Crystallization and preliminary X-ray diffraction analysis of the arginine repressor ArgR from *Bacillus halodurans*

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Received 8 November 2014

Accepted 15 January 2015

Keywords: ArgR; transcriptional regulator; dehydration.

The arginine repressor (ArgR) is a transcriptional regulator which regulates genes encoding proteins involved in arginine biosynthesis and the arginine catabolic pathway. ArgR from the alkaliphilic bacterium *Bacillus halodurans* was cloned and overexpressed in *Escherichia coli*. ArgR (Bh2777) from *B. halodurans* is composed of 149 amino-acid residues with a molecular mass of 16 836 Da. ArgR was crystallized at 296 K using 1,2-propanediol as a precipitant. Crystals of N-terminally His-tagged ArgR were obtained by the sitting-drop vapour-diffusion method. Dehydrated crystals showed a dramatic improvement in diffraction quality and diffracted to 2.35 Å resolution. The crystals belonged to the cubic space group *I*23, with unit-cell parameters $a = b = c = 104.68$ Å. The asymmetric unit contained one monomer of ArgR, which generates a trimer by the threefold axis of the space group, giving a crystal volume per mass (V_M) of $2.98 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 56.8%.

1. Introduction

The arginine-metabolism pathway is controlled by an arginine repressor (ArgR) in bacteria. ArgR regulates the transcription of nearby genes in the arginine-biosynthesis regulon in the presence of L-arginine (Maas, 1994). ArgR from *Bacillus subtilis* and *Salmonella typhimurium* also activates the transcription of genes involved in arginine catabolism (Smith *et al.*, 1986; Lu *et al.*, 1992). ArgR binds to DNA operators called ARG-box sequences in the promoter regions of the arginine-biosynthesis and arginine-catabolism genes in the presence of elevated arginine concentrations (Charlier *et al.*, 1992).

Several structures of ArgR homologues from bacterial species including *Escherichia coli*, *B. subtilis*, *B. stearothermophilus* and *Mycobacterium tuberculosis* and their DNA complexes have been determined (Garnett *et al.*, 2008; Tian & Maas, 1994; Cherney *et al.*, 2008; Ni *et al.*, 1999; Van Duyne *et al.*, 1996). Biochemical and structural analysis revealed that the ArgR monomer consists of two domains separated by a protease-accessible linker: an N-terminal winged helix–turn–helix DNA-binding domain and a C-terminal core domain which is responsible for oligomerization and arginine binding (Czaplewski *et al.*, 1992; Sunnerhagen *et al.*, 1997). ArgR monomers assemble into either trimers or hexamers, being controlled by the protein concentration and the presence of the arginine corepressor. In the presence of arginine, two trimers of ArgR dimerize with approximate 32 symmetry and the hexameric form of ArgR can bind to whole ARG boxes, while trimeric ArgR still binds to half of the ARG box in the absence of arginine (Miller *et al.*, 1997; Cherney *et al.*, 2008).

The ArgR homologue (Bh2777) from *B. halodurans* was identified and encodes a protein of 149 amino-acid residues

with 72% sequence identity to ArgR from *B. subtilis*. Further sequence comparisons of *B. halodurans* ArgR with other ArgR proteins from *B. stearothermophilus*, *E. coli* and *M. tuberculosis* showed 73, 28 and 33% sequence identity, respectively. Although crystal structures of ArgR from bacterial species have been reported, the biological roles of the oligomeric forms of ArgR are not entirely clear. It would be attractive to determine the apo structure of the ArgR orthologue from *B. halodurans*. As a first step, we here report the cloning, purification, crystallization and preliminary X-ray diffraction data of *B. halodurans* ArgR.

2. Materials and methods

2.1. Macromolecule production

The gene encoding ArgR (Bh2777) was amplified by the polymerase chain reaction using the genomic DNA of *B. halodurans* as a template. It was inserted into the NdeI/XhoI-digested expression vector pET-28b(+) (Novagen, Germany), resulting in a 20-residue hexahistidine-containing tag at its N-terminus. The recombinant *B. halodurans* ArgR protein was expressed in *E. coli* BL21(DE3) Star pLysS cells (Invitrogen, USA). The cells were grown at 310 K to an OD₆₀₀ of ~0.6 in Luria–Bertani medium containing 30 mg ml⁻¹ kanamycin and chloramphenicol. Protein expression was induced by 1.0 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). Cell growth was continued at 303 K for 4 h after IPTG induction and the cells were harvested by centrifugation at 7000g for 10 min at 277 K.

The cell pellet was resuspended in lysis buffer [20 mM Tris–HCl pH 8.0, 0.5 M NaCl, 10%(v/v) glycerol, 1 mM phenylmethylsulfonyl fluoride] and was then homogenized using an ultrasonic processor. The insoluble fraction including cellular debris was removed by centrifugation at 36 000g for 60 min at 277 K and the recombinant protein in the supernatant fraction was purified by two chromatographic steps. The first step was metal-chelate chromatography on Ni–NTA resin (GE Healthcare, UK). The His-tagged ArgR protein was eluted with buffer A [20 mM Tris–HCl pH 8.0, 0.5 M NaCl, 10%(v/v) glycerol] containing 300 mM imidazole and the eluted sample was concentrated. The next step was gel filtration on a

Figure 1

Purification of *B. halodurans* ArgR. Elution fractions from gel-filtration chromatography on a Superdex 200 column were loaded onto 12% SDS–PAGE and the gel was stained with Coomassie Blue. The left lane contains prestained protein ladder marker (labelled in kDa). The other lanes contain His-tagged *B. halodurans* ArgR (16.8 kDa).

Table 1

Macromolecule-production information.

Source organism	<i>B. halodurans</i>
DNA source	Genomic DNA from <i>B. halodurans</i>
Forward primer	CGCGGATCCATGAATAAAGGACAGCGACATAT
Reverse primer	CGCCTCGAGCTATAACATGTTAAAAATCGCTC
Expression vector	pET-28b(+)
Expression host	<i>E. coli</i> BL21(DE3) Star pLysS
Complete amino-acid sequence of the construct produced	MGSSHHHHHSSGLEVLFGQPHMKGQRHKIRE– TIANDVETQDELVEQLKAAGYNVTQATVSRD– IKELHLVKVPMDGRYKSLPADQRFNPLQKL– KRGLVDSFVSIIDRTDNLIVMKTLPNGAHAIGA– LIDNLDWTEIMGTICGDDTILICKDKQDGPV– VTERFLNML

Table 2

Crystallization.

Method	Sitting-drop vapour diffusion
Plate type	96-well CrystalQuick plate
Temperature (K)	296
Protein concentration (mg ml ⁻¹)	40
Buffer composition of protein solution	0.2 M NaCl, 10 mM Tris–HCl pH 7.5, 1 mM DTT, 2 mM MgCl ₂ , 5%(v/v) glycerol
Composition of reservoir solution	5%(v/v) 1,2-propanediol, 0.1 M bicine pH 8.0
Volume and ratio of drop	2.0 µl, 1:1
Volume of reservoir (µl)	80

Superdex 200 column (GE Healthcare, UK), employing an elution buffer consisting of 0.2 M NaCl, 10 mM Tris–HCl pH 7.5, 1 mM DTT, 2 mM MgCl₂, 5%(v/v) glycerol. The purified protein was concentrated to 40 mg ml⁻¹ using a Centricon YM-10 (Millipore) and aliquots of the protein were stored at 193 K. Macromolecule-production information is summarized in Table 1.

2.2. Crystallization

Initial crystallization was performed by the sitting-drop vapour-diffusion method using various commercial screening solution kits (Hampton Research, USA; Qiagen, Germany; Axygen, USA; Emerald Bio, USA) at 296 K. Crystals were obtained using 96-well CrystalQuick plates (Greiner Bio-One, Germany), and each crystallization drop was prepared by mixing 1.0 µl reservoir solution and 1.0 µl protein solution. Crystals were initially obtained in several conditions containing polyethylene glycol and alcohols, which were further optimized to 5%(v/v) 1,2-propanediol, 0.1 M bicine pH 8.0. Crystallization information is summarized in Table 2.

2.3. Data collection and processing

Prior to data collection, crystals were dehydrated by adding a cryoprotectant solution consisting of 50%(v/v) glycerol at 296 K for 7 h. After dehydration, the crystals were flash-cooled in liquid nitrogen. X-ray diffraction data were collected at 100 K on an ADSC Quantum 270 CCD image-plate detector using synchrotron radiation on beamline 7A at Pohang Accelerator Laboratory (PAL), Pohang, Republic of Korea. The data were collected using a 1° oscillation per image with a crystal-to-detector distance of 270 mm. The crystal was

exposed to X-rays for 3 s per image and a total of 360 frames were recorded. Data were processed with *MOSFLM* using the *iMosflm* interface (Leslie, 2006) and were scaled with *SCALA* (Evans, 2006) using the *CCP4i* interface (Potterton *et al.*, 2003).

3. Results and discussion

The recombinant *B. halodurans* ArgR protein was expressed in an *E. coli* system and purified to give a final yield of ~60 mg per litre of culture. The protein was purified by two steps of chromatography to homogeneity as estimated by electrophoresis using 12% SDS-PAGE (Fig. 1). The crystals (Fig. 2) initially diffracted to ~10 Å resolution using several cryoprotectant solutions (Fig. 3*a*) and diffraction was subsequently improved by the dehydration method (Heras *et al.*, 2003). We observed an intermediate improvement (~4 Å resolution) in

Figure 2

Cubic crystals of *B. halodurans* ArgR. The crystals with the best quality were obtained in 5% (v/v) 1,2-propanediol, 0.1 M bicine pH 8.0 using the sitting-drop vapour-diffusion method at 296 K. Their approximate dimensions are $0.3 \times 0.3 \times 0.2$ mm.

Table 3

Data collection and processing.

Values in parentheses are for the outer shell.

Diffraction source	Beamline 7A, PAL
Wavelength (Å)	0.9793
Temperature (K)	100
Detector	ADSC Quantum 270 CCD
Crystal-to-detector distance (mm)	270
Rotation range per image (°)	1.0
Total rotation range (°)	360
Exposure time per image (s)	3
Space group	<i>I</i> 23
<i>a</i> , <i>b</i> , <i>c</i> (Å)	104.68, 104.68, 104.68
α , β , γ (°)	90.0, 90.0, 90.0
Mosaicity (°)	1.28
Resolution range (Å)	50.0–2.35 (2.48–2.35)
Total No. of reflections	324217 (43376)
No. of unique reflections	8128 (1171)
Completeness (%)	100 (100)
Multiplicity	39.9 (37.9)
$\langle I/\sigma(I) \rangle$	44.3 (12.1)
$R_{\text{r.i.m.}}$ (%)	7.8 (38.4)
Overall <i>B</i> factor from Wilson plot (Å ²)	28.7

the diffraction of the dehydrated crystal using a cryoprotectant solution with glycerol by incubation in air for 10 min to 2 h. The final optimized dehydration condition consisted of adding 15 µl 50% (v/v) glycerol directly to the drop solution and incubating in air for 7 h at 295 K. The dehydrated crystals showed a dramatic improvement in diffraction quality and diffracted to 2.35 Å resolution (Fig. 3*b*).

A total of 8128 unique reflections were measured with a multiplicity of 39.9. The merged data set was 100% complete to 2.35 Å resolution and gives an R_{merge} (on intensities) of 7.7%. The crystals belonged to the cubic space group *I*23, with unit-cell parameters $a = b = c = 104.68$ Å. The asymmetric unit contained one monomer of ArgR, which leads to the trimer by the threefold axis of the space group, giving a crystal volume per mass (V_M) of $2.98 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 56.8%. The statistics of X-ray data collection are summarized in Table 3.

Figure 3

X-ray diffraction images from a crystal of *B. halodurans* ArgR. (*a*) Initial diffraction pattern measured from a *B. halodurans* ArgR crystal without dehydration. (*b*) The *B. halodurans* ArgR crystal after dehydration using 50% (v/v) glycerol diffracted to 2.35 Å resolution on beamline 7A at Pohang Accelerator Laboratory. Diffraction spots in the outer resolution shell are indicated in the box.

Molecular replacement was attempted using the *B. stearothermophilus* ArgR structure (PDB entry 1b4a, 73% sequence identity; Ni *et al.*, 1999) with *Phaser* (McCoy *et al.*, 2007). Molecular replacement was not successful when the whole chain of the model containing both the N-terminal and C-terminal domains (residues 4–149) was used as a search model. Two separate models of ArgR consisting of the N-terminal domain (residues 4–65) and the C-terminal domain (residues 68–149) were successfully located in the asymmetric unit, with the top solution having a log-likelihood gain of 209.2 and an *R* value of 53.6, and model building and structure refinement are under way.

Acknowledgements

We thank the staff members at Pohang Accelerator Laboratory beamline 7A for their help with data collection. This research was supported by the Agriculture Research Center (ARC; 710003-03-1-SB110) program of the Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

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