ION SENSITIVITY OF DNA GEL STRUCTURE

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Introduction

Biological systems operate at the cellular and subcellular levels. Understanding the behavior of these systems, material properties including structure, osmotic and mechanical properties must be determined to dimensions below 100 nm. Synthetic polymers have been recently designed to mimic the hierarchical structure and function of biological macromolecules. Knowledge of the structure at molecular and nanometer scale is necessary for engineering useful and novel properties. Examples of applications of engineered structures include materials for tissue engineering, medical implants for diagnosis and therapy, and *in-vivo* drug-delivery.

We developed a multiscale approach to examine the structural hierarchy and equilibrium properties of natural and synthetic polyelectrolyte gels.[1-3] In these systems different kind of interactions (electrostatic forces, van der Waals forces, hydrophobic interactions, etc.) drive structure formation. Many natural and synthetic polyelectrolytes (e.g., polyacrylic acid, DNA) exhibit a strong sensitivity to ionic strength and, in particular, to counterion valence. The complexity of the behavior of charged macromolecular systems makes necessary to investigate their structure and physical properties on all length scales from the atomic level up to the macroscopic level. Small-angle neutron scattering (SANS) and small-angle X-ray scattering (SAXS) are ideal techniques to study biopolymer molecules and assemblies in their natural environment and to correlate the changes in environmental conditions (e.g., ionic composition, solvent quality) with physical properties.

In the present work we report SANS and osmotic swelling pressure measurements made on covalently cross-linked DNA gels swollen in NaCl and $CaCl_2$ solutions.

Theory. The neutron scattering intensity of a neutral polymer solution can be described by a Lorentzian function[4]

$$I(q) = \frac{A}{\left(1 + q^2\xi^2\right)} \qquad (1)$$

where A is a constant, ξ is the polymer-polymer correlation length, and q is the scattering vector.

The intensity scattered by gels contains additional contributions due to structural features frozen in by the cross-links.[5] In the simplest case, the gel signal is given by a two term function

$$I(q) = \frac{A}{\left(1 + q^2\xi^2\right)} + B(q) \qquad (2)$$

where the second term depends on the gel structure.

The swelling pressure of a polymer gel Π_{sw} is the sum of elastic (Π_{el}), mixing (Π_{mix}) and ionic (Π_{ion}) pressure contributions[6]

$$\Pi_{sw} = \Pi_{el} + \Pi_{mix} + \Pi_{ion} \qquad (3)$$

For neutral polymer solutions the mixing pressure can be described by the Flory-Huggins theory[6]

$$\Pi_{mix} = \frac{\partial \Delta F_{mix}}{\partial n_1} = -\frac{RT}{v_1}\left[\ln(1-\varphi) + \varphi + \chi_0\varphi^2 + \chi_1\varphi^3\right] \qquad (4)$$

where φ is the volume fraction of the polymer, v_1 is the molar volume of the solvent, n_1 is the number of the moles of the solvent, R is the gas constant, T is the absolute temperature, and χ_0 and χ_1 are constants that depend on the polymer-solvent interactions.

The elastic term can be approximated by the theory of rubber elasticity.[6,7]

It is reasonable to assume that in polyelectrolyte gels, in the presence of large excess of added salt, the electrostatic interactions are screened, and the ionic term does not play a significant role. However, the ionic interactions may modify the mixing pressure of the gel.

Experimental

Gel Preparation. DNA gels were made from deoxyribonucleic acid sodium salt (Sigma). The molecular weight determined by ultracentrifugation was 1.3×10^6 Da. DNA gels were prepared[8] from a 3% (w/w) solution by cross-linking with ethyleneglycol diglycidyl ether at pH = 9.0 using TEMED to adjust the pH.

DNA gels were swollen in NaCl solution, and then the concentration of the $CaCl_2$ in the surrounding NaCl solution was gradually increased.

Small-angle Neutron Scattering. SANS measurements were made on gels using the NG3 instrument[9] at the National Institute of Standards and Technology (NIST, Gaithersburg, MD). Gel samples swollen in D_2O were placed into standard NIST sample cells. The q range explored was 0.003 $Å^{-1} \leq q \leq 0.2$ $Å^{-1}$, and counting times were varied from twenty minutes to two hours. After radial averaging, detector response and cell window scattering were applied. The neutron scattering intensities were calibrated using NIST absolute intensity standards.[9] All experiments were carried out at 25 ± 0.1°C.

Swelling Pressure Measurements. Swelling pressure measurements were made by equilibrating the gels with aqueous solutions of poly(vinyl pyrrolidone) (M_n = 29 kDa) of known osmotic pressure.[10,11] The penetration of the polymer into the swollen network was prevented by a semipermeable membrane.

Results and Discussion

Figure 1 shows the dependence of the polymer volume fraction on the $CaCl_2$ concentration for a DNA gel swollen in 10 mM NaCl solution. In the figure are also shown similar data obtained for a poly(acrylic acid) (PAA) gel. With increasing $CaCl_2$ concentration both systems display an abrupt volume change. The sharp variation of the polymer volume fraction indicates that this transition is a highly cooperative process.

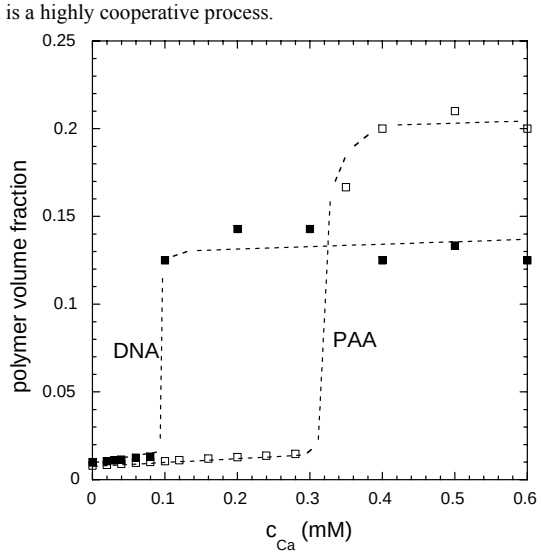

Figure 1. Variation of the polymer volume fraction with the calcium ion concentration for a DNA (filled symbols) and a PAA gel (unfilled symbols) swollen in 10 mM NaCl solutions.

We made osmotic pressure measurements on DNA gels at constant $CaCl_2$ concentrations (in 10 mM NaCl) below the volume transition. **Figure 2** shows the dependence of Π_{mix} on the DNA concentration. At constant polymer concentration the osmotic pressure gradually decreases with increasing calcium concentration.

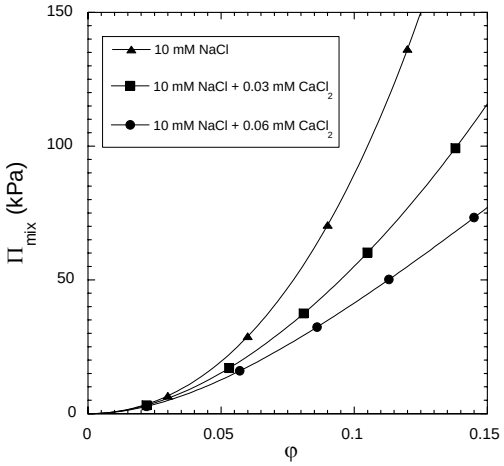

Figure 2. Dependence of the osmotic pressure on the polymer volume fraction for DNA gels swollen in salt solutions.

In **Figure 3** the SANS spectra of DNA gels measured in D_2O at different NaCl concentrations are shown. All the spectra display two common features: low-*q* clustering and high-*q* solvation. The upturn in *I(q)* at $q < 0.01$ Å^{-1} indicates cluster formation generally observed in polyelectrolyte solutions. The size of the clusters exceeds the resolution of the SANS experiment.

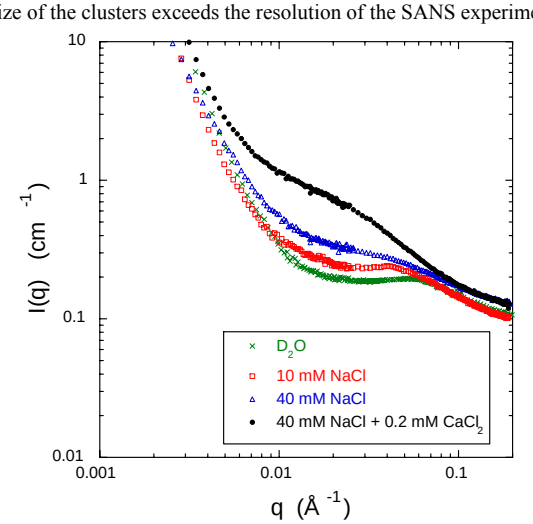

Figure 3. SANS spectra of DNA gels in pure D_2O and in salt solutions.

In the salt-free solution and at low salt concentration the SANS spectra display a correlation peak. In pure D_2O the peak occurs at $q_0 \approx 0.07$ Å^{-1} corresponding to an average distance of $d_0 = 2\pi/q_0 = 90$ Å. In salt solutions ions screen the charges, and the polyelectrolyte peak moves to lower values of *q* (≈ 0.04 Å^{-1} in 10 mM NaCl solution) indicating that the average size of the charged domains increases by roughly 80%. In 40 mM NaCl solution the polyelectrolyte peak has completely disappeared and the scattering curve only exhibits a shoulder at $q \approx 0.04$.

The upper curve in Figure 3 shows the SANS spectrum of a DNA gel measured in 40 mM NaCl containing 0.2 mM $CaCl_2$. Ca/Na ion exchange modifies the electrostatic interactions between the DNA strands and affects their organization. In the low-*q* and high *q*-regions the Ca ions only slightly influence the shape of the scattering curve. At intermediate length scales the scattering intensity from the Ca-containing gel significantly exceeds that from the other three samples. The increase of intensity is consistent with a system approaching phase separation. The present DNA gel undergoes phase separation at approximately 0.3 mM $CaCl_2$ concentration (in the surrounding 40 mM NaCl solution).

The SANS results indicate that introducing multivalent cations into DNA gels allows us to modify the nanoscale structure without significantly influencing the organization of the polymer segments on either large or short length scales.

Conclusions

Changes in the ionic environment induce structural changes in DNA gels. At low-*q* the SANS spectra indicate the formation of large domains which are only weakly influenced by the salt concentration of the solution. At low ionic strength a distinct correlation peak is observed in the SANS spectrum that disappears in 40 mM NaCl solution. Addition of $CaCl_2$ reduces the osmotic pressure, which ultimately results in a volume transition. The SANS intensity of DNA gels is enhanced as the volume transition is approached.

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