Crystallization and preliminary X-ray crystallographic studies of the ArsI C–As lyase from *Thermomonospora curvata*

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Arsenic is a ubiquitous and carcinogenic environmental element that enters the biosphere primarily from geochemical sources, but also through anthropogenic activities. Microorganisms play an important role in the arsenic biogeochemical cycle by biotransformation of inorganic arsenic into organic arsenicals and *vice versa*. ArsI is a microbial nonheme ferrous-dependent dioxygenase that transforms toxic methylarsonous acid to the less toxic inorganic arsenite by C–As bond cleavage. An ArsI ortholog from the thermophilic bacterium *Thermomonospora curvata* was expressed, purified and crystallized. The crystals diffracted to 1.46 Å resolution and belonged to space group $P4_32_12$ or its enantiomer $P4_12_12$, with unit-cell parameters $a = b = 42.2$, $c = 118.5$ Å.

1. Introduction

Arsenical compounds are the most toxic and carcinogenic substances and rank first on the US Environmental Protection Agency's (EPA) Superfund List of Hazardous Substances (<http://www.atsdr.cdc.gov/SPL/index.html>). They enter the biosphere primarily from geochemical sources such as volcanic emissions and also from anthropogenic sources such as monosodium methylarsonic acid [MSMA or MAs(V)], which has been used as a herbicide on golf courses in Florida (Whitmore *et al.*, 2008) and on agricultural crops such as cotton (Bednar *et al.*, 2002).

Microorganisms play an important role in the biogeochemical cycling of arsenic by biotransformation of inorganic arsenic into organic arsenic species and degradation of organoarsenicals back to inorganic arsenic. For example, prokaryotic and eukaryotic microbes methylate arsenite [As(III)] by ArsM [As(III) S-adenosylmethionine methyltransferase] to methylarsonic acid [MAs(V)], dimethylarsinic acid [DMAs(V)], trimethylarsine oxide [TMAs(V)O] and the nontoxic trivalent trimethylarsine [TMAs(III)] (Challenger, 1951; Cullen, 2005; Qin *et al.*, 2006, 2009). In the reverse direction, microbes demethylate the noncarcinogenic organoarsenical herbicide MSMA to the more carcinogenic inorganic As(III), which increases environmental exposure to this human carcinogen. Demethylating microbes have been isolated from soil and lake water (Von Endt *et al.*, 1968; Maki *et al.*, 2004; Maki, Takeda *et al.*, 2006; Maki, Watarai *et al.*, 2006). In a few cases, specific microorganisms such as *Mycobacterium neoaurum* (Lehr *et al.*, 2003), Montrachet wine yeast (Crecelius, 1977) and *Candida humicola* (Cullen *et al.*, 1979) have been associated with demethylation.

A novel pathway for MSMA degradation isolated from Florida golf course soil has two steps catalyzed by microbial communities; the first step is the reduction of MAs(V) to MAs(III) (methylarsonous acid) and the second step is the demethylation of MAs(III) to As(III) (Yoshinaga *et al.*, 2011). Recently, we identified the gene responsible for the second step, MAs(III) demethylation, from the MAs(III)-demethylating isolate *Bacillus* sp. MD1 (Yoshinaga & Rosen, 2014). *arsI* (for arsenic-inducible gene; Zhang *et al.*, 2009) encodes a nonheme ferrous-dependent extradiol dioxygenase with C–As lyase activity. Knowledge of the three-dimensional structure of this novel enzyme is necessary to understand the catalytic mechanism of C–As bond cleavage. We crystallized an ArsI ortholog (GenBank accession No. ACY99683.1) from the thermophilic Gram-positive bacterium

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Thermomonospora curvata DSM 43183 (TcArsI). Here, we report the crystallization, X-ray data collection and preliminary crystallographic analysis of TcArsI.

2. Materials and methods

2.1. Reagents

All chemicals were obtained from Sigma–Aldrich (St Louis, Missouri, USA) unless otherwise mentioned.

2.2. Cloning, expression and purification

Sequence alignment of TcArsI orthologs from various species revealed that the C-terminal regions are not conserved, and an ArsI construct of *Bacillus* sp. MD1 (B-ArsI) with a C-terminal truncation maintained catalytic activity (Yoshinaga & Rosen, 2014). There are two cysteine pairs at the nonconserved C-terminus that might prevent crystallization by forming intramolecular and/or intermolecular disulfide bonds. For this reason, a gene lacking the nonconserved C-terminus [25 residues from Thr124 to the end (Cys151)], designated *TcarsI*₁₂₃, was constructed for the purpose of crystallization.

*TcarsI*₁₂₃ was amplified by PCR using its genomic DNA (purchased from ATCC) as a template with forward primer 5'-GGGG**CATATG**-TCCCGCGTCCAGCTCGCCC-3' (*NdeI* site in bold) and reverse primer 5'-AAAAGGA**AATTC**CTAGTCTGCGTGCCTTTGAC-3' (*EcoRI* site in bold). A 30-cycle PCR reaction (367 K for 60 s, 328 K for 30 s and 345 K for 60 s) was run using *PfuTurbo* DNA polymerase (Agilent Biotechnologies, Santa Clara, California, USA). Because of the high GC content in *T. curvata* genomic DNA, 5% DMSO was added to the PCR reaction solution to improve amplification. The resulting PCR product was gel-purified using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) and then incubated with dATP, MgCl₂ and *Taq* polymerase (Invitrogen, Life Technologies, Carlsbad, California, USA) at 348 K for 1 h to add dATP to the 3'-end. The dATP-attached fragment was cloned into vector plasmid pGEM-T Easy (Promega, Madison, Wisconsin, USA) and the sequence was confirmed using a CEQ 2000 XL DNA Analysis System (Beckman Coulter, Pasadena, California, USA). The fragment containing *TcarsI*₁₂₃ was then isolated by digestion with the restriction enzymes *NdeI* and *EcoRI* (New England Biolabs, Ipswich, Massachusetts, USA) and ligated into doubly digested pET-28a(+)

vector plasmid (Novagen, Merck KGaA, Darmstadt, Germany) using T₄ DNA ligase (New England Biolabs).

The resulting plasmid, pET-28a-*TcarsI*₁₂₃, was transformed into *Escherichia coli* strain BL21 (DE3) for protein purification. The cells were grown in 1 l LB (Luria–Bertani) medium (Sambrook *et al.*, 1989) containing 50 µg ml⁻¹ kanamycin with shaking at 270 rev min⁻¹ at 310 K. Once the absorbance at 600 nm of the cell culture reached 0.5–0.6, 0.1 mM isopropyl β-D-1-thiogalactopyranoside (Research Products International, Mount Prospect, Illinois,

Figure 2

SDS–PAGE analysis of purified and crystallized TcArsI. Lane 1 contains marker proteins (labeled in kDa). Lane 2 contains purified histidine-tagged TcArsI. Lane 3 contains TcArsI after removal of the six-histidine tag by thrombin cleavage. Lane 4 contains crystals of TcArsI dissolved in water.

Figure 1

Crystals of TcArsI were grown in hanging drops. Their approximate dimensions are 0.1 × 0.05 × 0.05 mm.

Figure 3

An X-ray diffraction pattern (1° oscillation) of a native crystal of TcArsI using a MAR300 detector.

Table 1

Data-collection statistics.

Values in parentheses are for the last shell.

Wavelength (Å)	1.0088
Space group	$P4_32_12$ or $P4_12_12$
Unit-cell parameters (Å)	$a = b = 42.2$, $c = 118.5$
Resolution range (Å)	50.00–1.46 (1.51–1.46)
$R_{\text{merge}}^{\dagger}$ (%)	5.5 (44.0)
$R_{\text{r.i.m.}}$ (%)	5.6 (45.5)
$R_{\text{p.i.m.}}$ (%)	1.1 (11.5)
Average $I/\sigma(I)$	54.0 (5.3)
Unique reflections	19428 (1818)
Completeness (%)	99.6 (96.1)
Multiplicity	25.3 (12.0)
Crystal mosaicity (°)	0.55

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity and $\langle I(hkl) \rangle$ is the average intensity over symmetry-equivalent measurements.

USA) was added to induce protein expression. After 4 h, the cells were harvested and stored at 353 K. For purification, the cells were washed and resuspended in a buffer consisting of 50 mM MOPS pH 7.5, 0.5 M NaCl, 3 mM tris(2-carboxyethyl)phosphine (TCEP) (Gold Biotechnology, St Louis, Missouri, USA) (buffer A) containing 20 mM imidazole (5 ml per gram of wet cells). The cells were lysed by a single passage through a French pressure cell press at 138 MPa and were immediately treated with diisopropylfluorophosphate (2.5 μ l per gram of wet cells). The cell lysate was centrifuged at 25 000 rev min⁻¹ using an angle rotor (T865, Thermo Fisher Scientific, Waltham, Massachusetts, USA) for 60 min at 277 K. The supernatant solution was loaded onto a Ni–nitrilotriacetic acid (Ni–NTA) column (Qiagen) at a flow rate of 1.0 ml min⁻¹ and then washed with 100 ml buffer A. The target protein with a histidine tag (predicted molecular mass 15 320 Da) was eluted by elution with buffer A containing 0.2 M imidazole. The six-histidine tag was removed by digestion with thrombin (Calbiochem, Merck KGaA,

Darmstadt, Germany) for 16 h at 293 K. After enzymatic removal of the tag, three residues (Gly-Ser-His) from the tag remained, and the predicted mass of the protein is 13 438 Da. The tag-free protein was further purified using Ni–NTA chromatography and gel filtration on a Superdex75 column (Qiagen). TcArsI eluted from the gel-filtration column as a monomer. The protein yield was 40 mg ml⁻¹ from 1 l of cell culture.

2.3. Crystallization

The protein was concentrated to 15 mg ml⁻¹ using a 10 kDa ultracentrifugal filter (EMD Millipore Corporation, Billerica, Massachusetts, USA) in buffer A. Initial crystal screening was performed by the sitting-drop vapor-diffusion method (McPherson, 1990) using a variety of crystal screens from Hampton Research (Aliso Viejo, California, USA), Emerald Bio (Bainbridge Island, Washington, USA) and Jena Bioscience GmbH (Jena, Germany) in 96-well plates (Corning 3785, Corning, New York, USA) at 293 K. A crystalline precipitate was obtained with 0.2 M sodium acetate, 0.1 M Tris–HCl pH 8.5, 30% PEG 4000 (condition No. 22 of Crystal Screen from Hampton Research). Using this condition with replacement of sodium acetate by calcium acetate, diffraction-quality crystals were obtained by the hanging-drop vapor-diffusion method (McPherson, 1990) within 3 days. The diamond-shaped crystals with dimensions of approximately 0.1 $\times$ 0.05 $\times$ 0.05 mm (Fig. 1) were grown in 24-well Linbro plates (Hampton Research) with a hanging-drop volume of 4 μ l (2 μ l protein and 2 μ l well solution) and 300 μ l well solution. The crystals in a single drop were washed several times in 50% glycerol and dissolved in water for analysis by SDS–PAGE (Laemmli, 1970; Fig. 2). The stained band corresponded to the predicted mass of Arsl.

2.4. Data collection

The crystals were picked up from a hanging drop with cryoloops and flash-cooled in liquid nitrogen at 100 K for X-ray diffraction

Figure 4

Self-rotation function of TcArsI crystals. Sections of the self-rotation function calculated for the $P4_32_12$ native data set using *GLRF* (Tong & Rossmann, 1997) with an integration radius of 25.0 Å and data in the resolution range 9–4 Å with different κ angles. The $\kappa = 180^\circ$ section reflects the symmetry ($4/mmm$) expected for a tetragonal crystal. There were no additional peaks, indicating that the asymmetric unit consists of one molecule.

Figure 5

Patterson map of TcArsI crystals. The $z = 0$ section of the native Patterson map showing only origin peaks and no non-origin peaks suggests that only one molecule is present in the asymmetric unit. The map was calculated by FFT (Ten Eyck, 1973) and viewed with MapSlicer in the CCP4 suite (Winn *et al.*, 2011) using data in the resolution range 9–4 Å and contoured at 0.5σ intervals starting at 3σ .

experiments. 30% PEG 4000 was present in the mother liquor as a cryoprotectant. The data were collected on beamline 22-ID at the Advanced Photon Source (APS), Argonne National Laboratory using a MAR300 detector. The crystal-to-detector distance was 180 mm for 360 images collected using an oscillation angle of 1° and an exposure time of 1 s per frame (Fig. 3). The diffraction data were indexed and scaled using HKL-2000 (Otwinowski & Minor, 1997). The data-processing statistics are shown in Table 1.

3. Results and discussion

Crystallization of the TcArsI₁₂₃ protein was successful, and a complete data set was collected to a resolution of 1.46 Å. The crystal belonged to space group $P4_32_12$ or its enantiomer $P4_12_12$, with unit-cell parameters $a = b = 42.2$, $c = 118.5$ Å. The Matthews coefficient (Matthews, 1968) of $1.96 \text{ Å}^3 \text{ Da}^{-1}$ indicates that there is one molecule in the asymmetric unit, with 37% solvent content. The self-rotation map (Fig. 4) and native Patterson map (Fig. 5) support the existence of a monomer in the asymmetric unit. To solve the phase problem,

attempts to obtain ArsI crystals cocrystallized or soaked with heavy metals are in progress.

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