CROSS TOKAI **Experimental Report** J-PARC	承認日 Date of Approval 2017/5/3 承認者 Approver Jun-ichi Suzuki 提出日 Date of Report 2016/11/16
課題番号 Project No. 2015A0182 実験課題名 Title of experiment Hydration structures around proteins studied by small-angle scattering 実験責任者名 Name of principal investigator Satoru Fujiwara 所属 Affiliation National Institutes for Quantum and Radiological Science and Technology	装置責任者 Name of Instrument scientist Shinichi Takata 装置名 Name of Instrument/（BL No.） BL15 実施日 Date of Experiment 16/04/21～16/04/24

試料、実験方法、利用の結果得られた主なデータ、考察、結論等を、記述して下さい。（適宜、図表添付のこと）
Please report your samples, experimental method and results, discussion and conclusions. Please add figures and tables for better explanation.

1. 試料 Name of sample(s) and chemical formula, or compositions including physical form.

The small-angle neutron scattering curves of the following solution samples were measured.
(1) RNase A at pH 5.8 in H_2O (the concentration of the protein: 20.8 mg/ml, 14.9 mg/ml, and 10.5 mg/ml)
(2) RNase A at pH 5.8 in D_2O (the concentration of the protein: 21.0 mg/ml, 15.4 mg/ml, and 10.1 mg/ml)
(3) RNase A at pH 8.8 in H_2O (the concentration of the protein: 20.6 mg/ml, 14.9 mg/ml, and 10.5 mg/ml)
(4) RNase A at pH 8.8 in D_2O (the concentration of the protein: 20.7 mg/ml, 14.9 mg/ml, and 9.9 mg/ml)
(5) β-Lactoglobulin at pH 2.0 in H_2O (the concentration of the protein: 20.1 mg/ml, 15.8 mg/ml, and 10.5 mg/ml)
(6) β-Lactoglobulin at pH 2.0 in D_2O (the concentration of the protein: 18.1 mg/ml, 10.2 mg/ml, and 4.9 mg/ml)
(7) β-Lactoglobulin at pH 7.5 in H_2O (the concentration of the protein: 21.9 mg/ml, 16.1 mg/ml, and 10.6 mg/ml)

2. 実験方法及び結果（実験がうまくいかなかった場合、その理由を記述してください。）
Experimental method and results. If you failed to conduct experiment as planned, please describe reasons.

In order to obtain information on the hydration structure around the proteins, the small-angle neutron scattering (SANS) curves of the solution samples described above were measured. The measurements were carried out at the temperature of 20˚C. The exposure time of the samples was 1 – 4 hours, depending on the concentrations of the samples and solvent compositions (in H_2O or in D_2O). All the samples, which were planned to measured, were successfully measured. Data reduction was done us-

ing "Utsusemi". The two-dimentional scattering pattern of each sample was circularly averaged to obtain the one-dimentional scattering curve. The scattering curve of the buffer was subtracted from the curve of the sample to obtain the scattering curve of the protein. Figure 1 shows an example of these scattering curves.

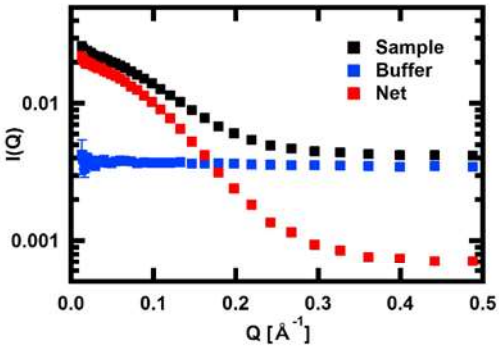

Figure 1. An example of the scattering curves. The curves of 21.0 mg/ml RNase A at pD 5.9 in D_2O are shown. The net scattering curve arising from the proteins were obtained by subtraction of the buffer curve from the sample curve.

The Guinier analysis of the net scattering curve of each sample was carried out to evaluate the radius of gyration of the protein. Figure 2 shows examples of the Guinier plots, from the slope of which the radius of gyration can be evaluated.

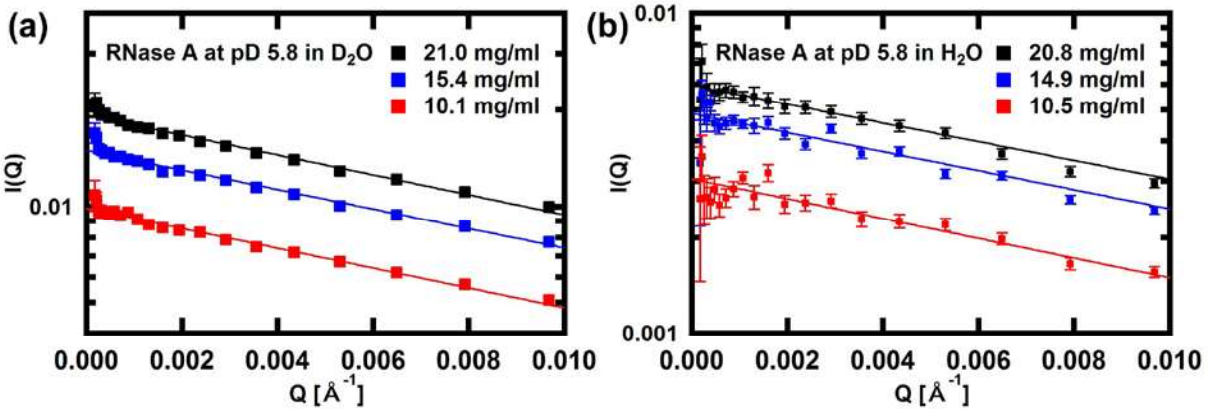

Figure 2. Examples of the Guinierplots. The plots of the scattering curves of (a) RNase A at pD 5.8 in D_2O and (b) RNase A at pH 5.8 in H_2O are shown.

Analysis by these Guinier plots showed that RNase A is monomeric in the experimental conditions employed, and β-Lactoglobulin are dimeric at pH 7.5 and in the equilibrium between the monomers and the dimers at pH 2.0. The radii of gyration evaluated for these samples were reasonable so that further analysis for obtaining the information on the hydration structure around these proteins is possible. This analysis should be done by combining these SANS data with the small-angle X-ray scattering data, and currently underway.