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Characterization of a large glycoprotein proteoglycan by SAXS

Yasushi Watanabe[1,*] and Yoji Inoko[2]
[1]NARO National Food Research Institute, Tsukuba, Ibaraki 305-8642, Japan
[2] Osaka University, Toyonaka, Osaka 560-8531, Japan

1 Introduction

Proteoglycans 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. Some of these functions are due to the proteoglycan solution properties that refer to the molecular structure. It is important to clarify the chain conformation of the conjugates in solution for understanding the physiological 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 physiological conditions, the chain conformation of the proteoglycan in solution were characterized by a SAXS method.

2 Experiment

Synchrotron radiation SAXS measurements were performed using an optics system at the beamline BL-10C station in the Photon Factory of the High Energy Accelerator Research Organization. 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-μm-quartz windows was maintained constant at 24 °Cusing the metallic cell holder through which constant temperature water was circulated. Data were collected for 5 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 this study, since we focused our attention on the structural characterization of the intact proteoglycan in solution, non-denatured sample preparations were used for the SAXS experiment. Since the sample concentration dependence of the molecular weights shows a positive second virial coefficient, that is, a non-aggregating phenomenon (not shown), the molecular weights of the proteoglycan will not be drastically changed in the present SAXS experiments. 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 (Rg) and molecular weight of this proteoglycan. Therefore, the Guinier analysis is not suitable for the proteoglycans in the present study.

The notion of 'fractal' has been quite successfully introduced in physical chemistry and has been used to interpret the scattering results. The scattering intensity will scale as: $I(q) \sim q^{-D}$. This scaling law has frequently been used to determine fractal dimensions (D) from the log-log plot of I versus q. The Gaussian chain has a fractal dimension of 2. On the other hand, the chain with an excluded volume has the value of 5/3 (=1.67) that is less than 2, but greater than 1 (corresponding to rods). The slopes of the log–log plot in the q-range of 0.1-1 nm^{-1} of the present proteoglycans were estimated to be 1.30-, 1.59. Furthermore, the "2" fractal feature appears in the low-q region in all cases. The obtained fractal dimension indicates that the shark cartilage proteoglycan molecule is nearly equal to a chain with an excluded volume.

Furthermore, the Kratky plots of the present SAXS data clearly reveal the transition from the q^{-2} (plateau) to the q^{-1} (a linear function relation) region. From the transition point, the persistence lengths of the main components of the proteoglycan were estimated to be 13.5 - 16.4 nm. The persistence length of the rod-shaped fd virus has been reported to be 880 nm. On the other hand, the persistence length of hyaluronic acid has been estimated to be 4 nm. Therefore, the chain of the shark cartilage proteoglycan in solution is suggested to be more rigid than hyaluronic acid and more flexible than rod-shaped fd virus molecules. This consideration is acceptable based on the fact that the bulk of the structure of proteoglycans is usually a large amount of carbohydrates that is attached to the protein core at very many sites. The elongated shape in solution will be similar to the shape that has been observed for other proteoglycans using an electron microscope. Studies are under way to define more clearly the nature of proteoglycans.

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* yasuw@naro.affrc.go.jp