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Glycerol-Specific Revertants of a Phosphoenolpyruvate Phosphotransferase Mutant: Suppression by the Desensitization of Glycerol Kinase to Feedback Inhibition

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Glycerol-specific revertants were isolated from a phosphoenolpyruvate phosphotransferase mutant lacking enzyme I activity. Sixteen of the eighteen separately derived revertants were found to synthesize a fully active glycerol kinase no longer subject to feedback inhibition by fructose 1,6-diphosphate. The suppressor mutation mapped at the known *glpK* locus. When the fructose 1,6-diphosphate-insensitive kinase allele was transduced into a strain producing the *glp* enzymes constitutively, cells of the resultant strain were susceptible to killing by glycerol if this compound was added to a culture growing exponentially in casein hydrolysate. This phenomenon had been previously described for a strain which had a constitutive glycerol kinase refractory to feedback inhibition, but isolated by a different procedure. It is suggested that the suppression of the growth defect on glycerol in the enzyme I^- mutant by the fructose 1,6-diphosphate-insensitive kinase is achieved by increasing the in vivo catalytic potential of glycerol kinase. This increased activity would allow more rapid conversion of glycerol to L-α-glycerophosphate, the true inducer of the *glp* system. The enzyme I defect in the parental strain impaired the inducibility of the *glp* system so that the normal basal catalytic activity of the kinase was insufficient to insure induction by glycerol.

The dissimilation of glycerol and L-α-glycerophosphate (L-α-GP) in *Escherichia coli* requires the induction of several gene products. A specific protein is postulated to mediate the facilitated diffusion of glycerol into the cell (25) where an adenosine triphosphate-dependent kinase traps the substrate by converting it to the phosphorylated derivative, L-α-GP (11). This product is converted to triosephosphate aerobically by a membrane-associated dehydrogenase and anaerobically by a soluble dehydrogenase (12). Exogenous L-α-GP can also be taken up by the cells through the mediation of an active transport system (9). Synthesis of these gene products is negatively controlled by a single regulatory locus, *glpR*, whose product is neutralized by the inducer L-α-GP (3, 10). In addition, the activity of the kinase is feedback-regulated by fructose 1,6-diphosphate (FDP; reference 34). The enzymes and the transport systems involved in glycerol and L-α-GP metabolism comprise the *glp* system (3) and their roles are schematically presented in Fig. 1. [Genetic symbols used in this paper are according to Taylor and Trotter (27).]

Induction of the enzymes for glycerol metabolism is impaired in certain mutants of *E. coli* which have lost enzyme I of the phosphoenolpyruvate-phosphotransferase system (7, 29, 30) discovered by Kundig, Ghosh, and Roseman (14, 24), although this phosphorylating system is not known to participate directly in the metabolic reactions of glycerol. In a previous paper (1) we reported that the ability of such an enzyme I^- mutant to grow on glycerol could be restored in two ways: (i) by converting the *glpR* allele from the inducible state to the constitutive state by transduction, or (ii) by the provision of cyclic 3',5'-adenosine monophosphate (cyclic AMP) to the growth medium.

Pastan and Perlman (20) reported earlier that an enzyme I^- mutant with broad defects in its carbohydrate metabolism, including the utilization of lactose, could be made to grow on lactose by the addition of cyclic AMP. Lactose rever-

tants from pleiotropic carbohydrate-defective mutants were isolated by Morse and Wang (Bacteriol Proc., p. 60, 1969), and in nearly two-thirds of the cases the suppressor mutations mapped in the *lac* region and showed altered inducibility. Similar findings were made in the case of maltose revertants (30).

The purpose of the present study was to find out whether specific glycerol "revertants" readily arise from an enzyme I^- mutant and whether these limited revertants have altered regulation of the *glp* system.

MATERIALS AND METHODS

Bacteria and phages. Strain 11 was selected for improved growth on mannitol by E. Solomon from *E. coli* K-12 AB313 [collection of A. L. Taylor (27)]. The original strain was auxotrophic for leucine, threonine, and thiamine, but during growth recycling the threonine requirement was lost [probably because of wasteful depletion of the limited supply by threonine deaminase (28, 31)]. Strain 223 was isolated by E. Solomon from strain 11 as a mutant lacking enzyme I (1). Strain 224 was isolated as a spontaneous revertant clone of 223 on agar containing mannitol, leucine, and thiamine, and strain 225 on agar containing glycerol, leucine, and thiamine. The isolation of strain 7 (constitutive in the *glp* system) and strain 43 (constitutive in the *glp* system and with a glycerol kinase insensitive to feedback inhibition) from *E. coli* K-12 Hfr C has already been reported (9, 34).

The following transductions were carried out by the method of Luria, Adams, and Ting (16). Phages grown on strain 225 were used to infect strain 27 which synthesizes the enzymes of the *glp* system constitutively but has no glycerol kinase activity. Several transductants selected on glycerol, among them strain 244, were examined for glycerol kinase activity. The enzyme was found to be restored in each.

Phages grown on strain 11 were used to infect strain 27. A transductant able to grow on glycerol, strain 271, was isolated.

To construct a strain carrying the markers *metB*$^-$, *glpK*$^-$, *glpR*$^-$, advantage was taken of the high frequency of cotransduction between the *mal A* and the *glpR* loci. Cells of strain 161 (*metB*$^-$, *glpK*$^-$, *glpR*$^+$, *mal A*$^-$) were infected with phages harvested after growth on strain 7 (*glpR*$^-$, *malA*$^+$), and transductants able to grow on maltose were selected. One of these, strain 162, was adopted after ascertaining that its *glp* system is constitutive. The genotypes of the various bacterial strains employed in this study are summarized in Table 1.

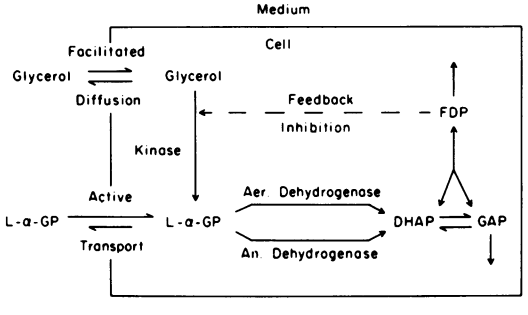

FIG. 1. *Metabolic scheme for the dissimilation of glycerol and* L-α-*GP by Escherichia coli.*

Culture media and growth conditions. The mineral medium used for growth was the same as previously specified (26). Carbon sources were added to give a final concentration of 0.02 M glycerol, 0.04 M DL-α-glycerophosphate, (DL-α-GP), 0.04 M pyruvate, 0.01 M mannitol, and 0.01 M arabinose singly or in combinations. For leucine and thiamine auxotrophs, the supplements were added to give a final concentration of 5 μg/ml and 2 μg/ml, respectively. Casein acid hydrolysate (Nutritional Biochemicals; salt-free, vitamin-free) was added to mineral medium to give a final concentration of 1%. To prepare solid media, 15 g of agar (Difco) was added to 1 liter of mineral medium. Growth rates were determined by following changes in turbidity (4). One Klett unit (no. 42 filter) corresponds to 4×10^6 cells/ml.

Mapping. Phages grown on strain 225 were used to transduce cells of strain 162 (*glpK*$^-$, *metB*$^-$, *glpR*$^-$); and *metB*$^+$ transductants were selected on arabinose mineral plates lacking the required amino acid. Possession of active glycerol kinase, the unselected marker, was scored by testing the ability of the transductants (harvested from an arabinose medium) to grow on glycerol mineral plates. The FDP sensitivity of the glycerol kinases of each of 10 transductants was determined indirectly by testing for susceptibility of cells fully derepressed in glycerol kinase to the bacteriocidal action of glycerol (33). The reliability of this technique of scoring was established by in vitro enzymatic assay.

Assays for enzyme I and HPr. Activities of enzyme I and HPr of the phosphoenolpyruvate phosphotransferase system were determined by the phosphorylation of ^{14}C-mannitol under a condition in which the other requisite components were in excess as described earlier (26), with the exception that the radioactive mannitol phosphate was adsorbed to diethylaminoethyl (DEAE) filter-paper discs (17) instead of adsorbed to and eluted from Dowex columns. Excess unreacted ^{14}C-mannitol was removed by rinsing the discs in distilled water for 30 min. After drying, the radioactivity on the filter discs was measured by scintillation counting.

Assay for enzymes of the glp system. Glycerol kinase was usually assayed spectrophotometrically at *p*H 9.5 (15), but, to test for sensitivity to inhibition by FDP, a spectrophotometric assay at *p*H 7.5 was used (34). An assay which measures the formation of ^{14}C-L-α-GP from ^{14}C-glycerol was used to determine *p*H profiles of the glycerol kinases of mutant and wild-type strains. The composition of the reaction mixture was as follows: 0.05 mM ^{14}C-glycerol (specific activity: 26 μCi/μmole), 10 mM $MgCl_2$, 10 mM adenosine triphosphate, and a buffer composed of 12.5 mM each of glycine, maleate, and triethanolamine adjusted to the desired *p*H. Appropriately diluted crude extract was added to the reaction mixtures, each buffered at a different *p*H. The final volume of the assay mixture was 0.4 ml. After 4 min of incubation at 25 C, 0.6 ml of an ice-cold solution of 0.02 M glycerol was added to stop the assay. The mix-

TABLE 1. *Genotype of strains used*

Strain	Parent	Reference	Relevant markers									
			glp System				Enz I[b]	*metB*	*malA*	*thr*	*leu*	*thi*
			K	T	D[a]	R						
AB313		27	+	+	+	+	+	+	+	−	−	−
11	AB313	1	+	+	+	+	+	+	+	+	−	−
223	11	1	+	+	+	+	−	+	(+)[c]	+	−	−
224	223		+	+	+	+	+	+	+	+	−	−
225	223		i	+	+	+	−	+	(+)	+	−	−
7		9	+	+	+	−	+	+	+	+	+	+
43	7	34	i	+	+	−	+	+	+	+	+	+
27	7		−	+	+	−	+	+	+	+	+	+
244	27		i	+	+	−	+	+	+	+	+	+
271	27		+	+	+	−	+	+	+	+	+	+
161		5	−	+	+	+	+	−	−	+	+	+
162	161		−	+	+	−	+	−	+	+	+	+

[a] Aerobic enzyme.
[b] Enzyme I.
[c] Maltose-negative due to the enzyme I lesion.

ture was then diluted 10-fold with tris(hydroxymethyl) aminomethane buffer (0.1 M, *p*H 7.5), and 25 μliters of the diluted mixture was placed on a disc of DEAE filter paper to adsorb the ^{14}C-L-α-GP formed. It is important to readjust the final diluted mixture to *p*H 7.5 to allow complete adsorption of the L-α-GP onto the DEAE filter paper. The filter-paper discs were washed in running distilled water for 30 min and dried, and the radioactivity on the discs was counted by scintillation. Phosphorylation is linear for more than 10 min, and the rate is proportional to the amount of enzyme over a 12-fold range.

The aerobic L-α-GP dehydrogenase was assayed by coupling to a tetrazolium dye (15) under a condition which does not allow the anaerobic enzyme to contribute significantly to the reaction (12).

For the measurement of L-α-GP transport, the washed cells were suspended at a density of 100 Klett units in mineral medium and incubated at 25 C in the presence of 10^{-6} M ^{14}C-L-α-GP. A sample of 0.5 ml was withdrawn after 15, 30, 45, and 60 sec of exposure and delivered immediately for filtration onto discs (0.45 μm pore size; Millipore Corp., Bedford, Mass.) previously wetted with mineral medium. After washing (9) and drying, the radioactivity on the discs was measured by scintillation counting. Self-adsorption of the cells was negligible.

Determination of FDP. Intracellular FDP was released instantly from an exponentially growing culture by the addition of perchloric acid. After removal of the debris and perchlorate, samples of the medium were assayed for FDP by an enzymatic method (33). FDP was not detectable in the medium of the culture if the cells were removed intact.

RESULTS

Different classes of revertants and their frequencies of occurrence. When cells of the enzyme I$^-$ mutant, strain 223, were plated on minimal mannitol-agar, spontaneous revertants were found at frequencies of two to six per 10^9 cells. When cells from the same culture were plated on minimal glycerol-agar, revertants appeared at a much higher frequency of approximately four per 10^7 cells. Higher reversion frequencies on the latter selective agar were consistently found in 10 separate comparisons in which the cells were derived from 10 separate clones of strain 223.

A mannitol revertant was isolated from each of 10 clones and tested for growth on minimal glycerol-agar. All grew normally. One mannitol revertant (strain 224) was tested further for growth on sorbitol, mannose, fructose, maltose, and galactose, compounds which the mutant strain could not utilize as carbon and energy source. Normal growth was observed in all cases. Enzymatic examination showed that the reversion restored most of the enzyme I activity expected of the wild-type strain (Table 2).

A glycerol revertant was isolated from each of 18 clones of strain 223. None grew on mannitol, and all but one were still unable to utilize sorbitol, mannose, fructose, maltose, and galactose. (The one exception grew on maltose and glycerol but not on the other compounds mentioned.) The enzyme I activity of one glycerol-specific revertant, strain 225, was measured and found not restored (Table 2).

Growth characteristics of suppressor, mutant, and parental strains. To compare more accurately the growth characteristics of strain 225 (the glycerol revertant), strain 224 (the mannitol revertant), strain 223 (the enzyme I$^-$ mutant), and strain 11 (the wild-type parent), cells were inoculated into liquid medium containing glycerol, L-α-GP, or arabinose. Strain 224 grew like the wild-type strain on all three media (Fig. 2). Strain 225,

after a long lag, grew as well as the wild-type strain on glycerol, but grew poorly on L-α-GP throughout the experiment. Growth of all four strains on arabinose was about the same.

Inducibility and catabolite repressibility of the enzymes of the glp system. Since the introduction of a constitutive allele of the *glpR* gene into the enzyme I⁻ mutant (strain 223) was reported earlier to restore growth on glycerol, the revertants obtained on glycerol-agar plates were first examined for the constitutive expression of the *glp* system. Of the 18 independent revertants, none was found to be genetically derepressed in this regulon. One of these, strain 225, was examined to see whether the degree of inducibility of its glycerol kinase, L-α-GP dehydrogenase, and L-α-GP transport system had been altered. Table 3 shows that the addition of glycerol to the reference medium with pyruvate as carbon source induced the *glp* system in this revertant almost to the extent attained in the wild-type strain under a similar condition, whereas induction hardly occurred at all in strain 223, the enzyme I⁻ mutant. L-α-GP was a poorer inducer in comparison to glycerol when tested with strain 225. (A considerable part of this inductive effect was subsequently found to be attributable to a small amount of glycerol present in the commercial preparation of DL-α-GP.) In contrast, the inducibilities of the gene products of the *glp* system in strain 224, the mannitol revertant, were normal whether glycerol or L-α-GP was used as the inducer. It should be noted also that the basal levels of the kinase, the dehydrogenase, and the transport system were lower in the cells containing the enzyme I⁻ mutation, i.e., strains 223 and 225.

TABLE 2. *Enzyme I and HPr activities of parent, mutant, and suppressor strains*

Strain[a]	Specific activity[b]	
	Enzyme I	HPr
11	6.8	4.8
223	0.1	2.7
224	4.3	2.9
225	0.05	2.8

[a] The cells were grown on casein hydrolysate plus mannitol.

[b] Expressed as nanomoles of product formed per minute per milligram of protein.

The facts that cyclic AMP has been shown to restore inducibility of the *glp* system in the mutant 223, and that this compound is implicated in the catabolite repression phenomenon (6, 18, 19, 21, 22, 32), suggest that a change in the balance of induction and catabolite repression acting on the *glp* system might underlie the suppression in strain 225. Therefore, the synthesis of two *glp* enzymes was compared in the various strains grown on arabinose with and without glycerol or L-α-GP as inducer. It might be recalled that glucose, the standard carbon source used to study catabolite repression, cannot be used in the present instance because of the enzyme I defects in certain strains. In addition, catabolite repression of the *glp* enzymes is noncoordinate in wild-type strains and synthesis of glycerol kinase is more sensitive to this control than is production of the dehydrogenase (13). Table 4 shows that arabinose was powerful in preventing the induction both in wild-type and enzyme I revertant strains. In the case of the glycerol revertant, however, arabinose was effective when L-α-GP but not glycerol served as the inducer.

Since the induction and the resistance to catabolite repression of the *glp* system in strain 225 were specifically released by the provision of glyc-

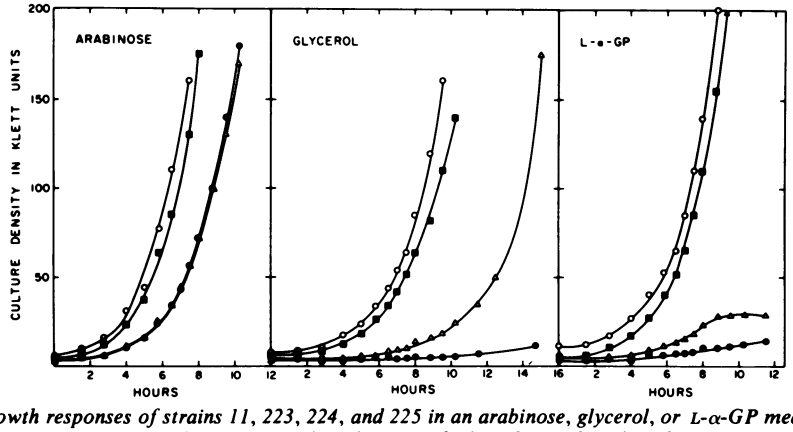

FIG. 2. *Growth responses of strains 11, 223, 224, and 225 in an arabinose, glycerol, or L-α-GP medium. Cells of each strain were pregrown on arabinose mineral medium, washed, and transferred to the various test media. Symbols: strain 11* (○), *strain 223* (●), *strain 224* (■), *strain 225* (△).

TABLE 3. *Activities of the glp enzymes and transport system of parent, mutant, and suppressor strains grown on pyruvate mineral medium*

Strain	Inducer added	Specific activity[a]		
		Glycerol kinase[b]	L-α-GP dehydrogenase[c]	L-α-GP transport[d]
11	None	0.06	1.6	.22
	Glycerol	0.29	5.2	.86
	L-α-GP	0.45	7.6	.76
223	None	0.01	<1.0	<.02
	Glycerol	0.02	<1.0	<.02
	L-α-GP	0.03	<1.0	<.02
224	None	0.06	1.0	.08
	Glycerol	0.41	6.5	.94
	L-α-GP	0.47	6.3	.72
225	None	0.02	<1.0	<.02
	Glycerol	0.17	4.3	.20
	L-α-GP	0.12[e]	1.3[e]	.06[e]

[a] The cells were grown for 6 to 10 generations under the specified conditions and harvested at a culture density of about 6×10^8 cells/ml.

[b] Expressed as micromoles of L-α-GP formed per minute per milligram of protein.

[c] Expressed as nanomoles of L-α-GP dehydrogenated per minute per milligram of protein.

[d] Expressed as nanomoles of L-α-GP accumulated per minute per milligram of dry cells.

[e] These slightly higher than expected values were subsequently found to be attributable to the glycerol contamination (1.6% on a molar basis) of commercial DL-α-GP (W. S. Kistler, *personal communication*).

erol but not L-α-GP, one is compelled to conclude that the suppressor mutation involved some aspect of synthesis or property of the glycerol kinase. Further examination revealed that the enzyme produced by strain 225 was completely insensitive to inhibition by FDP at concentrations trations of 1, 5, and 10 mM. Of the 18 glycerol revertants, 16 were found to synthesize kinases totally refractive to inhibition by 5 mM FDP. The remaining two revertants are still to be characterized.

Genetic locus for the altered kinase in strain 225. To verify that the suppressor mutation in strain 225 occurred in the known *glpK* locus, a transduction experiment was undertaken. Phage P1kc prepared from strain 225 was used to donate the $metB^+$ marker to the recipient strain 162, which is both $metB^-$ and $glpK^-$. About 60% of these transductants were found to be glycerol-positive, a frequency close to the expected value (5). The kinase activities from 10 glycerol-positive transductants were examined in vitro. All were FDP-insensitive.

pH optima of the glycerol kinases. A mutant (strain 43) with a feedback-insensitive kinase had been isolated previously by an autoradiographic procedure (34). It was observed (N. Zwaig, *personal communication*) that the activity of the kinase of this mutant was maximal at about *p*H 6.5 and sharply decreased as the *p*H increased from 7 to 9.5. This profile is in marked contrast to that observed with the wild-type enzyme which exhibits a broad *p*H spectrum from 7 to 10 (11). The essential differences in the two enzymes were confirmed under the assay conditions used in the present study. When a similar characterization was made for the FDP-insensitive kinase of strain 244, its *p*H profile was unexpectedly similar to that of the normal enzyme (Fig. 3).

Killing by glycerol. Cells of strain 43, which synthesize constitutively a glycerol kinase insensitive to feedback inhibition, are known to be vulnerable to killing by glycerol during growth on a carbon source permitting the synthesis of high levels of the abnormal kinase (33). To see whether this phenomenon is common to other strains producing constitutively a feedback-refractory kinase, strain 244 was constructed by transferring the $glpK^i$ allele from strain 225 to a $glpR^-$ strain by transduction. As a control, strain 271, a transductant which produces constitutively a normal kinase, was prepared with the same recipient strain. Figure 4 shows the ef-

TABLE 4. *Activities of the glp enzymes of parent, mutant, and suppressor strains grown on arabinose mineral medium*

Strain	Inducer added	Specific activity[a]	
		Glycerol kinase[b]	L-α-GP dehydrogenase[c]
11	None	0.02	<1.0
	Glycerol	0.03	2.4
	L-α-GP	0.02	1.7
223	None	0.02	<1.0
	Glycerol	0.02	<1.0
	L-α-GP	0.03	1.2
224	None	0.02	<1.0
	Glycerol	0.02	<1.0
	L-α-GP	0.02	<1.0
225	None	0.02	<1.0
	Glycerol	0.16	3.5
	L-α-GP	0.03[d]	1.2[d]

[a] Same conditions as in Table 3.

[b] Expressed as micromoles of L-α-GP formed per minute per milligram of protein.

[c] Expressed as nanomoles of L-α-GP dehydrogenated per minute per milligram of protein.

[d] These values were obtained from a separate experiment in which the DL-α-GP used as inducer was freed of contaminating glycerol.

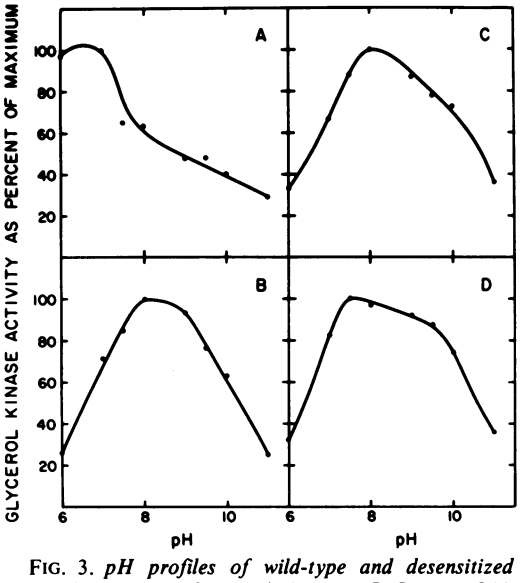

FIG. 3. *pH profiles of wild-type and desensitized glycerol kinases. A, Strain 43; B, strain 7; C, strain 244; D, strain 271.*

fect of added glycerol to cells of the two strains growing on casein hydrolysate. In the case of strain 244, the viable counts in the culture dropped within minutes after the addition of glycerol, and almost all the cells were dead within 20 min; whereas, in the case of strain 271, the rate of growth was stimulated by glycerol. In a separate test strain 244 was able to grow to normal stationary level in a medium containing casein hydrolysate without glycerol.

DISCUSSION

In view of the finding that the ability of an enzyme I^- mutant to grow on glycerol can be restored by introducing a constitutive allele of the *glpR* gene, it is surprising that, among the spontaneous "revertants" of the enzyme I^- mutant selected on glycerol, none was of the *glpR* class. A large variety of mutations in this gene should permit the constitutive expression of the *glp* system. In contrast, mutations in the structural gene of the kinase that abolishes feedback control are expected to be much more stringent and therefore less frequent.

A reconstruction experiment showed that cells with a feedback-insensitive kinase yielded visible clones on glycerol agar before those with a constitutive *glp* system. This differential growth rate on solid medium may very well explain the unexpected pattern of suppression.

The fact that a mutant producing a feedback-insensitive kinase can grow on glycerol despite increased catabolite repression caused by the enzyme I lesion seems at first readily explicable within the framework of existing knowledge. The inducer for the *glp* system is L-α-GP. In the enzyme I^- mutant, the basal level of glycerol kinase is reduced. Therefore, glycerol can no longer be converted to L-α-GP at a rate sufficient to initiate induction. Removal of the feedback control of the kinase would compensate for the reduction in the number of enzyme molecules provided that FDP is normally present in the cell at concentrations to cause partial inhibition of the kinase. Indeed, the intracellular pool of FDP in glycerol-grown cells of both strain 225 (enzyme I^-, desensitized glycerol kinase) and strain 11 (wild-type) was found to be 0.7 mM, high enough to exert significant inhibition on the wild-type enzyme which has a K_i of 1 mM. However, this simple picture does not account for the observation that cells of the revertant strain 225 grown on pyruvate could be induced in their *glp* system when presented with glycerol, whereas cells of the mutant strain 223 could not be. The FDP concentra-

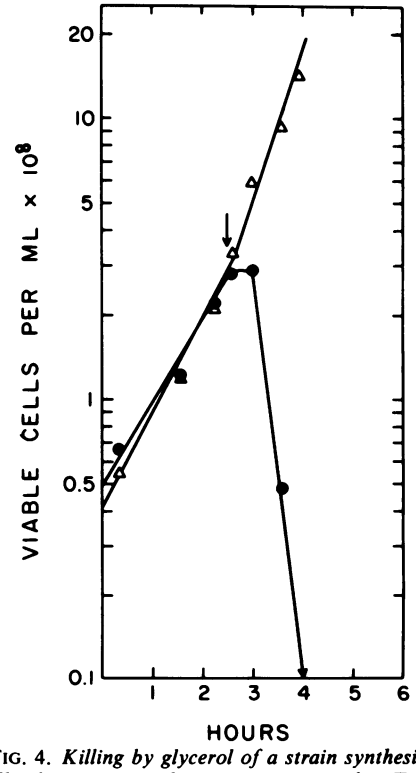

FIG. 4. *Killing by glycerol of a strain synthesizing a feedback-insensitive kinase constitutively. To cells growing exponentially on casein hydrolysate, glycerol was added at a concentration of 0.02* M *at the time indicated by the arrow. Viability was determined by plating out appropriately diluted samples of the culture onto agar containing 1% casein hydrolysate. Symbols: strain 271* (△), *strain 244* (●).

tion in cells growing on pyruvate was not measurable by the method used, which means that it could not be substantially above 0.1 mM. Hence, it is not obvious how a desensitization of glycerol kinase could tilt the balance; strain 223 and 225 should be isogenic, except for the physical properties of this enzyme. Relief from this difficulty is offered by a recent finding that the glycerol kinase activity of strain 225 is actually stimulated by low levels of FDP (J. Thorner, *personal communication*). The possibility that there is another feedback inhibitor of glycerol kinase, and that the altered kinase is no longer susceptible to this hypothetical compound has been made unlikely by a fruitless but exhaustive search.

[All the intermediates of the glycolytic pathway have been tested as possible feedback inhibitors (33). Tricarboxylic acid cycle intermediates and their related amino acids, pyruvate, phosphoenolpyruvate, cyclic AMP, formate, lactate, acetyl phosphate, as well as acetyl coenzyme A, nicotinamide adenine dinucleotide, and reduced nicotinamide adenine dinucleotide, each at a concentration of 1 mM have been shown to be ineffective in altering the activity of crystalline glycerol kinase (J. Thorner, *personal communication*).]

In our experiments, the alteration in sensitivity to FDP was not sufficient to restore the normal state of glycerol metabolism in the presence of the enzyme I lesion: the suppressed strain still showed a long lag period during the transition from an arabinose medium to a glycerol medium.

Pastan and Perlman (20) showed that a problem analogous to the one discussed exists for the induction of the *lac* operon in an enzyme I^- mutant. Lactose, like glycerol, must first be transported into the cell and then transformed into the true inducer by the action of β-galactosidase before the enzymes of the lactose operon are induced (2). The lactose analogue, isopropyl-thio-β-D-galactoside, however, can bind to the *lac* repressor without chemical transformation (8). It is probably for this reason that the analogue but not the natural substrate, lactose, can induce the *lac* operon in the mutant when used at a high concentration of 10^{-3} M (20).

It has been suggested that the increased severity of catabolite repression in enzyme I mutants is due to abnormally low levels of cyclic AMP (20). This hypothesis is strengthened by the properties of a pleiotropic mutant, isolated by Perlman and Pastan (23), lacking adenyl cyclase activity. The pattern of pleiotropy of the adenyl cyclase mutant is very similar to that of the enzyme I mutants; the defects also extend to the utilization of glycerol, galactose, maltose, and lactose. It may be worthwhile to see whether mutations in the *glp* system isolated as glycerol-specific revertants in an enzyme I^- strain can also suppress the growth defect in the adenyl cyclase mutant.

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