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 macromolecules), using equilibrium sedimentation (centrifugation) and a new model of macromolecular electrostatic interactions in a centrifuge field. We then demonstrate how to use this method to determine 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(r) within the centrifuge cell (Fig1), we calculate a profile of intermolecular spacing <d(r)> at thermodynamic equilibrium. At low ionic strengths, (a) 0.2M, and (b) 0.006M. Juxtaposed are the calculated PMAA concentration profiles at 17,000 RPM for solutions with ionic strengths, (a) 0.2M, and (b) 0.006M. The authors have not received anything of value from a commercial or other party related directly or indirectly to the subject of my presentation.

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 the 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, \( c_d(r)/\lambda \), is known to be less than 5 (4). For known GAG concentrations and physicochemical conditions in vivo, \( c_d(r)/\lambda \) is known to be less than 5 (9), 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, enriopic, 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" \( c_d(r) \); measured \( \Phi(r) \) in 0.2 & 0.006M NaCl 1929. 4.Basser, PJ et al., 1995 Bioengineering Conference, CO, 33, 1995. 5.Buschmann, MD et al., 1929. 4.Basser, PJ et al., 1995 Bioengineering Conference, CO, 33, 1995. 5.Buschmann, MD et al., J Biomech Engng, 117, 179, 1995. AJG was supported in part by NIH Grant AR33236

\[ \Phi(r) = \frac{M_1}{2\pi F} \frac{\omega^2 r^2}{2\pi} + \frac{RT}{2\pi F} \ln c(r) \]