JOURNAL OF BACTERIOLOGY, Apr. 1984, p. 351–353
0021-9193/84/040351-03$02.00/0

Vol. 158, No. 1

Interaction Between III^{Glc} of the Phosphoenolpyruvate:Sugar Phosphotransferase System and Glycerol Kinase of *Salmonella typhimurium*

P. W. POSTMA,[1]* W. EPSTEIN,[2] A. R. J. SCHUITEMA,[1] AND S. O. NELSON[1]

Laboratory of Biochemistry, University of Amsterdam, Amsterdam, The Netherlands,[1] and Department of Biochemistry, The University of Chicago, Chicago, Illinois 60601[2]

Received 14 November 1983/Accepted 16 January 1984

Purified III^{Glc} of the phosphoenolpyruvate:sugar phosphotransferase system of *Salmonella typhimurium* inhibits glycerol kinase. Phosphorylation of III^{Glc} via phosphoenolpyruvate, enzyme I, and HPr abolishes this inhibition. The glycerol facilitator is not inhibited by III^{Glc}. It is proposed that regulation of glycerol metabolism by the phosphoenolpyruvate:sugar phosphotransferase system is at the level of glycerol kinase.

The first steps in glycerol metabolism in *Escherichia coli* and *Salmonella typhimurium* involve a facilitator, required for entry of glycerol at low concentrations, and glycerol kinase (7). The resulting product, glycerol 3-phosphate, is oxidized subsequently by glycerol 3-phosphate dehydrogenase. These and other related enzymes are coded for by a number of genes that comprise the *glp* regulon (7). The inducer of the *glp* regulon is glycerol 3-phosphate, whereas cyclic AMP is required for full expression of these genes. The phosphoenolpyruvate (PEP):sugar phosphotransferase system (PTS) is involved in the regulation of glycerol metabolism (12). *pts* mutants, lacking the general PTS enzymes, enzyme I or HPr or both, are unable to grow on glycerol, in addition to a number of other non-PTS compounds such as lactose, melibiose, and maltose (class I compounds [11]). Experimentally, it was found that the cellular accumulation of radioactivity from labeled glycerol (often referred to as uptake or accumulation of glycerol) as well as the synthesis of glycerol kinase are impaired in *pts* mutants (14). Two mechanisms are thought to play a role in the regulation of glycerol metabolism (and other class I compounds) by the PTS:inducer exclusion, i.e., the inability of the inducer to enter the cell, and a defect in cyclic AMP synthesis (for a review, see references 12 and 13). It has been proposed that these phenomena are the consequence of the fact that one of the PTS proteins, the glucose-specific factor III^{Glc}, can exist in two forms. It was postulated that nonphosphorylated III^{Glc} inhibits non-PTS transport systems, whereas phosphorylated III^{Glc} activates adenylate cyclase (12, 13). When the cell is unable to phosphorylate III^{Glc} sufficiently, both the intracellular inducer as well as the cyclic AMP concentrations are low, resulting in impaired induction. Recently, it has been shown that purified nonphosphorylated III^{Glc} indeed binds to the purified lactose carrier in a fixed stoichiometry and inhibits it (9, 10). Phosphorylated III^{Glc} has no such effect. These results confirm that III^{Glc} is indeed the regulatory molecule in at least one system, the lactose transport system, and it seemed reasonable to extrapolate that inducer exclusion via the PTS indeed acts at the level of the respective transport systems. Here we report that the site of inhibition of glycerol metabolism by III^{Glc} is glycerol kinase, the first enzyme involved in glycerol metabolism, and not the membrane-bound glycerol facilitator.

Glycerol kinase was measured in cell extracts of *S. typhimurium* after passing the cells through a French pressure cell and removing intact cells and cell debris by centrifugation for 10 min at 10,000 × *g* (15). The activity of glycerol kinase was determined with 0.5 mM [*U*-^{14}C]glycerol (120 cpm/nmol; New England Nuclear Corp.) as substrate. The amount of glycerol 3-phosphate formed was determined by the ion-exchange method (6). Glycerol kinase from *Candida mycoderma* was obtained from Boehringer Mannheim Biochemicals. The purification of III^{Glc}, enzyme I, and HPr has been described elsewhere (15). Varying amounts of III^{Glc} were added (see below) and, if required, 0.003 IU each of enzyme I and HPr and 10 mM PEP. Swelling experiments to determine the activity of the glycerol facilitator were performed exactly as described by Heller et al. (4) with 250 mM xylitol. Cells were pregrown overnight in minimal lactate medium (8) and after dilution grown in minimal medium containing glycerol as a carbon source to achieve partial induction of the glycerol-metabolizing enzymes (8). *S. typhimurium* SB3507 (*trpB223 pts*$^+$), SB1476 (*ptsI17*), and PP800 [Δ(*ptsHI-crr*)*49 trpB223*] have been described elsewhere (8). Strain PP800 lacks the PTS enzymes, enzyme I, HPr, and III^{Glc}, completely.

Figure 1 shows that the glycerol kinase activity in an extract of wild-type SB3507 is inhibited progressively by the addition of increasing concentrations of purified III^{Glc}. Under the conditions used, half-maximal inhibition is reached at ca. 1 mg of III^{Glc} per ml. Similar results have been obtained with cell extracts of the Δ(*ptsHI-crr*) deletion strain PP800. We have found previously that the concentration of III^{Glc} required to saturate half of the lactose carriers is 5 to 15 μM, i.e., 100 to 300 μg/ml, assuming a molecular weight of 20,000 for III^{Glc} (9). It should be kept in mind that the intracellular concentration of III^{Glc} is estimated to be about 1 mg/ml (15). If a stoichiometric interaction is required, as is the case with the lactose (9) and the maltose permeases (8), it is in principle possible to "titrate out" the inhibitor. Assuming a similar turnover number of 11,600 for glycerol kinase from *S. typhimurium*, as was found for *E. coli* (7), and having ca. 60 μg of cell extract per ml, it can be calculated that under our experimental conditions, III^{Glc} (at 3 mg/ml) is present in at least a 1,000-fold excess compared with glycerol kinase. The somewhat higher amount of III^{Glc} required for inhibition of

* Corresponding author.

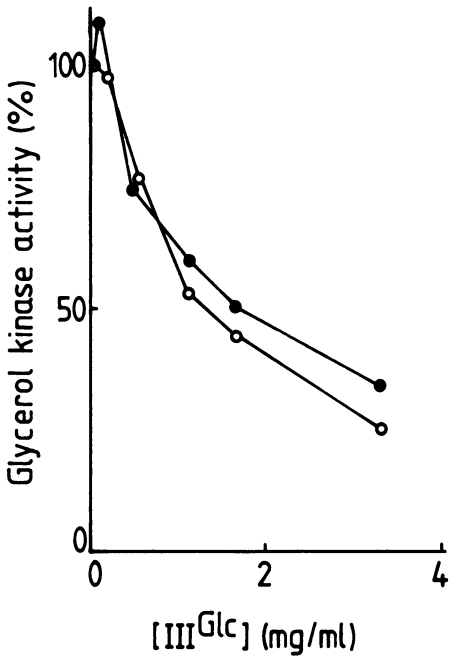

FIG. 1. Inhibition of glycerol kinase by III^{Glc}. Glycerol kinase was measured in a medium (final volume, 100 μl) containing 50 mM potassium phosphate, 2.5 mM dithiotreitol, 12.5 mM KF, 10 mM ATP, 5 mM $MgCl_2$, and 0.5 mM [^{14}C]glycerol (final pH 7.5). As a source of glycerol kinase, a cell extract of strain SB3507 (○) or PP800 (●) was used. A final concentration of ca. 60 μg of cell-free protein per ml was used. Purified III^{Glc} was added up to 3.3 mg/ml. The activity is expressed as the percentage of the activity in the absence of III^{Glc}. One-hundred percent equals 450 nmol of glycerol phosphorylated/min per mg of protein at 37°C in the case of strain SB3507 and 203 nmol of glycerol phosphorylated/min per mg of protein in the case of strain PP800.

glycerol kinase compared with the lactose permease may be due to the fact that we measured glycerol kinase in very dilute extracts. Under these conditions, part of the oligomeric glycerol kinase may be dissociated and not sensitive to inhibition by III^{Glc}.

Since strain PP800 is completely devoid of enzyme I, HPr, and III^{Glc}, we were able to test the effect of the phosphorylation state of III^{Glc} (Table 1). Neither PEP alone nor enzyme I plus HPr had any effect on the inhibitory action of III^{Glc}. However, when III^{Glc} was phosphorylated by adding enzyme I, HPr, and PEP together, the inhibition of glycerol kinase was largely abolished. This result is similar to our results obtained with the lactose permease (9). In both cases, phosphorylation of III^{Glc} abolished its inhibitory action. Preincubation of glycerol kinase with III^{Glc} was not required, the inhibition being similar with preincubation for either 30 or 0 min. Similarly, PEP completely abolished inhibition in the presence of enzyme I and HPr whether III^{Glc} and the cell extract were preincubated for 30 min in the absence of PEP or not at all. Finally, a fungal glycerol kinase used as a control and assayed under identical conditions was not significantly inhibited by III^{Glc} (Table 1).

In contrast with results obtained for the kinase, we found no inhibition of glycerol facilitator activity under conditions that markedly inhibited accumulation of radiolabel from glycerol (data not shown). Facilitator activity was measured by the rate of cell swelling in the presence of 250 mM xylitol as described by Heller et al. (4). These authors showed that xylitol was a substrate of the facilitator and was transported much more slowly than glycerol, so that initial rates of swelling could be measured with a spectrophotometer. First, we determined that wild-type *S. typhimurium* behaves like *E. coli*. Growth on glycerol indeed greatly enhanced xylitol-induced swelling compared with cells grown on lactate, as described for *E. coli* by Heller et al. (4). Next, we measured activity in glycerol-grown cells of strain SB1476 which was the leaky *pts-17* mutation and is very sensitive to inhibition by PTS sugars. It has been shown earlier that accumulation of radiolabel from glycerol in glycerol-induced cells of this strain is markedly inhibited by the PTS substrate methyl-α-glucoside (8, 14). No inhibition (<10% change) of the rate of xylitol entry by 0.5 mM methyl-α-glucoside was detected. In parallel experiments, this glycoside inhibited glycerol oxidation by over 95% (<4 nonatoms of O taken up/min per mg [dry weight] at 25°C in the presence of 0.5 mM methyl α-glucosidase compared with 75 nonatoms of O taken up/min per mg without methyl α-glucosidase) and accumulation of radiolabel from glycerol by over 90% (8, 14).

The results presented above suggest strongly that the regulation of glycerol metabolism by the PTS is at the level of glycerol kinase, not at that of the glycerol facilitator. Accumulation of radioactivity represents accumulation of glycerol phosphate and products derived from it in the cell, because glycerol equilibrates rapidly across the cell membrane and is lost during the washing procedures inherent in measuring accumulation. Thus, accumulation of label requires glycerol kinase activity (7) because only products after this step are retained. Inhibition of glycerol kinase also explains the inhibition of the *glp* regulon because the true inducer is glycerol 3-phosphate (2).

Interaction between III^{Glc} and various metabolic systems has been proposed in addition to its interaction with adenylate cyclase. In the case of the lactose permease, it has been shown that III^{Glc} interacts directly with the permease, and in the case of the melibiose and maltose transport systems, this seems likely. PTS sugars inhibit transport of thiomethylga-

TABLE 1. Inhibition of glycerol kinase by phosphorylated and nonphosphorylated III^{Glc}[a]

Expt.	Source of enzyme	Additions	Glycerol kinase activity[b] (%)
1	*S. typhimurium*	None	222 (100)
		PEP	260 (117)
		Enzyme I + HPr	186 (84)
		III^{Glc}	75 (34)
		Enzyme I + HPr + III^{Glc}	44 (20)
		Enzyme I + HPr + III^{Glc} + PEP	200 (90)
2	*C. mycoderma*	None	94,500 (100)
		III^{Glc}	86,200 (92)

[a] Glycerol kinase was measured as described in the legend to Fig. 1. As a source of glycerol kinase in experiment 1, a cell extract of strain PP800 was used. When present, III^{Glc} was added to a final concentration of 3 mg/ml. A total of 0.003 IU each of enzyme I and HPr and 10 mM PEP containing 5 mM $MgCl_2$ were added when tested. In experiment 2, glycerol kinase from *C. mycoderma* was measured. The amount of cell extract from PP800 (6 μg/100 μl) and purified glycerol kinase from *C. mycoderma* (12.5 ng/100 μl) were adjusted to yield comparable amounts of glycerol 3-phosphate.

[b] Glycerol kinase activity is expressed as nanomoles of glycerol phosphorylated per minute per milligram of protein at 37°C.

lactoside, a nonmetabolizable analog of melibiose (14), and mutations in the *malK* gene, coding for one of the maltose transport proteins, render this system resistant to PTS-mediated regulation (5). Surprisingly, we have now found that nonphosphorylated III^{Glc} is also able to interact with a soluble, cytoplasmic enzyme, glycerol kinase. Berman and Lin (1) have described mutations in the *glpK* gene of *E. coli pts* mutants that allow growth on glycerol but not on other class I compounds. The authors have shown that these mutants acquire an altered glycerol kinase, which is insensitive to inhibition by fructose-1,6-diphosphate. Our findings imply that they in fact selected for a mutant glycerol kinase, insensitive to inhibition by III^{Glc} which at the same time became resistant to fructose-1,6-diphosphate.

The present findings will greatly facilitate our studies on the interaction between III^{Glc} and its various targets since purification of glycerol kinase has been described (3, 16). We intend to study the properties of the altered glycerol kinases and the interaction between purified glycerol kinase and III^{Glc} to determine whether in this case as well (cf. the lactose permease described in reference 9) the glycerol substrate is required for interaction. It has been shown (17) that glycerol promotes a major conformational change in glycerol kinase. Finally, it should be interesting to find out what common structure in lactose permease and glycerol kinase is recognized by III^{Glc}.

LITERATURE CITED

1. **Berman, M., and E. C. C. Lin.** 1971. Glycerol-specific revertants of a phosphoenolpyruvate phosphotransferase mutant: suppression by the desensitization of glycerol kinase to feedback inhibition. J. Bacteriol. **105**:113–120.
2. **Cozzarelli, N. R., W. B. Freedberg, and E. C. C. Lin.** 1968. Genetic control of the L-α-glycerophosphate system in *Escherichia coli*. J. Mol. Biol. **31**:371–387.
3. **Hayashi, S., and E. C. C. Lin.** 1967. Purification and properties of glycerol kinase from *Escherichia coli*. J. Biol. Chem. **242**:1030–1035.
4. **Heller, K. B., E. C. C. Lin, and T. H. Wilson.** 1980. Substrate specificity and transport properties of the glycerol facilitator of *Escherichia coli*. J. Bacteriol. **144**:274–278.
5. **Hengge, R., and W. Boos.** 1983. Maltose and lactose transport in *Escherchia coli*. Examples of two different types of concentrative transport systems. Biochim. Biophys. Acta **737**:443–478.
6. **Kundig, W., and S. Roseman.** 1971. Sugar transport II. Characterization of constitutive membrane-bound enzymes II of the *Escherichia coli* phosphotransferase system. J. Biol. Chem. **246**:1407–1418.
7. **Lin, E. C. C.** 1976. Glycerol dissimilation and its regulation in bacteria. Annu. Rev. Microbiol. **30**:535–578.
8. **Nelson, S. O., B. J. Scholte, and P. W. Postma.** 1982. Phosphoenolpyruvate:sugar phosphotransferase system-mediated regulation of carbohydrate metabolism in *Salmonella typhimurium*. J. Bacteriol. **150**:604–615.
9. **Nelson, S. O., J. K. Wright, and P. W. Postma.** 1983. The mechanism of inducer exclusion. Direct interaction between purified III^{Glc} of the phosphoenolpyruvate:sugar phosphotransferase system and the lactose carrier of *Escherichia coli*. EMBO J **2**:715–720.
10. **Osumi, T., and M. H. Saier.** 1982. Regulation of lactose permease activity by the phosphoenolpyruvate:sugar phosphotransferase system: evidence for direct binding of the glucose-specific enzyme III to the lactose permease. Proc. Natl. Acad. Sci. U.S.A. **79**:1457–1461.
11. **Postma, P. W.** 1982. Regulation of sugar transport in *Salmonella typhimurium*. Ann. Microbiol. **133A**:261–267.
12. **Postma, P. W., and S. Roseman.** 1976. The bacterial phosphoenolpyruvate:sugar phosphotransferase system. Biochim. Biophys. Acta **457**:213–257.
13. **Saier, M. H., Jr.** 1977. Bacterial phosphoenolpyruvate:sugar phosphotransferase systems: structural, functional, and evolutionary relationships. Bacteriol. Rev. **41**:856–871.
14. **Saier, M. H., and S. Roseman.** 1976. Sugar transport. Inducer exclusion and regulation of the meliboise, maltose, glycerol and lactose transport systems by the phosphenolpyruvate:sugar phosphotransferase system. J. Biol. Chem. **251**:6606–6615.
15. **Scholte, B. J., A. R. Schuitema, and P. W. Postma.** 1981. Isolation of III^{Glc} of the phosphoenolpyruvate-dependent glucose phosphotransferase system of *Salmonella typhimurium*. J. Bacteriol. **148**:257–264.
16. **Thorner, J. W., and H. Paulus.** 1971. Composition and subunit structure of glycerol kinase from *Escherichia coli*. J. Biol. Chem. **246**:3885–3894.
17. **Thorner, J. W., and H. Paulus.** 1973. Catalytic and allosteric properties of glycerol kinase from *Escherichia coli*. J. Biol. Chem. **248**:3922–3932.