JOURNAL OF BACTERIOLOGY, Nov. 1978, p. 638-646
0021-9193/78/0136-0638$02.00/0

Vol. 136, No. 2
Printed in U.S.A.

Relationship Between Catabolism of Glycerol and Metabolism of Hexosephosphate Derivatives by *Pseudomonas aeruginosa*

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Received for publication 21 September 1977

The relationship between catabolism of glycerol and metabolism of hexosephosphate derivatives in *Pseudomonas aeruginosa* was studied by comparing the growth on glycerol and enzymatic constitution of strain PAO with these characteristics of glucose-catabolic mutants and revertants. Growth of strain PAO on glycerol induced a catabolic oxidized nicotinamide adenine dinucleotide-linked glyceraldehyde-phosphate dehydrogenase and seven glucose-catabolic enzymes. The results indicated that these enzymes were induced by a six-carbon metabolite of glucose. All strains possessed a constitutive anabolic Embden-Meyerhof-Parnas pathway allowing limited conversion of glycerol-derived triosephosphate to hexosephosphate derivatives, which was consistent with induction of these enzymes by glycerol. Phosphogluconate dehydratase-deficient mutants grew on glycerol. However, mutants lacking both phosphogluconate dehydrogenase and phosphogluconate dehydratase were unable to grow on glycerol, although these strains possessed all of the enzymes needed for degradation of glycerol. These mutants apparently were inhibited by hexosephosphate derivatives, which originated from glycerol-derived triosephosphate and could not be dissimilated. This conclusion was supported by the fact that revertants regaining only a limited capacity to degrade 6-phosphogluconate were glycerol positive but remained glucose negative.

Induction of various glucose-catabolic enzymes by growth of *Pseudomonas aeruginosa* on glycerol has been noted repeatedly (9, 10, 20). Similar, though less extensive, observations have been reported for *P. putida* (32) and *P. fluorescens* (19). This has been interpreted as indicating that these glucose-catabolic enzymes are induced by triosephosphate or a derivative thereof (9). However, this does not satisfactorily account for the fact that most glucose-negative mutants which are able to grow on lactate also fail to grow on glycerol (2, 18). Indirect evidence for additional unselected and as yet undefined defects associated with glycerol catabolism in such mutants has been presented (2).

This report specifically addresses the relationship between growth on glycerol and ability to dissimilate hexosephosphate derivatives in the wild type strain and glucose-negative mutants of *P. aeruginosa*.

MATERIALS AND METHODS

Bacterial strains. *P. aeruginosa* strain PAO (ATCC 15692, strain 131 of Stanier et al. [25]) was provided by B. W. Holloway, Monash University. *Escherichia coli* strain B (ATCC 11303) was from the collection of J. W. Drake, University of Illinois. All mutants unable to grow on glucose but capable of normal growth on lactate were derived from strain PAO. Strain 707 was isolated from a culture mutagenized with ethyl methane sulfonate (15) by replica plating without enrichment. All other mutants were obtained after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (1). The remaining glucose-negative mutants were isolated by replica plating either without enrichment (strain 720) or after enrichment with D-cycloserine alone (strains 728 and 732) or in combination with carbenicillin (16). Stock cultures were maintained by periodic transfer on slants of nutrient agar (Bacto, Difco Laboratories).

Media and cultural conditions. Nutrient broth (Bacto, Difco) was used for routine growth of cells. Where indicated, nutrient broth was supplemented with 0.5% (wt/vol) glucose (GNB). The composition of minimal medium has been described previously (29). Compounds used as sole sources of carbon and energy were added to a final concentration of 0.5% (wt/vol). All cultures were grown at 37°C; liquid cultures were aerated by reciprocal shaking. Growth was measured as absorbance at 540 nm.

Enzyme assays. Cells were grown to late-exponential phase in 200 ml of the indicated medium. Mutant cells unable to grow on a particular compound were grown to late-exponential phase in 200 ml of lactate-minimal medium, washed in minimal medium without substrate at approximately 22°C, resuspended to volume in minimal medium containing 0.5% (wt/vol) of

the desired compound, and incubated an additional 4 h for induction. Then, the cells were washed in saline at 0°C, resuspended in 5.0 ml of 20 mM 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris)-hydrochloride (pH 7.5), and ruptured by intermittent sonic oscillation. Residual cells and debris were removed by centrifugation at 27,000 × *g* for 10 min at 0°C.

All assays were performed at approximately 22°C. All substrates and coupling enzymes were tested for spurious contributions to reaction velocity by contaminating substances, and, when necessary, appropriate corrections were made as indicated. A unit of enzyme activity is defined as the amount required to convert 1.0 μmol of substrate(s) to product(s) per min. The following millimolar extinction coefficients were used to calculate enzyme activities: formazan product of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reduction at 550 nm, 8.1 (24); reduced form of nicotinamide adenine dinucleotide (phosphate) [NAD(P)H] at 340 nm, 6.2 (14); and phosphoenolpyruvate at 230 nm, 3.0 (36). Protein was determined by the method of Sutherland et al. (27). Specific activities are given in milliunits per milligram of protein.

The following enzymes were assayed according to the referenced procedure: glucokinase (28); glucose-6-phosphate dehydrogenase (28); phosphogluconate dehydrogenase (28); glycerol kinase (11); glycerol-3-phosphate dehydrogenase (11); triosephosphate isomerase (3); glyceraldehyde-phosphate dehydrogenase (28); glyceraldehyde-phosphate dehydrogenase (oxidized nicotinamide adenine dinucleotide phosphate [$NADP^+$]) (phosphorylating) (28); phosphoglycerate kinase (3); enolase (35); pyruvate kinase (30); fructose-bisphosphatase (7, 28); and glucosephosphate isomerase (7, 28).

Glucose dehydrogenase was measured as glucose-dependent reduction of MTT to the formazan. The reaction mixture contained 67 mM Tris-hydrochloride (pH 7.5), 10 mM KCN, 0.24 mM MTT, 2.5 mM glucose, and 0.33 mM phenazine methosulfate. The reaction was dependent on phenazine methosulfate which was used to initiate the reaction. Less than 10% of the activity was sedimented during the preparation of crude extracts. For determination of gluconate 2-dehydrogenase activity, 2.5 mM gluconate replaced glucose.

Gluconokinase was assayed under the same conditions as glucokinase except that phosphogluconate dehydrogenase (approximately 0.1 U) and 2.5 mM gluconate replaced glucose-6-phosphate dehydrogenase and glucose. The excess phosphogluconate dehydrogenase was provided by 0.1 ml of an extract of *E. coli* cells grown in GNB. Such extracts typically have only about 0.02 U of gluconokinase per U of phosphogluconate dehydrogenase for which the observed reaction velocities were corrected.

The combined activity of phosphogluconate dehydratase and phospho-2-keto-3-deoxygluconate (PKDG) aldolase was assayed under the conditions described by von Tigerstrom and Campbell (33). Iodoacetamide did not affect the reaction and was omitted. PKDG aldolase was assayed directly by substitution of PKDG for 6-phosphogluconate in the reaction mixture. In all cases where both activities were present, phosphogluconate dehydratase was shown to be rate limiting by addition of excess PKDG aldolase (approximately 0.16 U) which was provided by 0.1 ml of an extract of *E. coli* cells grown in GNB. Such extracts lack detectable phosphogluconate dehydratase activity and were included routinely in the reaction mixture to assure an excess of PKDG aldolase when assaying for phosphogluconate dehydratase activity in extracts of mutant cells. A sensitive semiquantitative test which detects glyceraldehyde 3-phosphate in addition to pyruvate produced by the action of PKDG aldolase was devised to screen mutants. The reaction conditions were modified to include phosphogluconate dehydratase (approximately 0.05 U), triosephosphate isomerase (approximately 1 U) and glycerol-3-phosphate dehydrogenase (approximately 6 U). Phosphogluconate dehydratase and additional triosephosphate isomerase were provided as 0.1 ml of an extract of lactate-grown, glucose-induced strain 720 cells which lack detectable PKDG aldolase activity. Results obtained by this method were interpreted qualitatively because the resulting activity was less than that observed in the direct PKDG aldolase assay.

Fructose-bisphosphate aldolase was measured as fructose-1,6-bisphosphate-dependent reduction of oxidized nicotinamide adenine dinucleotide (NAD^+) in the presence of excess glyceraldehyde-phosphate dehydrogenase. The reaction mixture contained 67 mM Tris-hydrochloride (pH 7.5), 100 mM KCl, 17 mM Na_2HAsO_4, 10 mM freshly neutralized cysteine, 0.25 mM NAD^+, approximately 10 U of glyceraldehyde-phosphate dehydrogenase, and 2.5 mM fructose-1,6-bisphosphate. Two moles of NAD^+ was assumed to be reduced per mole of fructose-1,6-bisphosphate cleaved because the excess triosephosphate isomerase (approximately 0.5 U) of the extract would convert dihydroxyacetone phosphate to glyceraldehyde 3-phosphate. The same consideration applies for determination of 6-phosphofructokinase as fructose-6-phosphate-dependent reduction of NAD^+ in the presence of excess fructose-bisphosphate aldolase and glyceraldehyde-phosphate dehydrogenase. This reaction mixture contained 67 mM Tris-hydrochloride (pH 8.0), 50 mM KCl, 17 mM Na_2HAsO_4, 5.0 mM $MgCl_2$, 2.5 mM ATP, 0.25 mM NAD^+, approximately 5 U of fructose-bisphosphate aldolase, approximately 10 U of glyceraldehyde-phosphate dehydrogenase, and 2.5 mM fructose 6-phosphate.

Chemicals and enzymes. The barium salt of PKDG, a generous gift from W. A. Wood of Michigan State University, was converted to the free acid by ion exchange with Dowex-50. DL-Glyceraldehyde 3-phosphoric acid was prepared from the barium salt of the diethylacetal derivative by ion exchange with Dowex-50 and subsequent hydrolysis for 3 min at 100°C. Carbenicillin was donated by Beecham Pharmaceuticals. Commercial enzymes were the highest quality available from Calbiochem or Sigma Chemical Co. All other chemicals were reagent grade or the highest purity available.

RESULTS

Growth phenotypes of glucose-negative mutants. Twenty independent glucose-negative

mutants were isolated. Each was also unable to grow on gluconate or 2-ketogluconate but was able to grow on lactate. As shown in Table 1, three classes of glucose-negative mutants could be distinguished by growth in glycerol-minimal medium and GNB. Only three strains were able to grow on glycerol; strain 707 grew well, but strain 732 grew poorly. The glycerol-negative strains could be grouped further by growth in GNB. Strain 728 is representative of 13 mutants that grew in GNB. Strain 720 is representative of four mutants that grew normally in NB but became severely inhibited and usually lysed in GNB. The other glycerol-positive strain and two additional representatives of each glycerol-negative group have been characterized thoroughly but were omitted here because they gave the same results as strains 707, 728, and 720, respectively.

Enzymatic defects of glucose-negative mutants. The inability of each mutant to grow on glucose, gluconate, or 2-ketogluconate suggested a defect in catabolism of 6-phosphogluconate (Fig. 1). Eight of the ten glucose-catabolic enzymes were assayed. As shown in Table 2, growth of strain PAO on glucose or gluconate induced all eight enzymes approximately threefold or more. Growth on glycerol also caused approximately threefold or more induction of all these enzymes except gluconokinase.

The glycerol-positive strains were specifically defective for phosphogluconate dehydratase (Table 2); strains 707 and 732 lacked detectable activity. Both of these mutants possessed phosphogluconate dehydrogenase, and the level of this activity correlated with their ability to grow on glycerol (cf. Table 1). In contrast to the wild type, growth of these mutants on glycerol also induced gluconokinase. Although glucokinase and gluconate 2-dehydrogenase were not induced during the poor growth of strain 732 on glycerol, these activities were increased to 25.2 and 54.5 mU/mg of protein, respectively, by exposure of lactate-grown cells to glycerol.

Strain 728 lacked detectable phosphogluconate dehydratase and phosphogluconate dehydrogenase activities (Table 2). Similar results have been obtained for cells induced with gluconate or glycerol. Again, unlike the wild type, glycerol induced gluconokinase to 60 mU/mg of protein in this strain.

Strain 720 specifically lacked detectable PKDG aldolase activity (Table 2). Low levels of glucose-6-phosphate dehydrogenase, gluconokinase, and phosphogluconate dehydrogenase also were observed upon induction with gluconate or glycerol.

Absence of additional defects from strains with impaired growth on glycerol. Because *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine often causes multiple mutations (13), strains with impaired growth on glycerol were examined for additional unselected defects specific for glycerol degradation. As shown in Table 3, glycerol kinase and glycerol-3-phosphate dehydrogenase were induced by growth of strain PAO on glycerol but not on glucose; triosephosphate isomerase was constitutive. Strain 732, which grew poorly on glycerol, and representative glycerol-negative strains 728 and 720 did not lack either inducible enzyme (Table 3). Triosephosphate isomerase activity was demonstrated for strain 728 (Table 3) and is inferred for the other mutants from normal growth on glucogenic substrates.

Enzymes of the triosephosphate pathway were also examined for additional defects that could affect degradation of glyceraldehyde 3-phosphate. Tiwari and Campbell (28) noted the presence of NAD^+- and $NADP^+$-linked glyceraldehyde-phosphate dehydrogenase activities in *P. aeruginosa*. As shown in Table 4, strain PAO possessed inducible glyceraldehyde-phosphate dehydrogenase activity (NAD^+-linked) in addition to a constitutive glyceraldehyde-phosphate dehydrogenase ($NADP^+$) (phosphorylating) activity. Phosphoglycerate kinase, enolase, and pyruvate kinase were also constitutive (Table 4). NAD^+-linked glyceraldehyde-phosphate dehydrogenase and pyruvate kinase are presumed to be uniquely catabolic; and, if either were defective, impaired growth on glycerol could result. However, none of the mutants defective for growth on glycerol lacked NAD^+-linked glyceraldehyde-phosphate dehydrogenase, pyruvate kinase, or any other enzyme of the triosephosphate pathway (Table 4). Although not assayed here, the presence of phosphoglyceromutase activity was inferred from normal growth on glucogenic substrates.

TABLE 1. *Growth phenotypes of glucose-catabolic mutants*

Strain	Growth[a] on:					
	Glucose	Gluconate	2-Ketogluconate	Glycerol	Lactate	GNB
PAO	+	+	+	+	+	+
707	−	−	−	+	+	+
732	−	−	−	±	+	+
728	−	−	−	−	+	+
720	−	−	−	−	+	−

[a] Absorbance (A) was determined at 12 h for GNB and 24 h for minimal media: +, $A > 0.9$; ±, $A < 0.3$; −, $A < 0.1$.

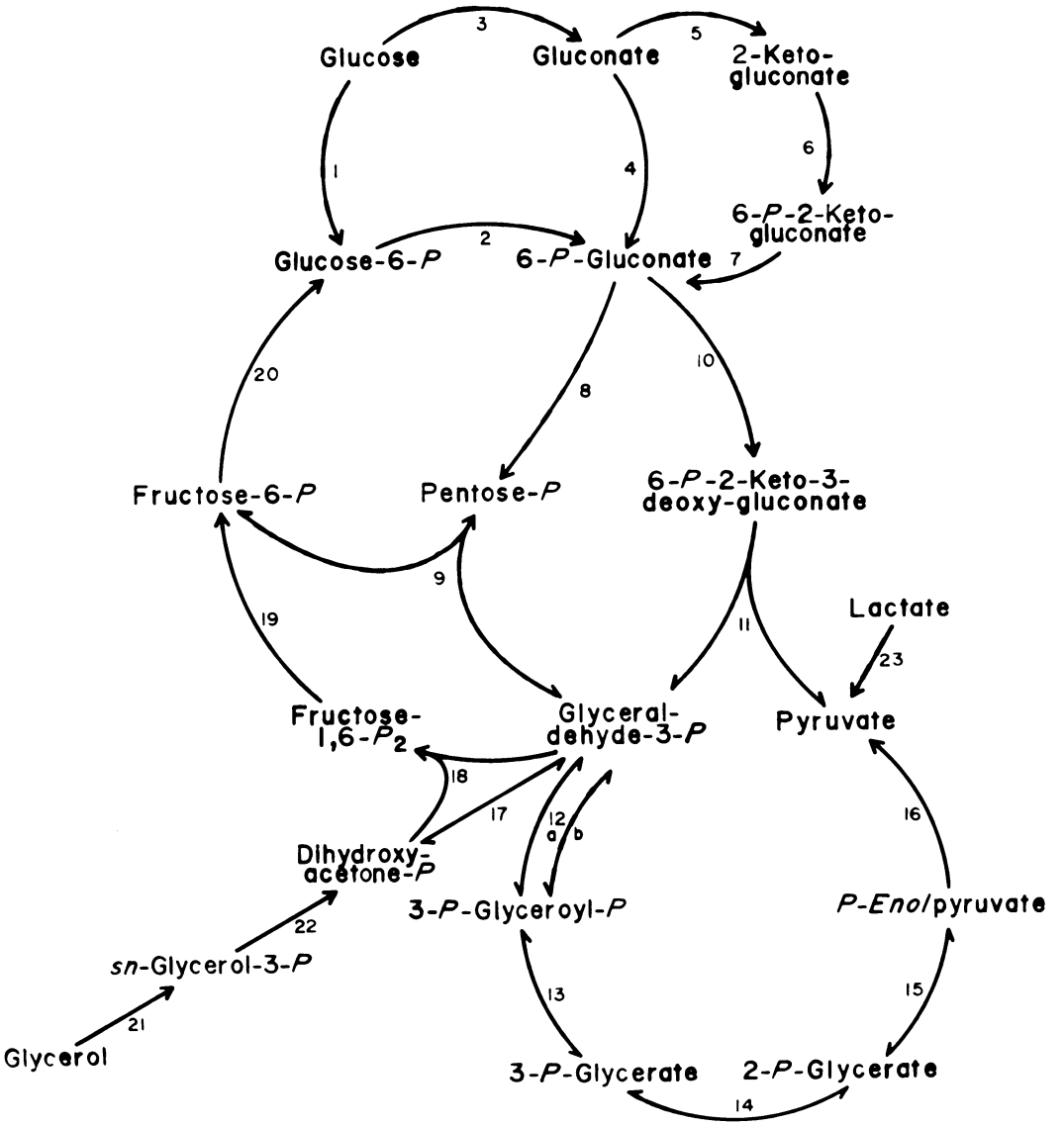

FIG. 1. *Metabolism of carbohydrate derivatives by P. aeruginosa. Numbers refer to the following enzymes: 1, glucokinase; 2, glucose-6-phosphate dehydrogenase; 3, glucose dehydrogenase; 4, gluconokinase; 5, gluconate 2-dehydrogenase; 6, ketogluconokinase; 7, 2-keto-6-phosphogluconate reductase; 8, phosphogluconate dehydrogenase; 9, reversible nonoxidative enzymes of the hexose monophosphate pathway; 10, phosphogluconate dehydratase; 11, phospho-2-keto-3-deoxygluconate aldolase; 12a, glyceraldehyde-phosphate dehydrogenase; 12b, glyceraldehyde-phosphate dehydrogenase (NADP+) (phosphorylating); 13, phosphoglycerate kinase; 14, phosphoglyceromutase; 15, enolase; 16, pyruvate kinase; 17, triosephosphate isomerase; 18, fructose-bisphosphate aldolase; 19, fructose-bisphosphatase; 20, glucosephosphate isomerase; 21, glycerol kinase; 22, glycerol-3-phosphate dehydrogenase; and 23, lactate dehydrogenase. The Entner-Doudoroff enzymes are 10 and 11. The triosephosphate pathway (lower portion of the EMP pathway) consists of enzymes 12a, 12b, 13, 14, 15, and 16. The anabolic portion of the EMP pathway is composed of enzymes 18, 19, and 20.*

Presence of the anabolic EMP pathway. The facts that growth of strain PAO on glycerol induced NAD^+-linked glyceraldehyde-phosphate dehydrogenase and all the glucose-catabolic enzymes examined except gluconokinase and that mutants with impaired growth on glycerol apparently had no additional defects suggested that growth on glycerol may actually depend on limited metabolism of hexosephosphate derivatives. Therefore, enzymes of the

TABLE 2. *Inducible glucose-catabolic enzymes and biochemical defects of glucose-negative mutants*

Strain	Growth substrate (inducer[a])	Sp act (mU/mg of protein)							
		Glucokinase	Glucose-6-phosphate dehydrogenase	Glucose dehydrogenase	Gluconokinase	Gluconate 2-dehydrogenase	Phosphogluconate dehydrogenase	Phosphogluconate dehydratase	PKDG aldolase
PAO	Lactate	14.8	5.8	6.0	0[b]	4.4	0	1.5	10.7
	Glucose	97.5	276	16.1	44.0	518	89.5	120	304
	Gluconate	67.7	199	42.6	37.4	127	31.3	61.3	≥61.3[c]
	Glycerol	82.2	194	126	0	12.7	18.2	80.1	458
707	Glycerol	91.2	363	53.3	74.7	32.1	7.6	0	630
732	Glycerol	18.9	145	178	4.0	2.6	0.7	0	≥42.3[d]
728	(Glucose)	34.4	50.0	23.0	68.5	10.3	0	0	≥13.3[d]
720	(Glucose)	90.9	2.9	28.1	0.5	31.0	0.6	53.2	0

[a] See text for induction procedure.

[b] Undetectable activity: <1.5 mU for gluconokinase, phosphogluconate dehydrogenase, and phosphogluconate dehydratase; <0.5 mU for PKDG aldolase.

[c] Minimum specific activity of PKDG aldolase because phosphogluconate dehydratase was rate limiting in the assay for the combined activity of these enzymes (see text).

[d] Minimum specific activity obtained by the semiquantitative test for PKDG aldolase (see text).

TABLE 3. *Formation of the glycerol-catabolic enzymes by strain PAO and mutants with impaired ability to grow on glycerol*

Strain	Growth substrate (inducer[a])	Sp act (mU/mg of protein)		
		Glycerol kinase	Glycerol-3-phosphate dehydrogenase	Triosephosphate isomerase
PAO	Lactate	0.3	0[b]	424
	Glucose	0.4	0	705
	Glycerol	6.3	3.8	556
732	Glycerol	5.2	2.7	NA[c]
728	(Glycerol)	2.9	12.2	916
720	(Glycerol)	4.9	2.1	NA

[a] See text for induction procedure.

[b] Undetectable activity (<1.2 mU).

[c] Not assayed.

Embden-Meyerhof-Parnas (EMP) pathway were examined in the wild type and glucose-negative mutants (Table 5). Lactate- or glucose-grown cells of strain PAO lacked detectable 6-phosphofructokinase activity. However, this activity was readily detectable in glucose-grown cells of *E. coli* (55.6 mU/mg of protein). Consequently, strain PAO lacks a catabolic EMP pathway. In contrast, each of the enzymes involved in conversion of triosephosphate to hexosephosphate was constitutive in strain PAO, and none of the mutants lacked any of these anabolic activities (Table 5).

Reversion of strain 728. Glucose-positive revertants occurred at a frequency of approximately 4×10^{-8} in cultures of strain 728. All glucose-positive revertants were also able to grow on glycerol. These revertants regained phosphogluconate dehydratase and phosphogluconate dehydrogenase activities as shown in Table 6 for one representative, strain A11. However, glycerol-positive revertants occurred at a frequency of approximately 3×10^{-6} in cultures of strain 728, and only about 1% of these were able to grow on glucose. Like the revertants selected on glucose, these glucose-positive revertants selected on glycerol regained both enzyme activities as shown in Table 6 for one representative, strain A6. In contrast, strain G1, a typical glycerol-positive revertant that was unable to grow on glucose, regained low phosphogluconate dehydrogenase activity but had less than 1% of the normal phosphogluconate dehydratase activity (Table 6). These revertants therefore resemble the glycerol-positive, glucose-negative mutants described initially. Similarly, asymmetric reversion to growth on glycerol or glucose has been observed for two other mutants lacking phosphogluconate dehydratase and phosphogluconate dehydrogenase.

DISCUSSION

It is clear that in *P. aeruginosa* strain PAO the inducible enzymes for catabolism of glycerol and of glucose constitute three regulatory groups based on nutritional inducers. Only growth on glycerol induced glycerol kinase and glycerol-3-phosphate dehydrogenase; growth on glycerol or glucose (or gluconate) induced glucose dehydrogenase, gluconate 2-dehydrogenase, glucokinase, glucose-6-phosphate dehydrogenase, phosphogluconate dehydrogenase, phosphogluconate dehydratase, PKDG aldolase, and NAD^+-linked glyceraldehyde-phosphate dehydrogenase; only growth on glucose (or gluconate) induced gluconokinase, ketogluconokinase, and 2-keto-6-phosphogluconate reductase (20). This study in-

TABLE 4. *Enzymes of the triosephosphate pathway in strain PAO and glucose-negative mutants*

Strain	Growth substrate (inducer[a])	Sp act (mU/mg of protein)				
		Glyceraldehyde-phosphate dehydrogenase	Glyceraldehyde-phosphate dehydrogenase ($NADP^+$) (phosphorylating)	Phosphoglycerate kinase	Enolase	Pyruvate kinase
PAO	Lactate	1.8	95.2	363	232	153
	Glucose	67.7	176	355	240	168
	Glycerol	74.9	98.4	276	114	101
732	(Glucose)	147[b]	114	399	147	63.9
728	(Glucose)	37.0	74.6	324	82.8	53.2
720	(Glucose)	109	98.7	460	172	113

[a] See text for induction procedure.
[b] Glycerol-grown cells.

TABLE 5. *Enzymes of the anabolic EMP pathway in strain PAO and glucose-negative mutants*

Strain	Growth substrate (inducer[a])	Sp act (mU/mg of protein)			
		Fructose-bisphosphate aldolase	Fructose-bisphosphatase	Glucosephosphate isomerase	6-Phosphofructokinase
PAO	Lactate	15.2	14.6	39.6	0[b]
	Glucose	16.0	22.9	88.4	0
	Glycerol	17.2	12.5	81.2	NA[c]
707	(Glucose)	19.3	19.6	45.6	NA
732	(Glucose)	13.1	12.9	72.8	NA
728	(Glucose)	9.5	9.3	61.8	NA
720	(Glucose)	13.8	18.9	83.2	NA

[a] See text for induction procedure.
[b] Undetectable activity (<0.8 mU). Glucose-grown cells of *E. coli* had 55.6 mU of 6-phosphofructokinase per mg of protein.
[c] Not assayed.

TABLE 6. *Reversion of strain 728*

Strain	Revertant phenotype[a]		Growth substrate	Sp act (mU/mg of protein)	
	Selected	Unselected		Phosphogluconate dehydrogenase	Phosphogluconate dehydratase
A11	Glu^+	Gly^+	Glucose	5.3	34.5
A6	Gly^+	Glu^+	Glucose	11.0	35.2
G1	Gly^+	Glu^-	Glycerol	1.5	0.7

[a] Phenotypic symbols: Glu and Gly, utilization of glucose and glycerol, respectively.

dicates that induction of these glucose-catabolic enzymes by growth on glycerol is due to a limited dependence of glycerol utilization on degradation of 6-phosphogluconate to glyceraldehyde 3-phosphate. Growth of phosphogluconate dehydratase-deficient mutants on glycerol is correlated with the level of phosphogluconate dehydrogenase observed (cf. strains 707, 732, and 728 in Tables 1 and 2). Moreover, reversion of glycerol-negative, glucose-catabolic mutants to growth only on glycerol is associated with recovery of a low phosphogluconate dehydrogenase level without an equivalent increase in phosphogluconate dehydratase activity.

The induction specificity of NAD^+-linked glyceraldehyde-phosphate dehydrogenase provides an explanation for this dependence. The constitutive glyceraldehyde-phosphate dehydrogenase ($NADP^+$) (phosphorylating) activity apparently is not sufficient for catabolism of glycerol or other compounds degraded at least partially via glyceraldehyde 3-phosphate because growth on such compounds induces NAD^+-linked glyceraldehyde-phosphate dehydrogenase. However, this enzyme appears to be induced by a six-carbon metabolite because it is induced

by glucose in mutant strains lacking a fission mechanism (i.e., catabolic EMP pathway, phosphogluconate dehydratase, and phosphogluconate dehydrogenase). Therefore, sufficient triose-phosphate derived from glycerol presumably is converted to hexosephosphate via the anabolic EMP pathway for induction of NAD^+-linked glyceraldehyde-phosphate dehydrogenase. This also accounts for induction of glucose-catabolic enzymes by growth on glycerol. Glycerol degradation proceeds then via the usual triosephosphate pathway to pyruvate. Any hexosephosphate produced is dissimilated via the Entner-Doudoroff pathway and to a lesser extent via the hexose monophosphate pathway.

This interpretation is supported by the following observations. In contrast to the wild type, glycerol induces gluconokinase in phosphogluconate dehydratase-deficient mutants, which cannot dissimilate 6-phosphogluconate well or at all. As previously noted, growth of phosphogluconate dehydratase-deficient mutants on glycerol correlates with their ability to dissimilate 6-phosphogluconate. In the extreme case of mutants completely lacking both phosphogluconate dehydratase and phosphogluconate dehydrogenase, failure to grow on glycerol in spite of the presence of all the enzymes needed for degradation to pyruvate is presumably due to inhibition by undegraded hexosephosphate derivatives. This is substantiated by the occurrence of glycerol-positive revertants that remained glucose negative and had regained only 8% of the normal phosphogluconate dehydrogenase level but less than 1% of the normal phosphogluconate dehydratase level. Whereas a limited capacity for dissimilation of 6-phosphogluconate is sufficient to prevent inhibition, thus permitting growth on glycerol via the usual triosephosphate pathway, it is clearly insufficient to support growth of phosphogluconate dehydratase-deficient mutants and revertants on glucose, gluconate, or 2-ketogluconate. Finally, although limited phosphogluconate dehydrogenase is necessary for growth of phosphogluconate dehydratase-deficient mutants on glycerol, it is not a sufficient condition for growth of PKDG aldolase-deficient mutants on glycerol. *P. aeruginosa*, like *E. coli* (6) and *P. putida* (31), apparently is extremely sensitive to accumulation of PKDG, as shown by the severe inhibition of mutants lacking PKDG aldolase in GNB. These mutants would also be expected to accumulate PKDG from glycerol and therefore fail to grow in spite of the presence of low phosphogluconate dehydrogenase activity and all the necessary enzymes for degradation of glycerol via the triosephosphate pathway.

It is clear that a single mutation caused the loss of phosphogluconate dehydratase and phosphogluconate dehydrogenase activities in mutants such as strain 728 because spontaneous revertants simultaneously recovering both activities are readily isolated by selecting for growth on glucose. However, it is equally clear that phosphogluconate dehydrogenase activity usually is preferentially restored in revertants selected for growth on glycerol. These observations are consistent with strain 728 containing a polar or perhaps regulatory mutation, but the organization and regulation of the genes encoding the glucose-catabolic enzymes are presently unknown. Consequently, the precise nature of the mutation in such strains is not yet clear. Nevertheless, the predominance of this pleiotropic mutation among the glucose-negative isolates probably reflects efficient selection against even slightly leaky growth in the enrichment procedure (16) used to obtain most of the mutants.

Phosphogluconate dehydrogenase plays a critical role in the interpretation of the results reported here, and its existence in the aerobic pseudomonads has been questioned recently. It has been suggested that this activity in crude extracts results from coupling of phosphogluconate dehydratase, PKDG aldolase, and glyceraldehyde-phosphate dehydrogenase (2). The strongest evidence against the existence of phosphogluconate dehydrogenase is circumstantial (21; P. V. Phibbs, Jr., and C. McNamee, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, K184, p. 167). As discussed above, simultaneous loss and recovery of phosphogluconate dehydrogenase and phosphogluconate dehydratase by mutation need not preclude the existence of phosphogluconate dehydrogenase. Likewise, inhibition of both activities by fluoride (21) is inconclusive. Fluoride inhibits a variety of enzymes by diverse and complex mechanisms (8), so phosphogluconate dehydrogenase of *P. aeruginosa* could conceivably be inhibited, whereas the enzyme from other sources is not. Finally, the lack of evidence for oxidative decarboxylation of 6-phosphogluconate may be due to choice of buffer (the enzyme from various sources is inhibited by phosphate [17, 22, 23]) or physiological state of the cells employed. In *P. aeruginosa*, radiorespirometric analyses indicate that the hexose monophosphate pathway accounts for up to 30% of the glucose degraded (26), but this pathway is not involved in degradation of gluconate (34). As shown in Fig. 1, glucose can be degraded via glucose-6-phosphate or gluconate. The relative contribution of these routes is unknown (20) but undoubtedly depends on the physiological state

of the cells studied. Thus, under certain conditions, glucose may be degraded primarily via gluconate and oxidative decarboxylation of 6-phosphogluconate might not be observed. Such conditions are in fact known. For example, uninduced resting cells quantitatively convert glucose to gluconate (Heath, unpublished data). Similar results were obtained for *P. putida* (formerly *P. fluorescens*) (5). Furthermore, exposure of fluorescent pseudomonads to high concentrations of glucose often results in quantitative conversion to gluconate (12). Moreover, Eisenberg et al. (4) have presented evidence that although *P. fluorescens* can transport and phosphorylate glucose and oxidize the resulting glucose-6-phosphate to 6-phosphogluconate, this organism will not grow on glucose unless it is oxidized to gluconate which is required for induction of 6-phosphogluconate dehydratase (19). Vicente and Canovas (31, 32) similarly concluded that glucose catabolism by *P. putida* is primarily via 2-ketogluconate in spite of the presence of glucokinase (4) and glucose-6-phosphate dehydrogenase in this species also. These considerations may affect the ability to observe oxidative decarboxylation of 6-phosphogluconate.

Several lines of evidence from this study are incompatible with the coupling hypothesis. Phosphogluconate dehydrogenase activity is detectable in mutants completely lacking phosphogluconate dehydratase or PKDG aldolase activity. Phosphogluconate dehydrogenase and phosphogluconate dehydratase activities are neither lost nor restored to the same extent by mutation. NAD^+ gives only 1% of the activity observed with $NADP^+$ in spite of the presence of high NAD^+- and $NADP^+$-linked glyceraldehyde-phosphate dehydrogenase activities. Moreover, these latter activities were totally dependent upon addition of Na_2HAsO_4 which was not present in the phosphogluconate dehydrogenase assays. Finally, phosphogluconate dehydrogenase activity was inhibited 86% by substitution of potassium phosphate buffer for Tris-hydrochloride of pH 7.5. If $NADP^+$-linked glyceraldehydephosphate dehydrogenase were involved in the activity, stimulation would have been expected because this enzyme requires phosphate (or arsenate), which had been excluded previously. It therefore seems premature to conclude that phosphogluconate dehydrogenase does not exist in the fluorescent pseudomonads. If it were an artifact, then the data presented here clearly indicate it is not due to the proposed coupling.

Clarification of the role of phosphogluconate dehydrogenase in degradation of glucose and related compounds by *P. aeruginosa* will require further study. However, it now seems clear that growth on glycerol depends on limited metabolism of hexosephosphate derivatives due to the induction specificity of the catabolic NAD^+-linked glyceraldehyde-phosphate dehydrogenase. This then accounts for glycerol-induced formation of the glucose-catabolic enzymes.

ACKNOWLEDGMENTS

We thank K. K. Brown, C. M. Cowen, and S.-S. Tsay for assaying glycerol kinase, glycerol-3-phosphate dehydrogenase, and triosephosphate isomerase in extracts of certain strains.

This work was supported in part by grants to E.T.G. from the National Science Foundation (GB-4195) and the Oklahoma State University Research Foundation and to H.E.H. from The University of Alabama Research Grants Committee (789). H.E.H. was a Public Health Service Trainee (GM01102) during part of this study.

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