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γ-ray Irradiation Effect on Structure of βB_2-Crystallin Observed by Small-Angle X-ray Scattering

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Introduction

Some proteins make a complex with the size considerably larger than normal aggregates under external stress, such as temperature change, UV, X-, γ-rays irradiation: we call these huge complex abnormal aggregates. It is supposed that abnormal aggregation by denatured proteins, induces a serious disease, such as variant Creutzfeldt-Jakob disease (by abnormal prion), Alzheimer's disease (by β-amyloid proteins), and so on. *Cataract* is induced by aggregated crystallins in eye lens, and then is considered as one of those abnormally protein-aggregated diseases. Because there is no metabolic system in eye lens, the denatured proteins by external stresses accumulate in whole life and then the denatured protein aggregates up to the huge size, namely abnormal aggregation occurs.

Eye lens mainly consists of three crystallin proteins, α-, β-, γ-crystallin. Among them, 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, and therefore maintain the transparency.

In our previous study, it was clarifies that there are two steps in the initial stage of abnormal aggregation of α-crystallin under UV-irradiation. Here, we are interesting of the aggragation mechanics of the other crystallins by external stress and the chaperon activity of α-crystallin for the other protein. In this report, we show the structural deformation of βB_2crystallin by γ-ray irradiation, observing with small-angle x-ray scattering (SAXS).

Experimental

Human βB_2-crystallin expressed by *E.Coli* was used as a sample. The concentrations of samples were tuned to be 3.0 mg/ml and the solvent was 20 mM Tris/HCl (pH 7.8) + 150 mM NaCl. One sample solution was γ-ray irradiated with 10Gy.

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 shows SAXS profiles of βB_2-crystallins with and without γ-ray irradiation. It is clearly observed the difference in the low *q*-region, indicated that γ-ray irradiated βB_2-crystallin has slightly larger size than no-irradiated one. It means that 10Gyγ-ray irradiation makes initial abnormal aggregation onβB_2-crystallin. In the next step, we will examine if there is the interaction between normal αA-crystallin and denaturedβB_2-crystallin.

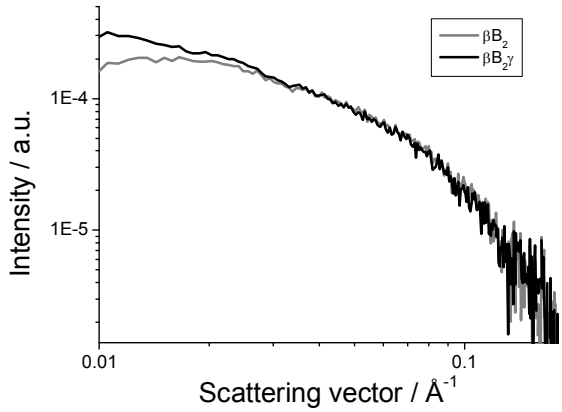

Figure 1. SAXS profiles of βB_2-crystallins with and without γ-ray irradiation.

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