Persistence Length of Cartilage Aggrecan Macromolecules Measured via Atomic Force Microscopy

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Summary: Tapping mode atomic force microscopy (TMAFM) was employed to directly calculate the persistence length of individual fetal bovine epiphyseal and mature nasal cartilage aggrecan monomers, as well as their constituent chondroitin sulfate glycosaminoglycan chains.

Keywords: aggrecan; atomic force microscopy; biopolymers; cartilage; glycosaminoglycan

Introduction

Cartilage is a highly specialized, dense connective tissue found between the surfaces of movable articular joints whose main function is to bear stresses during joint motion. The negatively charged disaccharide *chondroitin sulfate glycosaminoglycan* (CS-GAG) macromolecules are a major determinant of the tissue's ability to resist compressive and shear loading *in vivo*, e.g., responsible for >50% of the equilibrium compressive elastic modulus under normal physiological conditions (0.15 M salt concentration).[1] Approximately 100 CS-GAGs are covalently bound at extremely high densities (separated by ~2-4 nm) to a 250 kDa core protein forming the *aggrecan* molecule. Multiple aggrecan molecules self-assemble to form supramolecular *proteoglycan*

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aggregates by noncovalently end-attaching to a *hyaluronan* (HA) central filament, an interaction that is stabilized by the binding with *link protein*. These aggregates form a gel-like component enmeshed within a network of reinforcing collagen fibrils.

Experimental

Purified A1A1D1D1 aggrecan monomers from the fetal bovine epiphyseal growth plate region and mature bovine nasal region was dialyzed first against 500 volumes of 1 M NaCl and then against HOH to remove excess salts to 5-25 μg/mL. A positively-charged amine-functionalized muscovite mica surface (Pelco International, Redding, CA) was prepared with a treatment of 60 μL 0.01% 3-amino-propyltriethoxysilane (APS) for 20-30 min (Sigma Aldrich Co., St. Louis, MO) v/v MilliQ water (Millipore Corp, Bedford, MA.). 60 μL of 250 μL/mL aggrecan solution was allowed to incubate on the APS-mica for 20-30 min, then rinsed, and dried in air for 1 h before imaging in tapping mode in ambient conditions with a Nanoscope IIIa Multimode atomic force microscope (TMAFM) (Digital Instruments (DI), Santa Barbara, CA) and Olympus AC240TS-2 Si cantilevers (probe tip radius < 10 nm, spring constant 2 N/m).

Results and Discussion

TMAFM visualization of partially hydrated individual aggrecan monomers with unprecedented clarity and molecular-level resolution (Figure 1a) enables the direct quantitative measurement of the ultrastructure, dimensions, and conformation of both the protein core backbone and individual CS-GAG chains.

To calculate an effective persistence length, L_p, a series of equal length vectors were iteratively projected onto the digitized trace of the core protein and GAG contours in increments of l =1.2 nm (Figure 1b). For each l, the bend angle with respect to the previous vector, θ, was calculated for each vector (i.e. as a function of position along the polymer chain) yielding $\langle\theta^2(l)\rangle$. Using the assumptions of the Worm-Like Chain (WLC)

model, L_p was estimated from the linear relationship of the variance of θ as a function of *l* (Figure 2a). The kurtosis of θ at each *l* was plotted to assess the WLC behavior of the molecule (Figure 2b). Table 1 summarizes the results for L_p, the chain end-to-end distance (R_{ee}), the trace length (L_c), the aggrecan diameter (*w*), and GAG-GAG interchain spacing (*d*).

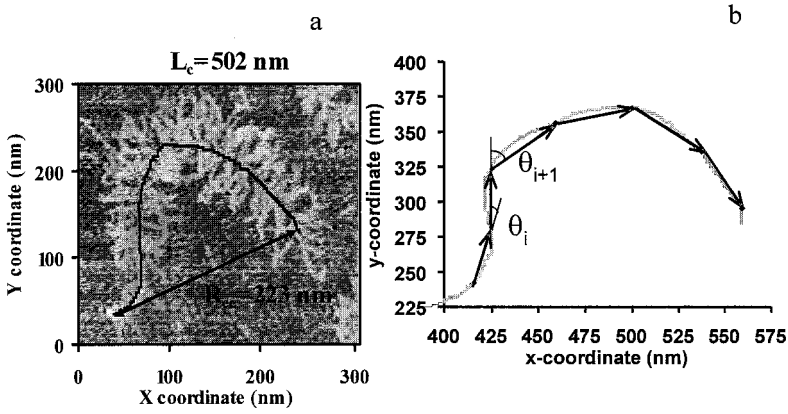

Figure 1. a) TMAFM height image taken of an aggrecan molecule and b) projection of a series of equal length vectors onto the digitized trace of the core protein backbone of the aggrecan molecule shown in a)

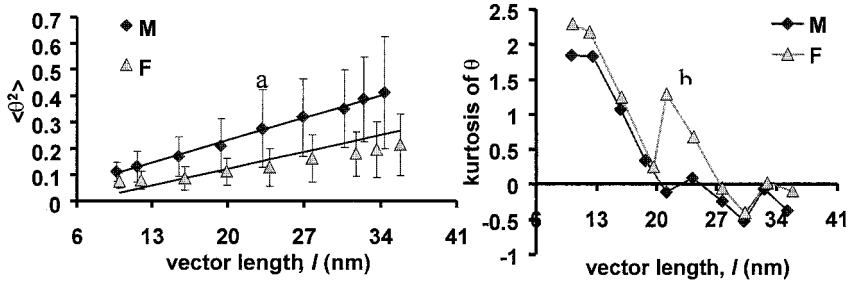

Figure 2. a) Calculation of L_p and b) kurtosis of θ both for aggrecan n_{mature}=15 (M), n_{fetal}=15 (F)

Table 1. Properties of aggrecan protein core and CS-GAG chains measured by TMAFM

cartilage	aggrecan protein core				CS-GAG			
units=nm	L_c	R_{ee}	L_p	w	L_c	R_{ee}	L_p	d
mature nasal	353±88	226±81	82	47±12	32±5	26±7	14	4.4±1.2
	n=40	n=40	n=15	n=108	n=49	n=49	n=17	n=40
fetal epiphyseal	398±57	257±87	110	57±14	41±7	32±8	21	3.2±0.8
	n=102	n=102	n=15	n=104	n=102	n=102	n=21	n=102

n = number of molecules used in calculation

Conclusion

Distinct differences in the nanoscale properties between two aggrecan populations (mature nasal versus fetal epiphyseal) have been clearly observed via TMAFM imaging. Hence, it is clear that such studies, as a function of age, disease, and injury, have great potential to yield new insights into the molecular origins of cartilage dysfunction.

[1] M.D. Buschmann, A.J. Grodzinsky, *J. Biomech. Eng.* **1995,** *117*, 179.