COMPUTATIONAL BIOMECHANICS OF AGGRECAN IN CARTILAGE: CONNECTING MOLECULAR COMPOSITION WITH MACROSCOPIC MECHANICAL PROPERTIES

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INTRODUCTION

Adult articular cartilage is an avascular tissue that serves to reduce the frictional load between contacting bones in load bearing joints. The tissue consists of a sparse population of chondrocytes (~ 2% by volume) embedded in an extracellular matrix (ECM). Aggrecan and type-II-collagen are the two primary load bearing macromolecules within the ECM and play distinct yet complementary biomechanical roles: The swelling pressure induced by the highly charged comb polymer aggrecan is balanced by tensile stresses in the interconnected, fibrillar scaffold formed by collagen [1].

Prior to the onset of osteoarthritis, an incurable and debilitating joint disease that affects more than 30 million Americans, aggrecan undergoes distinct changes in its molecular composition. Primary alterations include changes in the molecular weight and sulfation pattern of the anionic glycosaminoglycans (GAGs) that decorate its protein backbone [2]. Concurrently, the macroscopic biomechanical properties of cartilage are modified [3].

The goal of this research is to use molecular modeling and simulation techniques to relate the chemical composition of aggrecan to its bulk mechanical properties. Due to the high molecular weight of aggrecan, we are successively coarse-graining its mathematical description to obtain a computationally tractable model. In the initial stages of coarse-graining we are focused on the glycosaminoglycan constituents of aggrecan because they constitute the largest fraction (~ 90%) of its total molecular weight. Subsequent stages will be directed towards assembling the chains to form a complete aggrecan molecule, after which numerous interacting aggrecan molecules may be simulated to compute truly macroscopic properties. The aim of the current communication is to present our methodology for the initial level of coarse-graining as well as some preliminary results towards this end.

MODELING

Aggrecan consists of a linear core protein backbone (250 kDa) with approximately 100 covalently attached chondroitin sulfate (CS) chains. Chondroitin sulfate is a linear (unbranched) polysaccharide consisting of repeating disaccharide units of glucuronic acid and N-acetyl-galactosamine (sulfated at the 4- or 6-carbon), alternately linked in $\beta 1 \rightarrow 3$ and $\beta 1 \rightarrow 4$ glycosidic linkages, respectively (Fig. 1). 20-60 disaccharides constitute each CS chain, giving aggrecan a molecular weight of up to 3 MDa. Aggrecan associates with high molecular weight Hyaluronan (HA), another anionic glycosaminoglycan, to form large-scale aggregates in cartilage. HA differs chemically from CS in that N-acetyl-glucosamine (unsulfated) is substituted for N-acetyl-galactosamine-4- or -6-sulfate. In the current work we apply our coarse-graining procedure to both CS and the chemically similar HA for purposes of validation due to the lack of available experimental data for CS.

In defining the coarse-grained model we begin by treating each sugar monomer as a rigid chemical entity, fixed in its mean room temperature configuration. This assumption is consistent with the analysis of ring puckering parameters from molecular dynamics (MD) simulations of solvated hyaluronan tetrasaccharides that demonstrate that each residue (glucuronic acid and N-acetyl-glucosamine) exists in a stable, $^4C_1$ chair conformation [4]. The internal degrees of freedom of the biopolymer are thereby limited to the glycosidic torsion angles, (ϕ, ψ), defined by the glycosidic bond hydrogens (H1-C1-Ox-Cx) and (C1-Ox-Cx-Hx), respectively, where x depends upon the linkage type (13/14) (Fig. 1).

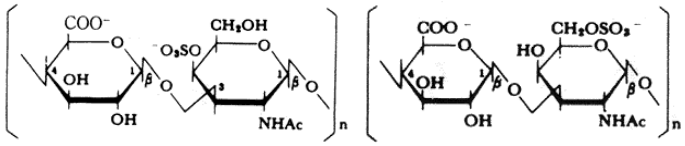

Fig. 1 Disaccharide repeat units of chondroitin-4-sulfate (left) and chondroitin-6-sulfate (right).

Energetic interactions are decomposed into short-range and long-range for 'bonded' and 'non-bonded' sugar monomers, respectively. Short-range interactions are limited to include nearest-neighbor

interactions across single glycosidic linkages and are denoted $U_{13}(\phi,\psi)$ and $U_{14}(\phi,\psi)$ for the $\beta 1 \to 3$ and $\beta 1 \to 4$ linkages, respectively. For computational efficiency we pre-compute and tabulate the short-range energies, $U_{13}(\phi,\psi)$ and $U_{14}(\phi,\psi)$, using all-atom models of solvated disaccharides and subsequently employ the tables in the coarse-grained GAG simulations.

Due to the highly charged nature of CS and HA, long-range interactions are limited to include solvent-mediated electrostatic interactions. For computational efficiency, the electrostatic potential due to the charge distribution of a sugar monomer is approximated by the monopole of its multi-pole expansion. The solvent-screened electrostatic interaction energy of two sugar monomers is then given by the Debye-Hückel interaction energy (in SI units),

$$U_{ij} = \frac{Q_i Q_j}{4\pi\varepsilon_0 \varepsilon r_{ij}} e^{(-\kappa r_{ij})} \qquad (1)$$

where Q_i is the net charge of sugar residue i, r_{ij} is the distance between the centers of charge of sugars i and j as uniquely defined by their mean, room temperature configurations and the set of glycosidic torsion angles $\{\phi,\psi\}$, ε is the dielectric of water at 298 K (78.5), ε_0 is the permittivity of vacuum, κ is the inverse of the Debye length ($\kappa^2 \equiv e^2 \sum_k n_k^0 z_k^2 / \varepsilon\varepsilon_0 k_B T$), where e is the elementary charge of an electron, n_k^0 and z_k are the bulk concentration and valency of the k^{th} ionic species (+1 and −1 for Na and Cl), respectively, k_B is the Boltzmann constant, and T is absolute temperature. The Metropolis Monte Carlo (MMC) algorithm is employed using the short- and long-range energy models above and the mean polysaccharide geometry to simulate isolated GAGs (infinitely dilute solution) in the canonical ensemble at room temperature. Structural and thermodynamic properties are computed for chains of up to 1024 sugar monomers.

RESULTS

To validate the coarse-grained GAG models we compute the persistence length, l_p, of HA as a function of ionic strength and compare it to experimentally determined values. Physically, the persistence length of a polymer measures the chain length over which conformational disorder destroys the chain's memory of its initial direction.

The persistence length computed for HA attains its infinite chain length limit for $N \sim 1024$ sugars. A linear fit to the computed persistence length data for HA gives

$$\begin{aligned} l_p &= 7.0 + 34.3 C_s^{-0.5} \quad \text{(Computational Model, } R^2 > 0.99\text{)} \\ l_p &= (9 \pm 1) + 31 C_s^{-0.52 \pm 0.1} \quad \text{(Experimental, [5])} \end{aligned} \qquad (2)$$

where C_s is the ionic strength in mM and the units of l_p are nm. We also computed the persistence length for CS4 and CS6 but do not have direct experimental data to compare with. We did, however, find a linear dependence of l_p on charge density, consistent with experimental findings for other polyelectrolytes. The computational intrinsic (or hypothetical infinite ionic strength) persistence length, l_{p0}, of HA is seen to be equal to 7 nm (about 7 disaccharides in length) in Eq. (2). The various experimental data in Table I exhibit a range from 4 – 9 nm, bracketing our result of 7 nm.

Table I HA experimental intrinsic persistence length.

l_{p0} (nm)	Experiment	Reference
9	LS	[5]
4	SAXS	[6]
4	Viscometry	
4	LS/Viscometry	[7]

CONCLUSION

A coarse-grained model of anionic glycosaminoglycans, the molecular building blocks of aggrecan, is presented. Treating each monosaccharide as a rigid chemical entity results in a reduced model in which the biopolymer conformation is determined solely by the set of glycosidic torsion angles. Our modeling assumptions enable the simulation of > 1000 sugar monomer isolated GAGs, approximately 100x higher molecular weight than would be attainable with an all-atom representation of the biopolymer. We validate the model by computing the persistence length of hyaluronic acid in dilute solution and find that it is in quantitative agreement with experimental light scattering data. Subsequent work will include bulk simulations of chondroitin-sulfate chains to investigate the effects of sulfation and molecular weight on their mechanical properties. The ultimate aim of this research is to predict the dependence of the meso- and macroscopic mechanical properties of cartilage on the underlying chemical composition of aggrecan to gain insight into the biomechanics of the tissue in its healthy and osteoarthritic states.

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