Crystallization and preliminary X-ray diffraction analysis of the *S*-adenosylhomocysteine hydrolase (SAHH) from *Thermotoga maritima*

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Received 28 March 2014
Accepted 10 June 2014

S-Adenosylhomocysteine hydrolase (SAHH) catalyzes the reversible conversion of *S*-adenosylhomocysteine into adenosine and homocysteine. The SAHH from *Thermotoga maritima* (*TmSAHH*) was expressed in *Escherichia coli* and the recombinant protein was purified and crystallized. *TmSAHH* crystals belonging to space group C2, with unit-cell parameters $a = 106.3$, $b = 112.0$, $c = 164.9$ Å, $\beta = 103.5^\circ$, were obtained by the sitting-drop vapour-diffusion method and diffracted to 2.85 Å resolution. Initial phase determination by molecular replacement clearly indicated that the crystal contains one homotetramer per asymmetric unit. Further refinement of the crystal structure is in progress.

1. Introduction

S-Adenosylhomocysteine hydrolase (SAHH; EC 3.3.1.1) is an important and ubiquitous cellular enzyme in various living organisms. It catalyzes the reversible hydrolysis of *S*-adenosylhomocysteine (SAH) to adenosine and L-homocysteine (Palmer & Abeles, 1976, 1979). The reaction equilibrium favours the synthetic direction *in vitro*. Several human diseases, including cardiovascular disease (Zaina *et al.*, 2005), white matter atrophy, delayed myelination, slowly progressive myopathy, retarded psychomotor development and mildly active chronic hepatitis (Honzi *et al.*, 2012), have been found to be associated with deficiency of SAHH. In addition, studies of SAHH inhibitors have encouraged the design of antiviral (De Clercq, 2005) and antiparasitic drugs (Henderson *et al.*, 1992; Bujnicki *et al.*, 2003). In addition to physiological regulation, SAH has also been identified as having several pharmaceutical effects, including sedative, sleep modulating and anticonvulsant activities (Lozada-Ramírez *et al.*, 2013).

Several SAHH structures from different organisms, including *Homo sapiens* (Turner *et al.*, 1998; Yang *et al.*, 2003; Lee *et al.*, 2011), *Rattus norvegicus* (Hu *et al.*, 1999; Takata *et al.*, 2002; Huang *et al.*, 2002; Yamada *et al.*, 2005, 2007; Komoto *et al.*, 2000), *Plasmodium falciparum* (Tanaka *et al.*, 2004), *Mycobacterium tuberculosis* (Reddy *et al.*, 2008), *Burkholderia pseudomallei* (PDB entry 3d64; Seattle Structural Genomics Center for Infectious Disease, unpublished work), *Trypanosoma brucei* (PDB entry 3h9u; Structural Genomics Consortium, unpublished work), *Leishmania major* (PDB entry 3glu; Structural Genomics Consortium, unpublished work) and *Lupinus luteus* (Brzezinski *et al.*, 2012), have been solved. However, none of them is from a thermophilic microorganism. Enzymes from extremophiles have drawn much attention because they provide benefits in industrial applications, which usually involve harsh operating conditions (e.g. high salt or high temperature; Liszka *et al.*, 2012). Moreover, thermostable enzymes often serve as models for studying catalytic mechanisms. Currently, the contributing factors and structural features responsible for the better thermal profiles of the thermophilic SAHs remain unexplored and constitute a topic of great interest.

Recently, an SAHH has been identified from the anaerobic hyperthermophilic bacterium *Thermotoga maritima* (*TmSAHH*). Recombinant *TmSAHH* expressed in *Escherichia coli* shows optimal activity at pH 6.5 and 348 K (Lozada-Ramírez *et al.*, 2013). The

enzyme exhibits extreme thermostability and retains 95% enzyme activity after incubation at 348 K for 2 h. Therefore, *TmSAHH* is considered to have a high potential for development for commercial utilizations.

2. Materials and methods

2.1. Protein preparation

The gene encoding SAHH (GenBank AAC01562.1) from *T. maritima* MSB8 was amplified by polymerase chain reaction (PCR) with forward primer 5'-CATGCCATGGCTAACACAGGT-GAAATGAAGA-3' and reverse primer 5'-CCGCTCGAGCTGCCAACTTCTCAGATA-3'. The PCR fragments encoding SAHH were digested with *NcoI* and *XhoI*, and ligated with *NcoI/XhoI*-digested pHsh (Shine-E, Nanjing, People's Republic of China; Wu *et al.*, 2010). An Ala residue is inserted right after the N-terminal Met and there are eight extra residues (LEHHHHHH) at the C-terminus, making a fusion protein of 413 residues (about 46 kDa). *E. coli* BL21-CodonPlus (DE3)-RIL (Novagen, USA) cells were transformed with the recombinant plasmid and the recombinant *E. coli* cell cultures were grown at 310 K to an OD₆₀₀ of about 0.8; protein expression was then induced for 8 h by heat shock at 315 K. The cells from 0.8 l culture were then harvested by centrifugation (5000g, 10 min, 277 K), resuspended in 20 ml 5 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl pH 7.9 and disrupted with a French press at 1.25×10^5 kPa. Cell extracts were obtained by centrifugation for 1 h at 100 000g and 277 K using a Beckman L8-M ultracentrifuge (Beckman Instruments Inc., Palo Alto, California, USA). The cell extracts were heat-treated (353 K, 1 h), cooled in an ice bath and centrifuged (10 000g, 277 K, 30 min). The resulting supernatants were loaded onto a nickel-affinity column (Novagen) and eluted with 100 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl pH 7.9. The purification step was performed at 298 K in the presence of 0.02%(w/v) sodium azide, which was added to prevent microbial growth. The overall protein yield was 8 mg l⁻¹. The protein was examined by SDS-PAGE and the protein bands were analyzed by density scanning with an image-analysis system (Bio-Rad). Protein concentration was determined using the Bradford assay.

2.2. Crystallization and data collection

Initial crystallization screening was performed manually using 768 different reservoir conditions from Hampton Research (Laguna Niguel, California, USA), including Crystal Screen, Crystal Screen 2, Crystal Screen Cryo, Crystal Screen Lite, MembFac, Natrix, Index, SaltRx, SaltRx 2, PEG/Ion Screen, PEG/Ion 2 Screen, Quick Screen and six different Grid Screens (ammonium sulfate, 2-methyl-2,4-pentandiol, sodium chloride, sodium malonate, PEG 6000 and PEG/LiCl) by the sitting-drop vapour-diffusion method. Prior to crystallization, *TmSAHH* was dialyzed against buffer consisting of 25 mM Tris-HCl, 150 mM NaCl pH 7.5, 10 mM DTT and the protein was concentrated by centrifugation using Centriprep (Millipore, USA). All crystallization experiments were conducted at 295 K. In general, 2 µl *TmSAHH*-containing solution (10 mg ml⁻¹ in 25 mM Tris-HCl, 150 mM NaCl pH 7.5, 10 mM DTT) was mixed with 2 µl reservoir solution in 24-well Cryschem plates (Hampton Research) and equilibrated against 300 µl reservoir solution. Crystals of *TmSAHH* appeared within 4 d using Crystal Screen Lite condition No. 46 [0.2 M calcium acetate hydrate, 0.1 M sodium cacodylate trihydrate pH 6.5, 9%(w/v) polyethylene glycol 8000]. The condition was optimized to obtain better crystals using 0.3 M calcium acetate hydrate, 0.1 M sodium cacodylate trihydrate pH 6.5, 8%(w/v) polyethylene glycol

Table 1

Data-collection statistics for the *TmSAHH* crystal.

Values in parentheses are for the highest resolution shell.

Beamline	BL-13C1, NSRRC
Wavelength (Å)	0.97622
Resolution (Å)	25.00–2.85 (2.95–2.85)
Space group	C2
Unit-cell parameters	
<i>a</i> (Å)	106.3
<i>b</i> (Å)	112.0
<i>c</i> (Å)	164.9
β (°)	103.5
No. of measured reflections	148719 (14160)
No. of unique reflections	43417 (4291)
Completeness (%)	99.4 (98.9)
$R_{\text{merge}}^{\dagger}$ (%)	9.7 (50.7)
$R_{\text{meas}}^{\ddagger}$ (%)	11.5 (60.5)
$R_{\text{p.i.m.}}^{\S}$ (%)	6.2 (32.6)
Mean $I/\sigma(I)$	15.2 (2.4)
Multiplicity	3.4 (3.3)
Detector	Q315r
X-ray beam size (µm)	200
Oscillation range (°)	0.5
Time of exposure (s)	40
Crystal-to-detector distance (mm)	350

$$\dagger R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}, \quad \ddagger R_{\text{meas}} = \frac{\sum_{hkl} \{N(hkl) / [N(hkl) - 1]\}^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}, \quad \S R_{\text{p.i.m.}} = \frac{\sum_{hkl} \{1 / [N(hkl) - 1]\}^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}.$$

8000. Within 3–5 d, the crystals reached dimensions of about $0.5 \times 0.07 \times 0.07$ mm. Prior to flash-cooling at 100 K, crystals were soaked in a reservoir-based cryoprotectant consisting of 0.4 M calcium acetate hydrate, 0.2 M sodium cacodylate trihydrate pH 6.5, 15%(w/v) polyethylene glycol 8000, 17%(w/v) glycerol for about 3 s. Subsequently, a crystal was mounted in a cryoloop and flash-cooled in liquid nitrogen. An X-ray diffraction data set was collected to 2.85 Å resolution on beamline BL13C1 of the National Synchrotron Radiation Research Center (NSRRC), Hsinchu, Taiwan. The diffraction data were processed using *HKL-2000* (Otwinowski & Minor, 1997). Data-collection statistics are given in Table 1.

3. Results and discussion

As shown in Fig. 1, the single *TmSAHH* crystal obtained under the optimized condition was rod-shaped. Based on the diffraction pattern (Fig. 2), the *TmSAHH* crystal belongs to the C-centred monoclinic space group C2, with unit-cell parameters $a = 106.3$, $b = 112.0$, $c = 164.9$ Å, $\beta = 103.5^\circ$. Assuming the presence of four molecules per

Figure 1

A crystal of *TmSAHH*. The dimensions of the crystal reached $0.5 \times 0.07 \times 0.07$ mm after 3–5 d.

Figure 2
A typical diffraction pattern of the *TmSAHH* crystal.

asymmetric unit, the Matthews coefficient V_M (Matthews, 1968) is $2.65 \text{ \AA}^3 \text{ Da}^{-1}$ and the estimated solvent content is 53.6%.

The structure of *TmSAHH* was solved by the molecular-replacement method with *Phaser* (McCoy *et al.*, 2007) from the *CCP4* suite (Winn *et al.*, 2011) using a hypothetical *TmSAHH* model as the search model. This model was generated from the structure of *Mycobacterium tuberculosis* SAHH (PDB entry 3dhy; 43% sequence identity; Reddy *et al.*, 2008) using the *Phyre2* server (Kelley & Sternberg, 2009). Preliminary structural refinement using *REFMAC5* (Murshudov *et al.*, 2011) and *CNS* (Brunger, 2007) resulted in an R_{work} and R_{free} of 39 and 44%, respectively. There are four monomers in the asymmetric unit and these four monomers form a homotetramer, which is consistent with previously solved SAHH structures (Lee *et al.*, 2011; Reddy *et al.*, 2008; Tanaka *et al.*, 2004; Brzezinski *et al.*, 2012). Further refinement of the crystal structure is under way. To fully understand the substrate-binding modes and catalytic mechanism, cocrystallization and soaking of the *TmSAHH* crystals with its substrates *S*-adenosylhomocysteine, adenosine and homocysteine are also in progress.

The synchrotron data collection was conducted on beamline BL13C1 of the NSRRC (National Synchrotron Radiation Research Center, Taiwan) supported by the National Science Council (NSC).

This work was supported by grants from the National Natural Science Foundation of China (31200053 and 31300615) and the Tianjin Municipal Science and Technology Commission (12ZCZDSY12500).

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