ION-DRIVEN VOLUME TRANSITIONS IN DNA GELS

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Introduction

In biopolymer systems, such as DNA or protein solutions, electrostatic interactions often play a central role in determining the phase behavior. Electrostatic interactions in aqueous polymer solutions can be controlled by changing the ionic composition, pH, etc. Anionic polymers interact with cations of different ionic valence. Cations mediate equilibrium properties and dynamics within the microenvironment of the polyelectrolyte chains. In addition, monovalent and divalent cations may act differently, and exhibit a behavior that cannot be attributed to electrostatic interactions alone.[1,2,3]

We study ion-biopolymer interactions in gels.[3,4] The gel state is intermediate between liquid and solid states. In gels the liquid prevents the collapse of the polymer molecules into a compact mass while the polymer material retains the liquid from flowing out of the system. The balance of the associated osmotic and elastic forces defines whether the gel tends to swell or shrink in response to changes in the environment. The solvent uptake (or loss) continues until the equilibrium is attained, i.e., the osmotic and elastic forces balance one another.

Dramatic changes in the gel volume can be induced by relatively small changes in the environmental conditions (e.g., pH, ionic strength).[1,2,3,4] The extent of the volume change strongly depends on the degree of ionization of the polyelectrolyte chains and can lead to a collapse. These observations are reminiscent of the well-known "coil-globule" transitions of flexible synthetic and biopolymers.

In the present work we compare the effect of monovalent ions, divalent ions and pH on the structure and thermodynamic properties of polyelectrolyte gels synthesized from DNA.

Experimental

Gel Preparation. DNA gels were made from deoxyribonucleic acid sodium salt (prepared from salmon testes, Sigma).[5] The % G-C content of the DNA was 41.2 %. The molecular weight determined by ultracentrifugation was 1.3×10^6 Da, which corresponds to approximately 2000 base pairs. DNA was dissolved in HEPES buffer (pH = 7.0) and the solutions were dialyzed against distilled water. DNA gels were made by crosslinking with ethyleneglycol diglycidyl ether in solution [pH = 9.0, c_{DNA} = 3% (w/w)]. TEMED was used to adjust the pH. DNA gels were equilibrated with NaCl solutions containing different amounts of $CaCl_2$.

Small Angle Neutron Scattering. SANS measurements were made on the NG3 and NG7 instruments at NIST, Gaithersburg MD, using an incident wavelength of 8 Å. The DNA gels swollen in D_2O were held in 2 mm thick quartz sample cells. The transfer wave vector explored in the experiments covered the range 2.8 10^{-3} Å^{-1} < q < 0.2 Å^{-1}. After azimuthal averaging, corrections for solvent background, detector response and cell window scattering were applied.[6]

Osmotic Swelling Pressure Measurements. The osmotic swelling pressure of DNA gels was measured as a function of concentration by bringing them to equilibrium with poly(vinyl pyrrolidone) solutions of known osmotic pressure.[7] A semipermeable membrane was used to prevent penetration of poly(vinyl pyrrolidone) molecules into the DNA gels. The reversibility of the swelling/shrinking process was checked by reswelling the deswollen gels in poly(vinyl pyrrolidone) solutions of known water activities.

All measurements were made at 25 ± 0.1 °C.

Results and Discussion

Figure 1 illustrates the effect of monovalent salt concentration (NaCl) on the SANS response of DNA gels at pH = 7 (in D_2O). Each SANS spectrum exhibits two common features: low-q clustering and high-q solvation. The clustering contribution points to large (micron size) gel-like structures. The solvation contribution provides information on the interaction between the polymer and the solvent molecules. Polymer solvation is characterized by either a polyelectrolyte peak at finite q or a forward scattering peak for the high-q signal. The peak position in salt-free D_2O indicates that the interdistance between the charged domains is approximately 100 Å. Addition of monovalent salt (NaCl) gradually decreases the height of the peak, and the peak position is slightly shifted towards lower values of q as the salt concentration increases. In the presence of 100 mM NaCl the polyelectrolyte peak is no longer distinguishable.

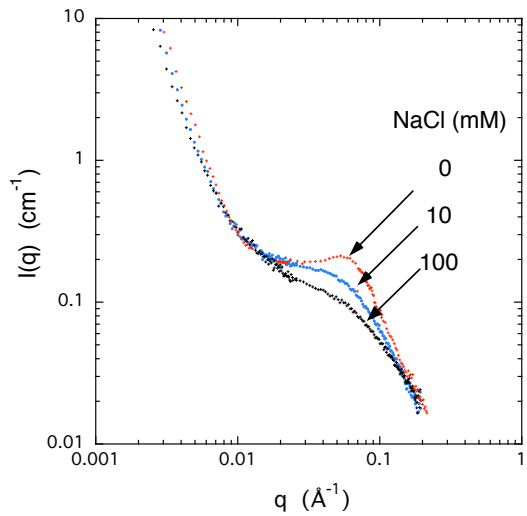

Figure 1. SANS spectra of DNA gels in NaCl solutions.

Figure 2 shows the effect of pH on the SANS spectra of DNA gels swollen in 40 mM NaCl solution. The shape of the scattering curves hardly changes in the pH range 3 to 7. However, below pH = 3 the scattering intensity is enhanced indicating that the system becomes unstable as the volume transition is approached. (In these gels volume transition occurs below pH = 2.) At low pH the phosphate backbone of DNA becomes protonated (the pK_a of the phosphate is approximately 2) and the electrostatic (Coulombic) repulsion is significantly reduced.[8] The DNA molecule behaves like a neutral polymer in a poor solvent.

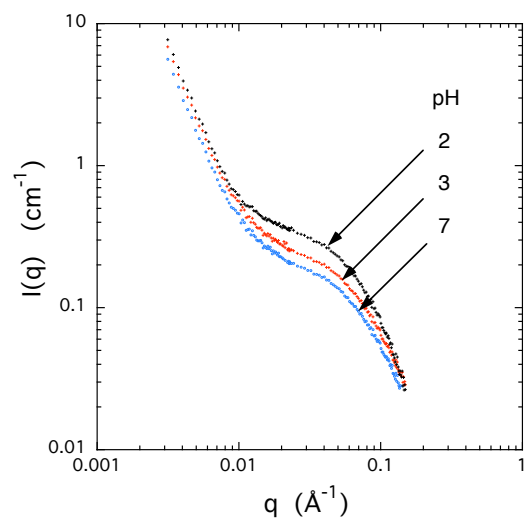

Figure 2. SANS spectra of DNA gels at different values of pH in 40 mM NaCl solution.

To estimate the effect of pH on the osmotic properties of DNA gels we made osmotic swelling pressure measurements in 40 mM NaCl solutions as a function of the pH. The data displayed in **Figure 3** show that the swelling

pressure ω decreases with decreasing pH. The continuous curves in Figure 3 are the least squares fits to the scaling expression[9,10]

$$\omega = A\varphi^n - G \qquad (1)$$

where A is a constant depending on the polymer solvent system, φ is the volume fraction of the polymer in the gels, n is a scaling exponent, and G is the elastic (shear) modulus of the gel. As expected theoretically, n gradually increases from its good solvent value (n = 2.2 at pH = 7) and exceeds its theta value (n = 3.2 at pH ≈ 2, i.e., just below the transition).[11] For polyelectrolytes, the first term in Eq. (1) should include both a mixing contribution, Π_{mix}, and an ionic contribution. It appears that in the presence of added salt the ionic contribution does not play a significant role. This finding is consistent with previous experimental observations made on other polyeletrolyte gels.[12,13]

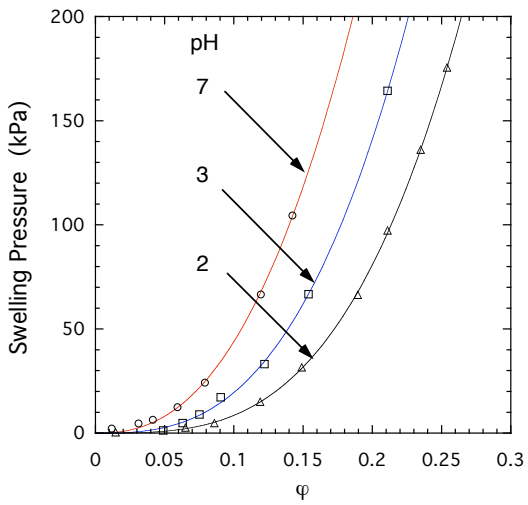

Figure 3. Osmotic swelling pressure as a function of DNA volume fraction for DNA gels at different values of pH.

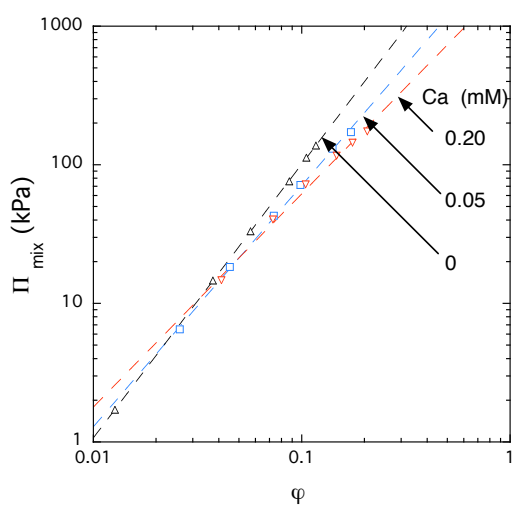

Figure 4. Osmotic mixing pressure (first term in eq. 1) as a function of DNA volume fraction at different Ca^{2+} concentrations.

The decrease of the swelling pressure with decreasing pH implies that the longitudinal osmotic modulus of the gel, M_{os}, also decreases since the latter is defined by[14]

$$M_{os} = (\partial\omega/\partial\varphi) + (4/3)G \qquad (2)$$

The SANS spectra of DNA gels shown in Figure 2 indicate that in the intermediate *q* range the scattering intensity is enhanced as the pH decreases. The low- and high-*q* regions of the spectra are only weakly affected by the pH, i.e., the large clusters (low-*q* feature) and the geometry of the chains (high-*q* feature) remain unchanged. The increased intensity reflects the decrease in M_{os}.

In **Figure 4** is illustrated the effect of divalent counter-ions on the osmotic pressure of DNA gels at three different calcium concentrations. In this gel a volume transition takes place at a 0.3 mM $CaCl_2$ concentration (in 40 mM NaCl solution). In the double logarithmic representation each of these curves is linear over the whole concentration range explored. The slope of the straight lines through the data points varies from 2.0 (no $CaCl_2$) to 1.5 (0.2 mM $CaCl_2$). The decrease in the power law exponent with increasing calcium concentration is opposite to what is observed in the same gel system upon decreasing pH. In the latter case the exponent increases, as the transition is approached.[12]

Conclusions

The difference between the effects of salt concentration, pH, and counter-ion valence on the structure and thermodynamic properties of DNA gels has been investigated using scattering techniques in combination with macroscopic osmotic measurements. Both the exchange of monovalent counter-ions to divalent counter-ions and the reduction of the pH produce drastic changes in the degree of swelling of DNA gels. Closer inspection of the effects of ion valence and pH on the osmotic swelling pressure reveals significant differences that cannot be attributed to changes in the electrostatic interactions alone.

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