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High resolution neutron diffraction on a tiny perdeuterated crystal

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Perdeuterated Type III Anti-Freeze Protein (AFP-D, M.W.=7kDa) has been overexpressed and crystallized in D2O in the Deuteration Laboratory at the Institut Laue Langevin (ILL) in Grenoble, France. Neutron Laue data collection up to 1.85A was performed at room temperature (RT) on the new LADI-III Diffractometer at ILL using a sub-cubic millimetric crystal volume (0.15mm3). The crystal quality was very good, as shown by the mosaicity (0.05 - 0.10° refined by Program HKL2000). Detailed data processing statistics will be given. The structural identity between the hydrogenated and perdeuterated proteins has been checked by comparing with the structure refined against X-ray diffraction data (RT, ID29, Synchrotron ESRF , 1.05 A resolution).

Keywords: neutron diffraction, protein crystallography, perdeuteration

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Proton polarization technique for neutron protein crystallography (NPC)

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The hydrogen atoms in a protein have a large neutron incoherent-scattering cross section, which makes the background signal large and the hydrogen atoms sometimes difficult to see. The current method for reducing this incoherent scattering is to deuterate the sample crystal, either partially by soaking it in heavy water solution or completely by culturing the protein-producting bacteria in deuterated media from the beginning. However, there always remains the question of whether or not the deuteration affects the folding structure of the protein, because the living cells that produce the protein never live in nature in a heavy water environment for so long. It might be possible to carry out a neutron protein crystallography experiment with a normal protein crystal, without deuterating it. The method would be to polarize the spins both of the neutrons and of the protons (hydrogen atoms) in the protein. This method, called the proton polarization method (PPM), would enable a very accurate determination of the hydrogen atoms in a protein without deuteration. The effective coherent-scattering length of a hydrogen atom (proton) is given as a function of the polarization. When the proton is not polarized, scattering length of hydrogen atom, $bH(0) = -0.375 \cdot 10^{-12}$ cm. When the proton is 100 % polarized and the polarization is parallel with the neutron, $bH(++) = 1.085 \cdot 10^{-12}$ cm. When the proton is 100 % polarized but the polarization is anti-parallel with the neutron, $bH(+-) = -1.835 \cdot 10^{-12}$ cm. Thus it is seen that the effective coherent-scattering lengths of hydrogen atoms with parallel and anti-parallel spins, relative to the neutron polarization, are very different from each other. If this nature could be exploited, it would be a unique way to determine the position of the hydrogen atoms in proteins. Firstly, all of the protons (hydrogen atoms) in all of the protein molecules in a single crystal must be polarized. A small amount of paramagnetic centers is added to the sample. In a moderately strong magnetic field of 2.5 T at temperatures less than 1 K, microwave irradiation will polarize the nuclear spins. Secondly, the neutron beam must be polarized parallel to the protein protons and then used to make a typical diffraction experiment. Thirdly, the neutron polarization must be reversed, and another diffraction experiment should be made. When the difference Fourier map, ||F(++)(hkl)|-|F(+-)(hkl)|, is calculated, only the hydrogen atoms (protons) appear in the difference map. Moreover, since the signs of bH(++) and bH(+-) are opposite, the difference between them becomes much larger. Indeed, the ratio of this difference to the scattering length of unpolarized hydrogen atoms is about eight ((bH(++) - bH(+-) / bH(0)≈ 8), so that the Fourier peak of hydrogen atoms in this method is enhanced eight times over that obtained in normal NPC. The technique of producing polarized neutrons is well established. The polarization of protons in protein molecules in solution has been tried successfully. [1] However, the polarization of protons in protein molecules in a single crystal has never been tried yet. The remaining significant hurdles are (1) how to realize the cooling of a protein crystal at 1 mK, and (2) how to dope the crystal with paramagnetic materials, which are essential to initialize the polarization of protons. The first hurdle might be overcome by the development of the technique of high-pressure cooling of protein crystals. [2] The second hurdle might be overcome by upgrading the technique of polarizing the protons in protein molecules in solution.

[1] H. Stuhrmann, et al. (1986), *Eur. Biophys. J.* 14,1-6. [2] C. Kim,C. et al. (2005), *Acta Cryst*. D61, 881-890.

Keywords: neutron protein crystallography, deuteration, polarized neutron

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Overview of the IBARAKI biological crystal diffractometer (iBIX) at J-PARC

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The IBARAKI Biological Crystal Diffractometer (iBIX), a new diffractometer for protein crystallography at the next generation neutron source at J-PARC (Japan Proton Accelerator Research Complex), has been constructed and has been operational since December 2008. Preliminary structure analyses of organic crystals and a protein crystal showed that iBIX has high performance even at 120 kW operation [1]. From November 2010, J-PARC proton power has increased up to 220 kW and full data sets of two protein crystals were collected successfully.

Now iBIX has 14 detector units, the basic part of data reduction software (STARGazer) and an equipment of cryostream cooler to 20K. According to the performance of iBIX measurements, it turned out that it is possible to collect a full data set of a protein in 3-4 days for a crystal of 1 mm^3 in volume and to reduce data from a crystal whose unit cell dimension is as long as about 200 Angstrom. When J-PARC reaches at 1MW, one can expect nearly a 10 times higher efficiency at iBIX than that at present because iBIX detector units will be added up

to twice at maximum.

Because of the 3.11 big earthquake in Japan, J-PARC was damaged as well as iBIX. They are being recovered and recovery is expected to take at least a year.

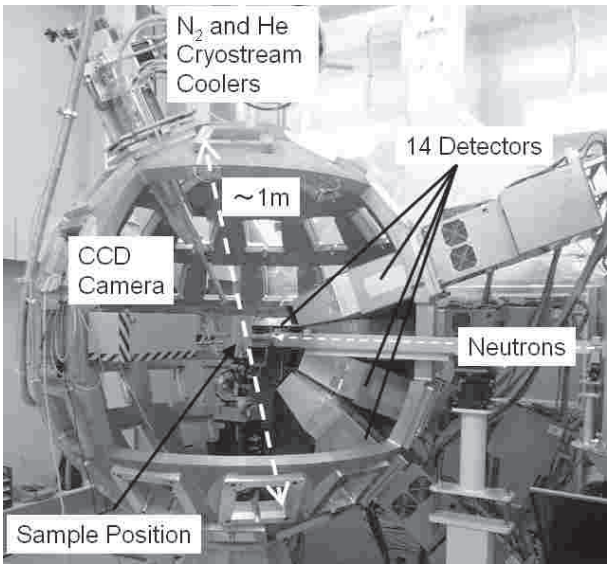

Fig. iBIX sample position and 14 detectors.

[1] I. Tanaka, K. Kusaka, T. Hosoya, N. Niimura, T. Ohhara, K. Kurihara, T. Yamada, Y. Ohnishi, K. Tomoyori and T. Yokoyama, *Acta Cryst. D* **2010**, *66*, 1194-1197.

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Large volume crystal growth in restricted geometery for neutron crystallography

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Neutron Macromolecular Crystallography (NMC) is the prevalent method that accurately determines the positions of hydrogen atoms in macromolecules. There are two major limiting factors in determining protein structure with neutron diffraction; one is obtaining large crystal volumes and the other is the extended experimental duration. We demonstrate that crystallizing hyperthermophilic proteins by counter-diffusion crystallization can produce suitable protein crystals for NMC. Counter-diffusion crystallization in restricted geometry, such as in capillary tubes, allows diffusion to govern mass transport processes so that a spatial-temporal gradient of supersaturation is formed along its length. Consequently, proteins can crystallize in an optimized supersaturated condition where a single crystal can fill up the diameter of the capillary. We have employed the same process in capillary vessels having diameters exceeding 1mm while minimizing the ratio between buoyant and viscous forces. As a result, protein crystals suitable for neutron crystallography can be grown in this configuration with volumes greater than 1mm^3 while eliminating invasive crystal manipulation. Using crystals grown by this method, we have undergone neutron diffraction analysis of recombinant proteins from a hyperthermophilic archaeon. These proteins are excellent crystallization targets because of their thermal and mechanical stability and high propensity to crystallize. As an initial proof of principle, a novel inorganic pyrophosphatase (IPPase) was among the first of these proteins to be grown in large diameter capillaries resulting in a 9mm^3 volume crystal. The next generation neutron beamlines at the Spallation Neutron Source (SNS) along with an effective counter-diffusion crystallization procedure can improve structures of a diverse range of proteins of interest to the crystallographic community. Thus the crystallization of macromolecules can grow to volumes never before achieved, potentially leading to enormous increases in the number of neutron crystallographic protein structures.

Keywords: neutron crystallography, counter-diffusion crystallization, large-volume protein crystals

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Neutron structure of retaining glycosyltransferase GTA

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With at most one electron, hydrogen atoms can be difficult to observe in protein structures by X-ray crystallography even at atomic resolution. This can pose a barrier to the critical evaluation of enzymatic mechanisms that involve proton translocation as the ionization states of active site residues cannot always be reliably determined by the chemical environment in which they reside.

One such class of enzyme is the glycosyltransferase. Glycosyltransferases catalyze the biosynthesis of oligosaccharides and glycoconjugates by the transfer of a monosaccharide unit from an activated donor molecule to an acceptor molecule with high stereospecificity. The anomeric stereochemistry of the donor sugar will either be inverted or retained upon formation of the new glycosidic linkage, depending on the enzyme. Although a consensus has been reached on a straightforward S_N2 mechanism used by inverting glycosyltransferases, the nature of the retaining mechanism is still a matter of debate.

The model retaining enzyme human ABO(H) blood group A α-1,3-N-acetylgalactosaminyltransferase (GTA) generates the blood group A antigen by the transfer of N-acetyl-galactosamine from UDP-GalNAc to the blood group H antigen. To understand better how specific active-site-residue protons and hydrogen-bonding patterns affect substrate recognition and catalysis, neutron diffraction studies were conducted at the Protein Crystallography Station (PCS) at Los Alamos Neutron Science Center (LANSCE).

This is the first study of a retaining glycosyltransferase using combined X-ray crystallographic data (to 1.9 Å resolution) and neutron Laue data (to 2.5 Å resolution) [1]. These data provide the first unambiguous assignment of protons and the causative hydrogen-bond patterns in a glycosyltransferase active site.