

OSMOTIC AND SMALL-ANGLE NEUTRON SCATTERING PROPERTIES OF DNA GELS

Ferenc Horkay¹, Peter J. Basser¹, Anne-Marie Hecht² and Erik Geissler²

¹Section on Tissue Biophysics and Biomimetics, Laboratory of Integrative and Medical Biophysics, NICHD, National Institutes of Health, 13 South Drive, Bethesda, MD 20892

²Laboratoire de Spectrométrie Physique CNRS UMR 5588, Université Joseph Fourier de Grenoble, B.P.87, 38402 St Martin d'Hères, France.

Introduction

In general, the description of polyelectrolyte systems (solutions and gels) requires a formalism that can take into account the existence of the multiple length scales that characterize the structure and reflect changes in the thermodynamic interactions. In the presence of high valence cations DNA undergoes a volume transition. The aim of the present study is to identify the main structural changes that occur in DNA gels swollen in nearly physiological NaCl solution upon the addition of Ca ions. An attempt is made to distinguish those structural features that affect the osmotic properties from the larger scale structures that are thermodynamically inactive.

Experimental

DNA gels were made from deoxyribonucleic acid sodium salt (salmon testes DNA, purchased from Sigma) according to a method described previously.¹ The molecular weight was 1.3×10^6 , which corresponds to approximately 2000 basepairs. The % G-C content of this DNA was 41.2 %.

DNA gels were made by cross-linking with ethyleneglycol diglycidyl ether at pH=9.0 using TEMED to adjust the pH. The gels were equilibrated in NaCl solutions containing different amounts of CaCl₂ (0-0.2 mM).

Osmotic swelling pressure measurements were made by equilibrating the DNA gels with poly(vinyl pyrrolidone) solutions (molecular weight: 29 kDa) of known osmotic pressure.^{2,3} A semipermeable membrane was used to separate the gel from the polymer solution. After attaining equilibrium (approximately 4-5 days), the concentrations in both phases were measured.

Small-angle neutron scattering (SANS) measurements were made at NIST (Gaithersburg, MD) on the NG3 instrument at two sample-detector distances, 2.5m and 13.1m, with incident wavelength 8Å. Corrections for incoherent background, detector response and cell window scattering were applied.⁴

Results and Discussion

The effect of Ca²⁺ ions on the swelling degree ($1/\phi$) of a DNA gel volume fraction is illustrated in **Figure 1**. At small CaCl₂ concentrations the swelling degree gradually increases with the addition of CaCl₂. At a critical concentration ($c_{Ca} \approx 0.3$ mM), a sudden volume change occurs. It is apparent that this volume transition is a highly cooperative process, i.e., it occurs over a relatively narrow concentration range of CaCl₂. Above $c_{Ca} \approx 0.4$ mM the swelling degree is practically independent of the CaCl₂ concentration of the surrounding liquid. We note that the swelling-shrinking process, including the volume transition induced by Ca²⁺ ions, is reversible.

In **Figure 2** the osmotic mixing pressure is plotted for DNA gels equilibrated with solutions containing 40 mM NaCl and different amounts of CaCl₂. We found that over the whole concentration range the osmotic pressure of DNA gels can be satisfactorily described by a Flory-type expression⁵

$$\Pi_{\text{mix}} = - (RT/V_1) [\ln(1-\phi) + \phi + \chi_0\phi^2 + \chi_1\phi^3] \quad (1)$$

where V_1 is the molar volume of the solvent and χ_0 and χ_1 are constants. The curves through the data points show the least squares fits to eq. (1). The dependence of the parameters, χ_0 and χ_1 , on the CaCl₂ concentration of the surrounding solution is displayed in **Figure 3**. It is apparent that χ_1 significantly increases with increasing calcium concentration. χ_0 only slightly changes in the CaCl₂ concentration range explored here. Qualitatively similar behavior has been reported for synthetic polyelectrolyte systems (e.g., polyacrylate gels) when multivalent ions were introduced into gels swollen in nearly physiological NaCl solution.^{6,7}

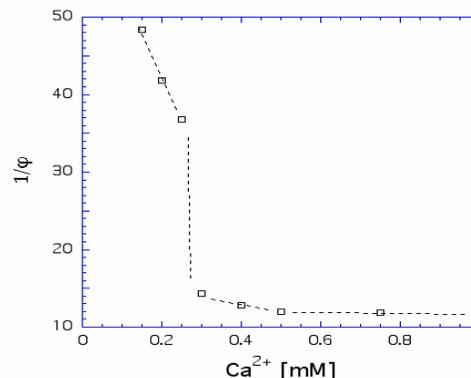


Figure 1. Dependence of the swelling degree of a DNA gel in 40 mM NaCl solution as a function of the CaCl₂ concentration.

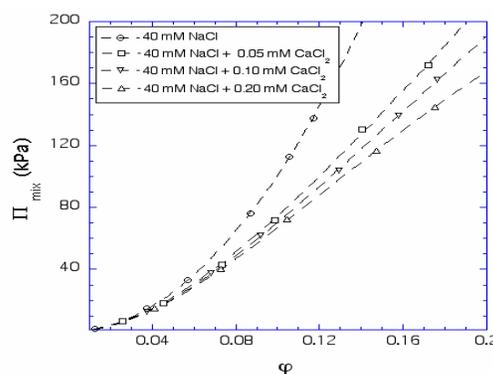


Figure 2. Osmotic pressure vs polymer volume fraction plots for DNA gels in 40 mM NaCl solutions at different CaCl₂ concentrations.

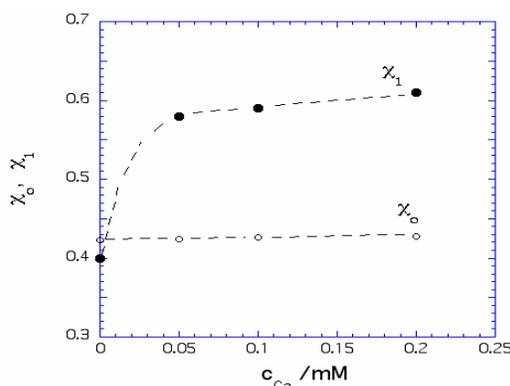


Figure 3. Variation of the Flory interaction parameter as a function of the CaCl₂ concentration in DNA gels swollen in 40 mM NaCl solution.

To identify the main effects of the calcium ions on the structure of the DNA we made SANS measurements at three different CaCl_2 concentrations (0, 5 mM and 30 mM) in the presence of 500 mM NaCl. It is reasonable to assume that at high NaCl concentration the electrostatic interactions between the charged groups of the DNA are screened. Thus, differences in the shape of the scattering curves can be entirely attributed to the effect of the calcium ions on the DNA structure. In **Figure 4** the main effect of the calcium ions is visible in the intermediate q range, where the slope of the SANS curves approaches -1 in the double logarithmic representation. This behavior is characteristic of linearly correlated structures.

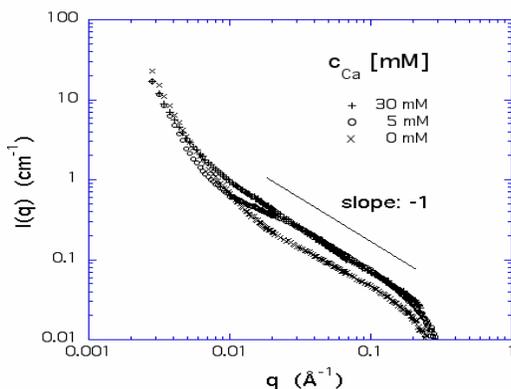


Figure 4. SANS spectra of DNA in 500 mM NaCl solution containing different amounts of CaCl_2 .

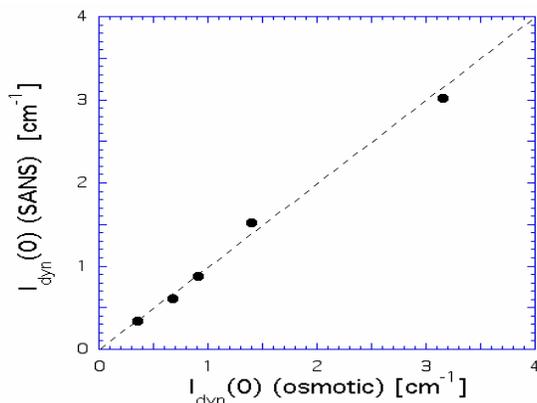


Figure 5. Comparison of the intensity of the thermodynamic component of the SANS response with the intensity calculated from macroscopic osmotic measurements.

Recently we proposed⁸ eq. 2 to describe the scattering from neutralized polyacrylate hydrogels swollen in nearly physiological salt solutions

$$I(q) = I_{\text{dyn}}(q) + I_{\text{sta}}(q) \\ = \Delta\rho^2 \left[\frac{kT\phi^2}{M_{\text{os}}} \frac{1}{(1+qL)} \frac{1}{(1+q^2\xi^2)} + A\phi^n \right] \quad (2)$$

where $\Delta\rho^2$ is a contrast factor, k is the Boltzmann constant, L and ξ are correlation lengths, q is the scattering vector, A and n are constants. The first term in eq 2 describes the thermodynamic concentration fluctuations the amplitude of which is governed by the longitudinal osmotic modulus M_{os} of

the gel, while the second term arises from concentration fluctuations frozen-in by the cross-links.

On the basis of eq 2, the thermodynamic fluctuations can be separated from the intensity scattered by large associations whose contribution to the osmotic properties is negligible. The applicability of eq 2 to DNA gels was studied by comparing the scattering intensity due to thermodynamic fluctuations derived from the SANS spectrum with that obtained from macroscopic measurements. In **Figure 5** the intensity determined from the analysis of the SANS spectra, $I_{\text{dyn}}(q=0)$ (SANS), is compared with that calculated from the concentration dependence of the osmotic swelling pressure and the shear modulus in conjunction with the neutron scattering contrast factors $I_{\text{dyn}}(0)$ (osmotic). The data points lie close to the theoretical straight line of slope 1, indicating that the scattering response of DNA gels are adequately described by eq. 2.

Conclusions

Osmotic swelling pressure measurements indicate that introducing calcium ions into DNA gels swollen in NaCl solution increases the third order Flory-Huggins interaction term and induces a reversible volume transition. This behavior is consistent with previous results obtained for synthetic polyelectrolyte gels swollen in nearly physiological salt solutions. SANS measurements indicate that calcium favors the alignment of DNA strands. It is found that the intensity of the thermodynamic component of the SANS response is in reasonable agreement with that calculated from macroscopic osmotic observations.

Acknowledgements. We acknowledge the support of the National Institute of Standards and Technology, U.S. Department of Commerce, in providing the neutron research facilities used in this work. This work utilized facilities supported in part by the National Science Foundation under Agreement No. DMR-9986442.

References

- (1) Horkay, F.; Basser, P.J. *Biomacromolecules* **2004**, *4*, 232.
- (2) Vink, H. *Europ. Polym. J.* **1971**, *7*, 1411.
- (3) Horkay, F.; Zrinyi, M. *Macromolecules* **1982**, *15*, 815.
- (4) NIST Cold Neutron Research Facility. NG3 and NG7 30-meter SANS
- (5) Instruments Data Acquisition Manual, January 1999.
- (6) Flory, P.J. *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, NY, 1953.
- (7) Horkay, F.; Tasaki, I.; Basser, P.J.: *Biomacromolecules* **2000**, *1*, 84.
- (8) Horkay, F.; Tasaki, I.; Basser, P.J.: *Biomacromolecules* **2001**, *2*, 195.
- (9) Horkay, F.; Hecht, A.-M.; Grillo, I.; Basser, P.J.; Geissler, E.: *J. Chem. Phys.* **2002**, *117*, 9103.