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Expression, purification and X-ray analysis of 1,3-propanediol dehydrogenase (*Aq*_1145) from *Aquifex aeolicus* VF5

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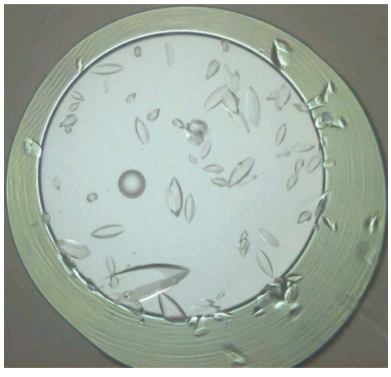

1,3-Propanediol dehydrogenase is an enzyme that catalyzes the oxidation of 1,3-propanediol to 3-hydroxypropanal with the simultaneous reduction of $NADP^+$ to NADPH. SeMet-labelled 1,3-propanediol dehydrogenase protein from the hyperthermophilic bacterium *Aquifex aeolicus* VF5 was overexpressed in *Escherichia coli* and purified to homogeneity. Crystals of this protein were grown from an acidic buffer with ammonium sulfate as the precipitant. Single-wavelength data were collected at the selenium peak to a resolution of 2.4 Å. The crystal belonged to space group $P3_2$, with unit-cell parameters $a = b = 142.19$, $c = 123.34$ Å. The structure contained two dimers in the asymmetric unit and was solved by the MR-SAD approach.

1. Introduction

Aquifex aeolicus is a hyperthermophilic bacterium with a growth-temperature maximum near 368 K. Its complete genome, which is only one-third of the size of the *Escherichia coli* genome, contains 1512 open reading frames (ORFs). *A. aeolicus* represents the earliest branch in bacterial phylogeny (Pitulle *et al.*, 1994) and is a hydrogen-oxidizing, microaerophilic and obligate chemolithoautotroph (Huber *et al.*, 1992). Many hyperthermophilic organisms utilize carbohydrates as carbon and energy sources (Stetter, 1996).

1,3-Propanediol (1,3-PD) has long been an important chemical and is used in the polymer and cosmetics industries. The synthetic production of 1,3-PD involves toxic intermediates (Marçal *et al.*, 2009; Raynaud *et al.*, 2003) and therefore 1,3-PD has been produced from microorganisms *via* microbial fermentation of glycerol as an alternative to chemical synthesis (Cameron *et al.*, 1998). 1,3-PD has successfully been produced in some anaerobic and microaerophilic bacteria such as *Klebsiella*, *Citrobacter* and *Clostridum* (Luers *et al.*, 1997; Bouvet *et al.*, 1995; Daniel *et al.*, 1995). 1,3-PD has also been produced by glycerol fermentation using a two-step sequential enzymatic reaction in the microorganism *Lactobacillus* (Veiga-da-Cunha & Foster, 1992; Veiga da Cunha & Foster, 1992; Talarico *et al.*, 1990). In the reductive pathway, glycerol is converted to the intermediate 3-hydroxypropionaldehyde by a vitamin-B_{12}-dependent glycerol dehydratase. The intermediate product 3-hydroxypropionaldehyde is then reduced to 1,3-PD by NADH-dependent 1,3-PD dehydrogenase (Ahrens *et al.*, 1998; Cheng *et al.*, 2006).

The *Aq*_1145 gene of *A. aeolicus* VF5 encodes a 1,3-propanediol dehydrogenase protein containing 387 amino-acid residues with a calculated molecular weight of 43 kDa and a predicted pI of 5.8. A *BLAST* (Altschul *et al.*, 1997) search of the *Aq*_1145 protein against the Swiss-Prot database indicated that its highest level of sequence similarity (35%) was to 1,3-propanediol oxidoreductase from *Klebsiella pneumoniae* (PDB code 3bfj; Marçal *et al.*, 2009) and an iron-containing alcohol dehydrogenase (TM0920) from *Thermotoga maritima* (PDB code 1o2d; Schwarzenbacher *et al.*, 2004). Accordingly, *Aq*_1145 was annotated as an iron-dependent 1,3-propanediol dehydrogenase. Here, we report the expression, purification, crystallization and preliminary X-ray crystallographic studies of a 1,3-propanediol dehydrogenase enzyme from the hyperthermophilic bacterium *A. aeolicus* VF5.

2. Materials and methods

2.1. Expression and purification

The *Aq*_1145 gene was amplified by polymerase chain reaction from the *A. aeolicus* VF5 genome and cloned into the expression plasmid pET-21a (Novagen). *Escherichia coli* BL21-CodonPlus (DE3)-RIL-X cells were transformed with the expression plasmid. The transformants were pre-cultured at 310 K for 6–7 h in a medium containing 1.0% polypeptone (Wako Co. Ltd, Japan), 0.5% yeast extract, 0.5% NaCl and 50 µg ml^{-1} ampicillin pH 7.0 and grown overnight at 310 K in SeMet core medium containing 0.13 µ*M* L-selenomethionine, 21 amino acids and bases (LeMaster & Richards, 1985), 1% pre-mixed vitamin solutions (Sigma), 1.0% lactose, 100 µg ml^{-1} ampicillin pH 7.0 and 25 µg ml^{-1} chloramphenicol. The cells were lysed by sonication in 20 m*M* Tris–HCl pH 8.0 containing 500 m*M* NaCl, 5 m*M* β-mercaptoethanol and 1 m*M* phenylmethylsulfonyl fluoride (PMSF). The lysate was incubated at 343 K for 10 min and centrifuged at 14 000 rev min^{-1} for 30 min at 277 K. After buffer exchange on a HiPrep 26/10 desalting column (GE Healthcare Biosciences) pre-equilibrated with 20 m*M* Tris–HCl pH 8.0, the protein sample was loaded onto a TOYOPEARL SuperQ-650M column (Tosoh) pre-equilibrated with 20 m*M* Tris–HCl pH 8.0. The *Aq*_1145 protein bound to the column and eluted at 210 m*M* NaCl in a linear gradient from 0 to 400 m*M* NaCl. The *Aq*_1145-containing fraction was subjected to buffer exchange with 20 m*M* Tris–HCl pH 8.0 and loaded onto a RESOURCE Q column (GE Healthcare Biosciences) pre-equilibrated with the same buffer. The protein bound to the column and eluted at approximately 190 m*M* NaCl in a linear gradient from 0 to 300 m*M* NaCl. The *Aq*_1145 protein was subjected to buffer exchange with 10 m*M* potassium phosphate buffer pH 7.0 and loaded onto a Bio-Scale CHT20-I column (Bio-Rad) pre-equilibrated with the same buffer. The protein bound to the column and eluted at 99.8 m*M* potassium phosphate in a linear gradient from 10 to 500 m*M* potassium phosphate pH 7.0. The *Aq*_1145 protein was subjected to gel filtration on a HiLoad 16/60 Superdex 200 pg column (GE Healthcare Biosciences) pre-equilibrated with 20 m*M* Tris–HCl, 200 m*M* NaCl pH 8.0. The gel-filtration elution profile indicated that the protein was a monomer in solution. The fraction containing the protein was collected and the purified *Aq*_1145 protein was concentrated using a Vivaspin device with a 10 kDa cutoff (Sartorius). The final preparation was dissolved in 20 m*M* Tris–HCl containing 200 m*M* NaCl and 1 m*M* DTT pH 8.0 and the yield of the purified protein was 21.0 mg per litre of culture medium. The purified SeMet-labelled *Aq*_1145 protein was homogeneous on native PAGE.

2.2. Crystallization

The 1,3-PD protein from *A. aeolicus* VF5 (*Aq*_1145) was crystallized at 291 K using the microbatch-under-oil method in Nunc HLA plates (Nalge Nunc International). The purified SeMet-substituted protein was screened for preliminary crystallization conditions using the TERA robotic automatic crystallization system (Sugahara & Miyano, 2002) with 144 conditions. The growth of SeMet-substituted 1,3-PD crystals was further optimized using a droplet consisting of 1 µl SeMet-substituted protein (21 mg ml^{-1} protein in 20 m*M* Tris–HCl pH 8.0, 200 m*M* NaCl and 1 m*M* DTT) mixed with 1 µl of a solution containing 1.22 *M* ammonium sulfate, 0.1 *M* citrate–HCl pH 5.1 and 10%(*w*/*w*) dioxane. The SeMet-substituted *Aq*_1145 protein crystals (Fig. 1) reached final dimensions of 0.1 × 0.1 × 0.2 mm after 20 d and were used for X-ray diffraction experiments.

Table 1
X-ray data collection for 1,3-propanediol dehydrogenase.

Values in parentheses are for the last resolution bin.

X-ray source	BL26B1, SPring-8
Wavelength (Å)	0.97932
Detector	Jupiter 210 CCD
Temperature (K)	100
Crystal-to-detector distance (mm)	200
Space group	$P3_2$
Unit-cell parameters (Å)	$a = b = 142.19$, $c = 123.34$
Resolution range (Å)	50–2.4 (2.49–2.4)
Total reflections	610409
Unique reflections	108448
Completeness (%)	100 (100)
$R_{\rm merge}$† (%)	7.9 (59.7)
$\langle I/\sigma(I)\rangle$	12.8 (1.2)
Redundancy	5.6
$V_{\rm M}$ (Å^3 Da^{-1})	4.1
Z	4
Solvent content (%)	70.3

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity and $\langle I(hkl)\rangle$ is the average intensity for multiple measurements.

2.3. Data collection and processing

Prior to data collection, the crystal was transferred to mother liquor containing 20%(*v*/*v*) glycerol for cryoprotection and cooled in a nitrogen-gas stream at 100 K using the SPring-8 Precise Automatic Cryo-sample Exchanger (SPACE), which was controlled using the *BSS* beamline-scheduling software (Ueno *et al.*, 2004, 2005). Diffraction data were collected from the SeMet-substituted 1,3-propanediol dehydrogenase protein crystal at the selenium peak ($\lambda_{\rm peak}$ = 0.97932 Å) on RIKEN Structural Genomics Beamline I (BL26B1) at SPring-8 (Hyogo, Japan) with a Jupiter 210 CCD detector (Rigaku MSC Co., Tokyo Japan). The crystal-to-detector distance was maintained at 200 mm. The crystal diffracted to 2.4 Å resolution. All intensity data were indexed and integrated using *DENZO* (Otwinowski & Minor, 1997) and scaled using *SCALEPACK* (Otwinowski & Minor, 1997). The SAD (single-wavelength anomalous diffraction) data statistics for the SeMet-labelled crystal are summarized in Table 1. Intensities were converted to structure-factor amplitudes using the program *TRUNCATE* (French & Wilson, 1978; Collaborative Computational Project, Number 4, 1994). The self-rotation function was calculated using the program *MOLREP*

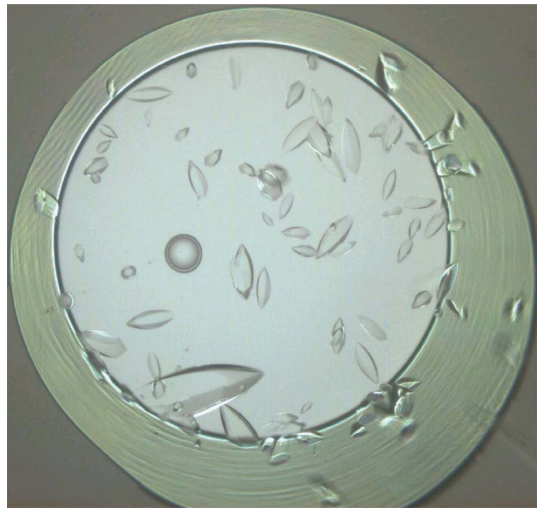

Figure 1
Single crystals of 1,3-propanediol dehydrogenase (*Aq*_1145) from *A. aeolicus* VF5.

(Collaborative Computational Project, Number 4, 1994; Vagin & Teplyakov, 1997).

3. Results and discussion

The SeMet-labelled 1,3-propanediol dehydrogenase protein from *A. aeolicus* VF5 was successfully overexpressed in *E. coli* strain BL21 (DE3) and purified to homogeneity. The protein contains 387 amino-acid residues with a molecular weight of 43 kDa. The Matthews coefficient (Matthews, 1968) was calculated to be 4.1 $Å^3$ Da^{-1}, with a solvent content of 70.3%, assuming the presence of four monomers in the asymmetric unit. Analysis of self-rotation peaks showed one high non-origin peak with one noncrystallographic twofold axis between the crystallographic *x* and *y* axes, confirming the presence of two dimers in the asymmetric unit. Gel filtration and nondenaturing polyacrylamide gel electrophoresis demonstrated that 1,3-PD exists as a monomer in solution. This enzyme shares 35% sequence identity with 1,3-propanediol oxidoreductase from *K. pneumoniae* (PDB code 3bfj; Marçal *et al.*, 2009) and 34% sequence identity with an iron-containing alcohol dehydrogenase (TM0920) from *T. maritima* (PDB code 1o2d; Schwarzenbacher *et al.*, 2004). The structure of the 1,3-PD protein (*Aq*_1145) was solved by employing the MR-SAD protocol as implemented in *Auto-Rickshaw* (Panjikar *et al.*, 2005, 2009) using a single monomer of the TM0920 structure (PDB code 1o2d) as a search model. Within *Auto-Rickshaw*, molecular replacement was performed using *MOLREP* and the program placed four monomers in the asymmetric unit. The resulting model was used for initial refinement, model phase calculations, location of selenium positions based on the model phase, SAD phasing, phase combination, density modification and model building in an automatic manner. Iterative manual building and refinement of the model are currently under way. Details of the structure determination and analysis will be published elsewhere. Comparison of the structure of 1,3-propanediol dehydrogenase with those of other dehydrogenases will provide new structural insights into the molecular mechanism of this enzyme.

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