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Analysis of Cloned Structural and Regulatory Genes for Carbohydrate Utilization in *Pseudomonas aeruginosa* PAO†

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Five of the genes required for phosphorylative catabolism of glucose in *Pseudomonas aeruginosa* were ordered on two different chromosomal fragments. Analysis of a previously isolated 6.0-kb *Eco*RI fragment containing three structural genes showed that the genes were present on a 4.6-kb fragment in the order glucose-binding protein (*gltB*)–glucokinase (*glk*)–6-phosphogluconate dehydratase (*edd*). Two genes, glucose-6-phosphate dehydrogenase (*zwf*) and 2-keto-3-deoxy-6-phosphogluconate aldolase (*eda*), shown by transductional analysis to be linked to *gltB* and *edd*, were cloned on a separate 11-kb *Bam*HI chromosomal DNA fragment and then subcloned and ordered on a 7-kb fragment. The 6.0-kb *Eco*RI fragment had been shown to complement a regulatory mutation, *hexR*, which caused noninducibility of four glucose catabolic enzymes. In this study, *hexR* was mapped coincident with *edd*. A second regulatory function, *hexC*, was cloned within a 0.6-kb fragment contiguous to the *edd* gene but containing none of the structural genes. The phenotypic effect of the *hexC* locus, when present on a multicopy plasmid, was elevated expression of glucokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydratase, and 2-keto-3-deoxy-6-phosphogluconate aldolase activities in the absence of inducer.

Glucose catabolism in *Pseudomonas aeruginosa* proceeds by either an oxidative or a phosphorylative pathway (Fig. 1; reviewed in reference 24). In the direct phosphorylative pathway, glucose is transported into the cell by a periplasmic glucose-binding protein (GLTB)-dependent active transport system. Intracellular glucose is phosphorylated by glucokinase (GLK) and converted to 6-phosphogluconate (6PG) by glucose-6-phosphate dehydrogenase (ZWF). The 6PG from this pathway and from the oxidative pathway (Fig. 1) is further metabolized to glyceraldehyde-3-phosphate and pyruvate by the Entner-Doudoroff enzymes 6PG dehydratase (EDD) and 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase (EDA). GLK, ZWF, EDD, and EDA are strictly co-inducible, and 6PG is thought to serve as the physiological inducer (2, 8, 19). The glucose transport functions are separately regulated (20).

All of the genes known to be required for direct phosphorylative catabolism of glucose to pyruvate and glyceraldehyde-3-phosphate are clustered in the 39-min region of the *Pseudomonas aeruginosa* chromosome (7, 8, 36). A 6.0-kb DNA fragment containing the structural genes for GLK, EDD, and GLTB was cloned from this region recently (8). This fragment also appeared to contain regulatory sites which affected the expression of four carbohydrate catabolic proteins. This report more precisely maps the region with respect to order and location of the structural genes and regulatory loci. We also report here the cloning and ordering of structural genes for EDA and ZWF on a separate 11-kb *Bam*HI chromosomal DNA fragment.

(Major portions of these results are from a Ph.D. dissertation submitted by L. Temple to the graduate faculty of Virginia Commonwealth University, Richmond, 1988, and from an M.S. thesis submitted by R. C. Bass to the same graduate faculty in 1985.)

MATERIALS AND METHODS

Bacterial strains and plasmids. All bacterial strains used in this study were derived from prototrophic *P. aeruginosa* PAO (16) and have been described previously. The *edd* lesions in strains PFB57 (*edd-8 hexR1*) and PFB2 (*edd-4 hexR2*) have been described previously (2, 8). Both of these mutant strains also were noninducible for the other glucose catabolic enzymes ZWF, GLK, and EDA (2, 8). We have designated these regulatory mutations *hexR1* and *hexR2*. Other strains used in this study were PFB9 (*edd-1*) and PFB52 (*edd-2*) (2), PFB362 (*gltB1*) (7), PRP444 (*glk-1*) (8), PFB98 (*zwf-1*) (36), PFB103 (*zwf-2*) (37), and PAO1838 (*eda-9001 met-9020*) (29).

Plasmids in this study were constructed from pRO1614 (32), a *P. aeruginosa* cloning vector with ampicillin and tetracycline resistance determinants obtained from pBR322. The β-lactam antibiotic carbenicillin was used in these studies in place of ampicillin because of inherent resistance to ampicillin in *P. aeruginosa*.

Cell growth, extract preparation, and enzyme assays. Basal salts medium (BSM), growth conditions, concentrations of antibiotics, extract preparation, and enzyme and glucose transport assays, with the exceptions mentioned below, have been described before (7, 8, 20). Activity of KDPG aldolase (EC 4.2.1.14) was measured by coupling the reac-

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† We dedicate this communication to the memory of Stephen M. Cuskey, our beloved colleague, coauthor, and friend, who conducted the initial experiments in this project.

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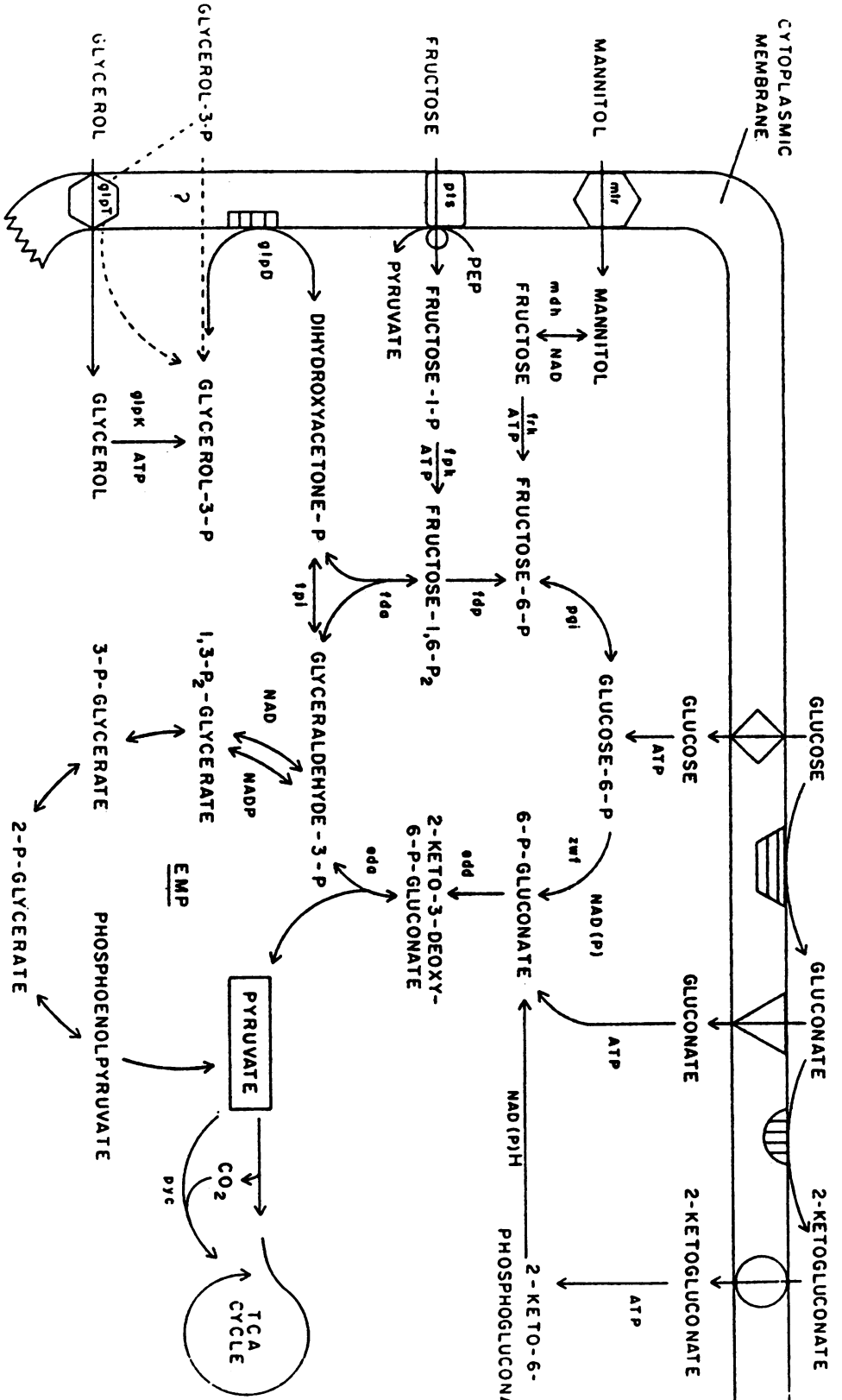

FIG. 1. Carbohydrate catabolism pathways in *P. aeruginosa*. *pgi*, Phosphoglucoisomerase; *zwf*, glucose-6-phosphate dehydrogenase; *edd*, 6-phosphogluconate dehydratase; *eda*, 2-keto-3-deoxy-6-phosphogluconate aldolase; *tpi*, triosephosphate isomerase; *fda*, fructose-1,6-diphosphate aldolase; *fdp*, fructose-1,6-diphosphatase; *fpk*, fructose-1-phosphate kinase; *pts*, phosphotransferase system; *mtr*, mannitol transport system; *mdh*, mannitol dehydrogenase; *frk*, fructokinase; *pyc*, pyruvate carboxylase; PEP, phosphoenolpyruvate; TCA, tricarboxylic acid; EMP, Embden-Meyerhof pathway. Reproduced from a previous communication (36).

tion (KDPG cleavage yielding pyruvate and glyceraldehyde-3-phosphate) with the reduction of pyruvate to lactate catalyzed by excess exogenous lactate dehydrogenase and monitored by oxidation of NADH, which is measured spectrophotometrically at 340 nm. The composition of a 1-ml reaction mixture was as follows: 7.6 IU of lactate dehydrogenase (type II; Sigma Chemical Co.), 0.3 mM NADH, 30 mM imidazole (pH 8.0), and appropriately diluted soluble cell extract. The reaction was initiated by the addition of 5 mM KDPG (kindly supplied by H. Paul Meloche of the Papanicolaou Cancer Research Institute, Miami, Fla.).

The activity of EDD was assayed in a combined reaction which depends on the presence of EDA in the cell extract. In strains used in this study, EDD can be assumed to be the rate-limiting enzyme in the combined reaction. The reaction mixture for the combined EDD-EDA activities was identical to that listed above, except that the reaction was initiated with the addition of 8 mM 6-PG. Protein was determined by the method of Lowry et al. (25), using bovine serum albumin as the standard, or spectrophotometrically by the method of Kalb and Bernlohr (23).

DNA preparation, restriction endonuclease cleavage, and transformations. Plasmid DNA for use in transformations was isolated as described previously (12, 15, 26). Plasmid DNA for restriction mapping and size determinations was isolated by the method of Holmes and Quigley (17) or the alkaline lysis method (26) and banded in CsCl. Chromosomal DNA from strain PAO was isolated by the procedure of Marmur (27).

DNA modifications were performed as recommended by the suppliers of the enzymes (Bethesda Research Laboratories, Rockville, Md.; New England BioLabs, Beverly, Mass.; or International Biotechnologies, Inc., New Haven, Conn.). Locations of restriction sites were determined by doing single and double digests with appropriate restriction endonucleases and comparing the respective electrophoretic banding patterns.

Recipient cells were transformed by using a previously described modification (32) of the procedure of Mercer and Loutit (30). Transformants were selected by acquisition of appropriate antibiotic resistance markers (growth in the presence of 500 μg of carbenicillin or 200 μg of tetracycline per ml).

Electrophoresis. Plasmids and their digestion products were analyzed by electrophoresis on 0.7% agarose gels in 40 mM Tris acetate (pH 8.0)–20 mM EDTA or on 5.0% polyacrylamide gels in 89 mM Tris borate (pH 8.3)–20 mM EDTA. DNA bands were visualized with a UV transilluminator after staining with ethidium bromide (0.5 μg/ml).

RESULTS

Construction of pRO1817. Plasmid pRO1816 contained a 6.0-kb fragment from the *P. aeruginosa* PAO chromosome inserted into the *Eco*RI site of vector plasmid pRO1769 (8). The chromosomal fragment carried the structural genes for EDD, GLK, and GLTB (8). To facilitate further analysis, the 6.0-kb fragment was subcloned into the *Eco*RI site of plasmid pRO1614 (32) and designated pRO1817 (Fig. 2). In the transfer process, approximately 2.4 kb of vector DNA which contained the tetracycline resistance determinant was spontaneously deleted. The 6.0-kb chromosomal fragment was recovered intact as determined by restriction endonuclease analysis and enzyme expression in transformed cells (data not shown). Reversed orientation of this fragment gave identical results, indicating that endogenous promoters were active. The remaining 3.7 kb of vector DNA in pRO1817 retained the carbenicillin resistance determinant of pRO1614.

Subcloning of the 6.0-kb fragment. Plasmids pRO1833, pRO1836, and pPZ198A were constructed by deletion of restriction fragments from pRO1817 (Fig. 2). Plasmid pRO1833 was cleaved with *Hin*dIII, treated with exonuclease *Bal*31, filled to blunt ends with Klenow fragment of DNA polymerase, and religated. Plasmids pRO1839 and pRO1840 were isolated by this method (Fig. 2). Plasmid pRO1836 was cleaved with *Eco*RI and *Bcl*I, and overhanging ends were filled with Klenow fragment of DNA polymerase and religated to form plasmid pPZ187 (not shown). Plasmid pPZ187 was cleaved with *Nco*I, releasing a 0.7-kb fragment including 200 bases form the vector, and religated, resulting in plasmid pPZ190, which contained 0.6 kb of PAO chromosomal DNA insert (Fig. 2). The 1.9-kb *Bcl*I fragment (1.6 to 3.5 kb in Fig. 2) from pRO1833 was ligated into the unique *Bcl*I site in plasmid pPZ190, resulting in plasmid pPZ197A.

Gene order on the 4.6-kb fragment in pRO1833. Strain PFB 362 (*gltB*) does not show glucose transport binding protein activity or glucose uptake activity (7). Plasmids pRO1833, pRO1839, and pPZ198A complemented the *gltB* mutation in PFB 362 (Fig. 2) and showed inducible glucose uptake activity (Table 1). These results demonstrated that the structural gene for the glucose-binding protein was located between *Eco*RI site at 0 kb and the *Bcl*I site at 1.6 kb.

Strain PRP 444 (*glk-1*) does not exhibit detectable levels of GLK activity (8). Plasmids pRO1833 and pRO1839 complemented this mutation (Table 2) and restored high enzyme specific activities (Fig. 2), whereas pPZ198A did not. These data indicated that the GLK structural gene was contained between the *Eco*RI site at 0 kb and the *Sal*I site at 2.15 kb, to the right of the gene encoding glucose-binding protein (Fig. 2).

Strains PFB52 (*edd-2*), PFB2 (*edd-4*), and PFB57 (*edd-8*) are deficient in EDD activity (2). This activity was restored by plasmids pRO1833 and pPZ197A, but not pRO1836 or pRO1839 (Table 3). These results located the EDD structural gene between the *Bcl*I site at 1.6 kb and the *Nco*I site at 4.1 kb (Fig. 2). Transformation of plasmid pRO1840 (but not pRO1839) into any of the *edd* mutant strains resulted in apparent recombinational repair of the lesion, as measured by colonies arising after 2 days, but displaying wild-type growth upon subculturing. These data showed that the mutations in these three strains were located between the *Bcl*I site at 1.6 kb and the *Sal*I site at 2.9 kb. The gene order on the 4.6-kb fragment was shown to be *gltB-glk-edd* (Fig. 2).

Cloning of genes for EDA and ZWF. Chromosomal DNA from *P. aeruginosa* PAO was partially digested with restriction enzyme *Sau*3A, and the resulting fragments were fractionated according to size by sucrose density gradient centrifugation. Fragments in the 7- to 15-kb size range were ligated into the unique *Bam*HI site in cloning vector pRO1614. The ligation mixture was transformed into mutant strain PFB98 (*zwf-1*), which is unable to utilize mannitol as a sole carbon source due to a specific defect in glucose-6-phosphate dehydrogenase activity. A single colony able to grow on BSM with mannitol was identified among 3,200 carbenicillin-resistant transformants. Plasmids pPZ300, containing a chromosomal insert of approximately 11 kb, was isolated from this colony (Fig. 3). This plasmid also allowed strain PAO1838 (*eda-9001*), which has a specific defect in KDPG aldolase, to utilize gluconate as sole carbon source. The presence of plasmid pPZ300 in mutant strains PFB98 and PAO1838 resulted in specific activities of ZWF and EDA

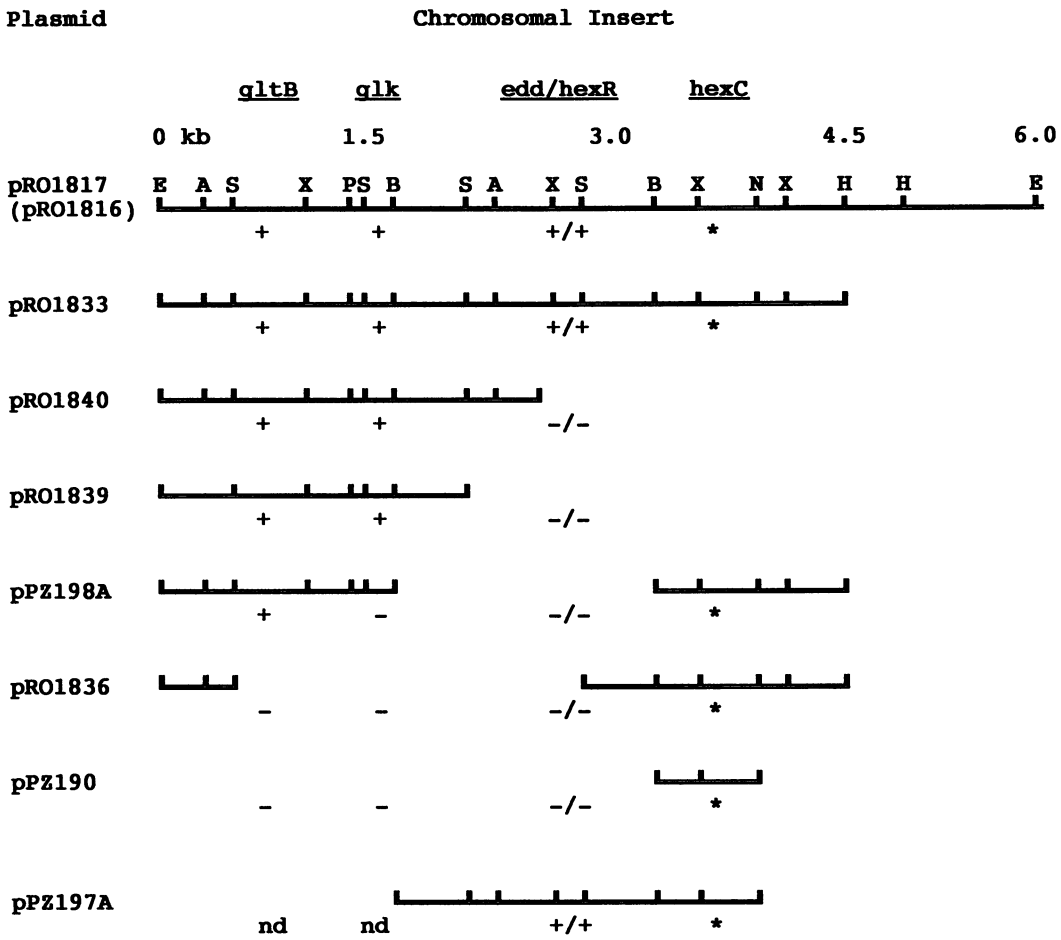

FIG. 2. Restriction enzyme cleavage and genetic map of the 6.0-kb *Eco*RI PAO chromosomal insert in plasmid pRO1817 and its subclones. Restriction enzymes are as follows: E, *Eco*RI; A, *Aval*; X, *Xhol*; P, *Pstl*; S, *Sall*; B, *Bcll*; N, *Ncol*; H, *Hin*dIII. Genetic notations: *gltB*, glucose transport binding protein; *glk*, glucokinase; *edd*, 6-phosphogluconate dehydratase; *hexR* and *hexC*, regulatory loci. + and − denote complementation patterns of the subclones. nd, Not determined. * denotes presence of *hexC* as shown by phenotype (see text).

which were elevated 10- to 20-fold over activities in wild-type strain PAO (Table 4).

Plasmids pPZ301 and pPZ305 were constructed by deletion of restriction enzyme fragments from pPZ300 (Fig. 3). Plasmid pPZ301 complemented the *zwf* mutation in PFB98, but not the *eda* mutation in PAO1838, while plasmid pPZ305 complemented neither mutation (Fig. 3). Plasmid pPZ300 was digested with *Eco*RI, yielding two fragments of approximately 7 and 11 kb. The 7-kb fragment included about 6.4 kb of insert DNA plus 375 bases of vector DNA. This fragment was ligated into the unique *Eco*RI site of vector pRO1614. The resulting plasmid, pPZ303, complemented both the *zwf* mutation in PFB98 and the *eda* mutation in PAO1838 (Fig. 3). These results located the structural gene encoding ZWF between the *Bam*HI site at 7.8 kb and the *Sau*3A site at 11 kb at the right end of the chromosomal insert and the EDA gene between the *Eco*RI site at 4.2 kb and the right end of the fragment, but to the left of *zwf* (Fig. 3).

TABLE 1. Effect of chimeric plasmids on glucose uptake activity in *P. aeruginosa* wild-type strain PAO and mutant strain PFB362

Strain	Relevant genotype	Glucose uptake (nmol of [U-^{14}C]glucose min^{-1} mg of protein^{-1})[a]	
		lct	lct + glc
PAO	Prototrophic	2	28
PFB362	(*gltB1*)	<1[b]	<1
PFB362(pRO1833)		<1	13
PFB362(pRO1839)		<1	9
PFB362(pPZ198A)		<1	12

[a] Glucose transport activity by washed whole cells (carbon-free BSM) (19). The results with plasmid pPZ198A are from one experiment. Cells were grown in BSM containing 20 mM lactate (lct) or lactate plus 10 mM glucose (lct + glc).

[b] No detectable activity.

TABLE 2. Effect of chimeric plasmids on expression of glucokinase in *P. aeruginosa* mutant strain PRP444[a]

Strain	Enzyme sp act (mIU/mg of protein)
PRP444	<1[b]
PRP444(pRO1833)	830
PRP444(pRO1839)	124
PRP444(pPZ198A)	<1

[a] Cells were grown to late logarithmic phase in BSM containing 20 mM lactate and 10 mM glucose. Relevant genotype for PRP444, *glk-1*.

[b] No detectable activity.

TABLE 3. Specific activity of 6PG dehydratase in *P. aeruginosa* *edd* mutant strains with and without plasmids[a]

Strain	Relevant genotype	Sp act (mIU/mg of protein)
PFB52	*edd-2*	<1[b]
PFB52(pRO1833)		596
PFB2	*edd-4 hexR2*	<1
PFB2(pPZ197A)		380
PFB2(pRO1836)		<1
PFB57	*edd-8 hexR1*	<1
PFB57(pRO1833)		131
PFB57(pRO1839)		<1
PFB57(pRO1836)		<1
PFB57(pPZ197A)		260

[a] Cells were grown to late logarithmic phase in BSM containing 20 mM lactate and 10 mM glucose.
[b] No detectable activity.

TABLE 4. Specific activities of EDA and ZWF in *P. aeruginosa* mutant strains PFB98 (*zwf-1*) and PAO1838 (*eda-9001*), with and without plasmid pPZ300[a]

Strain	Relevant genotype	Sp act (mIU/mg of protein)	
		ZWF	EDA
PAO	Prototrophic	11	17
PFB98	*zwf-1*	<1[b]	8
PFB98(pPZ300)		118	246
PAO1838	*eda-9001*	91	<1
PAO1838(pPZ300)		249	497

[a] Cells were grown to late logarithmic phase in BSM containing 40 mM lactate.
[b] No detectable activity.

Complementation of regulatory mutation(s) *hexR*. Mutant strains PFB57 (*edd-8 hexR1*) and PFB2 (*edd-4 hexR2*) contain lesions in the EDD structural gene (2, 8) (Table 3). These two strains are also noninducible for activities of ZWF, GLK, and EDA; the putative regulatory mutations have been designated *hexR*. Inducibility of ZWF, GLK, and EDD in strains PFB57 (Table 5) and PFB2 (data not shown) was restored by plasmid pRO1833 (which contained the EDD structural gene) but not by plasmids pRO1836 or pRO1839 (which did not contain the EDD structural gene). Strains PFB57 and PFB2, which had apparently undergone recombinational repair by plasmid pRO1840, were shown to have normally inducible enzyme activities (data not shown). These data established the location of the putative *hexR* locus between the *Bcl*I site at 1.6 kb and the *Sal*I site at 2.9 kb.

Mapping and evidence for regulatory function *hexC*. In a previous study, it had been noted that plasmid pRO1816 caused overexpression of four enzymes: ZWF, GLK, EDD, and EDA (8). We have designated this regulatory locus *hexC*, defined by the phenotype conferred when it is present on a multicopy plasmid. In this study, the presence of pRO1833, pRO1836, and pPZ190 in wild-type cells caused an elevation of basal levels of these enzymes (Table 6). Plasmid pRO1833 contained the structural genes for GLK and EDD, and overexpression of these two genes was predicted as a result of elevated copy number. However, the structural genes for ZWF and EDA were not contained in pRO1833, yet basal level activities of these enzymes were increased three- to fourfold over the activities in wild-type cells with no plasmids (Table 6). Plasmids pRO1836 and pPZ190 did not contain any of the structural genes; nonetheless, wild-type cells transformed with plasmids pRO1836 and pPZ190 showed 1.4- to 10-fold increases in activities of ZWF, EDA, EDD, and GLK, over basal activities in non-transformed wild-type cells (Table 6). In contrast, plasmids pRO1839, containing the structural gene for GLK, and pPZ300, containing the structural genes for EDA and ZWF, did not confer the *hexC* phenotype when present in wild-type strain PAO (Table 6). While elevated enzyme activities were apparent in wild-type strain PAO carrying plasmids pRO1833, pRO1836, and pPZ190, plasmids pRO1836 and pPZ190 did not restore inducibility of the same enzymes in *hexR* mutant strains PFB57 (Table 5) and PFB2 (data not shown).

These data confirmed the existence of a regulatory locus, *hexC*, which is physically and functionally separate from

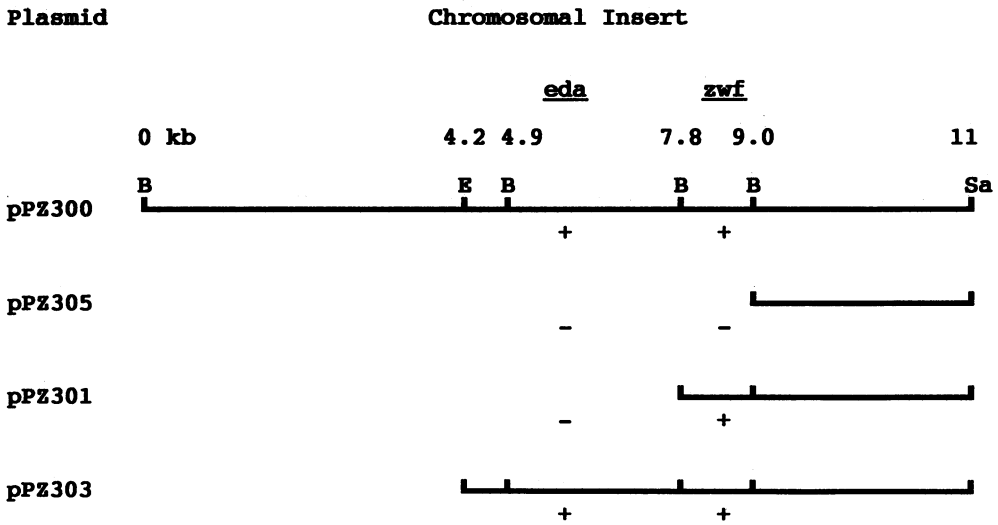

FIG. 3. Restriction enzyme cleavage map and complementation patterns of plasmid pPZ300 and its subclones. Restriction enzyme abbreviations: B, *Bam*HI; E, *Eco*RI; Sa, *Bam*HI/*Sau*3A hybrid no longer cut by *Bam*HI. Genetic notations: *eda*, 2-keto-3-deoxy-6-phosphogluconate aldolase; *zwf*, glucose-6-phosphate dehydrogenase. + and − denote complementation patterns of the subclones.

TABLE 5. Effect of chimeric plasmids on inducibility of ZWF, GLK, EDD, and EDA in *P. aeruginosa* mutant strain PFB57

Strain	Relevant genotype	Growth conditions[a]	Enzyme activities[b]			
			ZWF	GLK	EDD	EDA
PAO	Prototrophic	lct	0.13	0.23	0.05	0.15
		lct + glc	1.00	1.00	1.00	1.00
PFB57	*edd-8 hexR*	lct	0.09	0.10	0.07	0.17
		lct + glc	0.14	0.08	0.11	0.18
PFB57(pRO1833)	*hexC*$^+$ *hexR*$^+$	lct	0.45	3.32[c]	1.40[c]	0.67
		lct + glc	0.91	5.08[c]	3.82[c]	1.15
PFB57(pRO1836)	*hexC*$^+$ *hexR*$^-$	lct	0.21	0.14	ND[d]	0.39
		lct + glc	0.21	0.16	ND	0.41
PFB57(pRO1839)	*hexC*$^-$ *hexR*$^-$	lct	0.10	1.94[c]	0.04	0.16
		lct + glc	0.12	2.00[c]	ND	0.16

[a] Cells were grown to late logarithmic phase in BSM with 20 mM lactate (lct). To induce enzyme synthesis, 10 mM glucose (glc) was added to lactate-grown cultures in early log phase. Cultures were incubated for 1.5 to 2.0 generations after addition of glucose before harvesting.

[b] Results are expressed as a decimal fraction of specific activities determined in cells grown in BSM with lactate plus glucose.

[c] Structural genes for these proteins are present on the respective plasmids.

[d] ND, No detectable activity.

hexR. The presence of this locus on plasmid pPZ190 located *hexC* between the *Bcl*I site at 3.5 kb and the *Nco*I site at 4.1 kb on the 6.0-kb *Eco*RI fragment (Fig. 2).

DISCUSSION

The four enzymes ZWF, EDA, GLK, and EDD are strictly coinducible in the presence of glucose, gluconate, and mannitol, fructose, glycerol, or glycerol-3-phosphate (2, 33), and they are subject to catabolite repression by tricarboxylic acid cycle intermediates (31, 39). While these data indicate that these genes form a single regulatory unit, normally regulated expression of *glk*, *edd*, and *zwf* and *eda* from separate plasmids precludes the genes constituting a single transcriptional unit.

The parent plasmid, pRO1816, had been shown previously to have two distinct and separable regulatory functions, as follows: complementation of *hexR* mutations in PFB57 and PFB2, and increase in basal enzyme activity of ZWF, EDA, GLK, and EDD in the absence of inducer (8).

The regulatory defect(s) in PFB57 (*edd-8 hexR1*) and PFB2 (*edd-4 hexR2*) causes noninducibility of EDA, GLK, and ZWF. Present data indicate that this regulatory function is encoded by a locus (*hexR*) separate from but very tightly linked to the EDD structural gene. The regulatory mutation has never been isolated without a concomitant loss of EDD activity. Spontaneous glucose-positive revertants of PFB57 and PFB2 are extremely rare ($<10^{-11}$), suggesting the presence of more than one mutation. However, when these strains are transduced to glucose-positive phenotype, they show normal inducibility of ZWF and GLK (repair of *hexR*) (1). In this study, the regulatory mutations in PFB2 and PFB57 were complemented only by plasmids which also contained the structural gene for EDD (Table 5). These results indicated that perhaps the regulatory mutation, *hexR*, cannot be complemented in *trans* or that the regulatory effect (noninducibility) is a result of a structural gene mutation.

The *hexC* locus was functional on plasmids pRO1836 and pPZ190 which did not contain any known carbohydrate catabolism structural genes. Since both the chromosome and the plasmid were wild type for this gene, we believe that it was the presence of multiple copies of *hexC* which revealed the locus. The genes affected by the presence of *hexC* on a multicopy plasmid include *edd* and *glk*, which map within 1 to 3 kb of the *hexC* locus, and *eda* and *zwf*, which map at some greater distance from the locus. That independent clones containing *glk* (pRO1839) or *eda* and *zwf* (pPZ300) do not confer the *hexC* phenotype (Table 6) suggests that similar *hexC* loci are not present in the chromosomal inserts of those plasmids.

Preliminary nucleotide sequence data showed no open reading frames in the smallest *hexC*-containing fragment. Thus, we believe that the *hexC* locus probably is a binding site for a negative control protein, which represses expression of the four structural genes. The presence of such a site on a multicopy plasmid would titrate out a limited supply of the repressor, resulting in constitutive expression of these enzymes. A similar repressor titration causes constitutivity of the *gal* (21) *carAB* (34) operons in *Escherichia coli* and the *tynDEF* operon in *P. aeruginosa* (8). The exotoxin A gene (14, 40), the phospholipase C gene (13, 35), the tryptophan synthetic genes (5), the alginate biosynthetic genes (9–11), the elastase genes (1, 38), and the arginine biosynthetic genes (22) are examples of *P. aeruginosa* chromosomal genes whose control of expression has been studied at the molecular level. The present study indicates that regulation of the synthesis of proteins involved in carbohydrate catabolism in *P. aeruginosa* may be complex. Studies of the regulation of bacterial gene expression have shown that complex control schemes are not unusual and may occur in small regions of DNA (4, 18, 28).

Analysis of cloned fragments of DNA from the 39-min region of the *P. aeruginosa* chromosome has allowed us to order five structural genes and to define and approximately locate two determinants involved in the regulation of carbohydrate catabolism in this species. Additional subcloning and DNA sequence analysis of this region, currently being performed in this laboratory, will permit further analysis of gene regulation in the pseudomonads.

TABLE 6. Effect of chimeric plasmids on basal levels of expression of ZWF, EDA, GLK, and EDD in *P. aeruginosa* PAO[a]

Strain	Enzyme sp act[b]			
	ZWF	GLK	EDD	EDA
PAO	0.13	0.23	0.05	0.15
PAO(pRO1833)	0.34	2.30[c]	1.48[c]	0.58
PAO(pRO1836)	0.34	0.45	0.30	0.60
PAO(pPZ190)	0.55	0.33	0.54	0.61
PAO(pRO1839)	0.07	1.33[c]	0.09	0.11
PAO(pPZ300)	2.36[c]	0.10	0.11	1.98[c]

[a] Cells were grown to late logarithmic phase in BSM plus 2 mM lactate. Relevant genotype for PAO, prototrophic.

[b] Specific activity is expressed as a decimal fraction of specific activities determined in cells grown in BSM with lactate plus glucose.

[c] Structural genes for these proteins are present on the respective plasmids.

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