Biological Science

NW12A/2008G029

Crystal structure analyses of mouse *S*-adenosyl-L-homocysteine hydrolase

Masaaki ISHIHARA, Yoshio KUSAKABE, Nobutada TANAKA*, Tomonobu UMEDA, Yuki ODANAKA, Satoko MATSUBAYASHI, Yasuyuki KITAGAWA, and Kazuo T. NAKAMURA
School of Pharmacy, Showa University,
1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

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. The SAHH enzymes catalyze the reversible hydrolysis of *S*-adenosyl-L-homocysteine (SAH or AdoHcy) to adenosine (Ado) and L-homocysteine (Hcy). 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 a cellular accumulation of SAH, which is a potent feedback inhibitor of SAM-dependent biological methylation. Targets of SAM-dependent methyltransferase 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, tuberculosis, and virus infection.

Although the high-resolution structure of prokaryotic SAHH has recently been reported, that of mammalian SAHH complexed with either inhibitor or substrate analogue is not (the resolution limits are 2.0 and 2.8 Å for human and rat enzymes, respectively). In the absence of the high-resolution structure of mammalian SAHH complexed with an inhibitor (or substrate analogue), the structural image of the SAHH-substrate interactions including the active-site water molecules, which is essential for fully understanding the reaction mechanism of mammalian SAHH, is not clarified enough.

Furthermore the high-resolution structure of mammalian SAHH is essential for the structure-based design of novel selective inhibitors of pathogenic SAHHs, which may serve as anti-tuberculosis or anti-malarial drug leads. Here we report the preliminary crystallographic study of mouse (*Mus musculus*) SAHH (MmSAHH) in the presence of a reaction product Ado [1].

Experimental

Crystallization

Crystallization was carried out at 293 K by the hanging-drop vapour diffusion method. In the best case, a droplet was prepared by mixing equal volumes (2.0 + 2.0 μl) of the protein solution (12 mg/ml protein and 4 mM Ado) and the reservoir solution (500 μl) containing 22%(w/v) PEG3350 and 0.2 M calcium sodium formate in 0.1 M HEPES buffer at pH 7.0. Plate shaped crystals with typical dimensions of about 0.1 x 0.07 x 0.01 mm^3 were grown in 1 week [1].

X-ray data collection

The crystals belong to an orthorhombic space group *I*222 with cell dimensions of a = 100.64 Å, b = 104.44 Å, c = 177.31 Å. Assuming two subunits (1/2 tetramer) per asymmetric unit, we obtained a V_M value of 2.43 $Å^3$/Da, corresponding to a solvent content of 49 %. The data collection was performed at 100 K using an ADSC Q210r CCD detector with the synchrotron radiation of NW12A (λ = 1.00 Å). The current best diffraction data from a MmSAHH crystal were collected up to 1.55 Å resolution.

Results and Discussion

The initial phase determination was carried out by the molecular replacement (MR) method using the coordinate set of one protomer of human SAHH (PDB code: 1LI4) as a search model. The results showed clear initial solutions (correlation coefficient of 0.690 and R-factor of 0.356 in the resolution range of 30.0 - 3.0 Å), and reasonable molecular arrangement of MmSAHH tetramer in the unit cell. Structural details of MmSAHH and structural comparison with pathogenic SAHHs will be published elsewhere.

References

[1] M. Ishihara et al., Acta Cryst. F66, 313 (2010).

* ntanaka@pharm.showa-u.ac.jp