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Regulation of lactose permease activity by the phospho*enol*pyruvate:sugar phosphotransferase system: Evidence for direct binding of the glucose-specific enzyme III to the lactose permease

(inducer exclusion/sugar transport/allosteric control/protein effector/phosphorylation)

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ABSTRACT **Interaction between the glucose-specific enzyme III (enzyme III^{glc}) of the phospho*enol*pyruvate:sugar phosphotransferase system and the lactose permease was studied with membrane fragments from an *Escherichia coli* strain that overproduces the lactose permease. Substrates of the permease markedly and specifically stimulated binding of enzyme III^{glc} to the membranes. The sugar-stimulated binding of enzyme III^{glc} was concluded to be specific to the lactose permease because it (*i*) was dependent on the amount of the permease, (*ii*) was promoted only by sugar substrates of the permease, and (*iii*) was completely eliminated by treatment of the membranes with *N*-ethylmaleimide in the absence (but not the presence) of thio-β-D-digalactoside. The pH dependence of binding was similar to that reported for the binding of thio-β-D-digalactoside to the permease. Phospho*enol*pyruvate prevented the binding of enzyme III^{glc} to the lactose permease in the presence (but not the absence) of the other phosphate transfer components of the phosphotransferase system. These results support the hypothesis that enzyme III^{glc}, in its dephosphorylated form, modulates the activity of the lactose permease by a direct protein–protein interaction.**

In the 1940s, it was established that bacteria possess regulatory mechanisms that allow them to select preferred carbon sources when several are present in the growth medium simultaneously (1, 2). Thus, glucose is utilized by *Escherichia coli* or *Salmonella typhimurium* in preference to lactose, maltose, or glycerol. This phenomenon and its manifestations have been termed "the glucose effect," "catabolite repression," and "diauxic growth" by different investigators (3, 4). It is generally believed that these phenomena are attributable in large part to the lowering of the intracellular concentrations of cyclic AMP and inducer. The former process results primarily from the inhibition of the cyclic AMP synthetic enzyme, adenylate cyclase, but also from the stimulation of cyclic AMP efflux (5, 6), whereas the latter usually results from inhibition of permease function (inducer exclusion) (3, 7).

For over a decade our research efforts have been concerned with the mechanisms by which the rates of carbohydrate uptake are regulated. Extensive genetic evidence for the direct catalytic participation of the proteins of the phospho*enol*pyruvate:sugar phosphotransferase system (PTS) in the regulation of the uptake of certain carbohydrates such as maltose, lactose, and glycerol has been published (7–20). In 1975, we proposed a unifying mechanism explaining how the proteins of the PTS might control the activities of adenylate cyclase and several non-PTS carbohydrate permeases (10, 18, 20). By this mechanism a regulatory protein, termed RPr and thought to be the glucose-specific enzyme III (enzyme III^{glc}) of the PTS (8, 9, 12, 16, 18, 19, 21), was considered to bind to allosteric regulatory sites on the permeases. Such binding was thought to influence the conformation of the permease so that it exhibited decreased catalytic activity. It was further proposed that phosphorylation of enzyme III^{glc} by the PTS cascade [phospho*enol*pyruvate (*P-e*Prv) → phosphorylated enzyme I → phosphorylated heat-stable protein (HPr) → phosphorylated enzyme III^{glc}] prevented binding to the allosteric site. Substrates of the PTS were presumed to decrease the phosphorylation level of enzyme III^{glc} by removing phosphate groups from the PTS protein cascade and hence cause inhibition of permease activity.

Recently, we showed that lactose uptake into membrane vesicles prepared from *E. coli* strain ML308-225 was inhibited when partially purified enzyme III^{glc} was introduced into them by osmotic shock (22). Intravesicular *P-e*Prv relieved this inhibition. These results provided biochemical evidence that the regulatory protein controlling lactose permease activity is, in fact, identical with enzyme III^{glc}, as suggested by the earlier genetic data (7–20, 23).

To further characterize the regulatory interaction biochemically, we attempted to detect direct binding of enzyme III^{glc} to the permease. With the membrane fraction from an *E. coli* strain with enhanced *lacY* gene expression (24), we could demonstrate a cooperative binding relationship between the substrates of the lactose permease and enzyme III^{glc}. The results of these experiments are summarized in this communication.

MATERIALS AND METHODS

Purification of PTS Components. Enzyme III^{glc}, enzyme I, and HPr were purified from *S. typhimurium* strain LJ144, which contained increased amounts of these proteins due to the presence of the *E. coli* episome F'198 (10). Enzyme III^{glc} was purified by column chromatography on DEAE-cellulose, Sephadex G-75, and calcium phosphate-gel cellulose (25), and by isoelectric focusing. The purity of the final preparation was more than 90% on the basis of the result of polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. In the present study the preparation before the isoelectric focusing step was used. It was about 50% pure on the basis of specific

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Abbreviations: PTS, phospho*enol*pyruvate:sugar phosphotransferase system; *P-e*Prv, phospho*enol*pyruvate; enzyme III^{glc}, the glucose-specific enzyme III of the PTS; HPr, heat-stable phosphate carrier protein of the PTS; $SGal_2$, thio-β-D-digalactoside; MalNEt, *N*-ethylmaleimide.

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activity measurements relative to the highly purified enzyme and was free of detectable enzyme I or HPr activity. Enzyme I and HPr were partially purified as described (26). In some experiments highly purified HPr, which was kindly supplied by G. T. Robillard, was used.

Assays for PTS Components. Enzyme III^{glc} was assayed by following the *P-e*Prv-dependent phosphorylation of methyl α-glucoside for 30 min at 37°C in the presence of a crude extract of a *crrA* mutant strain (LJ138; *cpd-401 crrA3*) (10, 27) as a source of other PTS components. The reaction mixture contained, in a total volume of 0.1 ml: 0.1 mM [^{14}C]methyl α-glucoside (2.5 mCi/mmol; 1 Ci = 3.7×10^{10} becquerels), 5 mM *P-e*Prv, 1 mM dithiothreitol, 18 mM KF, 9 mM $MgCl_2$, 50 mM potassium phosphate (pH 7.6), crude extract of LJ138 (75 μg of protein), and enzyme III^{glc}. In each experiment the activity of enzyme III^{glc} was determined relative to calibration curves obtained with standard enzyme III^{glc} solutions. The calibration curve usually gave an approximately linear pattern up to about 0.5 μg of enzyme III^{glc}, giving about 18,000 cpm of radioactive sugar phosphate with 2000 cpm of background. Duplicate measurements were made for each sample and averaged. Enzyme I and HPr were determined in the presence of excess HPr and enzyme I, respectively, using the membrane fraction of an *E. coli* strain that contained a plasmid coding for the mannitol operon (28). [^{14}C]Mannitol was used as the sugar substrate. One enzyme unit was defined as the amount of enzyme that gave 1 nmol of sugar phosphate in 30 min under standard conditions at 37°C.

Preparation of Membrane Fractions. Lactose permease-rich membranes were prepared from an *E. coli* strain, T52RT, which was generously supplied by P. Overath (24). This strain contains a hybrid plasmid, pTE18, carrying the *lacY* gene. It has been reported to synthesize the lactose permease to a level of 12–16% of the total cytoplasmic membrane protein in the fully induced state (24). The presence of enhanced lactose permease activity in strain T52RT was verified by showing that lactose uptake activity in this strain was enhanced 9- to 10-fold relative to a *lacI*$^-$ constitutive *E. coli* strain, ML308-225. Cultivation of the bacteria and induction of the lactose permease were carried out as described (24). The harvested cells were stored frozen at −70°C until used. For the preparation of the membranes, the frozen bacteria were suspended in 50 mM Tris·HCl (pH 7.5) containing 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, and deoxyribonuclease at 10 μg/ml, and the cells were broken by two passages through a French pressure cell [11,000 pounds/inch^2 (76 MPa)]. Cell debris was removed by centrifugation at 10,000 × *g* for 5 min, and the membranes were collected by centrifugation at 100,000 × *g* for 90 min. They were washed once with 50 mM Tris·HCl (pH 7.5) containing 1 mM dithiothreitol, resuspended in the same solution at a protein concentration of 10–13 mg/ml, and used immediately for the experiments. Protein was estimated by the method of Lowry *et al.* (29).

Binding Experiments. Incubations were carried out at room temperature in cellulose nitrate tubes (5 × 20 mm) designed for use in the Beckman Airfuge ultracentrifuge. In a typical experiment, the incubation mixture contained, in a total volume of 120 μl, 30 mM Tris·HCl (pH 7.5), 0.6 mM dithiothreitol, 0.8 mg of membrane protein from strain T52RT, 125 units (20 μg) of enzyme III^{glc}, 50 units of HPr, 40 units of enzyme I, 5 mM $MgCl_2$, and other substances as needed. After 10 min of incubation, the membranes were collected by centrifugation at 100,000 × *g* for 10 min in an Airfuge. The supernatants were discarded, tubes were rinsed with 100 μl of ice-cold 20 mM Tris·HCl (pH 7.5) containing 1 mM dithiothreitol, and the pellets were resuspended in 100 μl of 50 mM Tris·HCl (pH 7.5) containing 1 M NaCl and 1 mM dithiothreitol. After centrifugation at 100,000 × *g* for 10 min, the supernatants were collected and assayed for the activities of the PTS components, usually using 10 μl for each measurement. Components that were bound to the membranes during the first incubation were almost quantitatively recovered in the final supernatants, and the final pellets were essentially lacking in these activities. Contamination of the final supernatants by the initial incubation mixtures was checked by adding [^{14}C]sucrose to the mixture at the beginning of the incubation and measuring the radioactivity in the final supernatant. This value was fairly constant at 2.7 ± 0.2% (12 measurements) and was subtracted from all the data obtained. Most experiments were run in duplicate, and the average values are presented below. Different membrane preparations of strain T52RT gave different (2.5-fold) basal (sugar-independent) enzyme III^{glc} binding activities. Consequently absolute binding values should be compared only within an experiment in which a single membrane preparation was used.

RESULTS

When the soluble PTS components were incubated with the membranes of the lactose permease-overproducing strain, T52RT, a small amount of enzyme III^{glc} pelleted with the membranes by centrifugation and was recovered in the final supernatant, which contained a high concentration of salt (1 M NaCl). When a substrate of the lactose permease was added, the association of enzyme III^{glc} to the membranes was markedly increased (Table 1). About 25% of the enzyme III^{glc} added was bound in the presence of thio-β-D-digalactoside ($SGal_2$) or melibiose, the two sugars that gave the largest stimulatory effects. Maltose, which is not a substrate of the lactose permease, had no effect. HPr was also bound to the membranes to some extent, but the amount of binding was much less than that of enzyme III^{glc} (less than 6%, which corresponds to 0.25 μg as calculated on the basis of the specific activity of highly purified HPr). The effects of sugar substrates of the lactose permease on HPr binding were variable. Enzyme I was not bound significantly. Therefore, only the binding of enzyme III^{glc} was measured in the later studies.

Enzyme III^{glc} binding was examined as a function of $SGal_2$ concentration (Fig. 1). The $SGal_2$ concentration that half-maximally promoted enzyme III^{glc} binding was about 15 μM. A similar experiment was carried out with lactose, and the half-saturating concentration was estimated to be about 1.5 mM (data not shown). It is noteworthy that the dissociation constant reported for lactose is much higher than that for TDG [9–18 mM for lactose (30) compared with 50–80 μM for $SGal_2$ (30–32)].

Table 1. Enzyme III^{glc} binding to the membranes in the presence of various substrates of the lactose permease

Addition	Enzyme III^{glc} bound, μg
None	1.3
Lactose	3.4
Thio-β-D-digalactoside	4.9
Isopropyl β-D-thiogalactoside	4.7
Melibiose	5.0
Methyl β-D-thiogalactoside	3.9
Maltose	1.1

The binding experiments were carried out in the presence of the indicated sugars at 10 mM. The amount of enzyme III^{glc} bound to the membranes was determined by enzyme assays and expressed in micrograms as calculated on the basis of the specific activity of the highly purified preparation of the enzyme obtained after the isoelectric focusing step (6.3 μmol/30 min per mg of protein under the standard conditions).

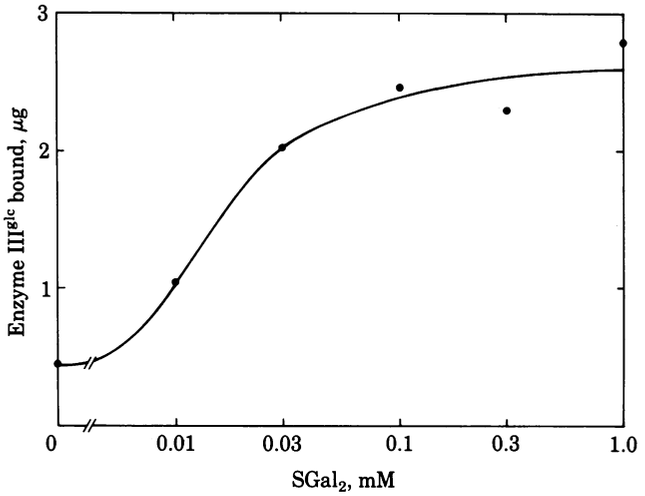

FIG. 1. Binding of enzyme III^{glc} as a function of $SGal_2$ concentration. The binding experiments were carried out in the presence of $SGal_2$ at the concentrations indicated and according to the procedure outlined in the text.

The dependence of enzyme III^{glc} binding on the lactose permease was examined (Fig. 2). In this experiment, an *E. coli* strain, T28RT, was used as the control. This strain is *lacY* deficient because it does not contain the plasmid pTE18, but it is otherwise isogeneic to T52RT (24). Membranes from T28RT and T52RT were mixed in various proportions, and enzyme III^{glc} binding was measured. The fraction of the binding that was dependent on $SGal_2$ showed a clear correlation with the amount of the T52RT membranes. The basal binding activity did not change significantly with the increase in T52RT membranes, suggesting that basal binding was largely nonspecific. Membranes isolated from strain T28RT bearing the plasmid pBR322, which does not carry the *lacY* gene, did not show $SGal_2$-promoted enzyme III^{glc} binding (data not shown).

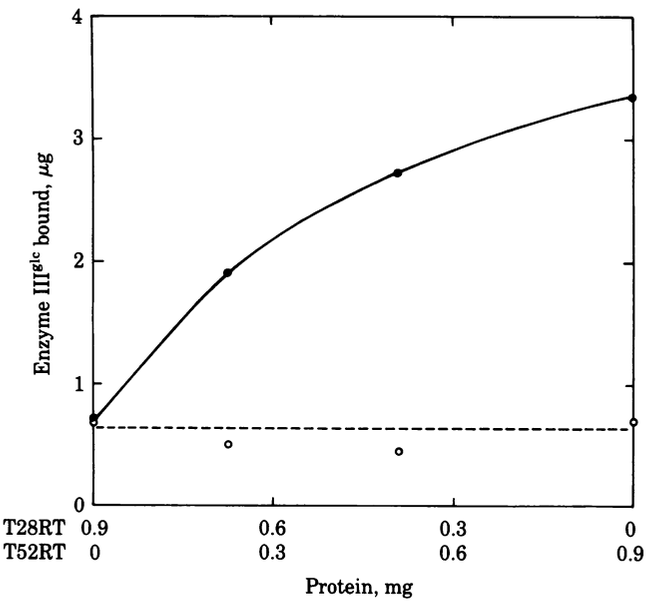

FIG. 2. Dependence of the enzyme III^{glc} binding on lactose permease. Membrane preparations from isogeneic strains T28RT (*lacY*$^-$) and T52RT (strain T28RT containing the *lacY* plasmid pTE18) were mixed in the proportions indicated, keeping the total amount of the protein in the incubation mixture at 0.9 mg. They were sonicated in the presence (●) or absence (○) of 1 mM $SGal_2$ for 15 s in a bath sonicator to facilitate the equilibration of $SGal_2$ across the membranes. The final $SGal_2$ concentration was 0.6 mM.

Inactivation of the lactose permease by modification of an essential sulfhydryl group with *N*-ethylmaleimide (MalNEt) and protection of the permease against derivatization by $SGal_2$ have been demonstrated (31). Accordingly, the effect of MalNEt on enzyme III^{glc} binding was studied in the presence or absence of $SGal_2$ (Table 2). MalNEt virtually abolished the $SGal_2$-dependent enzyme III^{glc} binding. When, however, $SGal_2$ was added prior to treatment with MalNEt, the binding capacity was largely retained. In separate experiments, we found that lactose (10 mM) had no protective effect (data not shown). These results agree well with the reported results (32, 33) that $SGal_2$, but not lactose (at 5 mM), protects the lactose permease from modification by MalNEt. Basal binding of enzyme III^{glc} was also diminished by MalNEt, and $SGal_2$ showed a slight protective effect.

The dependence of enzyme III^{glc} binding on pH is shown in Fig. 3. The optimal pH was about 6.0 for the $SGal_2$-dependent binding, and the curve obtained was very similar to that for $SGal_2$ binding reported by Kennedy *et al.* (31). Basal binding did not show appreciable pH dependence.

In our model for the regulatory action of Enzyme III^{glc} in inducer exclusion, only the dephosphorylated form of the enzyme inhibits the non-PTS permeases, and inhibition is eliminated by the phosphorylation of enzyme III^{glc} by the PTS cascade (10, 18, 19). Accordingly, the effect of *P-e*Prv on the binding of enzyme III^{glc} was examined (Table 3). In experiment I, in which KF was added to the reaction mixture, *P-e*Prv almost completely inhibited the binding of enzyme III^{glc}. 2-Phosphoglycerate, which is a structural analogue and a metabolic precursor of *P-e*Prv, had essentially no effect. On the other hand, in the absence of fluoride, 2-phosphoglycerate decreased the binding of enzyme III^{glc} to nearly the same extent as *P-e*Prv (data not shown). This is probably due to the conversion of 2-phosphoglycerate to *P-e*Prv by endogenous enolase (2-phospho-D-glycerate hydro-lyase, EC 4.2.1.11), which is strongly inhibited by fluoride. In the absence of enzyme I and HPr, enzyme III^{glc} bound normally to the lactose permease, but *P-e*-Prv had no effect on the binding (Exp. II). These results suggest that phosphorylated enzyme III^{glc} does not interact with the mem-

Table 2. Effect of MalNEt treatment of the membranes in the presence or absence of $SGal_2$ on enzyme III^{glc} binding

MalNEt treatment	$SGal_2$ during treatment	Enzyme III^{glc} bound, μg: Without $SGal_2$	Enzyme III^{glc} bound, μg: With $SGal_2$
−	−	0.5	2.4
−	+	0.5	2.2
+	−	0.2	0.4
+	+	0.4	1.7

Membranes (prepared without dithiothreitol) were incubated at room temperature for 10 min in the presence or absence of $SGal_2$ in 45 mM sodium phosphate (pH 7.0). They were then exposed to MalNEt, or an equivalent amount of water was added. Samples were then incubated at room temperature for 15 min. The final concentrations of $SGal_2$ and MalNEt were 5 mM and 0.2 mM, respectively, and the total incubation volume was 180 μl. After the incubation, dithiothreitol was added to each tube to a final concentration of 2 mM, and $SGal_2$ (final concentration 5 mM) was added to those samples that had not previously received this compound. Membranes were collected by centrifugation in an Airfuge and washed twice with 50 mM Tris·HCl (pH 7.5) containing 1 mM dithiothreitol. Resuspension and equilibration of $SGal_2$ across the membranes were facilitated by sonication for 15 s in a bath sonicator. Each pelleted membrane fraction was finally suspended in 160 μl of the above solution and divided into two portions, and either a solution of $SGal_2$ or water was added. After sonication as described above, the membranes were used for the binding experiments. The final concentration of $SGal_2$ was 0.7 mM.

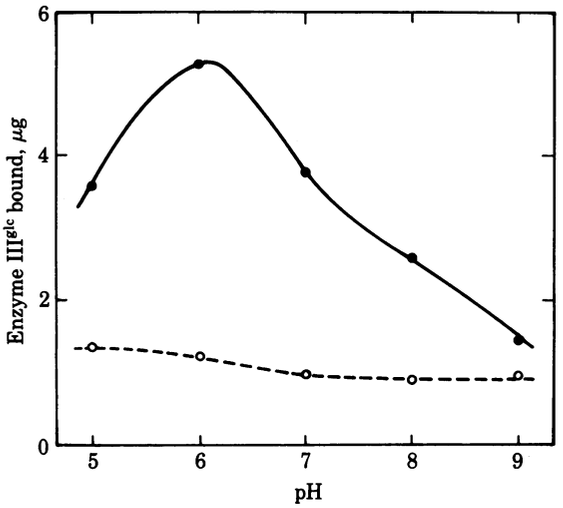

FIG. 3. Dependence of enzyme III^{glc} binding on pH. Membranes were first prepared in 50 mM Tris·HCl (pH 7.5) containing 1 mM dithiothreitol as described in *Materials and Methods*, except that they were washed and finally suspended in 10 mM Tris·HCl (pH 7.5) containing 1 mM dithiothreitol. Before the experiments, the membranes were divided into five equal aliquots and collected by centrifugation in an Airfuge. They were resuspended in the buffers, which consisted of 40 mM each of phosphoric, acetic, and boric acids, adjusted with NaOH to the pH values indicated. The final incubation mixtures contained all anions at 27 mM. Enzyme III^{glc} binding was carried out in the presence (●) or absence (○) of 1 mM $SGal_2$.

branes and that enzyme III^{glc} binding to the permease occurs independently of the other soluble PTS enzymes.

DISCUSSION

We have shown that enzyme III^{glc} can bind to membranes that contain an abnormally large amount of the lactose permease, provided that a substrate of the permease is present. We suggest that the sugar-dependent binding is specific to the lactose permease on the basis of (*i*) sugar specificity for the stimulatory effect (Table 1), (*ii*) its dependence on the amount of lactose permease (Fig. 2), and (*iii*) the effects of MalNEt treatment with and without $SGal_2$ (Table 2). The simplest interpretation of these observations is that enzyme III^{glc} directly interacts with the lactose permease by a mechanism that is dependent on the binding of a sugar substrate to the permease. The apparent half-saturating concentrations of $SGal_2$ and lactose for the stimulation of the enzyme III^{glc} binding were significantly lower than the reported dissociation constants (30–33). A possible explanation for this apparent discrepancy is provided by the cooperative effects of sugar and enzyme III^{glc} binding. The affinity of the permease for the sugar substrates should be enhanced by the binding of enzyme III^{glc} and vice versa.

Table 3. Effect of *P-e*Prv on enzyme III^{glc} binding

Exp.	Addition	Enzyme III^{glc} bound, μg
I	None	4.1
	*P-e*Prv	0.4
	2-Phosphoglycerate	3.6
II	None	4.4
	*P-e*Prv	4.5

Experiments were carried out in the presence of 150 units of HPr, 80 units of enzyme I, 1 mM $SGal_2$, 2.5 mM NADH, and 2 international units of lactate dehydrogenase (L-lactate:NAD oxidoreductase, EC 1.1.1.27). In Exp. I, 10 mM KF was added. HPr and enzyme I were omitted in Exp. II. Sodium phosphate buffer (pH 7.0) was used instead of Tris·HCl (pH 7.5), and the incubation temperature was 30°C. Other conditions were as described in *Materials and Methods*.

Phosphorylated enzyme III^{glc} appears not to bind to the lactose permease. When enzyme III^{glc} was incubated with excess quantities of *P-e*Prv, enzyme I, and HPr, no binding of enzyme III^{glc} to the membranes could be demonstrated. This observation is in agreement with the suggestion, based on genetic analyses, that binding of free enzyme III^{glc} is responsible for the regulation of the lactose permease. Our model for the mechanism of lactose permease regulation is illustrated in Fig. 4. When the intracellular pool of enzyme III^{glc} is largely dephosphorylated, binding of a substrate (lactose in the figure) to the permease induces the binding of enzyme III^{glc}, and the ternary complex (lactose–lactose permease–enzyme III^{glc}) exhibits low transport activity relative to the binary complex (lactose–lactose permease). When phosphorylated by the sequential reactions of the PTS, enzyme III^{glc} dissociates from the lactose permease, and hence the permease assumes its active conformation. It is possible, of course, that some other protein(s) is (are) required for, involved in, or influences the regulatory interaction. Further studies with highly purified proteins—enzyme III^{glc}, lactose permease, enzyme I, and HPr—in a reconstituted transport system will be required before a definitive conclusion regarding the number of components can be reached.

A dissociation constant between enzyme III^{glc} and the lactose permease in the presence of saturating concentrations of $SGal_2$ was calculated roughly on the basis of data in Fig. 2, assuming the molecular weight of enzyme III^{glc} to be 20,000 (21) and the content of the lactose permease protein in the total membrane fraction (inner plus outer membranes) to be 1–2 nmol/mg of protein (2.5–3.5 nmol/mg of cytoplasmic membrane protein according to ref. 24). The average value calculated from the available data was 20–45 μM. The true dissociation constant may be even smaller because a significant proportion of the lactose permease in the membrane vesicle fraction is probably in an orientation inaccessible to enzyme III^{glc}. Most of the mem-

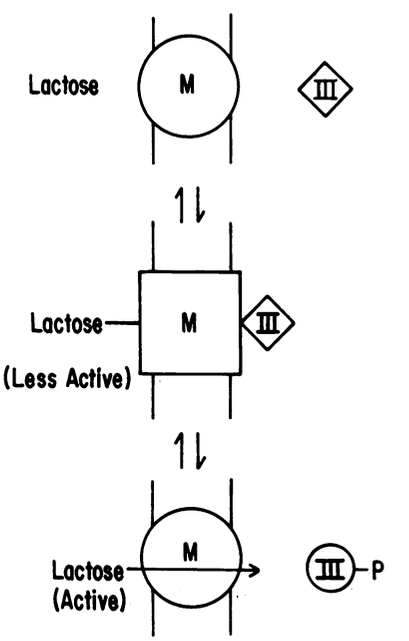

FIG. 4. Proposed mechanism of the regulatory interaction between enzyme III^{glc} and the lactose permease. M and III indicate lactose permease (M protein) and enzyme III^{glc}, respectively. Lactose and enzyme III^{glc} are shown to bind to the lactose permease on opposite sides (outer surface for lactose and inner surface for enzyme III^{glc}) of the plasma membrane (the area between the two vertical lines).

brane fragments, however, are expected to be in the form of inside-out vesicles (34). It is worth noting that our enzyme III^{glc} purification data indicate that this protein represents as much as 0.5% of the total soluble protein in the *E. coli* cell (unpublished result). This value corresponds approximately to an intracellular concentration of 20 μM. In view of these approximate values, it seems reasonable to assess physiological significance to the protein–protein interaction between enzyme III^{glc} and the lactose permease.

In this connection we have found that the inhibition of the uptake of glycerol by methyl α-glucoside in T52RT cells was relieved by adding $SGal_2$ to the incubation mixture (unpublished result). Inhibition of the glycerol permease also requires free enzyme III^{glc}, and this protein would be expected to bind to the lactose permease when $SGal_2$ is added to the cell suspension. These results indicate that the above regulatory mechanism may indeed operate *in vivo* to control the activities of non-PTS permeases.

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1. Monod, J. (1947) *Growth* **11**, 223–289.
2. Gale, E. F. (1943) *Bacteriol. Rev.* **1**, 139–173.
3. Magasanik, B. (1970) in *The Lactose Operon*, eds. Beckwith, J. & Zipser, D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), pp. 189–219.
4. Paigen, K. & Williams, B. (1970) *Adv. Microbiol. Physiol.* **4**, 251–324.
5. Pastan, I. & Adhya, S. (1976) *Bacteriol. Rev.* **40**, 527–551.
6. Leonard, J. E., Lee, C. A., Apperson, A., Dills, S. S. & Saier, M. H., Jr. (1981) in *Organization of Prokaryotic Cell Membranes*, ed. Ghosh, B. K. (Chemical Rubber, West Palm Beach, FL), Vol. 1, pp. 1–52.
7. Saier, M. H., Jr., & Moczydlowski, E. G. (1978) in *Bacterial Transport*, ed. Rosen, B. (Dekker, New York), pp. 103–125.
8. Saier, M. H., Jr., & Roseman, S. (1972) *J. Biol. Chem.* **247**, 972–975.
9. Roseman, S. (1972) in *Metabolic Pathways*, ed. Hokin, L. E. (Academic, New York), Vol. 6, p. 41.
10. Saier, M. H., Jr., & Feucht, B. U. (1975) *J. Biol. Chem.* **250**, 7078–7080.
11. Saier, M. H., Jr., Simoni, R. D. & Roseman, S. (1976) *J. Biol. Chem.* **251**, 6584–6597.
12. Saier, M. H., Jr., & Roseman, S. (1976) *J. Biol. Chem.* **251**, 6598–6605.
13. Saier, M. H., Jr., & Roseman, S. (1976) *J. Biol. Chem.* **251**, 6606–6615.
14. Castro, L., Feucht, B. U., Morse, M. L. & Saier, M. H., Jr. (1976) *J. Biol. Chem.* **251**, 5522–5527.
15. Saier, M. H., Jr., Feucht, B. U. & Hofstadter, L. J. (1976) *J. Biol. Chem.* **251**, 883–892.
16. Saier, M. H., Jr., Straud, H., Massman, L. S., Judice, J. J., Newman, M. J. & Feucht, B. U. (1978) *J. Bacteriol.* **133**, 1358–1367.
17. Saier, M. H., Jr., & Feucht, B. U. (1980) *J. Bacteriol.* **141**, 611–617.
18. Postma, P. & Roseman, S. (1976) *Biochim. Biophys. Acta* **457**, 213–257.
19. Dills, S. S., Apperson, A., Schmidt, M. R. & Saier, M. H., Jr. (1980) *Microbiol. Rev.* **44**, 385–418.
20. Saier, M. H., Jr., & Stiles, C. D. (1975) *Molecular Dynamics in Biological Membrane* (Springer, New York), pp. 99–104.
21. Kundig, W. (1974) *J. Supramol. Struct.* **2**, 695–714.
22. Dills, S. S., Schmidt, M. R. & Saier, M. H., Jr., *J. Supramol. Struct.*, in press.
23. Saier, M. H., Jr., in *Membranes and Transport* (1982) (Academic, New York), in press.
24. Teather, R. M., Bramhall, J., Riede, I., Wright, J. K., Furst, M., Aichele, G., Wilhelm, U. & Overath, P. (1980) *Eur. J. Biochem.* **108**, 223–231.
25. Koike, M. & Hamada, M. (1971) *Methods Enzymol.* **22**, 339–342.
26. Saier, M. H., Jr., Schmidt, M. R. & Lin, P. (1980) *J. Biol. Chem.* **255**, 8579–8584.
27. Feucht, B. U. & Saier, M. H., Jr. (1980) *J. Bacteriol.* **141**, 603–610.
28. Lee, C. A., Jacobson, G. R. & Saier, M. H., Jr. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7336–7340.
29. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
30. Wright, J. K., Teather, R. M. & Overath, P. (1979) in *Function and Molecular Aspects of Biomembrane Transport*, eds. Quagliariello, E., Falmieri, F., Papa, S. & Klingenberg, M. (Elsevier/North-Holland, Amsterdam), pp. 239–248.
31. Kennedy, E. P., Rumley, M. K. & Armstrong, J. B. (1974) *J. Biol. Chem.* **249**, 33–37.
32. Carter, J. R., Fox, C. F. & Kennedy, E. P. (1968) *Proc. Natl. Acad. Sci. USA* **60**, 725–732.
33. Fox, C. F. & Kennedy, E. P. (1965) *Proc. Natl. Acad. Sci. USA* **54**, 891–899.
34. Hertzberg, E. L. & Hinkle, P. C. (1974) *Biochem. Biophys. Res. Commun.* **58**, 178–184.