

実験報告書様式(一般利用課題・成果公開利用)

(※本報告書は英語で記述してください。ただし、産業利用課題として採択されている方は日本語で記述していただいても結構です。)

 <b>Experimental Report</b> 	承認日 Date of Approval 2013/9/18 承認者 Approver Kaoru Shibata 提出日 Date of Report 2013/6/26
課題番号 Project No. 2012B0137 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 Instrument scientist Kaoru Shibata 装置名 Name of Instrument/(BL No.) DNA/BL02 実施日 Date of Experiment January 23, 2013 ~ January 31, 2013

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) 12 mg/ml Hen egg white lysozyme (HEWL) in 80% deuterated ethanol (EtOD) (2) 9 mg/ml HEWL in 70% EtOD (3) 80% EtOD (4) 70% EtOD
---

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 $0.5$ meV and the momentum transfer range of $0.125 \text{ \AA}^{-1}$ and $1.775 \text{ \AA}^{-1}$ , at the energy resolution of $12 \text{ \mu eV}$ , using the instrument BL02 (DNA), run at 300 kW. The measurements were done at several temperature points between 280 K and 300 K. Exposure times of the measurements were between 6 hours and 10 hours. The obtained spectra were corrected for the vanadium standard, and the contribution of the empty cell was subtracted. The spectra of the background (the sample #3 and 4 in section 1) were then subtracted from those of the corresponding samples. These difference spectra contain information on the internal dynamics of HEWL in various structural states. 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.
---

## 2. 実験方法及び結果(つづき) Experimental method and results (continued)

### Results

The experiments carried out here were the continuation of the experiments done by the period 2012A. HEWL has been shown to form the amyloid fibrils as a function of ethanol concentration and HEWL concentration (Yonezawa et al. J. Mol. Biol., 323 (2002) 237). The pathway of the amyloid fibril formation is through the dimer formation, the protofilament formation, and the amyloid fibril formation. By the period 2012A, the data on the HEWL monomer as a standard and the amyloid fibrils were obtained. Here the measurements on other structural states were carried out. HEWL in 80% EtOD (sample #1) corresponds to the state where the protofilaments are formed, and HEWL in 70% EtOD (sample #2) corresponds to the state where the dimers of HEWL are formed. The EINS curves obtained from the measurements are shown in Fig. 1.

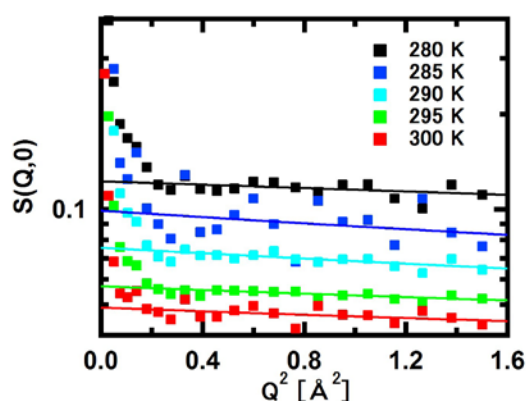


Figure 1. Examples of the EINS curves. The curves of HEWL in 80% EtOD at various temperatures between 280 K and 300 K are shown. The curves are displaced vertically for clarity.

As shown in the figure, there are curves, the signal-to-noise ratio of which is different: the curve at 285 K and to less extent, the curve at 300 K are more scattered than other curves. Some instability of the instrument that occurred during our machine time appears to be the cause of this noise problem. Because of the low concentrations of HEWL in the samples, such noise problems were exaggerated, and thereby preventing the full analysis of the data. The data of HEWL in 70% EtOD were more scattered than the data shown in Fig. 1 (data not shown). Unfortunately, however, the concentrations of HEWL in the samples cannot be increased because the structural states depend on the concentration of HEWL. The stability of the instrument and the higher power of the neutron source are thus required.

Although we should be cautious to carry out further analysis, the mean square displacements of HEWL in 80% EtOD were estimated from the slopes of the EINS curves. The force constant analysis of the temperature dependence of the mean square displacement provides a measure of flexibility of the protein (Zaccai, Science, 288 (2000) 1604). The effective force constant of HEWL in 80% EtOD obtained suggests that flexibility of HEWL in this state is similar to that in the monomer state. Increase in flexibility thus occurs during the formation of the amyloid fibrils. How this insight can be incorporated in elucidation of the mechanism of the amyloid fibrils is currently investigated.