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Crystal structure of *S*-adenosyl-L-homocysteine hydrolase from *Plasmodium falciparum* complexed with a selective inhibitor.

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Introduction

Malaria is one of the world's most serious parasitic diseases. There are estimated 300-500 million cases and up to 2.7 million deaths from malaria each year. Human malaria is caused by infection with intracellular parasites of the genus *Plasmodium* that are transmitted by *Anopheles* mosquitoes. *Plasmodium falciparum* is the most lethal among the four species of *Plasmodium* that infect humans. The emergence of strains of malarial parasite resistant to conventional drug therapy has stimulated searches for antimalarials with novel modes of action.

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 malaria, because neplanocin A, a strong inhibitor of SAHH, is reported to be a growth inhibitor of *P. falciparum*. The cDNA cloning of *P. falciparum* SAHH (PfSAHH) revealed that the PfSAHH contains a 41-amino acid insert in its sequence as compared with mammalian SAHH. The PfSAHH enzyme, in its active form, is a homo-tetramer of identical subunits, each of which comprises 479 amino acid residues, and contains a tightly but not covalently bound NAD cofactor and has a molecular mass of about 54 kDa. The structural difference between the PfSAHH and mammalian SAHH may give valuable clues for development of antimalarials.

In 2004, we reported the crystal structure of PfSAHH complexed with the reaction product adenosine (Ado) [1]. A structural comparison with human SAHH (HsSAHH) revealed that a single substitution between the PfSAHH (Cys59) and HsSAHH (Thr60) accounts for the differential interactions with nucleoside inhibitors. Recent studies suggested that introduction of a fluorine atom at the 2-position of an adenine nucleoside derivative improve the selective index between HsSAHH and PfSAHH. To obtain a insight into structural basis for selective inhibition of SAHHs by the inhibitors, structural analyses of PfSAHH complexed with the selective inhibitors are essential.

Here we report the crystal structure of PfSAHH complexed with a selective inhibitor, 2-fluoronoraristeromycin (2-F-NAM).

Experimental

Crystallization

The expression and purification of PfSAHH were performed as described [2]. Crystallization was carried out at 293 K by the hanging-drop vapor diffusion method. In the best case, a droplet was prepared by mixing equal volumes (1.5 + 1.5 μl) of the protein solution (4 mg/ml protein and 2 mM 2-F-NAM) and the reservoir solution (500 μl) containing 1.2 M sodium citrate in 100 mM Hepes buffer at pH 7.5. Plate shaped crystals with typical dimensions of about 0.5 x 0.2 x 0.05 mm^3 could be grown in 2 weeks.

X-ray data collection

The crystals belong to an orthorhombic space group $P2_12_12_1$ with cell dimensions of a = 76.76 Å, b = 88.11 Å, and c = 333.9 Å. Assuming four subunits (one tetramer) per asymmetric unit, we obtained a V_M value of 2.57 $Å^3$/Da, corresponding to a solvent content of 52%. Crystals in a droplet were transferred directly to a cryoprotectant Paratone-N (Hampton Research). The data collection was performed at 100 K using an ADSC Q210 CCD detector with the synchrotron radiation of NW12 in PF-AR (λ = 1.00 Å). The current best diffraction data from a PfSAHH/2-F-NAM complex crystal were collected up to 2.5 Å resolution.

Results and Discussion

The phase determination was carried out by the difference Fourier method using the coordinate set of the PfSAHH/Ado complex [1] (PDB code: 1V8B) as a starting model. Crystallographic refinement was performed with the program REFMAC. The crystal structure of PfSAHH/2-F-NAM complex revealed that the 2-F atom of 2-F-NAM is accommodated in the surface depression specifically found in PfSAHH, as expected from the crystal structure analysis of PfSAHH/Ado complex. Structural details of PfSAHH/2-F-NAM complex will be published elsewhere [4].

References

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