Poster Presentation

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Neutron crystallographic analysis of human α-thrombin using iBIX at J-PARC

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α-Thrombin is a serine protease, which plays the central role in the coagulation system. Investigating the protonation states of the enzyme is useful to reveal the reaction mechanism and to design anticoagulant drugs. We have studied the protonation states of the α-Thrombin-bivalirudin complex using neutron diffraction method. The complex is regarded as the enzyme-product complex, because the hydrolyzed bivalirudin fragments keep staying in the binding sites in the crystal. Previously we had performed a neutron crystallographic analysis of α-Thrombin-bivalirudin complex at pD 5.0 (space group P21212) at 2.8 Å resolution.[1] To observe the protonation states of the active site more clearly, we carried out time-of-flight neutron diffraction experiments for a different crystal form of this complex (space group C2 at pD 7.9) using IBARAKI biological crystal diffractometer, iBIX, installed in J-PARC. Using improved 30 neutron detectors with high-efficiency, we have succeeded in collecting the reflections at around 2.0 Å resolution. XN-joint refinements were performed using PHENIX program. The neutron scattering length OMIT map showed a density on the hydroxyl group of serine 195, which could be a deuterium. Since the density was not observed for P21212 crystal at pD 5.0 and the position was too far from an acceptor atom to form a stiff hydrogen bond, currently we are confirming the result. In this presentation, details of the neutron crystallographic analysis and the comparison between the structures, especially, the protonation states of amino acid residues in the active site of the complex at pD 5.0 and pD 7.9 will be given.

[1] *T. Yamada et al., Biochim Biophys Acta., 2013, 1834, 1532-8.*

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