Nuclear polarization neutron diffraction of protein single crystal
- Starting a proof with protein polycrystalline diffraction experiment -

Ibaraki University Ichiro Tanaka

1. Introduction

The dynamic nuclear polarization (DNP) technique in neutron diffraction can increase the hydrogen detection sensitivity in principle. This is because the relative scattering length of hydrogen becomes about 8 times larger at maximum can be obtained. This technique will be useful for obtaining the positional information of active hydrogen atoms in the molecules. The ultimate goal is to realize DNP experiments with the iBIX protein single crystal diffractometer. First, however, it is necessary to ascertain whether polarization is actually possible for protein crystals and, as a result, the diffraction intensity depends upon the polarization rate. Previously, the maximum proton polarization of 22.3% at a temperature of 0.5 K in a 2.5 T normal-conducting magnet without neutron beam was realized [1]. But the polarization was too low and it was very difficult for the system to be installed at a neutron diffractometer. Then, by using a super-conducting 7 T magnet installed at BL20 in MLF in J-PARC [2], nuclear polarization experiments of protein polycrystal could be conducted. With polycrystallized protein sample in H_2O buffer, it was found that neutron diffraction patterns had a dependence upon polarization rate and the maximum total polarization of 62% was realized. This time, as an NMR coil has a limitation on the minimum weight of sample, the aim is that nuclear polarization is applied to polycrystalline protein sample in D_2O buffer as well as H_2O, and that neutron powder diffraction is observed and quantifed even partly.

2. Experiment

As a protein and a radical molecule, lysozyme and 4-hydroxy-TEMPO (TEMPOL) were purchased from Merck (L6876 and 176141), respectively. A batch crystallization condition was lysozyme 60 mg/mL and NaCl 9% (wt/v) as a mixture concentration in 50 mM sodium acetate D_2O buffer (pD4.5) with TEMPOL 50 mM, whereas 100 mM in previous H_2O buffer case. Temperature and the volume were 293 K and 1.5mL, respectively. As a cryoprotectant, 30% (wt/v) glycerol-d8 (Sigma-Aldrich 447498) was added. From the precipitant of protein crystal suspension, about 100 mg was sealed into perfluoroerastomer (FFKM) cell which has windows of quartz (6 mmϕ, 1 mmt). This cell was set at the top of cryostat and installed at sample position of iMATERIA with 7 T magnet and microwave frequency of 188 GHz was applied under 1.2 K. J-PARC proton power was 830 kW and 3 to 9 Ang neutrons were used as incident in -93 % polarization. For sample polarizations, +52, 0, -25 and -56 % were applied, and total polarizations were -48, 0, 23 and 52 %, respectively. Each polarization took about 30-60 min, and each exposure time was 0.5 to 3 hrs.

3. Results

According to iMATERIA data processing method on SANS mode, raw TOF data was reduced into intensity of momentum transfer Q (= $4\pi \sin\theta/\lambda$), that is I(Q) table (Fig.1). The intensity was normalized per unit time of observation. From the pattern, it was found that typical powder

diffraction was observed, and the intensities depended upon polarizations. With a solver function of MS excel, crystal unit cell parameters were refined successfully, and it was found the protein crystal had a tetragonal form of a=b=80.289, c=36.924 Ang. From a calculation of structure factor for each I(hkl) where each atom scattering length becomes a function of polarization, the variation of intensity was confirmed. In addition, background levels have agreed to theoretical values.

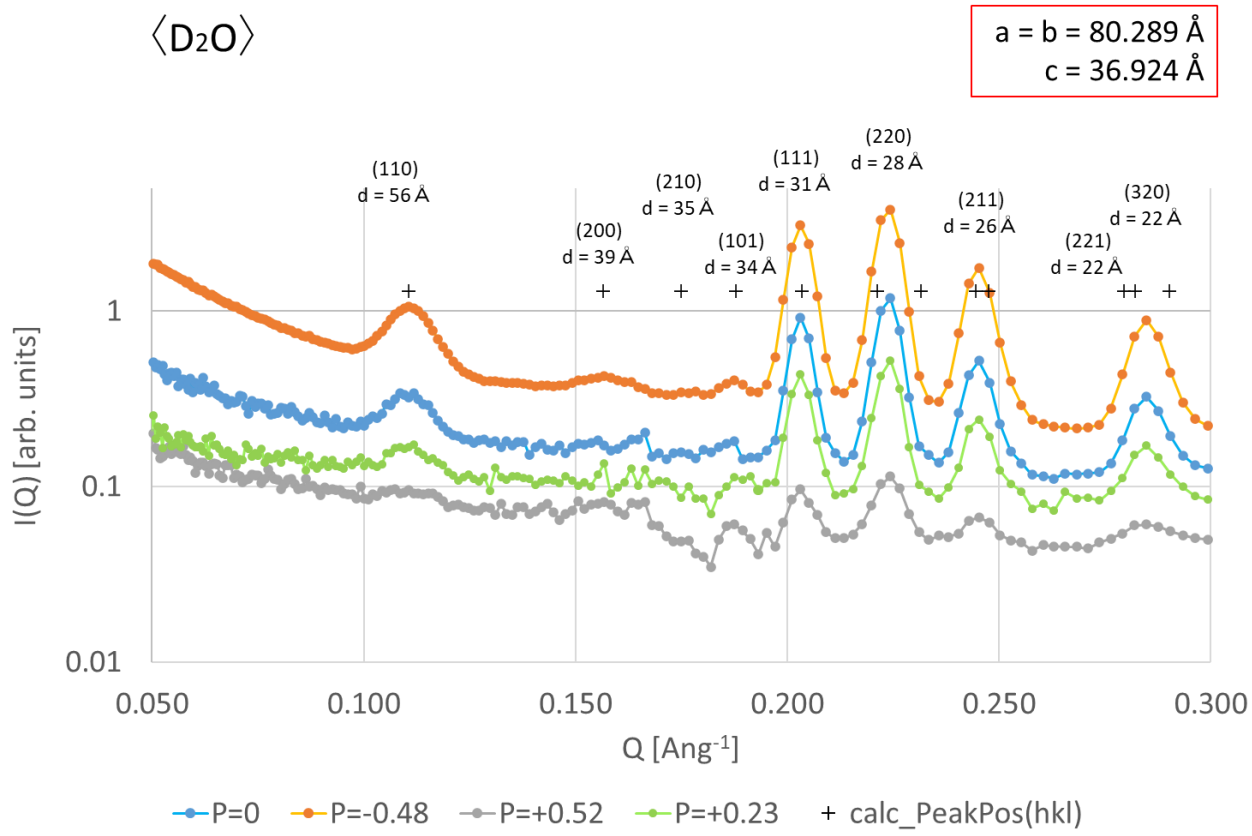

Fig.1: Protein powder diffraction profiles as I(Q) graph. P indicates total polarization; orange:-0.48, blue:0, green: 0.23 and gray:0.52. + shows calculated Q value position corresponding to (hkl) index with d-spacing. Red inset shows unit cell dimensions. Data were extracted from 0 to 0.8 in Q-range.

4. Conclusion

Using 7 T magnet and 50mM TEMPOL, the total maximum polarization of D_2O protein crystal reached +52% and D_2O crystal form could be determined as well as H_2O. Both the peak intensity and background dependence upon polarization agreed to theoretical calculation, which proves protein crystal polarization could improve hydrogen detecting sensitivity actually.

Reference:

[1] I. Tanaka et al., Acta Cryst. D74 (2018) 787-791.

[2] Y. Noda & S. Koizumi, Nucl. Instr. Meth. A 923 (2019) 127-133.