CROSS TOKAI **Experimental Report** J-PARC	承認日 Date of Approval Jan. 4, 2015 承認者 Approver J. Suzuki 提出日 Date of Report
実験課題番号 Project No. 2013P0501 実験課題名 Title of experiment Analysis of the structures of the proteins and protein complexes in solution by small-angle neutron scattering 実験責任者名 Name of principal investigator Satoru Fujiwara 所属 Affiliation Japan Atomic Energy Agency	装置責任者 Name of Instrument scientist Jun-ichi Suzuki 装置名 Name of Instrument/(BL No.) BL15 (TAIKAN) 利用期間 Dates of experiments April 2, 2014 ～ April 4, 2014

1. 研究成果概要(試料の名称、組成、物理的・化学的性状を明記するとともに、実験方法、利用の結果得られた主なデータ、考察、結論、図表等を記述してください。

Outline of experimental results (experimental method and results should be reported including sample information such as composition, physical and/or chemical characteristics.

Because of the limited machine time due to the accidents happened at the Hadron Experimental Facility, the experiments for the project "analysis of the structure-dynamics relationship of biological macromolecules" were focused on the crowding effects on proteins.

In order to investigate the effects of the crowding agents on proteins, the protein-glycerol system was chosen as an example. Small angle neutron scattering measurements for hen egg white lysozyme (Lys) in D_2O solution at various deuterated glycerol concentrations (g=0.0, 0.1, 0.3 and 0.5 w/w) and protein concentrations (100, 70, 50 and 10 mg/ml) were performed with TAIKAN. Thickness of the sample cell is 0.5 mm. The measurement time for the sample of 100mg/ml is about 30 min. For the samples with the lower concentrations, it took twice to five times longer measurement times. We also measured the empty cell and glassy carbon for normalization. We successfully obtained high quality data for every sample.

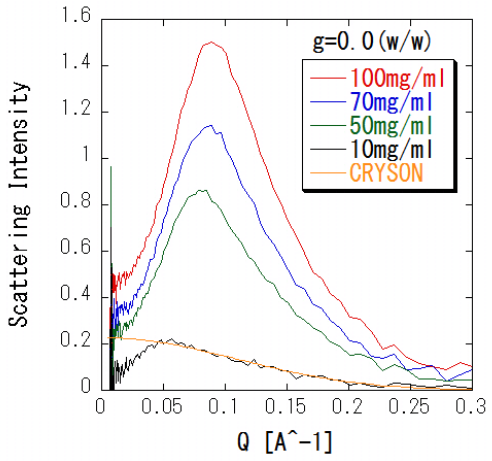

Figure 1
Scattering intensity of Lys at the glycerol concentration of g = 0.0 (w/w) at various protein concentrations. The form factor of Lys was calculated by CRYSON software [1] with 6LYZ.pdb.

We had measured the protein global diffusion and internal dynamics with BL02 (DNA). In this beam time at TAIKAN, we intended to examine the inter-particle interaction (structural factor) in order to characterize the protein-protein interaction in the same sample conditions at DNA instrument.

Ref
[1] Svergun D.I. et al., (1998) PNAS, 95, 2267

1．研究成果概要（つづき） Outline of experimental results (continued).

Figure 1 is the scattering intensity of Lys at various concentrations for g=0.0(w/w). At the lowest concentration, 10mg/ml, the scattering intensity is described by form factor predicted by crystal structure (6LYZ.pdb) above 0.05 $Å^{-1}$. The scattering below 0.05 $Å^{-1}$ decrease from the calculated form factor, indicating the repulsive inter-particle interaction.

Figure 2 shows the structural factor, S(Q). The first peaks are observed at around 0.1$Å^{-1}$. The peak positions move to higher Q values with increasing protein concentration. These results suggest that the protein is ordered in the high concentration solution and the correlation length is smaller at higher concentration. Figure 3 shows the glycerol concentration dependent S(Q) at 100mg/ml. It was found that the peak of S(Q) is shifted into higher Q value with glycerol concentration. This result suggests that the glycerol affects the protein-protein interaction.

Detailed analysis is in progress. We will discuss both the protein-protein interaction and protein dynamics in the context of molecular crowding by combination of SANS (TAIKAN instrument) with backscattering spectroscopy (DNA instrument).

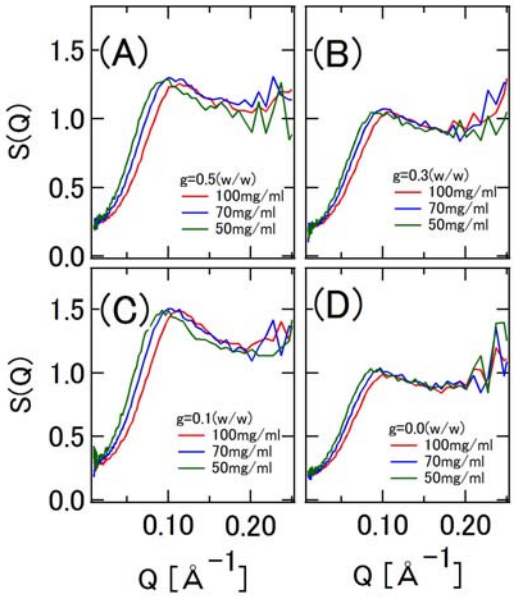

Figure 2
Structural factor of Lys at (A) g=0.5(w/w), (B) 0.3(w/w), (C) 0.1(w/w) and (D) 0.0(w/w) for 100, 70 and 50 mg/ml.

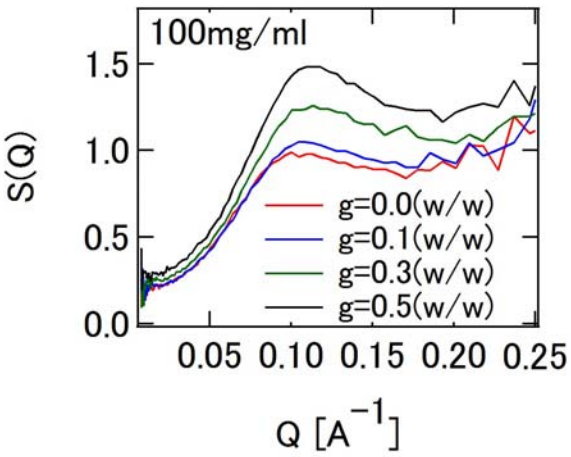

Figure 3
Structural factor of Lys at (A) g=0.5(w/w), (B) 0.3(w/w), (C) 0.1(w/w) and (D) 0.0(w/w) for 100, 70 and 50 mg/ml.

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