Cloning, expression, purification, crystallization and preliminary X-ray diffraction analysis of the regulator AcrR from Escherichia coli

Ming Li
Iowa State University

Xi Qiu
Iowa State University

Chih-Chia Su
Iowa State University, jacksu@iastate.edu

Feng Long
Iowa State University

Ruoyu Gu
Iowa State University

See next page for additional authors

Follow this and additional works at: http://lib.dr.iastate.edu/physastro_pubs

Part of the Biological and Chemical Physics Commons, Medicinal-Pharmaceutical Chemistry Commons, and the Other Chemistry Commons

The complete bibliographic information for this item can be found at http://lib.dr.iastate.edu/physastro_pubs. For information on how to cite this item, please visit http://lib.dr.iastate.edu/howtocite.html.

This Article is brought to you for free and open access by the Physics and Astronomy at Iowa State University Digital Repository. It has been accepted for inclusion in Physics and Astronomy Publications by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Cloning, expression, purification, crystallization and preliminary X-ray diffraction analysis of the regulator AcrR from Escherichia coli

Abstract
This paper describes the cloning, expression, purification and preliminary X-ray data analysis of the AcrR regulatory protein. The *Escherichia coli* AcrR is a member of the TetR family of transcriptional regulators. It regulates the expression of the AcrAB multidrug transporter. Recombinant AcrR with a 6×His tag at the C-terminus was expressed in *E. coli* and purified by metal-affinity chromatography. The protein was crystallized using hanging-drop vapor diffusion. X-ray diffraction data were collected from cryocooled crystals at a synchrotron light source. The best crystal diffracted to 2.5 Å. The space group was determined to be *P*32, with unit-cell parameters $a = b = 46.61$, $c = 166.16$ Å.

Keywords
AcrR, regulators

Disciplines
Biological and Chemical Physics | Medicinal-Pharmaceutical Chemistry | Other Chemistry

Comments

Authors
Ming Li, Xi Qiu, Chih-Chia Su, Feng Long, Ruoyu Gu, Gerry McDermott, and Edward Yu

This article is available at Iowa State University Digital Repository: http://lib.dr.iastate.edu/physastro_pubs/162
Cloning, expression, purification, crystallization and preliminary X-ray diffraction analysis of the regulator AcrR from Escherichia coli

This paper describes the cloning, expression, purification and preliminary X-ray data analysis of the AcrR regulatory protein. The Escherichia coli AcrR is a member of the TetR family of transcriptional regulators. It regulates the expression of the AcrAB multidrug transporter. Recombinant AcrR with a 6xHis tag at the C-terminus was expressed in E. coli and purified by metal-affinity chromatography. The protein was crystallized using hanging-drop vapor diffusion. X-ray diffraction data were collected from cryocooled crystals at a synchrotron light source. The best crystal diffracted to 2.5 Å. The space group was determined to be $P_3_2$, with unit-cell parameters $a = b = 46.61$, $c = 166.16$ Å.

1. Introduction

The increase in bacterial resistance to multiple drugs has emerged as a major clinical problem. One important mechanism that gives rise to multidrug resistance (MDR) in bacteria is the expression of multidrug transporters, which are often regulated at the transcriptional level by repressors and/or activators (Grkovic et al., 2001). Many of these transcriptional factors are multi-ligand-binding proteins which recognize the same array of toxic chemicals extruded by the transporters that they regulate (Ahmed et al., 1994). These transcriptional factors act as cytosolic chemical sensors and respond to threatening levels of toxic chemicals. The results are the overexpression of MDR transporters, which promote efflux from cells, thus protecting them from toxic substances.

Of all known MDR transporters, the Escherichia coli AcrB multidrug efflux pump, which belongs to the resistance–nodulation–division transporter family, shows the widest substrate specificity, ranging from most currently used antibiotics, disinfectants, dyes, bile salts, fatty acids and detergents to simple solvents (Nikaido, 1996; Zgurskaya & Nikaido, 2000a). This inner membrane efflux pump, AcrB, interacts with a periplasmic membrane-fusion protein, AcrA (Zgurskaya & Nikaido, 2000b), and an outer membrane channel, TolC (Koronakis et al., 2000), to mediate the extrusion of toxic compounds across both membranes of E. coli.

The AcrAB MDR efflux complex (Ma et al., 1995) is regulated by a global transcriptional activator MarA and a local transcriptional repressor AcrR (Ma et al., 1996). The $acrR$ gene is located 140 bp upstream of the $acrAB$ operon and transcribed divergently (Ma et al., 1996). It encodes a 215-amino-acid protein with a molecular weight of approximately 25 kDa, which shares sequence and structural similarities to members of the TetR family of transcriptional repressors (Ramos et al., 2005). Like other members of the TetR family, the N-terminal domain of AcrR contains a predicted DNA-binding helix–turn–helix (HTH) motif and its C-terminal domain is predicted to form a multi-ligand-binding site for its inducing ligands. Sequence alignment of the N-terminal HTH motif indicates that AcrR shares 28% identity and 61% similarity with TetR (Hirich et al., 1994). It also shows 53% identity and 78% homology to the MtrR repressor (Hoffmann et al., 2005). Like many other transcriptional regulators, AcrR can autoregulate its own expression.

The AcrR repressor recognizes a variety of structurally unrelated toxic compounds and regulates the transcription of the AcrAB transporter. The hypothesis is that binding of ligands to the
The recombinant plasmids were transformed into DH5α and then digested with NcoI and BamHI (New England Biolabs). The resulting product was ligated into the pET15b expression vector. The 680 bp PCR fragment of the \textit{acrR} gene with flanking sequences was cloned into pET15b (Novagen) to generate a recombinant protein and regulate gene expression, we here report the cloning, expression, purification and crystallization of the AcrR repressor.

### 2. Cloning, expression and purification

The ORF of \textit{acrR} from \textit{E. coli} K12 chromosomal DNA was amplified by PCR using the primers 5'-AAACCATGGCACGAAAAACC-3' and 5'-AAAGGATCCTTAATGGTGATGGTGATGA-3'. The resulting product was cloned into pET15b (Novagen) to generate a recombinant protein that contains a 6 × His tag at the C-terminus. Engineered restriction sites and the 6 × His-encoding sequence were designed in the primers. The 680 bp PCR fragment of the \textit{acrR} gene with flanking sequences was extracted from the agarose gel using a gel-extraction kit (Qiagen) and then digested with NcoI and BamHI (New England Biolabs). The digested products were ligated into the pET15b expression vector. The recombinant plasmids were transformed into DH5α cells and selected on LB plates containing 100 μg ml⁻¹ ampicillin. The construction was verified by DNA sequencing.

Native AcrR protein containing a 6 × His tag at the C-terminus was overproduced in \textit{E. coli} BL21(DE3) cells. Briefly, cells from a 5 ml overnight pre-culture were grown in 500 ml LB broth medium containing 100 μg ml⁻¹ ampicillin at 310 K and 210 rev min⁻¹. The culture was induced with 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) at an OD₆₀₀ value of approximately 0.5. Cells were harvested within 4 h of induction and were frozen and stored at 193 K until further processing.

For producing selenomethionyl-AcrR protein, a 50 ml LB culture containing 100 μg ml⁻¹ ampicillin was grown at 310 K and 210 rev min⁻¹. When the OD₆₀₀ value was around 1.2, cells were harvested by centrifugation at 6000 rev min⁻¹ for 10 min and then washed two times with 5 ml M9 medium containing 6.8 g l⁻¹ Na₂HPO₄, 3 g l⁻¹ KH₂PO₄, 0.5 g l⁻¹ NaCl, 4 g l⁻¹ glucose, 1 g l⁻¹ NH₄Cl, 10 ml l⁻¹ Gibco MEM vitamin solution, 1 mM MgSO₄ and 0.1 mM CaCl₂. The cells were resuspended in 5 ml M9 medium and then transferred into 500 ml M9 medium containing 100 μg ml⁻¹ ampicillin. The cell culture was incubated at 310 K and 210 rev min⁻¹. When the OD₆₀₀ reached 0.4, 50 mg lysine, phenylalanine and threonine, 25 mg isoleucine, leucine and valine and 30 mg l-selenomethionine were added. The culture was then induced with 1 mM IPTG after 15 min. Cells were harvested within 4 h and were frozen and stored at 193 K.

The purification procedures for native AcrR and SeMet-AcrR were the same. For purification of either the native or SeMet protein, cells were suspended in 40 ml ice-cold buffer containing 20 mM Na HEPES pH 7.2 and 200 mM NaCl. The cells were then lysed in a French pressure cell. Cell debris was removed by centrifugation for 45 min at 277 K and 20 000 rev min⁻¹. The crude lysate was filtered through a 0.2 μm membrane and was loaded onto a 5 ml Hi-Trap Ni²⁺-chelating column (Amersham Pharmacia Biosciences) which was pre-equilibrated with 20 mM Na HEPES pH 7.2 and 200 mM NaCl. To remove unbound proteins and impurities, the column was first washed with six column volumes of buffer containing 50 mM imidazole, 200 mM NaCl and 200 mM Na HEPES pH 7.2. The AcrR protein was then eluted with four column volume of buffer containing 400 mM imidazole, 200 mM NaCl and 20 mM Na HEPES pH 7.2. The purity of the protein was judged using 10% SDS–PAGE stained with

### Table 1

Data-collection and processing statistics for native and selenomethionyl-AcrR.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Native</th>
<th>Peak (λ₁)</th>
<th>Edge (λ₂)</th>
<th>Remote (λ₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (Å)</td>
<td>1.0020</td>
<td>0.9816</td>
<td>0.9818</td>
<td>0.9666</td>
</tr>
<tr>
<td>Space group</td>
<td>P₃₂</td>
<td>P₃₂</td>
<td>P₃₂</td>
<td>P₃₂</td>
</tr>
<tr>
<td>Unit-cell parameters (Å)</td>
<td>a (Å)</td>
<td>46.61</td>
<td>46.40</td>
<td>46.47</td>
</tr>
<tr>
<td>b (Å)</td>
<td>46.61</td>
<td>46.40</td>
<td>46.47</td>
<td>46.51</td>
</tr>
<tr>
<td>c (Å)</td>
<td>166.16</td>
<td>166.37</td>
<td>167.75</td>
<td>167.90</td>
</tr>
<tr>
<td>Resolution range (Å)</td>
<td>50–2.49</td>
<td>50–3.00</td>
<td>50–3.11</td>
<td>50–3.23</td>
</tr>
<tr>
<td>(2.59–2.49)</td>
<td>(3.11–3.00)</td>
<td>(3.22–3.11)</td>
<td>(3.35–3.23)</td>
<td></td>
</tr>
<tr>
<td>Total No. of reflections</td>
<td>471437</td>
<td>1541643</td>
<td>775457</td>
<td></td>
</tr>
<tr>
<td>No. of unique reflections</td>
<td>15849</td>
<td>7281</td>
<td>6497</td>
<td></td>
</tr>
<tr>
<td>Rmerge (%)</td>
<td>52.4 (24.7)</td>
<td>9.1 (18.8)</td>
<td>8.1 (19.8)</td>
<td>9.3 (19.9)</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>94.4 (90.0)</td>
<td>98.0 (87.1)</td>
<td>98.0 (87.1)</td>
<td>96.5 (78.4)</td>
</tr>
<tr>
<td>Average I/σ(I)</td>
<td>16.1 (3.9)</td>
<td>15.4 (7.1)</td>
<td>13.2 (3.1)</td>
<td>15.7 (3.1)</td>
</tr>
</tbody>
</table>

### Figure 1

\textit{E. coli} AcrR crystal. The dimensions of the crystal are approximately 200 × 100 × 100 μm.

### Figure 2

X-ray diffraction pattern of the native AcrR crystal. The crystal diffracted beyond a resolution of 2.5 Å.
Coomassie Brilliant Blue. The purified protein was extensively dialyzed against buffer containing 60 mM imidazole, 200 mM NaCl and 20 mM Tris pH 7.5 and was concentrated to 20 mg ml⁻¹. The 215-amino-acid AcrR contains seven methionines; the replacement of these methionine sulfur with selenium in the SeMet-AcrR protein was confirmed by MALDI time-of-flight mass spectrometry.

3. Crystallization, data collection and processing

The 6×His AcrR protein was crystallized in 24-well plates using the hanging-drop vapor-diffusion method at 293 K. The initial crystallization screening was performed using commercially available sparse-matrix screens (Jancarik & Kim, 1991) from Hampton Research. A 0.1 M drop consisting of 2 µl protein solution (20 mg ml⁻¹ AcrR in 20 mM Tris pH 7.5, 60 mM imidazole and 200 mM NaCl) and 2 µl well solution was equilibrated against 500 µl well solution. Crystals appeared within 3 d. After optimization, the best native 6×His AcrR crystals were obtained from well solution containing 32% PEG 4000, 0.2 M MgCl₂ and 0.1 M Tris buffer pH 8.5, with dimensions of about 200 × 100 × 100 µm. Fig. 1 illustrates a typical native crystal of the 6×His AcrR. SeMet crystals with approximate dimensions 120 × 80 × 80 µm were obtained from 29% PEG 4000, 0.2 M MgCl₂ and 0.1 M Tris buffer pH 8.5.

For data collection, a single native crystal was flash-cooled in a cryoprotectant solution containing 35% PEG 4000, 0.2 M MgCl₂ and 0.1 M Tris buffer pH 8.5 at 100 K. The best crystal diffracted to a maximum resolution of 2.5 Å (Table 1). Fig. 2 depicts one of the diffraction images of the native AcrR crystal. Multiple-wavelength anomalous diffraction (MAD) data were collected from a single SeMet-AcrR crystal to a maximum resolution of 3.0 Å (Table 1).

Diffraction data sets for both the native and SeMet-AcrR crystals were obtained at the Advanced Light Source (beamline 8.2.2) at cryogenic temperature (100 K) on an ADSC Quantum 315 CCD-based detector. The beam size was 140 × 150 µm. Diffraction data sets were processed with DENZO and scaled with SCALEPACK (Otwinowski & Minor, 1997). The native AcrR crystal belonged to space group P2₁₂₁ with unit-cell parameters a = b = 46.6, c = 166.2 Å. The SeMet-AcrR crystal belonged to the same space group, with very similar unit-cell parameters (Table 1). Based on the molecular weight of the protein (25.4 kDa, including the 6×His tag at the C-terminus) and the volume of the asymmetric unit, the Matthews parameters for one, two and three molecules of AcrR in the asymmetric unit were found to be 4.1, 2.0 and 1.4 Å³ Da⁻¹, respectively. The available structures of members of the TetR family of transcriptional repressors, including TetR (Hinrichs et al., 1994; Orth et al., 2000), QacR (Schumacher et al., 2001, 2002), CprB (Natsume et al., 2003) and EthR (Dover et al., 2004; Frenois et al., 2004), indicate that all these repressors assemble as dimers. This suggests the presence of two AcrR molecules per asymmetric unit, with a solvent content of 39.5%. Analysis of the structure is currently in progress.

We are grateful to Corie Ralston (Advanced Light Source, Lawrence Berkeley National Laboratory) for assistance during the X-ray diffraction experiment. This work was supported by NIH grant GM-074027 to EWY.

References


