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Pseudomonas aeruginosa AlgB, a Two-Component Response Regulator of the NtrC Family, Is Required for *algD* Transcription

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Most strains of *Pseudomonas aeruginosa* isolated from the respiratory tracts of cystic fibrosis patients have a mucoid colony morphology due to the synthesis of an exopolysaccharide called alginate. The *algB* gene product (AlgB) is necessary for the high-level production of alginate in mucoid *P. aeruginosa*. In this study, AlgB was shown to be involved in the transcription of *algD*, a gene previously demonstrated to be activated in mucoid *P. aeruginosa*. In vitro and in vivo expression studies reveal that *algB* encodes a protein with a molecular size of 49 kDa. The DNA sequence of a 2.2-kb *P. aeruginosa* fragment containing *algB* was also determined. The amino-terminal domain of AlgB was found to be conserved with the amino-terminal domains of the response regulator class of two-component regulatory proteins. The central domain of AlgB has sequences highly conserved with those in the NtrC subfamily of transcriptional activators (NtrC, NifA, HydG, DctD, FlbD, TyrR, and PgtA). The central domain of AlgB also contains a potential nucleotide binding site. AlgB is the first NtrC homolog described from *P. aeruginosa*. At the carboxy terminus of AlgB, a helix-turn-helix motif was observed, suggesting that AlgB is a DNA-binding protein. The strongly conserved NtrC-like central domain of AlgB is not present in AlgR, another alginate response regulator. This study therefore identifies and characterizes the second of at least two unique response regulators used by *P. aeruginosa* to control alginate gene expression.

Among the diverse range of infections caused by *Pseudomonas aeruginosa*, pulmonary infection is currently the major cause of morbidity and mortality by this organism (29). Cystic fibrosis (CF) patients are particularly prone to pulmonary infections with *P. aeruginosa* (29). Initially, colonization of the CF respiratory tract is with a nonmucoid strain of *P. aeruginosa*. However, mucoid variants of the original strain emerge and prevail, leading to a chronic infection and a poor prognosis for the patient (26, 29). Most of the *P. aeruginosa* CF isolates are mucoid as a result of the production of a slimy exopolysaccharide known as alginate (20, 39). The proposed roles of alginate in *P. aeruginosa* pathogenesis include resistance to phagocytosis and an adherence mechanism (1, 41, 45, 49).

Most of the genes encoding alginate biosynthetic enzymes are clustered at 34 min on the *P. aeruginosa* chromosome (9). The first gene in this cluster (*algD*) is transcriptionally activated in mucoid cells (11), and many of the studies regarding the regulation of alginate biosynthesis have focused on *algD* expression (2, 11–13, 17, 32, 33, 35). A number of regulatory genes have also been cloned and partially characterized. Two such genes, *algS* and *algT*, which map near *hisI* (68 min), are involved in a genetic switch which turns on alginate production (Alg^+). Depending on whether the alginate genes are activated or silent, this is referred to as *algS*(On) or *algS*(Off), giving *P. aeruginosa* the Alg^+ or Alg^- phenotype, respectively (22, 23). Other genes, such as *algR*, *algP*, and *algQ* (alternatively designated *algR1*, *algR3*, and *algR2*, respectively), map at 8 min and are required for expression of *algD* (7, 10, 14, 32, 33, 35). AlgR has homology at its amino terminus to a class of environmentally responsive bacterial proteins (10, 16). Another regulatory gene, designated *algB*, maps at 13 min on the *P. aeruginosa* chromosome. *algB* was cloned from *P. aeruginosa* FRD by complementation of an *alg-50*(Ts) (later designated *algB50*) alginate mutation (27). *algB*::Tn*501* mutants have a nonmucoid phenotype, yet they produce low levels of an alginate which is chemically indistinguishable from wild-type alginate (28). Therefore, *algB* does not appear to be directly involved in the synthesis of alginate, but instead promotes a more efficient, high-level production of alginate.

Here we report the DNA sequence of *algB* and its expression in *Escherichia coli*. The predicted AlgB amino acid sequence reveals that AlgB, like AlgR, has an amino-terminal domain which is structurally conserved in a large family of proteins that respond to environmental stimuli. AlgB is clearly distinct from AlgR, however, in that AlgB has a conserved central and C-terminal domain which is shared by members of the NtrC subclass of response regulators. On the basis of the sequence homology and gene fusion studies, AlgB was shown to be required for the transcriptional activation of *algD*.

(A preliminary report of a portion of this work was reported elsewhere [55].)

MATERIALS AND METHODS

Bacterial strains and plasmids. All *P. aeruginosa* strains used were derived from the CF isolate FRD and have previously been described (27, 28). FRD1 and FRD130 are Alg^+, the latter being a stable mucoid strain. FRD2 is a spontaneous *algS*(Off) nonmucoid derivative of FRD1. FRD444 and FRD439 are *algB*::Tn*501-2* mutants of FRD1 and FRD130, respectively (28). *E. coli* strains used were TB1 [*ara* Δ(*lac-proAB*) *rspL* F80 *lacZ* ΔM15 *hsdR*] (Bethesda Research Laboratories) and XL1-Blue [*recA1 endA1 gyrA96*

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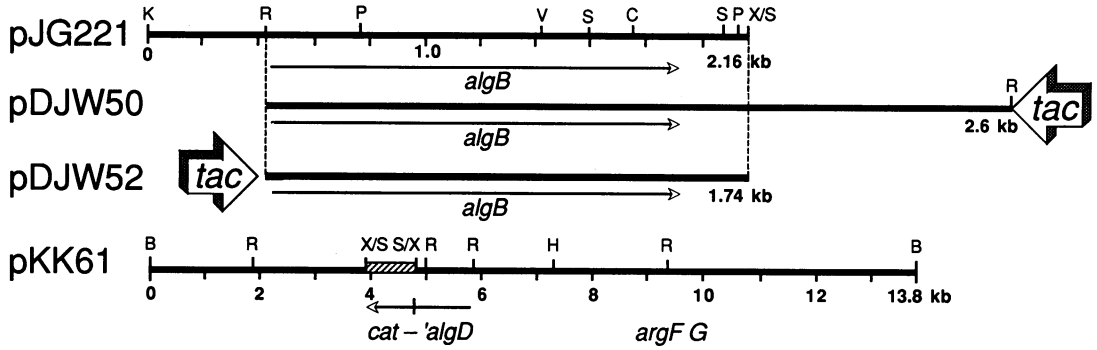

FIG. 1. Restriction maps of *P. aeruginosa* DNA in plasmids used throughout this study. The vector of pJG221 is pUC18. The vector of pDJW50 and pDJW52 is pKK223-3 with the *tac* promoter in the orientation indicated. The vector of pKK61 is pCP19 (22). The arrows under pJG221, pDJW50, and pDJW52 indicate the extent of the *algB* coding sequence. pKK61 was generated by cloning a promoterless *cat* gene (▨) into an *Xho*I site in the *algD* coding sequence. Restriction site abbreviations: K, *Kpn*I; R, *Eco*RI; P, *Pst*I; V, *Eco*RV; S, *Sal*I; C, *Cla*I; X, *Xho*I; H, *Hin*dIII; B, *Bam*HI.

thi hsdR17 supE44 relA1 lac(F' *proAB lacI*^{q} *lacZ* ΔM15 Tn*10*)] (Stratagene). For complementation analysis, *algB* subclones were generated in pLAFR1 (24) or pRK404 (18), and these plasmids were transferred to *P. aeruginosa* strains by using the conjugative plasmid pRK2013 (21). pJG221, which contains a 2.2-kb *Kpn*I-*Xho*I *algB*$^+$ insert in pUC18 (28; Fig. 1), was used for subcloning and DNA sequence analysis. For expression of *algB* in *E. coli*, two plasmids (pDJW50 and pDJW52) were constructed. These plasmids are similar except for the orientation of *algB* with respect to the *tac* promoter of pKK223-3 (Pharmacia); pDJW50 contains a 2.6-kb *Eco*RI-*Eco*RI fragment derived from pJG1 (27) cloned in pKK223-3, while pDJW52 has a 1.74-kb *Eco*RI-*Xho*I insert in pKK223-3 (Fig. 1). pCC27 is pCP19 with a 23-kb *P. aeruginosa* DNA insert containing the alginate biosynthetic gene cluster (6), including *algD*, which encodes GDP mannose dehydrogenase. One of the *Xho*I sites of pCC27 was previously shown to be contained within the *algD* coding sequence (12). Therefore, an *algD'-cat* (chloramphenicol acetyltransferase) fusion plasmid (pKK61) was constructed by replacing a 10-kb *Xho*I-*Xho*I fragment in pCC27 with a 780-bp promoterless *cat* cartridge, derived from pCM1 (Pharmacia). This cloning placed *cat* transcription under control of the *algD* promoter (Fig. 1).

DNA manipulations. Most routine genetic manipulations and plasmid extractions were performed as described by Maniatis et al. (40). Triparental matings were used to mobilize recombinant plasmids from *E. coli* to *P. aeruginosa* by the method outlined elsewhere (27). DNA sequences were determined from pJG221 by the chain termination technique (48). Reactions were carried out at 42°C by the recommended procedure with Sequenase (United States Biochemical), using 5'-[α-^{32}P]dCTP (>6,000 Ci/mmol, 10 mCi/ml; Amersham) and 7-deaza-dGTP. Universal and reverse M13 primers (United States Biochemical) were used for sequencing pUC18-derived *algB* subclones. Other oligonucleotides used for sequencing were synthesized on an Applied Biosystems 380B DNA synthesizer. DNA sequences were analyzed by using Genepro (version 4.0) or DNA Inspector (version 3.02) software. Homology searches were performed by using the ALIGN program with a Protein Identification Resource (PIR) protein data base.

Media and enzyme assays. *E. coli* and *P. aeruginosa* strains were cultured in L broth (10.0 g of tryptone, 5.0 g of yeast extract, and 5.0 g of sodium chloride per liter, pH 7.5). Antibiotics were used at the following concentrations (per milliliter): ampicillin, 100 μg; and tetracycline, 15 μg for *E. coli* or 100 μg for *P. aeruginosa*. The minimal medium used for selection of *P. aeruginosa* following triparental matings has been described elsewhere (53). Extracts for CAT assays were made from *P. aeruginosa* cells in logarithmic phase cultured in L broth to 5 × 10^8 cells per ml. Cells from a 50-ml culture were collected by centrifugation (10,000 × *g* for 20 min), washed twice in saline, suspended in 5.0 ml of 10 mM Tris hydrochloride (pH 8.0)–100 mM NaCl–1 mM $MgCl_2$–1μg of DNase per ml, and passed twice through a French press (12,000 lb/in^2). Unbroken cells were removed by centrifugation (5,000 × *g* for 10 min). The supernatant was then centrifuged at 100,000 × *g* for 1 h at 4°C. Dilutions of the supernatant from this centrifugation were analyzed for CAT, using a sandwich enzyme-linked immunosorbent assay (ELISA) technique as recommended by the manufacturer (5 Prime → 3 Prime, Inc). CAT levels in the extracts were extrapolated from a standard curve by using purified CAT. Protein concentrations were determined as described elsewhere (4).

Protein labeling and expression studies. Polypeptide products were visualized from *E. coli* XL1-Blue cells harboring recombinant plasmids derived from the expression vector pKK223-3. In vivo synthesis of the *algB* product was accomplished by culturing *E. coli* strains in L broth to an A_{580} of 0.6. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1.0 mM, and the cells were allowed to incubate with shaking for an additional 4 h at 37°C. A 0.1-ml portion was removed, centrifuged at 10,000 × *g* for 2 min, washed in saline, suspended in sodium dodecyl sulfate (SDS) sample buffer (60 mM Tris hydrochloride [pH 6.8], 2% SDS, 10% glycerol, 0.1 mg of bromphenol blue per ml, 5% 2-mercaptoethanol), and loaded onto a 10% SDS-polyacrylamide gel (38). The gel was stained with Coomassie brilliant blue R250 (Bio-Rad). For in vitro expression studies, genes coded on pDJW50, pDJW52, and pKK223-3 were expressed and polypeptides were labeled by using a commercially available (Amersham) bacterial cell-free coupled transcription-translation system in the presence of L-[^{35}S]methionine (>800 Ci/mmol; Amersham). The radioactive signal was enhanced by impregnating the gel with 2,5-diphenyloxazole (PPO) in dimethyl sulfoxide (3) prior to drying and fluorographic exposure. ^{14}C-labeled molecular mass markers were used as recommended by the manufacturer (Amersham).

Nucleotide sequence accession number. The nucleotide and

amino acid sequence data reported in this paper have been submitted to the EMBL, GenBank, and DDBJ nucleotide sequence data bases and assigned accession number M37765.

RESULTS

DNA sequence of *algB*. The *algB* gene had previously been localized to approximately 2.2 kb of DNA from strain FRD by Tn*501* mutagenesis, subcloning, and complementation analysis (28; Fig. 1). The 2.2-kb *Kpn*I-*Xho*I DNA fragment containing *algB* was sequenced on both strands (Fig. 2). Although several open reading frames (ORFs) were identified within this fragment, only the single ORF shown in Fig. 2 is likely to encode AlgB. This ORF initiates with an ATG at nucleotide 445 and terminates with a TGA at position 1792. The ORF encodes 449 amino acids and a polypeptide of 49,267 Da. Since the sequence contains more acidic residues than basic ones, AlgB should have a net negative charge at neutral pH. This ORF has a *Sal*I site (position 1597; Fig. 2), and a plasmid deleted of the 490-bp *Sal*I fragment (utilizing the *Sal*I site downstream of this ORF at position 2084; Fig. 2) was not able to complement *algB* mutations in *P. aeruginosa* (data not shown). Preceding the ATG codon, a sequence AGGA may represent a ribosome binding site (underlined in Fig. 2). The sequence and spacing of this putative ribosome binding site with respect to the ATG codon (12 bp) is very similar to that of *alg*R (10). Codon usage of this ORF is highly reflective of other G+C-rich genomes and *P. aeruginosa* genes (54). G+C-rich codons are preferred, with a strong bias for these residues at the third (wobble) position. This bias, however, is not apparent for glutamic acid codons, in which GAA is preferred over GAG (data not shown). Interestingly, this has also been reported for other *P. aeruginosa* genes (8, 12, 30).

Homology of AlgB to response regulators. To analyze the characteristics of AlgB, the predicted *algB* ORF was used to perform a homology search with other proteins in a PIR protein data base. The amino-terminal sequences of AlgB (residues 1 to 129; dashed box in Fig. 3) showed homology to sequences of other response regulators (e.g., CheY, NtrC, OmpR, AlgR, and VirG) of two-component regulatory systems. The response regulator component is usually involved in responding to a particular environmental stimulus by interacting with a sensor component and eliciting a response, generally through the transcriptional apparatus. These regulators and AlgB contain conserved regions of hydrophobic residues, a structural motif that is shared by most members of the response regulatory protein family (for a recent review, see reference 51). Although sequence alignment in the amino-terminal domain between two response regulators may show only 20 to 30% amino acid identity, residues corresponding to Asp-13, Asp-57, and Lys-109 in CheY are highly conserved among most members of this family. AlgB also contains these residues (triangles in Fig. 3). It has been postulated (51) that the sequence similarities defining the N-terminal domain of response regulators can be understood in terms of a common structure, conserved phosphotransfer enzymology, and the interaction of response regulators with a sensor histidine protein kinase component.

Members of the response regulator class of proteins can be further placed into subfamilies based on sequence similarities between their central and C-terminal domains. The sequences of several transcriptional activators of σ^{54} promoters (NtrC, NifA, FlbD, HydG, DctD, TyrR, and PgtA) show sequence homology within their central domains (51). This region has been referred to as domain D in NtrC and NifA (19). The central part of AlgB (residues 146 to 387) is highly similar to the central domains of NtrC (Fig. 3), NifA, and DctD, showing 44, 50, and 41% identity, respectively, in this domain. Within this conserved domain, a potential nucleotide binding site is present (heavy underline in Fig. 3). In NtrC, this ATP binding domain has been proposed to be involved in promoting open complex formation by RNA polymerase containing σ^{54} (36).

At the extreme carboxy terminus of AlgB (residues 426 to 445), there is a sequence which is similar to the helix-turn-helix motifs present in many DNA-binding proteins (Fig. 3). In the alignment of most helix-turn-helix motifs, positions 5, 9, and 15 are among the most highly conserved (44). Alanine is generally at position 5, glycine is at 9, and either valine, leucine, or isoleucine usually occupies 15. The glycine at position 9 forms a tight turn in these proteins and has been proposed to separate the two α helices (44). The predicted AlgB sequence between residues 426 and 445 has 100% identity with the consensus helix-turn-helix motif (Fig. 3). The only exception might be an alanine at position 4, rather than a bulky hydrophobic residue, normally occupying this position. However, an alanine at this position has also been reported (19) in the *Klebsiella pneumoniae* NifA and NtrC proposed DNA binding motif (Fig. 3).

In vivo and in vitro expression of *algB*. To identify the AlgB protein, two plasmids, pDJW50 and pDJW52 (Fig. 1), were constructed in the expression vector pKK223-3. This plasmid contains the strong *tac* (*trp-lac*) promoter and *E. coli* ribosome binding site located upstream of a multiple cloning site. The *tac* promoter is repressed in a *lacI*^{q} strain (XL1-Blue) but may be derepressed by the addition of IPTG. DNA sequence analysis of pJG221 demonstrated that the ORF encoding AlgB was located 21 bp downstream of a convenient *Eco*RI site (Fig. 2). Therefore, a 2.6-kb *Eco*RI-*Eco*RI fragment of pJG1 was subcloned into *Eco*RI-cleaved pKK223-3 (Fig. 1). Interestingly, plasmid DNA isolated from 30 independent clones all had the 2.6-kb *Eco*RI fragment oriented opposite the direction of *algB* transcription, with respect to the derepressed pKK223-3 *tac* promoter (Fig. 1, pDJW50). This finding suggests a preference for *algB* not to be cloned in the orientation of the ORF with regard to a heterologous promoter. To obtain a clone with the *tac* promoter and *algB* in the same orientation, a 1.8-kb *Eco*RI-*Xho*I fragment was cloned into pKK223-3 (utilizing the polylinker *Hin*dIII site next to *Xho*I in pJG221), resulting in pDJW52. This plasmid had the AlgB ORF cloned in the correct orientation with respect to the *tac* promoter (Fig. 1). XL1-Blue cells harboring pDJW50, pDJW52, and pKK223-3 were cultured and induced as described in Materials and Methods. A protein of approximately 49 kDa was detected in cells containing pDJW52 (Fig. 4A, lane 3); this protein was not present in induced XL1-Blue cells containing pKK223-3 or pDJW50 (Fig. 4A, lanes 1 and 2, respectively). Identical results were obtained when these plasmids were placed in an in vitro coupled transcription-translation system (Fig. 4B). A unique protein of approximately 49 kDa was selectively labeled with [^{35}S]methionine when pDJW52 was used as the template (Fig. 4B, lane 3) but not with pKK223-3 (Fig. 4B, lane 1) or pDJW50 (Fig. 4B, lane 2). In addition, a 49-kDa protein has been visualized from *algB* clones contained in T7 expression vectors (data not shown). The size of AlgB expressed from heterologous promoters (49 kDa) agrees very closely with the 49,267-Da value predicted from the nucleotide sequence (Fig. 2).

FIG. 2. DNA sequence of the 2.2-kb *Kpn*I-*Xho*I fragment containing *algB*. Numbers at the left represent nucleotides; those at the right represent amino acids. Pertinent restriction sites are shown. The underlined sequence represents a potential ribosome binding site (RBS). DNA was sequenced by the chain termination method (48), using Sequenase and 7-deaza-dGTP to avoid band compression (43). The asterisk indicates the AlgB termination codon.

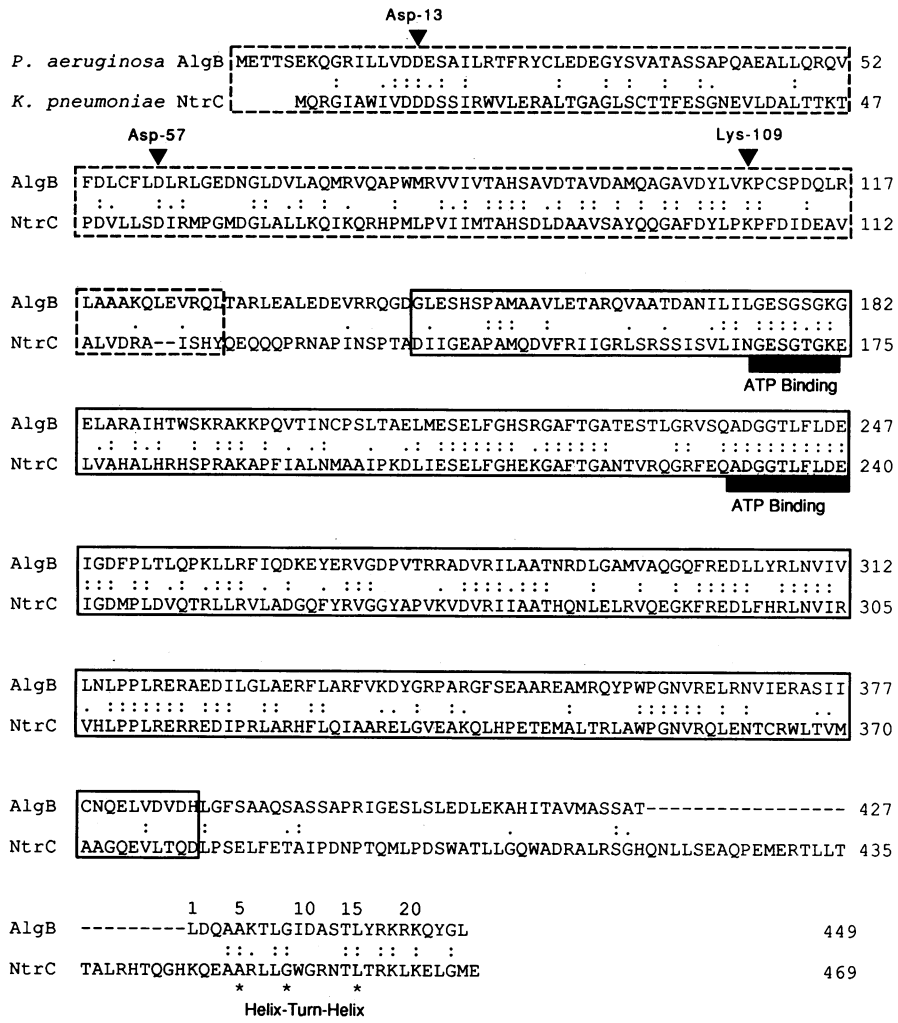

FIG. 3. Alignment of *P. aeruginosa* AlgB and *K. pneumoniae* NtrC. Numbers at the right correspond to the positions in the individual polypeptide sequences. For pairwise comparisons, two dots indicate identical residues and one dot indicates amino acids with similar properties (D and E; F, Y, and W; I, L, and V; K and R; N and Q; S and T; A and G). Dashes indicate gaps introduced to optimize alignment, particularly in the DNA binding domain. The dashed box represents the amino-terminal domain (residues 1 to 129 of AlgB and residues 1 to 122 of NtrC) which is conserved in several response regulators (51). The triangles depicted within this domain designate highly conserved residues and may be active sites within these domains (51). The numbering of these residues (i.e., Asp-13, Asp-57, and Lys-109) is based on the positions of these residues in CheY (50), not their positions in AlgB or NtrC. The central domains of AlgB and NtrC (residues 146 to 387 and 139 to 380, respectively) are boxed (solid line). A potential nucleotide binding site (25) conserved in these proteins, which may be involved in promoting open complex formation between σ^{54} holoenzyme and promoter sequences in NtrC (36), is shown by a heavy underline. The helix-turn-helix motif is indicated. The numbers above this region indicate positions of the residues within the helix-turn-helix motif and not their positions in the polypeptides. In the alignment shown, positions 5, 9, and 15 (asterisks) are the most highly conserved. Alanine (A) is predominant at position 5, glycine (G) is predominant at 9, and either isoleucine, leucine, or valine (I, L, or V) occupies position 15 (44).

Transcriptional regulation of *algD* by *algB*. Expression of *algD* is an absolute requirement for maintenance of the mucoid phenotype, and *algD* has been shown to be transcriptionally regulated in mucoid cells (11). Since AlgB has homology with other transcriptional activators (Fig. 3), and since AlgB is required for the high-level synthesis of alginate (28), we tested whether AlgB was involved in *algD* transcription. To accomplish this, an *algD'-cat* gene fusion was constructed in a low-copy-number (IncP) plasmid (Fig. 1). The *algD'-cat* gene fusion (pKK61) and a vector control were transferred to a variety of *P. aeruginosa* strains. CAT levels from dilutions of cell extracts were measured as described in Materials and Methods (Table 1). The Alg^+ *algS*(On) strains FRD1 and FRD130 containing pKK61 demonstrated high level synthesis of CAT. Synthesis of CAT from pKK61 in the nonmucoid strain FRD2 [*algS*(Off)] was absent, demonstrating that *algS* must be On in order to get transcription from the *algD* promoter. This has been noted elsewhere (11, 12). pKK61 was also transferred to two isogenic *P. aeruginosa* strains containing Tn*501-2* insertions in the *algB* gene (FRD444 and FRD439). Synthesis of CAT in these strains was reduced at least 20-fold (Table 1). FRD444 and FRD439 could be complemented to wild-type Alg^+ in *trans* with a 2.2-kb *Kpn*I-*Xho*I fragment (see Fig. 1) containing *algB*, demonstrating that the Alg^- defect of these strains is due to Tn*501-2* insertions in *algB* and not to spontaneous conversion to *algS*(Off). Taken together, these results indicate that AlgB, a member of the NtrC family of two-

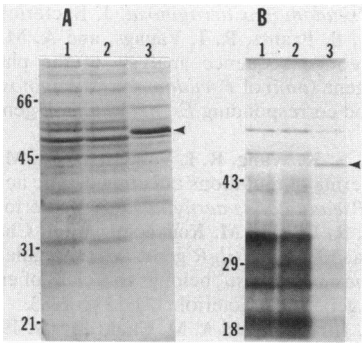

FIG. 4. In vitro and in vivo expression of *algB*. (A) SDS-polyacrylamide gel electrophoresis of proteins from IPTG-induced XL1-Blue cells harboring: pKK223-3 (lane 1), pDJW50 (lane 2), or pDJW52 (lane 3). See Fig. 1 and Materials and Methods for a description of these plasmids. Molecular size markers are shown along the left, indicated in kilodaltons. The arrowhead indicates the position of a unique protein of approximately 49 kDa expressed in induced XL1-Blue/pDJW52 cells. (B) In vitro expression of *algB* using a coupled transcription-translation assay. Lanes 1, 2, and 3 contain proteins synthesized and labeled with [^{35}S]methionine, using pKK223-3, pDJW50, and pDJW52, respectively, as templates. Positions of molecular size markers are shown on the left, indicated in kilodaltons. The arrowhead represents the position of a 49-kDa protein produced from pDJW52.

component response regulators, is involved in the transcriptional control of *algD*.

DISCUSSION

In this report, we have determined that the *algB* gene encodes a 49-kDa protein which has homology with the response regulator class of two-component regulatory proteins. This group of positive regulators is found in a wide variety of bacterial species, and they control complex bacterial regulons (42, 47, 51). The *ntrB/ntrC* nitrogen regulatory system in enteric bacteria may be one of the best-characterized two-component regulatory systems. Under appropriate conditions (nitrogen deprivation), NtrB, a histidine protein kinase, phosphorylates NtrC. Once phosphorylated, NtrC activates transcription of a number of genes involved in nitrogen metabolism. The *ntr* system also includes an alternative sigma factor (NtrA, also designated σ^{54} or RpoN), and phosphorylated NtrC can activate transcription of genes involved in nitrogen metabolism only in conjunction with the holoenzyme form of RNA polymerase containing NtrA. In addition to *ntrABC*, genes encoding accessory elements involved in protein modification are involved in activating nitrogen metabolic genes (37). In general, members of the NtrC subfamily of transcriptional activators have three primary domains (19): a conserved central domain which interacts with RNA polymerase containing σ^{54}, a carboxy-terminal domain which contains a DNA binding motif, and a homologous amino-terminal domain (with the exception of NifA, XylR, and TyrR) (51).

TABLE 1. CAT levels in cell extracts of *P. aeruginosa* strains[a]

Strain	Chromosomal genotype	ng of CAT/mg of protein[b]	
		pCP19 (vector)	pKK61 (*algD'-cat*)
FRD1	*algS*(On)	<20	2,824
FRD130	*algS*(On)	<20	4,319
FRD2	*algS*(Off)	<20	<20
FRD444	*algB*::Tn*501*	<20	122
FRD439	*algB*::Tn*501*	<20	112

[a] *P. aeruginosa* strains containing plasmids pCP19 (IncP vector) and pKK61 (pCP19 containing *algD'-cat*) were cultured to the same cell density in logarithmic phase. The cell extracts were prepared and assayed for CAT, using a sandwich ELISA technique.

[b] CAT levels in dilutions of the cell extracts were determined by extrapolation from a standard curve and normalized for protein content.

The regions of AlgB which share homology with response regulators and transcriptional activators (Fig. 3) are entirely consistent with the domain structure proposed for NtrC and NifA by Drummond et al. (19). According to the criteria stated above, the amino-terminal domain of AlgB (residues 1 to 129) is conserved with the amino-terminal domains of most response regulators. The amino-terminal domains of the response regulators contain highly conserved residues corresponding to Asp-13, Asp-57, and Lys-109 (in CheY), and of the proteins examined to date, these residues are located at the carboxyl end of β strands. Stock et al. (51) proposed that these residues of the response regulators form the active site. By analogy, these residues in AlgB (triangles in Fig. 3) would be involved in phosphorylation by an as yet unidentified sensor component, a hypothesis currently under investigation in this laboratory. This amino-terminal domain is separated from the central domain by a short hydrophilic segment (residues 130 to 145). This region of AlgB may be analogous to block C in NtrC and NifA, a segment proposed to be an interdomain linker joining the amino-terminal domain with the central domain of these proteins (19). The central domains of the NtrC subfamily of proteins are homologous with the central domain of AlgB (residues 146 to 387). Within this domain, regions strongly conserved among all members of the NtrC family are also highly conserved in the AlgB central domain. Following this conserved central core, a third domain containing a DNA binding motif is present at the carboxy terminis of these proteins (residues 426 to 445 in AlgB). The spacing between the central domain and the DNA binding domain varies between members of the NtrC family. AlgB is the only *P. aeruginosa* protein thus far identified which belongs to the NtrC family of transcriptional activators. Moreover, AlgB is the only member of the NtrC subfamily we are aware of that is directly involved in bacterial pathogenesis.

It has been proposed (51) that the conserved central domain in the NtrC subfamily is involved in the interaction with RNA polymerase containing σ^{54}. Of the alginate promoters mapped to date, two (from *algD* and *algR*) contain sequences which resemble those utilized by σ^{54}-activated promoters (14, 15). However, *algD* and *algR* promoters show only minor similarity to the consensus −26 CTGG YAYR-N4-TTGCA sequences recognized by σ^{54} (5), particularly in the location of the GG-GC dinucleotides with respect to the *algD* or *algR* transcriptional start sites. *P. aeruginosa* has a σ^{54} analog, and certain classes of genes (pilin, flagellin, and nitrogen assimilation) require σ^{54} for their transcription (31, 52). Yet an *rpoN* chromosomal mutation generated in a highly mucoid *P. aeruginosa* CF isolate (CF613) had no effect on the mucoid phenotype (52), suggesting that σ^{54} is not directly involved in the transcription of alginate-related genes. These results are in contrast with those of Kimbara and Chakrabarty (34), who recently demonstrated a decrease in *algD* and *algR* transcription in an RpoN-deficient mutant; however, these studies were done in a nonmucoid *P. aeruginosa* strain (PAK). Therefore, it is

unclear at this time whether AlgB may associate with RpoN to transcribe alginate genes or whether these genes are transcribed by RNA polymerase containing a sigma factor other than RpoN.

Although it remains to be shown, it seems reasonable to assume that sequences in AlgB resembling helix-turn helix motifs may enable AlgB to bind DNA and promote transcription of alginate-related genes. The residues in this presumptive DNA binding domain seem critical for AlgB function since a truncated AlgB lacking these residues (created by deletion of a *Sal*I fragment in pJG221) loses complementation ability. The involvement of AlgB in *algD* transcription (Table 1) makes *algD* sequences an attractive target for AlgB binding. The results shown in Table 1 should, however, be interpreted with caution. Although it is apparent that AlgB is involved in *algD* transcription, it is possible that this effect is mediated by the interaction of AlgB with other alginate regulatory genes or gene products in a cascade effect. DNA binding studies currently in progress should enable us to determine the specific binding site for AlgB.

The exact signals in the CF lung responsible for the conversion of *P. aeruginosa* from Alg^- to Alg^+ are unknown. Also, the signal to which AlgB responds has yet to be elucidated. However, some of the key components involved in regulating this process are beginning to be characterized. The conversion event probably involves (at a minimum) sensory transduction, covalent protein modification, transcriptional activation, and DNA rearrangements. Future management of pulmonary disease in CF patients, and other types of patients suffering from *P. aeruginosa* pneumonia, will benefit from a better understanding of these complex mechanisms controlling the synthesis of virulence factors by this opportunistic pathogen.

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