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

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Introduction

Human eye lens mainly consist of three proteins, α-, β-, γ-crystallin and water. High concentration of the proteins results in high transparency and refractive index of eye lens. Alpha-crystallin with the largest molecular weight of ca 800 kDa has a chaperone activity to prevent from anomalous aggregation of the crystallins in the human eye lens. Under external stresses, such as UV irradiation, low and/or high temperatures and so on, α-crystallin loses its function and makes higher molecular weight (HMW) aggregates involving the other crystallins. In fact, HMW aggregates are observed in aged eye lens. Therefore, it is considered that these HMW aggregates could make eye lens opaque because there is no metabolism in eye lens.

We are interested in the aggregation process of crystallin from the view point of its structural change. The most powerful technique to clarify a structure of protein is a single crystal X-ray structure analysis. However, we cannot use this method because crystallization of HMW crystallin has not been achieved. Therefore, with a small-angle X-ray scattering (SAXS) method, we are performing to find the size and shape of the HMW aggregates.

Experimental

HMW crystallin from human eye lens was used as a sample. The concentration of HMW crystallin was 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 ($q=(4\pi/\lambda)\sin(\theta/2)$, 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 3600 sec.

Results and discussion

Figure 1 (a) shows a SAXS profile of HMW-crystallin. The scattering intensity monotonically decreases with the increase of the magnitude of scattering vector, indicating that the scattering objects are not monodispersed. Here, we analyzed the scattering intensity with a double-Guinier formula: $I(q)=I_1\exp(-R_{g1}^2q^2/3)+I_2\exp(-R_{g2}^2q^2/3)+I_3$, where R_{g1} and R_{g2} indicate radii of gyration of scattering objects. As shown in Figure 1(b), a double-Guinier formula well reproduces the observed scattering intensity. R_{g1} and R_{g2} are found to be 210 Å and 59Å. R_{g2} corresponds to the size of crystallin which does not make further aggregation. On the other hands, R_{g1} corresponds to the huge aggregates composed with *ca.* 30 normal crystallins.

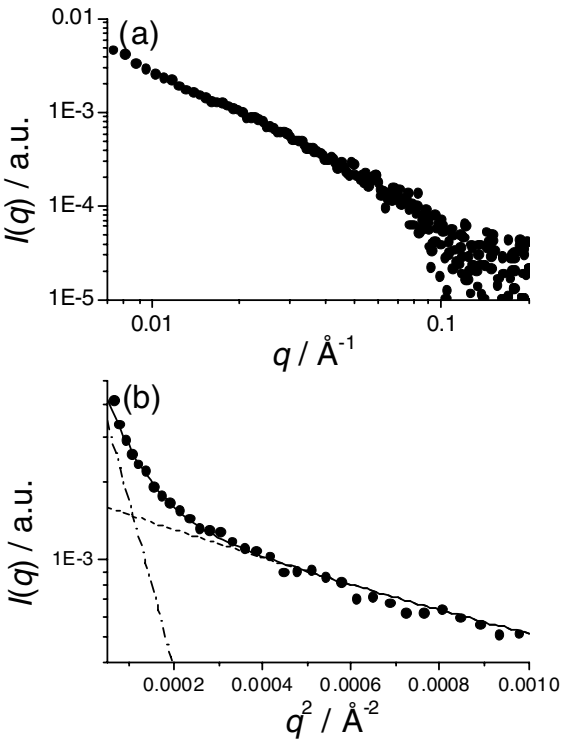

Figure 1. (a) Scattering profile of HMW-crystallin. (b) Guinier plot of the scattering intensity. Dash and dash-dot lines indicate Guinier formula with R_{g2} and R_{g1} of 59 Å and 210 Å, respectively. Solid line indicates the result of the least square fitting with double-Guinier formula.

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