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Crystallization of mouse *S*-adenosyl-L-homocysteine hydrolase

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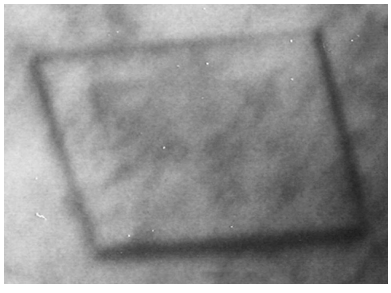

S-Adenosyl-L-homocysteine hydrolase (SAHH; EC 3.3.1.1) catalyzes the reversible hydrolysis of *S*-adenosyl-L-homocysteine to adenosine and L-homocysteine. For crystallographic investigations, mouse SAHH (MmSAHH) was overexpressed in bacterial cells and crystallized using the hanging-drop vapour-diffusion method in the presence of the reaction product adenosine. X-ray diffraction data to 1.55 Å resolution were collected from an orthorhombic crystal form belonging to space group *I*222 with unit-cell parameters a = 100.64, b = 104.44, c = 177.31 Å. Structural analysis by molecular replacement is in progress.

1. Introduction

S-Adenosyl-L-homocysteine hydrolase (SAHH or AdoHcy hydrolase; EC 3.3.1.1) is one of the most highly conserved enzymes from bacteria to mammals. The molecular mass of each subunit is 45–55 kDa; however, the quaternary structure varies among species. The SAHH enzymes from mammals (Turner *et al.*, 1998; Hu *et al.*, 1999), *Plasmodium falciparum* (Tanaka *et al.*, 2004) and *Mycobacterium tuberculosis* (Reddy *et al.*, 2009) are known to form homotetramers, whereas the enzymes from the plant *Lupinus luteus* (yellow lupin; Guranowski & Pawelkiewicz, 1977; Brzezinski *et al.*, 2008) and the bacterium *Alcaligenesis faecalis* (Matuszewska & Borchardt, 1987) have been reported to function as a homodimer and a homohexamer, respectively. The SAHH enzymes catalyze the reversible hydrolysis of *S*-adenosyl-L-homocysteine (SAH or AdoHcy) to adenosine (Ado) and L-homocysteine (Hcy) (Cantoni, 1975). SAH is produced from *S*-adenosylmethionine (SAM) as a by-product of SAM-dependent methyltransferase reactions and is degraded rapidly *in vivo* by SAHH. Inhibition of SAHH results in cellular accumulation of SAH, which is a potent feedback inhibitor of SAM-dependent biological methylation. Targets of SAM-dependent methyltransferases include a wide variety of cellular compounds such as DNA, mRNA, histones H3 and H4 and other proteins. Since SAHH plays a key role in the regulation of transmethylation reactions in all eukaryotic organisms, a number of SAHH inhibitors have been designed as drugs against a number of diseases, including cancer, malaria and viral infections (Chiang, 1998).

In order to clarify the reaction mechanism of SAHH, several crystal structure analyses and biochemical studies have been reported for mammalian SAHHs (Turner *et al.*, 1998; Hu *et al.*, 1999; Komoto *et al.*, 2000; Huang *et al.*, 2002; Takata *et al.*, 2002; Yang *et al.*, 2003; Yamada *et al.*, 2005, 2007). In addition, crystal structures of pathogenic SAHHs have been reported for the design of selective inhibitors against malaria (Tanaka *et al.*, 2004) and tuberculosis (Reddy *et al.*, 2009). Although a high-resolution structure of a prokaryotic SAHH has recently been reported (Reddy *et al.*, 2009), a high-resolution structure of mammalian SAHH complexed with either an inhibitor or a substrate analogue has not been obtained (the resolution limits are 2.0 and 2.8 Å for the human and rat enzymes,

respectively). In the absence of a high-resolution structure of a mammalian SAHH complexed with an inhibitor (or a substrate analogue), the structural image of the SAHH–substrate interactions, including the active-site water molecules, which is essential in order to fully understand the reaction mechanism of mammalian SAHH, is not sufficiently clarified. Here, we report the crystallization of mouse (*Mus musculus*) SAHH (MmSAHH) in the presence of the reaction product Ado. The crystals diffracted to at least 1.55 Å resolution and are suitable for X-ray structure analysis at high resolution.

2. Materials and methods

2.1. Expression and purification

The cDNA for mouse SAHH (MmSAHH) was PCR-amplified from the mouse liver cDNA library (Takara) with the forward and reverse primers 5′-CCG**GGATCC**ATGTCTGATAAACTGCCC-3′ and 5′-CGC**AAGCTT**TCAGTAGCGGTAGTGATCAGG-3′, respectively. The PCR product was cloned into pCold II expression plasmid (Takara) using the *Bam*HI and *Hin*dIII cloning sites (shown in bold). The resulting plasmid encoded an N-terminally His_6-tagged fusion protein in which the the sequence MNHKVHHHHHHMELGTLE-GS precedes that of full-length MmSAHH (1–432). The construct was verified by sequencing.

Escherichia coli BL21 (DE3) cells (Novagen) harbouring the expression plasmids were grown in YT medium (3 l shake flask containing 1 l medium) at 310 K to an OD_{600} of 0.5. Expression of MmSAHH was induced using 0.5 m*M* IPTG for 20 h at 288 K. Cells were then harvested by centrifugation at 8000*g* for 15 min, suspended in buffer *A* (50 m*M* Tris–HCl, 0.1 *M* NaCl pH 7.4) and disrupted by ultrasonication on ice for 4 × 30 s. The cell extract was obtained by centrifugation at 15 000*g* for 15 min and was applied onto a 4 ml TALON column (Clontech) equilibrated with buffer *A*. The column was successively washed with 25 column volume of buffer *A* and ten column volumes of wash buffer (5 m*M* imidazole in buffer *A*). After washing, MmSAHH was eluted with five column volumes of elution buffer (0.5 *M* imidazole in buffer *A*). The MmSAHH was further purified by gel chromatography using a Superdex 200pg column (GE Healthcare) equilibrated with buffer *A*. The fractions containing MmSAHH were pooled and concentrated to 15 mg ml^{-1} using a Centricon-30 (Millipore).

2.2. Crystallization

The protein solution was mixed with 20 m*M* adenosine (Ado) in buffer *A* at a volume ratio of 4:1. Initial sparse-matrix crystal screening (Jancarik & Kim, 1991) was conducted using Crystal Screen I and Index from Hampton Research and Wizard I, II and III and Cryo I and II from Emerald BioSystems. Crystallization was carried out by the hanging-drop method, in which 1 µl protein solution (12 mg ml^{-1} protein and 4 m*M* Ado) was mixed with the same volume of crystallization buffer and incubated at 293 K. The drops were suspended over 200 µl reservoir solution in 48-well plates.

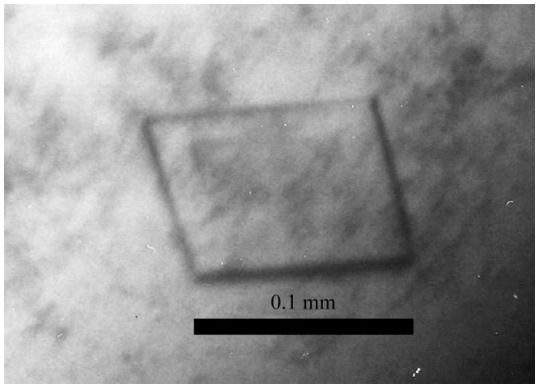

Figure 1
An orthorhombic crystal of MmSAHH.

Table 1
Data-collection statistics for MmSAHH.

Values in parentheses are for the outer shell.

Space group	*I*222
Unit-cell parameters (Å)	a = 100.64, b = 104.44, c = 177.31
No. of subunits per asymmetric unit	2 [half a tetramer]
Solvent content (%)	49.1
X-ray source	PF-AR NW12A
Detector	ADSC Q210r
Wavelength (Å)	1.000
Resolution (Å)	1.55 (1.58–1.55)
No. of observed reflections	544781
No. of unique reflections	132732
Multiplicity	4.1 (3.7)
Mean $I/\sigma(I)$	30.0 (2.9)
B factor (Wilson plot) (Å^2)	19.6
$R_{\rm merge}$† (%)	9.9 (57.4)
Completeness (%)	98.7 (99.8)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith measurement and $\langle I(hkl)\rangle$ is the weighted mean of all measurements of $I_i(hkl)$.

2.3. X-ray data collection

For data collection under cryogenic conditions, the crystals in the droplet were directly transferred to harvesting solution [0.2 *M* sodium formate, 22%(*w*/*v*) PEG 3350 and 20%(*v*/*v*) glycerol pH 6.9] for 1 min. Crystals were mounted in nylon loops and flash-cooled in a cold nitrogen-gas stream at 100 K just prior to data collection. Data collection was performed by the rotation method at 100 K using an ADSC Q210r CCD detector with synchrotron radiation [λ = 1.000 Å on beamline NW12A of the Photon Factory Advanced Ring (PF-AR), Tsukuba, Japan]. The Laue group and unit-cell parameters were determined using the *HKL*-2000 package (Otwinowski & Minor, 1997).

3. Results and discussion

3.1. Expression, purification and crystallization

MmSAHH was successfully cloned, expressed and purified to homogeneity. SDS–PAGE of the purified enzyme revealed a single 48 kDa protein band on Coomassie Brilliant Blue staining. Initial crystal screening produced several microcrystals in a week. Microplate crystals grew from condition Nos. 44 and 90 of Index [No. 44, 25%(*w*/*v*) PEG 3350 and 0.1 *M* HEPES pH 7.5; No. 90, 20%(*w*/*v*) PEG 3350 and 0.2 *M* sodium formate]. Trials to improve the crystallization conditions were performed by varying the pH, the buffer system and the precipitant concentration. To obtain crystals suitable for X-ray analysis, a droplet was prepared by mixing equal volumes (1 µl + 1 µl) of the working solution described above and reservoir solution [0.2 *M* sodium formate and 22%(*w*/*v*) PEG 3350 pH 6.9] and was suspended over 500 µl reservoir solution in 24-well plates. Plate-shaped crystals with typical dimensions of approximately 0.1 × 0.07 × 0.01 mm grew in one week (Fig. 1).

3.2. Data collection

The Laue group of the MmSAHH crystal was found to be *mmm*. Only reflections with $h + k + l = 2n$ were observed for *hkl* reflections, indicating that the crystal belonged to the orthorhombic space group $I222$ (or $I2_12_12_1$). Assuming the presence of two subunits (half a tetramer) in the crystallographic asymmetric unit led to an empirically acceptable V_M value of 2.43 Å^3 Da^{-1}, corresponding to a solvent content of 49.1% (Matthews, 1968). The current best diffraction data from an MmSAHH crystal were collected to 1.55 Å resolution. Data-collection statistics are summarized in Table 1.

3.3. Initial phase determination

Initial phase determination for the MmSAHH crystal was performed by the molecular-replacement (MR) technique using the coordinates of one protomer of human SAHH (PDB code 1li4; Yang *et al.*, 2003), which has approximately 97% amino-acid sequence identity to MmSAHH, as a search model. The bound inhibitor and water molecules were removed from the search model. Cross-rotation and translation functions were calculated using the program *MOLREP* (Vagin & Teplyakov, 1997) from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). The space-group ambiguity ($I222$ or $I2_12_12_1$) for the MmSAHH crystal was resolved by calculating the translation function for both cases. The results showed a clear solution [correlation coefficient of 0.690 (the first noise solution was 0.327) and R factor of 0.356 (the first noise solution was 0.519) in the resolution range 30.0–3.0 Å] and a reasonable molecular arrangement of two subunits of MmSAHH in the asymmetric unit for space group $I222$. Since the tetrameric SAHH molecule has 222 point-group symmetry (Turner *et al.*, 1998; Hu *et al.*, 1999), the two subunits of MmSAHH in the asymmetric unit are related by a crystallographic twofold rotation axis to form a tetramer with 222 point-group symmetry. The MR solution was supported by the observation that the directions of the noncrystallographic twofold axes determined by the self-rotation function (data not shown) were consistent with the MR solution obtained. Automatic model building and refinement using the programs *ARP*/*wARP* (Lamzin & Wilson, 1993) and *REFMAC*5 (Murshudov *et al.*, 1997) and further iterative manual model building and refinement with the programs *XtalView* (McRee, 1999) and *REFMAC*5 are currently in progress. In parallel with the refinement, we are preparing crystals of MmSAHH complexed with various nucleoside inhibitors in order to study their mode of interaction with the enzyme.

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