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Crystal structure analyses of a hydrolase from *Mycobacterium tuberculosis*

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Introduction

Mycobacterium tuberculosis is a main pathogen causing tuberculosis (TB) which is currently causing the death of more than two million people annually. TB has been treated with combination of several drugs. However, new cases of TB of multidrug resistance strain of *Mycobacterium tuberculosis* have been appeared. In the development of chemotherapeutic agents against TB, structure based design of selective inhibitors for the enzymes important for survive of *Mycobacterium tuberculosis* is essential.

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. Recently, SAHH inhibitors are expected to provide new-type chemotherapeutic agents against several pathogens. The SAHH enzyme from *Mycobacterium tuberculosis* (MtSAHH), in its active form, is a homo-tetramer of identical subunits, each of which comprises 495 amino acid residues, and contains a tightly but not covalently bound NAD^+ cofactor and has a molecular mass of about 55 kDa.

The X-ray crystal structures of mammalian (human and rat) and human malaria parasite (*Plasmodium falciparum*) SAHHs have been previously reported. However, crystal structure of MtSAHH has never been reported. Thus the three-dimensional structure of MtSAHH is essential for the structure-based design of novel selective inhibitors of MtSAHH, which may serve as anti-TB drug leads. Here we report the crystallization and preliminary X-ray crystallographic studies of recombinant MtSAHH.

Experimental

Crystallization

The expression and purification of MtSAHH will be published elsewhere. 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 (1.5 + 1.5 μl) of the protein solution (12 mg/ml protein and 4 mM adenosine) and the reservoir solution (500 μl) containing 15%(v/v) PEG400 and 0.2 M calcium acetate in 0.1 M HEPES buffer at pH 7.0. Plate shaped crystals with typical dimensions of about 0.4 x 0.4 x 0.1 mm^3 could be grown in 2 weeks [1].

X-ray data collection

The crystals belong to a monoclinic space group $P2_1$ with cell dimensions of a = 92.95 Å, b = 111.59 Å, c = 100.49 Å, and β = 98.19 deg. Assuming four subunits (one tetramer) per asymmetric unit, we obtained a V_M value of 2.46 $Å^3$/Da, corresponding to a solvent content of 50 %. The data collection was performed at 100 K using an ADSC Q4R CCD detector with the synchrotron radiation of BL17A (λ = 1.00 Å). The current best diffraction data from a MtSAHH crystal were collected up to 1.75 Å resolution.

Results and Discussion

The initial phase determination was carried out by the molecular replacement (MR) method using the coordinate set of *Plasmodium falciparum* SAHH (PfSAHH) tetramer (PDB code: 1V8B) [2] as a search model. The results showed clear initial solutions (correlation coefficient of 0.408 and R-factor of 0.475 in the resolution range of 15.0 - 3.0 Å), and reasonable molecular arrangement of MtSAHH tetramer in an asymmetric unit. Structural details of MtSAHH will be published elsewhere [3].

References

[1] Y. Kusakabe et al., in preparation.
[2] N. Tanaka et al., J. Mol. Biol. 343, 1007 (2004).
[3] Y. Kusakabe et al., in preparation.

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