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Crystallization and preliminary X-ray diffraction studies of the putative haloalkane dehalogenase DppA from *Plesiocystis pacifica* SIR-I

DppA from *Plesiocystis pacifica* SIR-I is a putative haloalkane dehalogenase (EC 3.8.1.5) and probably catalyzes the conversion of halogenated alkanes to the corresponding alcohols. The enzyme was expressed in *Escherichia coli* BL21 and purified to homogeneity by ammonium sulfate precipitation and reversed-phase and ion-exchange chromatography. The DppA protein was crystallized by the vapour-diffusion method and protein crystals suitable for data collection were obtained in the orthorhombic space group $P2_12_12$. The DppA crystal diffracted X-rays to 1.9 Å resolution using an in-house X-ray generator.

1. Introduction

Haloalkane dehalogenases (EC 3.8.1.5) are α/β -hydrolase fold enzymes that catalyze the hydrolytic conversion of a broad spectrum of halogenated alkanes to the corresponding alcohols (Janssen, 2004). This reaction is of great environmental interest and haloalkane dehalogenases have therefore been extensively studied in recent years. Attempts have been made to enhance the speed of the catalytic reaction, to expand the substrate range and to increase the enantioselectivity (Bosma *et al.*, 2002; Schanstra *et al.*, 1996; Gray *et al.*, 2001; Chaloupkova *et al.*, 2003; Pries *et al.*, 1994; Holloway *et al.*, 1998). To date, only a handful of haloalkane dehalogenases have been crystallized (Franken *et al.*, 1991; Newman *et al.*, 1999; Marek *et al.*, 2000; Stsiapanava *et al.*, 2008; Mazumdar *et al.*, 2008), of which the enzymes from *Xanthobacter autotrophicus* GJ10, *Sphingomonas paucimobilis* UT26 and *Rhodococcus rhodochrous* NCIMB13064 have been extensively investigated in several further studies. They all share the α/β -hydrolase fold, which is a hallmark of this class of enzymes (Ollis *et al.*, 1992).

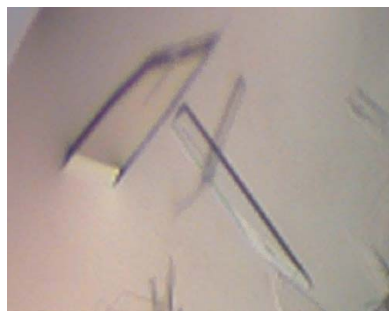
Plesiocystis pacifica SIR-1 is a myxobacterium which was isolated from the Japanese coast (Iizuka *et al.*, 2003). The strain is Gram-negative, chemoheterotrophic and strictly aerobic (Iizuka *et al.*, 2003). Its DNA sequence is available online at NCBI as a shotgun library (NCBI reference sequence NZ_ABCS000000000). The entry ZP_01908831.1 is tagged as a putative haloalkane dehalogenase with a calculated molecular weight of 32 kDa.

This haloalkane dehalogenase was chosen as it showed the highest sequence homology to another dehalogenase currently under study in an ongoing project and showed good alignment, with the residues of the catalytic triad (Asp-His-Asp) in reasonable positions.

2. Materials and methods

2.1. Protein expression and purification

The strain *P. pacifica* SIR-I was ordered from the German Collection of Microorganisms and Cell Cultures (DSMZ; DSM No. 14875) and was supplied as an actively growing culture on agar medium. Genomic DNA of *P. pacifica* SIR-I was amplified with the help of the GenomiPhi-Kit from GE Healthcare. The coding region of ZP_01908831.1 was cloned into a pET28a vector (without a His



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tag) and expressed in *Escherichia coli* BL21 Gold. The cells were grown at 310 K to an OD₆₀₀ of 0.5, induced with IPTG (final concentration 0.1 mM) and incubated at 293 K overnight. Subsequently, the cells were harvested and resuspended in buffer A (10 mM Tris-HCl buffer pH 8). The cells were disrupted by adding lysozyme (incubation time of 1 h at 280 K on an orbital shaker) and further treatment with a French press (three rounds of disruption). The cell lysate was centrifuged at 8000g for 45 min to remove cellular debris.

Ammonium sulfate precipitation was carried out using 15% (w/v) ammonium sulfate in buffer A with an incubation time of 2 h at 280 K. Afterwards, the solution was centrifuged at 8000g for 30 min. The following purification steps were all performed using an ÄKTA Purifier (GE Healthcare). The cell lysate was applied onto a Butyl Sepharose column equilibrated with buffer A and eluted using a linear gradient from 15 to 0% ammonium sulfate. Fractions containing DppA were pooled and desalted by gel filtration (buffer A). Subsequently, the sample was applied onto a Q Sepharose ion-exchange column with buffer A, eluted using a linear gradient from 0 to 1 M NaCl and desalted by gel filtration (buffer A). The sample was then applied onto a high-resolution Resource Q ion-exchange column equilibrated with buffer A and eluted with a linear gradient from 0 to 1 M KCl. Finally, the protein solution was desalted (buffer A) and concentrated with Amicon Ultra spin columns (exclusion size 10 kDa) from Millipore.

2.2. Crystallization

Initial screening for crystallization conditions was performed by the sitting-drop vapour-diffusion method using a crystallization robot (HTPC, CyBio) and 96-well plates (CrystalQuick LP, Greiner Bio-One). The drops contained 0.3 µl protein solution (at 8 mg ml⁻¹ in buffer A) and 0.3 µl reservoir solution and were equilibrated against 40 µl reservoir solution. From these initial screens (JBScreen Classic 1–10, Jena Bioscience) several hits were observed. The crystallization conditions were further optimized using 24-well crystallization plates (Greiner Bio-One) with the hanging-drop vapour-diffusion method. Each well contained 500 µl reservoir solution and the drop volume was a mixture of 1 µl protein solution and 1 µl reservoir solution.

2.3. Data collection and X-ray crystallographic analysis

For cryoprotection, the reservoir solution was mixed with 50% (v/v) PEG 400 to give a final concentration of 5% (v/v) PEG 400. Before

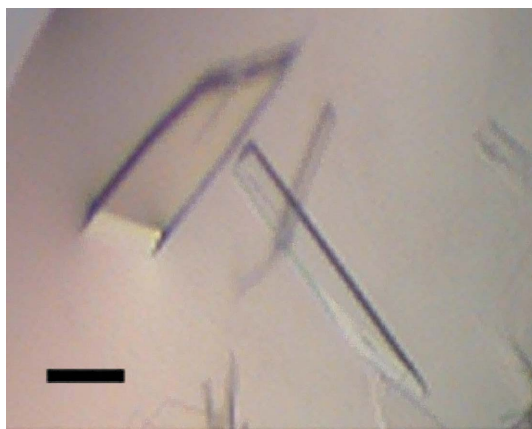


Figure 1
Crystals of the haloalkane dehalogenase DppA grown by the hanging-drop vapour-diffusion method. The crystal which was used for data collection (the leftmost crystal) has a thickness of approximately 40 µm. The solid bar represents 100 µm.

Table 1

X-ray data-collection statistics for DppA.

Values in parentheses are for the outer resolution shell.

X-ray source	Rigaku MicroMax-007
Detector	Saturn 92
Wavelength (Å)	1.5418
Resolution range (Å)	38.68–1.95 (2.02–1.95)
Space group	<i>P</i> 2 ₁ 2 ₁ 2
Unit-cell parameters (Å)	<i>a</i> = 47.3, <i>b</i> = 108.5, <i>c</i> = 67.3
Total reflections	139903
Unique reflections	25613
Completeness (%)	98.5 (97.4)
<i>R</i> _{merge} (%)	12.0 (43.7)
<i>R</i> _{r.i.m.} (%)	13.7 (57.9)
<i>R</i> _{p.i.m.} (%)	6.4 (32.7)
Average <i>I</i> /σ(<i>I</i>)	7.0 (2.3)
<i>d</i> -spacing (Å)	2.02–1.95
Mosaicity (°)	1.00
Multiplicity	5.46 (4.13)
Wilson <i>B</i> factor (Å ²)	26.0
No. of images	258

the crystal was mounted, it was transferred into and soaked in this cryoprotectant for 30 s. The crystal was then flash-cooled to 110 K in a stream of nitrogen (Oxford Cryosystems).

Diffraction data were collected using a rotating-anode X-ray generator (MicroMax007, Rigaku) with Osmic multiple-layer optics (beam size 0.3 × 0.3 mm) and a CCD detector (Saturn 92, Rigaku).

The diffraction data were integrated and scaled using the software *CrystalClear* v.1.3.6 (Pflugrath, 1999). To obtain values of *R*_{p.i.m.} and *R*_{r.i.m.} the data were scaled with *SCALA* (Evans, 2006).

3. Results and discussion

The DppA protein was expressed in *E. coli* BL21 Gold and purified to homogeneity by ammonium sulfate precipitation and reversed-phase and ion-exchange chromatography. A final concentration of 8 mg ml⁻¹ was used for crystallization experiments.

Crystals of DppA protein could be obtained with several precipitants from the initial screen using the vapour-diffusion method. The crystallization conditions were further optimized and tiny needles that were too small for data collection appeared after several days in most cases (0.1 M sodium acetate pH 4.6, 28–31% PEG 400, 0.1 M MgCl₂; 0.1 M sodium phosphate–citrate pH 4.2, 0.2 M K₂HPO₄, 1.6 M NaH₂PO₄; 0.1 M Na HEPES pH 7.5, 1.8–2.2 M ammonium sulfate, 2% PEG 400; 0.1 M Na HEPES pH 7.5, 1.5–1.8 M ammonium sulfate, 0.2 M NaCl; 0.1 M Na MES pH 6.5, 1.8–2.0 M ammonium sulfate, 5% PEG 400; 0.1 M Na MES pH 6.5, 1.5–1.8 M ammonium sulfate). In one condition, comprised of 0.1 M Na MES pH 6.5, 1.8 M ammonium sulfate and 5% PEG 400, one crystal with an appropriate size for data collection (40 × 110 × 200 µm) was obtained after 4 d (Fig. 1).

This crystal of DppA diffracted X-rays to a resolution of 1.95 Å on a rotating-anode X-ray generator. The integration limit was set to an average *I*/σ(*I*) of 2. The data statistics are reported in Table 1.

The crystal of DppA belonged to the orthorhombic space group *P*2₁2₁2 (No. 18), with unit-cell parameters *a* = 47.3, *b* = 108.5, *c* = 67.3 Å.

Assuming the presence of one molecule in the asymmetric unit, the Matthews coefficient *V*_M was calculated to be 2.6 Å³ Da⁻¹.

The structure could be solved by molecular replacement using the program *Phaser* (McCoy *et al.*, 2007) with the haloalkane dehalogenase from *X. autotrophicus* GJ10 (PDB code 1edb; Verschuere *et al.*, 1993), which has a sequence identity of 50%, as a search model. A unique solution was obtained with a monomer in the asymmetric unit

with a likelihood score of 483.81 and a Z score of 21.51. Refinement is currently in progress.

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