CROSS TOKAI Experimental Report J-PARC	承認日Date of Approval 2016/1/21 承認者Approver Kaoru Shibata 提出日Date of Report 2016/1/20
実験課題番号 Project No. 2014P0502 実験課題名 Title of experiment Analysis of the dynamics of the proteins and protein complexes by neutron inelastic 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 responsible person Kaoru Shibata 装置名 Name of Instrument/（BL No.） BL02 (DNA) 利用期間 Dates of experiments April 28, 2014 ～ May 1, 2014 May 13, 2014 ～ May 15, 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.

As the experiments for the project "analysis of the structure-dynamics relationship of biological macromolecules", the experiments on the following sub-projects using the neutron inelastic scattering techniques were carried out: (1) analysis of the proteins under the crowding environment, and (2) dynamics of the proteins under the normal and the disease-related states. The experiments conducted for each of these sub-projects are described below.

(1) Analysis of the proteins under the crowding environment

Inelastic neutron scattering measurements for hen egg white lysozyme (Lys) in H_2O and D_2O solution at various deuterated glycerol concentrations (g=0.0, 0.1, 0.3 and 0.5 w/w) were performed. The protein concentration is 50mg/ml for each sample. We have examined the effect of glycerol on the water dynamics at 300K. The energy resolution is about 12μeV. Samples are put in the hollow aluminum containers of 14.0mm external radius and defining a sample layer thickness of 0.2mm, and shielded with indium wire. The measurement time for each protein sample and bulk water is about 4 – 5 hours and 2 hours, respectively.

The signals of water molecules were obtained by subtraction of protein H_2O solution from the protein D_2O solution. The data were fitted by the following double Lorentzian function to separate the diffusive and localized dynamics (see Figure 1),

$$S(Q,\omega) = e^{-Q^2\langle u^2\rangle}\left[S_{diffusion}(Q,\omega) \otimes S_{local}(Q,\omega)\right] \qquad (1)$$

The data fitting is reasonable. and then we have successfullv obtained two dynamical components.

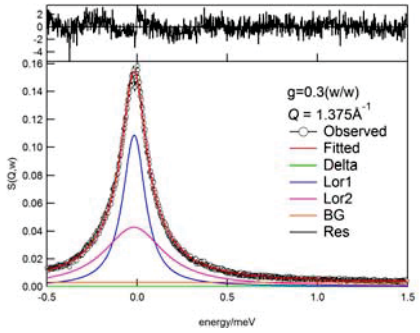

Figure 1. Data fitting by two Lorentzians with at g=0.3 (w/w).

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

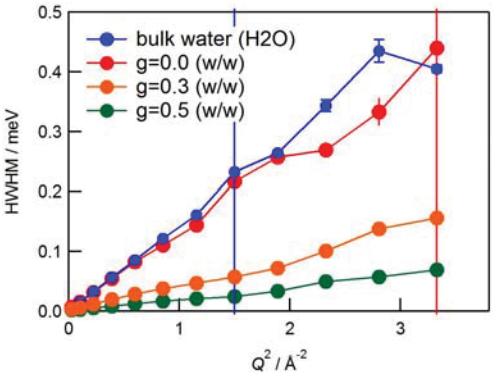

Figure 2. HWHM of slower dynamical component obtained by double Lorentzians fitting with some glycerol concentration samples and bulk water (H_2O).

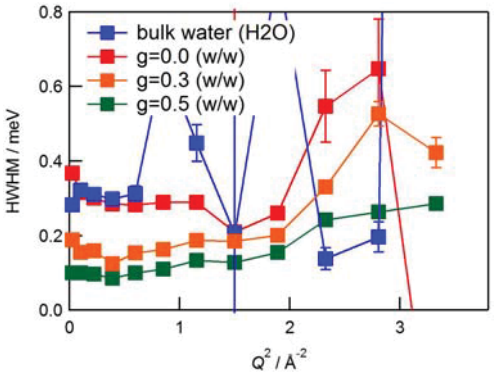

Figure 3. HWHM of faster dynamical component obtained by double Lorentzians fitting with some glycerol concentration samples and bulk water (H_2O).

Figure 2 shows the HWHM of the slower dynamical component of water (H_2O). HWHM linearly increases as a function of Q^2, which indicates the diffusion dynamics. HWHM of g=0.0(w/w) is slightly smaller than that of bulk water. This suggests that the protein affect the water dynamics. The HWHM significantly decrease with glycerol concentration. This indicates that the glycerol restricts the water diffusion dynamics in glycerol-water mixture.

Figure 3 shows the HWHM of the faster dynamical component of water (H_2O). The HWHM is independent of Q^2, suggesting that the faster component is localized motion. The faster HWHM decreases with glycerol concentration. The data analysis is on progress.

(2) Dynamics of the proteins under the normal and the disease-related states

The protein α-synuclein (α-Syn) is an intrinsically disordered protein of 14 kDa, and forms filamentous aggregates called amyloid fibrils. The amyloid fibrils of α-Syn are closely related to pathogenesis of a severe neuro-degenerative disorder, Parkinson's disease. Elucidation of the mechanism of the amyloid fibril formation of α-Syn is thus important for understanding the mechanism of pathogenesis of Parkinson's disease. Since the amyloid fibril formation involves partial denaturation of the proteins, the dynamics of the proteins should play some role in the fibril formation mechanism. We have thus been investigating the "dynamic" behavior of α-Syn at various structural states using quasielastic neutron scattering (QENS). As one of a series of the experiments, we measured the QENS spectra of α-Syn in the fibril state. Figure 4 shows examples of the QENS spectra arising from α-Syn. These spectra were obtained by subtracting the spectra of the buffer from those of the sample.

The spectra were fit well with the equation containing two Lorentzians corresponding to the global motions and the local motions, respectively. Analysis of the widths of these Lorentzians and the elastic incoherent structure factors were performed. Comparison of the results obtained here with those obtained for the monomeric state of α-Syn show that diffusive global motions observed in the monomeric state are largely suppressed in the fibril state, and that the amplitude of the side chain motion is larger in the fibril state than in the monomeric state. This implies that significant solvent space exists within the fibrils, which is attributed to the α-Syn molecules within the fibrils having a distribution of conformations. The larger amplitude of the side chain motion in the fibril state than in the monomeric state implies that the fibril state is entropically favorable.

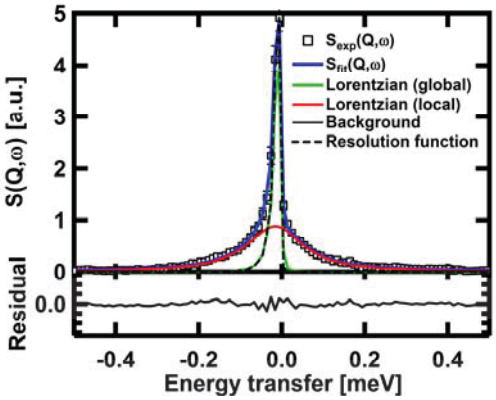

Figure 4. Examples of the QENS spectra of α-Syn in the fibril state. The spectra at Q = 1.225 $Å^{-1}$ at 280 K are shown.

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