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# Allosteric Regulation of the Glucose:$H^+$ Symporter of *Lactobacillus brevis*: Cooperative Binding of Glucose and HPr(ser-P)

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***Lactobacillus brevis* transports glucose and the nonmetabolizable glucose analog 2-deoxyglucose via a proton symport mechanism that is allosterically inhibited by the seryl-phosphorylated derivative of HPr, the small phosphocarrier protein of the phosphotransferase system. We here demonstrate that S46DHPr, a mutant analog of HPr which conformationally resembles HPr(ser-P) but not free HPr, specifically binds to membranes derived from glucose-grown *L. brevis* cells if and only if a substrate of the glucose permease is also present.**

*Lactobacillus brevis* is a heterofermentative, low-GC, gram-positive bacterium that transports glucose and lactose via proton symport mechanisms (11). In these same bacteria grown anaerobically, fructose is utilized via the phosphoenolpyruvate: sugar phosphotransferase system (3a). In early studies, uptake of nonmetabolizable lactose analogs via the lactose:$H^+$ symporter was shown to be subject to inhibition in the presence of a metabolizable sugar such as glucose (11). HPr of the phosphoenolpyruvate:sugar phosphotransferase system and an ATP-dependent, metabolite-activated HPr(ser) kinase (2) were identified in extracts of these bacteria (7). It was hypothesized that these two proteins might play a role in the regulation of lactose permease activity (7, 8, 12, 15).

In recent communications, we have described the use of membrane vesicle preparations to gain direct evidence of involvement of the seryl-phosphorylated derivative of HPr in the regulation of the activities of both the lactose and the glucose permeases of *L. brevis* (18, 19). A similar approach using vesicles of *L. lactis* (16) led to the conclusion that in this organism, HPr and the HPr(ser) kinase regulate uptake of lactose and glucose via the phosphotransferase system (20, 21). In both bacteria, the phenomenon of inducer expulsion (5, 6), as well as inducer exclusion (13), could be explained by a mechanism involving these same proteins (8, 9, 18–21). Moreover, with *Bacillus subtilis*, we obtained evidence that HPr(ser-P) together with a putative transcription factor, the CcpA protein (3), mediates catabolite repression (1). The phosphoenolpyruvate: sugar phosphotransferase system is similarly involved in catabolite repression and the control of cytoplasmic inducer levels in gram-negative enteric bacteria such as *Escherichia coli*, but the mechanism employed to bring about these regulatory phenomena is entirely different (4, 13, 14).

In a recent communication, we provided evidence that the lactose:$H^+$ symport permease of *L. brevis* binds HPr(ser-P) but not unphosphorylated HPr (22). Binding was greatly enhanced by the presence of any one of several sugar substrates of the permease. This fact suggested that binding of the sugar on the outer surface of the membrane induced a transmembrane conformational change in the permease that caused it to gain increased affinity for the phosphorylated allosteric regulatory protein HPr(ser-P) or its mutant analog S46DHPr on the cytoplasmic surface of the permease (9, 17). We report here that the same cooperative interaction can be demonstrated between S46DHPr and glucose in membranes induced for synthesis of the glucose:$H^+$ symporter. The effect is shown to be specific for both sugar substrates of the glucose permease and the S46DHPr mutant form of HPr.

The results presented in Table 1 summarize the sugar transport properties of *L. brevis* cells grown on either glucose or galactose. Glucose-grown cells take up glucose and the two nonmetabolizable glucose analogs 2-deoxyglucose and methyl-α-glucoside at rates that are 1.3- to 2.2-fold greater than those observed for galactose-grown cells, depending on the sugar used for measurement of the initial rate of uptake. Uptake of the glycoside is minimal compared to that of the two hexoses. By contrast, galactose-grown cells take up the lactose analogs thiomethyl-β-galactoside and isopropyl-β-D-thiogalactopyranoside (IPTG) at rates and to extents that are about fivefold greater than those observed for glucose-grown cells. These results and other unpublished results suggest that the glucose permease of *L. brevis* is inducible by growth in the presence of glucose while the lactose permease is inducible by growth in the presence of galactose (see also reference 22).

The S46DHPr mutant derivative has been shown to resemble wild-type HPr that is phosphorylated on serine 46 both conformationally (17) and functionally (9, 10, 18–21), while the S46AHPr derivative resembles the free, nonphosphorylated form of wild-type HPr. Purified S46DHPr was iodinated on tyrosyl residues, generating a radioactive derivative of the protein (22). As shown in Table 2, $^{125}$I-labeled S46DHPr was found to bind to membranes prepared from glucose-grown *L. brevis* cells in the presence of glucose or a glucose analog but it did not bind appreciably in the presence of either of two high-affinity lactose analogs, thio-β-digalactoside or IPTG. By contrast, the lactose analogs promoted $^{125}$I-labeled S46DHPr binding to membranes prepared from galactose-grown cells but not to those prepared from glucose-grown cells (Table 2). The specificity for sugar-promoted binding agrees with the inducer and substrate specificities of the transport systems as suggested by the data in Table 1.

Glucose and 2-deoxyglucose uptake as a function of sugar concentration and the ability of various concentrations of these two sugars to promote $^{125}$I-labeled S46DHPr binding to membranes from glucose-grown *L. brevis* cells are recorded in Fig. 1A and B respectively. Uptake of both glucose and 2-deoxyglucose followed monophasic curves with calculated $K_m$ values of 4 and 8 μM, respectively (Fig. 1A). Moreover, a hyperbolic

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TABLE 1. Uptake of radioactive sugars by *L. brevis* cells grown in glucose- or galactose-containing medium[a]

| Transport substrate[b] | Mean sugar uptake (μmol/mg [dry wt]/min) ± SEM of cells grown with: | |
|---|---|---|
| | Galactose | Glucose |
| Glucose | 1.3 ± 0.2 | 2.6 ± 0.6 |
| 2-Deoxyglucose | 1.6 ± 0.3 | 2.5 ± 0.5 |
| Methyl-α-glucoside | 0.4 ± 0.1 | 1.0 ± 0.2 |
| Thiomethyl-β-galactoside | 5.3 ± 0.4 | 1.0 ± 0.1 |
| IPTG | 5.0 ± 0.6 | 1.3 ± 0.4 |

[a] Growth and preparation of cells for $^{14}$C-labeled sugar uptake were conducted as described previously (18, 19). The final cell density was 2 mg (dry weight)/ml. Aliquots of 30 μl were removed for filtration after 1- and 2-min periods of incubation at 30°C. Rates of uptake were linear with time for more than 2 min.

[b] The radioactive sugars were used at the following concentrations and specific activities: glucose, 1 mM, 5 mCi/mmol; 2-deoxyglucose, 2 mM, 20 mCi/mmol; methyl-α-glucoside, 0.4 mM, 3 mCi/mmol; thiomethyl-β-D-galactopyranoside, 1 mM, 2 mCi/mmol; IPTG, 1 mM, 28 mCi/mmol.

curve was obtained when the binding of $^{125}$I-labeled S46DHPr was plotted as a function of either the glucose or the 2-deoxyglucose concentration (Fig. 1B). The binding constants estimated from double-reciprocal plots of the data (not shown) were the same, within experimental error, as the $K_m$ values calculated from the transport data for both substrates. The lack of biphasic uptake kinetics revealed in Fig. 1A suggests that a single glucose transport system is present in *L. brevis*.

In Table 3, the results of binding competition experiments are summarized. Inclusion of a large excess of nonradioactive S46DHPr in the incubation mixture prevented binding of $^{125}$I-labeled S46DHPr to the membranes, as expected. By contrast, wild-type HPr and S46AHPr were ineffective as competitors for S46DHPr binding to glucose-grown cell membranes. This fact agrees with our published results showing that only S46DHPr, not S46AHPr, inhibits the activity of the glucose permease in *L. brevis* membrane vesicles (18).

TABLE 2. Binding of $^{125}$I-labeled S46DHPr to membranes of glucose-grown *L. brevis* cells[a]

| Addition | Mean amt of $^{125}$I-labeled S46DHPr (μg/mg of membrane protein) ± SEM bound by membranes from cells grown with: | |
|---|---|---|
| | Glucose | Galactose |
| *N*-ethylmaleimide | 0.0[b] | 0.0[b] |
| None | 0.25 ± 0.03 | 0.06 ± 0.02 |
| Glucose | 1.44 ± 0.21 | 0.36 ± 0.09 |
| 2-Deoxyglucose | 0.95 ± 0.34 | —[c] |
| Methyl-α-glucoside | 0.88 ± 0.12 | — |
| Thio-β-digalactoside | 0.28 ± 0.05 | 0.68 ± 0.05 |
| IPTG | 0.27 ± 0.02 | 0.64 ± 0.10 |

[a] Experimental procedures were as described previously (22). The concentration of sugar present in the assay tube was 10 mM. Membrane protein was added to final concentrations of 1.4 mg/ml for glucose-grown cells and 1.3 mg/ml for galactose-grown cells. The concentration of $^{125}$I-labeled S46DHPr in these and the other experiments reported here was 4 μM. The final volume was 50 μl.

[b] The values obtained in the presence of 0.5 mM *N*-ethylmaleimide were 0.32 ± 0.08 and 0.29 ± 0.02 μg of S46DHPr associated with the glucose-grown cell membrane pellet and the galactose-grown cell membrane pellet per mg of membrane protein, respectively. These values were subtracted from all of the values shown here, in Table 3, and in Fig. 1 to provide an estimate of the amount of S46DHPr bound specifically to the permease.

[c] —, not determined.

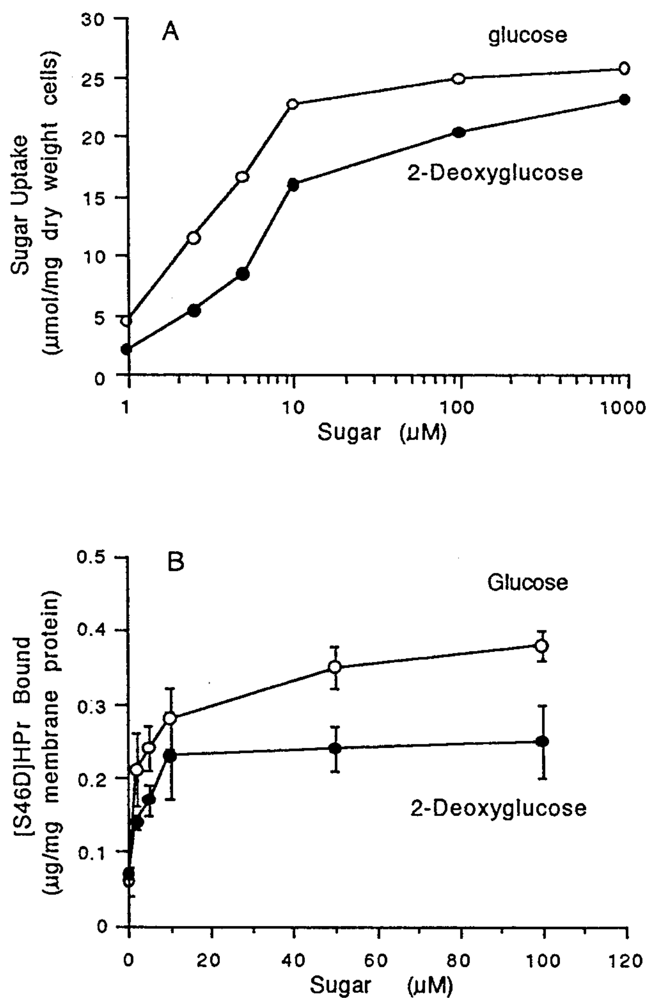


FIG. 1. Kinetics of sugar transport (A) and binding of $^{125}$I-labeled S46DHPr to membranes derived from glucose-grown *L. brevis* cells (B) as a function of the glucose (○) or 2-deoxyglucose (●) concentration. Note that a logarithmic scale is used in panel A while a linear scale is used in panel B. In panel A, the amount of radioactive sugar taken up in a 10-min period is indicated. The calculated apparent $K_m$ values were as follows: glucose, 4 μM; 2-deoxyglucose, 8 μM. Experimental procedures were essentially the same as those described in footnote *a* of Table 1 or footnote *b* of Table 3.

The results reported in this communication clearly suggest that S46DHPr [and therefore, by inference, HPr(ser-P)] binds with high affinity and specificity to an allosteric site on the cytoplasmic side of the glucose:H$^+$ symporter of *L. brevis* in a

TABLE 3. Effects of nonradioactive HPr and mutant HPr derivatives on $^{125}$I-labeled S46DHPr binding to *L. brevis* membranes containing the glucose permease[a]

| Addition | Mean amt of $^{125}$I-labeled S46DHPr bound (μg/mg of membrane protein) ± SEM |
|---|---|
| None | 1.44 ± 0.21 |
| S46DHPr | 0.12 ± 0.09 |
| S46AHPr | 1.23 ± 0.12 |
| Wild-type HPr | 1.11 ± 0.26 |

[a] The binding experiments were carried out as described previously (22), in the presence of the nonradioactive HPr derivative indicated at a concentration of 100 μM. Glucose was present in the assay mixture at a final concentration of 1 mM. The background activity measured in the presence of 0.5 mM *N*-ethylmaleimide was subtracted from all of the values shown (see Table 2, footnote *b*).

process that is cooperative with sugar substrate binding. They are in full agreement with the proposed allosteric mechanism whereby HPr(ser) phosphorylation by the metabolite-activated, ATP-dependent protein kinase inhibits sugar accumulation (22). Such a mechanism allows feedback regulation of sugar permease activity in response to cytoplasmic metabolite levels and consequent control of sugar uptake rates in accordance with the needs of the cell.

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