Hydrogen and hydration in proteins observed by high resolution neutron crystallography

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One of the most important fields today is structural genomics, in which the functions of proteins are analyzed using the results from synchrotron X-ray and NMR protein structure analysis. However, it is difficult in an NMR or X-ray crystallographic analysis of a protein to identify all of the hydrogen atoms and the water molecules of hydration, even though they play important roles in innumerable biological processes.

In contrast, the neutron diffraction method has the ability to locate hydrogen position absolutely. We have recently developed a neutron imaging plate (NIP) and a neutron monochromator, and have successfully applied them to construct a neutron diffractometer dedicated for biological macromolecules (BIX-2, BIX-3, BIX-4:) in the JRR-3M reactor. The performance of BIX type diffractometer has been certified as one of the best in the world.

By using BIX type diffractometer, all the hydrogen atoms and most of the solvent molecules of hydration of lysozyme (Hen Egg-white L. at different pH, Human L.), insulin, myoglobin and rubredoxin (wild type and mutant), which are small but fundamentally important proteins, have been unambiguously identified in 1.5 Å resolution. These structural results have provided new and important discoveries such as the bifurcated hydrogen bonds in alpha-helices, the fine structure of methyl group, details of hydrogen/deuterium exchange reactions, the role of hydrogen atoms in enzyme reaction and the dynamic behavior of hydration in proteins.