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Regulation of Glycerol Catabolism in *Klebsiella aerogenes*

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The utilization of glycerol as a carbon source for growth by *Klebsiella aerogenes*, strain 2103, involves separate aerobic (*sn*-glycerol-3-phosphate or G3P) and anaerobic (dihydroxyacetone or DHA) pathways of catabolism. Enzyme and transport activities of the aerobic pathway are elevated in cells grown under oxygenated conditions on glycerol or G3P. Anaerobic growth on G3P as carbon source requires the presence of an exogenous hydrogen acceptor such as fumarate; cells thus grown also are highly induced in the G3P pathway. Anaerobic growth on glycerol requires no exogenous hydrogen acceptors; cells thus grown are highly induced in the DHA pathway but almost uninduced in the G3P pathway and the addition of fumarate electron acceptors has no effect on the relative levels of the two pathways. When both glycerol and G3P are provided anaerobically with fumarate, the DHA pathway is still preferentially induced, which probably accounts for the exclusive utilization of glycerol until its exhaustion. These observations suggest the presence of a regulatory control of G3P pathway imposed by the operation of the DHA pathway.

Klebsiella aerogenes, strain 1033, dissimilates glycerol by two separate pathways. In one pathway, the nutrient is phosphorylated by an adenosine 5′-triphosphate (ATP)-dependent kinase to *sn*-glycerol-3-phosphate (G3P). G3P is then converted to dihydroxyacetone phosphate by dehydrogenases characteristic of flavoenzymes (Fig. 1). In the other pathway, glycerol is converted to dihydroxyacetone (DHA) by an NAD-linked dehydrogenase. DHA is then phosphorylated to DHA phosphate (DHAP) by an ATP-dependent kinase. Evidence from in vivo isotopic tracer studies, as well as in vitro measurements of enzyme activities, indicates that the G3P pathway is responsible for aerobic, and the DHA pathway is responsible for anaerobic, degradation of glycerol (7, 9, 13). The presence of the latter pathway in *K. aerogenes* apparently permits the cell to grow anaerobically on glycerol in the absence of exogenous hydrogen acceptors, since *Escherichia coli*, lacking the enzymes of this pathway, is unable to do so.

In the work to be described below, it is shown that *K. aerogenes*, like *E. coli*, can grow directly on G3P without prior hydrolysis, and that anaerobic growth on G3P requires exogenous hydrogen acceptors. All the enzymes of the *glp* regulon (a collection of operons, the expression of which is inducible by G3P) described for *E. coli* (2), including G3P permease and two flavin-dependent G3P dehydrogenases (5, 6), seem to be present and inducible in *K. aerogenes*. Additional evidence for the existence of separate kinases for glycerol and DHA has also been obtained. Finally, data will be presented to show that the expressions of the genes of the G3P and DHA pathways are regulated by different mechanisms.

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MATERIALS AND METHODS

Chemicals. Casein acid hydrolysate (salt free, vitamin free) was obtained from Nutritional Biochemicals, Cleveland, Ohio; glycerol and sodium citrate was from Fisher Chemical Co., Boston, Mass.; and dihydroxyacetone was from Mann Chemical Co., New York, N.Y. Fumaric acid, purchased from Eastman Chemical Co., Rochester, N.Y., was twice recrystallized from water. Piperazine-*N*,*N*′-bis-2-ethanesulfonic acid (PIPES); phenazine methosulfate (PMS); 3(4,5 dimethylthiazolyl 1-2)2,5-diphenyl tetrazolium bromide (MTT); D,L-α-glycerophosphate (Grade X), β-glycerophosphate, *p*-nitrophenyl phosphate, sodium phosphoenolpyruvate, ATP, nicotinamide adenine dinucleotide (NAD), reduced NAD (NADH), Fast Blue RR, naphthol AS-MX phosphate, and tris(hydroxymethyl)aminomethane (Tris) were from Sigma Chemical Co., St. Louis, Mo. The rabbit muscle enzymes, G3P:NAD oxidoreductase, L-lactate:NAD oxidoreductase, ATP:pyruvate phosphotransferase, and *Candida mycoderma* ATP:glycerol phosphotransferase (glycerol kinase) were products of C. F. Boehringer and Soehne, Mannheim, Germany. Bovine serum albumin (crystallized) was from Pentex

Corp., Kankakee, Ill. Uniformly labeled [^{14}C]G3P (20 mCi/mmol) and [^{14}C]dihydroxyacetone (60 mCi/mmol) were purchased from ICN Chemicals and Radioisotope Division, Irvine, Calif., and uniformly labeled [^{14}C]glycerol (134 mCi/mM) was from New England Nuclear Corp., Boston, Mass.

Bacteria. The *E. coli* K-12 used was strain 1 carrying a deletion of the alkaline phosphatase gene, pho A^- (8). The *K. aerogenes* 1033 (formerly designated *Aerobacter aerogenes* 1033 prior to its reassignment to the genus *Klebsiella*) used was strain 2103 lacking alkaline phosphatase and was isolated in the following way.

A wild-type clone was grown in liquid glucose medium and mutagenized with ethyl methane sulfonate (4). After recycling the culture once in low phosphate Tris medium (LPM-Tris) containing 10 mM glucose in soft agar as an overlay on Tris agar plates, 2,000 cells per plate were suspended containing 10 mM glucose and 2 μg of KH_2PO_4 per ml. After the top layer had gelled, a second layer of soft agar without cells was added. This technique allowed the screening of up to 5,000 small colonies of uniform size per plate for alkaline phosphatase activity. After incubation for 24 h at 37 C, the surface of the agar was first rinsed with 10 ml of 1 M Tris-hydrochloride at pH 8.0 and then covered with 5 ml of a solution containing naphthol AS-MX phosphate (2 mg/ml) in 1 M Tris-hydrochloride, pH 8.8, for 10 min at 25 C. The solution was then removed and the agar was rinsed again with Tris-hydrochloride buffer and covered with a mixture of 4 ml of 1 M Tris-hydrochloride at pH 8.8 and 1 ml of an aqueous solution of 20 mg of Fast Blue RR dye per ml (10). The reaction was followed until the wild-type colonies turned dark brown, at which time white colonies were picked with sterilized capillary tubes and streaked on rich agar plates.

Strain 2103, detected as a white colony by this procedure, produces less than 6% of parental wild-type alkaline phosphatase activity as measured by the extent of orthophosphate inhibitable *p*-nitrophenyl phosphate hydrolysis at pH 8.8 (16). This mutant was further shown to be unable to grow on β-glycerophosphate in liquid media either as a carbon or as phosphate source.

Growth of cells. All cultures were grown in a 37 C warm room. Growth was monitored with a Klett-Summerson colorimeter by using a no. 42 filter (1 Klett unit corresponds to approximately 4×10^6 cells per ml for both *K. aerogenes* and *E. coli*).

Growth of aerobic liquid cultures was conducted in Erlenmyer flasks in volumes of 10 to 15% of the total capacity of the container. The flasks were shaken vigorously at approximately 240 cycles per min on rotary shakers. Anaerobic cultures were grown either in flasks filled to capacity and stoppered to exclude air, or in partially filled flasks fitted with stop cocks containing 95% nitrogen and 5% carbon dioxide under a positive pressure to permit the expulsion of culture samples. The cultures were gently stirred magnetically.

Samples of growth media withdrawn for analysis of nutrients remaining were immediately passed through membrane filters (0.45 μm pore size, Millipore Corp., Bedford, Mass.) and the filtrate was stored frozen at -20 C until assayed.

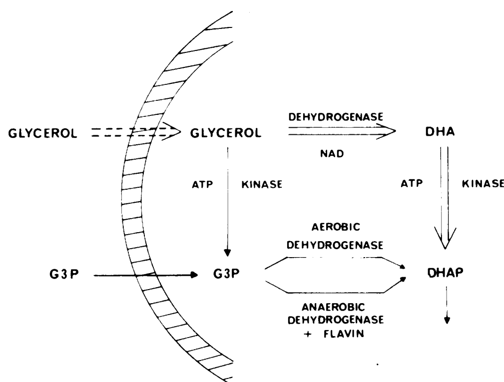

FIG. 1. *Pathways for glycerol and sn-glycerol-3-phosphate utilization in K. aerogenes 2103. The dissimilation of glycerol and G3P aerobically and that of G3P anaerobically are conducted via the pathway indicated by single-line arrows. The catabolism of glycerol anaerobically is depicted by double-lined arrows. The entry of glycerol is represented by a broken-lined arrow because it is not clear whether two separate mechanisms, one functioning aerobically, and the other anaerobically, are involved. G3P stands for sn-glycerol-3-phosphate, DHA for dihydroxyacetone, DHAP for dihydroxyacetone phosphate, NAD for nicotinamide adenine dinucleotide, and ATP for adenosine triphosphate.*

Culture media. Unless indicated otherwise, the standard mineral medium used in these experiments was a high phosphate (0.1 M) basal medium described previously (15). Where a high inorganic phosphate concentration was undesirable, one of the following media was employed.

LPM-Tris contained the following components in the concentrations listed: 1 mM KH_2PO_4 80 mM NaCl, 20 mM KCl, 20 mM NH_4Cl, 1 mM $MgCl_2$, 2 μM $FeCl_3$, 0.2 mM $CaCl_2$, 5 μM $ZnCl_2$, 0.25 mM Na_2SO_4, and 0.12 M Tris. The pH was adjusted to 7.5 by the addition of HCl.

LPM-PIPES contained the following components in the concentrations listed: 1 mM KH_2PO_4, 87 mM KOH, 40 mM KCl, 34 mM NaCl, 20 mM $(NH_4)_2SO_4$, 1 μM $FeSO_4$, 3 mM $MgSO_4$, 1 μM $ZnCl_2$, 10 μM $CaCl_2$, and 75 mM PIPES. The pH was adjusted to 6.9 by the addition of HCl.

LPM-citrate contained the same salts as LPM-PIPES medium except that sodium citrate, 25 mM, was substituted for PIPES buffer and the pH was adjusted to 6.8.

Preparation of cells and cell extracts. Cultures were harvested at a density of 4×10^8 cells/ml. For permeation assays, cells were washed twice and resuspended in Tris medium at a density of 8×10^8 cells/ml. The temperature was maintained at 25 C throughout. For enzyme assays, cells were washed twice with 0.1 M KH_2PO_4, pH 7.0, at 4 C and disrupted in 4-ml batches (70 mg of fresh cells/ml) for 2

min in a 60 W ultrasonic disintegrator (Measuring and Scientific Equipment).The disrupted cell preparations were centrifuged at 4 C for 15 min at 5,000 $\times$ g to remove the debris. Protein concentrations were determined by the biuret method (11).

Permeation and enzyme assays. G3P transport was measured by incubating cells in phosphate-free Tris medium in the presence of 0.01 mM [^{14}C]*sn*-glycerol-3-phosphate (4). Cell samples were collected, washed with a 50-fold excess of Tris medium at 25 C on membrane filters (0.6 μm pore size; Millipore Corp.) at 1-, 2-, and 3-min intervals, and counted in 10 ml of Bray's solution (1).

Aerobic G3P dehydrogenase was assayed by measuring the coupled reduction of a tetrazolium dye at pH 7.5 without the addition of coenzymes (3). Anaerobic G3P dehydrogenase activity was assayed by measuring the increment in the reduction rate of the tetrazolium dye resulting from the addition of 10 mM flavin adenine dinucleotide (FAD) and 1 mM flavin mononucleotide (FMN) (5).

Glycerol kinase and DHA kinase activities were measured by the binding of radioactive phosphorylated derivatives to diethylaminoethyl cellulose filters (H. Reeve Angel & Co., Clifton, N.J.). The presence of both kinases in the same extract and the reactivity of each with the substrate of the other (*E. coli* glycerol kinase has a K_m of 0.01 mM for glycerol and a K_m of 0.5 mM for DHA) required a modification of the assay procedure previously described (12). The activity of each enzyme was measured by the addition of radioactive substrate to a final concentration of 0.01 mM in the presence of unlabeled nonphysiological substrate. The presence of a 10-fold excess of unlabeled DHA in assays of glycerol kinase inhibited by 10 to 20% the phosphorylation of [^{14}C]glycerol in extracts of cells grown either aerobically or anaerobically on glycerol as carbon source. The phosphorylation of [^{14}C]DHA catalyzed by these same extracts was inhibited 30 to 35% by the addition of a 10-fold excess of unlabeled glycerol. Bovine serum albumin was added to a final concentration of 1 mg/ml to stabilize the kinases.

Glycerol dehydrogenase activity was measured for 2 to 5 min by the linear increase in absorbance at 340 nm produced by the addition of cell extract to a reaction mixture containing 0.1 M potassium carbonate buffer, pH 9.0, 30 mM ammonium sulfate, 0.6 mM NAD, and 0.1 M glycerol. NADH oxidase activity was found to be negligible under the experimental conditions.

All spectrophotometric assays were conducted at 30 C, while kinase and permeation assays were measured at 25 C.

Purification of D,L-α-glycerol-3-phosphate. The presence of significant levels (2 to 8%) of glycerol in commercially available reagent grade D,L-α-glycerol-3-phosphate necessitated the following purification. One volume of 1.5 M solution of the racemic mixture was added slowly with stirring at 25 C to four volumes of 80% ethanol. After 1 h of cooling at 4 C with stirring, the precipitate was collected, partially dried under vacuum, and dissolved in the original volume of water. The ethanol precipitation was repeated. The final residue was dried under vacuum at 25 C to remove excess aqueous alcohol. G3P twice purified by this procedure was found by enzymatic assay to contain 0.2 to 0.4% glycerol. The yield was approximately 80%.

Quantitation of glycerol and G3P. The concentration of glycerol in culture media was measured spectrophotometrically in a system dependent upon the coupling of glycerol kinase activity to those of L-lactic dehydrogenase and pyruvate kinase (3). The concentration of G3P in culture media was determined enzymatically by G3P-dependent reduction of MTT (3).

RESULTS

Growth properties of strain 2103. Aerobically, *K. aerogenes* 2103, a mutant possessing low alkaline phosphatase activity, can grow readily on glycerol or G3P as a sole source of carbon and energy (Table 1). Growth on glycerol (20 mM) is slightly faster than on G3P (20 mM). The slower utilization of G3P is not attributable to competitive inhibition by orthophosphate in the medium, since lowering concentration of inorganic phosphates from 100 to 1 mM did not alter the situation (data not presented in the table). For comparison, the corresponding growth rates of *E. coli* strain 1 were determined under similar conditions.

Anaerobically, *K. aerogenes* can grow on glycerol and DHA without exogenous hydrogen acceptors. Anaerobic growth on G3P, however, requires such acceptors.

Dissimilation of a mixture of glycerol and G3P. To test whether there is a preference of utilization when glycerol and G3P are provided together, cells were inoculated into a medium containing 2 to 5 mM of each nutrient. At this low concentration, the entry of G3P into the cell was found to be impeded by orthophosphate at a high concentration (100 mM). PIPES or citrate was therefore employed as the buffer, and phosphate was added only at 1 mM. The subsitution of the buffer did not have a pronounced effect on the growth rates studied (compare data in Tables 1 and 2).

Aerobic growth on the mixture of glycerol and G3P occurred at a steady rate until carbon source exhaustion (Fig. 2A). The two nutrients were utilized simultaneously, although the rate of glycerol depletion was faster than that of G3P depletion (Fig. 2B). In contrast, anaerobic growth on glycerol and G3P, in the presence of 40 mM fumarate as the hydrogen acceptor was diauxic (Fig. 3A). Analysis of the culture medium revealed no appreciable utilization of G3P before 90% of glycerol was consumed (Fig. 3B). When a similar set of experiments was carried out with *E. coli*, no biphasic growth was observed either aerobically or anaerobically (Fig. 2C and D, 3C and D). It might be noted, however, that aerobically glycerol was used

TABLE 1. *Doubling times of K. aerogenes 2103 on various carbon sources*[a]

Growth condition	Glycerol	G3P	Glycerol and fumarate	G3P and fumarate	DHA
Aerobic	43 (90)[c]	56 (90)			NG[b] (NG)
Anaerobic	58 (NG)	NG (NG)	48 (170)	155 (130)	91 (NG)

[a] Doubling times are given in minutes. Carbon sources were present at concentrations of 20 mM. G3P was provided as the mixture D,L-α-GP (40 mM). Sodium fumarate was added as an exogenous electron acceptor at a concentration of 20 mM.

[b] NG, No growth.

[c] Values in parenthesis represent the doubling times of *E. coli* strain 1 under similar growth conditions.

TABLE 2. *Doubling times of K. aerogenes 2103 and E. coli 1 in LPM-PIPES medium*[a]

Strain	LPM-PIPES	
	Glycerol	G3P
Aerobic		
1	94	130
2103	48	62
Anaerobic		
1	156	120
2103	57	144

[a] Doubling times are given in minutes. Glycerol and G3P were provided at concentrations of 8 mM as single carbon sources. Anaerobic cultures were provided with 40 mM sodium fumarate as an exogenous electron acceptor.

more rapidly by *E. coli* cells, and anaerobically the reverse was true.

Enzyme levels in cells grown aerobically and anaerobically on various carbon sources. When cells grown on various carbon sources under aerobic and anaerobic conditions were examined for transport and enzyme activities, two patterns became evident (Fig. 4). Aerobic growth, whether on glycerol or G3P, resulted in high G3P pathway activities and low activities of the DHA pathway (Table 3). The opposite pattern resulted from anaerobic growth on glycerol alone, DHA alone, or glycerol plus fumarate. An interesting case is anaerobic growth on G3P plus fumarate. G3P transport and anaerobic G3P dehydrogenase are found at high levels, whereas the rest of the enzymes (with the exception of aerobic G3P dehydrogenase), which are gratuitous under such a growth condition, are found in rather low levels (Table 4).

The glycerol permeability of cells grown under a variety of conditions was checked by the osmotic method employed for the study of *E. coli* (12, 14). The data are not presented because of inadequacy of this technique in discriminating between rapid rates of equilibration. It should nevertheless be mentioned that cells of strain 2103 grown on glycerol, either aerobically or anaerobically, were highly permeable to the compound, showing a half-time of equilibration of less than 2.5 s when exposed to 1 M glycerol. Glucose-grown cells, when assayed under these same conditions, showed a half-time of equilibration of 7.0 s.

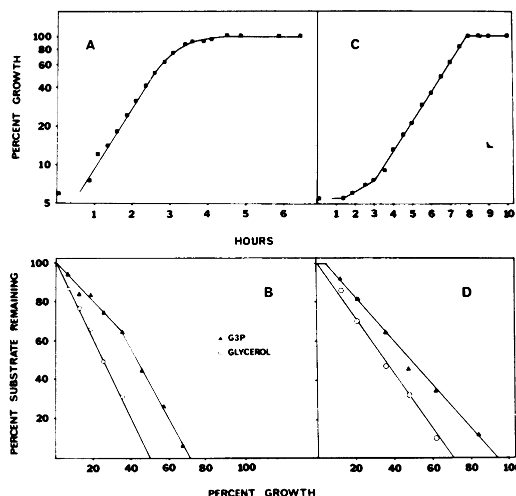

FIG. 2. *Aerobic growth and carbon source utilization by K. aerogenes 2103 (panels A and B) and E. coli (panels C and D) on a limiting mixture of glycerol and G3P. The turbidimetric increase in cultures (A and C) was followed together with the amounts of glycerol and G3P remaining in the media (B and D). An equimolar (2.0 mM in A and 3.0 mM in C) mixture of glycerol and the L-isomer of G3P was used as the carbon source and the yield of cells was 150 Klett units for both cultures. K. aerogenes was grown in LPM-PIPES medium and E. coli in LPM-Citrate. The cells used were grown overnight in their respective LPM medium containing glucose as carbon source, washed with the appropriate medium without carbon source, and resuspended at densities of 5 to 8 Klett units for growth measurements. (See Materials and Methods for further details.)*

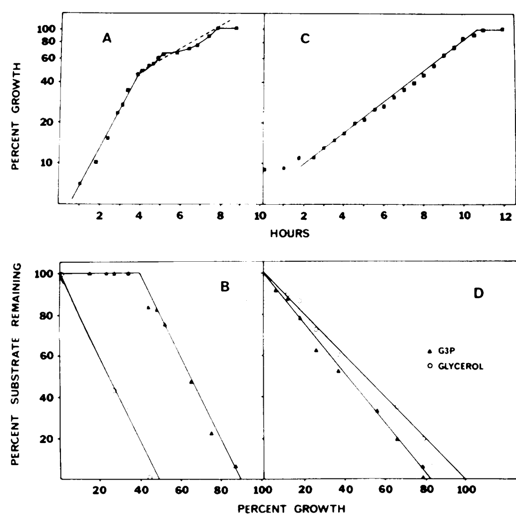

FIG. 3. *Anaerobic growth and carbon source utilization by K. aerogenes 2103 (panels A and B) and E. coli (panels C and D) on a limiting mixture of glycerol and G3P. The turbidimetric increase in cultures (A and C) was monitored together with the amounts of glycerol and G3P remaining in their culture media (B and D). An equimolar (4.0 mM in A and 5.0 mM in C) mixture of glycerol and the L-isomer of G3P supplemented with 0.03% casein hydrolysate and 40 mM fumarate was used in both cultures. A yield of 143 Klett units was observed for K. aerogenes and 103 Klett units for E. coli. The cells used were grown aerobically overnight on glucose as a carbon source, washed with the medium without carbon source and resuspended at densities of 5 to 8 Klett units for growth measurements. LPM-PIPES medium was used throughout.*

Enzyme and transport levels during anaerobic growth on a mixture of glycerol and G3P. Enzyme and transport activities of *K. aerogenes* cells were measured at midpoints of the primary and secondary phases of the diauxie during anaerobic growth. The data in Table 5 indicate that glycerol dehydrogenase and DHA kinase levels were high in cells during growth on glycerol. Proteins of the G3P system are only slightly induced at the time of the initial sampling when 71% of the glycerol was consumed. By the time both carbon sources were exhausted, the levels of the enzymes in the DHA pathway had decreased, whereas those of the G3P pathway had increased.

DISCUSSION

K. aerogenes seems to have all of the enzymes of the *glp* system found in *E. coli* with the possible exception of the anaerobic G3P dehydrogenase, the existence of which was inferred from the circumstantial evidence that anaerobic growth of *K. aerogenes* on G3P is dependent upon an exogenous hydrogen acceptor, such as fumarate, and that the dehydrogenation of G3P catalyzed by crude cell extracts can be stimulated by flavins, a requirement demonstrated

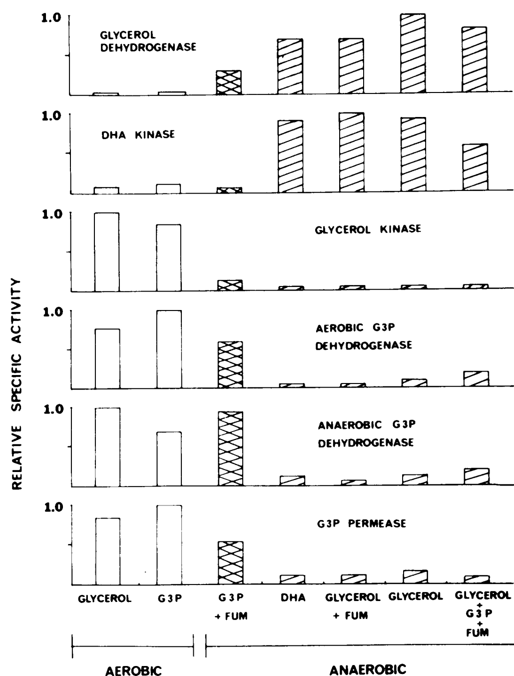

FIG. 4. *Relative specific activities of glycerol-catabolizing enzymes in K. aerogenes 2103 grown under a variety of conditions. The variables, enumerated under the abscissa, were carbon sources and hydrogen acceptors. Cultures were harvested at a density of 100 Klett units and specific enzyme and permease activities were determined using freshly prepared cells. G3P stands for sn-glycerol-3-phosphate, DHA for dihydroxyacetone, and FUM for sodium fumarate.*

TABLE 3. *Specific enzyme activities of aerobically grown cells of strain 2103*

Enzyme	Carbon source		
	Glycerol[a]	G3P	Glucose
Glycerol dehydrogenase	<1	<1	<1
DHA kinase	7.1 ± 0.2	12.4 ± 0.4	0.4 ± 0.1
Glycerol kinase	807 ± 51	697 ± 23	2.7 ± 0.6
Aerobic G3P dehydrogenase	110 ± 1	139 ± 5	0.3 ± 0.1
Anaerobic G3P dehydrogenase	57 ± 3	38 ± 5	0.2 ± 0.1
G3P transport	26 ± 0.2	28 ± 2	0.2 ± 0.1

[a] Glycerol and G3P were provided at 20 mM each and glucose at 10 mM. Specific activities are expressed as nanomoles per minute per milligram of protein. Means are presented with their standard errors. Two to four separate cultures were assayed with each carbon source.

TABLE 4. *Specific enzyme activities of anaerobically grown cells of strain 2103*

Enzyme	Carbon source and hydrogen acceptor[a]					
	Glycerol	Glycerol + fumarate	Glycerol + G3P + fumarate	G3P + fumarate	DHA	Glucose
Glycerol dehydrogenase	1,870 ± 200	1,300 ± 27	1,580 ± 50	591 ± 14	1,330 ± 25	< 1
DHA kinase	86 ± 10	92 ± 13	53 ± 2	4.1 ± 0.3	83 ± 6	0.5 ± 0.2
Glycerol kinase	34 ± 6	15.7 ± 0.1	20.4 ± 0.4	77 ± 4	15 ± 1	0.3 ± 0.1
Aerobic G3P dehydrogenase	13.4 ± 5.3	4.1 ± 0.2	30 ± 5	83 ± 0.5	6.8 ± 0.5	0.3 ± 0.1
Anaerobic G3P dehydrogenase	5.5 ± 2.6	3.0 ± 0.2	10 ± 2	55 ± 6	5.2 ± 0.2	0.2 ± 0.1
G3P transport	4.3 ± 1.0	2.9 ± 0.0	1.4 ± 0.1	15.3 ± 0.2	2.5 ± 0.2	0.1 ± 0.1

[a] Carbon sources were provided at 20 mM when employed singly and 10 mM when employed doubly. Fumarate, used as a hydrogen acceptor, was added at 40 mM. All anaerobic cultures were supplemented with 0.03% casein hydrolysate. Specific activities are expressed as nanomoles per minute per milligram of protein. Means of two to four determinations in each case are presented with their standard errors.

TABLE 5. *Specific enzyme activities of strain 2103 cells grown anaerobically on a mixture of glycerol and G3P*[a]

Enzyme	Sp act	
	34% Total growth (71% glycerol consumed 0% G3P consumed)	87% Total growth (97% glycerol consumed 100% G3P consumed)
Glycerol dehydrogenase	1,750.0	189.0
Dihydroxyacetone kinase	51.5	14.2
Glycerol kinase	15.0	74.4
Aerobic G3P dehydrogenase	22.2	47.9
Anaerobic G3P dehydrogenase	5.9	21.4
G3P transport	0.5	2.3

[a] Strain 2103 growing anaerobically in a low phosphate medium containing 4.0 mM glycerol and 4.0 mM G3P, and 20 mM sodium fumarate was sampled during each of its biphasic growth periods. Specific activities were measured as nanomoles per minute per milligram of protein.

for the anaerobic enzyme of *E. coli* (5, 6). In addition, *K. aerogenes* possesses an NAD-linked glycerol dehydrogenase and an ATP-dependent DHA kinase which apparently enable the organism to grow anaerobically on glycerol without exogenous hydrogen acceptors. Interestingly, the anaerobic growth rate on glycerol is three times higher than on G3P, indicating that the NAD-dependent pathway has a distinct advantage over the flavoprotein pathway. Perhaps under anaerobic conditions, hydrogens are more readily removed from NADH than from reduced flavins. Other differences, however, such as permeation mechanisms or kinetic regulatory mechanisms, cannot yet be excluded.

In *K. aerogenes*, enzymes of the *glp* system are repressed when the cells are grown anaerobically on glycerol or on DHA. The presence of G3P and fumarate does not reverse this effect. Anaerobic growth on G3P plus fumarate in the absence of glycerol, however, results in high levels of the *glp* enzymes with the exception of glycerol kinase. Hence, molecular oxygen is not necessary for the induction of the *glp* system.

How, under anaerobic conditions, glycerol prevents the induction of the G3P pathway by G3P is not at all clear. Mutants that lack glycerol dehydrogenase or DHA kinase should help to determine whether metabolites, such as DHA or DHAP, affect the expression of the *glp* system.

The low levels of glycerol dehydrogenase in cells grown aerobically on glycerol may be the result of two kinds of control mechanisms. This enzyme was previously found to undergo a rapid energy-dependent inactivation when cells were shifted from anaerobic to aerobic growth on glycerol (7). Specific repression of the gene for glycerol dehydrogenase may also be taking place, but this could be demonstrated only if the specific inactivation process were prevented. DHA kinase, in contrast, is relatively stable after the transition from anaerobic to aerobic growth on glycerol (Ruch, unpublished observations). Thus, its low level in aerobically grown cells is probably due to repression.

Experiments are now in progress to determine whether DHA is the inducer of the DHA pathway. If this is so, the rapid inactivation of glycerol dehydrogenase during aerobiosis would prevent glycerol from being converted to the inducer and thus would also serve as a mecha-

nism for selectively shutting down the DHA pathway at the level of transcription.

ACKNOWLEDGMENTS

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