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	提出日 Date of Report 11/27/2012
課題番号 Project No. 2012A0003 実験課題名 Title of experiment Dynamics-structure relationship in the amyloid fibril formation studied by 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 responsible person Kaoru Shibata 装置名 Name of Instrument/(BL No.) DNA/BL02 実施日時 Date and time of Experiment 15 June 2012,10:00 ~ 21 June 2012, 21:00 (143h) (20 June: half-day operation)

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. (1) 50 mg/ml Hen egg white lysozyme (HEWL) in D <sub>2</sub> O (2) 8 mg/ml Hen egg white lysozyme (HEWL) in 70% deuterated ethanol (EtOD) (3) 10 mg/ml Hen egg white lysozyme (HEWL) in 85% deuterated ethanol (EtOD) (4) 10 mg/ml Hen egg white lysozyme (HEWL) in 90% deuterated ethanol (EtOD) (5) D <sub>2</sub> O (6) 70% EtOD (7) 85% EtOD (8) 90% EtOD
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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. <u>Experimental method</u> The neutron scattering spectra of the samples described in section 1 were measured in the energy transfer range of -0.5 meV and 1.0 meV and the momentum transfer range of 0.125 Å <sup>-1</sup> and 1.725 Å <sup>-1</sup> , at the energy resolution of 14 μeV, with BL02 (DNA), run at 200 kW. The measurements were done at several temperature points between 280 K and 300 K. Exposure times of the measurements were between 2 hours and 4 hours. The obtained spectra were corrected for the vanadium standard, and the contribution of the empty cell was subtracted. The spectra of the background (samples # 5, 6, 7, or 8 in section 1) were then subtracted from those of the corresponding samples. These difference spectra contain information on the internal dynamics of HEWL. The spectra thus obtained were then integrated over the region corresponding to the energy resolution around the elastic peak at each Q. These integrated curves are the elastic incoherent neutron scattering (EINS) curves.
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## 2. 実験方法及び結果(つづき) Experimental method and results (continued)

### Results

The purposes of the experiments were threefold. Each purpose and the results of the corresponding measurements are described below.

#### (1) Confirmation of the results obtained from the experiments during the period 2011B.

The experiments done here is a continuation of the experiments done during the period 2011B. Because of some technical reason, the sample containers employed in 2011B and here were different (flat containers in 2011B and cylindrical containers here). We measured the spectra of the same samples to those done during the period 2011B in cylindrical containers (the samples #1 and #4). Comparison of the spectra obtained here with those obtained from the measurements done during the period 2011B confirmed reproducibility of the results and correctness of the method of the data reduction such as correction for the different sample thickness in the flat containers.

#### (2) Additional measurements to the measurements during the period 2011B.

The measurements done during the period 2011B were on HEWL in D<sub>2</sub>O and in 90% EtOD. These samples correspond to HEWL under the monomer state and HEWL under the amyloid fibril state. Unfortunately, however, the sample of 90% EtOD was not measured because of the technical difficulty of putting such a high concentration of EtOD in a flat container which has a large opening. Here the measurements of this sample in a cylindrical container were done. The measurements were successful, and as the method of the data reduction was shown to be correct as above, the spectra of the background for those of HEWL in 90% EtOD done during the period 2011B were obtained here. Thus, together with the data taken during the period 2011B, the measurements on HEWL under the two typical structural states, the monomer state and the amyloid fibril state, have completed.

#### (3) Measurements on HEWL under other structural states than those done during the period 2011B.

The pathway of the amyloid fibril formation of HEWL starts from the monomer state, through the dimer state and the protofilament state, to the amyloid fibril state. Since the measurements on the monomer state and the amyloid fibril state completed as above, we tried to measure the spectra of HEWL under other structural states, the dimer state and the protofilament state, the corresponding samples of which were the samples #2 and #3 in section 1, respectively. We have obtained the EINS curves of these states. Unfortunately, however, the data did not have enough statistics to analyze further. This was because of insufficient exposure times for the data collection. Since the concentration of HEWL cannot be increased to keep the structural states, the exposure time have to be increased. Together with the experience during the period 2011B, the exposure times for the samples in dilute solutions were shown to be at least 6 hours. This is very important information for the future experiments of the protein solutions.