Electrostatic Interactions Between Glycosaminoglycan Molecules *

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The electrostatic interactions between nearest-neighbouring chondroitin sulfate glycosaminoglycan (CS-GAG) molecular chains are obtained on the bottle brush conformation of proteoglycan aggrecan based on an asymptotic solution of the Poisson-Boltzmann equation the CS-GAGs satisfy under the physiological conditions of articular cartilage. The present results show that the interactions are associated intimately with the minimum separation distance and mutual angle between the molecular chains themselves. Further analysis indicates that the electrostatic interactions are not only expressed to be purely exponential in separation distance and decrease with the increasing mutual angle but also dependent sensitively on the saline concentration in the electrolyte solution within the tissue, which is in agreement with the existed relevant conclusions.

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Glycosaminoglycans (GAGs), which are the most abundant heteropolysaccharides in the body, play an important role in the physiological and physical functions of biological tissues. In particular, the chondroitin sulfate glycosaminoglycans (CS-GAGs) of the proteoglycan aggrecan contain one negatively charged carboxylate and sulfate group per disaccharide that is completely ionized under normal physiological conditions, as shown in Figs. 1(a) and 1(b). Therefore, they have high negative charge density in physiological solution of articular cartilage. Based on the electrostatic repulsive effects, CS-GAGs have the tendency of extension and rod-like conformation rather than random coil in articular cartilage in vivo.[1−3] The previous studies demonstrated that the electrostatic repulsive effect existed between CS-GAGs that was responsible for 50–75% of the equilibrium compressive resistance of articular cartilage.[2,4−10] However, the aggrecan in vivo is the three-dimensional structure that is known as a bottle brush conformation, in which the CS-GAG and keratan sulfate glycosaminoglycan (KS-GAG) molecular chains are extended as much as possible from the core protein to minimize interactions between negative charges.[1,11−13] Therefore, two arbitrary nearest-neighbouring polysaccharide chains in the aggrecan, each of which radiates away from the core protein, in general, are not in the same geometrical plane, as shown in Fig. 1(c). It is the three-dimensional conformation that brings on a lot of difficulties to study both the interactions between CS-GAGs and the biological functions of aggrecan itself.

In the study of the GAGs of articular cartilage on the molecular level, Buschmann and Grodzinsky[4] and Jin and Grodzinsky[14] used the Poisson–Boltzmann (PB) cell model to investigate the swelling pressure and the shear modulus of articular cartilage. Their results showed that the swelling pressure of proteoglycan solution increases with the increasing solute concentration. Seog *et al.*[2] and Dean *et al.*[15] 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. Then they indicated that the interaction force between the GAG brush layer and the probe tip decreases with the increasing saline concentration in the solution. However, the real 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, still remain unclear. In this Letter, we focus on the intermolecular electrostatic interactions between the nearest-neighbouring 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 to the core protein can be approximated as a cylindrical rod having a surface charge density and a fixed radius. By solving the PB equation that the molecular cylinders satisfy in the physiological solution, we firstly obtain the electrical potential of the CS-GAGs, and then obtain the interactions between the molecular cylinders including the interaction potential, force and torque. Further analyses indicate that the presented results are in good agreement with the existed relevant conclusions.

Each of the GAG molecules can be modelled as locally rigid even though its global structure is

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flexible,[16,17] 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 nearest-neighbouring CS-GAGs can be modelled on the average by employing the PB equation.[2,4,14,15] 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^-.[1−10] Therefore, the PB equation satisfied by an individual CS-GAG chain is written as[2,4,14,15]

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

where φ is the electrical potential derived from the charged surface of the CS-GAG chain in the electrolyte solution; ε_0 ($= 8.85 \times 10^{-12}\mathrm{C^2J^{-1}m^{-1}}$) and ε_r (=80) are the vacuum permittivity and the relative dielectric constant of the solvent, respectively; F ($= 9.65 \times 10^4\mathrm{C/mol}$) is the Faraday constant; c_b is the bulk concentration of ions ($\mathrm{mol/m^3}$); R ($= 8.314\,\mathrm{J/mol{\cdot}K}$) is the universal gas constant; T is the absolute temperature (taken as 298 K in the present study). In this equation, the solvent is approximated as an incompressible fluid dielectric with a relative dielectric constant, ε_r is the potential of the mean force on an arbitrary ion and is equated with the electrostatic potential.

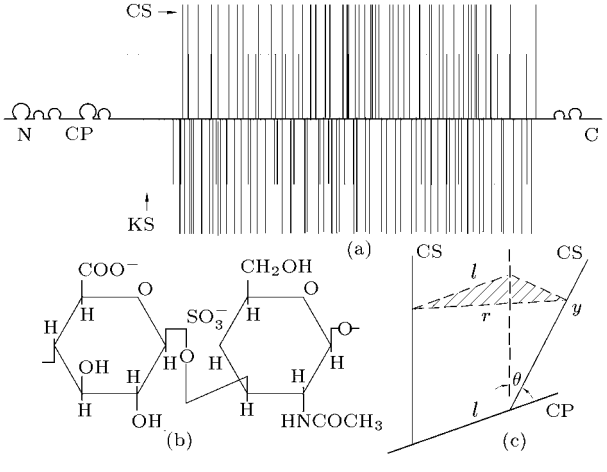

Fig. 1. (a) The planar model of aggrecan: glycosaminoglycans are separately indicated by solid line (chondroitin sulfate, CS) and wavy line (keratan sulfate, KS), and NH_2 and COOH ends of core protein (CP) are denoted by N and C, respectively. (b) Chemical structure of the disaccharide repeating unit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

Using the cylindrical coordinates, we can rewrite Eq. (1) as

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

and the corresponding boundary conditions are given by

$$\left.\frac{dy}{dr}\right|_{r=a} = -\frac{F\sigma}{\varepsilon_r\varepsilon_0 RT}, \tag{3}$$

$$y|_{r=\infty} = \left.\frac{dy}{dr}\right|_{r=\infty} = 0, \tag{4}$$

where $\sigma = -e/2\pi ab$ is the surface charge density of a CS-GAG chain; e ($= 1.6 \times 10^{-19}\mathrm{C}$) is the electronic charge; a ($= 0.55\,\mathrm{nm}$) is the radius of the CS-GAGs; b ($= 0.64\,\mathrm{nm}$) is the intercharge distance;[4] $y = F\varphi/RT$ is a scaled potential; and κ^{-1} is the Debye length of the solution and defined by

$$\kappa^2 = \frac{2F^2c_b}{\varepsilon_r\varepsilon_0 RT}. \tag{5}$$

We employ an approximate asymptotic solution of the nonlinear PB equation presented by Ohshima[18] to determine the electrostatic potential of the individual CS-GAG chain. Under the boundary conditions, an asymptotic solution of Eq. (2) can be written as

$$y(c) = 2\ln\left\{\frac{[1+(1+\beta)Y_c/8][1+(1-\beta)Yc/8]}{[1-(1+\beta)Yc/8][1-(1-\beta)Yc/8]}\right\}, \tag{6}$$

where

$$\beta = \frac{K_0(\kappa a)}{K_1(\kappa a)}, \quad c = \frac{K_0(\kappa r)}{K_0(\kappa a)}, \tag{7}$$

Y is the effective surface potential of the cylindrical molecules,

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

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. Using Eq. (3), we obtain the relationship between the surface charge density and surface potential for the cylindrical CS-GAG chains in an electrolyte solution,

$$\frac{\sigma}{\varepsilon_r\varepsilon_0} = \frac{2\kappa RT}{F}\sinh\left(\frac{y_s}{2}\right)\left[1+\left(\frac{1}{\beta^2}-1\right)\frac{1}{\cosh(y_s/4)}\right]^{1/2}. \tag{9}$$

Equation (6) gives the electrostatic potential of a CS-GAG chain in the solution. Note that in the present study, we have $(\kappa a)^{-1} \sim 1.4$. However, the comparison between the result of Eq. (6) and the exact numerical result of Eq. (2) indicates that the relative error is less than 1% for $(\kappa a)^{-1} \sim 1$.[18]

In the bottle brush conformation of the aggrecan, the average length of each CS-GAG is about $L = 35\,\mathrm{nm}$ and the minimum interaxial separation distance between any two nearest-neighbouring

CS-GAGs on the core protein is approximately 2–4 nm.[2,3] We roughly estimate the average separation distance between two nearest-neighbouring CS-GAGs to be about 18.5–19.6 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 is less than the minimum separation distance between two nearest-neighbouring CS-GAGs. Therefore, we can employ the methods presented by Brenner and Parsegian[19] to obtain the electrostatic interactions between two arbitrary nearest-neighbouring CS-GAG molecular chains.

For two nearest-neighbouring and skewed CS-GAG chains of minimum interaxial separation l, the mutual angle θ of rotation from parallel configuration is shown in Fig. 1(c), and the same surface potential φ_s, i.e. the interaction potential between the two chains per unit area, can be written as[18,19]

$$V(l,\theta) = \frac{\pi^2 \varepsilon_r \varepsilon_0}{2\pi a L \kappa} \left(\frac{RT}{F}\right)^2 \frac{Y^2}{K_0^2(\kappa a)} \frac{e^{-\kappa l}}{\sin\theta}, \tag{10}$$

where Y is the effective surface potential of a rod-like molecule and determined by Eq. (8). In Eq. (10), we have assumed that all the CS-GAGs have the same surface charge density and geometrical conformation, and we have deemed that each of the CS-GAG molecular chains is a radial eradiated from the core protein and the interaxial separation distance between two chains is 2 nm $\leq l \leq$ 4 nm on the core protein.

According to Eq. (10), the electrostatic force and torque between two chains per unit area are separately given by

$$\begin{aligned} p(l,\theta) &= -\frac{\partial V(l,\theta)}{\partial l} \\ &= \frac{\pi^2 \varepsilon_r \varepsilon_0}{2\pi a L} \left(\frac{RT}{F}\right)^2 \frac{Y^2}{K_0^2(\kappa a)} \frac{e^{-\kappa l}}{\sin\theta}, \end{aligned} \tag{11}$$

$$\begin{aligned} \tau(l,\theta) &= -\frac{\partial V(l,\theta)}{\partial \theta} \\ &= \frac{\pi^2 \varepsilon_r \varepsilon_0}{2\pi a L \kappa} \left(\frac{RT}{F}\right)^2 \frac{Y^2}{K_0^2(\kappa a)} \frac{\cos\theta}{\sin^2\theta} e^{-\kappa l}. \end{aligned} \tag{12}$$

Equations (10)–(12) show that the electrostatic interactions are purely exponential in the separation distance and decease with the increasing mutual angle, for example, Eq. (11) is illustrated in Fig. 2(a). In particular, because all the CS-GAGs have been assumed to hold the same distribution and sign of charge, Eq. (12) indicates 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. Note that when the mutual angle between two arbitrary molecular chains is more than 90°, the interactions between the two chains, in general, are not important on the bottle brush conformation of aggrecan.

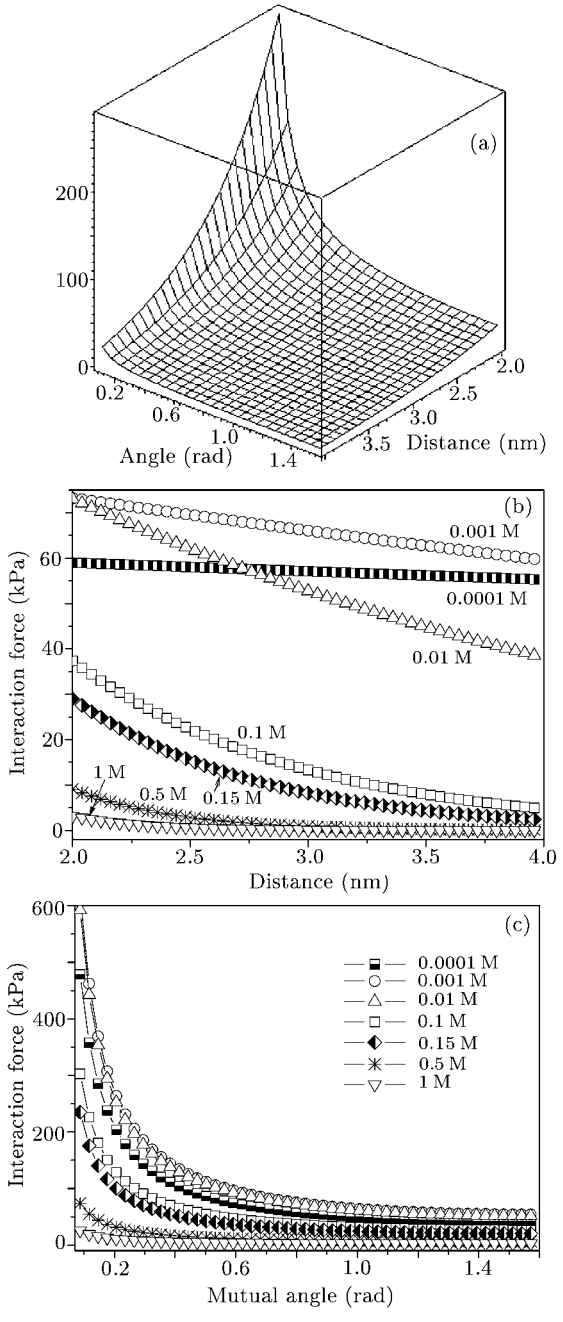

Fig. 2. (a) Illustration of Eq. (11) showing the relations among the interaction force (kPa), the vertical axis, and the variables including the separation distance (2 nm $\leq l \leq$ 4 nm) and the mutual angle (5° $\leq \theta \leq$ 90°). (b) Relations between the interaction force and the separation distance, 2 nm $\leq l \leq$ 4 nm, under a fixed mutual angle ($\theta = 45°$) and different saline concentrations. (c) Relations between the interaction force and the mutual angle, 5° $\leq \theta \leq$ 90°, under a fixed separation distance ($l = 2$ nm) and the different saline concentrations.

For the sake of simplicity, here we only discuss the

relations between the electrostatic interaction force and the saline concentration of solution. However, similar discussions can be readily given between either the potential or the torque and the saline concentration. Firstly, at an arbitrary given mutual angle, we obtain the relations among the interaction force, the separation distance and the saline concentration, as shown in Fig. 2(b). This figure indicates that in the range of the separation distance 2–4 nm, if the saline concentration is not less than about 0.001 M, the interaction force will monotonically increases with the decreasing saline concentration. However, if the saline concentration is less than the concentration, the relation between the interaction force and the saline concentration will fail to be a monotonic response. This result is in agreement with the relevant conclusion given previously by Seog *et al.*[2] and Dean *et al.*[15] Secondly, at an arbitrary given separation distance, we show the relations between the interaction force and the saline concentration in Fig. 2(c). Obviously, the same results stated above will be obtained again.

From Eqs. (10)–(12), all the electrostatic interactions seem to diverge when the mutual angle is close to zero, which is due to the reason that the CS-GAGs are assumed to be infinite length during solving the PB equation.[19] In fact, it could been hardly observed that two nearest-neighbouring CS-GAG molecular chains are exactly parallel to each other on the bottle brush conformation of aggrecan.[3] Therefore, we approximately consider two CS-GAG molecular chains to be parallel to each other when the mutual angle between them is less than 5°, and we define the average value of Eq. (11) in the mutual angle of 4°–5° as the interaction force that two arbitrary molecular chains will be yielded if their mutual angle is in 0°–5°. When the minimum separation distance is taken to be in the range 2–4 nm, we calculate the average interaction force on the range of the angle and distance to be about 136 kPa under normal physiological conditions (0.15 M). It is in good agreement with the results about the CS-GAGs parallel to each other.[2,4]

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