PROTEIN STRUCTURE REPORT

The X-ray crystal structure of PA1607 from *Pseudomonas aureginosa* at 1.9 Å resolution—a putative transcription factor

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Abstract

The structure of the PA1607 protein from *Pseudomonas aureginosa* was determined at 1.85 Å resolution using the Se-Met multiwavelength anomalous diffraction (MAD) technique. PA1607 forms a dimer and adopts a winged-helix motif similar to the MarR family of transcription regulators, though it has an unusual dimerization profile. The DNA-binding regions and a putative metal-binding site are not conserved in PA1607.

Keywords: transcription factor; winged-helix; crystal structure

The PA1607 gene encodes a small, conserved protein of unknown function, with a predicted molecular weight of 16.2 kDa (148 amino acids). The protein belongs to a family of conserved proteins believed to be transcription factors (COG 1733) (Fig. 1A), but has low sequence identity to any known structures. There is an annotational link to transcription factors (pfam 01638) (Marchler-Bauer and Bryant 2004).

PA1607 was chosen as a target protein for the Ontario Structural Proteomics project due to the low sequence identity to any known structures. In order to gain further insight into the function of PA1607, we have determined the crystal structure of PA1607. The crystals diffracted to a resolution of 1.85 Å and the structure was solved by the multiwavelength anomalous diffraction technique (Hendrickson 1985), using selenomethionine (SeMet)-labeled protein crystals.

The structure consists of a dimer and shows similarities to the winged-helix motif adopted by other transcription factors, including the MarR family of proteins (Aleksun et al. 2001). All of these proteins form a biological dimer, and the structure of PA1607 is consistent with this, though the formation of the dimer is altered in PA1607. Additionally, some proteins with similar structure have been proposed to have a metal-binding site (Cook et al. 1998). This structure suggests that the metal-binding site is not conserved in PA1607. The potential roles for PA1607 as a DNA-binding proteins as either a metallo-regulatory protein or an oxidative stress sensor are discussed with respect to the similar structures.

Results and Discussion

Structure

A monomer of PA1607 has dimensions of 58 Å × 46 Å × 33 Å and assumes an α/β motif, containing six α-helices, four β-strands, and one ₃₁₀ helix (Fig. 1B). This fold is representative of the winged-helix repressor DNA-binding domain (CATH no. 1.10.10.10) (Orongo et al. 1997). Helices α5 and α6 form one long helix, with

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Figure 1. Crystal structure of PA1607. (A) Sequence alignment of PA1607 with its closest homologs (based on a BLAST search). Diagram showing the secondary structure elements in PA1607 superimposed on the sequence alignment. Aligned sequences are PA1607 (*Pseudomonas aeruginosa*), *Burkholderia cenocepacia*, *Rhodopseudomonas palustris*, *Pseudomonas fluorescens* Pf-5, *Shewanella baltica*, and *Mycobacterium tuberculosis*. The blue bars represent the regions that structure alignment suggests are involved in DNA binding. (B) Stereo ribbon diagram of *Pseudomonas aeruginosa* PA1607 dimer, color coded for monomer A (red) and monomer B (blue). α -helices ($\alpha 1$ – $\alpha 6$), β -strands ($\beta 1$ – $\beta 4$), and 3_{10} -helices ($\eta 1$) are indicated on monomer A and correspond to elements in A. Alignment figures were drawn using ESPript (Gouet et al. 1999) and other figures were made using PYMOL (Delano 2002).

a 35° kink in the middle, likely induced by a highly conserved Pro at the N terminus of helix 6. The first two β -strands, along with $\alpha 4$ and $\alpha 5$ form the “winged-helix” motif (wHTH), with the two β -strands forming a short anti-parallel β -sheet. The other two strands form part of the dimer interface as described below. There are three β -turns located in the protein, as defined by DSSP (Kabsch and Sanders 1983). All three turns are classical type I turns, as defined by Hutchinson and Thornton (1994).

The structure contains four sulfate molecules, all associated with arginine residues (Arg39 and Arg117 of chain A, and Arg39 and Arg125 of chain B) on the surface of the protein. The sulfate at Arg125 is located on the twofold axis. Also located close to this position is sugar residue, modeled as α -D-glucose, likely from the sucrose used as a cryoprotectant, though the other portion of the sucrose could not be located in any density.

Dimer formation

PA1607 protein crystallizes as a dimer, consistent with the other members of this structural superfamily and many other transcription factors. The dimerization surface buries 31% of the solvent accessible surface area (ASA) of each monomer and includes 27 hydrogen bonds and two salt bridges (Jones and Thornton 1996; Table 1). The contacts consist mostly of hydrophobic atoms (67%) and the dimer is predominantly stabilized by contacts made by α -helices 1, 2, 5, and 6. Additional contacts are made by the extended loop at the C-terminal end of the protein. These contacts include the formation of two short anti-parallel β -sheets between the $\beta 3$ strands from each monomer and the $\beta 4$ strands ($\beta 3$ and $\beta 3'$ form one sheet and $\beta 4$ and $\beta 4'$ form another sheet) (Fig. 1B).

The dimer interface has contributions from 70 residues in each monomer, 12 of which are absolutely conserved.

Table 1. Comparison of dimerization parameters

Protein	Area ^a	% Area ^a	Nonpolar ^a	# Residues ^a	H-bond ^a	Salt ^a	RMSD (Å)
PA1607	3204	31	67	70	27	2	—
1jgs	2078	21	70	44	10	2	35.3
1lj9	2573	25	67	57	15	2	32.6
1smt	1970	27	70	46	9	2	16.3
1z91	2517	26	70	47	14	0	41.4

^aColumn headings refer to the following: (Area) The solvent accessible surface area of an individual monomer buried in the dimer interface (Å²); (% Area) the percentage of total monomer solvent accessible surface area found in dimer interface; (Nonpolar) the percentage of interface composed of nonpolar atoms; (# Residues) the number of residues of a monomer involved in dimerization; (H-bond) the number of H-bonds formed across dimer interface; (Salt) the number of salt bridges formed across dimer interface; (RMSD) the root mean squared deviation of the conserved core of monomer B of the other proteins compared with PA1607 monomer B. RMSD between monomer A of each protein and PA1607 is less than 2.50.

There are three arginine residues (Arg16, Arg117, and Arg142) in the dimer interface that each contribute three hydrogen or ionic bonds. Only Arg16 is conserved between the similar proteins. This arginine forms a salt bridge with the highly conserved Glu119 of the other monomer. The other arginines are primarily making hydrogen bonds with carbonyls of the main chain. The side chain of Arg117 is also involved with stabilizing one of the sulfates. The absolutely conserved residue Trp99 is the most highly buried residue, burying 144 Å³ of the residue, accounting for 4.5% of the interface (Jones and Thornton 1996). This residue forms a hydrophobic pocket across the dimer interface, with the conserved hydrophobic N terminus of helix α6 of the other monomer. The α5 and α6 helices form an extensive “anti-parallel” two-helix bundle across the dimer interface with α5' and α6' (Fig. 1B). The helix α6 in particular maintains a high degree of hydrophobic conservation (Fig. 1A) that appears to be essential for dimer formation.

Comparison to other structures

A search of the Protein Data Bank (Berman et al. 2000) using the Dali server (Holm and Sanders 1993) yields many structures with a Z-score over 7, almost all identified as transcription factors. Eight of these structures have a Z-score over 9, but none greater than 9.8. The best matches are MarR (PDB code: 1JGS), a regulator of antibiotic resistance in *Escherichia coli* (Aleksun et al. 2001), a SlyA transcriptional regulator (PDB code: 1LJ9) from *Enterococcus faecalis* (Wu et al. 2003), SmtB (PDB code: 1SMT), a metallothionein repressor protein from *Synechococcus* PCC7942 (Cook et al. 1998), and OhrO (PDB Code: 1Z91), a MarR-like protein from *Bacillus subtilis* (Hong et al. 2005). The sequence identity between PA1607 and each of these proteins is <20%. The DALI search shows

that all of the similar structures have <23% identity with PA1607 (Fig. 2A). In each monomer, the section from α1 to α6 (residues 20–100) is the most highly conserved structurally, including the kinked helix at α5/α6, which in some of the reports is considered to be one helix.

Variable dimerization

Although the structures of these proteins are quite similar (Fig. 2B; CA-RMSD = 2.0–2.5 Å; Holm and Sanders 1993), the dimerization of these proteins is quite different, resulting in a different arrangement of the dimer (Fig. 2C). PA1607 contains a much more extensive dimerization interface, the others burying between 2000 and 2500 Å² ASA of each monomer, compared to the over 3200 Å² ASA buried for PA1607. There is also a corresponding decrease in the number of hydrogen bonds in these other structures as well (see Table 1). A structure-based alignment of these five proteins show that there are no residues directly corresponding to each other in the sequences. The hydrophobic helix α6 that plays a critical role in the dimer interface of PA1607 is not conserved as a hydrophobic helix in any of the similar structures and is not utilized in dimer formation in all structures. This results in very different orientations of the dimers for all of the structures (Fig. 2C). The structures where this helix does make extensive dimer contacts (1smt and 1z91) still do not retain similar overall dimer geometries. This can be seen by comparing the α-carbon RMSD values of each monomer of PA1607 with the corresponding monomer from the other proteins. The conserved structural core of PA1607 in monomer A (superimposed structures) fits well with each of the other structures (RMSD < 2.5 Å). However, when this superposition (Fig. 2C) is used to compare the same structural core of monomer B from each dimer, the RMSD values increase to between 16 Å and 41 Å (Table 1, last column).

DNA binding

Hong et al. (2005) determined the structure of OhrO bound to a 29-base-pair oligonucleotide and give insight into the binding of these types of protein to DNA. OhrO undergoes a conformational shift in the DNA-bound structure, with the majority of the changes taking place in the wHTH region (in PA1607, this is α4, β1, β2, and α5). These changes result in a 25° rotation and a 16-Å movement in the tips of the wings. In OhrO, the specific contacts between the DNA and protein are quite limited—there are two regions that appear to be conserved as DNA-binding regions. These regions are also consistent with regions proposed to be DNA-binding regions for SmtB (Cook et al. 1998). In OhrO, the DNA-binding regions consist of side-chain contacts between Tyr65, Leu66, Asp67, Ser68, Thr70, Thr72,

B

C

Figure 2. (Legend on next page)

Lys76, Arg77 (see Fig. 2C, blue triangles), and the DNA, plus the carbonyl of Gly69 for region 1 and Arg86, Arg88, Asp92, Glu93, and Arg94 for region 2 (Fig. 2A, blue triangles). Asp92 and Arg94 are highly conserved in the MarR family members, and Arg94 has been shown to play an important role in DNA binding for MarR and MexR (Aleksun et al. 2001; Saito et al. 2003).

In PA1607, neither of these regions is conserved in the proteins similar to PA1607 (Fig. 1A), although there are conserved residues found in these regions (Fig. 1A, blue underlined regions), particularly in region 1. In region 2, the arginine residues that are important for DNA binding in the MarR family of proteins are missing from the PA1607 family. The lack of conserved residues, particularly arginines, in the tip of the wing of PA1607 that are seen in other wHTH proteins suggests that if PA1607 is actually a DNA-binding protein, the mode of recognition of and binding to DNA will be different than is seen in other members of this structural family.

Metal binding

In the report on the structure of SmtB (Cook et al. 1998), it was proposed that there are several Zn^{2+} -binding sites located in the protein. SmtB was crystallized without Zn^{2+} ; however, soaks of the crystals with Hg^{2+} revealed the presence of four Hg^{2+} -binding sites in the derivative structure and these sites are suggested to be the location of the Zn^{2+} -binding sites. There is one independent site per dimer, consisting of residues Cys61, Asp64, and His97. The other two sites are found in the dimer interface, these sites being composed of residues Asp104 and His106 from one monomer and His117 and Glu120 from the other monomer (Fig. 2A, red stars). The structural alignment of the similar proteins shows that these residues are not conserved in PA1607.

Additionally, PA1607 contains only a single cysteine residue (Cys12) that, although highly conserved among similar proteins (Fig. 1A), is located away from the regions in SmtB that are believed to form the metal-binding sites. This cysteine residue is within 3.3 Å of a

conserved arginine residue (Arg31) from the other monomer, making it more likely that this residue is playing a role in dimerization rather than metal binding. There is also a second conserved arginine from the other monomer (Arg97) located 5.0 Å from Cys12. This combination makes it unlikely that Cys12 is playing a role in metal binding.

Oxidative stress sensor

A cysteine residue near the N terminus in OhrO (Cys15) is a putative sensor of reactive oxygen species and acts as a regulator of the DNA binding of OhrO (Hong et al. 2005). In OhrO, the high reactivity of Cys15 is attributed to the presence of two conserved tyrosine residues (29 and 40—from the other monomer) that are thought to lower the pK_a , in conjunction with the positively charged end of the helix dipole (Wada 1976; Hol et al. 1978; Kortemme and Creighton 1995). In PA1607, the conserved Cys12 (Fig. 1A) could play a similar role in sensing oxidative stress. This cysteine residue is located between the two monomers and interacts with conserved arginine residues from the other monomer. These arginines would act to stabilize the negatively charged thiolate that would be the sensor residue. Oxidation of Cys12 could therefore act to signal in a manner analogous to Cys15 in OhrO.

Conclusions

Without any biochemical data, it is difficult to suggest any roles for PA1607 other than that it is likely to be a DNA-binding protein, based on the structure and sequence alignment. From the structural comparisons, it seems unlikely that PA1607 has a metal-binding site similar to SmtB; however, it is possible that this protein plays a role in sensing oxidative stress in a manner analogous to OhrO.

Materials and methods

Protein production and crystallization

The PA1607 gene was subcloned, expressed, and its product purified and screened for crystallization as described previously

Figure 2. Structural comparison of PA1607 with other proteins. (A) Sequence alignment of PA1607 with structural homologs (based on a DALI; Holm and Sanders 1993). The alignment was carried out by aligning the structural elements in Chimera (Pettersen et al. 2004). The structural homologs are MarR (PDB code: 1JGS), a SlyA transcriptional regulator (PDB code: 1LJ9) from *Enterococcus faecalis*, SmtB from *Synechococcus* PCC7942 (PDB code: 1SMT), and OhrO (PDB code: 1Z91), a MarR-like protein from *Bacillus subtilis*. The colored highlighted sequence for each one represents the regions that are making dimer contacts. The blue triangles indicate the positions of residues in OhrO (1Z91) that contact the DNA and the red asterisks indicate the residues that form the putative metal-binding sites in SmtB (1SMT). (B) Cartoon representation of the superposition of monomer A from PA1607 with the corresponding monomers from each structure in A. The color scheme corresponds to the highlighted regions in A. Structure alignment is from DALI (Holm and Sanders 1993). (C) Stereo ribbon diagram of a superposition of PA1607 dimer with the other proteins from A. Monomer A from each of the structures was superimposed on PA1607 monomer A (as in part B) and are displayed as a colored line (color scheme as highlighted regions in A). Monomer B from each protein is then shown as a ribbon cartoon in the appropriate color for each structure. The orientation is the same as in B.

(Zhang et al. 2001). Crystals for X-ray diffractions data collection were obtained from hanging drop vapor diffusion conditions containing 2 μ L of Se-Met derivative of PA1607 plus 2 μ L of well buffer, containing 0.1 M Tris (pH 8.6), 0.2 M LiSO₄, and 28% PEG 3350. The crystallization was carried out at 21°C. The crystals were flash-frozen with 13.2% sucrose in crystallization buffer.

Data collection

Diffraction data (Table 2) were collected at beamline 19ID of APS, Argonne National Laboratory, following the approach described earlier (Walsh et al. 1999). The two-wavelength inverted beam MAD inflection and peak wavelengths up to 1.85 Å were collected from one Se-Met labeled protein crystal at 100 K with 4-sec exposure/1°/frame using a 180-mm crystal-to-detector distance. The total oscillation range was 180°. All data were processed and scaled with HKL2000 (Otwinowski and Minor 1997). The space group was determined to be C2 with unit-cell dimensions of $a = 46.88$, $b = 78.87$, $c = 78.94$, $\alpha = \delta = 90$, and $\beta = 91.64$. Calculation of the Matthews volume (Matthews 1968) indicated that the unit cell contained two monomers ($V_m = 2.25$ Å³Da⁻¹, solvent content = 45.4%), assuming a molecular weight of 16.2 kDa.

Structure solution and refinement

The selenium sites were located and refined using BnP (Weeks et al. 2002). Four Se sites were located, consistent with two

monomers in the unit cell. RESOLVE (Terwilliger 2000) followed by ARP/wARP 6.1 (Lamzin et al. 2001) resulted in the automatic building of 228 residues (of 296). The remaining residues were built by superimposing the two monomers to fill in missing stretches and manual rebuilding using COOT (Emsley and Cowtan 2004). All programs not individually referenced are part of the CCP4 package (Collaborative Computational Project, Number 4 1994).

The peak wavelength datum was chosen for structure refinement as it had slightly better statistics than the inflection point wavelength (Table 2). Refinement was carried out using REFMAC5, using TLS and isotropic B-factor refinement. The final R -factor was 0.181 and the free R was 0.251 (Table 2). The N termini of both monomers were missing from any electron density maps and the missing N-terminal residues were not included in the structure refinement. Monomer A therefore starts from residue 5 (threonine) and monomer B from residue 6 (serine).

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Table 2. Data collection and refinement statistics

	Peak	Inflection
Data collection		
Wavelength (Å)	0.97932	0.97948
Resolution (Å)	50–1.85 (1.92–1.85)	50–1.85 (1.92–1.85)
Space group	C2	
Unit-cell parameters	$a = 46.88$, $b = 78.87$, $c = 78.94$, $\alpha = \delta = 90$, $\beta = 91.64$	
Total measurements	144,492	143,159
Unique reflections	43,324	43,292
$I/\sigma(I)$	29.1 (2.4)	27.6 (1.9)
Average redundancy	5.9 (5.0)	3.3 (2.4)
Completeness (%)	89.7 (51.5)	89.1 (48.9)
R_{merge} (%) ^a	4.0 (42.8)	4.3 (48.8)
Refinement		
$R_{\text{work}}/R_{\text{free}}$ (%) ^b	18.1/25.1	
No. protein atoms	2215	
No. water atoms	329	
B factor (all) (Å ²)	37.2	
RMSD bond lengths (Å)	0.019	
RMSD bond angles (°)	1.696	
Ramachandran plot (%)		
Most favored regions	96.8	
Allowed regions	3.2	
Disallowed regions	0.0	

Values in parentheses refer to highest-resolution shell.

^a $R_{\text{merge}} = \sum \sum I(h)_j - I(h) / \sum \sum I(h)_j$, where $I(h)$ is the measured diffraction intensity and the summation includes all observations.

^b R_{work} is the R -factor $= \sum |F_o - F_c| / \sum |F_o|$. R_{free} is the R -factor calculated using 5% of the data that were excluded from the refinement.

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