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6-Phosphogluconate Dehydratase Deficiency in Pleiotropic Carbohydrate-Negative Mutant Strains of *Pseudomonas aeruginosa*

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Mutants of *Pseudomonas aeruginosa*, strain PAO, have been isolated that are unable to grow on mannitol, glucose, gluconate, or 2-ketogluconate, but that exhibit wild-type growth on pyruvate, lactate, citrate, succinate, or acetate. Although some of these mutants were also unable to grow on glycerol, the mutations formed a single linkage group by quantitative transductional analysis with phage F116 on glucose minimal agar medium. Cell extracts of all mutant strains were either lacking or severely deficient in 6-phosphogluconate dehydratase activity. Glu$^+$ transductants of each mutant strain recovered 6-phosphogluconate dehydratase activity, and Glu$^+$ transductants derived from mutant strains that retained the wild-type ability for growth at the expense of glycerol also regained the ability to grow on all C_6 compounds. Although a role for the pentose phosphate pathway in the catabolism of C_6 substrates was not found, a functional Entner-Doudoroff pathway appears to be essential for the catabolism of mannitol, glucose, gluconate, and 2-ketogluconate.

The pathways of hexose catabolism in *Pseudomonas aeruginosa* are complex, and the regulatory mechanism(s) associated with hexose catabolic enzymes in this species are different from those found in the fermentative enteric bacteria and aerobic sporeforming bacteria. Various strains of this species have been found to lack phosphofructokinase (EC 2.7.1.11) (26) or fructosediphosphate aldolase (EC 4.1.2.13) (12) and therefore lack a functional catabolic Embden-Meyerhof pathway. Enzymes associated with the Entner-Doudoroff pathway are present at high specific activities in cell extracts prepared from *P. aeruginosa* when grown on glucose, gluconate, 2-ketogluconate, fructose, mannitol, glycerol, or glycerate, but not in extracts from cells grown on succinate or citrate, indicating that these enzymes are inducible in this species (10, 12, 17, 26). The induction of hexose catabolic enzymes has been shown to be repressed citrate (17), succinate (10), or their metabolic products. Because glucose, glycerol, and glycerate are each effective nutritional inducers of several hexose catabolic enzymes, it is probable that one or more common metabolic intermediate of hexose and triose catabolism may serve as the inducer molecule(s) for these enzymes (10).

Analogous to observed differences between *P. aeruginosa* and other bacteria, with respect to hexose catabolic pathways and their regulation, major differences have also been observed with respect to mechanism of hexose transport. The glucose transport system in *Escherichia coli* has been shown to be constitutive and is associated with the phosphoenolpyruvate:hexose phosphotransferase system (1, 11). In contrast, the glucose transport system has been found to be induced by D-glucose in *P. aeruginosa* and to be repressed by citrate, succinate, acetate, fumarate, or malate (4, 10, 15, 16). Furthermore, phosphoenolpyruvate:hexose phosphotransferase system activity has not been demonstrated in *P. aeruginosa* (19), or in other aerobic oxidative bacteria (22).

Because it is difficult to assess the role of alternative hexose catabolic pathways in wild-type strains of this species, we have utilized defective mutants as a means of ascertaining the role of an enzyme or group of enzymes in these complex metabolic pathways. In a previous report (20), we have described the isolation of pleiotropic carbohydrate-negative mutants of *P. aeruginosa*, strain PAO, after pulse nitrosoguanidine mutagenesis, presented genetic

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evidence for two unlinked transductional groups within these mutant derivatives (referred to as A and B), and demonstrated that the genetic basis for the observed pleiotropic phenotype of the group B strains is due to a common deficiency for the enzyme pyruvate carboxylase (EC 6.7.1.1). In this report, we demonstrate that the genetic basis for the group A phenotype resides in a common deficiency for the enzyme 6-phosphogluconate dehydratase (EC 4.2.1.12).

MATERIALS AND METHODS

Organisms and cultural conditions. The wild-type strain was *P. aeruginosa*, strain PAO (7). The methods for pulse nitrosoguanidine mutagenesis, isolation of pleiotropic carbohydrate-negative mutant strains, and transductional analyses of these strains with phage F116 have been described (20). The composition of growth media and cultural conditions for cells employed in phenotype determinations and for induction of enzyme activities were previously described (20).

Assays. The preparation of cell extracts and enzyme assays were as described previously (9, 10, 20). The specific activities of all enzymes were calculated as international enzyme units per milligram of extract protein. Membrane-associated glucose dehydrogenase (EC 2.7.1.2), nicotinamide adenine dinucleotide phosphate (NADP)-linked glucose-6-phosphate dehydrogenase (EC 1.1.1.44), and 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase (EC 4.1.2.14) activities were determined as previously described (10). The combined activities of 6-phosphogluconate dehydratase (EC 4.2.1.12) and KDPG aldolase were determined as described previously (9), by replacing KDPG with 6-phosphogluconate as the reaction substrate. Glucose-6-phosphate isomerase (EC 5.3.1.9) activity was determined as described previously (20). Gluconokinase (EC 2.7.1.12) activity was measured spectrophotometrically by coupling 6-phosphogluconate formation to excess commerical 6-phosphogluconate dehydrogenase (PGDH) as described previously (20).

Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.13) activity was determined spectrophotometrically as described by Tiwari and Campbell (26). Barium was removed from the substrate by treatment with Dowex 50× resin, and the solution was adjusted to pH 7.0 immediately before use. The activities of 3-phosphoglycerate kinase (EC 2.7.2.3), enolase (EC 4.2.1.11), and fructose-1,6-diphosphate aldolase (EC 4.1.2.13) were determined as described by McFadden and Schuster (14), Westhead (30), and Sibley and Lehninger (23), respectively. Pyruvate kinase (EC 2.7.1.40) activity was measured by the method of Valentine and Tanaka (27).

The initial velocities of pyridine nucleotide-linked enzyme reactions were determined at 22 C in a Gilford recording spectrophotometer at 340 nm. Nonspecific oxidation of NADH and NADPH in the soluble fraction of crude extracts was not detectable under any of the assay conditions described above. Protein estimations were performed as described by Lowry et al. (13), with crystalline bovine serum albumin as the standard.

Chemicals and commercial enzymes. All pyridine nucleotides and sodium adenosine 5'-triphosphate were of the highest chemical purity available from P-L Biochemicals, Inc., Milwaukee, Wis. All phosphorylated intermediates of carbohydrate catabolism employed in enzyme assays and all commerical enzymes employed in assays were of the highest purity available from either Calbiochem, Los Angeles, Calif., or Sigma Chemical Co., St. Louis, Mo. All inorganic salts and other organic chemicals were of reagent grade. KDPG was a generous gift of W. A. Wood, Michigan State University, East Lansing.

RESULTS

Wild-type phenotype. *P. aeruginosa* strain PAO grows in liquid minimal medium containing 20 mM glucose, gluconate, 2-ketogluconate, glycerol, or mannitol, with generation times that are different but characteristic for each of these substrates. Like other strains of *P. aeruginosa* (10, 12, 15, 27), strain PAO exhibited diauxic growth patterns when inocula from 18-h starter cultures of minimal medium containing 10 mM succinate were transferred to fresh minimal medium containing 2 mM succinate and 10 mM of one of the above carbohydrates. The lag period observed after apparent depletion of succinate was different but characteristic for each substrate. Growth of the wild-type strain on succinate exhibited a generation time of 40 to 45 min followed by a lag period of 30 min after the apparent depletion of succinate (Fig. 1). Growth then resumed at the expense of glucose with a generation time of approximately 90 min. Strain PAO failed to grow within 72 h at 37 C with vigorous aeration in minimal medium containing 20 mM ribose, mannose, galacturonate, or glucuronate. Growth in liquid minimal medium containing 20 mM fructose was extremely unpredictable in that lag periods >24 h were common, and cells that appeared to be in mid-log phase growth on this substrate exhibited similiarly long lag periods when transferred to fresh minimal fructose medium.

Group A phenotype. Pleiotropic carbohydrate-negative mutant derivatives of strain PAO, which are referred to as group A mutants, failed to grow on mannitol, glucose, gluconate, or 2-ketogluconate. These strains formed a single linkage group as determined by transductional analysis with phage F116 and, unlike the group B strains described previously (20), were able to grow on both pyruvate and lactate. The phenotypes of these strains are depicted in Table 1; strains PFB 9, 52, and 66 were able to

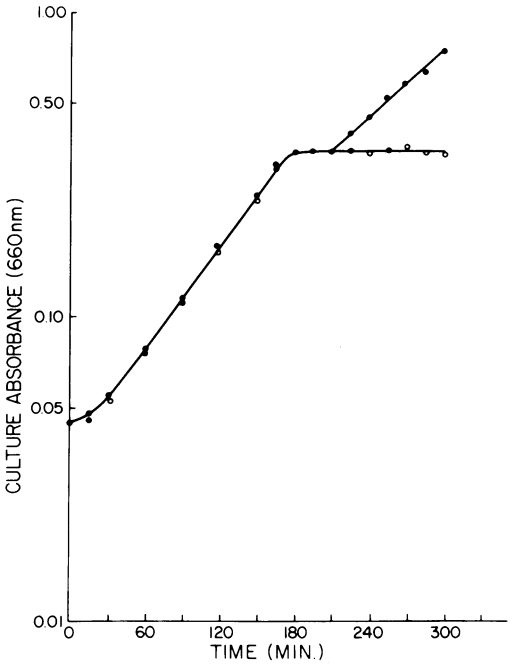

FIG. 1. *Diauxic growth responses of P. aeruginosa in succinate-glucose minimal medium. Starter cultures were grown 18 h at 37 C in a shaking water bath in minimal medium containing 10 mM succinate and were used to prepare 5% inocula into fresh minimal medium containing 2 mM succinate and 10 mM glucose prewarmed to 37 C. Symbols: ●, Wild-type strain (PAO); ○, group A mutant (strain PFM-9).*

grown on glycerol, whereas strains PFB 2, 11, 25, 39, and 57 were incapable of growth on this compound. The diauxic growth response of strain PFB 9 on succinate-glucose, shown in Fig. 1, is typical of all group A and B strains. These strains grew at wild-type rates on succinate; after the apparent depletion of succinate, however, they were unable to reinitiate growth at the expense of any hexose tested.

Specific activities of hexose catabolic enzymes. The specific activities of enzymes associated with hexose catabolism in this species were determined in cell extracts of the wild-type and each group A mutant strain. None of the mutant strains were found to be deficient for enzymes of the lower portion of the Embden-Meyerhof pathway (Table 2): glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, enolase or pyruvate kinase (columns 10–13), or for fructose diphosphate aldolase (column 9). All of the group A mutants exhibited greatly reduced or nondetectable activity for the combined activities of 6-phosphogluconate dehydratase and KDPG aldolase (column 5). However, wild type or slightly reduced specific activities for the enzyme KDPG aldolase (column 6) were detected when this enzyme was assayed separately. All of the mutant strains exhibited greatly reduced levels of 6-PGDH activity; the possible significance of this observation is discussed below. Strain PFB-39 lacked detectable glucose-6-phosphate isomerase activity. Strain PFB-57 lacked detectable activities for glucose dehydrogenase, glucokinase, and gluconokinase and exhibited noninduced levels of glucose-6-phosphate dehydrogenase activity. Specific activities of all enzymes assayed, except for gluconokinase, were essentially identical to those shown in Table 2 when glycerol was used in place of glucose as the nutritional inducer in the growth medium (unpublished data).

Phenotypes of group A spontaneous revertants and F116 transductants. Spontaneous revertants of strains PFB 9, 52, and 66 were observed at frequencies of 10^{-8} to 10^{-9} on minimal agar medium containing 20 mM of a single hexose substrate. All revertants of these strains regained the wild-type ability to grow on all hexose substrates independently of the substrate used for their selection. In contrast, spontaneous reversions of strains PFB 2, 11, 25, 39, and 57 rarely occurred on any substrate; when they did occur, they frequently failed to exhibit the wild-type abilities for growth on all substrates. For example, 27, 10, and 11 spontaneous revertants of strains PFB-2, 11, and 25, respectively, obtained on glycerol minimal agar medium failed to grow on any hexose substrate. Moreover, spontaneous revertants of strain PFB-39 obtained on glucose, gluconate, or 2-ketogluconate minimal agar media failed to grow on mannitol; four spontaneous revertants of strain PFB-57 obtained on glucose were found to be capable of growth on glycerol but not on any hexose substrate other than glucose.

The transductional analysis of these mutants described previously (20), with phage F116, and which led to delineation of two unlinked transduction groups, A and B, were carried out on glucose minimal agar medium. With the exception of strains PFB 39 and 57, all Glu^+ transductants of group A mutants regained the ability to grow on all hexose substrates (Table 1). Glu^+ transductants of strain PFB-39 were unable to grow on mannitol, whereas Glu^+ transductants of strain PFB-57 were unable to grow on any other hexose substrate.

Phage F116, grown on the wild-type donor, was tested for its ability to produce wild-type transductants of each group A mutant that could be selected on glycerol or each hexose

TABLE 1. *Growth of P. aeruginosa strain PAO, group A carbohydrate-negative mutant strains, and Glu⁺ transductants of group A strains in minimal medium containing single sources of carbon and energy*

Class	Strain designation	Lactate	Pyruvate	Glycerol	Mannitol	Glucose	Gluconate	2-Keto-gluconate
Parent	PAO	+	+	+	+	+	+	+
Group A carbohydrate-negative mutants[a]	PFB 9, 52, and 66	+	+	+	−	−	−	−
	PFB 2, 11, 25, 39, and 57	+	+	−	−	−	−	−
Glu⁺ transductants of group A mutants[b]	PFB 2, 9, 11, 25, 52, and 66	+	+	+	+	+	+	+
	PFB 39	+	+	+	−	+	+	+
	PFB 57	+	+	+	−	+	−	−

[a] Growth recorded in liquid medium containing 20 mM substrate after 24 and 48 h at 37 C. Final absorbance (660 nm) <0.05 as no growth (−); absorbance >0.50 as wild-type growth (+). These patterns of growth were confirmed when cells were plated on minimal agar medium containing the same individual carbon sources.

[b] 100 Glu⁺ transductants of each mutant strain obtained on glucose minimal agar plates were transferred to a series of master plates of the same medium and subsequently replica plated to a series of minimal agar plates each containing a single substrate at 20 mM; growth (+) or absence of growth (−) was recorded after 48 h at 37 C.

TABLE 2. *Specific activities of enzymes associated with carbohydrate catalism in P. aeruginosa PAO and group A glucose-negative mutants*[a]

Strain designation	Sp act (IU per mg of extract protein)[b]												
	1 GUDH	2 GUK	3 GOK	4 G6PDH	5 EDD-EDA	6 EDA	7 6PGDH	8 GPI	9 FDPA	10 G3PDH	11 PGK	12 EN	13 PK
PAO (30 mM pyruvate)	ND	0.023	<0.001	0.021	<0.001	ND	<0.001	0.058	0.043	0.011	0.127	0.450	0.080
PAO (20 mM pyruvate and 10 mM glucose)	0.141	0.084	0.066	0.252	0.114	0.152	0.049	0.047	ND	ND	ND	ND	ND
9	0.101	0.060	0.084	0.313	0	0.161	0.003	0.050	0.023	0.018	0.227	0.400	0.078
52	0.110	0.057	0.067	0.323	0.003	0.228	0.008	0.052	0.024	0.008	0.189	0.137	0.117
66	0.173	0.075	0.038	0.335	0.005	0.280	0.008	0.054	0.051	0.008	0.116	0.546	0.051
2	0.084	0.032	0.046	0.095	0	0.024	0.003	0.087	0.026	0.015	0.220	0.694	0.068
11	0.112	0.012	0.037	0.022	0	0.029	0.002	0.081	0.018	0.018	0.226	0.547	0.043
25	0.026	0.103	0.061	0.617	0.020	0.127	0.011	0.095	0.012	0.006	0.228	0.667	0.070
39	0.224	0.007	0.021	0.013	0	0.084	0.002	0	0.013	0.013	0.196	0.315	0.056
57	0	0	0	0.018	0	0.016	0	0.036	0.046	0.014	0.160	0.600	0.063

[a] Specific activities of enzymes in columns 1 through 8 were determined in crude extracts of cells grown on 20 mM pyruvate plus 10 mM glucose except as specifically indicated for strain PAO. Those in columns 9 to 13 were determined in crude extracts of cells grown on 40 mM succinate alone.

[b] GUDH, Glucose dehydrogenase; GUK, glucokinase; GOK, gluconokinase; G6PDH, glucose-6-phosphate dehydrogenase; EDA, KDGP aldolase; GPI, glucose-6-phosphate isomerase; FDPA, fructose-1,6-diphosphate aldolase; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; PGK, 3-phosphoglycerate kinase; EN, enolase; PK, pyruvate kinase; ND, not determined; 0, no detectable activity.

substrate individually. With the exception of strains PFB-2, 39, and 57, transductants were obtained in number comparable to those obtained on glucose when selection was made on gluconate, 2-ketogluconate, mannitol, or glycerol. No transductants were obtained with strain PFB-2 on glycerol, with strain PFB-39 on mannitol, or with strain PFB-57 on gluconate, 2-ketogluconate, or mannitol. Glycerol-positive transductants of strains PFB-11 and 25 failed to grow on any hexose substrate; however, hexose-positive transductants of these two strains were

capable of growth on glycerol. Transductants of strain PFB-39 obtained on glucose, gluconate, or 2-ketogluconate all failed to grow on mannitol, whereas glycerol transductants of this strain also failed to grow on any hexose substrate.

Recovery of combined Entner-Doudoroff enzyme activities in group A Glu$^+$ transductants. The specific activities of glucose-6-phosphate dehydrogenase and the combined activities of 6-phosphogluconate dehydratase and KDPG aldolase were measured in cell extracts of the wild-type strain, each group A mutant, and two Glu$^+$ transductants of each mutant grown in 20 mM lactate plus 10 mM glucose. It is clear that wild type induced specific activities of the combined enzymes unique to the Entner-Doudoroff pathway were recovered in Glu$^+$ transductant (Table 3); Glu$^+$ transductants of strain PFB-57 also regained wild type-induced specific activity for glucokinase, but lacked detectable activity for glucose dehydrogenase and gluconokinase (data not shown).

TABLE 3. *Specific activities of glucose-6-phosphate dehydrogenase and Entner-Doudoroff enzymes in cell extracts of Glu$^+$ transductants of group A mutant strains*[a]

Strain no. (mutant-transductant)	Sp act (IU per mg of extract protein)	
	G6PDH	Combined EDD-EDA enzymes
PAO	0.171	0.087
PFB-2	0.181	0.001
2T1	0.139	0.073
2T2	0.082	0.132
PFB-9	0.142	0
9T1	0.171	0.105
9T2	0.254	0.094
PFB-11	0.207	0.003
11T1	0.223	0.125
11T2	0.288	0.082
PFB-25	0.221	0.010
25T1	0.161	0.082
25T2	0.203	0.073
PFB-39	0.099	0.001
39T1	0.146	0.077
39T2	0.206	0.092
PFB-52	0.358	0.009
52T1	0.198	0.125
52T2	0.173	0.126
PFB-57	0.025	0
57T1[b]	0.173	0.138
57T2[b]	0.169	0.090

[a] Enzyme activities were determined in cell extracts of wild-type, mutant, and Glu$^+$ transductant strains grown on 20 mM lactate plus 10 mM glucose.

[b] Glu$^+$ transductants of strain PFB-57 also regained wild type-induced activity for glucokinase.

DISCUSSION

The known and suspected pathways of hexose and glycerol catabolism in *P. aeruginosa* are summarized in Fig. 2. Direct oxidation of glucose to gluconate and 2-ketogluconate has been demonstrated in this species (25) and the phosphorylation of 2-ketogluconate to 2-keto-6-phosphogluconate and the conversion of this compound to 6-phosphogluconate by the enzymes 2-ketogluconate kinase and 2-ketogluconate-6-phosphate reductase, respectively, has recently been established in strain PAO (21). Although glucose, gluconate, and 2-ketogluconate have been shown to serve as nutritional inducers of the two enzymes unique to 2-ketogluconate catabolism (21), glycerol and mannitol, as well as glucose, gluconate, and 2-ketogluconate, serve as nutritional inducers of several enzymes associated with hexose catabolism including: glucose dehydrogenase, glucokinase, glucose-6-phosphate dehydrogenase, and the two enzymes unique to the Entner-Doudoroff pathway, 6-phosphogluconate dehydratase and KDPG aldolase (10, 12, 17, 26). Although the enzyme sequence of the lower portion of the Embden-Meyerhof pathway has been reported to be constitutive in this species (26), no direct demonstration of the specific enzyme sequences required for glycerol or mannitol catabolism has been published. The ability of the mutant strains described in this report to grow at wild-type rates in minimal succinate, citrate, or acetate media indicates that there was no genetic or physiological impairment of biosynthetic or gluconeogenic abilities in these strains.

The data obtained in this study indicate that all of the group A mutants share a common deficiency for the combined EDD-EDA (combined activities of 6-phosphogluconate dehydratase and KDPG aldolase) activity and an apparent common deficiency for 6-PGDP activity. Since all of the mutants within this linkage group were found to form varied, but significant, levels of KDPG aldolase, the genetic basis for loss of the combined EDD-EDA activity appears to be due specifically to a mutation

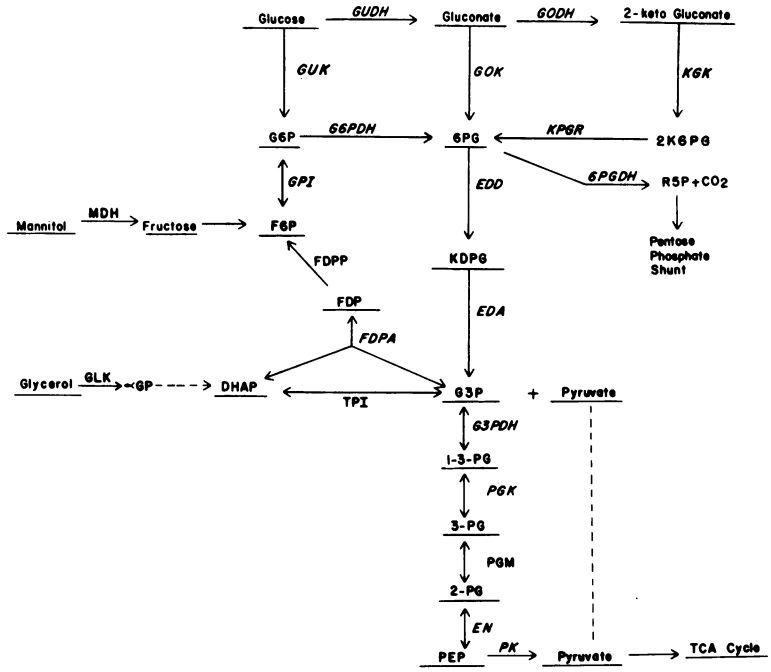

FIG. 2. *Carbohydrate catabolic pathways in P. aeruginosa. Abbreviations: footnote b, Table 2; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; 6PG, 6-phosphogluconate; 2K6PG, 2-keto-6-phosphogluconate; R5P, ribose-5-phosphate; FDP, fructose-1,6-diphosphate; αGP, α-glycerol phosphate; DHAP, dihydroxyacetone phosphate; G3P, glyceraldehyde-3-phosphate; 1,3-PG, 1,3-diphosphoglycerate; 3-PG, 3-phosphoglycerate, 2-PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate; TCA, tricarboxylic acid. Enzymes: MDH, mannitol dehydrogenase; FDPP, fluctose diphosphate phosphatase; FDPA, fructose diphosphate aldolase; GLK, glycerokinase; TPI, triosephosphate isomerase; PGM, phosphoglycerate mutase; KGK, 2-ketogluconate kinase; KGPR, 2-keto-6-phosphogluconate reductase.*

leading to a deficiency in 6-phosphogluconate dehydratase activity. Radiorespirometric studies (24, 29) indicate that up to 30% of glucose carbon is catabolized via the pentose cycle in *P. aeruginosa*; however, no direct proof of the participation of the enzyme 6-PGDH is available. The specificity of the 6-PGDH assay in crude extracts is questionable since other enzymes are present which could interfere with the pyridine nucleotide-linked reaction. In addition to pyruvate, the combined EDD-EDA enzyme activities also generate glyceraldehyde-3-phosphate which serves as the substrate for endogenous glyceraldehyde-3-phosphate dehydrogenase. In the assay used, the 6-PGDH reaction was initiated by the addition of NADP because activity in crude extracts of the wild-type strain displayed linear initial reaction velocities only after preincubation (2 min) with 6-phosphogluconate (10). Significantly, apparent induced 6-PGDH activities were detected only in the wild-type strain and in group B mutant strains which formed induced levels of the combined EDD-EDA activities (20). Moreover, several attempts to increase the specific activity of this enzyme through partial purification from extracts of wild-type cells have proved unsuccessful (Hylemon and Phibbs, unpublished data). These observations are consistent with the proposition that the apparent 6-PDGH activity observed in crude extracts of the wild-type strain and group B mutants may be due to glyceraldehyde-3-phosphate dehydrogenase activity present in these extracts (20). The apparent severe deficiency of 6-PGDH activity observed in cell extracts of the group A mutant strains could therefore be due to an inability to generate glyceraldehyde-3-phosphate because of their common deficiency in 6-phosphogluconate dehydratase activity.

Characterization of the three mutant strains PFB 9, 52, and 66 demonstrates that a functional Entner-Doudoroff pathway is essential to hexose catabolism in *P. aeruginosa*, and lends support to the proposition that each of these strains contains a single genetic lesion specific for the observed 6-phosphogluconate dehydratase deficiency. Although cell extracts of each of

these strains also exhibited a severe reduction in apparent 6-PGDH activity, spontaneous revertants of each strain, all of which regained the ability to grow on all hexose substrates, occurred at frequencies higher than would be expected if these strains also carried a second mutation specifying a defect in 6-PGDH activity. Glu$^+$ transductants of these strains were shown to regain simultaneously wild type-induced activities for the two enzymes unique to the Entner-Doudoroff pathway and to regain the ability to grow on all hexose substrates. Characterization of these strains also demonstrates that a functional Entner-Doudoroff pathway may not be essential for glycerol catabolism in this organism, even though glycerol serves as a nutritional inducer of this enzyme sequence. It is of interest that in *Pseudomonas putida*, mutants defective in phosphoglycerate kinase activity retain wild-type ability for growth at the expense of glycerol (2), indicating that the enzyme sequence unique to the lower portion of the Embden-Meyerhof pathway also may not be essential for glycerol catabolism in the pseudomonads.

Characterization of group A mutant strains PFB 2, 11, 25, 39, and 57 clearly indicates that each of these strains contains more than one genetic defect associated with hexose and glycerol catabolism. However, with the exception of strain PFB-57, all Glu$^+$ transductants of these mutants simultaneously regained wild type-induced specific activities for the combined Entner-Doudoroff enzymes and the ability to grow on glucose, gluconate, and 2-ketogluconate. Although Glu$^+$ transductants of strains PFB 11 and 25 also regained the ability to grow on glycerol and mannitol, the inability of glycerol transductants of these strains to grow on any hexose substrate indicates that these strains, as well as strains PFB 2 and 39, contain undefined defect(s) associated with glycerol catabolism. The inability of Glu$^+$ transductants of strain PFB 39 to grow on mannitol is ascribed to nondetectable levels of glucose-6-phosphate isomerase activity which has been shown to be an essential enzyme for mannitol catabolism (S. M. McCowen, P. V. Phibbs, Jr., W. T. Blevens, and T. W. Feary, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1974, P72, p. 156). This characterization of strain PFB 39 indicates that the genes coding for glucose-6-phosphate isomerase and 6-phosphogluconate dehydratase are not co-transducable, because no transductants of PFB 39 were obtained with phage grown on the wild-type donor by selection on mannitol medium, and Glu$^+$ transductants of this strain were unable to grow on mannitol.

Among the group A mutants, strain PFB 57 is of particular interest because it exhibits multiple enzyme defects relative to hexose catabolism. In addition, this strain cannot be induced for gluconate transport (6) or mannitol transport (unpublished data), although wild-type transport activities for glucose and glycerol have been retained (6; unpublished data). This strain resembles defective glucolytic mutants of *P. putida* obtained by Vicente and Canovas (28) after nitrosoguanidine mutagenesis. Nitrosoguanidine is well known for its ability to preferentially mutagenize the replication region of actively replicating bacterial chromosomes (3), and frequently induces closely linked, multiple mutations in synchronously growing bacteria (5). Accordingly, the altered phenotype of strain PFB 57 may reflect multiple, closely linked mutations. Alternatively, the pleiotropic phenotypes exhibited by such strains may prove to be associated with alterations in membrane structure (8). The observation that Glu$^+$ transductants of strain PFB 57 simultaneously regain glucokinase and 6-phosphogluconate dehydratase activities is consistent with co-transduction of closely linked loci. However, we have recently observed simultaneous reaquisition of these two enzyme activities in cell extracts of spontaneous Glu$^+$ revertants of this strain (unpublished data). Perhaps this indicates that the genetic defect specific to glucose catabolism in this mutant may be related to a defective regulatory function.

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