実験報告書様式(一般利用課題・成果公開利用)

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CROSS TOKAI **Experimental Report** J-PARC	承認日 Date of Approval 2013/11/23 承認者 Approver Jun-ichi SUZUKI 提出日 Date of Report 2013/04/10
課題番号 Project No. 2012B0090 実験課題名 Title of experiment Structural Study of alpha-Crystallin with deuterated subunits 実験責任者名 Name of principal investigator Masaaki Sugiyama 所属 Affiliation Kyoto University	装置責任者 Name of Instrument scientist Dr. Jiun-ichi Suzuki 装置名 Name of Instrument/(BL No.) BL-15 実施日 Date of Experiment 2012/03/22-2012/03/26

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. (Native) Human recombinant αA-Crystallin in H2O buffer solution
#2. (Native) Human recombinant αA-Crystallin in D2O buffer solution
#3. (Native) Human recombinant αB-Crystallin in H2O buffer solution
#4. (Native) Human recombinant αB-Crystallin in D2O buffer solution
#5. Deuterated human recombinant αB-Crystallin in H2O buffer solution
#6. Deuterated human recombinant αB-Crystallin in D2O buffer solution

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.

α-Crystallin is a major protein in human eye lens. It is believed that this protein is a. huge protein complex with 20-30 subunits which are two kinds of similar subunits, αB-Crystallin and αB-Crystallin. In other words, α-Crystallin is a hetero-oligomer with two kinds of subunits. But there is no clear evidence .that α-Crystallin is the hetero-oligomer. It means that there is a possibilities that α-Crystallin is mixture of two homo-oligomers each of which consists of αA-Crystallin or αB-Crystallin. because αA-Crystallin and αB-Crystallin make homo-oligmeres, respectively. Therefore, we supposed that homo-ologmerss of αA-Crystallin or αB-Crystallin could have a kinetics of subunit exchange if hetero-oligomer of α-Crystallin does exist. The subunit exchange is very important to understand the function and its loss of α-Crystallin by aging.

To examine the existences of subunit exchange between αA-Crystallin and αB-Crystallin and also between αB-Crystallins, we adopted the following procedure.

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

#1:We prepared for homo-oligomers of native αA-Crystallin , native αB-Crystallin and deuterated αB-Crystallin.

#2: With those homo-oligomers, we prepared for two mixed 80% D_2O solutions, native αA-Crystallin and deuterated αB-Crystallin., and native αB-Crystallin and deuterated αB-Crystallin.

#3: We observed time-evolutions of SANS intensities of two solutions.

(If the subunit exchange exists, the SANS intensity exhibits decrease because the scattering contrast hetero-oligomer of native and deuterated subunits becomes lower than that of mixture of native oligmers)

The time-resolved SANS experiment shows that the intensities of both mixtures gradually decrease. (Not shown: Because recently DAQ program on BL-15 has bugs, our observed SANS spectrum could be changed .). This result clearly indicated that the subunit exchange kinetics does exist. In addition, interestingly, the exchange speed between native αA-Crystallin and deuterated αB-Crystallin. is faster than that between native αB-Crystallin and deuterated αB-Crystallin.. This result could relate with the conformation change of α-Crystallin by aging.

In addition, this is the first trial to prepare for deuterated protein for us and we succeeded it. Another meaning of this result is that the protein deuteration is a technique open for everyone.