NE3A, NW12A/2008G029

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

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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 and 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.

Here we report crystal structures of mouse (*Mus musculus*) SAHH (MmSAHH) complexed with ADO, 3'-keto-aristeromycin (3KA), and noraristeromycin (NRN). These results provide insights into the catalytic mechanism of SAHH. In addition, we have determined a crystal structure of MmSAHH complexed with ribavirin (RBV), which is a potent inhibitor of SAHH. Although RBV is a well-known guanosine analogue, our crystal structure analysis clearly shows that RBV can also act as an adenosine analogue.

2 Experiment

The purification and crystallization of MmSAHH were carried out as described previously [1]. The crystals of the MmSAHH/NAD^+/ADO (or analogue) complexes were prepared as follows. ADO or its analogues (aristeromycin, ARI; NRN; or RBV) were dissolved in a standard buffer (0.1-M sodium chloride in 0.05-M Tris-HCl buffer at pH 7.4) to a concentration of 20 mM. The 15-mg/ml MmSAHH solution was mixed with aliquots of the respective ligand solutions in a volume ratio of 4:1 with a final ligand concentration of 4 mM and a MmSAHH concentration of 12 mg/ml. A droplet was prepared by mixing an equal volume of the working solution described above and the reservoir solution containing 0.2-M sodium formate and 22 %(w/v) PEG3350 in Hepes/NaOH buffer at pH 7.0. The droplet was suspended over 500 μl of reservoir solution in a 24-well plate. The crystals belong to an orthorhombic space group *I*222 with cell dimensions of a = 100.64 Å, b = 104.44 Å, and c = 177.31 Å for the ADO complex. Crystals of the ADO analogue complexes were isomorphous with the crystals of the ADO complex. For data collection under cryogenic conditions, the crystals in a droplet were directly transferred to a harvesting solution [0.2-M sodium formate, 22 %(w/v) PEG3350, and 20 %(v/v) glycerol in Hepes/NaOH buffer at pH 7.0] for 1 minute. Crystals were mounted in nylon loops and flash-frozen in a cold nitrogen-gas stream at 100 K immediately prior to data collection. Data collection was performed at 100K using a CCD detector with the synchrotron radiation of the beam lines of PF-AR (NE3A and NW12A).

The initial phase determination was performed with the molecular replacement method using one protomer of HsSAHH (PDB code: 1LI4) as a search model. The refined structure of the MmSAHH/NAD^+/ADO complex was then used for the structure determination of the other ADO analogue complexes by the difference Fourier method.

3 Results and Discussion

In this study, we report the high-resolution crystal structures of MmSAHH co-crystallized with ADO, ARI, and NRN at resolutions of 1.55, 1.55, and 1.65 Å, respectively. Co-crystallization with ARI resulted in the oxidation of the 3'-OH group of the nucleoside to generate the 3'-keto form (3KA). The NRN complex is the first crystal structure of SAHH to be solved with NRN, and a water molecule is identified as the candidate donor in a Michael addition to the reaction intermediate 3'-keto-4',5'-didehydroadenosine. These structures constitute the structural snapshots of the last three molecular stages in ADO formation and provide insights into the catalytic mechanism of SAHH [2].

References

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