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The importance of polysaccharide configurational entropy in determining the osmotic swelling pressure of concentrated proteoglycan solution and the bulk compressive modulus of articular cartilage

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Abstract

One important contribution to the osmotic swelling pressure of concentrated proteoglycan and hence the elasticity of articular cartilage arises from the configurational entropy of the polysaccharide chains in the extracellular matrix. The work presented here provides a theoretical determination of this entropy and an analysis of its effect on the equilibrium osmotic swelling pressure of concentrated proteoglycan solutions. This effect is calculated in a manner similar to the Flory–Huggins technique where the solution is treated as a lattice (P. Flory, Principles of polymer chemistry (Cornell Univ. Press, Ithaca, 1953); J. Chem. Phys. 12 (1944) 425). In addition, the charge-related contribution to the elasticity of these materials is reviewed in the form of a Donnan equilibrium model (T. Hill, Faraday Soc. Discussions 21 (1956) 31; A.G. Ogston and J.D. Wells, Biochem. J. 119 (1970) 67; C. Tanford, Physical Chemistry of Macromolecules (Wiley, New York, 1961)). It is found that the configurational entropy of the glycosaminoglycan (GAG) chain polysaccharides together with the charge effects reproduce the equilibrium swelling pressure of concentrated proteoglycan solutions as experimentally determined by J.P.G. Urban et al., Biorheol. 16 (1979) 447. In addition this theoretical model is manifestly independent of the proteoglycan molecular weight, consistent with prior experimental findings (J.P.G. Urban et al., Biorheol. 16 (1979) 447). The model is also extended to include polydispersity of proteoglycan size and to predict the equilibrium bulk compressive modulus of articular cartilage. This work represents the first comprehensive theoretical description of the equilibrium elastic properties of proteoglycan solutions. It establishes the important role for polysaccharide configurational entropy in determining the equilibrium elastic properties of proteoglycan solutions, and serves to conceptually link their molecular structure with their important mechanical properties.

Keywords: Proteoglycan; Polysaccharide; Swelling pressure; Configurational entropy; Volume exclusion; Mean field theory

1. Introduction

Most of the theoretical work on proteoglycan and cartilage mechanics has focused on dynamic properties such as poro-elastic creep, electromechanical transduction ion induced swelling, and shear viscosity, [1–12]. The equilibrium stress-strain behavior of cartilage has been the subject of some recent work in which the important role of thermodynamics was recognized and explored [13]. In that work however,

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the equilibrium properties of cartilage were explained in terms of an empirical free energy whose significance and form were not clearly related to the internal structure and underlying molecular interactions. Collectively, these efforts provide significant insight into the physical properties of concentrated proteoglycan and cartilage. All of this work, however, depends on macroscopic parameters which do not incorporate the molecular architecture of extracellular matrix.

An exception is a recent study by Bushmann which incorporated tissue ultrastructure into the description of charge interaction in cartilage [30]. Bushmann treated the glycosaminoglycan (GAG) chains in cartilage as charged parallel cylinders, and using this model, predicted the contribution to the bulk longitudinal compressive modulus which arises from charge interaction. This was accomplished by means of a Poisson–Boltzmann-type model [14–16]. There have been no attempts to quantitatively relate the charge-independent component of the swelling pressure of proteoglycan or the bulk longitudinal modulus of cartilage to tissue ultrastructure, although the importance of "thermal motion of matrix macromolecular segments" and "excluded volume effects" has been recognized [17,18].

The equilibrium osmotic swelling pressure of concentrated proteoglycan solution and cartilage has been the subject of extensive experimental work [17,18, 32]. In Urban and Maroudas' work the majority of the swelling pressure of proteoglycan is explained in terms of charge interactions by means of a Donnan equilibrium theory [19–21]. The existence of entropic or volume exclusion effects are recognized and their magnitude extrapolated to be that component of the swelling pressure which can not be explained in terms of the charge effects [18]. To date there is, however, no precise theoretical explanation of the nature and magnitude of these effects.

In this work, the charge independent contribution to the equilibrium swelling pressure of concentrated proteoglycan solution is explained in terms of the configurational entropy of the GAG chains. Changes in the concentration of proteoglycan solution result in changes in configurational entropy of the material. These entropic changes give rise to a volume dependent entropic term in the free energy, and a swelling pressure which can be calculated.

The vast majority of configurational entropy associated with the extracellular cartilage matrix resides in the terminal GAG chains. The entropy of mixing associated with the aggrecans is minimal due to their large molecular weight, while the hyaluronic acid (HA) backbones and core proteins are vastly outnumbered by the terminal GAGs. In cartilage the entropic contribution from the collagen fibrils is minimal due to their large size and correspondingly relatively small number density when compared to proteoglycan chains.

The configurational entropy is calculated using a lattice model of the extracellular matrix [22]. Volume exclusion effects are included through the use of a mean field theory approach [22–24]. The associated swelling pressure is found to be independent of the coordination number of the lattice. The charge-dependent contribution to the elasticity will be explained in terms of a Donnan equilibrium model [19–21]. The charge-dependent and charge-independent contributions taken together accurately reproduce the available experimental data with no free parameters. The result is manifestly independent of the molecular weight of the aggrecan, as has been experimentally noted to be the case [18].

2. Charge effects

The Donnan equilibrium method can be used to calculate the charge-dependent contribution to the swelling pressure [19–21]. This method has already been applied to concentrated proteoglycan, and for further detail regarding this method the reader is referred to Urban's paper [18,20].

The premise of this method is that in the bulk macromolecular and bath phases there must be charge neutrality, and that equilibrium is established across the boundary between these phases. We state the results of this theory. For its derivation and further explanation the reader is referred to the above mentioned original sources,

$$\Pi_{\mathrm{Don}} = RT\left\{\underline{\Phi}\left[Z^2 + 4m^2\left(\gamma^2/\underline{\gamma}^2\right)\right]^{1/2} - 2m\Phi\right\}, \tag{1}$$

where Π_{Don} is the Donnan contribution to the osmotic pressure, $\underline{\Phi}$ is the osmotic coefficient in the macromolecular phase, Φ is the osmotic coefficient

in the bath phase, m is the ionic strength of the bath, γ and $\underline{\gamma}$ are the activity coefficients in the bath and macromolecular phases respectively, R is the gas constant, and T the temperature.

At higher ionic strength, i.e. 1 M, Eq. (1) is well approximated by the more familiar result

$$\Pi_{\mathrm{Don}} = RT(Z^2/4m), \tag{2}$$

where Z is the fixed charge density. This can be written alternatively as

$$\Pi_{\mathrm{Don}} = \frac{c^2\nu^2 RT}{4mM_{\mathrm{w}}^2}, \tag{3}$$

where c is the concentration in mg/ml, ν is the charge per macromolecule, and other symbols are as previously defined. This can be reexpressed – the reasons for doing so will be evident later – by noting that the vast majority of charge and molecular weight arise from the terminal GAG chains [25]. That is $\nu/M_{\mathrm{w}} = \nu_0/M_0$, where ν_0 is the charge per monomeric subunit of the GAG chains and M_0 is the molecular weight per monomeric subunit of the GAG chains. This allows us to express the Donnan contribution in a molecular weight independent fashion, i.e.

$$\Pi_{\mathrm{Don}} = \frac{c^2\nu_0^2 RT}{4mM_0^2}. \tag{4}$$

The simplification of Eq. (1) occurs because at higher ionic strength the activity coefficients and osmotic coefficients do not significantly change at the boundary between macromolecular and bath phases. For the purpose of this work we will confine ourselves to the higher ionic strength regime where Eq. (4) can be used to good approximation. Although this does not represent the physiologic regime, it allows us to examine the entropic contribution to swelling pressure without introducing the complexity and error associated with approximating non-ideal solvent behavior.

3. Entropic contributions

The contribution to the swelling pressure which arises from the mixing entropy of the aggrecans is readily given by the standard result [26],

$$\Pi_{\mathrm{mix}} = RT(c/M_{\mathrm{w}}). \tag{5}$$

As will become evident, this result is vastly overshadowed by the pressure arising from the internal, configurational entropy of the GAG chains within the aggrecan. In order to determine the swelling pressure and modulus associated with the GAG configurations it is first necessary to calculate the configurational entropy of the GAG chains.

The problem of determining the configurational entropy of the terminal GAG chains differs somewhat from the problem in more traditional polymer systems. In the aggrecan, the structural unit of proteoglycan aggregate in cartilage, the proximal end of the GAG chain is attached to the core protein, which in turn is fastened to the HA backbone. One terminus of the terminal GAG chain is thus attached to an object of immense size on the molecular scale (MW $\approx 10^8$ dalton). As a result of this configuration, the proximal end of the GAG chain can be treated as fixed in space. The extracellular matrix will be treated as an ensemble of such GAG chains fixed at one end. A lattice model type approach, like that of Flory, is still applicable [22].

The number of possible configurations a polymeric GAG chain fixed at one end can adopt, Ω_i, is readily determined using a lattice model,

$$\Omega_i = z(z-1)^{n-1}, \tag{6}$$

z is the coordination number of the lattice and n is the number of subunits within the chain. As will be shown, the value of z does not enter into the swelling pressure.

Eq. (6) must be modified to include volume exclusion effects which result from adjacent GAG chains. Consistent with a mean field theory, the corrected version of Eq. (1) can be written approximately as

$$\Omega_i \approx z^n(1-\phi)^n, \tag{7}$$

where ϕ is the excluded volume fraction [22–24]. The corresponding entropy of a single such chain is obtained from the statistical definition of entropy in terms of the number of allowed states, $S = k \ln \Omega$.

$$S_{\mathrm{c}} = nk[\ln z + \ln(1-\phi)], \tag{8}$$

where k is Boltzmann's constant and other symbols are as previously defined. Because entropy is an extensive thermodynamic parameter, the configura-

tional entropy of an ensemble of such chains, as in an aggrecan, is simply

$$S_a = N_c nk[\ln\ z + \ln(1-\phi)], \tag{9}$$

where N_c is the number of chains in the aggrecan. Volume dependence of the entropy is introduced through the volume exclusion term, ϕ, which can be written as

$$\phi = \frac{V_0}{V}\phi_0, \tag{10}$$

where subscripts denote the initial state. Making use of Eq. (10), the entropy in a given volume of solution can be written with manifest volume dependence as

$$S = N_a N_c nk\{\ln\ z + \ln[1-\phi_0(V_0/V)]\}, \tag{11}$$

where N_a is the number of aggrecans within the volume.

The pressure exerted by such an ensemble of GAG chains can be readily determined from the Helmholtz free energy, F. The free energy can be broken into charge-dependent and charge-independent portions,

$$F = F_e + F_{ne}, \tag{12}$$

where F_e is charge dependent and F_{ne} is charge independent.

This partitioning of the free energy into charge dependent and charge independent portions allows for the pressure and bulk longitudinal modulus to also be subdivided along these lines. The pressure is determined by taking the volume derivative of the free energy [26],

$$\Pi = -\frac{\partial F}{\partial V} = -\frac{\partial F_e}{\partial V} - \frac{\partial F_{ne}}{\partial V}. \tag{13}$$

The charge-dependent component of the free energy in Eq. (12) will give rise to the charge-dependent component of the swelling pressure, to be approximated by the Donnan result. F_{ne} is taken to be due to the standard mixing entropy as well as the configurational entropy of the GAG chains, i.e. $F_{ne} = F_{mix} + F_{config}$. The mixing component gives rise to the well known result, Eq. (5). The configurational component can be written as

$$F_{config} = -TS_{config} = -N_a N_c nkT\{\ln\ z + \ln[1-\phi_0(V_0/V)]\}. \tag{14}$$

The pressure arising from the ensemble of GAG chains by virtue of changes in configurational entropy associated with volume changes is determined using Eq. (13),

$$\Pi_{config} = -\frac{\partial F_{config}}{\partial V} = \frac{N_a N_c nkT}{V_0}\left(\frac{\phi_0(V_0/V)^2}{1-\phi_0(V_0/V)}\right). \tag{15}$$

This can be reexpressed and simplified by noting that,

$$\phi_0 V_0 = \frac{N_a M_w}{\rho_0} \quad \text{and} \quad \frac{N_a}{V} = \frac{c}{M_w}, \tag{16}$$

where ρ_0 is the density of desiccated proteoglycan, c is the concentration of proteoglycan in mg/ml, and other symbols are as previously defined. This allows us to reexpress Eq. (15) as,

$$\Pi_{config} = \frac{c^2 N_c nRT}{M_w(\rho_0 - c)}. \tag{17}$$

The majority of the molecular weight of the proteoglycan molecule comes from the terminal GAG chains. As a result of this we can further simplify Eq. (17) by noting that

$$M_w = N_c nM_0, \tag{18}$$

where M_0 is the average molecular weight of a monomeric subunit. This allows us to rewrite the internal configurational entropic contribution to the osmotic swelling pressure as

$$\Pi_{config} = \frac{c^2 RT}{M_0(\rho_0 - c)}. \tag{19}$$

The fact that the internal configurational entropic contribution to the swelling pressure dominates over the aggrecan mixing component is readily seen by noting the quotient

$$\frac{\Pi_{mix}}{\Pi_{config}} = \frac{M_0(\rho_0 - c)}{M_w c}, \tag{20}$$

which for typical, large aggrecans, is much less than one except in the extreme dilute environment, i.e. volume fractions of less than 10^{-5}. This makes sense conceptually as the number of configurations available to the GAGs plateaus in the dilute limit while the mixing entropy continues to increase as the concentration decreases for a fixed number of solute.

4. Swelling pressure of concentrated proteoglycan

We are in a position to combine the predictions regarding the charge-dependent and charge-independent contributions to the equilibrium swelling pressure of concentrated proteoglycan and to compare these results with experimental data in the literature. The expression obtained is

$$\Pi = \Pi_{\text{config}} + \Pi_{\text{mix}} + \Pi_{\text{Don}}, \tag{21}$$

or

$$\Pi = \frac{c^2 RT}{M_0(\rho_0 - c)} + RT(c/M_w) + \frac{c^2 \nu_0^2 RT}{4mM_0^2}. \tag{22}$$

By expanding the configurational contribution to this expression it can be recast as a virial expansion in terms of powers of the concentration [31],

$$\Pi = RT\left[c(1/M_w) + c^2\left(\frac{1}{M_0\rho_0} + \frac{\nu_0^2}{4mM_0^2}\right) + c^3\left(\frac{1}{M_0\rho_0^2}\right) - \ldots\right]. \tag{23}$$

This interesting result demonstrates that both the ideal Donnan effect and the configuration entropy of the terminal GAGs enter into the osmotic equation of state in the second order virial coefficient. The Donnan effect, when treated as ideal, only enters into the equation of state as a second order correction while the configuration entropy contributes higher order terms as well.

The validity of this approach rests on its ability to reproduce experimental data. In Fig. 1 Eq. (22) is plotted with data obtained by Urban et al. [18]. The data are for chondroitin sulfate with molecular weight about 10^6 in a 1.5 M NaCl bath. The data were obtained by equilibrium dialysis against standardized polyethylene glycol as described by Urban et al. [18]. Of particular importance is the finding of Urban that the results were not dependent on the molecular weight of the proteoglycan aggregates. The theory presented here is manifestly independent of the molecular weight except at extreme dilution, far from the physiologic and experimental range.

As can be seen in Fig. 1 this theory accurately reproduces Urban et al.'s data [18]. The dots represent their data. The dotted line is the Donnan theory,

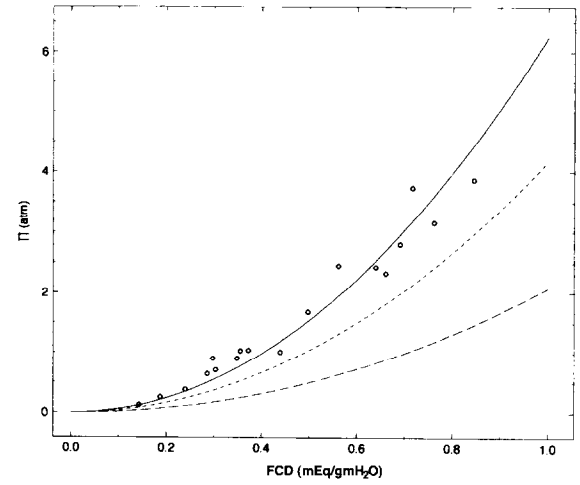

Fig. 1. Maroudas' experimental data for the osmotic swelling pressure of concentrated chondroitin sulfate are plotted as a function of fixed charge density (mEq/gmH_2O) in 1.5 M NaCl. The theoretical contributions to the swelling pressure from the ideal Donnan model (dotted line) and the configurational entropic contribution (dashed line) are also plotted. The solid line is the sum of the contributions from the Donnan and entropic effects. There are no free parameters in this model. See text for details.

the dashed line is the contribution from configurational entropic effects, and the solid line is the sum of the two contributions. The partial specific gravity of proteoglycan is taken to be 2.2 and the molecular weight per monomer 228.5. There are no free parameters in this theory [33].

5. Polydispersity of GAG molecular weight

The model presented here can be extended to encompass proteoglycan solutions with a polydispersity of molecular weight, to include free terminal GAG chains. The Donnan contribution remains unchanged, where c is understood to represent the total concentration of proteoglycan, including contributions from all molecular weights. The mixing contribution is also readily adapted to a polydisperse system, keeping in mind the analogous partial pressure notion in ideal gas mixtures, i.e.

$$\Pi_{\text{mix}} = RT\sum_{M_w}\frac{c(M_w)}{M_w}. \tag{24}$$

The configurational pressure can also be generalized to a polydisperse system as follows:

$$\Pi_{\text{config}} = \frac{RT}{M_0}\left(\frac{\phi_0 V_0}{V-\phi_0 V_0}\right)\sum_{M_w} c(M_w), \tag{25}$$

or

$$\Pi_{\text{config}} = \frac{c^2 RT}{M_0(\rho_0 - c)}, \tag{26}$$

where c here represents the total concentration in mg/ml, comprised of all different molecular weights. The fact that this expression and the Donnan expression remain unchanged in the polydisperse scenario should not come as a surprise. They are both molecular weight independent. When performing the summing involved in the polydisperse model the entire expression, with the exception of the concentration being summed over, factors out of the sum, leaving its form unchanged.

In summarizing the polydisperse model, the form of the expressions for the contribution from charge effects and configurational entropy are unchanged except that the concentration, c, is understood to be comprised of contributions from various molecular weights. The mixing contribution is changed. As demonstrated above however, this contributes negligibly in the scenario under study. In the extremes of very dilute solution or very small molecular weights (i.e. a preponderance of monomers or oligomers) the relative importance of the mixing term and its molecular weight dependence will be enhanced.

6. Bulk compressive modulus of cartilage

The model of the configurational entropy of concentrated proteoglycan solution and its effect on osmotic swelling pressure can be adapted to the problem of cartilage elasticity. By redefining the volume exclusion term in Equation (7) to mean the volume fraction occupied by both the proteoglycan and the collagen, the swelling pressure of cartilage can be obtained,

$$P^{\text{ne}} = -\frac{\partial F^{\text{ne}}}{\partial V} = \frac{N_c nkT}{V_0}\left(\frac{\phi_0(V_0/V)^2}{1-\phi_0(V_0/V)}\right). \tag{27}$$

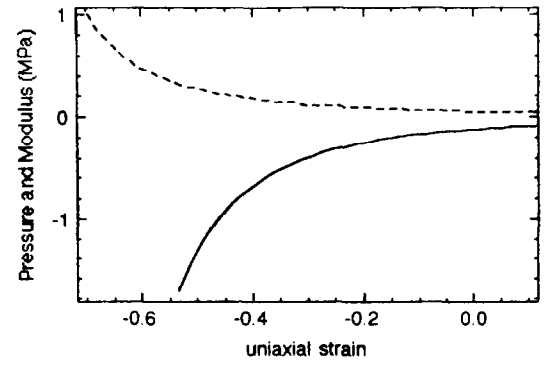

Fig. 2. The entropic contribution to the swelling pressure (dashed line) and compressive bulk longitudinal modulus (solid line) of cartilage is plotted as a function of uniaxial compressive strain. The parameters used are given in Table 1.

In the case of uniaxial confined compression, the volume can be written in terms of the strain as $V = V_0(\epsilon + 1)$, where negative strain corresponds to compression. Making use of this expression, the charge-independent contribution to the pressure can be expressed in terms of the uniaxial strain as

$$P^{\text{ne}} = \frac{N_c nkT}{V_0}\left(\frac{\phi_0}{(\epsilon+1)(\epsilon+1-\phi_0)}\right). \tag{28}$$

This allows for the determination of the charge independent contribution to the bulk longitudinal modulus H^{ne}, which is simply the derivative of the pressure with respect to strain,

$$H^{\text{ne}} = -\frac{\partial P^{\text{ne}}}{\partial \epsilon} = \frac{N_c nkT}{V_0}\left(\frac{\phi_0(2\epsilon+2-\phi_0)}{(\epsilon+1)^2(\epsilon+1-\phi_0)^2}\right). \tag{29}$$

The results of Eqs. (28) and (29) are plotted in Fig. 2 with the values of constants taken to describe cartilage at room temperature. The parameters used are adapted from Hunziker and presented in Table 1 [25]. A detailed comparison of these results to experiment is as of yet not complete. The high degree of success of this approach obtained in the isolated

Table 1

n	50
ϕ_0	0.15
N_c / V_0	1.6×10^{24} m^{-3}
T	298 K

proteoglycan problem, together with the experimental evidence that proteoglycan is responsible for the compressive stiffness of cartilage give hope that this approach may prove fruitful.

7. Conclusion

This work provides a theoretical framework for understanding the role of proteoglycan configurational entropy in determining the mechanical properties of concentrated proteoglycan solution and articular cartilage. The importance of understanding the physical properties of cartilage and proteoglycan in terms of molecular architecture can not be overstated. Ultimately, pathologic change occurs at the molecular level. A sound theory relating the mechanical properties of proteoglycan to its ultrastructure is essential in order to understand and alter the pathogenesis of numerous debilitating disorders which afflict articular cartilage and other tissues in which proteoglycan plays a key structural role.

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