regulatory mechanism controlling PTS sugar uptake that was independent of HPr(ser) phosphorylation. Although several possible mechanisms exist, we believe that 2-deoxyglucose probably inhibited fructose uptake by depleting the cell of cytoplasmic phosphoenolpyruvate. This suggestion is in agreement with the results for *E. coli* recently published by Kornberg and Lambourne (11). The results reported here clearly show that metabolizable sugars that generate phosphoenolpyruvate during glycolytic metabolism control the rates of sugar uptake by mechanisms that are completely dissimilar from those used when nonmetabolizable analogs are employed. This observation should serve as a cautionary note for investigators wishing to mimic the effects of metabolizable sugars by using nonmetabolizable analogs.

HPr(ser-P) apparently regulates four distinct processes: (i) sugar uptake via the PTS (inducer exclusion of PTS sugars), (ii) cytoplasmic sugar phosphate hydrolysis and efflux (inducer expulsion of PTS sugars), (iii) the uncoupling of H^+ symport from sugar transport via certain non-PTS permeases (inducer exclusion and expulsion of non-PTS sugars), and (iv) catabolite repression mediated by the CcpA protein and the catabolite-responsive element DNA binding sequence (21, 22). We have recently shown that inducer expulsion of cytoplasmic sugar phosphates, accumulated via the PTS and hydrolyzed by an HPr(ser-P)-activated sugar-phosphate phosphatase (34), is present in some, but not other, low-G+C gram-positive bacteria (28). Moreover, the regulation of sugar:H^+ symport via the non-PTS permeases specific for lactose and glucose in *Lactobacillus brevis* (30, 31, 33, 34) could not be demonstrated for the gluconate:H^+ symport permease of *B. subtilis* (5, 6). Finally, the preliminary results reported in Fig. 5 and Table 1 suggest that HPr(ser-P) allosterically inhibits a high-efficiency, low-affinity, broad-specificity sugar facilitator in *B. subtilis*. We propose that the HPr(ser-P)-mediated regulation of PTS sugar uptake (this communication) and of catabolite repression (7, 8) will prove to be general for all low-G+C gram-positive bacteria that possess the metabolite-activated HPr(ser) kinase. On the other hand, the regulation of non-PTS sugar uptake and the sugar-phosphate phosphatase-initiated inducer expulsion phenomenon are likely to prove to be both species specific and permease specific (5, 28–30). We predict that only some non-PTS permeases in select low-G+C gram-positive bacteria will prove to be subject to this type of regulation. Further work will be required to verify or refute this important proposal.

ACKNOWLEDGMENTS

We thank Paula Boerner for independently conducting some of the experiments reported in this study, Jonathan Reizer for providing the purified *B. subtilis* HPr and S46A HPr used, Irene F. Fu and Betty Tseng for helpful technical assistance, and Mary Beth Hiller for excellent assistance in the preparation of the manuscript.

This work was supported by Public Health Service grant 5RO1AI 21702 from the National Institute of Allergy and Infectious Diseases.

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