Structure Formation and Interactions in Aggrecan Solutions

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INTRODUCTION

Aggrecan is a high molecular weight $(1x10^6<M<3x10^6)$ proteoglycan. It exhibits a bottlebrush structure, in which chondroitin sulfate and keratan sulfate chains are attached to an extended protein core. In the presence of hyaluronic acid (HA) and link protein, aggrecan molecules self-assemble into a supermolecular structure with as many as 100 aggrecan monomers attached as side chains on a filament of hyaluronan.[1] In cartilage these complexes are interspersed in the collagen matrix. This supermolecular array forms a hydrated, viscous gel that provides the osmotic properties required for the cartilage to resist deswelling under compressive load.[2]

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. The static properties are studied by osmotic pressure measurements, and small angle neutron scattering (SANS)), while the dynamics are probed by dynamic light scattering (DLS).

EXPRIMENTAL

Sample Preparation. Aggrecan (from bovine cartilage, Sigma) was dissolved in 100 mM NaCl solution. The aggrecan concentration was varied in the range 0.0002 - 0.03 g/cm^3. A solution of aggrecan was also prepared in which the ratio of aggrecan to HA (Sigma, M_w = 1.2 10^6) was set equal to 100. For the SANS measurements D_2O with 100 mM NaCl was used as a solvent. All measurements were made at 25 °C.

Osmotic Pressure Measurements. The osmotic pressure of the aggrecan solutions was measured as a function of concentration by equilibrating them with polyvinyl alcohol (PVA) gels of known osmotic swelling pressure.[3.4] The size of the gel filaments was measured by optical microscopy after equilibration in the solution (approximately 24 h). The large size of the aggrecan molecule prevented penetration into the PVA gels.

Small Angle Neutron Scattering. SANS measurements were performed on the NG3 instrument at NIST, Gaithersburg MD at three sample-detector distances, 1.35 m, 4 m and 13.1 m, using an incident wavelength of 8 Å. This configuration allowed us to make measurements in the transfer wave vector range 2.8 10^{-3} Å^{-1} < q < 0.35 Å^{-1}. The transfer wave vector $q = 4\pi n/\lambda \sin(\theta/2)$, where λ is the wavelength of the incident radiation, θ the angle of observation and n the refractive index of the scattering medium. The aggrecan solutions were placed in 2 mm thick quartz sample cells and the temperature during the experiments was maintained at 25° ± 0.1°C. After azimuthal averaging, corrections for solvent background, detector response and cell window scattering were applied.[5]

Dynamic Light Scattering. The DLS measurements were made with an ALV DLS/SLS 5022F goniometer equipped with a fiber optic coupling and an avalanche diode, working with a 22 mW HeNe laser and an ALV 5000E multi-tau correlator. The temperature of the refractive index matching toluene bath was maintained at 25.0 °C with a precision of better than 0.1 °C. Measurements were made in the angular range 20° to 150° with accumulation times of 200 s.

RESULTS AND DISCUSSION

Figure 1 shows the concentration dependence of the osmotic pressure Π both for the pure aggrecan solution and for that containing aggrecan-HA complexes.

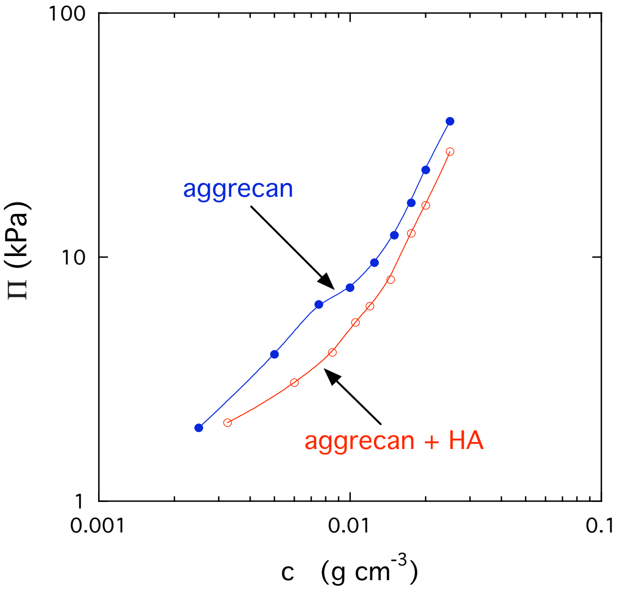

Figure 1. Concentration dependence of the osmotic pressure in aggrecan and aggrecan-HA solutions. The NaCl concentration was 100 mM in both systems. Ratio of aggrecan to HA was 100:1.

At low aggrecant concentration, Π increases linearly for both systems, typical of dilute solution behavior, but is smaller by roughly 30% when HA is present. This reduction in Π is direct evidence for the formation of complexes between aggrecan bottlebrushes and HA molecules. The difference between the two systems progressively disappears as the aggrecan concentration approaches the physiological concentration range, 0.04 g/cm^3 ≤ c ≤ 0.08 g/cm^3. In the aggrecan-HA system $\Pi(c)$ varies smoothly with a continuously increasing slope. By contrast, in the vicinity of 0.01 g/cm^3 the pure aggrecan solution exhibits a characteristic 'kink', resembling micelle formation.[6] This feature, indicating self-assembly among the aggrecan bottlebrushes, can be attributed to the difference in water affinity between the N-terminal domain of the protein core in the aggrecan molecule and the highly charged hydrophilic polysaccharide side chains. In such associating solutions, individual bottlebrush subunits coexist with microgel-like assemblies. At higher concentration, above 0.012 g/cm^3, the osmotic pressure rises more steeply owing to the densification of the overlapping aggrecan assemblies.

In **Figure 2** is plotted the intensity $I(q)$ measured by SANS for aggrecan solutions at 3 different concentrations, 0.06, 0.12 and 0.25 % w/w. The SANS curves lie parallel to each other and the intensity increases linearly with increasing concentration. These results imply that the extent of mutual penetration of the aggrecan brushes is independent of the overall concentration.[6] In each case, power-law behavior is distinguishable in the q range above 0.01 Å^{-1}, with a slope approximately –2.7. The value of a fractal exponent of 2.5 or slightly above is typical of branched polymer structures,[7] such as an association of a small number of aggrecan molecules. The diameter of these clusters, calculated from the position of the knee at $q \approx 0.01$ Å^{-1} in the scattering curve, is approximately 50 nm, which is consistent with a single aggrecan bottlebrush. In the lower q-range of the figure, the slope of the $I(q)$ plots falls to approximately -2.

Information on the dynamics of the aggrecan assemblies was obtained from DLS measurements. The non-exponential correlation functions of the aggrecan solutions were analyzed using the cumulant procedure[8,9], where the average decay rate is given by the first cumulant Γ

$$-d\left[\ln(G(\tau)-1)\right]/d\tau \,\Big|_{\tau\to 0} = 2\Gamma \qquad (1)$$

where $G(\tau)$-1 is the intensity correlation function and τ is the delay time.

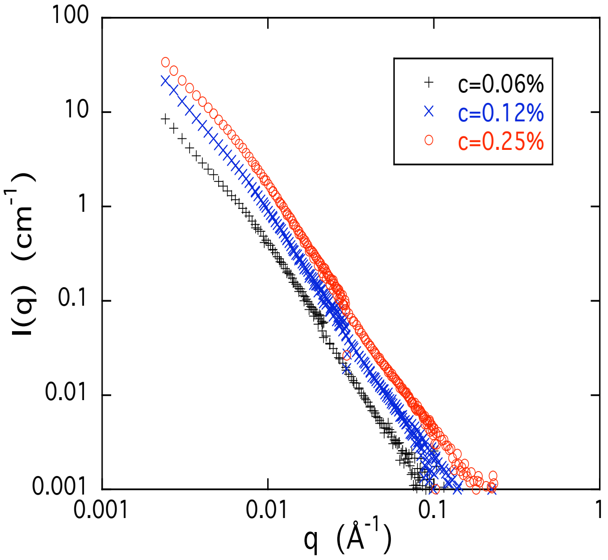

Figure 2. SANS spectra of aggrecan at different concentrations in 100 mM NaCl solutions with D_2O.

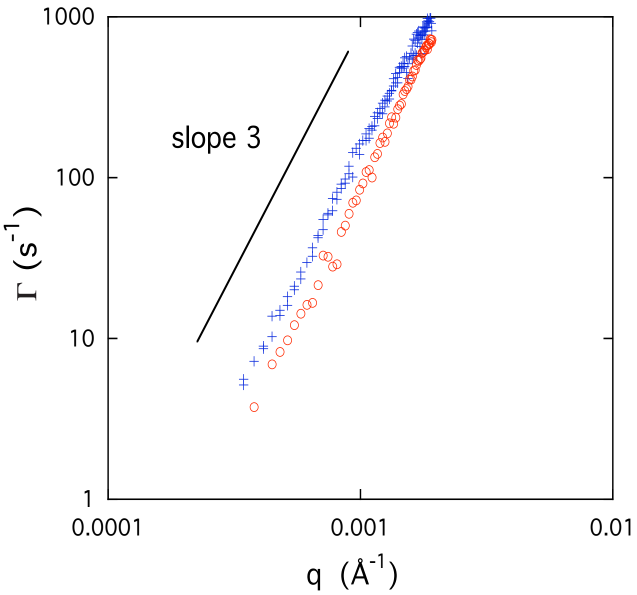

Figure 3. Mean relaxation rate Γ as a function of wave vector q, for two aggrecan concentrations, 0.02 % (+) and 0.14 % (o).

Figure 3 shows the q-dependence of Γ at two aggrecan concentrations (0.02 and 0.14 % w/w). At the lower concentration, the relaxation rate Γ of the intensity correlation function obeys a power law in q,

$$\Gamma = \frac{k_B T}{6\pi\eta} q^3 \qquad (2)$$

where k_B is Boltzmann's constant, T the absolute temperature and η is the viscosity of the solvent.

The q^3 dependence of the relaxation rate is characteristic of internal motions in polymer solutions under free draining conditions in the regime $qR_G >> 1$, where R_G is the radius of gyration.[10,11] In such systems, where no characteristic length scale can be associated with the structural element that defines the dynamics, the internal motion yields non exponential correlation functions $G(\tau)$. For the higher concentration sample shown in Fig. 3 the smaller value of Γ implies a decreased molecular mobility within the associations.

CONCLUSIONS

Osmotic pressure measurements, SANS and DLS results show that aggrecan molecules self-assemble into loose microgels. At length scales of the order of 50 nm an apparent fractal dimension of 2.7 was found. No change is detected in the degree of mutual penetration of the aggrecan bottlebrushes with increasing concentration. The relaxation rates measured by DLS are proportional to q^3, which is the signature of internal modes in large loosely connected assemblies of aggrecan molecules.

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