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Solution X-ray scattering studies on a large glycoprotein under a denaturing condition

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1 Introduction

Proteoglycans are important constituents of the extracellular matrix of multi-cellular organisms. They are high molecular weight glycoproteins consisting of a large amount of carbohydrates that is bound to the protein core of the proteoglycan at very many sites. They play an important role in protein binding, cell signaling, modulation of cell growth, differentiation and physical functions in articular tissue [1]. Some of these functions are due to the proteoglycan solution properties that refer to the molecular structure. It is important to clarify the conformation of the conjugates in solution for understanding their functions. Since most of the cartilage proteoglycans have high molecular weights and high carbohydrate contents, it is very difficult to form an overall structural characterization by gel electrophoresis and NMR measurements. In this study, in order to elucidate the solution properties of shark cartilage proteoglycan molecules under a denaturing condition, the conformation of the proteoglycan in protein-denaturant surfactant micelles were characterized by a preliminary small-angle X-ray scattering (SAXS) measurement.

2 Experiment

The synchrotron radiation SAXS measurements were performed using an optics system at the BL-10C station in the Photon Factory of the High Energy Accelerator Research Organization as previously described [2]. The wavelength (λ) of 0.1488 nm was used. The temperature of the cell with a 1-mm light path and a pair of 20-nm-quartz windows was maintained constant at 24 C° using the metallic cell holder through which constant temperature water was circulated. Data were collected for 10 min by a position sensitive proportional counter at the sample to detector distance of 1.98 m. The obtained signals were corrected for solvent scattering and normalized to the beam intensity to yield the net scattering intensity $I(q)$, where q ($=(4\pi/\lambda)\sin\theta$, 2θ is the scattering angle) is the modulus of the scattering vector. The q-value was calibrated using a diffraction pattern of dried chicken collagen.

3 Results and Discussion

In the previous study [2], the molecular weight and chain conformation of a proteoglycan derived from shark cartilage in solution were characterized by size-exclusion chromatography combined with low-angle laser light scattering and small-angle X-ray scattering methods. The total molecular weight of the proteoglycan was 3.9±0.2 million and the molecular weight of the main component was about 2.0±0.2 million. The X-ray scattering data revealed that the main components of the proteoglycan are nearly equal to a chain with excluded volume and their persistence lengths range from 13.5 to 16.4 nm.

In this study, we focused our attention on the structural characterization of the proteoglycan in sodium dodecyl sulfate micelle solution. The molecular weights of the proteoglycan in the presence of sodium dodecyl sulfate will not be changed in a preliminary light scattering experiment. Although the measuring limit may depend on the SAXS instrumentation, the small-angle resolution of the present SAXS measurement system was not sufficient for the estimation of the radius of gyration and molecular weight of this proteoglycan. Therefore, the Guinier analysis is not suitable for the proteoglycans in the present study. Although the typical SAXS patterns of denatured water-soluble proteins in the presence of sodium dodecyl sulfate micelles show a single peak at the q-value of 1.6 nm^{-1}, the SAXS pattern of the proteoglycan in the surfactant micelles didn't show any peaks and was slightly different from the pattern of the native proteoglycan in a preliminary experiment (not shown). The structure of the complex of the glycoprotein with surfactants remains to be investigated.

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References

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