ELECTROSTATIC FORCES BETWEEN CHARGED MACROMOLECULES MEASURED BY EQUILIBRIUM SEDIMENTATION: RELEVANCE TO CARTILAGE MECHANICS

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Introduction: Molecular interactions between glycosaminoglycans (GAGs) affect the behavior of extracellular matrix in health and disease. However, their heterogeneous composition, distribution, and physical-chemical properties complicate their characterization *in situ*. Here we present a new approach to measuring intermolecular electrostatic repulsive forces between GAGs (and between other matrix macro-molecules), using equilibrium sedimentation (centrifugation) and a new model of macromolecular electrostatic interactions in a centrifugal field. We then demonstrate this method using a well-characterized molecular analog of GAGs, polymethacrylic acid (PMAA).

Theory, Materials and Methods: With a centrifuge, we apply a known, spatially varying mechanical force to a macromolecular solute (e.g., see (1)). From the resulting measured macroscopic solute concentration profile, $c_i(r)$ within the centrifuge cell (Fig1), we calculate a profile of intermolecular spacing $\langle d(r)\rangle$. At thermodynamic and mechanical equilibrium, the electrostatic, centrifugal, and diffusive forces, respectively, balance, leading to:

$$\Phi(r) - \Phi_0 = \frac{M_i(1-v_i\rho)\omega^2 r^2}{2z_iF} - \frac{RT}{z_iF}\ln c_i(r) \quad . \qquad (1)$$

where $\Phi(r)$=*macroscopic* electrostatic potential, $M_i(1-v_i\rho)$=solute effective mass, ω=angular velocity, RT/F=thermal voltage. At the resolution of the measured macroscopic solute concentration, (~10 μm), $\Phi(r)$ is a smoothly varying function of r, (Fig1(a)). However, at the molecular length scale, the microcontinuum electrostatic potential, $\phi(r')$, varies rapidly and even discontinuously, (Fig1(b)), producing large intermolecular forces. We can calculate this molecular scale electrostatic repulsive force using a unit-cell model and a relationship between the macroscopic and molecular scale potential distributions.

In this study, we use 110,000 MW monodisperse (M_w/M_n = 1.02) PMAA-Na (Polysciences, Lot. #403372) as an analog for GAG chains. PMAA is an appropriate model system since a) the intramolecular spacing between adjacent charged groups on PMAA (approximately 9 x 10^{-10} m) and GAGs is similar, b) the α and β glycoside (C-O-C) bonds along the backbone of the disaccharide chain in GAGs and the C-C bonds along the backbone of PMAA are both rotationally mobile (2), c) the negative charged groups on GAG disaccharides and MAA monomers are completely dissociated above pH 7, and d) the number of monomers per polymer of PMAA and GAG is large enough for end effects to be negligible.

We loaded the centrifuge cells with PMAA solutions at pH 8 with varying ionic strengths from 0.002M to 1.0M, which were prepared by adding varying amounts of NaCl and NaOH to a 1.82 x 10^{-5} M PMAA (2mg/ml) stock solution. Macromolecular solutions were spun in a Beckman XL-A ultracentrifuge at 8-20 KRPM. After equilibrium was reached, PMAA concentration profiles were measured spectrophotometrically. We compared them to concentration profiles predicted by the analytical solution to the Lamm equation (3) for an ideal uncharged (equivalent) polymer with the same molecular weight as PMAA.

Results and Discussion: Fig. 2 shows the measured equilibrium PMAA concentration profiles at 17,000 RPM for solutions with ionic strengths, (a) 0.2M, and (b) 0.006M. Juxtaposed are the calculated ideal concentration profiles (for the uncharged equivalent polymer), as well as the calculated macroscopic potential, $\Phi(r)$. Measured PMAA profiles progressively deviate from that of an (ideal) uncharged solute (with same molecular weight as PMAA) as solvent ionic strength decreases. This nonideal behavior is clearly due to electrostatic repulsion. This conclusion is supported by inspection of Eq. (1) and of the slope of the measured macroscopic potential distribution in Fig 2, which is proportional to the macroscopic electric field. We also estimated the importance of intermolecular electrostatic interactions using the molecular scale Poisson-Boltzmann model. Intermolecular electrostatic interactions are significant when the mean local intermolecular distance, $\langle d(r)\rangle$ (calculated from the measured PMAA profile) divided by the Debye length, λ is less than 5 (4). For known GAG concentrations and physicochemical conditions *in vivo*, $\langle d(r)\rangle/\lambda$ is known to be less than 5 (5), and thus electrostatic repulsive forces are predicted to be significant. This condition was satisfied in all experiments in which we observed non-ideal behavior and was not satisfied in all experiments in which we observed ideal behavior of the PMAA solution.

Although the measured macroscopic $\Phi(r)$ was small, its molecular scale counterpart produced large intermolecular electrostatic repulsive forces. When using $\Phi(r)$ as a thermodynamic variable, one must specify the characteristic length over which the electrostatic potential is measured since different approximations to Maxwell's equations apply at different length scales. We showed that one may measure different electrostatic potential distributions and intermolecular forces at different characteristic length scales.

Conclusion: Our goal is to understand the nature of the interactions between GAGs in cartilage and in extracellular matrix *in vivo*. By choosing appropriate centrifuge operating conditions we can now subject solutions of matrix macromolecules to physicochemical conditions comparable to those encountered *in vivo*. A more general goal is to use sedimentation to investigate structure/function relationships among matrix macromolecules. This approach is applicable to examining how a structural change (e.g., due to a point mutation or enzymatic modification) associated with age or degeneration affects the measured electrostatic, entropic, and enthalpic forces between matrix macromolecules. Changing these forces may, in turn, affect the molecule's biological function.

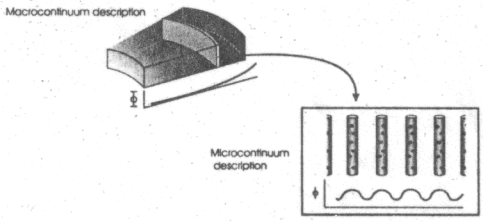

Fig 1: (a) macro- and (b) micro-continuum view of the solute and potential distributions in a centrifuge cell.

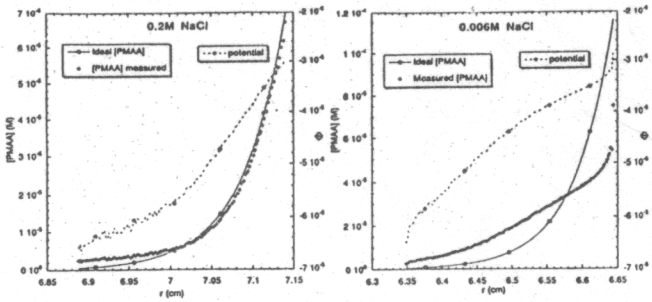

Fig 2: measured & "ideal" $c_i(r)$; measured $\Phi(r)$ in 0.2 & 0.006M NaCl

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