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Structural Study on Human Origin Crystallin by Small-Angle X-ray Scattering

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Introduction

Crystallin is a major protein in a human eye lens. It is well known that there are three kinds of crystallins, namely, α-, β- and γ-crystallins. By size exclusion chromatography of a homogenized human eye lens, four peaks are observed. The second, third and fourth peaks correspond to α-, β- and γ-crystallins, respectively. The first one, which is called as HMW (High Molecular Weight) fraction, is considered to be the complex consisting of α-, β- and γ-crystallins. In addition, the interesting point is that the HMW fraction is not observed in a eye lens from youth sample.

In the previous SAXS experiments, we revealed that aggregates of recombinant αA-, αB-crystallins and their complex ([αA]:[αB]=1:1) have almost same gyration radii, which are around 58 Å: αA- and αB-crystallins are polypeptides consisting of native α-crystallin. Moreover, HMW has two components which are the aggregates with the size of ca 60Å and those with the size over 210Å. The aggregates with the size of ca 60Å could be normal α-crystallin dissociating from HMW components.

In this reports, with a small-angle X-ray scattering (SAXS) method, we are performing to find the size of the HMW and α-crystallin obtained from same human origin eye lens.

Experimental

HMW aggregates and α-crystallin ones from cataract same human eye lens were used as the samples. The both concentrations were tuned to be 1 mg/ml.

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=(4π/λ)sin(θ/2), where λ is the wavelength and θ is the angle of scatter) ranged from 7.0 × 10^{-3} to 2.1×10^{-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 samples was 3600 sec.

Results and discussion

Figure 1 shows guinier plots of SAXS profiles of HMW and α-crystallin. The straight lines indicate the SAXS intensities approximated by Guinier formula, $I(q)=I_0\exp(-R_g^2q^2/3)$ with R_g of 60Å, corresponding to normal α-crystallin aggregate. As shown in Figure 1, the SAXS profiles are deviated from the Guinier formula in the lower Q-range and the deviation range of HMW is wider than that of α-crystallin. It means that both samples include the higher molecular weight components and the normal size components. The higher molecular weight aggregates in HMW are more than those in α-crystallin. In addition, it is supposed that the dissociation-equilibrium kinetics exists in both samples.

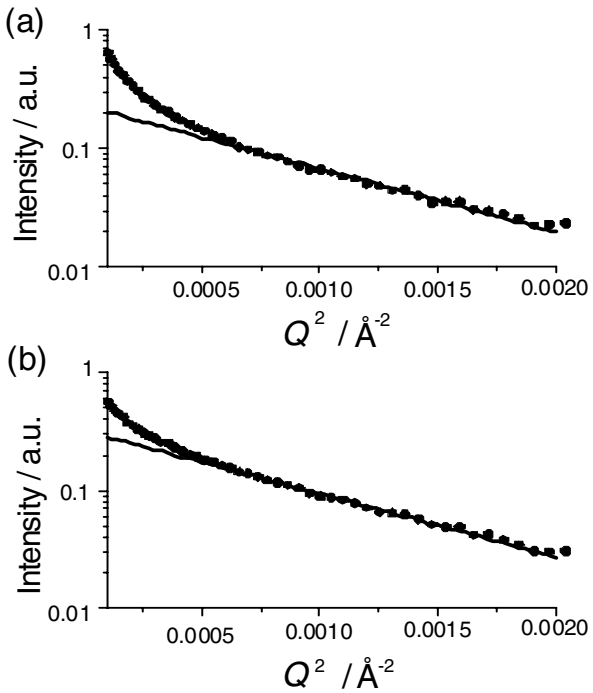

Figure 1. Guinier plot of the scattering intensity of (a) HMW and (b) α-crystallin. Straight lines indicate guinier formula with R_g of 60 Å.

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