Practical study of D/H contrast neutron protein crystallography

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1. Introduction

Neutron protein crystallography (NPC) has an advantage on determining positions of hydrogen (H) atoms in proteins and water molecules. X-ray scattering factor of H atom is too small to detect electron densities of H atoms in X-ray Fourier maps. On the other hand, neutron scattering lengths of H atom and its isotope (deuterium (D)) are comparative to those of non-H atoms, and therefore, the densities corresponding to H and D atoms can be observed at the same level of the other atoms in neutron Fourier maps. Nowadays, there are neutron diffractometers specialized for determining hydrogen and hydration structures of proteins in neutron research facilities. However, NPC requires at least ~2.0 Å resolution diffraction to detect hydrogen atoms in neutron Fourier maps, and higher resolution data are necessary to elucidate precise hydrogen and hydration structures of proteins. This limitation due to the resolution is a major issue on NPC.

The D/H contrast technique is expected to improve the quality of neutron Fourier maps without the improvement of the resolution of diffraction on NPC. The large difference in the scattering length between H and D atom (b_D = 6.67 fm and b_H = -3.74) leads to high density contrast in neutron Fourier maps. The D/H exchangeable atoms in proteins (amino and hydroxyl groups) and water molecules can be significantly enhanced in the difference Fourier map between a crystal containing D_2O solvent (d-crystal) and one containing H_2O solvent (h-crystal). The first application of this D/H contrast technique was carried out by Kossiakoff and coworkers in the early period of NPC (1,2). However, their protocol designed as an additional refinement was thought to be unsuitable for standard structure determination, which involves iteration of model building and atomic parameter refinement. We developed an alternative protocol for the D/H contrast technique, and performed the second D/H contrast analysis in NPC (3).

In this study, the new technique: spallation neutron source , was employed to establish the newest technique of NPC.

2. Experiment

Hen-egg-white lysozyme (HEWL) was purchased from Hampton Research, and was used in crystallization without further purification. Large h-crystals of HEWL were grown in H_2O solution containing 15~25 mg/mL HEWL, 50 mM sodium acetate (pH4.5), and 10% (w/w) $NiCl_2$ at 293 K using a concentration gradient method (4). The d-crystals were prepared by vapor diffusion using D_2O solution (pD 4.1) containing the same components used in crystallization for 3 months. The X-ray diffraction data of the d-crystal was collected at room temperature using BL5A at Photon Factory, and the initial model for NPC was determined at 1.42 Å resolution with the R-factor of 0.160 (R_{free} = 0.189). At the same time, the X-ray data set of the h-crystal was collected at 1.43 Å resolution, and the isomorphous between the two crystals was confirmed.

Neutron diffraction images of the h- and d-crystals were collected using iBIX diffractometer installed at BL03 of J-PARC. These images were processed by the program, STARGazer. Neutron structure determination was carried out by the procedure reported previously (3). In the refinement of atomic parameters and the map calculation, the program, Phenix, was used instead of the program, nCNS.

The GUI program was developed using Python3 for the calculation of the D/H contrast maps.

3. Results

The d- and h-crystals of the sizes of ~10 mm^3 were used in neutron experiments. The diffraction spots of d- and h- crystals could be visually recognized on the graphic workstation up to 1.7 Å and 1.8~1.9 Å, respectively. Both of the diffraction images were processed up to 1.8 Å resolution. The refinement of atomic parameters was carried at 2.0 Å resolution, and the neutron Fourier maps were calculated using all reflections (up to 1.8 Å). The statistics of data collection and structure determination at the moment (2019.8.23) summarized in Table 1.

Table 1. Statistics of Diffraction experiment

	X-ray (BL5A PF)		neutron (BL03 J-PARC)	
Space group	$P4_32_12$			
Cell dimensions (Å, °)	a = b = 79.14, c = 37.38, α = β = γ = 90			
d_{min} (Å)	1.42	1.43	1.80	1.80
Crystal	d-crystal	h-crystal	d-crystal	h-crystal
R_{pim} (%)	1.5 (23.6)	1.7 (18.2)	7.1 (28.4)	7.62 (41.8)
Completeness (%)	100 (99.5)	100 (99.5)	97.9 (95.4)	970 (89.5)
d_{min} for refinement (Å)	1.42	1.43	2.00	2.00
R_{work} / R_{merge} (%)	16.0 / 18.9	16.5 / 18.3	16.8 / 21.2	

Figure 1(a) shows typical pictures of the D/H contrast maps. Distinct densities corresponding to the D/H exchangeable atoms could be clearly observed on the amino and hydroxyl groups of the protein and a solvent molecule (D_2O). An interesting picture could be found in the side chain of Trp111. There is no significant denisty correspoinding to the D/H atom of the amino group in the side chain (red circle in Fig. 1(b)), suggesting that the rate of D/H exchange at this site was so slow that sufficent contrast did not emerged in the D/H contrast map. In the other areas, the considerable number of D/H exchcangeable atoms could be also identified.

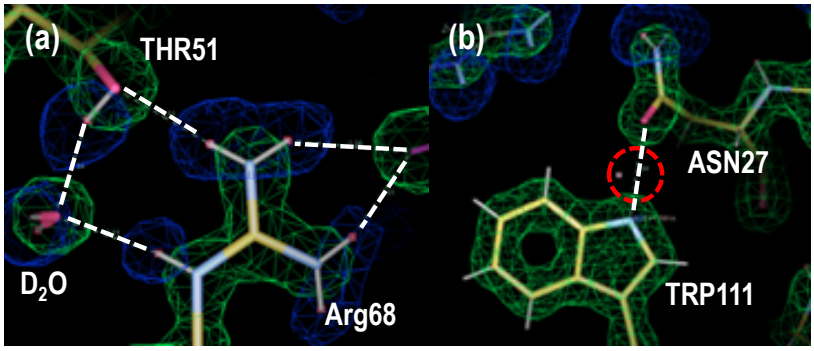

Figure 1. Two types of 2|*F*o|-|*F*c| maps (blue: neutron D/H contrast, green: X-ray) superimposed on the stick model of HEWL. Broken line indicates hydrogen bonds.

4. Conclusion

The present neutron D/H contrast Fourier maps demonstrated the feasibility and the high performance of the D/H contrast technique on the newest NPC system. We are finishing the structure determination using the D/H contrast technique, and now developing the method for investigating the detailed hydrogen and hydration structures using the D/H contrast map. We are planning to publish a few rapid communications within a year, and a full paper within a few years.

References

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