10C, 2002G094

Structural change of dehydrated agarose gel by penetration of trehalose

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Introduction

Recently, trehalose has attracted a great deal of attention since this disaccharide seems to confer a lot of living organisms an ability to survive under extreme conditions such as dehydration, freezing and so on, where the cells are usually destructed. It is well known that the living organisms in temporary state of apparent death have high concentration of trehalose. Therefore, it is considered that trehalose is exchanged water in the cells under dehydration and/or freezing conditions and protects from the destruction of the cell. However, the molecular mechanisms underlying the bioprotective effectiveness of trehalose have not yet been completely understood.

Here, in order to understand the function of trehalose in the cell, it is inevitable to investigate structural features of the cell including a lot of trehalose. We adopted agarose gel with trehalose as a simple model of living biomaterial system and performed small-angle X-ray scattering (SAXS) experiments of the agarose gel with trehalose under dehydration condition for clarifying the structural features.

Experimental

Firstly, we dissolved powder of agarose (0.3 g) into H_2O (50 ml) at 150 °C. The solution was cooled down to RT and then we obtained the agraose gel with concentration of 0.6 wt%. The gel was cut with cylindrical shape of 6 (diameter) × 3 (length) mm. Secondly, we immersed the shaped gel into 10 wt% trehalose aqueous solution for 24 hrs. Thirdly, the gels were dehydrated in air atmosphere at 30 °C for 3 days.

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 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 6.0×10^{-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 and irradiation beam intensity. Typical irradiation time for sample was 600 sec and the transmissions were found to be 0.65 and 0.45 for the samples immersed into the trehalose solution and not, respectively.

Results and discussion

Figure 1 shows SAXS profiles of the dehydrated agarose gel with and without trehalose. As you can see, in double legalism plot, the dehydrated agarose gel without trehalose does not show a peak but the SAXS profile is almost expressed with a straight line. This indicates that the dehydrated agarose gel without trehalose has fractal-like structure in the observed scale range (from 60 Å to 1000 Å). The fractal dimension of the gel was estimated to be 2.3 from the result of the least square fitting.

The dehydrated agarose gel with trehalose shows the different feature in the SAXS profile: The region of straight line in the double legalism plot ends around 0.03 Å $^{-1}$ (pointed with an arrow), which corresponds to be 200 Å in a real space. From this result, we speculate that the trehalose makes a structure in the dehydrated agarose gel with the scale of 200 Å.

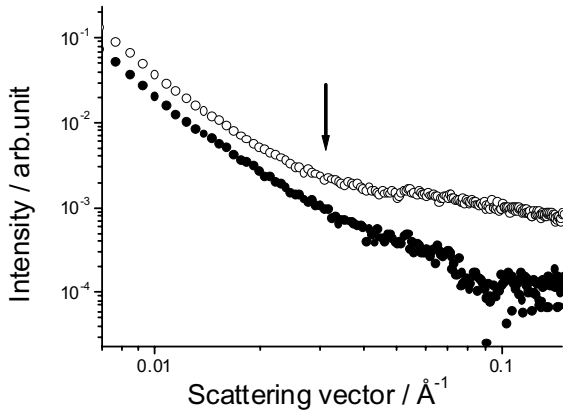

Fig.1. SAXS profiles of dehydrated agarose gel with and without trehalose. Closed circle and open circle indicate the profiles of the dehydrated agarose gel without trehalose and with trehalose, respectively. The inset arrow indicates the lower limit of scale of fractal structure in the agraose gel with trehalose (see text).

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