Biological Science

10C/2005G082

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

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Introduction

A major protein in human eye lens is crystallin, which has an important part to play in high transparency and refractive index of eye lens. There are three types of crystallins in human eye lens: α-, β- and γ-crystallin. α-Crystallin with the largest molecular weight of ca 800 kDa has a chaperone activity to prevent from anomalous aggregation of the crystallins under the high concentration of the crystallins in the human eye lens. Therefore, a denatured α-crystallin looses the chaperone activity and then begins to make aggregation of the crystallin (incl. itself).

It is very important to reveal the structure in order to understand the function of α-crystallin. 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 α-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 α-crystallin.

Samples

α-Crystallin consists of ca 20 subunits: there are two kinds of the subunits, named αA-Crystallin and αB-crystallins. The ratio of two subunits [αA]/[αB] is varied depending upon age: [αA]/[αB] is 2:1 in an infant and that is 1:1 in the elder person (over 60 years old).

We prepared for two solution samples: the solutes are a complex ([αA]/[αB]=2:1), and a complex ([αA]/[αB]=1:1), of which αA- and αB-crystallins are recombinant ones expressed in Escherichia coli. Both samples are D_2O solution with the concentration of 1mg/ml.

Small-angle X-ray scattering experiment

The SAXS experiments were carried out at 37 C 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 detector. 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 1.5×10^{-1} Å^{-1}. The observed X-ray intensity was corrected for the cell 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 600 sec.

Results and discussion

Figure (a) shows a SAXS profile of α-crystallin with the [αA]/[αB] ratio of 1:1. In order to find the size of the complex, we analyzed the scattering intensity with a Guinier formula: $I(q)=I_0\exp(-R_g^2q^2/3)$, where R_g indicates a radius of rotation of a solute. As shown in Figure (b), a Guinier plot ($\log(I(q))$ vs q^2) of the scattering intensity, a clear straight line is observed. From the slope of the straight line, R_g is found to be 58.4A.

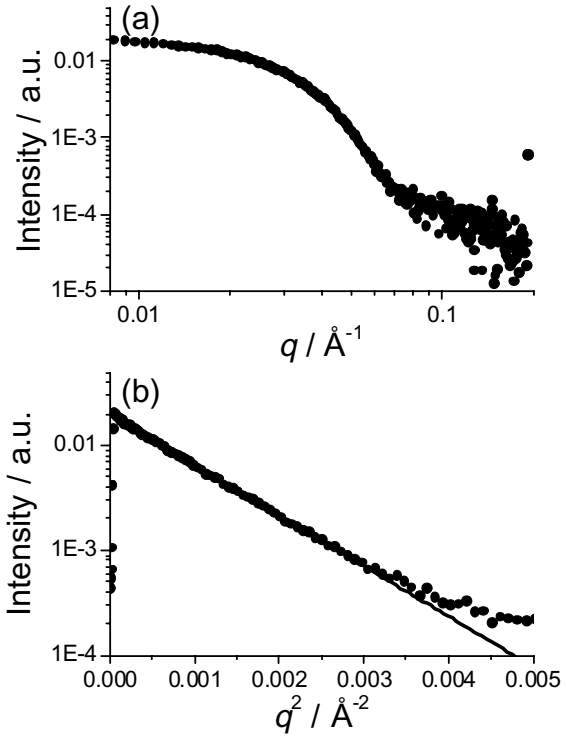

Figure 1. (a) Scattering profile of α-crystallin with the [αA]/[αB] ratio of 1:1. (b) Guinier plot of the scattering intensity. Straight line indicates the result of the least square fitting with Guinier formula.

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