Purification, crystallization and preliminary X-ray diffraction analysis of ArsH from *Synechocystis* sp. strain PCC 6803

Xiao Zhang,^{a,b} Xi-Mei Xue,^{a,b} Yu Yan^{a,b} and Jun Ye^{a*}

^aKey Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, 1799 Jimei Road, Xiamen 361021, People's Republic of China, and

^bUniversity of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

Correspondence e-mail: jye@iue.ac.cn

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ArsH is an NADPH-dependent flavin mononucleotide reductase and is frequently encoded as part of an *ars* operon. The function of the *arsH* gene remains to be characterized. Crystallization and structural studies may contribute to elucidating the specific biological function of ArsH associated with arsenic resistance. ArsH from *Synechocystis* sp. strain PCC 6803 was overproduced, purified and crystallized. Crystals were obtained by the sitting-drop vapour-diffusion method. Diffraction data were collected and processed to a resolution of 1.6 Å. The crystals belonged to the tetragonal space group $I4_122$, with unit-cell parameters $a = b = 127.94$, $c = 65.86$ Å and one molecule in the asymmetric unit. Size-exclusion chromatography and molecular-replacement results showed that the ArsH formed a tetramer. Further structural analysis and comparison with ArsH from *Sinorhizobium meliloti* will provide information about the oligomerization of ArsH.

1. Introduction

Arsenic is one of the most toxic substances in the environment. It can exist as a variety of species such as arsenate [arsenic(V)], arsenite [arsenic(III)] and organic arsenicals. Nearly all bacteria and archaea harbour the arsenic-resistance (*ars*) operon which encodes at least three components: ArsR, ArsC and ArsB (Qin *et al.*, 2006; Bhattacharjee & Rosen, 2007); among these ArsR is a repressor protein acting as a negative regulator of the *ars* operon, ArsB is an arsenite-specific efflux pump protein and ArsC is an enzyme that reduces arsenic(V) to arsenic(III). In *Synechocystis* and other organisms, ArsH is also part of the *ars* operon (López-Maury *et al.*, 2003; Chang *et al.*, 2007; Cai *et al.*, 2009).

The function of the *arsH* gene remains to be characterized. ArsHs from *Yersinia enterocolitica*, *Sinorhizobium meliloti*, *Ochrobactrum tritici* SCII24 and *Lactobacillus acidophilus* confer resistance to arsenicals (Neyt *et al.*, 1997; Yang *et al.*, 2005; Branco *et al.*, 2008; Sinha *et al.*, 2011), while ArsH from *A. ferrooxidans* or *Synechocystis* shows no arsenic resistance (Butcher *et al.*, 2000).

The structural analyses of ArsH from *S. meliloti* and an apo form of ArsH from *S. flexneri* (Vorontsov *et al.*, 2007; Ye *et al.*, 2007), as well as enzymatic assays and protein sequence analysis, have demonstrated that ArsH is a member of the NADPH-dependent FMN (flavin mononucleotide) reductase family. It has been demonstrated that ArsH from *S. meliloti* acts as an azoreductase, while ArsH from *A. ferrooxidans* has ferric reductase activity and ArsH from *Synechocystis* is a quinone reductase (Mo *et al.*, 2011; Hervás *et al.*, 2012). To date, there is no direct biochemical evidence to show how ArsH confers arsenic resistance. Since ArsH is part of the *ars* operon and is widely distributed in bacteria, we hypothesize that ArsH provides a specific biotransformation mechanism in arsenic resistance.

The ArsH protein from *Synechocystis* sp. strain PCC 6803 has 74% sequence identity to ArsH from *S. meliloti* which crystallizes as a tetramer. It has been proposed that the termini play an important role in tetramer formation, and that tetramer formation may be needed for functioning. The salt bridges or hydrogen bonds between helix $\alpha 5$ and the terminal residues of another subunit may be essential for tetramer formation. However, *Synechocystis* ArsH is 27 residues shorter at the N-terminus compared with *S. meliloti* ArsH. This raises

the question of whether the *Synechocystis* ArsH still forms a tetramer. If not, will this affect its function?

In this study, we report the overproduction, purification, crystallization and preliminary X-ray studies of ArsH from the widely used experimental model organism *Synechocystis* sp. strain PCC 6803. ArsH is a 206-residue protein with a mass of 23.7 kDa. Diffraction data were collected to a resolution of 1.6 Å.

2. Materials and methods

2.1. Overproduction and purification

The *arsH* gene from the cyanobacterium *Synechocystis* sp. PCC 6803 was amplified by PCR, cloned into the plasmid pET-22b and then expressed with a 6×His tag in the *Escherichia coli* strain Rosetta. Cells were grown overnight at 16°C after adding 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at an OD₆₀₀ of 0.6–0.8

to induce the overproduction of ArsH. Cells were harvested by centrifugation at 4000g and 4°C for 15 min and resuspended in buffer A (20 mM HEPES, 500 mM NaCl pH 7.2). After sonication, the lysate was centrifuged at 13 000g and 4°C for 1 h. The supernatant was loaded onto an Ni²⁺-charged resin (GE Healthcare), washed with 20 mM imidazole in buffer A and then eluted with 15 ml buffer A containing 500 mM imidazole. ArsH was further purified by size-exclusion chromatography using a HiLoad16/600 Superdex 75 prep-grade column (GE Healthcare) equilibrated with buffer A. The elution volume of ArsH was then compared with the elution profile of molecular-mass standards to investigate the possibility of oligomerization (Fig. 1). The purified ArsH was analyzed by SDS-PAGE (Fig. 2). The purified protein was concentrated using an Amicon Ultra centrifugal filter (10 kDa molecular-mass cutoff).

2.2. Crystallization

Purified ArsH was concentrated to 15 mg ml⁻¹ in buffer A for crystallization. Crystallization experiments were set up in 96-well plates at 18°C using the sitting-drop vapour-diffusion method by mixing equal volumes of protein solution and reservoir solution (0.5 µl of each) and equilibrating against 50 µl reservoir solution from a variety of crystallization screens from Hampton Research, Molecular Dimensions and Emerald Bio. After 3 d of incubation, crystals appeared in several conditions from Wizard, Index, Salt and BioXtal, and the best diffraction-quality crystals (Fig. 3) with maximum dimensions of 0.2 × 0.15 × 0.15 mm were obtained from condition No.3 of BioXtal (1.5 M ammonium sulfate, 0.1 M MES pH 6.5).

2.3. Data collection and processing

Crystals were cryoprotected by soaking in well solution supplemented with 20% glycerol and then flash-cooled in liquid nitrogen before data collection. Diffraction data were collected at a wavelength of 0.9718 Å at -173°C on beamline BL17U of the Shanghai Synchrotron Research Facility (SSRF). A total of 360 images were collected with a crystal-to-detector distance of 200 mm and 0.5° oscillation range. Data were indexed and integrated using *iMosflm*

Figure 1
Analytical size-exclusion chromatography of purified ArsH on a HiLoad 16/600 Superdex 75 prep-grade column. The standard curve for molecular mass is shown. V_e of ArsH is 58 ml.

Figure 2
The SDS-PAGE profile of ArsH is shown. Lane 1, molecular-mass markers (labelled in kDa). Lane 2, whole cells after induction by IPTG. Lanes 3 and 4, purified protein after Ni-NTA chromatography and size-exclusion chromatography, respectively. The arrow indicates the position of ArsH.

Figure 3
Crystals of ArsH grown using 1.5 M ammonium sulfate, 0.1 M MES pH 6.5 as a crystallizing agent (maximum dimensions 0.2 × 0.15 × 0.15 mm).

Table 1

Data-collection and processing statistics for ArsH.

Values in parentheses are for the highest resolution shell.

Diffraction source	SSRF
Wavelength (Å)	0.9718
Crystal-to-detector distance (mm)	200
Resolution (Å)	35.8–1.60 (1.63–1.60)
Space group	$I4_122$
Unit-cell parameters (Å)	$a = b = 127.94$, $c = 65.86$
Total No. of reflections	274360 (14652)
Total No. of unique reflections	36159 (1883)
Multiplicity	7.6 (7.8)
Solvent content (%)	56.72
$R_{\text{merge}}^{\dagger}$ (%)	8.5 (36.7)
Completeness (%)	99.9 (100)
$\langle I/\sigma(I) \rangle$	14.2 (4.8)

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the i th observation of unique reflection hkl and $\langle I(hkl) \rangle$ is the mean recorded intensity of reflection hkl over multiple recordings.

(Battye *et al.*, 2011) and scaled and merged using *SCALA* (Potterton *et al.*, 2002).

2.4. Molecular replacement

The structure of *Synechocystis* ArsH was determined by the molecular-replacement method using *AMoRe* (Navaza, 2001) from *CCP4* (Winn *et al.*, 2011) and the published crystal structure of the ArsH protein from *S. meliloti* (PDB entry 2q62; Ye *et al.*, 2007) as a search model.

3. Results and discussion

A C-terminally His-tagged ArsH from *Synechocystis* was over-produced in *E. coli* Rosetta strain and purified, yielding approximately 20 mg protein per litre of culture. The purified protein was tested for crystallization using a variety of commercial screens.

Figure 4
X-ray diffraction image from an ArsH crystal.

Diffraction-quality crystals were obtained in 3 d from a drop consisting of 15 mg ml^{−1} protein, 1.5 M ammonium sulfate, 0.1 M MES pH 6.5. X-ray diffraction data were collected to a resolution of 1.6 Å (Fig. 4). The data-collection statistics are provided in Table 1. The ArsH crystals belonged to space group $I4_122$, with unit-cell parameters $a = b = 127.94$, $c = 65.86$ Å. Assuming the presence of a single molecule of ArsH in the asymmetric unit, a V_M of 2.84 Å³ Da^{−1} and a solvent content of 56.72% were calculated (Matthews, 1968). Using one molecule from the crystal structure of *S. meliloti* ArsH as a template, molecular replacement at 3.0 Å resolution yielded a solution with a correlation coefficient of 49.6% and an initial R factor of 45.4%. The model obtained by molecular replacement showed reasonable crystal packing and good electron density. Further refinement of the structure is in progress.

Size-exclusion chromatography of the purified *Synechocystis* ArsH resulted in a single peak at 58 ml. Comparison with molecular-mass standards yielded a molecular mass of 95 kDa (Fig. 1), while SDS-PAGE indicated that the monomer molecular mass was about 24 kDa (Fig. 2). Thus, ArsH probably exists as a tetramer in solution. In agreement with a tetramer configuration, the molecular-replacement solution also showed that ArsH formed a tetramer by four crystallographic related molecules in the crystal structure. These four monomers showed crystallographic 222 symmetry, while the tetramer of *S. meliloti* ArsH shows noncrystallographic 222 symmetry (Ye *et al.*, 2007). It has been previously proposed that the termini may play a role in maintaining the tetramer configuration, which might be functionally relevant (Ye *et al.*, 2007). Our preliminary results showed that the *Synechocystis* ArsH still formed a tetramer, despite being 27 amino acids shorter at the N-terminus than the *S. meliloti* ArsH. The *Synechocystis* ArsH structure may also shed light on the detailed mechanism involved in arsenic biotransformation.

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