DNA Gels: pH Mediated Structural Changes

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INTRODUCTION

In hydrogels different kind of interactions (electrostatic, van der Waals, hydrophobic interactions, etc.) determine the formation of complex hierarchical structures. These interactions are governed by a combination of structural properties at the micro- and nanoscale as well as by macroscopic physical parameters such as ionic strength and solvent quality. Changes in the ionic environment (pH, ion concentration and ion valence) impact the structure and physical properties of these polymer systems. The main focus of our research is on the roles that nanoscale structures and interactions play in determining the macroscopic properties of polyelectrolyte gels in near physiological ionic condition. The complexity of the behavior of charged macromolecular assemblies necessitates an investigation of the structure and physical properties on all length scales from the atomic to the macroscopic level. We apply a multiscale approach to examine the structural hierarchy, phase behavior and equilibrium properties of DNA gels. Here we report SANS and osmotic pressure measurements that probe the structure over a wide range of length scales and provide insight into the effects of structural characteristics on the macroscopic properties of these gels.

THEORY

The osmotic swelling pressure ω associated with the swelling of a charged polymer network is given as a sum of three terms [1]

$$\omega = \Pi_{el} + \Pi_{mix} + \Pi_{ion} \qquad (1)$$

where Π_{el} is the elastic, Π_{mix} is the mixing, and Π_{ion} is the ionic free energy contribution.

The elastic term is described by the Gaussian theory of rubber elasticity [1,2]

$$\Pi_{el} = -G \qquad (2)$$

where G is the shear modulus.

For semi-dilute polymer solutions the mixing pressure can be approximated by a simple power law [3]

$$\Pi_{mix} = A\varphi^{n} \qquad (3)$$

where A is a constant and φ is the volume fraction of the polymer. The value of the exponent *n* depends on the thermodynamic quality of the solvent. In good solvent condition $n \approx 9/4$ while in a theta solvent $n = 3$.

In the presence of large amount of added salt, the electrostatic interactions are screened, and Π_{ion} is not expected to play a significant role [4].

The neutron scattering intensity of a neutral polymer solution can be described by a Lorentzian function [3]

$$I(q) = \frac{B}{\left(1 + q^2\xi^2\right)} \qquad (4)$$

where *B* is a constant, ξ is the polymer-polymer correlation length, and *q* is the scattering wave vector [$q = (4\pi/\lambda)\sin(\theta/2)$ and θ the scattering angle].

The scattering intensity from polyelectrolyte gels usually contains another contribution due to large clusters frozen in by the cross-links [5, 6]. Thus, the gel signal is given by

$$I(q) = \frac{B}{\left(1 + q^2\xi^2\right)} + Cq^{-m} \qquad (5)$$

where *C* is another constant and the exponent *m* is defined by the details of gel structure.

MATERIALS AND METHODS

Gel Preparation. DNA gels were made from deoxyribonucleic acid sodium salt (Sigma) [7]. DNA solutions (concentration: 3% w/w) were crosslinked with ethyleneglycol diglycidyl ether at pH = 9.0 using TEMED to adjust the pH. After gelation, gels were washed with deionized water, and swollen in NaCl solution (nearly physiological condition). The pH in the surrounding liquid phase was adjusted with HCl solution.

Small-angle Neutron Scattering. SANS measurements were made on DNA gels on the NG3 instrument at the National Institute of Standards and Technology (NIST, Gaithersburg MD) [8]. Gel specimens swollen in D_2O were placed into standard NIST sample cells. The sample cell consisted of 1 mm thick quartz windows separated by a 2 mm thick spacer. The *q* range explored was $0.002\ \text{Å}^{-1} \le q \le 0.2\ \text{Å}^{-1}$, and counting times from twenty minutes to two hours were used. After radial averaging, detector response and cell window scattering were applied. The neutron scattering intensities were calibrated using absolute intensity standards. All experiments were carried out at 25 ± 0.1°C.

Osmotic Swelling Pressure and Elastic Modulus Measurements. Swelling pressure measurements were made by equilibrating the gels with aqueous poly(vinyl pyrrolidone) solutions of known osmotic pressure. The penetration of the polymer into the swollen network was prevented by a semipermeable membrane [9,10].

Elastic modulus measurements were carried out on cylindrical gel samples using a TA.XT2I HR Texture Analyser (Stable Micro Systems, UK) [4]. Swollen networks were uniaxially compressed (at constant volume) between two parallel flat plates. The stress-strain isotherms were determined in the range of the deformation ratio $0.7 < \Lambda < 1$. The shear modulus was obtained from the relation

$$G = \frac{\sigma}{\Lambda - \Lambda^{-2}} \qquad (6)$$

where σ is the nominal stress (related to the undeformed cross-section of the gel cylinder). The absence of volume change and barrel distortion was checked by measuring the dimensions of the deformed and undeformed gel cylinders.

RESULTS AND DISCUSSION

Figure 1 illustrates the variation of the DNA volume fraction as a function of the pH in 40 mM NaCl solution. The data clearly indicate that decreasing the pH induces volume transitions in DNA gels. The transition occurs around pH ≈ 1.2. In the vicinity of the transition the gel volume sharply changes with the pH. In the figure is also shown the dependence of the DNA volume fraction on the NaCl concentration of the equilibrium solution (lower curve). With increasing NaCl concentration the swelling degree decreases smoothly and continuously, no abrupt volume change is detectable.

Figure 2 shows the SANS spectra of a 3 % w/w gel measured in D_2O at different NaCl concentrations. At high values of *q* the shape of the scattering curve strongly depends on the NaCl concentration. In the salt-free solution a distinct correlation peak can be observed. This behavior is typical of polyelectrolyte solutions and gels [11]. Ions screen the charges, and with increasing NaCl concentration the polyelectrolyte peak disappears. In the low *q* region $I(q)$ decreases with increasing *q* according to the negative fourth power law, $I(q) \propto q^{-4}$. This *q* dependence corresponds to the so-called Porod law [12].

Figure 2 also shows the SANS spectrum of the same DNA gel at pH = 1.5 (in 40 mM NaCl). The increased intensity reflects increased thermodynamic concentration fluctuations as the

transition is approached.

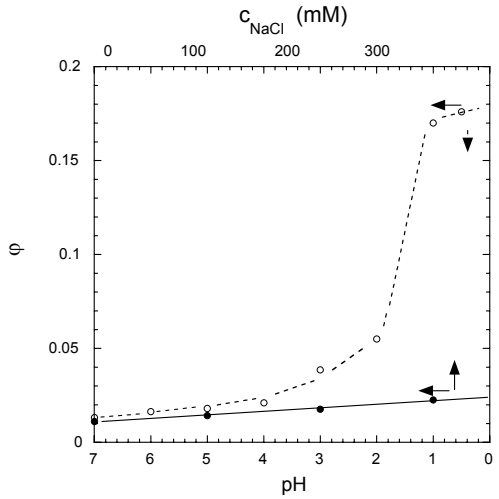

Figure 1. Variation of the DNA volume fraction φ with the pH (lower x-axis) in 40 mM NaCl solution. The continuous curve shows the dependence of φ on the NaCl concentration (upper x-axis) at pH = 7.

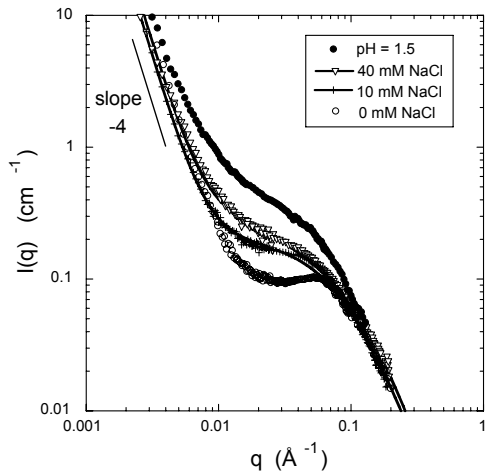

Figure 2. SANS intensity from DNA gels in equilibrium with salt solutions. The continuous curves through the data points of the gels swollen in 40 mM NaCl and 100 mM NaCl solutions show the least squares fits to eq. 5 with Cq^{-4}. The highest curve shows the SANS spectrum of a DNA gel below the volume transition at pH = 1.5 (40 mM NaCl).

We made osmotic pressure measurements to gain insight into the thermodynamic changes occurring in the course of the volume transition. In **Figure 3** is shown the variation of the osmotic pressure as a function of the polymer volume fraction for DNA gels swollen in 40 mM NaCl solution at different values of pH. In the double logarithmic representation all the curves are linear over the whole concentration range explored, indicating that the power law (eq. 3) holds for all gels.

The value of the power law exponent *n* varies in the range 2.1 (pH = 7) < *n* < 2.9 (pH = 1.5). This behavior is similar to the transition from good to poor solvent condition in neutral gels. In weak polyelectrolytes the small hydrogen ions are relatively strongly bound. As the pH decreases the degree of protonation increases and the polyelectrolyte gel behaves almost like an uncharged polymer in poor solvent.

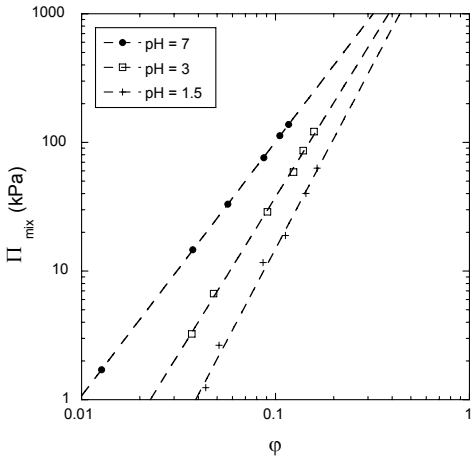

Figure 3. Dependence of the osmotic pressure of DNA gels on the polymer volume fraction at different values of pH in 40 mM NaCl solution. The dashed lines are least squares fits to eq. 2.

CONCLUSIONS

DNA gels swollen in near physiological NaCl solution undergo a volume transition by decreasing the pH. The SANS spectrum of the DNA gel in the absence of added salt displays a correlation peak, characteristic of weak polyelectrolyte systems. As the salt concentration increases the peak is shifted towards lower values of *q*. At constant pH the dependence of the osmotic pressure on the DNA concentration exhibits a power law behavior. The power law exponent increases with decreasing pH as expected for the transition from good to poor solvent condition in neutral polymer systems.

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