Neutron crystallographic analysis of Ribonuclease A by using iBIX at J-PARC

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Ribonuclease A (RNase A) catalyzes the hydrolysis of a ribonucleic acid by a two-step reaction. It is important to know the protonation states of two key histidine residues (His12 and His119) in order to understand the hydrolysis mechanism of the cleavage reaction catalyzed by RNase A. Neutron crystallographic analysis 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 order to clarify the protonation states of two active site histidine residues, and then to elucidate the detailed mechanism of the cleavage reaction of RNase A, neutron diffraction data of bovine pancreatic RNase A with high resolution and completeness were collected by using iBIX at J-PARC. The structure model was refined to joint neutron and X-ray data set. The final values of R_{cryst} and R_{free} for neutron data 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].

|Fo|-|Fc| neutron scattering length density map was calculated after omitting $D^{\delta 1}$ and $D^{\varepsilon 2}$ of His12 and His119 These omit maps indicated that in the protonation states of them as above. His12 and 119 are completely singly and doubly protonated respectively. These results are consistent with the protonation states in the first step of the putative mechanism of catalysis by RNase A. A deuterium atom of a water molecule which is hydrogen bonded to $N^{\varepsilon 2}$ of His12 is could be also observed.

References

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