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Regulation of Glycerol Kinase by Enzyme III^{Glc} of the Phosphoenolpyruvate:carbohydrate Phosphotransferase System

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Wild-type glycerol kinase of *Escherichia coli* is inhibited by both nonphosphorylated enzyme III^{Glc} of the phosphoenolpyruvate:carbohydrate phosphotransferase system and fructose 1,6-diphosphate. Mutant glycerol kinase, resistant to inhibition by fructose 1,6-diphosphate, was much less sensitive to inhibition by enzyme III^{Glc}. The difference between the wild-type and mutant enzymes was even greater when inhibition was measured in the presence of both enzyme III^{Glc} and fructose 1,6-diphosphate. The binding of enzyme III^{Glc} to glycerol kinase required the presence of the substrate glycerol.

The phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS) regulates in *Escherichia coli* and *Salmonella typhimurium* the uptake and metabolism of a number of non-PTS carbohydrates, including lactose, maltose, melibiose, and glycerol, by inhibiting both the actual uptake process and the expression of the various operons involved (for a recent review, see reference 8). A central role in this regulation is played by the glucose-specific enzyme III^{Glc} of the PTS, which can exist in two forms, of which the nonphosphorylated form inhibits the various uptake systems. In the case of lactose permease, this has been demonstrated with the purified components (5). The phosphorylated form of enzyme III^{Glc} is involved in the activation of adenylate cyclase and, thereby, indirectly activates the expression of certain operons. Thus, the actual phosphorylation state of enzyme III^{Glc} is important in determining the activity and the number of the various uptake systems.

We have recently shown that in *S. typhimurium* the uptake of glycerol is regulated at the level of glycerol kinase, rather than at the level of the glycerol facilitator (7). Nonphosphorylated enzyme III^{Glc} inhibits glycerol kinase, but phosphorylated enzyme III^{Glc} does not. These results have been confirmed by Novotny et al. (6). *ptsI* mutants of *E. coli* and *S. typhimurium* do not grow on glycerol and several other non-PTS carbohydrates. Berman and Lin (1) have described suppressor mutations in the *glpK* gene, coding for glycerol kinase, that allow *E. coli ptsI* mutants to grow on glycerol (but not on the other non-PTS carbohydrates mentioned above). These *glpK*^{i} mutants contain an altered glycerol kinase which is insensitive to feedback inhibition by fructose 1,6-diphosphate (1). We have suggested the possibility that glycerol kinase in these mutants has become insensitive to inhibition by nonphosphorylated enzyme III^{Glc} (7).

Here we wish to report that at least one of these *glpK*^{i} mutants has indeed a glycerol kinase that is less sensitive to enzyme III^{Glc}. The difference between the wild-type and mutant enzymes seems even greater when the inhibition by fructose 1,6-diphosphate is also taken into account. Finally, we show that the interaction between glycerol kinase and enzyme III^{Glc} requires the substrate glycerol, analogous to the binding of enzyme III^{Glc} to lactose permease, which requires β-galactoside (5).

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E. coli 11 (*leu thi*) and 225 (*leu thi ptsI glpK*^{i}) (1) were kindly donated by E. C. C. Lin (Harvard Medical School). Plasmid pK3, containing the *E. coli glpK*$^{+}$ gene, was a gift of W. Boos (University of Konstanz). It was transformed into *E. coli* WM648 (*asnA asnB thi recA1*). Strains were grown on a rotary shaker in Luria broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl) or in minimal salts medium (9). Cell-free extracts were prepared as described elsewhere (7, 10). Glycerol kinase was purified from *E. coli* WM648 (pK3) by the method of Thorner and Paulus (11) with slight modifications. The purified glycerol kinase had a specific activity of 21 μmol/min per mg of protein. Glycerol kinase activity was measured as described by Postma et al. (7) by using [U-^{14}C]glycerol (8 Ci/mol; New England Nuclear Corp.). Enzyme III^{Glc} was purified from *S. typhimurium* SB3507 (pBCP2) as described elsewhere (4, 10). The binding of enzyme III^{Glc} to the *E. coli* lactose carrier was measured as described by Nelson et al. (5).

We have shown before that glycerol kinase from *S. typhimurium* is inhibited by the addition of purified enzyme III^{Glc} (7). Since the *glpK*^{i} mutation that allows the growth of *ptsI* mutants on glycerol has been isolated in *E. coli* (1), we first determined whether *E. coli* glycerol kinase behaves similarly to *S. typhimurium* glycerol kinase with respect to inhibition by enzyme III^{Glc}. Figure 1A shows that this is the case, confirming the results of Novotny et al. (6). Glycerol kinase, synthesized from plasmid pK3 and present at approximately fivefold-elevated levels, was also inhibited by enzyme III^{Glc}.

Next we investigated whether *glpK*^{i} strains contain a glycerol kinase that is less sensitive to inhibition by enzyme III^{Glc}. *E. coli* 225 (derived from strain 11 [1]) contains a *glpK*^{i} mutation that allows the growth of this *ptsI* mutant on glycerol but not on other non-PTS carbohydrates and results in an altered glycerol kinase which is insensitive to feedback inhibition by fructose 1,6-diphosphate (1). Figure 1B shows that glycerol kinase from the wild type (strain 11) was indeed strongly inhibited by fructose 1,6-diphosphate, whereas the mutant (strain 225) glycerol kinase was insensitive towards this inhibitor, confirming the results reported by Berman and Lin (1). Figure 1A shows that the glycerol kinase in mutant strain 225 was less sensitive to inhibition by enzyme III^{Glc} than was the wild-type enzyme.

Table 1 shows the percent inhibition of glycerol kinase from both wild-type and mutant strains at various concen-

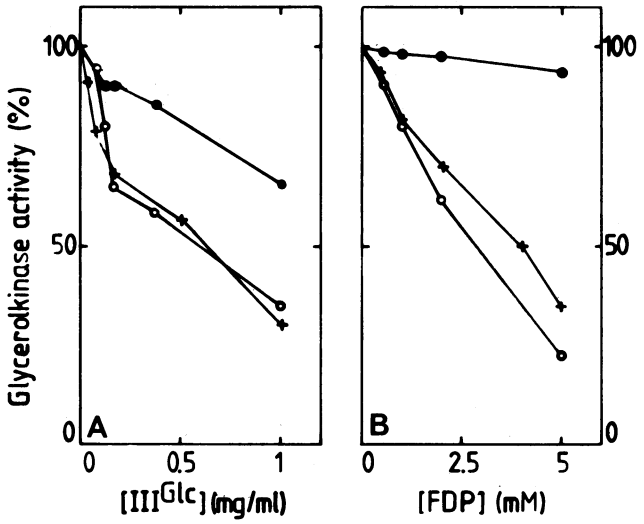

FIG. 1. Inhibition of glycerol kinase by enzyme III^{Glc} and fructose 1,6-diphosphate. Activity is expressed as the percent activity remaining in the absence of an inhibitor. (A) Inhibition by enzyme III^{Glc}. (B) Inhibition by fructose 1,6-diphosphate (FDP). Symbols: ○, *E. coli* 11 supernatant; ●, *E. coli* 225 supernatant; ×, *E. coli* WM648 (pK3) supernatant. The specific activities of glycerol kinase in these three strains in the absence of an inhibitor were 0.5, 0.2, and 2.4 μmol of glycerol phosphorylated per min per mg of protein, respectively.

trations of both inhibitors. From Fig. 1A and B we calculated the expected inhibition if both effects were additive. From Table 1 it is clear that at all concentrations except the highest enzyme III^{Glc} concentration, the inhibition of both wild-type and mutant glycerol kinases was greater than expected.

One of our goals is to define the interaction between enzyme III^{Glc} and its various target proteins in more detail. An important question is whether the formation of a complex between glycerol kinase and enzyme III^{Glc} requires a substrate of the kinase, as is the case for the binding of purified enzyme III^{Glc} to the isolated lactose permease, which has been shown to occur only when β-galactoside is present (5). Since the substrate of glycerol kinase is required for the measurement of its enzymatic activity, we cannot simply determine whether the binding of enzyme III^{Glc} to glycerol kinase requires the presence of its substrate glycerol. For this reason we determined whether glycerol kinase can compete with the lactose carrier for binding to enzyme III^{Glc}.

TABLE 1. Inhibition of glycerol kinase by enzyme III^{Glc} and fructose 1,6-diphosphate

Enzyme III^{Glc} (mg/ml)	Fructose 1,6-diphosphate (mM)	% Inhibition of glycerol kinase activity in *E. coli*[a]:			
		11		225	
		Measured	Calculated	Measured	Calculated
0.072	0.5	19	17	9	8
0.072	1	46	27	22	10
0.144	1	60	38	34	15
0.144	2	70	52	44	17
1.0	0.7	78	85	38	35

[a] The calculated percent inhibition was derived from the data in Fig. 1A and B by adding the percent inhibition of glycerol kinase activity at the particular concentraitons of enzyme III^{Glc} and fructose 1,6-biphosphate used.

TABLE 2. Competition between glycerol kinase and the lactose carrier for enzyme III^{Glc}[a]

Glycerol kinase (mg/ml)	Amt of enzyme III^{Glc} bound (μg/mg of membrane) when glycerol was:	
	Absent	Present
0	8.6	8.6
0.5	8.6	6.8
1.5	7.5	4.6

[a] Glycerol kinase was purified from *E. coli* WM648(pK3). The binding of enzyme III^{Glc} to the lactose carrier was determined as described by Nelson et al. (5). Glycerol, when present, was added to a final concentration of 1 mM. Incubations (25 mM Tris hydrochloride, 4.2 mM $MgSO_4$; pH 7.5; final volume, 60 μl) contained 0.365 nmol of lactose carrier (Ribi vesicles, 17.4 mg/ml; 1.4 nmol of lactose carrier per mg of protein), 0.38 nmol of enzyme III^{Glc}, and various amounts of glycerol kinase. Membrane vesicles containing 1.4 nmol of lactose carrier per mg of protein were isolated from *E. coli* T206 containing a *lacY* plasmid, kindly donated by J. K. Wright (Max-Planck Institut, Tubingen).

Table 2 shows a competition experiment in which we measured the binding of a limited amount of enzyme III^{Glc} to a fixed amount of lactose permease as a function of increasing concentrations of purified glycerol kinase and the presence or absence of glycerol. From the results we concluded that glycerol is required for the interaction of enzyme III^{Glc} and glycerol kinase.

The regulatory enzyme III^{Glc} of the PTS can interact with many different proteins, including the PTS proteins HPr and enzyme II^{Glc} (and enzyme II^{Scr}, the enzyme II for sucrose [2]) and a number of non-PTS proteins, including components of the lactose, melibiose, glycerol, and maltose uptake systems. Finally, enzyme III^{Glc} is involved in the regulation of adenylate cyclase (for a review, see reference 8). In the case of glycerol, enzyme III^{Glc} was shown to react not with the membrane-bound glycerol facilitator but rather with the first enzyme in glycerol metabolism, glycerol kinase (7). Nonphosphorylated enzyme III^{Glc} inhibits the glycerol kinases from *S. typhimurium* (7), *E. coli* (6; Fig. 1A), and *Klebsiella aerogenes* (B. Pover and P. W. Postma, unpublished results).

The binding of enzyme III^{Glc} to the lactose carrier has been studied in detail (5). Nonphosphorylated enzyme III^{Glc} binds only in the presence of β-galactoside. From experiments with intact *S. typhimurium* cells, we concluded previously that the interaction between enzyme III^{Glc} and glycerol kinase also requires the presence of the substrate glycerol (3). In this paper, we report an experiment with the two purified enzymes that confirms this result. The interaction between enzyme III^{Glc} and glycerol kinase required the presence of the substrate glycerol. From the data presented in Table 2, one can calculate that at a glycerol kinase concentration of 1.5 mg/ml, 0.1 to 0.15 nmol of enzyme III^{Glc} is bound per nmol of glycerol kinase. One should remember that the enzyme III^{Glc} concentration used (0.12 mg/ml) was far below saturation and was selected for experimental reasons. From Fig. 1A, one can calculate that at this enzyme III^{Glc} concentration, glycerol kinase is inhibited less than 20%, indicating incomplete binding.

In a previous paper (7), we suggested a possible explanation for the $glpK^1$ mutation isolated by Berman and Lin (1): an altered glycerol kinase insensitive to inhibition by enzyme III^{Glc}. Figure 1A shows that the inhibition of the mutant enzyme by enzyme III^{Glc} is indeed less than that of the wild-type enzyme. It has been shown before that the mutant glycerol kinase is still able to bind fructose 1,6-diphosphate,

although it is resistant to inhibition by this compound (12). This might explain why the cooperative effect of enzyme III^{Glc} and fructose 1,6-diphosphate is observed even in the mutant. *S. typhimurium* mutants that are resistant to enzyme III^{Glc} but still sensitive to fructose 1,6-diphosphate have been reported by Novotny et al. (6).

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