JOURNAL OF BACTERIOLOGY, June 1971, p. 724-731

Vol. 106, No. 3
Printed in U.S.A.

Promoter-Like Mutant with Increased Expression of the Glycerol Kinase Operon of *Escherichia coli*

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Received for publication 3 March 1971

A glycerol-specific phenotypic revertant isolated from a mutant of *Escherichia coli* missing enzyme I of the phosphoenolpyruvate phosphotransferase system was studied. This revertant is capable of producing higher levels of glycerol kinase and the protein mediating the facilitated diffusion of glycerol (facilitator) than wild-type cells. The kinase of the revertant is indistinguishable from the wild-type enzyme with respect to its sensitivity to feedback inhibition by fructose-1,6-diphosphate, its *p*H optimum, and its turnover number. The synthesis of glycerol kinase in strains bearing the suppressor locus is resistant to catabolite repression. The suppressor mutation mapped at the known *glpK* locus. Thus, it is suggested that the mutation occurred in the promoter of the operon specifying the kinase and the facilitator.

The structural genes coding for the transport systems and enzymes required for the metabolism of glycerol and L-α-glycerophosphate (L-α-GP; Fig. 1) are found in three clustered regions of the *Escherichia coli* map (Fig. 2). The locus *glpF* controlling the facilitated diffusion of glycerol (the protein will be referred to as facilitator) across the cell membrane (26) and the locus *glpK* specifying the structure of kinase, the first enzyme of the pathway (17), are situated at minute 76 on the Taylor map (7, 32; Y. Sanno, *unpublished data*). The structural gene specifying the aerobic L-α-GP dehydrogenase is located at minute 66 (5, 11). Mutations affecting the L-α-GP transport system map in yet another region at minute 42 (5). This locus is close or adjacent to that controlling the anaerobic L-α-GP dehydrogenase (16; Kistler and Lin, *manuscript in preparation*). The synthesis of all of these proteins is regulated negatively by the product of a single gene, *glpR* (5), which apparently interacts with L-α-GP as the inducer (13). The *glp* system is thus a regulon comprising at least three operons.

The fact that the utilization of both glycerol and L-α-GP in each case requires the expression of genes scattered in two separate chromosomal regions has made it difficult to select mutations that affect the control of any individual operon. An unexpected opportunity for isolating a class of *cis* control mutations affecting the glycerol kinase operon has been provided by the fact that induction of the *glp* system is impaired in certain mutants lacking enzyme I of the phosphoenolpyruvate phosphotransferase system (18), and that a further mutation resulting in the increase in the basal activity of glycerol kinase could specifically restore the ability of the cells to grow on glycerol.

Previous reports described how specific restoration of the capacity of an enzyme I^- mutant to grow on glycerol was achieved by a mutation that abolished the feedback inhibition of the kinase by fructose-1,6-diphosphate (FDP; reference 1) or by the conversion of the *glp* regulon from inducibility to constitutivity (2). Two other classes of mutations in the *glp* system might be expected to have similar suppressing effects: the conversion of the kinase operon to the O^c state and alterations of its promoter leading to increased gene expression. In this report, we describe a suppressor mutant of the latter kind.

MATERIALS AND METHODS

Terminology. The term "suppression" is used increasingly frequently by microbiologists in connection with genetic changes that rectify nonsense mutations. In this report, the term is used to designate, in the classical sense, a second site genetic alteration (the p allele of *glpK*, *see* Table 1) that reverses a particular phenotype caused by the original mutation, and the term "revertant" is used in this phenotypic sense.

Bacteria and phages. *E. coli* strain 11, with normal phenotype for glycerol and L-α-GP metabolism and used as the wild-type parent in this study, was derived from strain AB313 of K-12. Strain 223, an enzyme I^- mutant incapable of growth on glycerol, was isolated from strain 11; and strain 225, which produces a kinase

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refractive to inhibition by FDP, was in turn isolated as a glycerol-specific revertant of strain 223 (reference 1). Strain 240 was also isolated as a glycerol-specific revertant but was found to have a normal glycerol kinase. All of the remaining strains were derived from *E. coli* K-12 Hfr C (11).

The following transductions were carried out with the method of Luria, Adams, and Ting (20). Phages grown on strain 240 were used to infect strain 27 which synthesizes the enzymes of the *glp* system constitutively but has no glycerol kinase activity. Strain 242 was isolated as a transductant capable of growth on glycerol. Phages grown on strain 11 were used to infect strain 240, and the transductant, strain 248, capable of growth on mannitol, was isolated and found to have wild-type enzyme I activity. The genotypes of the various bacterial strains employed in this study are summarized in Table 1.

Culture media and growth conditions. Carbon sources were added to a mineral medium to give a final concentration of 0.02 M glycerol, 0.04 M DL-α-GP, and 0.01 M mannitol, arabinose, glucose, and glucose-6-phosphate, singly or in combinations, as previously specified (31). Leucine and thiamine, when required, were supplemented at 50 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 a liter of mineral medium. Growth rates were determined by measuring changes in turbidity (6). One Klett unit (no. 42 filter) corresponds to 4×10^6 cells/ml.

Mapping. Phages grown on strain 240 were used to transduce cells of strain 162 (*glpK*$^-$, *glpR*$^-$, *metB*$^-$), 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 purified transductants (harvested from an arabinose medium) to grow on glycerol plates. The uninduced levels of glycerol kinase activity of each of 10 transductants was determined by the uptake of ^{14}C-glycerol by whole cells (*see below*). The reliability of this technique for scoring the promoter marker was established by in vitro enzymatic assay by using known mutants.

Mutagenesis. Five separate clones of strain 242 and strain 271 were grown for more than 10 generations on casein hydrolysate in the presence of 2-aminopurine at 5 mg/ml for mutagenesis, 5 generations in a rich medium (Antibiotic Medium 3, Difco) for phenotypic expression of the mutations, and 5 generations in a minimal glucose medium to eliminate auxotrophs. Finally, two successive penicillin selections in a minimal glycerol medium were made to enrich for glycerol kinase$^-$ mutants (10). After the second treatment with the antibiotic, the survivors were plated onto MacConkey agar in which glycerol was substituted for lactose (7). Pale colonies, which upon subsequent screening grew on L-α-GP but not glycerol, were collected for direct examination of the kinase activity. To test the likelihood of

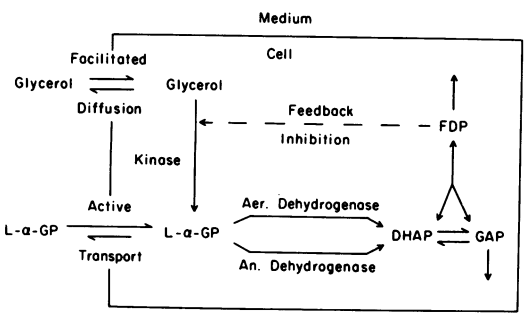

FIG. 1. *Pathways for glycerol and L-α-GP dissimilation in E. coli.*

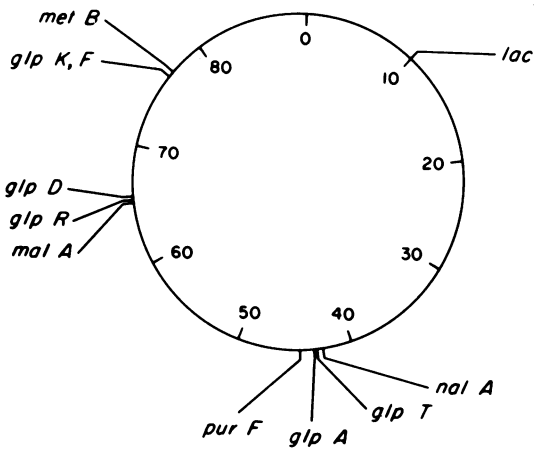

FIG. 2. *Map of E. coli. Pertinent markers on the genetic map of E. coli after Taylor (32). GlpA is the locus controlling the anaerobic L-α-GP dehydrogenase (W. S. Kistler, unpublished data), and glpF is the locus controlling the facilitated diffusion of glycerol (Y. Sanno and D. Richey, unpublished data).*

TABLE 1. *Genotype of strains used*

Strain	Parent	Reference	Relevant markers[a]					
			glp-				En-zyme I	*metB*
			K	*T*	*D*	*R*		
11	AB313	2	+	+	+	+	+	+
223	11	2	+	+	+	+	−	+
240	223	2	p	+	+	+	−	+
225	223	1	i	+	+	+	−	+
248	240		p	+	+	+	+	+
7		11	+	+	+	−	+	+
27	7	1	−	+	+	−	+	+
242	27		p	+	+	−	+	+
271	27	1	+	+	+	−	+	+
162	161	1	−	+	+	−	+	−

[a] The marker *glp* designates the L-α-glycerophosphate (L-α-GP) regulon. The symbols *K*, *T*, *D*, *R* represent, respectively, glycerol kinase, the L-α-GP transport system, the aerobic L-α-GP dehydrogenase, and the regulator. Under the *glpK* column, the notations p and i designate, respectively, the promoter-like allele and the allele specifying a *fructose-1,6-diphosphate*-desitized kinase.

obtaining spontaneously occurring *glpK*$^-$ mutants in the selection process, a clone of each strain was subjected to the entire procedure without the use of the mutagen.

Assay of enzyme I. The activity of enzyme I of the phosphoenolpyruvate phosphotransferase system was determined by the phosphorylation of ^{14}C-mannitol under a condition in which the other requisite components were in excess as described earlier (1, 31).

Assays of enzymes of the glp system. Glycerol kinase activity was usually determined by a continuous spectrophotometric assay at *p*H 9.5 based on the coupled reduction of nicotinamide adenine dinucleotide (19); however, to test for sensitivity to inhibition by FDP, the enzyme was assayed at *p*H 7.5 by a spectrophotometric method based on the glycerol-dependent formation of adenosine diphosphate from adenosine triphosphate (38). An assay based on the formation of ^{14}C-L-α-GP from ^{14}C-glycerol was used to determine *p*H profiles of the enzyme from mutant and wild-type strains (1).

In mapping experiments, glycerol kinase activity was scored by a rapid semiquantitative procedure based on the uptake of radioactive glycerol by whole cells, since it has been shown that glycerol is not accumulated by active transport and that it can be retained only after it is converted to L-α-GP (12). Cells grown exponentially on casein hydrolysate to a density of 4×10^8 cells/ml were assayed by mixing 0.2 ml of the culture directly with 0.8 ml of a solution of ^{14}C-glycerol (2×10^{-6} M), chloramphenicol (20 μg/ml), tris(hydroxymethyl)aminomethane (0.1 M at *p*H 7.4), and orthophosphate (0.3 mM). After 30 sec at 25 C, 0.5 ml of the suspension was withdrawn and delivered immediately for filtration onto a membrane filter disc (0.45-μm pore size; Millipore Corp.) previously wetted with mineral medium. After washing and drying, the radioactivity on the disc was measured by scintillation counting.

The aerobic L-α-GP dehydrogenase was assayed by coupling to a tetrazolium dye (19) under a condition which does not allow the anaerobic enzyme to contribute significantly to the reaction (16).

The facilitated diffusion of glycerol across the cell membrane was assayed by an optical method which depends on transient changes in the cell volume upon sudden exposure to hypertonic glycerol (26). The activity is expressed as $T_{1/2}$, the time in seconds for one-half of the final optical density change to occur.

Immunochemistry. For quantitative estimation of the glycerol kinase protein, precipitin tests were carried out (15). Specific antiserum from rabbits immunized against crystalline *E. coli* glycerol kinase was from a previous study (13). Antibodies cross reacting with *E. coli* proteins other than glycerol kinase were eliminated from the antiserum by centrifugation after treatment with an *E. coli* extract devoid of the enzyme. A mixture of the clarified serum and the bacterial extract to be assayed was incubated at 25 C for 2 hr. The precipitate formed was collected, washed, suspended, and measured turbidimetrically. The amount of precipitate per unit of enzyme activity obtained with induced wild-type cells was the same as that obtained with the pure enzyme (13).

RESULTS

Growth characteristics and inducibility of the glp enzymes in the suppressor strain 240. Like the more common glycerol-specific revertants of strain 223 that produce a desensitized glycerol kinase (1), strain 240 grew on glycerol but not on L-α-GP (Fig. 3). Unlike the other revertants, strain 240 was found to produce a wild-type glycerol kinase fully sensitive to feedback inhibition by FDP. The enzyme activity, however, was present at levels significantly higher than those found in wild-type cells whether growth occurred in the presence or absence of either glycerol or L-α-GP as inducers (Table 2). By contrast, the activity of the aerobic L-α-GP dehydrogenase in strain 240 was not abnormally elevated.

Genetic locus for the suppressor mutation. The fact that only the activity of the kinase and not of the dehydrogenase was elevated in strain 240 suggested that a mutation had occurred in the glycerol kinase operon, a conclusion borne out by the further finding that the suppressor mutation mapped in the region of the *glpK* locus. When phage P1kc grown on strain 240 was used to donate the *metB* marker to the recipient strain 162, which is both *metB*$^-$ and *glpK*$^-$, about 60% of the transductants were found to be glycerol kinase positive, a frequency close to the expected value. All 10 glycerol kinase-positive transductants examined exhibited activities of this enzyme which were several-fold higher than wild-type values.

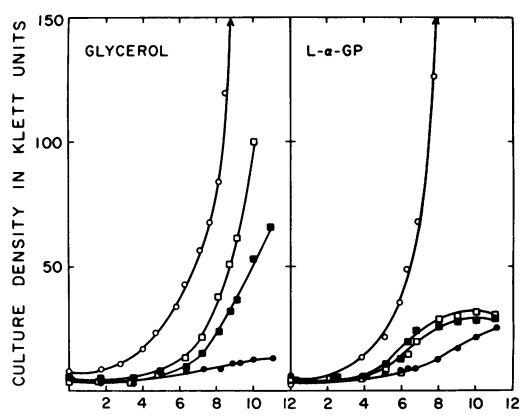

FIG. 3. *Growth responses of strains 11, 223, 225, and 240 in a glycerol or L-α-GP medium. Cells of each strain were pregrown on arabinose mineral medium, washed, and transferred to the various test media. Symbols: strain 11* (○), *strain 223* (●), *strain 225* (□), *and strain 240* (■). *The slightly greater growth of strains 225 and 240 over that of strain 223 in the L-α-GP medium was found to be due to contamination of commercial D L-α-GP with glycerol.*

TABLE 2. *Enzyme levels of the glp system in wild type, mutant, and revertant cells*

Strain	Inducer[a]	Specific activity[b]	
		Glycerol kinase	L-α-GP dehydrogenase[c]
11	None	0.04	<0.005
	Glycerol	1.0	0.052
	L-α-GP	1.2	0.048
223	None	0.07	<0.005
	Glycerol	0.51	0.018
	L-α-GP	0.89	0.024
240	None	0.45	<0.005
	Glycerol	2.8	0.050
	L-α-GP	3.6	0.027

[a] All growth media contained 1% casein hydrolysate.
[b] Units of enzyme activities are expressed as micromoles of substrate converted per minute per milligram of protein.
[c] L-α-GP, L-α-glycerophosphate.

Properties of glycerol kinase from strain 240. The localization of the suppressor mutation of strain 240 in the *glpK* region necessitated further examination of the properties of glycerol kinase to determine whether some unexpected structural change in the protein had also taken place. As already mentioned in a previous section, the sensitivity of the enzyme to inhibition by FDP was not distinguishable from that of wild-type enzyme. Figure 4 shows that the *p*H optimum of the enzyme from strain 240 is also indistinguishable from the wild-type enzyme. Finally, the turnover number of the glycerol kinase of strain 240 was compared with that of the wild-type enzyme with the aid of the precipitin test. For this comparison, the suppressor marker was transduced into an enzyme I^+ cell constitutive in the L-α-GP system (*glpR*$^-$). Extracts of the transductant, strain 242, contained 2.5 times more glycerol kinase activity than extracts of strain 271, an isogenic line with the wild-type *glpK* allele, when casein hydrolysate served as the carbon source during growth. Extracts of strain 242 also contained 2.4 times more kinase protein than those of strain 271, as measured by cross-reacting material (Fig. 5).

Expression of the glycerol kinase operon in a suppressor strain bearing glp R$^-$. The absence of any physical difference between the glycerol kinase synthesized by strain 240 and the wild-type enzyme suggests that the suppressor mutation in the *glpK* locus of strain 240 affects the expression of the operon. The data in Table 2 show that the synthesis of the enzyme remained inducible in the suppressor strain, indicating that an O^c mutation was not involved. To confirm this, the level of the kinase was compared in two isogenic *glpR*$^-$ strains, one with the wild-type *glpK* allele (strain 7), the other (strain 242) with the *glpK* allele transduced from strain 240. As can be seen from Table 3, the kinase level was higher in the suppressor-containing strain even when the regulatory protein was lacking. In another experiment, it was found that the kinase activity in strain 242 was more than twice that of a mutant with a deletion in the *glpR* region (not shown in table).

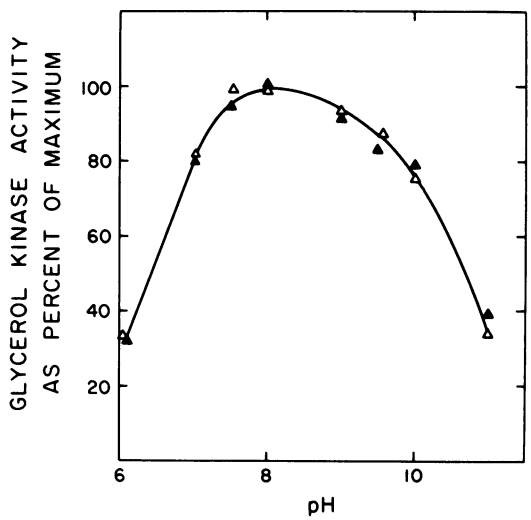

FIG. 4. *pH profiles of glycerol kinase from strain 242 and 271. Symbols: strain 242 (▲) and strain 271 (△).*

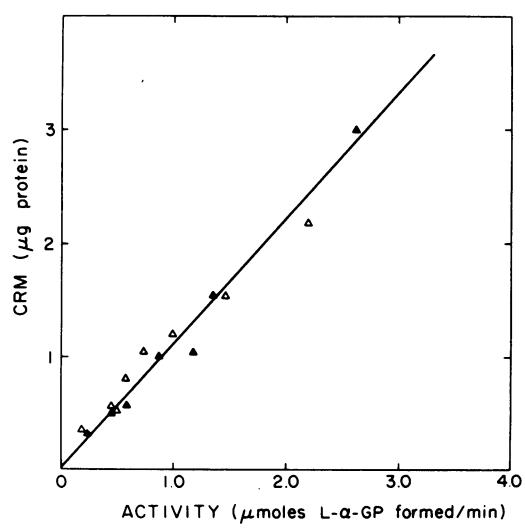

FIG. 5. *Correlation of glycerol kinase activity with the amount of CRM in crude extracts. Symbols: strain 242 (▲) and strain 271 (△).*

TABLE 3. *Effect of the suppressor mutation on the constitutive level of glycerol kinase*

Inducer and/or repressor[a]	Strain 7	Strain 242
None	3.4	7.7
L-α-GP[b]	2.5	3.7
Arabinose	0.36	3.6
Arabinose + L-α-GP	0.38	3.8

[a] All growth media contained 1% casein hydrolysate.
[b] L-α-GP, L-α-glycerophosphate.

Nature of the suppressor in strain 240. Several explanations for the increased enzyme levels in strain 240 might be entertained: (i) appearance of an extrachromosomal element bearing the *glpK* locus, (ii) tandem duplication or triplication of the gene on the chromosome itself, (iii) an alteration of the promoter.

Mechanism 1 can be excluded if glycerol kinase-negative derivatives could be readily obtained from the suppressor strain, especially as a result of mild mutagenesis induced by a chemical that causes single base substitutions, since the probability of inactivating both the chromosomal and the extrachromosomal genes should be exceedingly low. Five separate clones of strain 242 were therefore subjected to mutagenesis by growth in the presence of 2-aminopurine. Kinase-negative mutants were readily isolated in each case at frequencies close to those obtained in a parallel experiment in which five separate clones of strain 271, with wild-type *glpK* allele, were treated similarly. No such mutants were found when the experiment was repeated without applying mutagenesis. The further finding that all of these kinase-negative derivatives readily reverted to kinase-positive state at frequencies between 10^{-8} and 10^{-6} confirmed the belief that only point mutations were involved.

Tandem copies of the kinase operon are difficult to exclude. The ready isolation of K^- mutants would rule out duplication of genetic region containing the complete *glpK* operon but not duplication of the cistrons that code for the kinase within the operon. The topological requirements for the latter kind of duplication are likely, however, to be highly stringent so that a fruitful event would be extremely rare.

An "up" promotion thus becomes the most attractive explanation by elimination. Positive evidence in support of this model came from the following experiments. Recently, it was shown that mutations altering the promoter region for the *lac* operon also brought about reduced sensitivity to catabolite repression (28). The synthesis of glycerol kinase was therefore tested by growing cells of strains 240, 223, and 11 on casein hydrolysate under several conditions. L-α-GP was employed as the inducer and arabinose as the agent for catabolite repression. [It might be recalled that glucose, the standard carbon source used to study catabolite repression, can not be used in the present instance because the enzyme I defect prevents proper utilization of this sugar (30).] Table 4 shows that strain 240 was less powerfully repressed in its synthesis of glycerol kinase by arabinose in comparison to strain 223, its enzyme I^- parent or the wild-type strain 11. What effect arabinose had in preventing the induction of the kinase by L-α-GP in strain 240 was probably attributable to interference with the entry of the inducer, because, when the *glpK*^{p} locus of this strain was transduced into a strain positive in enzyme I strain and constitutive in the *glp* regulon, the expression of this allele was found to be highly resistant to glucose, mannitol, arabinose, and glucose 6-phosphate. The levels of enzyme activity attained with these carbon sources by the transductant (strain 242) were, respectively, 3.2, 4.8, 3.6, and 7.8 mmoles of product per min per mg of protein. Cells of strain 7, which is constitutive but synthesizes the wild-type kinase, contained only about one-tenth of these activities when grown under similar conditions (see also Table 3). The levels of the dehydrogenase of the strains studied, on the other hand, were similar to each other for each growth condition.

Facilitator synthesis in a suppressor-bearing strain. Synthesis of the facilitator has been shown to be regulated also by the *glpR* product (26). Moreover, a number of mutants missing

TABLE 4. *Basal, induced, and repressed enzyme levels of the glp system in wild type, mutant, and revertant cells*

Strain	Inducer and/or repressor[a]	Specific activity	
		Glycerol kinase	L-α-GP dehydrogenase[b]
11	None	0.04	0.005
	L-α-GP	1.2	0.048
	Arabinose	0.02	0.005
	Arabinose + L-α-GP	0.01	0.005
223	None	0.07	0.005
	L-α-GP	0.89	0.024
	Arabinose	0.01	0.005
	Arabinose + L-α-GP	0.01	0.005
240	None	0.45	0.005
	L-α-GP	3.60	0.027
	Arabinose	0.10	0.005
	Arabinose + L-α-GP	0.12	0.005

[a] All growth media contained 1% casein hydrolysate.
[b] L-α-GP, L-α-glycerophosphate.

glycerol kinase has been found to be also defective in the facilitated diffusion of glycerol (Y. Sanno and D. P. Richey, *unpublished data*). Thus, *glpK* and *glpF* appear to belong to the same operon (or share a protein in common). If the promoter mutation hypothesis to account for the increased level of glycerol kinase in strain 240 is correct, elevation in the synthesis of the facilitator would be expected. This possibility was tested by growing cells of strains 11 (wild type) and 248 (wild type, except for the glycerol kinase operon which was from strain 240) on casein hydrolysate with various amounts of arabinose. The levels of the facilitator and kinase under each growth condition were measured. It may be seen from Fig. 6 that in all cases more facilitator and kinase were synthesized in strain 248 than in strain 11.

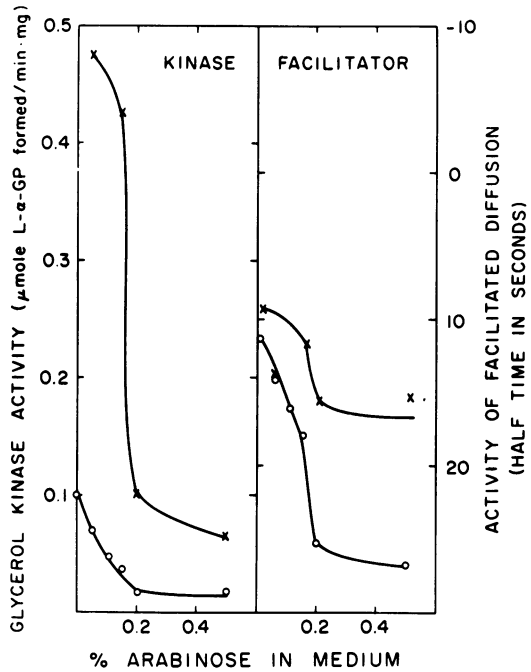

FIG. 6. *Parallel interference of the synthesis of facilitator and glycerol kinase in a suppressor-bearing strain. Uninduced cells of each strain were grown on casein hydrolysate medium with various amounts of arabinose. At a density of 1.2 × 10⁹ cells/ml, the cells were harvested and assayed for facilitator and kinase activities. Symbols: strain 11* (○) *and 248* (×).

DISCUSSION

The promoter controls the maximal level of expression for an operon (14, 27). The genetic location of the promoter (14) and results from studies of an in vitro protein-synthesizing system (3, 36) indicate that this region of the deoxyribonucleic acid (DNA) is the site of ribonucleic acid (RNA) polymerase binding and messenger initiation. In cell-free systems in which β-galactosidase formation was catalyzed by an extract of *E. coli*, the DNA from a mutant with a defective promoter in the *lac* system was only 10% as effective as normal DNA in serving as the template (36). Cyclic adenosine monophosphate (AMP) stimulated the synthesis (8, 35). Zubay, Schwartz, and Beckwith, and Pastan and his coworkers each isolated a cyclic AMP-binding protein which plays an important role in the in vitro system for β-galactosidase synthesis (9, 25, 37). Furthermore, the promoter has been identified as the catabolite-sensitive site of the *lac* operon. Silverstone and co-workers reported that deletions in the promoter region of the *lac* operon rendered the residual β-galactosidase synthesis resistant to glucose repression (28). Small deletions in this area rendered synthesis of β-galactosidase insensitive to stimulation by cyclic AMP (24, 28). Hence, it was suggested that expression of catabolite repressible enzymes is controlled by the internal level of cyclic AMP which limits the initiation of messenger RNA synthesis at the promoter site.

All of the known characteristics of the glycerol-specific suppressor mutation in strain 240 are compatible with the present notion of promoter mutants. (i) The mutation is in the *glpK* region; (ii) the inducibility of the operon is not affected in a major way; (iii) enzyme synthesis is resistant to catabolite repression; (iv) the synthesis of the facilitator, controlled by a nearby locus, is jointly affected. Mutants with an altered promoter of the *lac* operon respond to inducer, but when fully induced they synthesize only a fraction of the enzymes expected of the wild-type strain (14). Similar features were shown for presumed promoter mutants of the histidine degrading enzymes (*hut*) of *Bacillus subtilus* (4) and *Salmonella typhimurium* (21). In contrast, the change in strain 240 results in greater enzyme synthesis. In this respect, the characteristic resembles that of the putative promoter mutations of the *lac* i operon (23) and the *zwf* operon (glucose-6-phosphate dehydrogenase; D. G. Fraenkel and S. Banerjee, *in press*).

Although the genetic change in strain 240 is regarded as most likely a promoter mutation, there are also possible cases of missense mutations in structural genes which result in several-fold elevation of enzyme activity in cell-free preparations. One example is the high level of human glucose-6-phosphate dehydrogenase in the Hektoen variant. The enzyme differs from the normal protein by only one amino acid (34). Another example is the increased level of dihydrofolate reductase in amethopterin-resistant

mutants of *Diplicoccus pneumoniae* associated also with altered properties of the enzyme (29). Since these altered enzymes are neither more resistant to breakdown nor more effective in catalysis, the elevated activity was attributed to an increased rate of its synthesis.

The true nature of the suppressor mutation in strain 240 can be ultimately tested by fine structure genetic analysis of the kinase operon. However, for this undertaking, a number of kinase-negative mutations would have to be mapped.

The delivery of glycerol from the external medium to the central metabolic network requires not only the facilitator and the kinase, but L-P-GP dehydrogenase as well. Yet, in the presence of the enzyme I lesion, no genetic changes in the *glpD* operon are necessary for the restoration of the ability of cells to grow on glycerol. The answer apparently lies in the fact that the synthesis of the dehydrogenase is ordinarily not as sensitive to catabolite repression as is that of the kinase (17).

The study of limited phenotypic revertants of enzyme I^- mutants will likely lead to the isolation of other interesting classes of suppressors. For example, if indeed enzyme I^- mutants have abnormally low levels of cyclic AMP, there may exist mutations in the promoter region of the kinase operon that allow successful initiation of messenger synthesis without the aid of the cyclic AMP-complex of the "catabolite gene activator protein." There are also phenotypic revertants, the genetic change of which is not at all understood, such as mutations that simultaneously restore the ability of enzyme I^- cells to grow on two or more of the carbohydrates not phosphorylated by the phosphoenolpyruvate system (33). One might seek revertants with even broader restoration of the defect in carbohydrate utilization. Among such mutants, there may be those with an altered catabolite gene activator protein that no longer needs association with cyclic AMP to be active in the initiation of messenger RNA synthesis. This kind of mutation should endow general resistance to catabolite repression. A mutant in the cyclic AMP phosphodiesterase should have a similar phenotype (22). Thorough analysis of the revertants of many different enzyme I^- and HPr^- mutants thus may increase our understanding of the catabolite repression phenomenon.

ACKNOWLEDGMENTS

M. B-K was supported by a Public Health Service Training grant from the National Institutes of Health, E.C.C.L. was supported by a Public Health Service Research Career Development Award from the National Institute of General Medical Sciences, and D.P.R. was supported by a postdoctoral fellowship from the American Cancer Society.

This investigation was supported by grant GB8509 from the National Science Foundation, grant GM11983 from the National Institute of General Medical Sciences, and grant AHA69-991 from the Heart Fund.

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