Poster Presentation

MS82.P06

Neutron diffraction study to elucidate reaction mechanism of RNase A

K. Kusaka[1], T. Yamada[1], K. Tomoyori[2], T. Hosoya[1], T. Ohhara[3], I. Tanaka[1], N. Niimura[1]

[1]Ibaraki university, iFRC, Tokai, Japan, [2]JAEA, Quantum Beam Science Directorate, Tokai, Japan, [3]JAEA, J-PARC Center,Tokai, Japan

Ribonuclease A (RNase A) is a pyrimidine-specific endoribonuclease that claves and hydrolyzes single-stranded RNA in two distinct steps. The mechanism of the cleavage reaction catalyzed by RNase A involves two key histidine residues, His12 and His119. It is important to know the protonation states of them in order to understand the hydrolysis mechanism of RNase A. Neutron protein crystallography is a powerful technique for solving the problems. In previous reports, the protonation states of them for RNase A complexed with phosphate ion, uridine vanadate and phosphate free one have been investigated by neutron diffraction analysis [1-3]. In this study, neutron diffraction analysis of phosphate free RNase A has been carried out with high resolution and completeness data set in order to clarify the protonation states of two active site histidine residues, and to elucidate the detailed mechanism of the cleavage reaction. Neutron diffraction data of bovine pancreatic RNase A were collected by IBARAKI biological crystal diffractometer iBIX in J-PARC. The structure was determined by joint neutron and X-ray structure refinement. The final values of Rcryst and Rfree were 19.5% and 22.0%, respectively, for completeness of 86.7% to a resolution of 1.4Å. The structure with high reliability and good data statistics could be obtained by comparing with the already-reported one [3]. We calculated |Fo|-|Fc| neutron scattering length density map after omitting Dδ1 and Dε2 of His12 and His119 in order to confirm the protonation states of them. These omit maps indicated that His12 is completely singly protonated and Hi119 is doubly protonated. The protonation states of them are consistent with those in the first step of the putative mechanism of catalysis by RNase A. We could also observed a D atom of water molecule which is hydrogen bonded to Nε2 of His12.

[1] *A. Wlodawer, M. Miller, L. Sjölin, Proc. Natl Acad. Sci. USA, 1983, 80, 3628–3631.,* ***[2]*** *A. Wlodawer, L. Sjölin, Biochemistry, 1983, 22, 2720–2728.,* ***[3]*** *D. Yagi, T. Yamada, K. Kurihara et. al, 2009, D65, 892-899.*

Keywords: RNase A, Neutron diffraction, protonation state