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Neutron Diffraction Study of ADP-ribose pyrophosphatase from Thermus thermophilus HB8

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ADPRase, ADP ribose pyrophosphatase, catalyzes the hydrolysis of ADP ribose to ribose 5'phosphate and AMP. The postulated role of ADPRase is to control the cellular concentration of toxic nucleoside diphosphate derivatives or physiological methabolites. Yoshiba et al [1] have reported crystal structures of ADPRase derived from *Thermus thermophilus* HB8. To explain the catalytic mechanism including ionization state of catalytic residues, role of water molecules for the catalytic function and substrate recognition of ADPRase. However, hydrogen atoms of the enzyme, substrate and water molecules have not been detected directly because of lower scattering length of hydrogen atom by X-ray compared to other atoms. It is necessary to obtain information of hydrogen atoms by neutron crystallography for analysis of the catalytic mechanism in detail. For neutron crystallography of protein, large crystal in size is required for data collection. We prepared 13 mm3 ADPRase crystal in volume by macro seeding method after purification recombinant ADPRase from E. coli. PEG4000 was used as a precipitant and the crystal took about two months for its growth at 20 degrees. The crystal was soaked into the deuterated solution including inhibitor of AMPCPR(the nonhydrolyzable ADPR analogue) and Mg2+ ion. Neutron diffraction data were collected to 2.1 Å resolution on the BIX-3 neutron diffractometer in JAEA, and X-ray diffraction data were collected to 1.2 Å resolution at BL6A in PF using the same crystal. The crystal structure has been refined using X-ray and neutron data simultaneously (joint X-ray and neutron refinement) to R-factor 17% and free R-factor 24%. Because density maps of the inhibitor and Mg2+ ion were weak, occupancy of the inhibitor was adjusted to 0.3 and 0.2, respectively. The density maps obtained by neutron data suggested ionization status of active site residues. Glu82, Glu85 and Glu86 involved in binding of Mg2+ ions and catalytic reaction were deprotonated. Between Glu108 and Ser102 recognizing the ribose of substrate, there is a hydrogen atom forming hydrogen bonding interaction. Glu108 is found to be deprotonated. These observations are important to understand catalytic mechanism of ADPRase.

Reference

[1] Yoshiba S, Ooga T, Nakagawa N, Shibata T, Inoue Y, Yokoyama S, Kuramitsu S, Masui R. (2004). JBC 279(37), 163-174,