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# A Two-Component Response Regulator, *gltR*, Is Required for Glucose Transport Activity in *Pseudomonas aeruginosa* PAO1

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**A 729-bp open reading frame (*gltR*) was identified in *Pseudomonas aeruginosa* PAO1 that encodes a product homologous to the two-component response regulator family of proteins. Disruption of *gltR* caused loss of glucose transport activity. Restoration of *gltR* resulted in wild-type levels of glucose transport. These findings indicate that *gltR* is required for expression of the glucose transport system in *P. aeruginosa*.**

In *Pseudomonas aeruginosa*, dissimilation of glucose to the intermediate 6-phosphogluconate proceeds via at least three different pathways (9). During aerobic growth, glucose is preferentially oxidized in the periplasm to gluconate or 2-ketogluconate by the direct oxidative pathway prior to uptake and conversion to 6-phosphogluconate for dissimilation via the Entner-Doudoroff pathway (5, 7). When these bacteria are grown anaerobically or under a limited oxygen concentration, a separate glucose active transport system binds glucose directly and transports it into the cell (7) for conversion to 6-phosphogluconate and catabolism via the Entner-Doudoroff pathway.

Previously, mutants with a lesion in the putative glucose-binding protein (GBP) gene (*gltB*) were reported (3) that failed to bind and directly transport glucose. Glucose uptake in these mutants could be restored by treatment of the bacteria with purified GBP (18). Subsequently, the *gltB* locus was localized to a 1.1-kb fragment of DNA that restored glucose chemotaxis, binding, and transport activity to the *gltB* mutants (4, 19).

To further analyze the *gltB* locus, the 1.1-kb fragment was sequenced. An open reading frame was identified which did not encode GBP but instead exhibited identity with the OmpR family of two-component response regulators (14). Here we demonstrate that the gene, redesignated *gltR*, is required for glucose transport by *P. aeruginosa.*

The 1.1-kb fragment and an adjacent 0.5-kb segment of DNA were sequenced by the dideoxy chain termination method (16) by using the TaqTrack kit (Promega, Madison, Wis.) with the M13/pUC universal primers or synthetic oligonucleotides. Sequence analysis was performed with the University of Wisconsin Genetics Computer Group software package (6).

A 729-bp open reading frame beginning with a GTG start codon and encoding a peptide consisting of 242 amino acid residues was identified. The G+C content (69.2%), codon usage, and third-base GC bias were typical of *P. aeruginosa* (20). An EMBL-GenBank search showed that the predicted peptide exhibited 46.6% identity and 70.1% similarity to OmpR from *Escherichia coli* (Fig. 1) (22). Significantly, Asp-13, Asp-56, and Lys-108 are conserved in GltR. These residues have recognized functions in the phosphorylation of response regulator proteins and are highly conserved (12). GltR also exhibited 32 to 35% identity to other OmpR family members, such as VirG of *Agrobacterium tumefacians* (13), PhoB of *P. aeruginosa* (1), and VanR of *Enterococcus faecium* (2). Neither ATP binding nor DNA helix-turn-helix motifs were evident in GltR. It should be noted that GltR exhibited no homology with either the N-terminal sequence obtained for purified *P. aeruginosa* GBP (21) or GBP from *Pseudomonas putida* (8).

In the OmpR family of two-component systems, the gene encoding the sensor histidine kinase is located downstream of the response regulator (12). No such open reading frame was found encoded in the 404 bp of sequence downstream of *gltR*. Such a result was not surprising as the N termini of sensor histidine kinases exhibit poor sequence conservation.

To determine if *gltR* is important in glucose uptake, *gltR* was insertionally disrupted and the resulting mutants were examined for loss of glucose transport activity. Briefly, a 0.5-kb *Sph*I-*Sal*I internal fragment of *gltR* was cloned into the suicide vector pUC19*mob* (10) to produce pPZ468. pPZ468 was transferred into *P. aeruginosa* PFB311 by biparental mating with *E. coli* S17-1 (pPZ468). *E. coli* S17-1 provided transfer functions for pPZ468 (17). Strain PFB311 is blocked in the periplasmic direct oxidative pathway and can utilize glucose only through the uptake and glucokinase pathway (3). Transconjugants were selected by growth on basal salts medium (BSM) plates supplemented with 0.5% citrate and 500 μg of carbenicillin per ml. Since pPZ468 cannot replicate in *P. aeruginosa*, carbenicillin-resistant ($Cb^r$) isolates presumably contained a pPZ468 insertion in *gltR*. All selected transconjugants failed to grow on BSM plates supplemented with 20 mM glucose and 500 μg of carbenicillin per ml. In contrast, all of them grew when 20 mM fructose, glycerol, or mannitol was substituted for glucose, indicating that glucose utilization was specifically affected. Two transconjugants, designated PAO8021 and PAO8025, were randomly selected for further study. Glucose-positive revertants of each of these transconjugants were then obtained by growing each mutant on 20 mM glucose–BSM without carbenicillin. After 3 days, glucose-positive, $Cb^s$ revertants arose at an approximate frequency of 1 per $10^7$ bacteria plated. One revertant of each mutant strain was selected, and they were designated PAO8021R and PAO8025R.

Chromosomal DNA from strain PFB311, the insertional mutants, and the revertants was analyzed by Southern blotting with $^{32}P$-labelled pPZ468 as the probe to confirm that the plasmid had inserted into *gltR* (15). The 1.1-kb *gltR*-containing *Sal*I fragment in strain PFB311 was shifted in the mutants and resolved at a size consistent with insertion of pPZ468 into *gltR*.

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GltR   1 VSANGRSILLVDDDQEIRELLETYLSRAGFQVRSVSRGADFRQALCEEEA 50
OmpR   1 .MQENYKILVVDDDMRLRALLERYLTEQGFQVRSVANAEQMDRLLTRESF 49

GltR  51 SLAILDVMLPDEDGFSLCRWIRSHQRLACMPIIMLTASSDEADRVIGLEL 100
OmpR  50 HLMVLDLMLPGEDGLSICRRLRSQS..NPMPIIMVTAKGEEVDRIVGLEI 97

GltR 101 GADDYLGKPFSPRELLARIKALLRRAQFTQVRGG.....DVLAFEDWRLD 145
OmpR  98 GADDYIPKPFNPRELLARIRAVLRR.QANELPGAPSQEEAVIAFGKFKLN 146

GltR 146 TVSHRLFHEDGEEFFLSGADFALLKLFLDHPQQILDRDTIANATRGREVL 195
OmpR 147 LGTREMFRED.EPMPLTSGEFAVLKALVSHPREPLSRDKLMNLARGREYS 195

GltR 196 PLERIVDMAVSRLRQRLRDTGKAPRLIQTVRGSGYLLAAQVRPHLQP* 243
OmpR 196 AMERSIDVQISRLRRMVEEDPAHPRYIQTVWGLGYVFVPDGSKA*... 239
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FIG. 1. Comparison of the *P. aeruginosa gltR* product with *E. coli* OmpR. A comparison of the predicted peptide sequence of *gltR* with the *E. coli* OmpR sequence was performed by using the GAP program of the University of Wisconsin Genetics Computer Group (6). Highly conserved amino acid residues in the effector domain, involved in activation of DNA-binding functions, are highlighted in boldface. Vertical lines indicate identities between amino acid residues, while colons and periods indicate amino acid similarities.

In contrast, the revertant strains lost the larger band present in the glucose-negative, $Cb^r$ mutants, leaving the original *Sal*I fragment; this indicated that pPZ468 had been lost (data not shown).

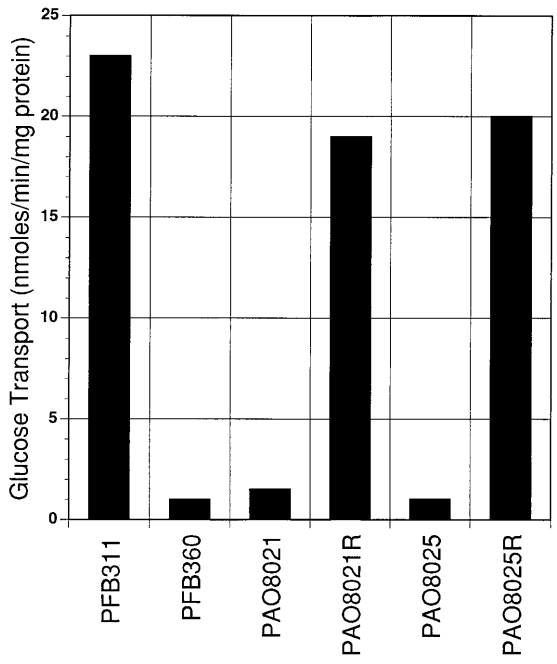


FIG. 2. Effect of pPZ468 insertion in *gltR* on D-[$^{14}$C]glucose transport by strains PAO8021 and PAO8025. Bacteria grown to mid-log phase in BSM containing 20 mM lactate and 20 mM glucose were pelleted, washed twice with carbon-free BSM to remove residual glucose, and preincubated in BSM alone for 15 min at 37°C. D-[$^{14}$C]glucose (0.2 mM) was added to 1.5 ml of each bacterial suspension, and samples were taken at 30- to 60-s intervals for up to 5 min. The samples were immediately filtered, the filters were washed with BSM to remove unassociated D-[$^{14}$C]glucose, and the radioactivity accumulated by the bacteria was determined by liquid scintillation spectrometry. The protein content of each cell suspension was measured by the procedure of Lowry et al. (11). Glucose transport specific activities are presented as nanomoles of D-[$^{14}$C]glucose accumulated per minute per milligram of protein.

To confirm that glucose transport activity was lost in the *gltR* mutants, glucose uptake assays were performed (5). Both PAO8021 and PAO8025 exhibited barely detectable D-[$^{14}$C] glucose transport activity (Fig. 2), comparable to that of the previously described glucose transport mutant PFB360 (3). The glucose-positive revertants, PAO8021R and PAO8025R, exhibited glucose transport activities similar to that of strain PFB311, demonstrating restoration of glucose transport activity.

These results demonstrate that *gltR*, whose product is homologous to the two-component response regulator family of proteins, is required for expression of the inducible glucose active transport system in *P. aeruginosa*. The full complement of genes and protein components of this system has not been described. Presumably, two of the genes regulated by GltR are *oprD*, which encodes a glucose-specific porin (23, 24), and the yet to be identified gene for GBP (*gltB*). The OmpR subfamily of these proteins control a variety of systems in different bacteria, including those involved in response to medium osmolarity, phosphate uptake, virulence, and antibiotic resistance (12). Further analysis will be directed toward the characterization of the *gltR* gene product and the identification of a putative sensor kinase gene.

**Nucleotide sequence accession number.** The nucleotide sequence data reported here appear in the EMBL and GenBank databases under accession number U50932.

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## REFERENCES


1. **Anba, J., M. Bidaud, M. L. Vasil, and A. Lazdunski.** 1990. Nucleotide sequence of the *Pseudomonas aeruginosa phoB* gene, the regulatory gene for the phosphate regulon. J. Bacteriol. **172:**4685–4689.
2. **Arthur, M., C. Molinas, and P. Courvalin.** 1992. The VanS-VanR two-component regulatory system controls synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. J. Bacteriol. **174:**2582–2591.
3. **Cuskey, S. M., and P. V. Phibbs, Jr.** 1985. Chromosomal mapping of mutations affecting glycerol and glucose catabolism in *Pseudomonas aeruginosa* PAO. J. Bacteriol. **162:**872–880.
4. **Cuskey, S. M., J. A. Wolff, P. V. Phibbs, Jr., and R. H. Olsen.** 1985. Cloning of genes specifying carbohydrate catabolism in *Pseudomonas aeruginosa* and *Pseudomonas putida*. J. Bacteriol. **162:**865–871.
5. **Eagon, R. G., and P. V. Phibbs, Jr.** 1971. Kinetics of transport of glucose, fructose and mannitol by *Pseudomonas aeruginosa*. Can. J. Biochem. **49:** 1031–1041.
6. **Genetics Computer Group.** 1991. Program manual for the GCG package, version 7. Genetics Computer Group, Madison, Wis.
7. **Hunt, J. C., and P. V. Phibbs, Jr.** 1983. Regulation of alternate peripheral pathways of glucose catabolism during aerobic and anaerobic growth of *Pseudomonas aeruginosa*. J. Bacteriol. **154:**793–802.
8. **Lateef, A., and E. Worobec.** 1995. Personal communication.
9. **Lessie, T. G., and P. V. Phibbs, Jr.** 1984. Alternative pathways of carbohydrate utilization in pseudomonads. Annu. Rev. Microbiol. **38:**359–387.
10. **Lory, S., M. S. Strom, and K. Johnson.** 1988. Expression and secretion of the cloned *Pseudomonas aeruginosa* exotoxin A by *Escherichia coli*. J. Bacteriol. **170:**714–719.
11. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
12. **Parkinson, J. S., and E. C. Kofoid.** 1992. Communication modules in bacterial signaling proteins. Annu. Rev. Genet. **26:**71–112.
13. **Powell, B. S., P. M. Rogowsky, and C. I. Kado.** 1989. *virG* of *Agrobacterium tumefaciens* plasmid pTiC58 encodes a DNA-binding protein. Mol. Microbiol. **3:**411–419.
14. **Sage, A., L. Temple, G. E. Christie, and P. V. Phibbs, Jr.** 1993. Presented at the Fourth International Symposium on *Pseudomonas* Biotechnology and Molecular Biology, Vancouver, British Columbia, Canada, 8 to 12 August 1993.
15. **Sambrook, J., E. F. Frisch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
16. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
17. **Simon, R., U. Pfiefer, and A. Pühler.** 1983. A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram

negative bacteria. Bio/Technology **5:**784–791.
18. **Sly, L. M., E. A. Worobec, R. E. Perkins, and P. V. Phibbs, Jr.** 1993. Reconstitution of glucose uptake and chemotaxis in *Pseudomonas aeruginosa* glucose transport defective mutants. Can. J. Microbiol. **39:**1079–1083.
19. **Temple, L., S. M. Cuskey, R. E. Perkins, R. C. Bass, N. M. Morales, G. E. Christie, R. H. Olsen, and P. V. Phibbs, Jr.** 1990. Analysis of cloned structural and regulatory genes for carbohydrate utilization in *Pseudomonas aeruginosa* PAO. J. Bacteriol. **172:**6396–6402.
20. **West, S. E., and B. Iglewski.** 1988. Codon usage in *Pseudomonas aeruginosa*. Nucleic Acids Res. **16:**9323–9335.
21. **Worobec, E. A.** Unpublished data.
22. **Wurtzel, E. T., M. Y. Chou, and M. Inouye.** 1982. Osmoregulation of gene expression. I. DNA sequence of the *ompR* gene of the *ompB* operon of *Escherichia coli* and characterization of its gene product. J. Biol. Chem. **257:**13692–13698.
23. **Wylie, J. L., and E. A. Worobec.** 1994. Cloning and nucleotide sequence of the *Pseudomonas aeruginosa* glucose-selective OprB porin gene and distribution of OprB within the family *Pseudomonadaceae*. Eur. J. Biochem. **220:**505–512.
24. **Wylie, J. L., and E. A. Worobec.** 1995. The OprB porin plays a central role in carbohydrate uptake in *Pseudomonas aeruginosa*. J. Bacteriol. **177:**3021–3026.