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Purification, crystallization and preliminary X-ray diffraction studies of the arsenic repressor ArsR from *Corynebacterium glutamicum*

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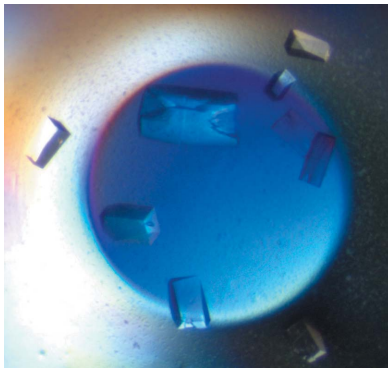

ArsR is a member of the SmtB/ArsR family of metalloregulatory proteins that regulate prokaryotic arsenic-resistance operons. Here, the crystallization and preliminary X-ray diffraction studies of a cysteine-free derivative of ArsR from *Corynebacterium glutamicum* (CgArsR-C15/16/55S) are reported. CgArsR-C15/16/55S was expressed, purified, crystallized and X-ray diffraction data were collected to 1.86 Å resolution. The protein crystallized in a tetragonal space group ($P4$), with unit-cell parameters $a = b = 41.84$, $c = 99.47$ Å.

1. Introduction

Arsenic is a toxic metalloid that is ubiquitous in the earth's crust. It is a human carcinogen (Sharma & Sohn, 2009) and is considered to be one of the most highly hazardous substances owing to the combination of its prevalence and severe health effects. Arsenic exposure is associated with dermal lesions, peripheral neuropathy, haematopoietic depression, anhydraemia, liver damage characterized by jaundice, portal cirrhosis and ascites, sensory disturbance and peripheral neuritis, anorexia and loss of weight (Duker *et al.*, 2005). Arsenic readily interacts with the sulfhydryl groups of proteins and of cofactors such as glutathione (GSH) and lipoic acid, leading to inhibition of the activity of many enzymes (Duker *et al.*, 2005). In addition to geochemical sources, anthropogenic activities add to environmental arsenic (Bhattacharya *et al.*, 2007). Many microbes are highly resistant to arsenic and can grow in arsenic-rich environments (Rensing & Rosen, 2009).

The mechanisms of arsenic resistance have been studied extensively in *Escherichia coli* (Wu & Rosen, 1993). The *ars* operon of *E. coli* plasmid R773 contains five genes *arsRDABC* that confer arsenic resistance, in which *arsR* encodes the negative regulator ArsR. It is a homodimeric winged-helix repressor of molecular weight 26 kDa (Wu & Rosen, 1991). In the absence of arsenic, ArsR binds to the *ars* promoter and prevents transcription. When ArsR is induced by binding arsenic(III) it dissociates from the DNA, allowing transcription to proceed. ArsR belongs to the SmtB/ArsR family of metalloregulatory proteins (Busenlehner *et al.*, 2003) that respond to transition metals, heavy metals and metalloids, including arsenic(III), antimony(III), cadmium(II), lead(II), zinc(II), cobalt(II) and nickel(II). A few structures of members of the ArsR/SmtB family of proteins have been determined, including the structures of the cadmium(II)/lead(II)/zinc(II)-responsive repressor CadC (Ye *et al.*, 2005) and the zinc repressors ZntR (Changela *et al.*, 2003) and SmtB (Eicken *et al.*, 2003). Recently, the structures of two members of the ArsR/SmtB family from *Rhodococcus* sp. RHA1 have been determined (PDB entries 3f6o and 3f6v; Midwest Center for Structural Genomics, unpublished work). However, these proteins lack the cysteine residues characteristics of arsenic(III)-responsive repressors and are probably not ArsR orthologues.

Ordóñez *et al.* (2005) investigated ArsR from the Gram-positive nonpathogenic soil bacterium *Corynebacterium glutamicum*. Wild-type CgArsR and its cysteine-free derivative (the cysteine residues at positions 15, 16 and 55 were mutated to serine residues) were cloned in pET28 and expressed in *E. coli* BL21 (DE3). EXAFS studies

reported that CgArsR has a three-sulfur (S3) coordination site for binding trivalent inorganic arsenic. There are three cysteine residues in CgArsR: Cys15, Cys16 and Cys55. Mutational analysis demonstrated that all three cysteine residues contribute to forming the S3 arsenic(III)-binding site (Ordóñez *et al.*, 2008). The results of cross-linking studies and homology modelling based on the CadC structure (Ordóñez *et al.*, 2008) suggested that Cys15 and Cys16 from one subunit and Cys55 from the other subunit form the arsenic-binding site. We have undertaken X-ray crystallographic studies to experimentally determine the structure of CgArsR and to characterize the inducer-binding sites. In this paper, we report the expression, purification, crystallization and preliminary X-ray studies of the triple cysteine mutant CgArsR-C15/16/55S from *C. glutamicum*.

2. Materials and methods

2.1. Expression and purification of CgArsR-C15/16/55S

The construction of clones and plasmids of the CgArsR cysteine-free mutant with a C-terminal hexahistidine tag has been described previously (Ordóñez *et al.*, 2008). *E. coli* strain BL21 (DE3) transformed with pETCgArsR-C15/16/55S was grown in Luria–Bertani (LB) medium with 30 µg ml^{-1} kanamycin overnight at 310 K. The overnight culture was diluted 1:100 in 1 l LB medium with the same concentration of kanamycin and grown until the OD_{600} reached 0.6. To induce expression, 0.3 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG) was added to the culture and growth continued for a further 4 h. IPTG-induced cells were centrifuged at 9000*g* for 5 min at 277 K. The pellet was washed with 50 m*M* 3-(*N*-morpholino)propanesulfonic acid (MOPS)–KOH pH 7.5 buffer containing 20%(*v*/*v*) glycerol, 0.5 *M* NaCl, 20 m*M* imidazole and 10 m*M* *β*-mercaptoethanol (buffer *A*). A Complete protease-inhibitor cocktail tablet (Roche Diagnostics India Pvt Ltd, India) was added to the cells prior to lysis by passing them twice through a French press at 138 MPa. The lysed cells were centrifuged at 60 000*g* for 30 min. The supernatant solution was applied at a flow rate of 0.5 ml min^{-1} onto a column containing 5 ml nitrilotriacetic acid saturated with nickel (Ni–NTA) that had been pre-equilibrated with buffer *A*. The column was then washed with several column volumes of buffer *A* to remove unbound protein. The protein was eluted at a flow rate of 0.5 ml min^{-1} with a step gradient of buffer *A* containing 0.02, 0.1, 0.13 and 0.2 *M* imidazole. The eluted protein was collected in 1.5 ml fractions, with 5 m*M* EDTA and 2 m*M* DTT pre-added to the collection vials. The fractions were analyzed by SDS–PAGE on a 16% acrylamide gel. A portion of the purified protein that eluted at 0.2 *M* imidazole was further purified by gel filtration using an S200 column with buffer *A* lacking imidazole at a flow rate of 0.4 ml min^{-1}. The eluted protein was again analyzed by SDS–PAGE. The purified protein was concentrated using an Amicon Ultra centrifugal filter (10 kDa molecular-weight cutoff). 1 l bacterial culture yielded about 40 mg CgArsR-C15/16/55S. The concentrated protein was buffer-exchanged to 50 m*M* MOPS pH 7.5, 0.5 *M* NaCl with 20% glycerol and stored at 193 K until further study. The protein samples that eluted at 0.13 and 0.2 *M* imidazole and protein that was subjected to further purification by gel filtration were individually screened for crystallization.

2.2. Crystallization of CgArsR-C15/16/55S

Initial crystallization screens were performed using PEG/Ion and Index screens from Hampton Research using the microbatch method. Two different protein concentrations (50 and 30 mg ml^{-1}) were used. 2 µl protein solution was mixed with an equal volume of screen reagent after exchanging the storage buffer to 50 m*M* MOPS pH 7.5 or 50 m*M* HEPES pH 7.5 or 50 m*M* bis-tris pH 7.5 with 0.1 *M* NaCl in various trials. Small crystals grew after 7 d from trials using the protein batch eluted with 0.13 *M* imidazole buffer and exchanged into 50 m*M* MOPS pH 7.5 and 0.1 *M* NaCl. Crystals grew in a drop consisting of 0.2 *M* ammonium iodide, 20%(*w*/*v*) PEG 3350 (reagent No. 12 of PEG/Ion screen from Hampton Research). To optimize crystal growth, the trials were repeated with different protein concentrations (65, 52, 39, 26 and 13 mg ml^{-1}) and/or different volume ratios of protein solution to screen solution. Diffraction-quality crystals of dimensions 0.2 × 0.1 × 0.05 mm (Fig. 1) were obtained in 10 d from a drop consisting of 52 mg ml^{-1} CgArsR-C15/16/55S in 50 m*M* MOPS pH 7.5 and 0.1 *M* NaCl, 0.2 *M* ammonium iodide, 20% PEG 3350.

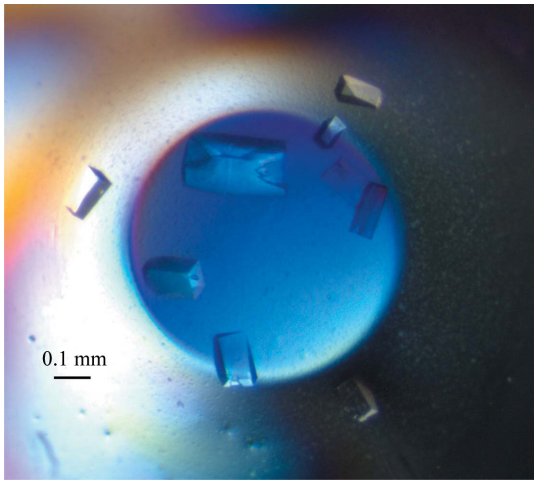

Figure 1
Crystals of CgArsR-C15/16/55S grown in the presence of 0.2 *M* ammonium iodide, 20% PEG 3350.

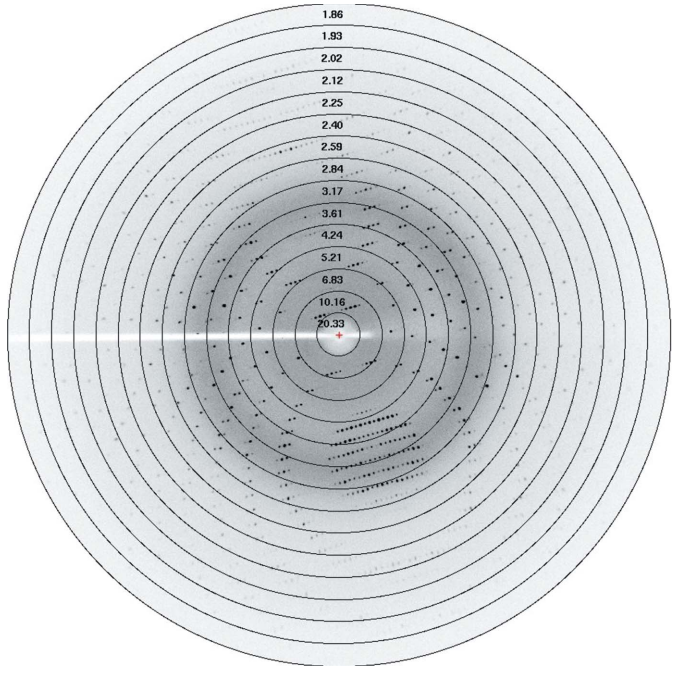

Figure 2
X-ray diffraction pattern of a CgArsR-C15/16/55S crystal. The crystal diffracted to 1.86 Å resolution.

Table 1
X-ray data statistics.

Values in parentheses are for the last shell.

Space group	*P*4
Unit-cell parameters (Å)	$a = b = 41.84$, $c = 99.47$
Wavelength (Å)	1.5418
Resolution (Å)	1.86 (1.93–1.86)
Completeness (%)	96.6 (80.3)
No. of unique reflections	13912
Multiplicity	2.9 (2.1)
Mean $I/\sigma(I)$	7.9 (2.8)
V_M† (Å^3 Da^{-1})	3.25
Solvent content† (%)	62.2
R_{merge}‡ (%)	7.9 (28.6)
R_{meas}§ (%)	11.2 (40.4)
$R_{p.i.m.}$¶ (%)	7.9 (28.5)

† Assuming the presence of one monomer in the asymmetric unit. ‡ $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$. § $R_{meas} = \sum_{hkl}[N/(N-1)]^{1/2} \times \sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$. ¶ $R_{p.i.m.} = \sum_{hkl}[1/(N-1)]^{1/2}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and $\langle I(hkl)\rangle$ is the average intensity of all observations of reflection hkl.

2.3. X-ray data collection and data processing

The X-ray diffraction experiments were carried out using the DBT Macromolecular X-ray diffraction facility at the School of Biotechnology, Madurai Kamaraj University, India. The X-ray diffractometer is equipped with a Bruker rotating-anode generator that produces Cu $K\alpha$ radiation and a MAR 345 image-plate detector. A single crystal of CgArsR-C15/16/55S of dimensions $0.2 \times 0.1 \times 0.05$ mm was soaked in a solution consisting of 15% glycerol and 23% PEG 3350 for 30 s and tested for X-ray diffraction. The crystal diffracted to a maximum resolution of 1.86 Å at 100 K (Fig. 2). 75 frames of diffraction data were collected at a crystal-to-detector distance of 150 mm with 1° φ oscillation and 60 s exposure time. The collected data set was processed using the *automar* software suite (Klein & Bartels, 2009).

3. Results and discussion

A C-terminally His-tagged triple cysteine mutant (C15/16/55S) of CgArsR from *C. glutamicum* was expressed in *E. coli* BL21 (DE3) and purified. The purified protein was screened for crystallization using commercial screens. Initial crystal hits obtained using the PEG/Ion screen from Hampton Research were optimized to grow larger diffraction-quality crystals. Diffraction-quality crystals were obtained in 10 d from a drop consisting of 52 mg ml^{-1} CgArsR-C15/16/55S in 50 m*M* MOPS pH 7.5 buffer containing 0.1 *M* NaCl, 0.2 *M* ammonium iodide and 20% PEG 3350. X-ray diffraction data were collected to a maximum resolution of 1.86 Å and processed using the *automar* software suite (Klein & Bartels, 2009). The data statistics are given in Table 1. Attempts to solve the structure by molecular replacement using the structure of the cadmium repressor CadC (PDB entry 1u2w; Ye *et al.*, 2005), the zinc repressor ZntR (PDB entry 1r1t; Eicken *et al.*, 2003) and other known structures of the SmtB/ArsR family of proteins as a template are in progress.

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