Conformational change of the AcrR regulator reveals a possible mechanism of induction

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Conformational change of the AcrR regulator reveals a possible mechanism of induction

The Escherichia coli AcrR multidrug-binding protein represses transcription of acrAB and is induced by many structurally unrelated cytotoxic compounds. The crystal structure of AcrR in space group $P2_12_12_1$ has been reported previously. This $P2_2_2_1$ structure has provided direct information about the multidrug-binding site and important residues for drug recognition. Here, a crystal structure of this regulator in space group $P3_1$ is presented. Comparison of the two AcrR structures reveals possible mechanisms of ligand binding and AcrR regulation.

1. Introduction

The disturbing increase in multidrug resistance (MDR) observed in bacteria is a growing problem associated with the use of antibiotics for treating bacterial infections. Bacterial multidrug resistance is to a large extent attributed to the expression of multidrug efflux transporters that are capable of extruding a wide variety of toxic compounds from bacterial cells (Levy, 1992, 2001; Saier et al., 1998; McKeegan et al., 2003). Escherichia coli AcrB is one of the prototypical members of the resistance–nodulation–division (RND) family of transporters (Tseng et al., 1999; Ma et al., 1995). This inner membrane protein recognizes a variety of structurally dissimilar agents, including most currently used antibiotics, chemotherapeutic agents, detergents and dyes (Nikaido, 1996). In association with a periplasmic membrane-fusion protein, AcrA (Zgurskaya & Nikaido, 2000), and an outer membrane channel, TolC (Koronakis et al., 2000), the AcrB multidrug efflux pump is capable of exporting these structurally dissimilar compounds directly to the external medium.

The transcription of acrAB is regulated by a global transcriptional activator, MarA, and a local transcriptional repressor, AcrR (Ma et al., 1996). AcrR is a 215-amino-acid protein that belongs to the TetR family of transcriptional repressors (Ramos et al., 2005). Recently, the ligand-free structure of AcrR has been determined in our laboratory (Li et al., 2007). The crystal structure revealed that AcrR is a dimeric two-domain molecule with an entirely helical architecture, similar to other members of the TetR family. Each subunit of AcrR is composed of nine helices ($\alpha_1$–$\alpha_9$ and $\alpha_1'$–$\alpha_9'$, respectively). The smaller N-terminal domain comprises helices $\alpha_1$–$\alpha_5$, with $\alpha_2$ and $\alpha_3$ forming the helix–turn–helix (HTH) DNA-binding motif. The larger C-terminal domain consists of helices $\alpha_4$–$\alpha_9$. The C-terminal domain forms a large internal cavity that was suggested to serve as the multidrug-binding pocket. Inside the drug-binding pocket, a completely buried negatively charged residue, Glu67, was found to be critical for drug recognition (Li et al., 2007).

In E. coli, AcrR acts as a moderator to maintain sufficient expression levels of AcrB for bacterial survival in varying environments. The induction of AcrR is triggered by many structurally unrelated compounds, which are also substrates of the AcrB efflux pump. How AcrR binds inducing ligands and regulates the expression of AcrB is still not clear. The hypothesis is that binding of drugs to the C-terminal ligand-binding domain of AcrR triggers a conformational
Refinement
Phasing

These crystal structures provide novel insight into the mechanisms of two structures represent different conformational states of AcrR. In both the N-terminal and C-terminal regions, suggesting that these of these two structures reveals considerable conformational changes between the previously reported P2221 structure of AcrR in molecular replacement, we determined the new P31 structure of AcrR using single-wavelength anomalous dispersion (SAD). The native and SeMet crystals both belonged to space group P31; unit-cell parameters are summarized in Table 1. Following refinement of all 14 selenium sites of the AcrR dimer, initial phases were obtained by SAD using the program BnP (Weeks et al., 2005). The electron-density map was then subjected to density modification (DM) and the initial model was obtained by the auto-interpretation routine function in the program RESOLVE (Terwilliger, 2001). After obtaining the initial model, the program O (Jones et al., 1991) was used to perform manual model building. The model was then refined against the native data at 2.5 Å resolution using the programs CNS (Brünger et al., 1998) and REFMAC5 (Collaborative Computational Project, Number 4, 1994; Murshudov et al., 1997). Solvent atoms were initially built using the program ARP/wARP (Collaborative Computational Project, Number 4, 1994; Lamzin & Wilson, 1993) and subsequently added or removed by manual inspection. The final Rwork and Rfree (calculated using 5% of reflections omitted from the refinement) were 21.4% and 26.8%, respectively.

Table 1
Data-collection, phasing and structural refinement statistics.

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<th>Native</th>
<th>SeMet, peak</th>
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change in the N-terminal DNA-binding region, which results in the release of AcrR from its operator DNA. Here, we report a new crystal structure of AcrR with space group P31, which is distinct from our previously reported P2221 space-group structure. A comparison of these two structures reveals considerable conformational changes in both the N-terminal and C-terminal regions, suggesting that these two structures represent different conformational states of AcrR. These crystal structures provide novel insight into the mechanisms of ligand binding and AcrR regulation.

2. Materials and methods

2.1. Cloning, expression, purification, crystallization and data collection

Recombinant AcrR containing a 6×His tag at the C-terminus was expressed in E. coli using the pET15b vector. The cloning, expression and purification procedures have been described previously (Li et al., 2006, 2007). For crystallization of native AcrR, a 4 μl drop consisting of 2 μl protein solution (20 mM Tris pH 7.5, 60 mM imidazole and 200 mM NaCl) and 2 μl well solution (34% PEG 3350, 3% MPD, 0.2 M MgCl2 and 0.1 M Tris buffer pH 8.5) was equilibrated against 500 μl well solution. Under these conditions, a new bipyramidal crystal shape was obtained. The crystals were subsequently found to belong to space group P31. The SeMet-AcrR protein was crystallized under conditions identical to those used for native AcrR. The crystallization conditions provided sufficient cryoprotection and crystals were frozen directly in liquid nitrogen at 100 K. Diffraction data sets for both native and SeMet-AcrR were obtained at the Advanced Photo Source (APS, beamline 24IDC) at cryogenic temperature (100 K).

2.2. Structural determination and refinement

Diffraction data sets were processed with DENZO and scaled with SCALEPACK (Otwinowski & Minor, 1997). In order to avoid any model bias from using the P2221 structure of AcrR in molecular replacement, we determined the new P31 structure of AcrR using single-wavelength anomalous dispersion (SAD). The native and SeMet crystals both belonged to space group P31; unit-cell parameters are summarized in Table 1. Following refinement of all 14 selenium sites of the AcrR dimer, initial phases were obtained by SAD using the program BnP (Weeks et al., 2005). The electron-density map was then subjected to density modification (DM) and the initial model was obtained by the auto-interpretation routine function in the program RESOLVE (Terwilliger, 2001). After obtaining the initial model, the program O (Jones et al., 1991) was used to perform manual model building. The model was then refined against the native data at 2.5 Å resolution using the programs CNS (Brünger et al., 1998) and REFMAC5 (Collaborative Computational Project, Number 4, 1994; Murshudov et al., 1997). Solvent atoms were initially built using the program ARP/wARP (Collaborative Computational Project, Number 4, 1994; Lamzin & Wilson, 1993) and subsequently added or removed by manual inspection. The final Rwork and Rfree (calculated using 5% of reflections omitted from the refinement) were 21.4% and 26.8%, respectively.

2.3. Protein Data Bank accession code

Coordinates and structural factors for the structure of AcrR have been deposited in the RCSB Protein Data Bank with accession code 3bcg.

3. Results and discussion

3.1. N-terminal DNA-binding domain

The overall structure of AcrR in space group P31 is very similar to the previously reported P2221 space-group structure (PDB code 2qop) at 2.5 Å resolution determined recently in our laboratory (Li et al., 2007). Superimposition of these two dimeric structures gives an overall r.m.s.d. of 1.4 Å calculated over the Cα atoms. However, a detailed comparison reveals a significant conformational change in the N-terminal DNA-binding domain. Fig. 1 illustrates a super-

Figure 1
Structural comparison of the P3$_1$ and P2$_{222}$, structures of AcrR. Superimposition of the dimeric AcrR structures was performed using the program ESCET (orange, P3$_1$, structure; green, P2$_{222}$, structure).
position of these two AcrR structures using the program ESCET (Schneider, 2002). A plot of the distance between corresponding \( C^\alpha \) atoms in the two dimeric AcrR structures with respect to residue number is shown in Fig. 2(a). The plot indeed suggests that the main difference in conformation between these two structures originates from helix \( a1 \) through the N-terminal half of helix \( a4 \). This change results in an overall r.m.s.d. of 2.8 \( \AA \) for the \( C^\alpha \) atoms in the N-terminal domains (residues 7–65), in contrast to the \(<0.7 \) \( \AA \) r.m.s.d. of the C-terminal domains (residues 73–210). Fig. 2(b) illustrates an error-scaled distance difference matrix (ESCET plot; Schneider, 2002) calculated between the \( P_{31} \) and \( P_{222_1} \) structures.

Judging from the two crystal structures of AcrR, the conformational changes between the \( P_{31} \) and \( P_{222_1} \) structures seem to be predominantly rigid-body translation and rotation of the N-terminal domain. These movements lead to a downward shift of the entire N-terminal DNA-binding domain of the \( P_{31} \) structure (with respect to the orientation shown in Fig. 1) by 2.6 \( \AA \) and a rotation of 10° towards the subunit interface of the dimer when compared with that of the \( P_{222_1} \) structure (Fig. 1). As a consequence of these movements, the two N-terminal domains of the AcrR dimer in the \( P_{31} \) structure...

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**Figure 2**

Structural rearrangement of the N-terminal domain of AcrR. (a) Plot of the distance between corresponding \( C^\alpha \) atoms in AcrR after superposition of the dimeric \( P_{31} \) and \( P_{222_1} \) structures. (b) Error-scaled distance difference matrix (ESCET plot) calculated between the dimeric \( P_{31} \) and \( P_{222_1} \) AcrR structures.

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**Figure 3**

Electron-density maps of the \( P_{31} \) structure. (a) The \( 2F_{obs} - F_{calc} \) electron-density map (blue), contoured at 1.0\( \sigma \), of the recognition helix \( a5 \) (orange, \( P_{31} \) structure; green, \( P_{222_1} \) structure). (b) The \( 2F_{obs} - F_{calc} \) electron-density map (blue), contoured at 1.0\( \sigma \), of the flexible loop between helices \( a4a \) and \( a4b \) (orange, \( P_{31} \) structure; green, \( P_{222_1} \) structure).
come closer to each other by approximately 2 Å. The center-to-center distance between recognition helices α3 and α3′ (as measured by the distance between the Cα atoms of Tyr49 and Tyr49′) decreases from 42 Å in the P2221 structure to 39 Å in the P31 structure. To bind two consecutive major grooves of B-DNA, the center-to-center distance has to be ~34 Å. This distance is thought to increase upon drug binding, which in turn inhibits the binding of the regulator to its operator DNA. Thus, this center-to-center distance can reflect different conformational states of the regulator. For CmeR, the corresponding center-to-center distance observed in CmeR suggested that CmeR was in a ligand-bound state (Gu et al., 2007). In the case of QacR, the center-to-center distance is 39 Å for apo-QacR (Schumacher et al., 2001). Upon DNA and drug binding to QacR, the corresponding center-to-center distances become 37 and 48 Å, respectively (Schumacher et al., 2002). Because of these center-to-center distances, it is likely that the structure of the DNA-bound form of AcrR is more similar to the P31 structure, while its drug-bound form is more closely related to the P2221 conformation.

In addition to these differences, Arg45, an N-terminal amino acid which has been identified to be indispensable for DNA binding and important for AcrR regulation (Webber & Piddock, 2001), undergoes a significant conformational change. The Cα−Cα distance between Arg45 and Arg45′ decreases from 40 Å in the P2221 structure to 35 Å in the P31 structure. A comparison of the conformation of helix α3 in the two AcrR structures is shown in Fig. 3(a).

We analyzed the normal modes of vibration of the P31 structure of AcrR using the program ElNeMo (Suhre & Sanejouand, 2004a,b). The results suggest that the lowest frequency nontrivial vibrational mode indeed corresponds to the swinging motion of the entire N-terminal DNA-binding domain. This swinging motion highlights the transition between the P31 and P2221 structures. Accompanied by this N-terminal swinging motion, the COOH-terminus of helix α4a and the NH2-terminus of helix α6 perform helix-to-coil transitions in this vibrational mode. These shifts in conformation of helices α4 and α6 are also consistent with the observed difference between the two AcrR structures, as discussed below.

### 3.2. C-terminal regulatory domain

One unique feature found in the crystal structure of AcrR is the presence of a short loop in the middle of helix α4. To better facilitate comparison with the structures of other TetR members, residues 55–65 and 69–80 in the P2221 structure were assigned as helices α4a and α4b, respectively (Li et al., 2007). The short loop between α4a and α4b acts as a transition region connecting the N-terminal and C-terminal domains. In the P2221 structure, this flexible loop consists of residues 66−68. In the P31 structure, however, this loop contains four more residues (66–72). Unlike the conformational change in the N-terminal domain, which is mainly an overall shift in position of the entire domain, the changes in the C-terminal domain of these two conformations are predominately associated with local movements of the flexible loop between α4a and α4b and helices α4 and α6.

It has been found that Glu67, a completely buried residue in the drug-binding pocket of the P2221 structure, is critical for drug recognition (Li et al., 2007). In the P31 structure, Glu67 is expelled from the hydrophobic core into the solvent (Figs. 1 and 3b). The shift in the Cα atom of this residue reaches 4.2 Å when compared with that of the P2221 structure. In addition, the side chain of Glu67 flips away from the hydrophobic core of the drug-binding pocket. This results in a positional shift of the two carboxylate O atoms (OE1 and OE2) in the side chain of Glu67 by more than 10 Å when compared with those of the P2221 structure. The nearby flexible-loop residues between α4a and α4b also shift considerably in response to this large movement (Fig. 3b). Coupled with the movement of Glu67, helix α4a shifts toward the N-terminal DNA-binding domain by 2.3 Å in the P31 structure when compared with the P2221 structure (Fig. 1).

Comparison of the P2221 and P31 structures also reveals conformational changes in helix α6. Arg105 and Arg106 in the P31 structure shift in position, leading to an unwinding of the helical residues Glu104–Arg106 at the N-terminus of α6. Thus, helix α6 is shortened by one turn in the P31 structure. This change may be attributed to the movement of the flexible loop between α4a and α4b. In the P2221 structure, Arg106 is hydrogen bonded to Glu67 (Fig. 4). This hydrogen bond is missing in the P31 structure owing to the expulsion of Glu67 from the hydrophobic core. As a consequence, this motion induces a helix-to-coil transition of helix α6. In addition to the above change, it was found that Arg105 is hydrogen bonded to residues Gln14 and Asp18 in the P2221 structure (Fig. 4). These hydrogen bonds are also missing in the P31 structure.

Based on the P31 and P2221 structures of AcrR, we suspect that the changes in the conformation of the N-terminal DNA-binding and C-terminal drug-binding domains of AcrR are cooperative owing to the formation of hydrogen bonds at the interface between these two domains (Fig. 4). In the DNA-bound form of AcrR, the structure of the regulator may be closer to the P31 structure. Thus, the side chain of Glu67 may point outside the drug-binding pocket and be exposed to the solvent. Drug binding to the C-terminal domain may induce conformational changes that result in a conformation more closely related to the P2221 structure, in which the side chain of Glu67 flips into the interior of the hydrophobic core. This change may also be accompanied by the formation of new hydrogen bonds between Glu67 and Arg106, Arg105 and Gln14, and Arg105 and Asp18. The
crystal structures of both DNA-bound and drug-bound AcrR would be necessary to confirm the change in conformation upon DNA and drug binding.

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References


