

X-ray/neutron joint refinements of serine proteases

Taro YAMADA^{*1}, Kenji KAWAMURA¹, Yuki OHNISHI¹, Takeshi YOKOYAMA¹,
 Ichiro TANAKA¹, Kazuo KURIHARA², Taro TAMADA², Ryota KUROKI², Nobuo NIIMURA¹
¹Ibaraki University, Tokai, Ibaraki 319-1106, Japan
²JAEA, Tokai, Ibaraki 319-1195, Japan

Introduction

Neutron scattering is a useful technique to observe hydrogen atoms, such as polar hydrogen in polar amino acid residues and solvent water molecules in protein crystals. However, availability of neutron source and intensity of neutron beam are limited. Currently the technique needs large sizes of protein crystals and quite a long experimental time. It was reported that using of x-ray diffraction data with neutron data for structure refinement gave better quality of neutron scattering length density maps.

Serine proteases are interesting targets to study the reaction mechanism with the neutron diffraction technique because the reaction is related with hydrogen bonds, hydrogen migration, and activation of water solvent. In this project, we deal with human bovine β -trypsin-BPTI complex, human α -thrombin-bivalirudin complex, and *Achromobacter* protease I. At BL-5A, 6A, AR-NE3, we were able to obtain the decent quality of x-ray diffraction data for those serine proteases. This report will give a quick review of the results of the x/n joint refinements.

Results*Bovine Trypsin-BPTI complex*

The crystal structure of the trypsin-BPTI complex has been refined, and the deuterium and hydrogen atoms in the complex have been identified, using both 1.6 Å resolution X-ray diffraction data and 2.15 Å resolution neutron diffraction data. The protonation states of the catalytic triad—Asp102, His57, and Ser195—in the trypsin-BPTI complex have been observed and are discussed on the basis of the average structure of the initial state (before the catalytic reaction of the trypsin), the acyl-enzyme intermediate state (“during” the reaction), and the final state (after the reaction). The result was published in ref. [1]

Human α -thrombin-bivalirudin complex

The crystal structure of the complex has been refined using both 1.6 Å resolution X-ray diffraction data and 2.75 Å resolution neutron diffraction data obtained at room temperature. The x/n joint refinement showed that H57/H is partially protonated and could interact with a water molecule near H57/H. The presence of the solvent water is not so clear from the electron density and the nuclear scattering length map. For confirmation, 1.26 Å x-ray diffraction data was collected at BL-5A at 100 K

and the structure refinement was carried out. The structure analysis showed that the solvent water molecule surely exists although the occupancy could be partial. Now we are trying to interpret the observation in terms of the reaction mechanism of the α -thrombin.

Achromobacter Protease I

The crystal structure of the apo enzyme has been refined using both 1.6 Å resolution x-ray diffraction data and 2.0 Å resolution neutron diffraction data obtained at room temperature [2]. The x/n joint refinement showed that H57 in the active site is slightly protonated, 15% in occupancy. To support the result, a 0.95 Å resolution x-ray diffraction data was collected at BL-5A and analyzed. Figure 1 shows the electron density map around H57. Between H57 and D113, electron density of HD1 was observed, although the clear electron density for HE2 between H57 and S194 was not observed. S194 having a disordered structure, seems to be caused by the protonation states of H57. More precise analysis is now underway.

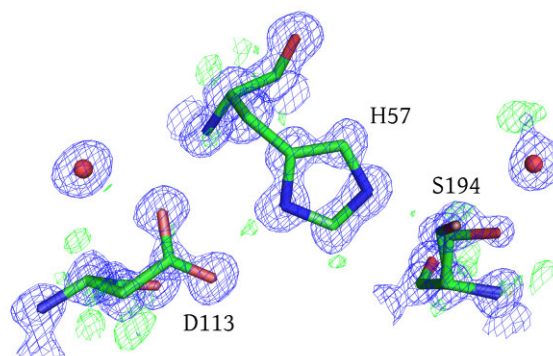


Figure 1. Electron density maps around H57 of API. 1.5σ level of $2fo-fc$ map was shown in blue mesh. 2.4σ level of $fo-fc$ map shows in green and red. Between H57 and D113, electron density for HD1 was observed, although the clear electron density for HE2 between H57 and S194 was not observed.

References

- [1] K. Kawamura et al., *Acta Cryst.* D67, 140 (2011).
 [2] Y. Ohnishi et al., *J. Physics Conference Series* 251, 012032 (2010).

* taro@mx.ibaraki.ac.jp