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Two Forms of D-Glycerate Kinase in *Escherichia coli*

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Escherichia coli K-12 synthesizes two chromatographically distinct forms of glycerate kinase which differ both in their thermolability and in the dependence of their activity upon *pH*. One enzymatic form, GK I, is found in cells grown with glycerate, glucarate, or glycolate. Of these compounds, glycolate is the only carbon source that elicits the synthesis of the second enzymatic form, GK II.

D-Glycerate kinase can play two metabolic roles in *Escherichia coli* K-12. When glyoxalate or a metabolic precursor thereof is the sole growth substrate, glycerate kinase catalyzes a reaction in the synthesis of three-carbon fragments by the "glycerate pathway" (5, 8-10). On the other hand, the enzyme performs a strictly catabolic function when cells are grown with glycerate, glucarate, or galactarate (1). Glycerate kinase is inducible (3) i.e., it is present at extremely low levels in the absence of any of the aforementioned carbon sources. Kornberg (7) observed that glyoxalate carboligase, the initial enzyme of the glycerate pathway, is strongly repressed if acetate is added to cultures of *E. coli* growing with glycolate. In accordance with his results, we found that glycerate kinase in *E. coli* is subject to severe repression when cells are grown with glycolate. In contrast, repression of glycerate kinase is relatively slight when cells are exposed to glycerate.

These differences in metabolic role and repressive control suggest that *E. coli* might form two glycerate kinases, subject to independent biosynthetic regulation. The purpose of this communication is to present evidence in support of this view, to describe some properties of the two forms of glycerate kinase, termed GK I and GK II, and to indicate how the relative concentration of the two forms is dictated by growth conditions.

MATERIALS AND METHODS

The strains of *E. coli* K-12 employed in this study were kindly provided by H. L. Kornberg. Strain K-1 requires methionine and thymine for growth; strain R-4 requires methionine. Cultures were grown in mineral medium (10) supplemented with 40 mg of methionine and 40 mg of thymine (Sigma Chemical Co., St. Louis, Mo.) per liter. The carbon source was 10 mM disodium glucarate, 20 mM sodium DL-glycerate, or 40 mM sodium glycolate. The medium was supplemented with 1.0% Casamino Acids (Oxoid) when indicated. Growth flasks were aerated by mechanical agitation in a Gallenkamp rotary shaker at 37 C. Cells were harvested from cultures in exponential growth; they were washed and suspended in buffer A: 10 mM potassium phosphate (*p*H 7.0) containing 0.1 M NaCl, 2 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid (EDTA). They were then stored in a freezer until used. Extracts were prepared by thawing cells, subjecting them for 2 min to the output of a 60W ultrasonic oscillator (Measuring and Scientific Equipment Co. Ltd., London) operating at 1.5 amp, and removing particulate material by centrifugation at 10,000 × *g* for 10 min. Although the extract was chilled to below 5 C during sonic treatment, the centrifugation and subsequent steps were performed at room temperature (19 to 22 C).

Glycerate kinase (ATP: D-glycerate 3-phosphotransferase; EC 2.7.1.31) was purified to homogeneity and crystallized from extracts of the Crookes strain of *E. coli* by Doughty, Hayashi, and Guenther (3). Their purification procedure was greatly facilitated by diethylaminoethyl (DEAE)-Sephadex A-50 chromatography. A modification of this technique was used as an analytical tool for the present study. Columns of DEAE-Sephadex A-50 (2.0 by 20 cm) were equilibrated with buffer A before use. Crude extract (120 mg of protein in 3- to 5-ml volume) was added to an equilibrated column and eluted with buffer A, modified only in that the NaCl concentration was increased from 0.1 to 1.1 M over a linear gradient of 500 ml. The flow rate was adjusted to 0.4 ml/min.

The activity of glycerate kinase was measured as the rate of D-glycerate-dependent formation of adenosine diphosphate (ADP) from adenosine triphosphate (ATP: from Sigma Chemical Co., St. Louis, Mo.). The assay was performed in the presence of an excess of phosphoenolpyruvate (PEP) and pyruvate kinase (from C. F. Boehringer and Soehne, Mannheim, Germany), and reduced β-nicotinamide adenine dinucleotide (NADH) and lactic dehydrogenase (from Sigma Chemical Co., St. Louis, Mo.). Under these conditions, formation of ADP resulted in the oxida-

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tion of an equimolar concentration of NADH. In all cases a unit is defined as the amount of enzyme that catalyzes the removal of 1 μmole of substrate per min under standard assay conditions. Assays were performed in silica cuvettes of 1.0-cm light path which contained: potassium phosphate buffer (*p*H 7.3), 50 μmoles; $MgCl_2$, 10 μmoles; EDTA, 1.0 μmole; disodium ATP, 0.5 μmole; potassium PEP, 2.5 μmoles; pyruvate kinase, one unit; lactic dehydrogenase, one unit; disodium NADH, 0.1 μmole; D-glycerate, 0.5 μmole, and enzyme in a final volume of 1.0 ml. The rate of decrease in absorbance at 340 nm was measured in a Unicam SP800 (Unicam Instruments Ltd., Cambridge, England) recording spectrophotometer at 30 C. Since extracts of *E. coli* contain adenosine triphosphatase and NADH oxidase, measurements were made against control cuvettes which contained all components of the reaction mixture except D-glycerate. Under these conditions, a decrement of 6.21 absorbance units at 340 nm corresponds to the D-glycerate-dependent formation of 1 μmole of ADP per cuvette. When the *p*H dependence of the activity of GK I and GK II were determined, all components of the reaction mixture remained constant, except the buffer which was varied according to the legend in Fig. 5. Michaelis constants (K_m) were determined under standard assay conditions by varying only the D-glycerate or ATP concentration.

Although the standard assay permits accurate measurement of partially purified glycerate kinase, the activity of the enzyme in crude extracts is obscured by NADH oxidase. Accordingly, a modified assay procedure, in which pyruvate was trapped and detected as its phenylhydrazone, was used for detecting the level of glycerate kinase in whole extracts (Tables 1 and 2). This assay was based on a procedure suggested by H. L. Kornberg. The modified assay mixture contained the following: potassium phosphate buffer (*p*H 7.1), 50 μmoles; $MgCl_2$, 10 μmoles; EDTA, 1.0 μmole; ATP, 0.5 μmole; PEP, 2.5 μmoles; pyruvate kinase, one unit; phenylhydrazine hydrochloride (Sigma Chemical Co., St. Louis, Mo.), 3.0 μmoles; and D-glycerate, 0.5 μmole, in a final volume of 1.0 ml. Control cuvettes contained no D-glycerate. Under these conditions, an increment of 13.5 absorbance units at 312 nm corresponded to the D-glycerate-dependent conversion of 1.0 μmole of PEP to pyruvate phenylhydrazone per cuvette.

RESULTS

Influence of growth substrate on the synthesis of glycerate kinase. The influence of several growth substrates upon the induced level of glycerate kinase is summarized in Table 1. After growth with glucarate, glycerate, or glycolate, cultures of *E. coli* possess glycerate kinase at a level over 100 times that observed in cultures grown with glucose, propionate, or Casamino Acids. Thus, as is often the case with enzymes that are only occasionally necessary for growth, glycerate kinase is synthesized at high levels only when it is required.

Although growth at the expense of either glycerate or glycolate elicits the synthesis of high levels of glycerate kinase, the data (Table 2) indicate that the synthesis of enzyme is repressed by Casamino Acids when glycolate, but not when glycerate, is the source of inducer. For the experiment reported in Table 2, cultures growing with Casamino Acids were exposed to glycerate or to glycolate for three generations. Exposure to glycerate under these conditions elicited the synthesis of glycerate kinase to a level equal to 85% of that observed in cells grown with glycerate in the absence of Casamino Acids. In contrast, exposure to glycolate during growth with Casamino Acids resulted in the synthesis of glycerate kinase at only 19% of the level ob-

TABLE 1. *Influence of carbon source on the level of glycerate kinase in extracts of E. coli K-12*

Carbon source[a]	Glycerate kinase[b]
Glycolate	0.26
Glucarate	0.40
Glycerate	0.41
Glucose	<0.002
Propionate	<0.002
Casamino Acids	<0.002

[a] Cultures of strain K-1 were grown for at least three generations at the expense of carbon sources at the following concentrations: 40 mM glycolate; 10 mM glucarate; 20 mM DL-glycerate; 10 mM glucose; 20 mM propionate; and 1.0% Casamino Acids.

[b] Activity of glycerate kinase was determined in crude extracts by the phenylhydrazine assay described in Materials and Methods. Expressed as units per milligram of protein.

TABLE 2. *Influence of Casamino Acids on the induced synthesis of glycerate kinase*

Carbon source[a]	Casamino Acids[b]	Glycerate kinase[c]
Glycerate	−	0.54
Glycerate	+	0.46
Glycolate	−	0.26
Glycolate	+	0.05
Glucarate	−	0.40
Glucarate	+	0.36

[a] Cultures of strain K-1 were grown for at least three generations at the expense of carbon sources at the following concentrations: 40 mM glycolate; 20 mM DL-glycerate; and 10 mM glucarate.

[b] Casamino Acids at 1.0%.

[c] Activity was determined in crude extracts by the phenylhydrazine assay described in Materials and Methods section. Expressed as units per milligram of protein.

served in glycolate-grown cells. Thus, it appears that the repressive effect of Casamino Acids upon glycerate kinase is in large part governed by the source of the inducer. This effect might be attributed to the presumed metabolic proximity of glycerate to the true inducer of glycerate kinase. Several sequential inductive steps might be required before glycolate could give rise to the metabolite that elicits the synthesis of this enzyme. If this were the case, the apparent repression of glycerate kinase during exposure to both glycolate and Casamino Acids might be due to the absence of an internally formed inducer. This interpretation is less likely because induction of glycerate kinase by growth with glucarate is not significantly repressed by exposing cells to Casamino Acids (Table 2), despite the fact that several metabolic steps are required to convert glucarate to glycerate (1). Since extremely low levels of glycerate kinase were observed after three generations of exposure to glycolate, it appears that the enzyme is, at least in part, repressed by metabolites derived from Casamino Acids. Strict repressive control is not exerted when glycerate is the source of the inducer.

Separation of two forms of glycerate kinase on DEAE-Sephadex A-50. The foregoing results suggested that glycerate kinase in *E. coli* is subject to two kinds of repressive control. Such independent regulation could be exerted by cells that can synthesize two forms of glycerate kinase: one induced by growth with glycerate and insensitive to catabolite repression, and a second induced by growth with glycolate and relatively sensitive to catabolite repression. To see whether this mechanism is operative in *E. coli*, we examined the glycerate kinase produced by these bacteria under different growth conditions. An extract of glycolate-grown strain K-1 was placed on a column of DEAE-Sephadex A-50; the protein was eluted with buffer A containing NaCl in an increasing linear gradient. The glycerate kinase from this extract was eluted as two peaks (Fig. 1B), the second somewhat larger than the first. The enzyme contained in the smaller, initially eluted, peak was designated GK I; the enzyme in the second peak was termed GK II. Chromatography of extract from cells grown with glycerate and Casamino Acids yielded a different result (Fig. 1A). Such extracts contained high levels of GK I, but very little GK II. Hence, it appears that *E. coli* can synthesize two forms of glycerate kinase and that the relative levels of the two enzymatic forms are dictated by the growth substrate.

Influence of growth substrate on the relative levels of GK I and GK II. The elution patterns in Fig. 1 show that growth with glycolate can elicit

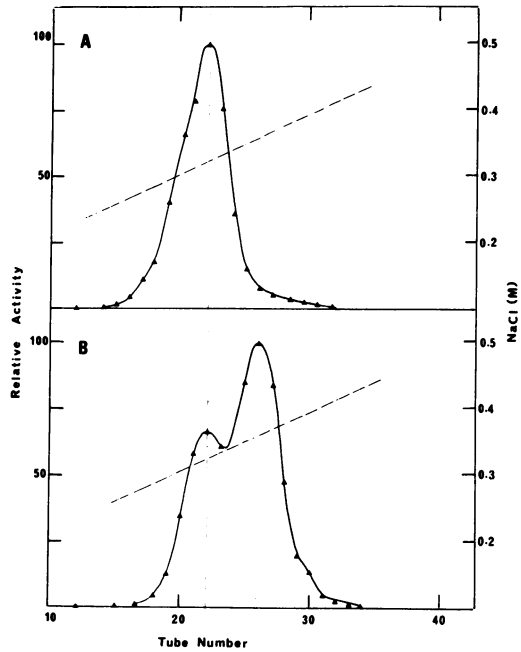

FIG. 1. *Chromatography of glycerate kinase from strain K-1. Crude extract (120 mg of protein) of cells grown either with 30 mM DL-glycerate and 1.0% Casamino Acids (A) or with 40 mM glycolate (B) was eluted from a column of DEAE-Sephadex A-50. Fractions of 5.0 ml were collected. More than two-thirds of the added activity was recovered from both columns.*

the formation of GK II. The absence of this enzymatic form in the glycerate-induced culture could be interpreted in one of two ways: (i) the synthesis of GK II might be repressed by Casamino Acids, or (ii) it might not be elicited by growth with glycerate. To establish which compounds effect the synthesis of GK II, cultures of strain R-4 were grown at the expense of three carbon sources: glycolate, DL-glycerate, or glucarate. Casamino Acids were not added to the growth medium. Extracts of these cultures were placed on columns of DEAE-Sephadex A-50 and eluted under conditions similar to those described in Fig. 1.

Glycerate kinase from glycerate- and glucarate-grown cells was eluted as a single peak corresponding to GK I (Fig. 2A and 2B). Only the extract prepared from glycolate-grown cells contained substantial levels of GK II (Fig. 2C). Thus it appears that the synthesis of GK II is not elicited by glycerate or glucarate.

The level of GK II in glycolate-grown cells varies. It is the major form in glycolate-grown strain K-1 (Fig. 1A) and the minor form in glycolate-grown strain R-4 (Fig. 2C). Although

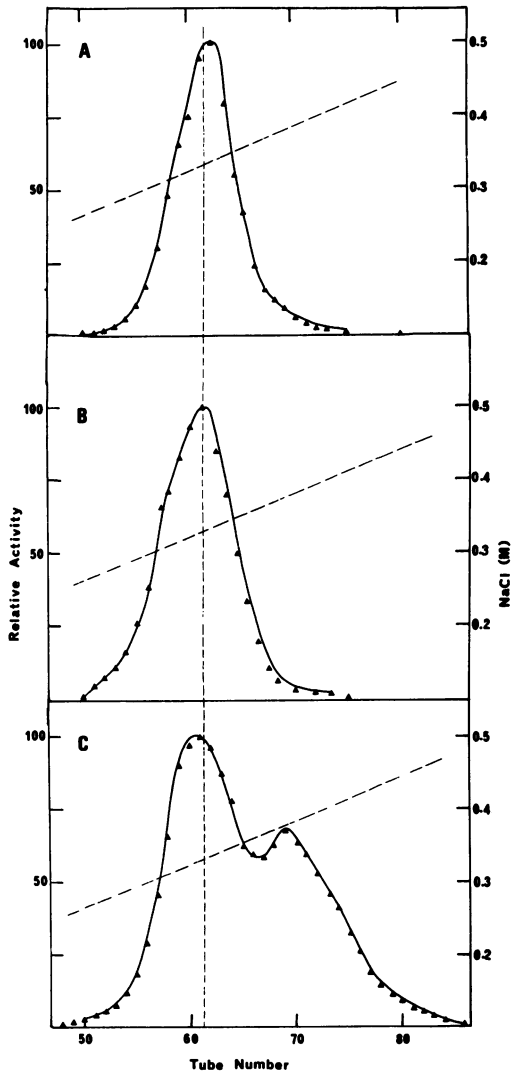

FIG. 2. *Chromatography of glycerate kinase from strain R-4. Crude extract (120 mg of protein) of cells grown with 30 mM DL-glycerate (A), 20 mM glucarate (B), or 40 mM glycolate (C) was eluted from a column of DEAE-Sephadex A-50. Fractions of 3.0 ml were collected. More than two-thirds of the added activity was recovered from all three columns.*

we observed wide variations in the level of GK II in glycolate-grown cells, substantial amounts of this enzymatic form were always present. GK II was never detected in extracts of glycerate- or glucarate-grown cells.

Thermolability of GK I and GK II. The dissimilar chromatographic properties of GK I and GK II are paralleled by differences in the thermolability of the two enzymes. GK II (half-life of 11 min) was inactivated far more rapidly than GK I (half-life of 92 min) at 49 C (Fig. 3). GK I and GK II are inactivated independently when mixed in the same tube (Fig. 3).

The identity of glycerate kinase from glucarate- and glycerate-grown cells as GK I was further established by measurement of its lability at 55 C. Material eluted in fraction 60 from each of the DEAE-Sephadex A-50 columns described in Fig. 2 was heated at this temperature. The glycerate kinase was inactivated at an identical rate (Fig. 4), regardless of whether it was obtained from glycerate-, glucarate-, or glycolate-grown cells. At 55 C, all three preparations have a half-life of 40 min, thus supporting the conclusion that they are all of the same enzymatic form, GK I.

Other properties of GK I and GK II. The two forms of glycerate kinase are similar in many ways. Under standard conditions of assay, the K_m for ATP was 5×10^{-5} M for both GK I and GK II; the K_m for D-glycerate was 7×10^{-5} M for both enzymatic forms. Both forms are cold-sensitive. They lost more than 90% of their activity when stored for 24 hr at 0 C, but re-

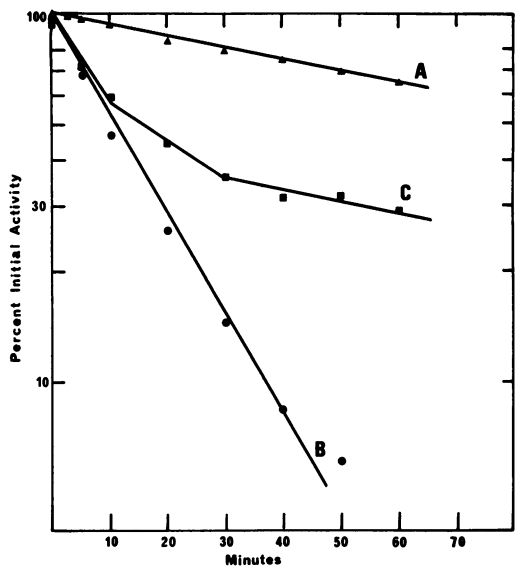

FIG. 3. *Thermolability of GK I and GK II from glycolate-grown cultures of E. coli. Fractions 21 and 27 from the column described in Fig. 1B were used as a source of GK I and GK II, respectively. The enzyme in buffer A, at a concentration of 0.3 units/ml, was heated at 49 C. Samples were removed at 10-min intervals and cooled to room temperature; the activity in the cooled samples was determined. The per cent of initial activity remaining is plotted against time of heating at 49 C for GK I (curve A), GK II (curve B), and a mixture of equal amounts of GK I and GK II (curve C).*

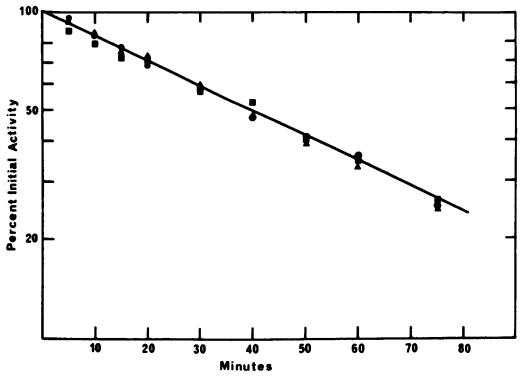

FIG. 4. *Thermolability of GK I from glycerate-, glucarate-, and glycolate-grown cells. Fraction 60 from each of the columns described in Fig. 2 was used as a source of GK I. Heat inactivation was conducted under the conditions described in Fig. 3, except that the inactivating temperature was raised to 55 C. Symbols:* (●) *GK I from glycerate-grown cells;* (▲) *GK I from glucarate grown cells;* (■) *GK I from glycolate-grown cells.*

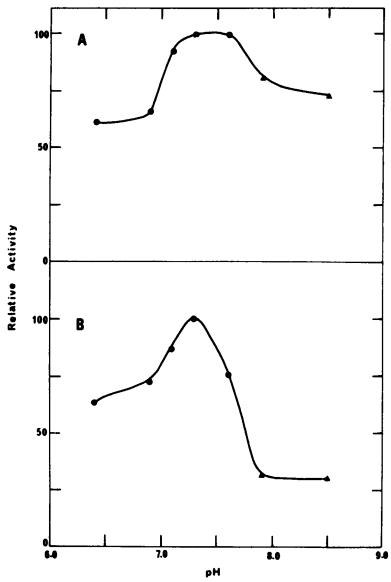

FIG. 5. *Influence of pH on the activity of GK I and GK II. Fractions 21 and 27 from the column described in Fig. 1B were used as a source of GK I and GK II, respectively. Buffers were 0.1 M KPO_4* (●) *and 0.1 M Tris-hydrochloride* (▲). *In all other respects, the D-glycerate-dependent conversion of PEP to lactate was measured by the procedure described in Materials and Methods. The relative activity of 0.010 units of enzyme was determined with GK I (A) and with GK II (B).*

tained at least 90% of their activity after 24 hr at room temperature. The glycerate kinases are relatively stable when frozen. Over 80% of the original activity could be recovered after 1 week at −20 C.

Neither GK I nor GK II catalyzed production of ADP in the presence of L-glycerate and ATP. Thus the enzyme is specific for the D-isomer of glycerate. The reaction catalyzed by GK I and GK II is stoichiometric. In the presence of a limiting concentration of D-glycerate and excess ATP, the enzymes catalyzed the formation of a concentration of ADP equimolar to D-glycerate.

GK I and GK II can be distinguished by the manner in which their activities vary with *p*H. The activity of GK I remained high as the *p*H was increased above *p*H 7.3 (Fig. 5A); the activity observed at *p*H 7.9 was 78% of the optimum. In contrast, the activity of GK II (Fig. 5B) at *p*H 7.9 was under 30% of the optimum.

DISCUSSION

E. coli K-12 can elaborate two kinds of glycerate kinase. The two enzymatic forms, GK I and GK II, differ in thermolability (Fig. 3), in strength of binding to DEAE-Sephadex A-50 (Fig. 1 and 2), and in the dependence of their activity upon *p*H (Fig. 5). Under standard assay conditions, the kinetic characteristics of GK I and GK II are similar. The K_m of both enzymes for D-glycerate and ATP are indistinguishable. Hence, we cannot readily assign a unique catalytic role to either enzymatic form.

When cells growing with glycolate or glyoxalate are simultaneously exposed to a compound that can be readily converted to acetyl coenzyme A (CoA), phosphoglycerate and metabolites derived therefrom can be formed independently of the glycerate pathway. Acetyl CoA and glyoxalate can condense to form malate which readily gives rise to pyruvate, phosphoenolpyruvate, and phosphoglycerate. The studies of Kornberg (7) suggest that three-carbon acids actually are formed via malate during these growth conditions. The synthesis of glyoxalate carboligase, the first enzyme of the glycerate pathway, is sharply repressed by acetate (7). Presumably the metabolic role of the glycerate pathway is reduced commensurately. The results (Table 2) indicate that sharp repressive control may extend to another enzyme of the glycerate pathway, glycerate kinase.

During exposure to glucarate or glycerate, cells must use glycerate kinase in order to utilize all of the carbon contained in these compounds, which cannot be completely assimilated via any known alternative pathway. Hence, weak repressive control of GK I renders these compounds

utilizable even in the presence of acetogenic metabolites.

Thus, the synthesis of two glycerate kinases might enable the cell to exert a refined regulatory control. In accord with this hypothesis, the two enzymatic forms might be the products of distinct structural genes in *E. coli*: one gene which dictates the synthesis of GK I during growth with glycerate, glucarate, or glycolate, and a second gene which directs the synthesis of GK II only in the presence of glycolate. The expression of the latter but not of the former gene might be postulated to be extremely sensitive to catabolite repression, thus enabling the cells to lower the level of glycerate kinase when utilizing glyoxalate via the malate pathway.

This interpretation is similar to that offered by Falmagne, et al. (4), who showed that *E. coli* K-12 can elaborate two forms of malate synthase. These authors proposed that this enzymatic duplication permitted cells to subject each malate synthase to independent biosynthetic control. One enzyme is induced during growth with acetate, and the other is induced in the presence of glycolate. Cánovas and Stanier (2) have shown the formation of three succinyl CoA-β-ketoadipate transferases in *Moraxella calcoacetica*. This apparent genetic redundancy permits cells to contain the enzymes in separate regulatory units, each of which is induced by a different compound.

In principle, interpretations which postulate the presence of independent genes governing the synthesis of isofunctional enzymes should be amenable to genetic analysis. The validity of such interpretations can be tested by studying the physiological consequences of genetic defects which prevent the synthesis of one of the enzymatic forms. Cánovas and Stanier (2) used this approach in their analysis of the β-ketoadipate pathway in *M. calcoacetica*. More recently, Vanderwinkel and De Vlieghere (11) applied a similar approach to describe the mechanisms governing the synthesis of the two malate synthases in *E. coli*. In other systems, the necessary mutant strains may be difficult to obtain, because mutants devoid of one enzyme may achieve slow growth with the other and thus escape selection or detection.

In a search for mutants lacking GK I, we isolated several mutant strains that grow very poorly with D-glycerate. All of these mutants grow well with glycolate and make substantial quantities of both GK I and GK II while doing so. This lack of success cannot be regarded as evidence in favor of two structural genes for glycerate kinase. We may have systematically selected for mutants altered in a regulatory gene governing glycerate kinase. Hence, we cannot exclude the possibility that the amino acid sequence of GK I and GK II are dictated by a single gene. Kingdon et al. (6) showed that the glutamine synthetase of *E. coli* can be modified enzymatically after synthesis of the polypeptide chain by covalent bonding of AMP. Similar modification could be responsible for interconversion of GK I and GK II. Indeed, GK I and GK II might be merely conformational isomers of the same protein. If this is the case, the two conformational states are not in ready equilibrium, because the synthesis of GK II appears to be dictated directly by growth conditions. Moreover, heat treatment of chromatographically separated GK I and GK II has shown that the enzymes are not interconvertible under normal conditions of storage.

In some respects, the glycerate kinases from *E. coli* K-12 differ greatly from the one obtained in crystalline form from the Crookes strain by Doughty, Hayashi, and Guenther (3). The K-12 enzymes are cold-sensitive, whereas the Crookes enzyme is not; the K-12 enzymes have a K_m for D-glycerate of 0.7×10^{-4} M compared with the value of 2.4×10^{-4} M reported for the enzyme from Crookes strain (3). Furthermore, both of the glycerate kinases from the K-12 strain are retained on columns of DEAE-Sephadex A-50 at ionic strengths which would permit rapid elution of the enzyme described by Doughty et al. (3). If, as these differences suggest, the enzymes from the two strains have different primary sequences, then we must conclude that *E. coli* K-12 and the Crookes strain used by Doughty et al. are separated by an evolutionary gap. Consequently, enzymological and physiological conclusions derived from the study of one strain may not be applicable to the other.

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LITERATURE CITED

1. Blumenthal, H. J., and D. C. Fish. 1963. Bacterial conversion of D-glucarate to glycerate and pyruvate. Biochem. Biophys. Res. Commun. 11:239–243.
2. Cánovas, J. L., and R. Y. Stanier. 1967. Regulation of the enzymes of the β-ketoadipate pathway in *Moraxella calcoacetica*. I. General aspects. European J. Biochem. 1: 289–301.
3. Doughty, C. C., J. A. Hayashi, and H. L. Guenther. 1966. Purification and properties of D-glycerate 3-kinase from *Escherichia coli*. J. Biol. Chem. 241:568–572.
4. Falmagne, P., E. Vanderwinkel, and J. M. Wiame. 1965. Mise en evidence de deux malate synthases chez *Escherichia coli*. Biochim. Biophys. Acta 99:246–258.

5. Hansen, R. W., and J. A. Hayashi. 1962. Glycolate metabolism in *Escherichia coli*. J. Bacteriol. 83:679–687.
6. Kingdon, H. S., B. M. Shapiro, and E. R. Stadtman. 1967. Regulation of glutamine synthetase. VIII. ATP:glutamine synthetase adenylyl transferase, an enzyme that catalyzes alterations in the regulatory properties of glutamine synthetase. Proc. Natl. Acad. Sci. U.S. 58:1703–1710.
7. Kornberg, H. L. 1961. Selective utilization of metabolic routes by *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. 26:257–260.
8. Kornberg, H. L. 1966. Anaplerotic sequences and their role in metabolism, p. 1–31. *In* P. N. Campbell and G. D. Greville (ed.), Essays in Biochemistry, vol. 2. Academic Press Inc., New York.
9. Kornberg, H. L., and A. M. Gotto. 1961. The metabolism of C_2 compounds in microorganisms. VI. Synthesis of cell constituents from glycolate by *Pseudomonas* sp. Biochem. J. 78:69–82.
10. Kornberg, H. L., and J. R. Sadler. 1961. The metabolism of C_2 compounds in microorganisms. VIII. A dicarboxylic acid cycle as a route for the oxidation of glycolate by *Escherichia coli*. Biochem. J. 81:503–513.
11. Vanderwinkel, E., and M. De Vlieghere. 1968. Physiologie et genetique de l'isocitritase et des malate synthases chez *Escherichia coli*. European J. Biochem. 5:81–90.