Cartilage Proteoglycans: Structure, Assembly and Organization

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INTRODUCTION

Aggrecan is a large charged biopolymer whose complexes with hyaluronic acid provide the compressive resistance of cartilage.[1-4] It exhibits a bottlebrush structure, in which chondroitin sulfate and keratan sulfate (linear sulfated polysaccharides) chains are attached to an extended protein core. At physiological concentrations (4-7 % w/w) the high osmotic swelling pressure of aggrecan-hyaluronic acid assemblies, which are enmeshed in the collagen matrix, provides resilience to compressive load, controls joint lubrication and protects bone surfaces from wear during articular movement. This supermolecular array yields a hydrated, viscous gel that provides the osmotic properties necessary for the cartilage to resist deswelling under compressive load with minimum deformation. Aggrecan also participates in cartilage/skeletal metabolism, playing an active role in bone mineralization by accumulating calcium ions.

The structure of aggrecan (e.g., length of bristles, charge density and distribution) varies with age and state of health. Owing to the complexity of the aggrecan-hyaluronic acid assembly the determination of the relationship between its structure and function requires a range of experimental approaches combining both physical and biochemical techniques. The aim of this work is to investigate the spatial organization both in solutions of pure aggrecan and in solutions containing aggrecan-hyaluronic acid complexes in a length scale range between 10 and 5000 Å.

EXPERIMENTAL

Sample Preparation. Aggrecan (bovine articular cartilage, Sigma) solutions were prepared in 100 mM NaCl with different amounts of $CaCl_2$ (0 - 100 mM). The concentration of the aggrecan was varied in the range 0.01%–4% by weight. A solution of aggrecan at 0.03% was also prepared in which the ratio of hyaluronic acid (Sigma, $M_w = 1.2\ 10^6$) to aggrecan was set equal to 0.01. The pH (= 7) was identical in all samples. At this pH the charged groups on the side-chains of the aggrecan molecule are dissociated.

Osmotic Pressure Measurements. Osmotic pressure measurements were made on aggrecan solutions by bringing them to equilibrium with polyvinyl alcohol gel filaments of known swelling pressure.[5,6] The large size of the aggrecan bottlebrush prevented penetration into the swollen gel. After equilibration the size of the PVA samples was determined by optical microscopy.

Scattering Measurements. Aggrecan solutions were investigated in near physiological salt solutions by small angle neutron scattering (SANS) on the NG3 instrument at the National Institute of Standards and Technology (NIST). For these measurements aggrecan solutions were prepared in D_2O. Solutions were placed in 2 mm thick sample cells. The measurements were made at an incident wavelength 8 Å. After azimuthal averaging, corrections for incoherent background, detector response and cell window scattering were applied.[7]

Small angle X-ray scattering (SAXS) measurements were made at the insertion device of Sector 5 at the Advanced Photon Source, Argonne National Laboratory, with an incident wavelength $\lambda = 1.55$ Å.

The combined SANS and SAXS measurements spanned the wave vector range 4×10^{-3} Å$^{-1} \le q \le 0.4$ Å^{-1}, where $q = 4\pi/\lambda \sin(\theta/2)$, λ is the wavelength of the incident radiation and θ the scattering angle.

Both osmotic pressure and scattering measurements were performed at 25 °C.

RESULTS AND DISCUSSION

Osmotic Pressure Measurements. In Figure 1 is shown the variation of the osmotic pressure Π as a function of the concentration for aggrecan and chondroitin sulfate (CS)[8] solutions. In the figure is also displayed the concentration dependence of Π for the solution of the aggrecan/hyaluronic acid complex. Since the bristles in the aggrecan molecule are mainly composed of CS, the CS solution models the side chains of the bottlebrush. The osmotic pressure of the CS solution is greater than that of the aggrecan solution by a factor of approximately two. The loss of entropy due to the bottlebrush arrangement of the CS molecules reduces Π. Further reduction is observed in the aggrecan/hyaluronic acid system due to complex formation between the aggrecan bottlebrushes and the hyaluronic acid (HA) chain.

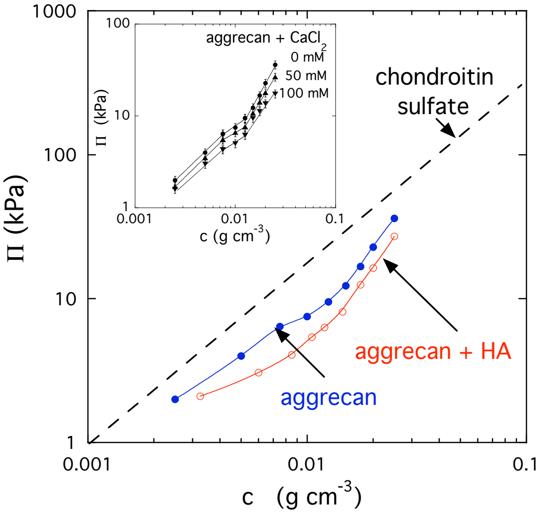

Figure 1. Dependence of the osmotic pressure Π as a function of polymer concentration in 100 mM NaCl solution. Dashed line is the osmotic pressure of chondroitin sulfate solutions in 150 mM NaCl from reference [8]. Inset shows the effect of $CaCl_2$ on the osmotic pressure of aggrecan solutions.

The osmotic response of the aggrecan solution exhibits three concentration regions. At low concentration ($c < 0.005$ g/cm^3) Π increases linearly with the aggrecan concentration. This behavior is typical for dilute systems in which the osmotic pressure is governed by independent particles. In the concentration range 0.006 to 0.015 g/cm^3 the slope of the double logarithmic plot decreases from 1 to about 0.6 indicating self-assembly among the aggrecan bottlebrushes. Above 0.015 g/cm^3 the slope increases to 2, corresponding to increasing packing density of the clusters.[9]

The effect of calcium ions is to decrease Π (see inset in Figure 1). The shape of the three curves, however, remains practically identical over the entire concentration range investigated. Addition of 100 mM $CaCl_2$ reduces Π by a factor of approximately 2. The change in the solvent quality and the modification of the electrostatic interactions associated with the calcium-sodium exchange promote association among the aggrecan molecules.

From the osmotic pressure curves an estimate of the osmotic compression modulus $K = c\partial\Pi/\partial c$ can be made. The greater slope in the aggrecan and aggrecan/hyaluronic acid systems in Figure 1 illustrates the fact that at physiological concentrations the bottlebrush architecture significantly enhances the osmotic modulus relative to the linear chondroitin sulfate solution. Extrapolation of the power law dependence to the physiological aggrecan concentration ($c = 0.07$ g/cm^3) yields $K = 520$ kPa, which is consistent with values reported by Treppo et al.[10]

Scattering Observations. Osmotic pressure measurements reveal that aggrecan bottlebrushes start to assemble in the dilute concentration range and display three different power law regimes. Scattering measurements provide information on the structure of a system in a range of length scales defined by $1/q$. Combination of scattering techniques using different wavelengths enables a wide range of characteristic distances to be explored.

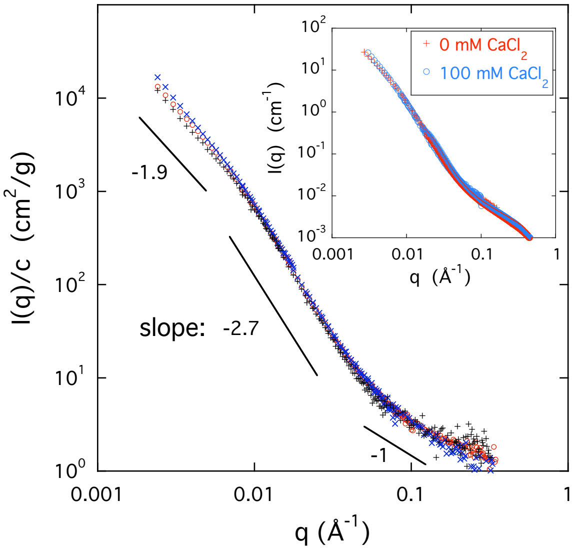

Figure 2. Variation of the reduced SANS and SAXS intensity $I(q)/c$ of aggrecan solutions at different concentrations (c = 0.006, 0.012, 0.025 gcm^{-3}) in 100 mM NaCl. Inset: SANS and SAXS intensity $I(q)$ for aggrecan solutions in 100 mM NaCl with 100 mM $CaCl_2$ and without $CaCl_2$.

Figure 2 shows the combined SANS and SAXS spectra of aggrecan solutions at three different concentrations. The measured intensities $I(q)$ are normalized by the aggrecan concentration. At low q the intensity decreases according to a power law, the exponent of which is about -1.9. This implies that the packing density of aggrecan assemblies is close to that of Gaussian clusters, for which the intensity varies as q^{-2}. Absence of a plateau regime at low q shows that the size of these objects exceeds the resolution of the scattering measurements. The normalized intensity decreases with increasing aggrecan concentration indicating that the packing density of the large assemblies increases. The power law behavior extends up to $q \approx 0.008$ $Å^{-1}$, where a change in slope is observed. In the range 0.012 $Å^{-1}$ $< q <$ 0.08 $Å^{-1}$, the scattering response exhibits a stronger power law behavior, with slope –2.7, characteristic of percolating branched polymer clusters.[11] This length scale range, from about 500 Å to 100 Å, is of the order of the length of the side-chains (bristles).[4,12] In this region the normalized intensity is practically independent of the aggrecan concentration, i.e., the degree of interpenetration between neighboring bottlebrushes does not vary. In the high-q range (corresponding to 5-10 Å) measured by SAXS, the intensity decreases as q^{-1} reflecting the linear (rod-like) structure of the side chains.

In the inset in Figure 2 are shown the results of similar SANS and SAXS measurements made in the presence of 100 mM calcium chloride. Within experimental error, the data sets with and without calcium chloride coincide. The lack of appreciable change in scattering intensity over the entire q range signifies that calcium ions induce neither phase separation nor densification of the clusters.[6] Thus, replacing sodium counter-ions on the polyanion with calcium ions, i.e., changing the electrostatic interactions, does not alter the structure. Even under conditions where the electrostatic interactions are screened, the random orientation of each bottlebrush axis and its rigid bristles limits interpenetration and prevents the development of compact structures. The most striking result from these scattering observations is that in aggrecan assemblies the ion-induced structural changes do not propagate to higher length scales.

CONCLUSIONS

The osmotic pressure of the aggrecan solution is significantly smaller than that of the corresponding solution of free chondroitin sulfate chains. SANS and SAXS measurements indicate that in the length scale range from about 100 Å to 500 Å, i.e., the order of the length of the side-chains the normalized scattering intensity is practically independent of the aggrecan concentration. This finding implies that the degree of interpenetration between neighboring bottlebrushes does not vary. Calcium ions reduce the osmotic pressure of aggrecan solution by promoting association among the aggrecan molecules. However, precipitation (phase separation) has not been observed even in the presence of 100 mM $CaCl_2$. Replacing sodium counterions on the polyanion with calcium ions, i.e., changing the electrostatic interactions, does not alter the structure of aggrecan assemblies.

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