（ ※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。 ）

CROSS TOKAI Experimental Report J-PARC	承認日Date of Approval 2015/9/15 承認者Approver Jun-ichi Suzuki 提出日Date of Report 2015/2/2
課題番号 Project No. 2014A0035 実験課題名 Title of experiment Conformation of β-crystallin with the existence of α-crystallin as studied by SANS 実験責任者名 Name of principal investigator Rintaro Inoue 所属 Affiliation Research Reactor Institute, Kyoto University	装置責任者 Name of responsible person Shinichi Takata 装置名 Name of Instrument/(BL No.) BL-15 実施日 Date of Experiment 2014/1114-2014/11/17

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.

Hydrogenated recombinant αB-crystalline dissolved in D_2O buffer

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.

The transparency of eye lens is maintained by the ordered arrangement of crystallins, which is a major component in the eye lens proteins. The well-ordered structure is disrupted by the formation of *abnormal* aggregation. The accumulation of *abnormal* aggregates can easily trigger the serious disease such as Alzheimer's disease, Creutzfeldt-Jacob disease, cataract and so on. Hence the mechanism to prevent the formation of *abnormal* aggregation of the crystallins is crucial for keeping lens transparency due to a extremely low protein turnover in eye lens.

One proposed mechanism for keeping the transparency is the chaperone activity of α-crystallin, which is one of three types of crystallins, α-, β- and γ-crystallins, and protects itself and the other crystallins from *abnormal* aggregation. α-crystallin is a huge macromolecule with a molecular weight of 800 kDa and is comprised of two types of subunits, αA- and αB-crystallins, of which amino acid sequences share about 55% homology. Considering the molecular weight of each subunit is approximately 20 kDa, α-crystallin contains about 40 subunits. In order to understand the unresolved mechanism of α-crystallin the clarification of α-crystallin is considered to offer the better opportunity, however even the spatial distribution of each subunit or quaternary structure of α-crystallins have not been determined due to no crystal structure of both hetero- and homo-aggregates. Here we should aim to disclose the quaternary structure of α-crystallins in

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

solution state, which is close to in-vivo environment compared to the crystal structure. For this purpose we performed solution scattering on hydrogenated αB-crystallin, which is one of the subunits in α-crystallin through small angle neutron scattering (SANS).

Fig.1 shows the scattering profile from hydrogenated αB-crystallin in D_2O buffer at the concentration of 1.60 mg/ml and no trace of upturn originating from aggregation was observed, supporting the mono-dispersity of prepared sample. In addition to check the stability of prepared sample we also compared the scattering profile obtained from just after the preparation (0h) to that from 48h later from the sample preparation. Both the scattering profiles overlapped nicely, implying the stability of sample at least within 48h measurements.

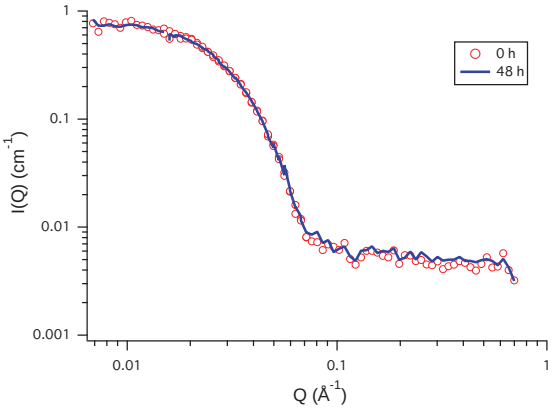

Fig.1 SANS profiles of hydrogenated alpha-crsytallin in D_2O buffer at the concentration of 1.60 mg/ml for 0h (just after preparation) and 48h.

As a next step for the evaluation of size we performed Guinier analysis on the obtained scattering curves and the Guinier plot is shown in Fig.2. The evaluated radius of gyration (R_g) was 56Å and this value is consistent with the previously reported one [1]. Since the scattering profiles are converted to the absolute intensity we also tried to estimate the number of subunits included in presently prepared hydrogenated αB-crystallin. By comparing the expected $I(0)$ value from monomer unit of hydrogenated αB-crystallin based on the amino acid sequence it was found that the number of subunits was 24. This number of subunits is quite similar with the other heat shock protein and is also consistent with recent transmission electron microscope (TEM) works [2]. The detailed structural analysis including ab inito modeling is now on the progress.

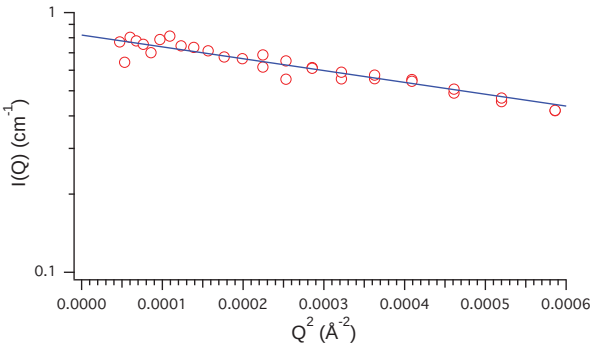

Fig.2 Guinier plot of hydrogenated α-crsytallin in D_2O buffer

References

[1] M. Sugiyama et al., Biomacromolecules 9, 431 (2008).
[2] J. Peschek et al., PNAS 106, 13272 (2009).