CROSS TOKAI **Experimental Report** J-PARC	承認日 Date of Approval: June.09.2014. 承認者 Approver: Kaoru Shibata 提出日 Date of Report June.06.2014
実験課題番号 Project No. 2013P0502 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 Instrument scientist Kaoru Shibata 装置名 Name of Instrument/（BL No.） BL02 利用期間 Dates of experiments March 5, 2014～March 10, 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 dynamics of the proteins related to pathogenesis of diseases.

As an important example of proteins related to pathogenesis of the diseases, the protein α-synuclein (α-Syn) was selected, and its dynamics was investigated. α-Syn is an intrinsically disordered protein of 14 kDa, of unknown function. Filamentous aggregates of α-Syn were found in brains of patients of a severe neuro-degenerative disorder, Parkinson's disease. These amyloid fibrils of α-Syn are thought to be involved with pathogenesis of 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. *In vitro*, amyloid fibril formation of α-Syn depends on environmental conditions such as pH, temperature, and mechanical stresses. For example, α-Syn at low pH is more prone to form fibrils than at neutral pH (Uversky et al. J. Biol. Chem., 2001). Comparison of the behavior of α-Syn at low and neutral pH should thus provide insights into the mechanism of the amyloid fibril formation of α-Syn. 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 thus investigated the "dynamic" behavior of α-Syn at low and neutral pH. For this purpose, neutron scattering was employed. Neutron scattering provides a unique tool to measure directly the dynamics of proteins at picosecond time and ångstrom length scales. We prepared the solution samples of α-Syn at the concentrations of about 10 mg/ml at pH 3.8 and 7.4. The samples were prepared in D_2O to measure the dynamics of the proteins. The fibrils were yet to be formed in these conditions. We carried out the quasielastic neutron scattering (QENS) experiments on these samples, using a high energy resolution near-backscattering spectrometer, BL02 (DNA), at the energy resolution of 12 μeV. The measure-

ments were carried out at several temperatures between 280 K and 300 K.

Figure 1 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.

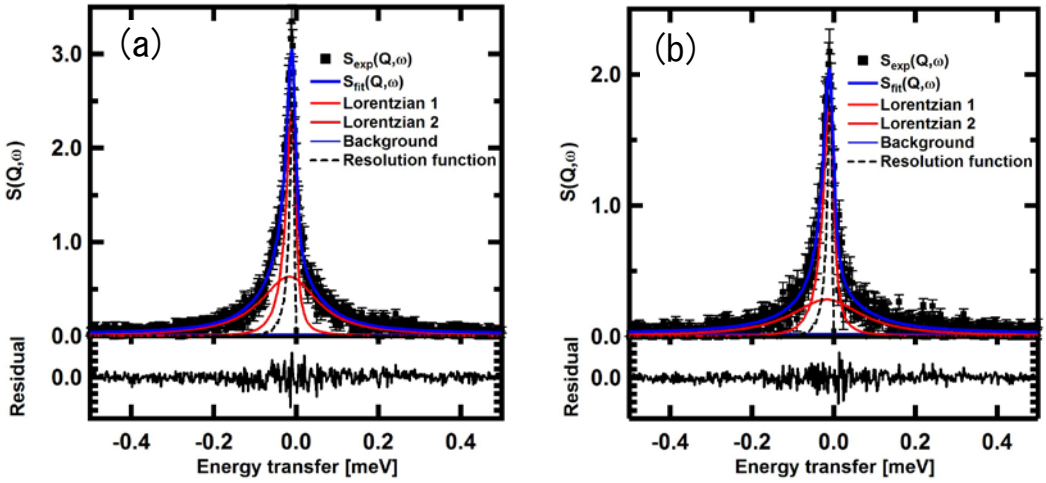

Figure 1. Examples of the QENS spectra of a-Syn.(a) at pH 7.4 and (b) at pH 3.8. The spectra at Q = 1.025 $Å^{-1}$ at 280 K are shown.

The spectra were fit well with the equation containing two Lorentzians corresponding to the global motions and the local motions, respectively. The Lorentzian corresponding to the global motions induces significant broadening of the elastic peak as observed in Fig. 1, From the half-width at half maximum (Γ) of this Lorentzian, translational diffusion coefficients (D_T) can be estimated. Figure 2 shows temperature dependence of D_T. Differences in the values of D_T were observed. It is noted that since the D_T values of whole molecules of α–Syn are in an order of 10^{-7} cm^2/s, the values observed here reflect the intramolecular motions rather than the motions of the whole molecule. The differences in these D_T values suggest the faster motions of α–Syn in low pH. The Γ values from the Lorentzian corresponding to the local motions also indicated faster fluctuations in low pH.

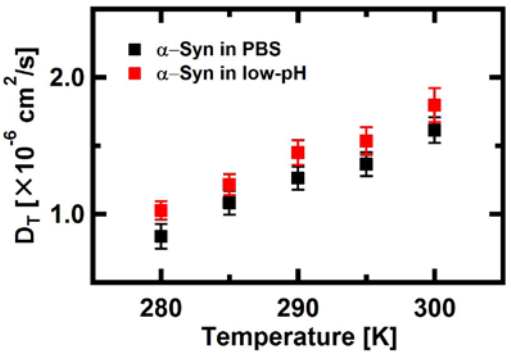

Figure 2. Temperature dependence of the translation diffusion coefficients D_T

These results indicate that the differences between in neutral and low pH were in flexibility of the protein, suggesting that the difference in propensity for amyloid fibril formation between different pH arises from the difference in kinetics. Since the increased flexibility is likely to arise from a wider distribution of conformational substates of α-Syn, the results obtained suggest an entropy-driven mechanism of the amyloid fibril formation.