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Interactions between Nearest-neighboring Glycosaminoglycan Molecules of Articular Cartilage

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Abstract: The electrostatic interaction effects including the interaction potential, force and torque between the neighboring chondroitin sulfate glycosaminoglycan (CS-GAG) molecular chains in the bottle brush conformation of proteoglycan aggrecan are obtained as the functions of the minimum separation distance and the mutual angle between the molecular chains based on an asymptotic solution of the Poisson-Boltzmann equation that the CS-GAGs satisfy under the normal physiological conditions of articular cartilage. The present study indicates that the electrostatic interactions are not only associated intimately with the separation distance and the mutual angle, which are shown as purely exponential in separation distance and decrease with increasing mutual angle, but also dependent sensitively on the saline concentration in the electrolyte solution within the tissue. Further analysis shows that in the range of the separation distance between two neighboring CS-GAG molecular chains in the bottle brush conformation of the aggrecan in vivo ($2 \sim 4$ nm), if the saline concentration in the electrolyte solution is not less than a value of concentration (~ 0.1 M), the interactions between the molecular chains will monotonically increase with decreasing the saline concentration, however, if the saline concentration is less than the value of concentration, the relationship between the interactions and the saline concentration will not be simply monotonic. Some present results are in agreement with the existed relevant conclusions.

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1 Introduction

Glycosaminoglycans (GAGs), which are the most abundant heteropolysaccharides in the body, play an important role in the physiological and physical functions of biological tissues such as cartilaginous tissue [Comper and Laurent, (1978); Maroudas, (1979); Ng et al. (2003); Seog et al. (2002); Mow and Guo, (2002); Iozzo and Murdoch, (1996)]. Their molecules are long unbranched polysaccharides made of repeating disaccharide units that contain either of two modified sugars, N-acetylgalactosamine (GalNAc) or N-acetylglucosamine (GlcNAc) and a uronic acid such as glucuronate or iduronate. The family of GAG molecules are usually composed of chondroitin sulfate (CS), keratin sulfate (KS), heparan sulfate (HS) and hyaluronic acid (HA). In particular, the proteoglycan aggrecan in articular cartilage of animals, the central region of which contains approximately 200 polysaccharide chains of CS and KS, each with a sugar unit motif repeated up to 100 times, plays a crucial role in the structures, the biological and physical properties of extracellular matrix [Comper and Laurent, (1978); Ng et al. (2003); Seog et al. (2002); Yanagishita and Hascall, (1992); Jin and Grodzinsky, (2001)].

Aggrecan, whose molecular mass is about 250 kDa, is made of core protein, CS and KS proteoglycan, as shown in Fig.1a. In particular, the chondroitin sulfate glycosaminoglycans (CS-GAGs) of aggrecan, which contain one negatively charged carboxylate and sulfate group per disaccharide that is completely ionized under normal physiological conditions, as shown in Fig.1b, have high negative charge density in articular cartilage. Based on the electrostatic repulsive effects, CS-GAGs have the tendency to assume ex-

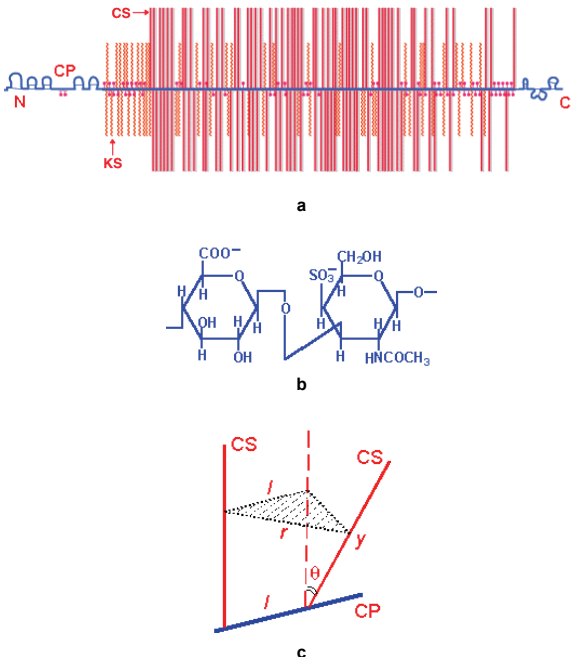


Figure 1: The planar model of aggrecan: glycosaminoglycans are separately indicated by solid line (chondroitin sulfate, CS) and wavy line (keratan sulfate, KS), and NH_2 and COOH ends of core protein (CP) are denoted by N and C, respectively. **b.** Chemical structure of the disaccharide repeating unit in chondroitin sulfate glycosaminoglycan (CS-GAG). **c.** The configuration of two neighboring CS-GAGs in the bottle brush conformation of aggrecan. l and θ are the minimum separation distance and mutual angle between the neighboring CS-GAGs, respectively, and $r^2 = l^2 + (y\sin\theta)^2$.

tended and rodlike conformation rather than random coil in physiological solution of articular cartilage. The previous studies demonstrated that it is the electrostatic repulsive effect between CS-GAGs that is responsible for 50∼75% of the equilibrium compressive resistance of articular cartilage [Maroudas, (1979); Seog et al. (2002); Mow and Guo, (2002); Buschmann and Grodzinsky, (1995); Maroudas, (1968); Mow et al. (1998); Lai et al. (1991); Lai et al. (2000)]. However, the aggrecan in vivo is the three-dimensional structure that is known as a bottle brush model, in which the CS-GAG and KS-GAG molecular chains are extended as much as possible from the core protein to minimize interactions between negative charges [Sajdera and Hascall, (1969); Wellauer et al. (1972); Muir and Hardingham, (1975)]. Therefore, arbitrary two neighboring polysaccharide chains of the CS and KS in the aggrecan conformation, each of which radiate away from the core protein, in general, are not in the same geometrical plane, as shown in Fig.1c. It is the three-dimensional conformation of aggrecan molecules that brings on a lot of difficulties to study both the interactions between neighboring CS-GAGs and the biological functions of aggrecan itself.

Each of the GAG molecules can be modelled as locally rigid even though its global structure is flexible [de Gennes, (1978); de Gennes, (1979)], so that each of the CS-GAG molecules can be approximated as a cylindrical rod having a known surface charge density and a fixed radius and the interactions between neighboring CS-GAGs can be modelled on average by employing the Poisson-Boltzmann (PB) equation [Seog et al. (2002); Jin and Grodzinsky, (2001); Buschmann and Grodzinsky, (1995); Dean et al. (2003)]. On the one hand, the PB equation has been widely used to characterize the intermolecular interactions of linear polyelectrolytes (e.g. DNA molecule) surrounded by aqueous electrolyte and to calculate the relevant electrical potential and mobile ion distributions. In particular, solving the PB equation to obtain the interaction between charged rodlike biopolymer has been presented in the past [Fuoss et al. (1951); Katchalsky, (1971); Brenner and Parsegian, (1974); Gur et al. (1978); McCaskill and Fackerell, (1988); Isarelachvili, (1992); GrØnbech-Jensen et al. (1997); Ospeck and Fraden, (1998); Harries, (1998); Shkel et al. (2000); Shkel et al. (2002)]. However, no single approximation is expected to hold true for the whole range of intermolecular separations. Some of these rely on the use of the linearized version of the PB equation (in the case that the cylinders are immersed in a salt solution) [Brenner and Parsegian, (1974)]. This is an appropriate approximation in cases of low surface charge densities. However, when dealing with macromolecules of high surface charge density, and the radius comparable to the Debye length, such as the present

study on the CS-GAGs, this approximation is no longer valid. On the other hand, in terms of the study of the GAGs in articular cartilage on the molecular level, Buschmann and Grodzinsky (1995) and Jin and Grodzinsky (2001) used the PB cell model to investigate the swelling pressure and the shear modulus on articular cartilage. Their results showed that the swelling pressure of proteoglycan solution increased with increasing the concentration of the solute; Seog et al. (2002) and Dean et al. (2003) experimentally and theoretically examined the interactions between the CS-GAGs of the grafted proteoglycan brush systems and between the grafted GAG layer and the chemical functionalized probe tip. And then they indicated that the interaction force between the GAG brush layer and the probe tip decreased with increasing the saline concentration in the solution. However, the interactions between the CS-GAGs on the bottle brush conformation of aggrecan, which are intimately associated with the physiological and physical properties of articular cartilage in vivo, have still been remained unclear so far.

In the present paper, we focus on the intermolecular electrostatic interactions between the neighboring CS-GAGs in the bottle brush conformation of aggrecan under the physiological conditions of articular cartilage. It is assumed that each of the CS-GAG molecular chains attached on the core protein can be approximated as a cylindrical rod having a surface charge density and a fixed radius. By solving the PB equation satisfied by the molecular cylinders in the physiological solution, we firstly obtain the electrical potential of the CS-GAGs, and then get the interactions between the molecular cylinders including the interaction potential, force and torque as the functions of the separation distance and mutual angle between the CS-GAGs. Our analyses indicate that the electrostatic interaction effects are not only associated intimately with the separation distance and the mutual angle between the CS-GAGs, but also dependent sensitively on the saline concentration in the physiological solution within the tissue. Some results presented in this paper are in good agreement with the existed relevant conclusions.

2 Electrostatic Effects of CS-GAGs

2.1 Poisson-Boltzmann Equation

Under the normal physiological conditions of articular cartilage, the mobile ions within the physiological electrolyte solution are generally considered to contain only two monovalent ions, i.e. Na^+ and Cl^- [Comper and Laurent, (1978); Buschmann and Grodzinsky, (1995); Mow and Guo, (2002); Song et al. (2005)]. Therefore, the PB equation satisfied by individual CS-GAG chain of aggrecan is written as

$$\nabla^2\varphi = \frac{2Fc_b}{\varepsilon_r\varepsilon_0}\sinh\left(\frac{F\varphi}{RT}\right) \tag{1}$$

whereφ is the electrical potential derived from the charged surface of the CS-GAG chain in the electrolyte solution; ε_0 (= 8.85×10^{-12} $C^2J^{-1}m^-1$) and ε_r (= 80) are the vacuum permittivity and the relative dielectric constant of the solvent, respectively; F (= 9.65×10^4C/mol) is the Faraday constant; c_b the bulk concentration of ions (mol/m^3); R (= 8.314 J/mol·K) the universal gas constant; T the absolute temperature (taken as 298K in the present study).

The boundary conditions of eq.(1) are given by

$$(\nabla\varphi\cdot n)|_{r=a} = -\frac{\sigma}{\varepsilon_r\varepsilon_0} \tag{2}$$

$$\varphi|_{r=\infty} = \left.\frac{d\varphi}{dr}\right|_{r=\infty} = 0 \tag{3}$$

where n is the unit exterior normal vector of the surface of the CS-GAG; σ is the surface charge density of a CS-GAG chain and can be written as

$$\sigma = \frac{-e}{2\pi ab} \tag{4}$$

where e(= 1.6×10^{-19} C) is the electronic charge; a(= 0.55 nm) the radius of the CS-GAGs and b(= 0.64 nm) the intercharge distance.

Using the cylindrical coordinates, we can separately write the Poisson-Boltzmann equation, eq.(1), the boundary conditions, eqs.(2) and (3), as

$$\frac{d^2y}{dr^2} + \frac{1}{r}\frac{dy}{dr} = \kappa^2\sinh(y) \tag{5}$$

and

$$\left.\frac{dy}{dr}\right|_{r=a} = -\frac{F\sigma}{\varepsilon_r\varepsilon_0 RT} \tag{6}$$

$$y|_{r=\infty} = \left.\frac{dy}{dr}\right|_{r=\infty} = 0 \tag{7}$$

where y is a scaled potential and expressed as

$$y = \frac{F\varphi}{RT} \tag{8}$$

and $\kappa^{-1}$is the Debye length of the solution and defined by

$$\kappa^2 = \frac{2F^2 c_b}{\varepsilon_r\varepsilon_0 RT} \tag{9}$$

The PB eq.(1) or (5) describes the mean effect of electrostatic potential. In the PB equation, the solvent is approximated as an incompressible fluid dielectric with a relative dielectric constant, ε_r, the potential of the mean force on an arbitrary ion is equated with the electrostatic potential. The mean field, point ion approximation inherent to PB theory has been verified to be valid, provided the ion size does not exceed the Debye length of the solution, eq.(9) [McLaughlin, S. (1989)].

2.2 Solution of the PB equation

In general, there is no known general analytic solution to PB eq.(5), and numerical solutions can be divergent in the nonlinear region, even with very small steps [Gur et al. (1978); McCaskill and Fackerell, (1988)]. Under some special boundary conditions, a few asymptotic solutions of the nonlinear PB eq.(5) on charged rodlike biopolymers immersed in a salt solution have been presented recently. For example, Shkel et al. (2000, 2002) gave an asymptotic solution for eq.(5) under the condition: $(\kappa a)^{-1} \leq 1$, and then they obtained another asymptotic solution in the range of electrolytic concentration, c_b, is 0.01∼0.1 M. However, the conditions required in articular cartilage are $(\kappa a)^{-1} \sim 1.44$ and the physiological electrolyte concentration $\sim$ 0.15 M. Therefore, we employ an approximate asymptotic solution of the nonlinear PB equation presented by Ohshima (1998) to determine the electrostatic potential of individual CS-GAG chain.

Under the boundary conditions, eqs.(6) and (7), an asymptotic solution of eq.(5) can be written as

$$y(c) = 2\ln\left\{\frac{[1+(1+\beta)Yc/8][1+(1-\beta)Yc/8]}{[1-(1+\beta)Yc/8][1-(1-\beta)Yc/8]}\right\} \tag{10}$$

where

$$\beta = \frac{K_0(\kappa a)}{K_1(\kappa a)} \tag{11}$$

and

$$c = \frac{K_0(\kappa r)}{K_0(\kappa a)} \tag{12}$$

and Y is the effective surface potential of the cylindrical molecules and given as

$$Y = \frac{8\tanh(y_s/4)}{1+[1-(1-\beta^2)\tanh^2(y_s/4)]^{1/2}} \tag{13}$$

In the above equations, $K_n(x)$ denotes the modified Bessel function of the second kind of order n; and $y_s = y|_{r=a}$ is the scaled surface potential.

Further, using the boundary condition (6), we obtain the relationship between the surface charge density and surface potential for cylindrical CS-GAG chains in an electrolyte solution to be

$$\frac{\sigma}{\varepsilon_r\varepsilon_0} = \frac{2\kappa RT}{F}\sinh\left(\frac{y_s}{2}\right)\left[1+\left(\frac{1}{\beta^2}-1\right)\frac{1}{\cosh(y_s/4)}\right]^{\frac{1}{2}} \tag{14}$$

Eq.(10) gives a approximate asymptotic solution of the cylindrical PB equation, i.e. the electrostatic potential of the CS-GAG chain in the solution. In addition, The comparison between the results of eq.(10) and the exact numerical results of eq.(5) indicates that the relative error is less than 1% for $(\kappa a)^{-1} \sim 1$ [Ohshima, H. (1998)].

2.3 Interactions between CS-GAGs

The conformation of the aggrecan in vivo is modelled as a three-dimensional bottle brush, in which the average length of each of the CS-GAGs is

about 35nm and the minimum separation distance between two neighboring CS-GAGs on the core protein is approximately 2∼4nm [Ng et al. (2003); Seog et al. (2002)], so that two arbitrary CS-GAG molecular chains on the core protein, in general, are not in the same plane, as shown in Fig.1c. We readily estimate the average separation distance between the two neighboring CS-GAGs to be roughly 18.5 $\sim$ 19.6 nm. Obviously, it is much greater than the average radius of the CS-GAGs, a= 0.55 nm. Furthermore, under the normal physiological conditions of articular cartilage (c_b = 0.15 M), the Debye length of the solution, κ^{-1}, is about 0.79 nm and much less than the minimum separation distance between two neighboring CS-GAGs. Therefore, we can employ the methods presented by Brenner and Parsegian (1974) and Ohshima (1998) to obtain the electrostatic interactions between two arbitrary neighboring CS-GAG molecular chains in the bottle brush conformation of aggrecan.

For two neighboring and skewed CS-GAG chains of minimum interaxial separation, l, mutual angle of rotation from parallel configuration,θ, and the same surface potentialφ_s, as shown in Fig.1c, the interaction potential between the two chains is written by

$$V(l,\theta) = \frac{\pi^2 \varepsilon_r \varepsilon_0}{\kappa} \left(\frac{RT}{F}\right)^2 \frac{Y^2}{K_0^2(\kappa a)} \frac{e^{-\kappa l}}{\sin\theta} \qquad (15)$$

where Y is the effective surface potential of a rodlike molecule and determined by eq.(13). In eq.(15), we have assumed that all the CS-GAG molecular chains on core protein have the same surface charge density and geometrical conformation, and we have also considered each of the CS-GAG molecular chains as a radial eradiated from the core protein. Note that the separation distance, $l = r - 2a$, between two neighboring CS-GAG molecular chains is taken on the average as 2 nm $\leq l \leq$ 4 nm. However, it is not taken as less than $2a$= 1.1 nm from the geometrical structure of the bottle brush conformation of aggrecan.

According to eq.(15), the electrostatic force between the two chains is given by

$$f(l,\theta) = -\frac{\partial V(l,\theta)}{\partial l} = \pi^2 \varepsilon_r \varepsilon_0 \left(\frac{RT}{F}\right)^2 \frac{Y^2}{K_0^2(\kappa a)} \frac{e^{-\kappa l}}{\sin\theta} \qquad (16)$$

and the torque between the two chains is read as

$$\tau(l,\theta) = -\frac{\partial V(l,\theta)}{\partial \theta} = \frac{\pi^2 \varepsilon_r \varepsilon_0}{\kappa} \left(\frac{RT}{F}\right)^2 \frac{Y^2}{K_0^2(\kappa a)} \frac{\cos\theta}{\sin^2\theta} e^{-\kappa l} \qquad (17)$$

Employing eqs.(15) $\sim$ (17), we can readily obtain the electrostatic interactions between two arbitrary CS-GAG molecular chains in the aggrecan.

3 Results and Discussion

Eqs.(10)∼(14) give the scaled potential produced by each of the CS-GAG molecular chains in the bottle brush conformation of aggrecan. Substituting these equations into eq.(8), we can write the electrical potential of each of the CS-GAGs. Our study indicated that the electrical potential is not only determined predominately by the distance from the surface of the CS-GAG molecular chain, but also associated intimately with the saline concentration in the electrolyte solution within articular cartilage, as shown in Fig.2. This figure shows that the electrical potential rapidly limits to zero with increasing the distance and the saline concentration.

Fig.3 shows the graphs of the electrostatic interactions between two arbitrary neighboring CS-GAG molecular chains under the normal physiological condition of articular cartilage, which are separately defined by eqs.(15), (16) and (17) as purely exponential in the separation distance and decease with increasing the mutual angle. Because all the CS-GAGs have been assumed to hold the same distribution and sign of charge, eq.(17) shows that the torque acts to twist the molecular chains away from the parallel orientation toward a perpendicular configuration, i.e. repulsion tends to minimize contact between the molecular chains.

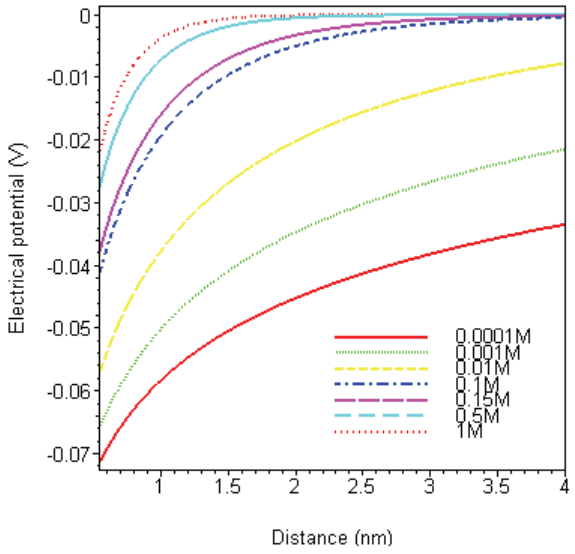


Figure 2: Graphs showing the relations between the electrical potential yielded by a CS-GAG molecular chain and the distance from the surface of the chain under the different saline concentrations in the solution.

Similar to the electrical potential, the electrostatic interactions between the CS-GAGs are also intimately associated with the saline concentration in the electrolyte solution. For the sake of simplicity in the following discussion, we only use eq.(16) to analyze the relations between the electrostatic interaction force and the saline concentration in the electrolyte solution, together with the separation distance and mutual angle. However, the relations between either the interaction potential or the interaction torque and the saline concentration can be similarly discussed via the same method.

First of all, at an arbitrary mutual angle, for example, $\theta = 45°$, we obtain the relations among the interaction force, the separation distance and the saline concentration as shown in Fig.4. This figure indicates that in the range of the minimum separation distance between two neighboring CS-GAG molecular chains, $2 \sim 4$ nm, if the saline concentration is not less than about 0.1 M, the interaction force between the molecular chains will monotonically increase with decreasing the saline concentration, however, if the saline concentration is less than the concentration, the relation between the interaction force and the saline con-

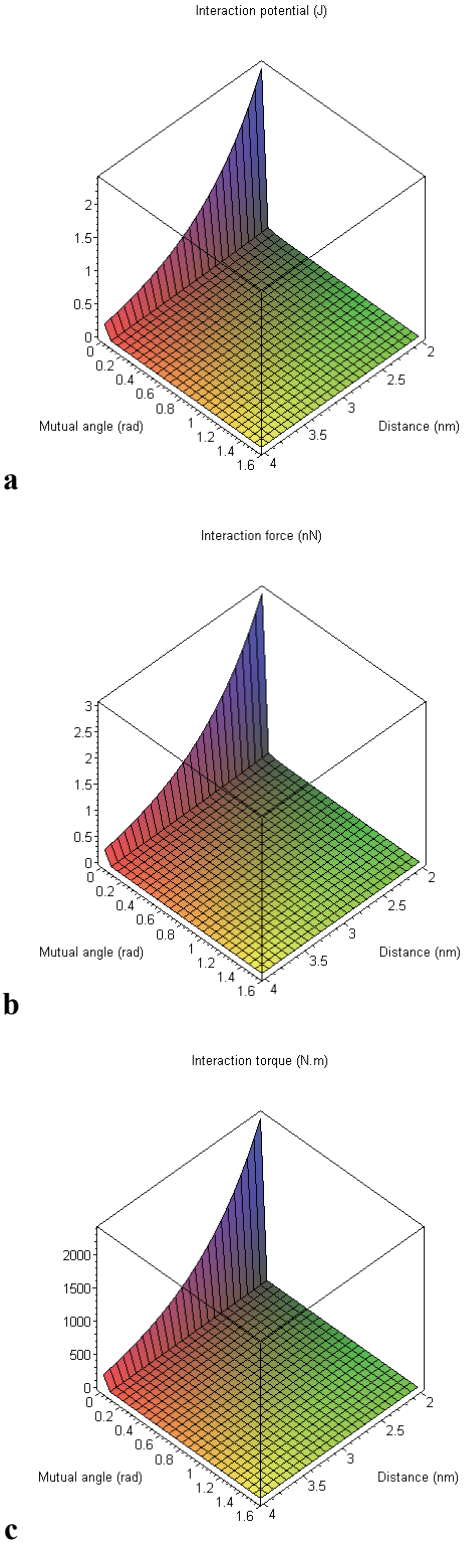


Figure 3: Graphs of eqs.(15), (16) and (17) showing the relations between the interactions and the variables including the separation distance and the mutual angle, **a.** interaction potential, **b.** interaction force and **c.** interaction torque, respectively.

centration will fail to be a monotonic response. In terms of the saline concentration greater than 0.0001 M, when the separation distance is greater than about 5.2 nm, the interaction force between the CS-GAGs will monotonically increase with decreasing the saline concentration. This result is in agreement with the relevant conclusion given previously by Seog et al. (2002) and Dean et al. (2003). Secondly, at a fixed separation distance, for example, l = 2 nm, we show the relations between the interaction force and the saline concentration as in Fig.5. Obviously, the same result is given. In fact, under the different saline concentrations, mutual angles and separation distances, all of our analyses indicated that the present results are true.

Note that all the electrostatic interactions seem to diverge as the mutual angle goes to zero. It is because the CS-GAGs are assumed to be infinite length during solving the PB equation [Brenner and Parsegian, (1974)]. However, when two neighboring CS-GAG molecular chains are parallel to each other, the electrical double layer interaction potential between the two chains per unit length is written as [Ohshima, H. (1998)]

$$\overline{V}(l) = \pi\varepsilon_r\varepsilon_0\left(\frac{RT}{F}\right)^2 Y^2 \frac{K_0(\kappa l)}{K_0^2(\kappa a)} \tag{18}$$

The relevant interaction force between the two molecular chains per unit length at the separation is given by

$$\overline{f}(l) = \pi\varepsilon_r\varepsilon_0\kappa\left(\frac{RT}{F}\right)^2 Y^2 \frac{K_1(\kappa l)}{K_0^2(\kappa a)} \tag{19}$$

Both eqs.(18) and (19) indicate that the interactions between the neighboring parallel CS-GAG chains are monotonicaly decrease with increasing the separation distance under normal physiological conditions, as shown in Fig.6.

If we define $\overline{p}(l) = \overline{f}(l)/2\pi a$ to be approximately the applied pressure per unit area on each of the CS-GAG molecular chains, when the separation distance is taken as 2∼4 nm, we calculate the average pressure on the range of the distances to be about 136 kPa under normal physiological conditions. It is in good agreement with the conclusion

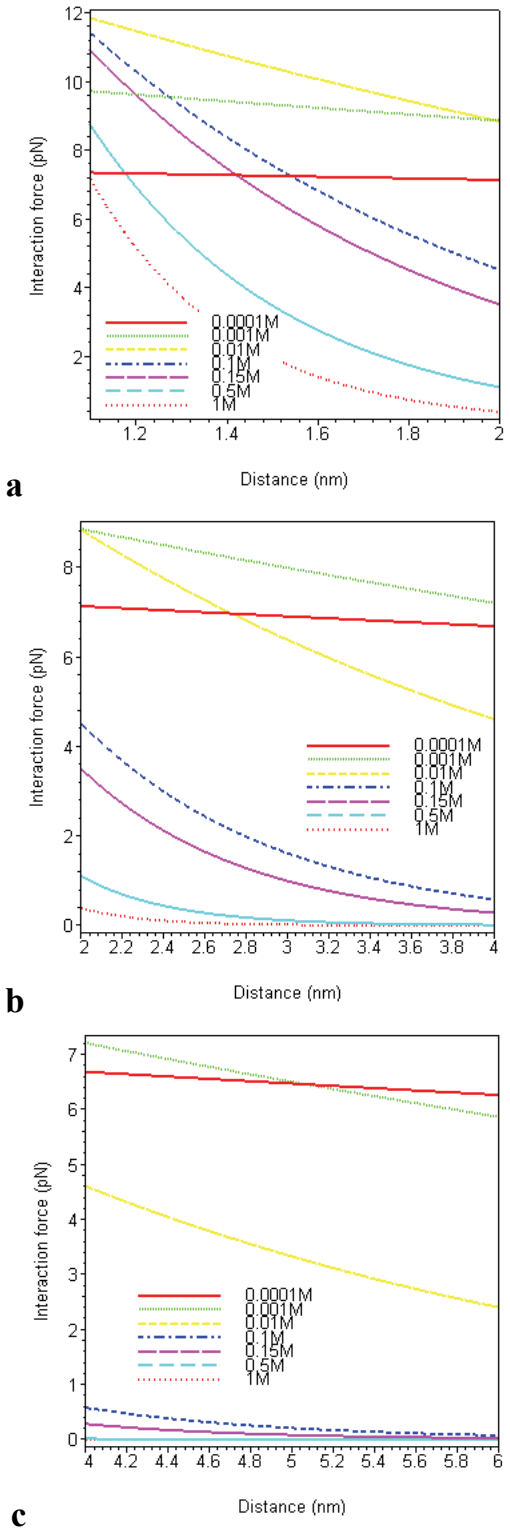
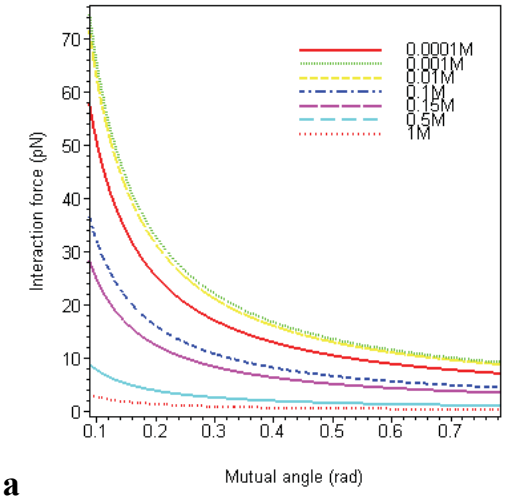


Figure 4: showing the relations between the interaction force and the separation distance under a fixed mutual angle (45°) and different saline concentrations. **a.** 1.1nm $\leq l \leq$ 2nm; **b.** 2nm $\leq l \leq$ 4nm; and **c.** 4nm $\leq l \leq$ 6nm.



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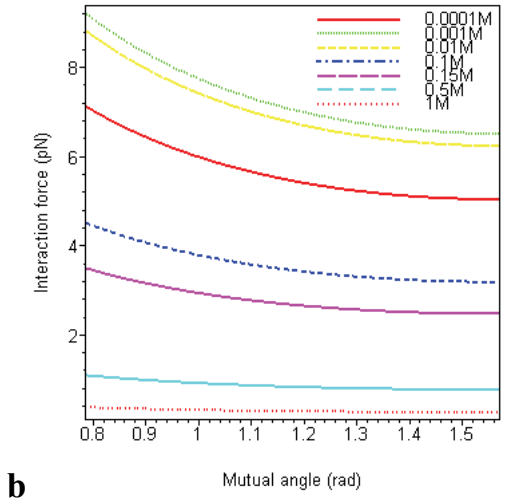

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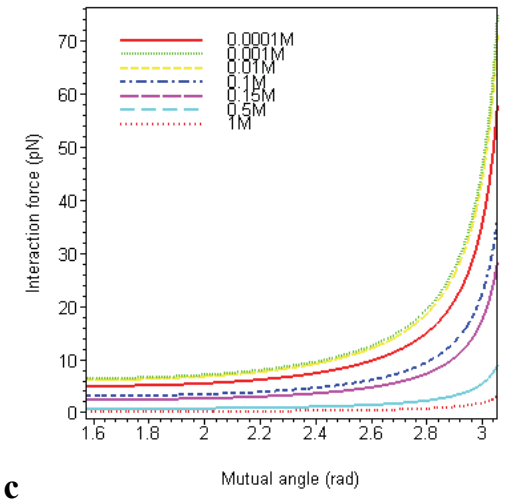

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Figure 5: showing the relations between the interaction force and the mutual angle under a fixed separation distance (2nm) and the different saline concentrations. **a.** $5° \leq \theta \leq 45°$; **b.** $45° \leq \theta \leq 90°$; and **c.** $90° \leq \theta \leq 175°$.

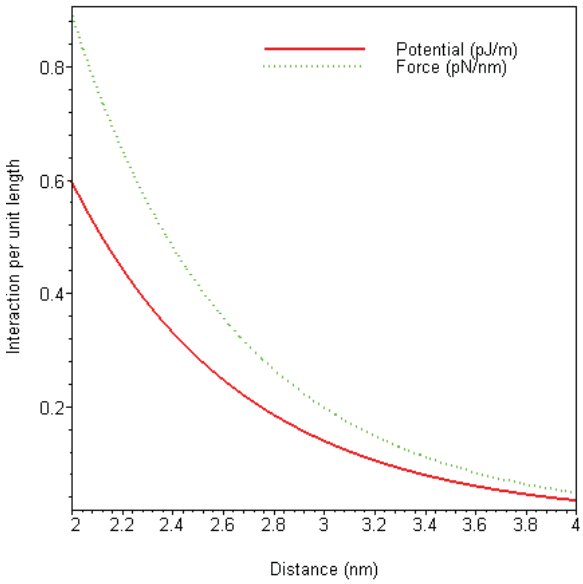
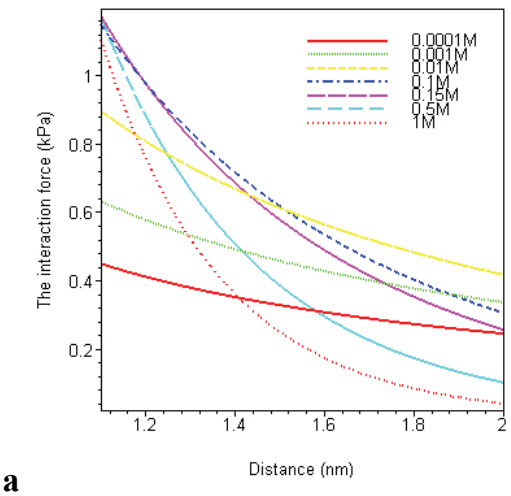


Figure 6: showing relations between the interactions per unit length and the separation distance when two CS-GAGs are parallel to each other.

given by Buschmann and Grodzinsky (1995) and Song et al. (2005). Further, the analyses on the relations among the interaction, the separation and the saline concentration give the same results as the stated above again, as shown in Fig.7.

In addition, relevant analyses demonstrate that if we approximately consider two CS-GAG molecular chains to be parallel to each other when the mutual angle between the two molecular chains is less than 5° in the bottle brush conformation of aggrecan, and we define the average value of eq.(16) in the mutual angle of 4°∼5° as the interaction force that two arbitrary molecular chains yielded when the mutual angle between them is in 0°∼5°, then eqs.(16) and (19) are roughly equivalent as long as the length of each of the CS-GAG molecular chains was taken to be $L = 35$nm. For example, when the fixed separation distance is $l = 2$ nm, eq.(16) gives the average interaction force in the mutual angle of 4°∼5° to be about 31.6pN while eq.(19) yields the interaction force to be about 31.2 pN. Also, we define $p(l,\theta) = f(l,\theta)/2\pi aL$ as roughly the pressure per unit area applied on each of the CS-GAG molecular chains. According to the discussed here, if we denote



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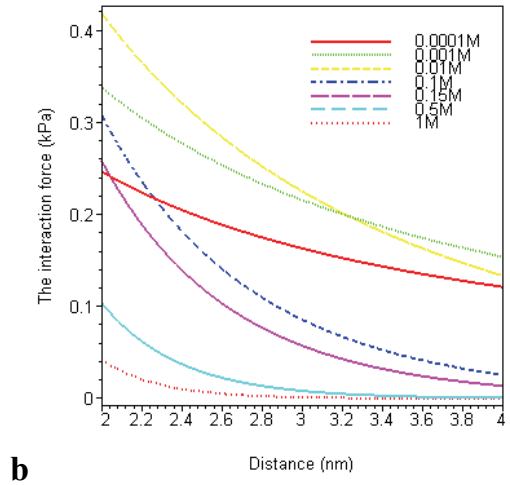

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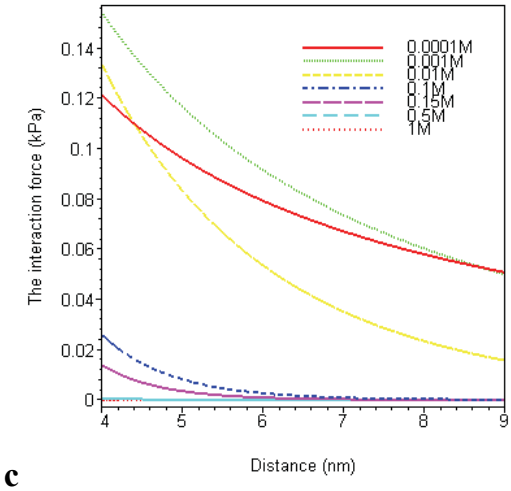

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Figure 7: showing the relations between the interaction pressure and the separation distance under different saline concentrations when the CS-GAGs are parallel to each other. **a.** 1.1nm $\leq l \leq$ 2nm; **b.** 2nm $\leq l \leq$ 4nm; and **c.** 4nm $\leq l \leq$ 9nm.

$p(l,\overline{\theta})$ to be the average value of $p(l,\theta)$on the range of $4° \leq \theta \leq 5°$, then we have $p(l,\overline{\theta}) \approx \overline{p}(l)$ when the mutual angle is $0° \leq \theta \leq 5°$. Such, the two equations, eqs.(16) and (19), have been unified.

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