OSMOTIC AND STRUCTURAL PROPERTIES OF BIOPOLYMER SOLUTIONS AND GELS

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Introduction

Interactions between cations (e.,g., Ca^{2+}, Na^{+}, K^{+}) and charged biopolymers are implicated in a wide-range of physiological processes including nerve excitation, muscle contraction, ion channel dynamics, etc.[1,2] Study of the thermodynamic and structural aspects of ion/polymer interactions has the potential to contribute to a more comprehensive understanding of ion mediated structural organization of charged biomacromolecules (e.g., nucleic acids, proteins, proteoglycans, etc.) within cells or extracellular matrix. When all charges on the polyion are identical, the repulsive electrostatic interactions between the charged monomers tend to expand the chain compared to the conformation of the corresponding uncharged polymer. In the presence of added salt, the repulsive interactions are reduced and so is the expansion. The effect of salt concentration on the polyelectrolyte conformation and on the osmotic properties of the solution is not yet fully understood. We are performing fundamental experimental and simulation studies on model polyelectrolyte solutions and gels.[3-5]

In the present work we report measurements made on three different biopolymers (DNA, hyaluronic acid and polyaspartic acid) in nearly physiological salt solutions. We also compare the scattering and osmotic properties of crosslinked and uncrosslinked polymers to estimate the effect of chemical crosslinks on the structure and macroscopic properties. The results suggest that hydrogels made from biopolymers are well-suited model systems to study ion/biopolymer interactions.

Experimental

Solution and Gel Preparation . DNA gels were prepared from DNA solutions by crosslinking with ethyleneglycol diglycidyl ether (Sigma) in solution [pH = 9.0, c_{DNA} = 3% (w/w)].[5] TEMED was used to adjust the pH. DNA solutions were made from deoxyribonucleic acid sodium salt (Sigma). The % G-C content of this DNA was 41.2 %. The molecular weight determined by ultracentrifugation was 1.3×10^6 Da, which corresponds to approximately 2000 basepairs.

Polyaspartic acid solutions were prepared from polyaspartic acid sodium salt (PASP, Sigma M_w = 42 kDa) in 100 mM NaCl. The pH (= 7) was identical in all samples.

Hyaluronic acid solutions were prepared form the sodium salt of hyaluronic acid (HA, Sigma $M_w = 1.2\ 10^6$ Da) in 100 mM NaCl. The pH (= 7) was identical in all samples.

HA gel was made from HA solution by crosslinking with ethyleneglycol diglycidyl ether (Sigma) in solution [pH = 9.0, c_{DNA} = 3% (w/w)]. TEMED was used to adjust the pH. After gelation the HA gel was equilibrated with 100 mM NaCl solution at pH=7.

Methods

Swelling pressure measurements were made by equilibrating the gels with aqueous solutions of poly(vinyl pyrrolidone) (PVP, M_n = 29 kDa) of known osmotic pressure.[6] A semipermeable membrane was used to prevent penetration of the PVP molecules into the swollen network.[7] At equilibrium the swelling pressure of the gel is equal to the osmotic pressure of the PVP solution. After attaining equilibrium (approximately 4-5 days), the polymer concentrations in both phases were measured. This procedure yields for each gel the dependence of ω upon the polymer volume fraction, φ.

Elastic (shear) modulus measurements were carried out on cylindrical gel samples using a TA.XT2I HR Texture Analyser (Stable Micro Systems, UK). The absence of volume change and barrel distortion was checked.

Small angle neutron scattering (SANS) measurements were made at the National Institute of Standards and Technology (NIST), Gaithersburg MD, on the NG3 instrument.[8] Corrections for incoherent background, detector response and cell window scattering were applied.

All experiments were carried out at 25 ± 0.1°C.

Results and Discussion

In **Figure 1** the variation of the osmotic pressure on the polymer volume fraction is shown for a DNA gel (open squares) together with that of a DNA solution (filled circles). (The osmotic pressure of the gel was obtained from the swelling pressure, ω, and the shear modulus, G, by equation $\Pi = \omega + G$.) The data illustrate that the osmotic pressure of the gel is lower than that of the solution by roughly 50%. Similar reduction of Π was reported for other polymer solvent systems. In the semi-dilute concentration region (φ_{DNA} > 0.001) both data sets can be fairly well described by a simple power law. The slopes of the straight lines are 2.36 ± 0.06 (gel) and 2.52 ± 0.05 (solution). Apparently, the gel behaves like a semi-dilute solution in which the polymer concentration is reduced.[5]

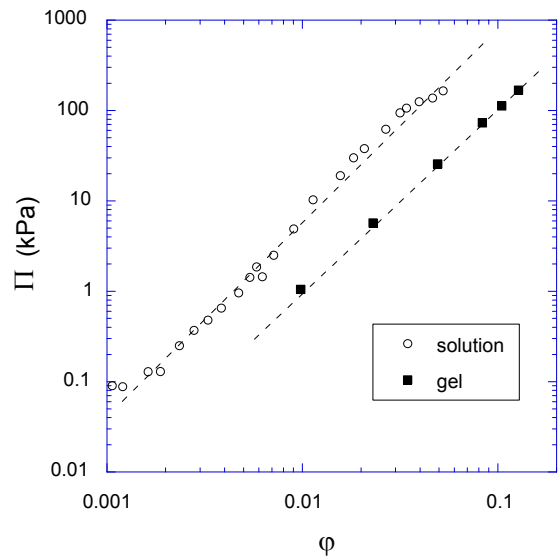

Figure 1. Dependence of the osmotic pressure Π on the polymer volume fraction φ for DNA solution[9] (in 10 mM tris-EDTA buffer) and DNA gel (in 10 mM NaCl).

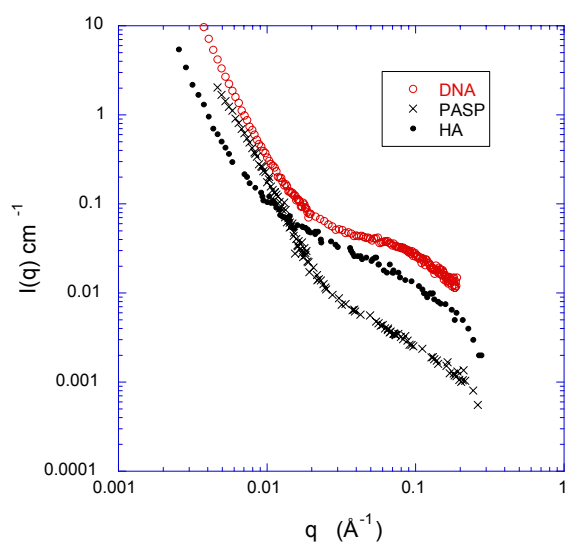

Figure 2. SANS spectra of DNA, HA and PASP solutions. Polymer concentration: 3% (w/w).

Figure 2 shows the neutron scattering intensity *I(q)* as a function of the scattering vector q [$= (4\pi/\lambda)\sin(\theta/2)$, where λ is the incident wavelength and θ the scattering angle] for three biopolymer solutions: DNA, PASP and HA. The scattering curves display similar characteristic features. At low q (< 0.005 Å^{-1}) power law behavior is observed of the form $I(q) \propto q^{-m}$, where $m \approx 4$. This response (Porod scattering[10]) is characteristic of sharp smooth surfaces, and can be attributed to scattering from large clusters of size greater than several thousand ångströms. Similar scattering behavior has been reported for other polyelectrolyte solutions.[11-13] It is quite remarkable that such large clusters can exist in solutions of charged polymers. Clusters can only be stable if there is a net attraction. For example, in DNA solutions the clusters consisting of closely packed phosphate residues posses an enormous negative charge. Schmitz[11] introduced the concept of "temporal aggregates" and attributed the attractive interactions to fluctuating dipolar interactions due to an asymmetric distribution of counterions around the polyelectrolyte chain. It is likely that attractive interactions of nonelectrostatic origin also play a role in solutions of highly charged polyelectrolytes.[12,13]

In the intermediate q range a linear region can be distinguished with a slope of -1. This behavior is typical of solutions containing rod-like structural elements.[14] The shoulder at high q (> 0.1 Å^{-1}) indicates the presence of a second length, which is defined by the geometry of the polymer molecule. The striking similarity among the SANS spectra of these entirely different biopolymers indicates that the thermodynamic interactions that govern the larger scale organization of the dissolved molecules are practically independent of the chemical details (size and chemical structure of the monomer unit, type of polymer backbone, etc.).

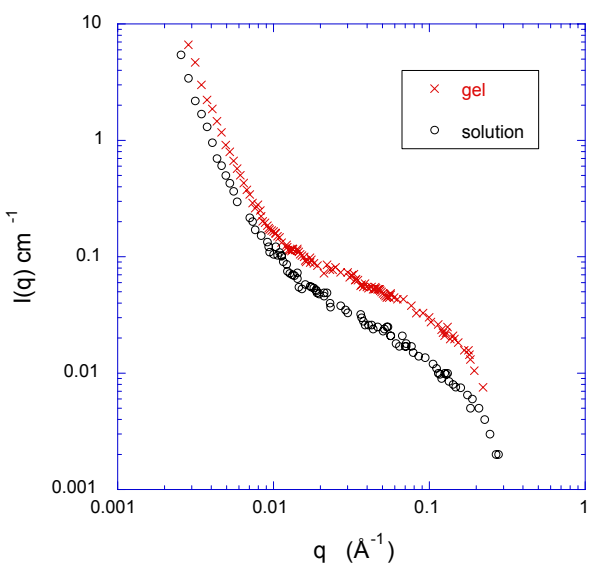

Figure 3. SANS spectra of a HA gel (o) and the corresponding HA solution (x) in 100 Mm NaCl solution.

In **Figure 3** the scattering intensity from a HA gel is compared to that from the corresponding (uncrosslinked) HA solution. At low values of q the intensity from the gel is slightly increased indicating that crosslinking produces clusters. At the high end of the SANS spectra the downturn occurs at the nearly identical q. The main difference between the solution and gel data can be observed in the intermediate q range. In this region the increase in the scattering intensity reflects the effect of crosslinking on the thermodynamic concentration fluctuations. In general, gel formation is accompanied by reorganization of the polymer molecules: in the vicinity of the crosslinks, the local polymer concentration is increased, while between the crosslinked zones the polymer concentration is reduced ("solution-like" region).[15] Previous studies performed on various polymer gels suggest that the osmotic properties of the gel are governed by the solution-like part.[16] This picture is consistent with the reduction of the osmotic pressure due to crosslinking as illustrated for DNA in Figure 1.

Conclusions

Weakly crosslinked gels and polymer solutions exhibit similar structural and osmotic properties. Introducing crosslinks into a biopolymer solution significantly reduces the osmotic pressure. However, the concentration dependence of the osmotic pressure of the crosslinked and uncrosslinked polymers is practically identical. In nearly physiological salt solutions specific interactions between the monovalent counterions and the negatively charged polyanions are negligible.

Small angle neutron scattering measurements show that the organization of biopolymer molecules at larger length scales is only weakly affected by the fine details of the polymer architecture (e.g., size and shape of monomers, type of chemical backbone). The scattering intensity of a crosslinked polymer exceeds that of the corresponding solution. The increased intensity from the gel reflects the reorganization of the polymer strands. The SANS results suggest that the interactions between the polyelectrolyte chains and the surrounding small ions govern the equilibrium conformation of the molecules and the osmotic concentration fluctuations of the solutions.

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References

(1) Tasaki, I.; Byrne, P. M.: *Biopolymers* **1994**, *34*, 209
(2) Tasaki, I.: *Jpn. J. Physiol*. **1999**, *49*, 125
(3) Horkay, F.; Tasaki, I.; Basser, P.J.: *Biomacromolecules* **2000**, *1*, 84
(4) Horkay, F.; Tasaki, I.; Basser, P.J.: *Biomacromolecules* **2001**, *2*, 195
(5) Horkay, F.; Basser, P.J.: *Biomacromolecules* **2004**, *5*, 232.
(6) Vink, H. *Europ. Polym. J*. **1971**, *7*, 1411
(7) Horkay, F.; Zrinyi, M.: *Macromolecules* **1982**, *15*, 1306.
(8) NIST Cold Neutron Research Facility. NG3 and NG7 30-meter SANS
(9) Instruments Data Acquisition Manual, January 1999.
(10) 9. Raspaud, E.; Conceicao, M.; Livolant, F. *Phys. Rev. Lett*., **2000**, *84*, 2533.
(11) 10. Porod, G. In Small Angle X-ray Scattering. Eds. Glatter, O. and Kratky O.
(12) Academic Press, London, 1982.
(13) 11. Schmitz, K.S.; Lu, M.; Gaunnt, J.: *J. Chem. Phys.* **1983**, *78*, 5059
(14) 12. Tanahatoe, J.J.; Kuil, M.E.: *J. Phys. Chem. B*. **1997**, *101*, 9233.
(15) 13. Zhang, Y.; Douglas, J.F.; Ermi, B.; Amis, E.: **2001**, *J. Chem. Phys. 114,*3299.
(16) 14. Higgins, J.S.; Benoit, H.C.:Polymers and Neutron Scattering (Clarendon Press, Oxford 1994).
(17) 15. Horkay, F.; Hecht, A.M.; Geissler, E.: *J. Chem. Phys.* **1989**, *91*, 2706.
(18) 16. Horkay, F.; Burchard, W.; Hecht A.M.; Geissler, E.: *Macromolecules* **1993**, *26*, 3375