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Crystal structure analyses of a hydrolase from the human malaria parasite

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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.

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

Experimental

Crystallization

The expression and purification of PfSAHH were performed as described [1]. 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 4 mM adenosine) 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 [2].

X-ray data collection

The crystals belong to an orthorhombic space group $P2_12_12_1$ with cell dimensions of a = 77.09 Å, b = 86.15 Å, and c = 333.8 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. The current best diffraction data from a PfSAHH crystal were collected up to 2.4 Å resolution.

Results and Discussion

The initial phase determination was carried out by the molecular replacement method using the coordinate set of the human SAHH (HsSAHH) tetramer (PDB code: 1LI4) as a search model. Crystallographic refinement was performed with the program CNS. The size of the tetrameric PfSAHH molecule is 65 x 85 x 100 Å and the four subunits are related by a 222 point group symmetry. The subunit of PfSAHH consists of two large domains separated by a cleft containing a deep pocket, and a small C-terminal domain that is separated from the main body of the subunit. The insert specifically found in PfSAHH is separated from the main body of the tetramer, whereas nucleoside inhibitors of SAHH bind to the crevice of the substrate-binding domain. It is therefore difficult to directly utilize structural information for the insert for a rational design of selective PfSAHH inhibitors. However, a structural comparison with HsSAHH revealed that a single substitution between the PfSAHH and HsSAHH accounts for the differential interactions with inhibitors. Structural details of PfSAHH will be published elsewhere [3].

References

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