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Structural Study on αA-Crystallin and βB_2-crystallin Complex by Small-Angle X-ray Scattering

Masaaki SUGIYAMA*[1], Norihiko FUJII[1], Shunsuke Kawabe[2], Keiji ITOH[1], Kazuhiro MORI[1], Toshiharu FUKUNAGA[1], Noriko FUJII[1]

[1]Research Reactor Institute, Kyoto University, Kumatori, Osaka 590-0494, Japan

[2] Graduate School of Engineering, Kyoto University, Yoshida, Kyoto 606-8501, Japan

Introduction

Main optical functions of eye lens are high refractive index and high transparency. For former feature, there are high concentrated proteins, α-, β-, γ-crystallin, in eye lens. Among the proteins, α-crystallin with the largest molecular weight of ca 800 kDa has a chaperone activity to prevent from anomalous huge aggregation of the crystallins in the human eye lens. Therefore, it is considered that α-crystallin plays an important role to maintain the transparency.

Recently, it is focused that protein aggregated deceases such as variant Creutzfeldt-Jakob disease (caused by abnormal prion), Alzheimer's disease (caused by β-amyloid proteins) and so on. The denatured protein makes the abnormal and huge aggregates: in the case of crystallin, the aggregated crystallin finally causes *Catarct*, which could be considered as one of protein aggregated deceases. Therefore, it is very important to reveal a repairing mechanism of α-crystallin. It is supposed that α-crystallin makes a complex with a denatured protein, for example denatured β-crystallin, when the α-crystallin fix the denatured protein. In order to observe making a complex with α-crystallin and the denatured protein, we adopt a small-angle X-ray scattering (SAXS). As the first step, we observe the solution with normal α-crystallin and normal β-crystallin. Here, we report the result of this preliminary experiment.

Experimental

Sample proteins, human αA-crystallin and βB_2-crystallin, were expressed by *E.Coli*. In addition, both mixed solution was also prepared. The concentrations of samples were tuned to be 1.0 mg/ml and the solvent was 20 mM Tris/HCl (pH 7.8) + 150 mM NaCl

The SAXS experiments were carried out at room temperature with a SAXS apparatus (SAXES) installed at BL10C of Photon Factory in Institute of Materials Structure Science (IMSS), High Energy Accelerator Research Organization (KEK), Tsukuba, Japan. An X-ray beam (1.488 Å in wavelength) was used as a light source of SAXES and the intensity distribution of the scattered X-ray was measured by a one-dimensional position sensitive proportional counter. The magnitude of the scattering vector ($q=(4\pi/\lambda)\sin(\theta/2)$, where λ is the wavelength and θ is the angle of scatter) ranged from 7.0 × 10^{-3} to 1.5×10^{-1} Å $^{-1}$. The observed X-ray intensity was corrected for the buffer scattering and absorption, and then normalized with respect to the thickness of the sample (1 mm) and irradiation beam intensity. Typical irradiation time for sample was 1800 sec.

Results and discussion

Figure 1 (a) shows SAXS profiles of αA-, βB_2-crystallins and their mixed solution, and Figure (b) also shows their Guinier plots, respectively. The gyration radii of αA- and βB_2-crystallins are 53 Å and 25 Å, respectively. These values are reasonable for their molecular weight. The mixed sample has an intermediates gyration radius (40Å) and the SAXS profile is expressed with a linear combination of those of αA and βB_2-crystallins. It means that there is no interaction between normal αA-and βB_2-crystallins. In the next, we will examine if there is the interaction between normal αA-crystallin and denatured βB_2-crystallin.

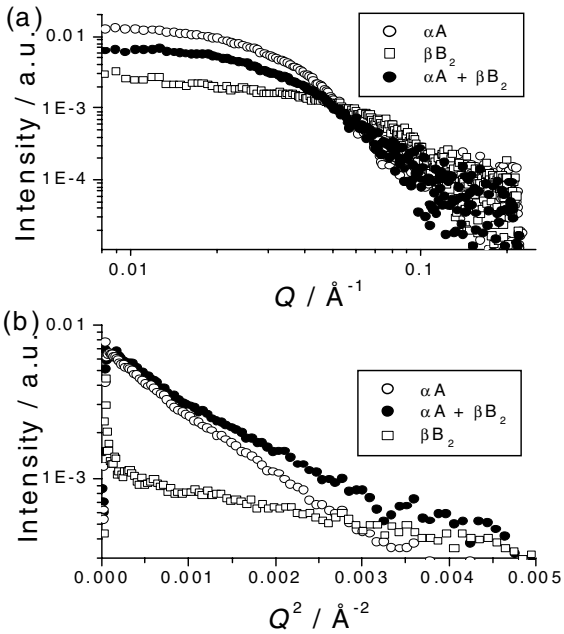

Figure 1. (a) Scattering profiles of αA, βB_2-crystallins and the mixed solution. (b) Guinier plots of their scattering intensities. Open circles, squares and closed squares exhibit αA, βB_2-crystallins and the mixed solution.

* sugiyama@rri.kyoto-u.ac.jp