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Crystal structure of *Pseudomonas aeruginosa* transcriptional regulator PA2196 bound to its operator DNA

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ABSTRACT

Pseudomonas aeruginosa is a major opportunistic human pathogen. PA2196 from *P. aeruginosa* is a member of TetR family of transcriptional repressors, which is involved in adaptation to environmental changes as well as bacterial antibiotic resistance. PA2196 consists of nine α-helical bundles divided into two separate domains. The N-terminal domain, called the DNA-binding domain, is composed of helices α1–α3 and has a helix-turn-helix motif. The C-terminal domain, called the ligand-binding domain, has a hydrophobic pocket for ligand binding. Here, PA2196 was shown to bind to a 25 bp semi-palindromic dsDNA located in the upstream region of its own gene. The crystal structure of the PA2196–25mer dsDNA complex determined at a resolution of 2.9 Å revealed that two dimers of PA2196 bound to one dsDNA, with each monomer interacting with the major groove of DNA. Especially, residues in helix α3, including Lys41, Gly42, Ser43, and Tyr45, interacted mainly with the base and phosphate backbone of dsDNA. PA2196 underwent large conformational changes upon DNA binding, as the distances between DNA-binding domains measured between two G42s in subunits A and B decreased from 41.7 Å to 36.8 Å. Our crystal structure of PA2196–25mer dsDNA complex revealed that PA2196 is similar to QacR in that two dimers bound to one dsDNA through specific interactions.

1. Introduction

Bacteria respond rapidly to variations in the environment by using regulatory proteins capable of detecting the changes [1]. *Pseudomonas aeruginosa* is a major opportunistic human pathogen, and its resistance to antibiotics and disinfectants are responsible for many diseases [2]. *P. aeruginosa* possesses TetR family repressors, which respond to specific environmental changes and induce target gene expression by being released from operator DNA upon ligand binding [1]. TetR family proteins share a common structural feature in the form of 10 α-helical bundles divided into two domains. The N-terminal domain, called the DNA-binding domain, is composed of helices α1–α3 and binds to palindromic operator DNA. Helix α3 plays an important role by binding specifically to the major groove of DNA. The C-terminal domain, called the ligand-binding domain, is composed of helices α4–α10 and contains a ligand-binding pocket of diverse size [3,4]. For example, the C-terminal domain of TetR repressor proteins recognizes tetracyclin and induces conformational changes in the N-terminal domain leading to the release of the repressor [5]. After the TetR repressor is released from operator DNA, downstream genes are activated to remove tetracyclin by an efflux pump [6].

We have previously determined the structure of apo PA2196 that contains a HTH-motif in the DNA-binding domain and a hydrophobic pocket in the ligand-binding domain [4]. To examine the exact binding mechanism of PA2196 to DNA, we determined the structure of PA2196 bound to 25 bp semi-palindromic dsDNA region located upstream of the PA2196 gene. The structure revealed that two dimers of PA2196 bound to one semi-palindromic DNA, which is similar to the binding mode of QacR repressor [7]. Our structure also elucidated critical residues in DNA binding and conformational changes upon DNA binding.

2. Materials and methods

2.1. Cloning and protein expression

The PA2196 gene was amplified from *P. aeruginosa* genomic DNA by polymerase chain reaction (PCR). The purified PCR product was cloned into pET28b vector using NheI and EcoRI restriction enzymes with an N-terminal His_6-tag and thrombin-cut site. The construct was transformed into BL21 (DE3) *Escherichia coli* strain (Novagen). Cells were grown in LB media containing 30 μg/ml of

Abbreviations: TetR, Tet repressor; HTH, α-helix-turn-α-helix motif; RMSD, root-mean-square deviations.

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kanamycin at 37 °C until an OD_{600} of 0.8. Protein expression was induced at 18 °C with 1 mM isopropyl β-D-1-thiogalacto pyranoside (IPTG). Cells were lysed by sonication in 20 mM Tris–HCl, pH 7.5 and 250 mM NaCl buffer (lysis buffer). The lysate was cleared by centrifugation, after which the supernatant was loaded onto a Ni-Sepharose 6 affinity column and eluted with a stepwise gradient of 100–800 mM imidazole pH 8.0 in lysis buffer. After the N-terminal His_6-tag from the vector was cut by thrombin at 4 °C, PA2196 was further purified using a Superdex75 size-exclusion column (GE Healthcare) equilibrated with buffer composed of 20 mM Tris–HCl pH 7.5, 250 mM NaCl, 2 mM dithiothreitol (DTT), and 2 mM EDTA. Purity of the protein was analyzed by SDS–PAGE.

2.2. Crystallization, data collection, and structure determination

Purified PA2196 was concentrated to 10 mg/ml by centrifugal ultrafiltration (Amicon). The 25 bp semi-palindromic DNA (5′-GTTTCTAGACGACTGGTCTAATTCA-3′) in the upstream region was synthesized. After the 25 bp DNA was mixed with PA2196 at a molar ratio (protein dimer/DNA) of 2, the sample was incubated for 1 h on ice. Crystal of the PA2196–DNA complex was obtained by the hanging-drop vapor-diffusion method at 20 °C using a well solution composed of 0.79 M sodium citrate. Crystals were transferred into 0.79 M sodium citrate and 25% glycerol as a cryoprotectant solution and then flash-frozen in liquid nitrogen. X-ray diffraction data were collected at 2.9 Å resolution at PAL beamline 5C (Korea). Data were processed with HKL2000 [8], and the model of the PA2196–DNA complex was obtained using the molecular replacement program of the CCP4 package [9] using the apo PA2196 structure (PDB ID: 3RD3) as a search model. The space group was $P3_221$, and the asymmetric unit contained 16 subunits of PA2196 that formed eight homodimers and four 25 bp dsDNAs (Table 1). The Matthews' coefficient (*V*m) was 4.46 Å3/Da, and the estimated solvent content was 72.4%. The model was refined with PHENIX [10], and manual model building was performed using the COOT program [11]. Three residues (Met1–Thr3) in the N-terminal region as well as the last residue (Ile194) were not observed in the electron density and thus not included in the final model of nine subunits, whereas six residues (Met1–Asp6) in the N-terminal region as well as the last residue (Ile194) were not included in the final model of seven subunits out of 16 subunits. The Ramachandran plot produced by MolProbity [12] showed that there was no Ramachandran plot outlier in the structure of the PA2196–DNA complex.

Table 1
Data collection and refinement statistics.

Data collection statistics	
Space group	$P3_221$
Unit cell dimensions	a = 212.0, b = 212.0, c = 282.8 Å
Resolution (Å)[a]	20.0–2.90 (2.95–2.90)
Observed reflections	2,228,983
Unique reflections	162,384
Completeness (%)	100.0 (100.0)
R_{sym} (%)[b]	0.082 (0.824)
I/σ (I)[c]	17.0 (2.6)
Refinement statistics	
Number of protein residues	3016
Number of DNA bases	200
Number of waters	209
R_{cryst} (%)/R_{free} (%)[d]	19.75/ 23.83
RMSD bonds (Å)	0.013
RMSD angles (°)	1.913

[a] Resolution range of the highest shell is listed in parentheses.
[b] $R_{sym} = \sum|I - \langle I\rangle|/\sum I$, where I is the intensity of an individual reflection and $\langle I\rangle$ is the average intensity over symmetry equivalents.
[c] I/σ (I) is the mean reflection intensity/estimated error.
[d] $R_{cryst} = \sum||F_o| - |F_c||/\sum|F_o|$, where F_o and F_c are the observed and calculated structure factor amplitudes, R_{free} is equivalent to R_{cryst} but calculated for a randomly chosen set of reflections that were omitted from the refinement process.

2.3. Protein data bank accession number

The coordinate and structure factors for the *P. aeruginosa* PA2196–DNA complex have been deposited in the RCSB Protein Data Bank with accession code 4L62.

3. Results and discussion

3.1. Overall structure of PA2196 with 25mer dsDNA

The crystal structure of PA2196 with 25mer dsDNA was determined at a 2.9 Å resolution by molecular replacement. The crystal structure revealed a protein–DNA complex composed of two pairs of dimers (cyan and green; magenta and yellow) bound to one 25mer dsDNA each (Fig. 1A and B). There were four such complexes in the asymmetric unit (total of eight dimers and four 25mer dsDNAs). The two dimers bound to the opposite side of the DNA with an angle of about 135° between them (Fig. 1B). The overall structures of the two dimers were quite similar with an RMSD of 0.282 Å. Similar to other TetR family proteins, the N-terminal DNA-binding domain (from α1 to α3) was shown to be involved in interactions with the major groove of DNA, as helix α3 played a major role in DNA interactions. For one dimer pair (cyan and green, for example), one monomer (cyan, for example) interacted with both strands (orange and blue) of the 25mer dsDNA (Fig. 1C). Of the two dimers, one dimer pair (cyan and green) displayed 2-fold symmetry (indicated in Fig. 1C as a diamond), which coincides with the semi 2-fold symmetry of the palindromic DNA (indicated in Fig. 1C and D as a diamond). The 2-fold symmetry of the other dimer pair (magenta and yellow) did not overlap with that of the semi-palindromic DNA. This dimer pair (magenta and yellow) also contained a lower number of hydrogen bonds with DNA than the cyan and green dimer pair. This binding mode is different from that of QacR, in which the 2-fold symmetries of the two dimers and palindromic DNA overlap [7].

3.2. Details of the PA2196–DNA interaction

Each monomer of the two dimers interacted similarly with DNA, although the exact number of interactions differed. The cyan monomer of the cyan and green dimer pair displayed the most number of interactions with DNA (eleven; six hydrogen bonds with the blue DNA strand and five with the orange DNA strand) (Fig. 2A). More specifically, His46 from helix α3 formed hydrogen bonds with the phosphate group of C5 of the blue DNA strand. Residue Thr8 from helix α1 and Ser43 and Thr47 from α3 formed hydrogen bonds with the phosphate group of T6. The amide group of Gly42 from helix α3 acted as a hydrogen bond donor for the N7 nitrogen of G8. Further, the amino group of Lys41 from helix α3 acted as a hydrogen bond donor for the O6 oxygen of G16 from the other (orange) strand of DNA. Tyr45 interacted with the phosphate group of G16 from the orange DNA strand. The amide group of Leu30 (from helix α2) and amino group of Lys51 (from helix α4) interacted with the phosphate group of C15 from the orange DNA strand. Multiple sequence alignment with homologous proteins showed that seven out of the nine residues involved in DNA binding were completely conserved, and six of them were located in helix α3 (Fig. 2B).

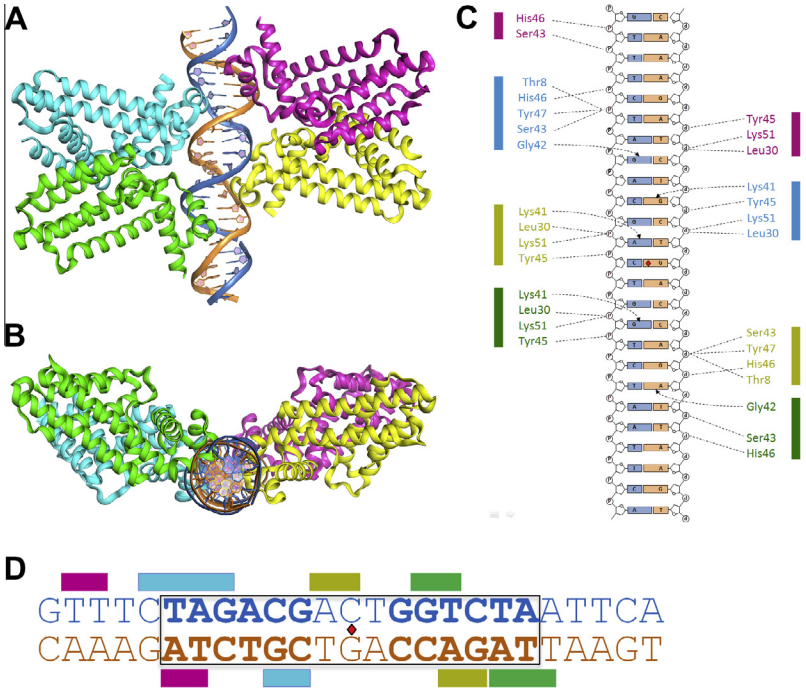

Fig. 1. Overall structure of PA2196: DNA complex (A) Two PA2196 dimers bind to the opposite side of one 25mer double stranded DNA. (B) The two homodimers form about 135° angle with each other. (C) Hydrogen bonds between the protein and DNA are indicated by arrows. The color scheme is same as in (A). The semi 2-fold axis of the 25mer semi palindromic DNA is indicated by red diamond. (D) Schematic view of (C). The semi palindromic region of the DNA is boxed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

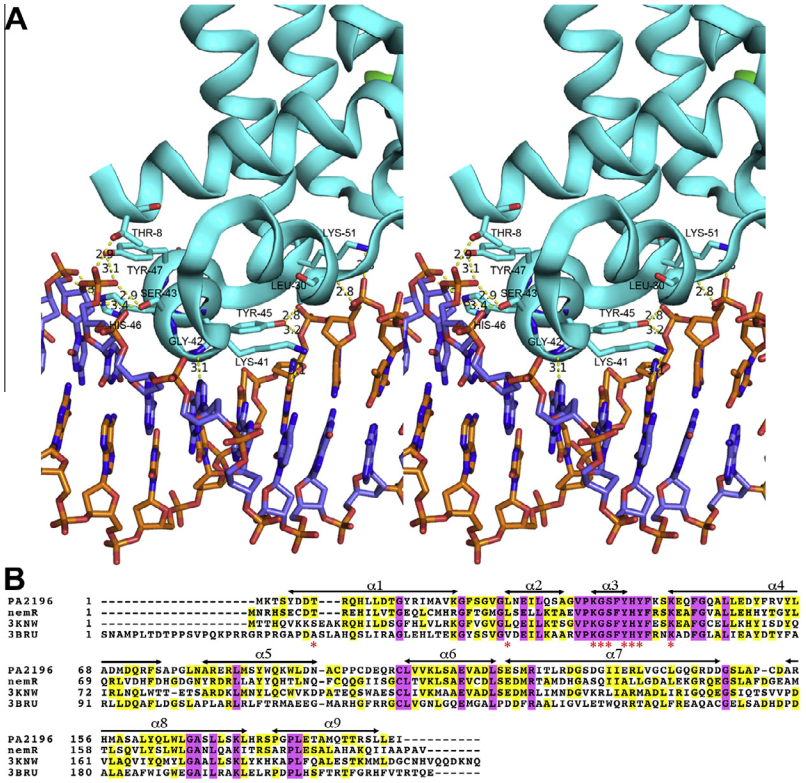

Fig. 2. Detailed view of the protein–DNA interaction. (A) Stereoview of PA2196 (chain J)–DNA interaction. Hydrogen bonds are indicated with yellow dashed lines with distances in Å. (B) Multiple sequence alignment of PA2196 and homologues, *Escherichia coli* nemR [17], *Acinetobacter baylyi* 3KNW (PDB ID) and *Rhodobacter sphaeroides* 3BRU (PDB ID). Completely conserved residues are colored in magenta and highly conserved residues in yellow. Residues of PA2196 participating in the interaction with DNA are indicated with red asterisk. Secondary structures of PA2196 are shown above the sequence. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

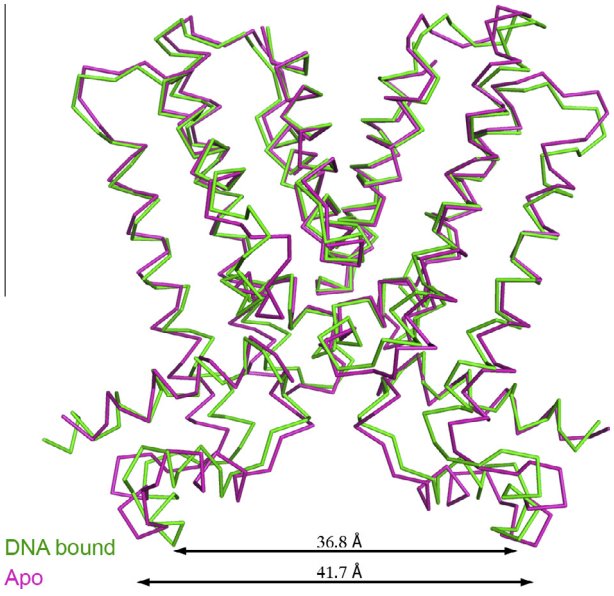

Fig. 3. Conformational changes of PA2196 upon DNA binding. Superposition of apo PA2196 (magenta) and DNA bound form (green) shows conformational changes leading to the adjustment of distance between helix-turn-helix DNA binding motif (distance between α3 helices are shown). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Conformational changes of the PA2196: DNA complex

Structural comparison between PA2196 in its apo form and bound to the 25mer semi-palindromic DNA showed conformational changes upon DNA binding. The distance between the two helix-turn-helix DNA-binding domains measured between two G42s in a dimer decreased from 41.7 Å. (apo form) to 36.8 Å (DNA bound) (Fig. 3). Analysis of the 25mer DNA bound by PA2196 using the Curves program [13] indicated that the DNA was significantly distorted from standard B-form DNA. The width of the major grooves bound by the helix-turn-helix DNA-binding motif widened to 23.4 Å compared to 22.0 Å of standard B-form DNA. Further, analysis of the DNA region bound by PA2196 (bp 2–20) showed that the average twist angle was 33.8° with 10.7 bp per turn, in contrast to a twist angle of 34.3° and 10.5 bp per turn in standard B-form DNA. These distortions altered the distance between adjacent major grooves to 35.7 Å, which is close to the center-to-center distance of the helix-turn-helix DNA-binding domain of PA2196 (36.8 Å). This lengthening of the major groove of DNA by unwinding has also been observed in the QacR–DNA complex structure [7], which will be discussed in the next section.

3.4. Structural comparison with transcription factor QacR

QacA is a multidrug transporter that confers resistance to antimicrobial agents via protein force-dependent efflux of the compound [14]. QacR regulator represses transcription of the qacA gene by binding to palindromic sequences in the upstream region [15,16]. Previous studies showed that two QacR dimers bind one operator site while one TetR dimer binds to one operator site [1]. Like QacR, two PA2196 dimers were shown to bind to one DNA. We superimposed the structures of QacR bound to its operator DNA and PA2196 in complex with DNA for structural comparison (Fig. 4). The overall mechanisms of interaction were very similar between QacR and PA2196 with an RMSD of 1.95 Å when helices α1–α3 were used for calculation. In both cases, two dimers bind to the opposite side of DNA, and helix α3 plays a major role in specific interactions with DNA by inserting into the center of the major groove.

Acknowledgments

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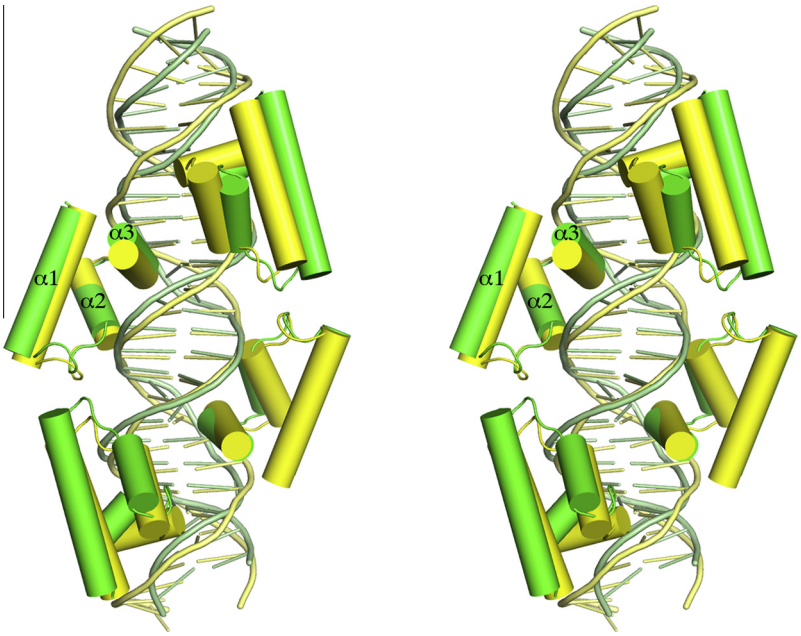

Fig. 4. Stereoview of the HTH DNA binding domains of PA2196 and QacR bound to DNA. Like QacR, two homodimers of PA2196 binds to one semi palindromic DNA. Comparison of the DNA binding elements (α1–α3) of PA2196 (green) and QacR (yellow) shows the overall similarity in binding mode. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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