Crystal structure of 5'-deoxy-5'-methylthioadenosine phosphorylase from the archaeon *Aeropyrum pernix* K1

Yasuhito Iizuka1,2, Makiko Kikuchi2, Takahiro Yamauchi1,3, Masaru Tsunoda1,2

1 Graduate School of Life Science and Technology, 2 Faculty of Pharmacy, Iryo Sosei University, Iwaki, Fukushima, Japan 3 Department of Pharmacy, Fukushima Rosai Hospital, Iwaki, Fukushima, Japan, yasuhito.iizuka@isu.ac.jp

Keywords: 5'-deoxy-5'-methylthioadenosine phosphorylase, polyamine biosynthesis, *Aeropyrum pernix*

[Background] 5'-Deoxy-5'-methylthioadenosine phosphorylase (MTAP) catalyzes the reversible phosphorolysis of MTA to free adenine and 5-methylthio-D-ribose-1-phosphate (Fig. 1). MTA is a byproduct of polyamine biosynthesis. *Aeropyrum pernix* is a member of hyperthermophilic archaea. Although the thermophilic archaea have not yet been clearly defined, hyperthermophilic archaea are those with optimal growth temperatures above 80°C. Because the proteins (including enzymes) produced are thermostable. Research on heat-stable enzymes will contribute to drug discovery and industrial applications. Here, we report the crystal structures of an unliganded ApMTAP and a complex of ApMTAP bound to MTA.

[Experimental procedures] ApMTAP was expressed in *Escherichia coli*. Proteins were purified using heat treatment, ammonium sulfate precipitation, and column chromatography. These proteins were crystallized via hanging-drop vapor diffusion at 20°C. Two microliters of protein solution containing 10 mg/mL ApMTAP was mixed with an equal amount of reservoir solution (20% (v/v) polyethylene glycol and 0.1 M phosphate-citrate; pH 4.8). D(+)-Galactose was added at a final concentration of 3%. X-ray diffraction data of crystals obtained in reservoir solutions under optimized conditions were collected at the Photon Factory BL-5A (High Energy Accelerator Research Organization, Japan). Ligand-bound ApMTAP crystals were prepared using the soaking method. ApMTAP crystals were soaked in 100 mM MTA/dimethyl sulfoxide solution to induce MTA/MTAP complex formation. The data were processed using the CCP4 program suite. Both non-soaked ApMTAP crystals and ApMTAP crystals soaked in MTA were identified using molecular replacement with ApMTAP (PDB entry code:1WTA). Both crystal structures belong to the space group *R*32.

[Results and discussion] The overall structure of ApMTAP was similar to that of human MTAP (PDB entry code:1CB0 [1]). In the non-soaked crystal structure, adenine and either phosphate or sulfate electron densities were observed even though the crystals were not soaked in the substrate or product. A comparison of the non-soaked and soaked crystal structures revealed a structural change in the active site loop region containing Ser20. The loop region followed the first β-strand in the middle of the β-sheet. The short helix present in the non-soaked crystal structure was replaced by large loops in the soaked crystal structure (Fig. 2). This rearrangement was also observed in hMTAP [2]. A flip in the side chain of Phe59 could be seen. A shift in Tyr23 was also observed, with the hydroxyl group forming hydrogen bonds with the main chain of Gly21 and the side chains of His68 and Glu234. These conformational changes may contribute to the stabilization of the complex.
