Proteoglycan Assemblies in Cartilage

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INTRODUCTION

Aggrecan is a high molecular weight proteoglycan. Its primary role is to provide the osmotic properties necessary for cartilage to resist compressive stress. The aggrecan molecule is composed of an extended protein core to which many chondroitin sulfate and keratan sulfate (linear sulfated polysaccharide) chains are attached. This array forms a bottlebrush structure. In the presence of hyaluronic acid aggrecan molecules self-assemble into a supermolecular structure with as many as 100 macromonomers bound to a filament of hyaluronic acid. [1-6]

The aim of our work is to obtain information on the mechanism of the structure formation and the relationship between the structure and the dynamic properties in this system.

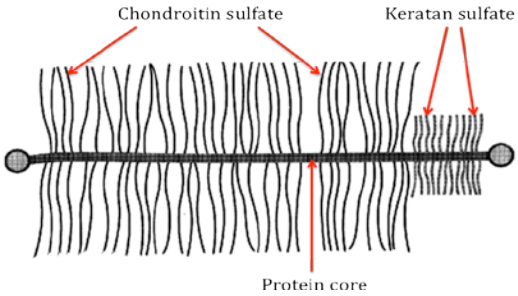

Figure 1. Diagram of the predominant cartilage proteoglycan, aggrecan.

EXPERIMENTAL

Sample Preparation. Aggrecan (bovine cartilage, Sigma) solutions were made in 100 mM NaCl solution. The concentrations of the aggrecan solutions were 0.25 mg/ml. 0.5 mg/ml and 1 mg/ml. Aggrecan-hyaluronic acid solutions were also prepared in which the ratio of aggrecan to hyaluronic acid was set equal to 100.

Light Scattering. Static and dynamic light scattering measurements (SLS and DLS) were made with an ALV 5022F goniometer equipped with a fibre optic coupling and an avalanche diode, working with a 22 mW HeNe laser and an ALV 5000E multi-tau correlator.[7] 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 between 20° and 150° with accumulation times of 200 s.

RESULTS AND DISCUSSION

In solutions containing extended objects such as macromolecular assemblies, light scattering provides information both on the size and dynamic properties of the assemblies.[8] First we focus on the dynamic behavior of aggrecan in nearly physiological salt solutions. Then we investigate the effect of hyaluronic acid on the self-assembly of aggrecan molecules.

Aggrecan Solutions. In Figure 2 are shown the intensity correlation functions for three solutions of aggrecan at concentration 0.25 mg/ml, 0.50 mg/ml and 1.0 mg/ml. These curves can be described satisfactorily by a two exponential decay, in which the relaxation times τ_1 and τ_2 differ by an order of magnitude, i.e.,

$$G(\tau) - 1 = [a \exp(-\tau/\tau_1) + (1-a) \exp(-\tau/\tau_2)]^2 \qquad (1)$$

where *a* and (1-a) are the relative intensities of the fast and slow relaxation modes. The two relaxation times reveal the presence of molecular structures whose hydrodynamic radius, which is inversely proportional to the relaxation time, is approximately 370 Å and 5000 Å, respectively. Both τ_1 and τ_2 increase with increasing aggrecan concentration, which is consistent with the increasing viscosity of these solutions. The inset in Figure 1 shows the intensity correlation function of an aggrecan solution at c = 0.25 mg/ml and scattering angle 90°, expressed by the stretched exponential form

$$G(\tau) - 1 = \beta \exp[-(\Gamma\tau)^{\mu}] \qquad (2)$$

where β (≈ 1) is the coherence factor and the value of the exponent μ is found to be 0.65. The initial part of the relaxation curve is adequately described by the stretched exponential (equation 2), while at longer times translational diffusion effects dominate.

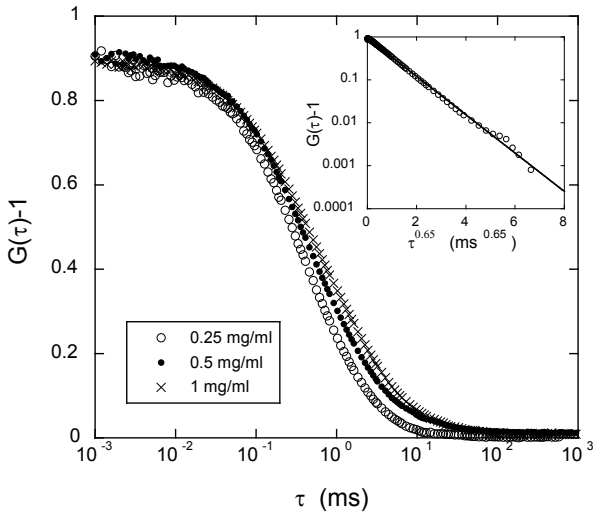

Figure 2. Light scattering intensity correlation function G(τ) - 1 for aggrecan solutions of different concentrations (scattering angle 90°). Inset: G(τ) - 1 for an aggrecan solution at 90°, *c* = 0.25 mg/ml with fit to eq 2, where μ = 0.65.

Aggrecan-Hyaluronic Acid Solutions. In the presence of the negatively-charged linear polymer hyaluronic acid (HA), aggrecan forms large aggregates. Previous studies of this system have not explored the effect of hyaluronic acid on the relaxation properties.

Figure 3 shows the DLS response of a solution containing aggrecan-hyaluronic acid aggregates at a molar ratio 100:1. It can be seen that the relaxation properties of the aggrecan-hyaluronic acid system become slower than that of the pure aggrecan solution. Analysis of the intensity correlation functions using eq 1, however, reveals that τ_1 becomes shorter, i.e. the mobility of the smaller units increases, while that of the larger assemblies remains roughly constant. This corresponds to a reduction in the hydrodynamic radius of the first component from approximately 370 Å to 250 Å, while the radius of second component (approximately 5000 Å) does not change. The results imply that the longer relaxation component dominates the dynamic response of the system, thus causing an overall slowing.

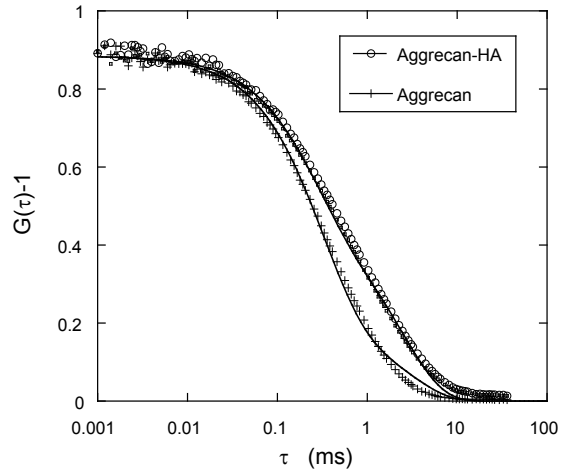

Figure 3. Light scattering intensity correlation function G(τ) - 1 for aggrecan (+) and aggrecan-HA (o) solutions at scattering angle 90°.

In the presence of HA ordering of the aggrecan monomers occurs, with the formation of elongated complexes several microns in length and about half a micron in diameter.[9,10] As this complex provides the compressive resistance in cartilage, a knowledge of its osmotic response is essential to understand its biological function. Complexation is expected to affect both the structure and the osmotic properties of the solution. Light scattering is well suited to quantify these changes.

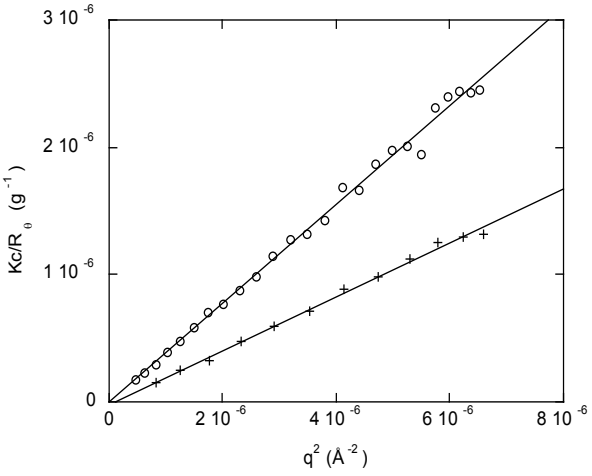

Figure 4. Zimm representation of the inverse static scattering intensity as a function of q^2 for solutions containing pure aggrecan (+) and aggrecan-HA complexes (o). Aggrecan concentration 0.25 mg/ml, aggrecan : HA ratio 1:100. In the aggrecan-HA system, the light scattering measurements were made within 1 h after sample preparation.

Figure 4 compares Kc/R_θ from an aggrecan solution with that of the aggrecan-HA complex. The latter data were collected soon after adding HA at a mass ratio 1:100 to the aggrecan solution. In Figure 4 the Zimm representation is used, i.e., Kc/R_θ vs q^2, where

$$\frac{Kc}{R_\theta} = \frac{\partial \Pi / \partial c}{kT} \frac{1}{S(q)} \qquad (3)$$

In equation 3, K is an optical constant, R_θ is the Rayleigh ratio, Π is the osmotic pressure, $S(q)$ is the structure factor of the complex, k is the Boltzmann constant and T is the absolute temperature. The linearity of Kc/R_θ in this representation corresponds to the q^{-2} dependence of the scattered intensity. In both samples the intercept at the origin is zero within experimental error, indicating that the molecular weight is too large to be resolved by SLS. The scattering intensity is inversely proportional to the osmotic modulus.[11] In the present experiment, the difference in slope of the two lines is not due to a difference in radius of gyration, as for polymer solutions, but to a difference in the osmotic modulus. Even at this low concentration the osmotic modulus $c\partial\Pi/\partial c$ of the aggrecan-HA complex exceeds that of the pure aggrecan microgels.

CONCLUSIONS

An understanding of the basic physical chemical interactions between aggrecan and hyaluronic acid is biologically important because their complexes provide the osmotic resistance of cartilage. In the presence of hyaluronic acid aggrecan self-assembles into large complexes of size greater than 1000 nm. This is reflected in the hydrodynamic radius, R_H. With increasing aggrecan concentration R_H. increases, which is a consequence of the steric hindrance produced by densification of the aggrecan aggregates. Hyaluronic acid, however, slows the relaxation rate in agreement with an increase of the friction coefficient due to the rearrangement of the aggrecan molecules along the hyaluronic acid chain. Static light scattering measurements reveal that the osmotic modulus of the aggrecan-hyaluronic acid complex is greater than that of the pure aggrecan solution.

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