Counterion Diffusion Reveals Coil-to-Helix Transition in a Polyelectrolyte

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The conformation of polyions, both naturally occurring and synthetic, critically influences their properties, including chemical reactivity.[1] We have verified that Manning's theory of counterion condensation[2] predicts values of diffusion coefficients for counterions of highly charged polyions, and that experimental measurements of diffusion coefficients of counterions can be used together with this theory to obtain values of charge spacing.[3] Here we show that similar measurements can be used to identify the coil-to-helix transition. The experimental example is the transformation from coil to double helix of the polysaccharide κ-carrageenan (κ-car, Figure 1) induced by a decrease in temperature. The technique employed, steady-state voltammetry, is simple, robust, rapid, and inexpensive in comparison with conventional methods such as circular dichroism, NMR, and X-ray diffraction. It can be employed in unmodified solutions of polyelectrolytes and provides quantitative structural information.

Manning's line charge model[2] describes quantitatively the effect of the polyion on the diffusion coefficient of a singly charged counterion as a function of charge spacing and electrolyte concentration. For highly charged polyions, in the absence of other electrolyte:

$$D_0/D_0{}^\circ = 0.867/\lambda \qquad \text{for } \lambda > 1 \qquad (1)$$

where

$$\lambda = e_0{}^2/4\pi\epsilon_0\epsilon b k_B T = \lambda_B/b \qquad (2)$$

and e_0 is the elementary charge, ϵ_0 the permittivity of vacuum, ϵ the dielectric constant of the solvent, b the (uniform) spacing between charges of the polyion, k_B the Boltzmann constant, T the absolute temperature, and λ_B the Bjerrum length, i.e., the spacing between two singly charged ions for which the electrostatic energy of interaction is equal to $k_B T$ ($\lambda_B = 0.71$ nm in H_2O at 25 °C). In eq 1, D_0 is the value of the diffusion coefficient of the counterion in the solution of polyelectrolyte (without added electrolyte), and $D_0{}^\circ$ is the reference value, measured under the same conditions, except without the polyion. We have shown that eqs 1 and 2 are obeyed quantitatively with respect to variation of both dielectric constant of the medium[4] and linear charge spacing.[3] We have also investigated some aspects of Manning's more complete theory, which predicts the diffusion coefficient ratio for smaller values of λ and for any ratio of added singly charged electrolyte.[2] The rather complicated prediction for smaller values
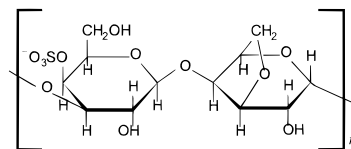

Figure 1. Repeating monomer unit of κ-carrageenan (κ-car).

of λ is less well established than is that of eqs 1 and 2.[3] Manning's extended theory predicts the dependence on the electrolyte ratio, $\gamma = X_E/X_C$, where X_E and X_C are the mole fractions of electrolyte and counterion, respectively.[2a] However, we have found that a simple, semiempirical equation describes accurately the dependence on electrolyte, as follows:[5]

$$D_\gamma/D_\gamma{}^\circ = X_E + (D_0/D_0{}^\circ)X_C \qquad (3)$$

As X_E approaches unity, the value of the diffusion coefficient becomes less sensitive to the properties of the polyion. However, at sufficiently low values of X_E, the measurement of D_γ yields an accurate value of the charge spacing, b, and changes in conformation can be followed by measuring D_γ. The ratio $D_0/D_0{}^\circ$ can be interpreted as the fraction of counterions in the unbound state; the quantity $(1 - D_0/D_0{}^\circ)$ corresponds to the condensed ions in Manning's terminology.[2a] Adding electrolyte frees counterions by ion exchange. However, the excess of ions of the opposite charge bound to the polyion does not change.

The cooperative nature of conformational transitions of polymers has been understood for three decades through the theoretical work of Zimm and Bragg[6] and later refinements. The present understanding of electrostatic interactions in the case of polyions is, however, less satisfactory. Measurement of counterion diffusion coefficients yields the charge spacing and thus should reveal the conformation of a polyion. Changes in diffusion coefficients with, for example, temperature, should reveal changes in conformation.

Diffusion of simple ions in solutions of polyelectrolytes has been studied using radioactive tracers[7] and by NMR spectroscopy.[8] Recently we have probed interactions between small ions and macroions (polyanions,[5,9] colloids,[10] micelles, and liposomes[10c]) by measuring diffusion coefficients of the small ions using steady-state voltammetry at microdisk electrodes, for which the diffusion-limited current, i_d, is[11]

$$i_d = 4nFCDr \qquad (4)$$

where C is the concentration of electroactive ion, r is the radius of the microelectrode, n is number of electrons transferred, and F is the Faraday constant. Thus, the diffusion-controlled current, together with eqs 1, 2, and 4, yields accurate values of the charge spacing.

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† The City University of New York.
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(1) Dautzenberg, H.; Jaeger, W.; Kötz, J.; Philipp, B.; Seidel, C.; Stscherbina, D. *Polyelectrolytes: Formation, Characterization and Application*; Hanser/Gardner Publications: Cincinnati, OH, 1994.
(2) (a) Manning, G. S. *J. Chem. Phys.* **1969**, *51*, 934. (b) Manning, G. S. *Acc. Chem. Res.* **1979**, *12*, 443.
(3) Ciszkowska, M.; Osteryoung, J. G. *J. Phys. Chem. B* **1998**, *102*, 291.
(4) Ciszkowska M.; Osteryoung, J. G. *J. Phys. Chem.* **1996**, *100*, 4630.

(5) (a) Ciszkowska M.; Osteryoung, J. G. *J. Phys. Chem.* **1994**, *98*, 3194. (b) Morris, S. E.; Ciszkowska, M.; Osteryoung, J. G. *J. Phys. Chem.* **1993**, *97*, 10453.
(6) Zimm, B. H.; Bragg, J. K. *J. Chem. Phys.* **1959**, *31*, 526.
(7) Ander, P. In *Water-Soluble Polymers*; Shalaby, S. W., McCormick, C. L., Butler, G. B., Eds.; ACS Symposium Series 467; American Chemical Society: Washington, DC, 1991.
(8) Nilsson, L. G.; Nordenskiold, L.; Stilbs, P. *J. Phys. Chem.* **1987**, *91*, 6210.
(9) (a) Ciszkowska M.; Osteryoung, J. G. *J. Phys. Chem.* **1994**, *98*, 11791. (b) Ciszkowska, M.; Zeng, L.; Stejskal, E. O.; Osteryoung, J. G. *J. Phys. Chem.* **1995**, *99*, 11764. (c) Scordilis-Kelley C.; Osteryoung, J. G. *J. Phys. Chem.* **1996**, *100*, 797.
(10) (a) Roberts, J. M.; Linse, P.; Osteryoung, J. G. *Langmuir* **1998**, *14*, 204. (b) Roberts, J. M.; O'Dea, J. J.; Osteryoung, J. G. *Anal. Chem.* **1998**, *70*, 3667. (c) Roberts, J. R. Ph.D. Dissertation, North Carolina State University, Raleigh, NC, 1998.
(11) Wightman R. M. *Anal. Chem.* **1981**, *53*, 1125A.

In this work, we use the anionic polysaccharide κ-car,[12] a charged, sulfated polysaccharide with one charge per repeating (disaccharide) unit (Figure 1). This polyanion undergoes a transition from coil to double helix on cooling and with increases in ionic strength.[13] This transition has been studied by optical rotation,[14] calorimetry,[15] and conductivity[16] and analyzed within the framework of Manning's limiting laws[17] and the Poisson–Boltzmann cylindrical cell model,[14b,18] yet there are considerable discrepancies among interpretations, especially regarding the multiplicity of the helical conformation and the charge density of conformers.[19]

We have monitored conformational transitions of κ-car, as effected by changes in the temperature, by using steady-state voltammetry[20] to measure diffusion coefficients of a probe ion, Tl^+. Of course, the diffusion coefficient of Tl^+ depends on temperature and viscosity according to the Stokes–Einstein equation,

$$D = k_B T/6\pi r_0 \eta \tag{5}$$

where r_0 is the radius of the diffusing species and η the viscosity of the medium. We measured the diffusion coefficient of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO), a stable, uncharged, electroactive free radical. The diffusion coefficient of Tl^+, properly normalized by diffusion coefficients of TEMPO, reflects only the electrostatic effects described by Manning's theory. The quantity for analysis, then, is the ratio $D_{Tl}(T)/[D_{TEMPO}(T)/D_{TEMPO}(25)] \equiv D_{Tl}'(T)$, the diffusion coefficient of Tl^+ at 25 °C, but with the electrostatic interactions with κ-car characteristic of temperature T. We employ also the similarly normalized current, i_d'. We determined D_{TEMPO} by steady-state voltammetry at a platinum electrode[3] over the range $1 \leq t/°C \leq 50$, and found $D(t)/cm^2\ s^{-1} = (2.46 \times 10^{-9})t^2 + (5.30 \times 10^{-8})t + (3.10 \times 10^{-6})$.

Steady-state voltammograms for reduction of Tl^+ in an aqueous solution of 20 mM $Na_n\kappa$-car are very well defined and similar to those obtained in 20 mM NaCl; the standard deviation of the limiting currents is 2% or less. Voltammograms for oxidation of TEMPO were of comparable quality, with standard deviation not exceeding 2.5%. Thus, the uncertainty in the values of normalized current or diffusion coefficient for Tl^+ does not exceed 7%.

Figure 2 shows the normalized current value for Tl^+ reduction in 20 mM $Na_n\kappa$-car as a function of temperature for two independent experiments, together with results of traditional circular dichroism measurements. For both circular dichroism and
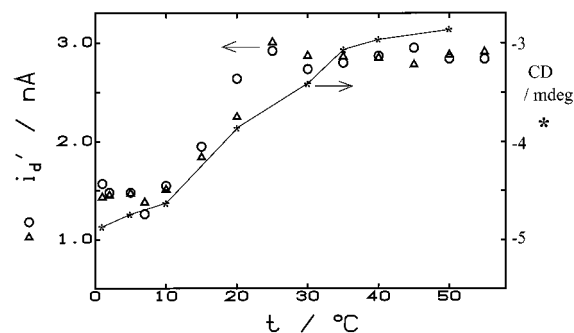

Figure 2. Dependence of the normalized reduction current,[20] i_d', of 0.3 mM Tl^+ (○,△) and CD response[21] measured at 300 nm (∗; points joined by straight-line segments) on temperature of 20 mM κ-car solution.

voltammetry, lower values correspond to the double helix and higher ones to the random coil. The transition is completely reversible, as the results do not depend on the direction of temperature change or on repeated cycling. The transition between the two forms (reported to occur in the range 10–30 °C[14a]) is more clearly defined by voltammetry than by circular dichroism. The normalized limiting current at low temperature for 0.30 mM Tl^+ in 20 mM $Na_n\kappa$-car is 1.48 nA, whereas that at high temperature is 2.83 nA; the value for limiting current in 20 mM NaCl at 25 °C is 3.13 nA. Therefore, the current ratios for helix and coil, the same as diffusion coefficient ratios (eq 4), are 0.473 and 0.904, respectively. From eq 1, the corresponding value of λ for the helix is 1.833. From the rather complicated equation[2a] for values of $\lambda < 1$, the λ value for the coil is 0.818. The average low-current value is obtained from data in the range 1–10 °C, so for this value we choose $T = 278.2$ K and $\epsilon = 85.5$ ($\lambda_B = 0.703$ nm). For the high-temperature range, 25–50 °C, we choose $T = 313.16$ K and = 73.2 ($\lambda_B = 0.729$ nm). Together with eq 2, these values yield $b = 0.38$ and 0.89 nm, respectively.

Considering that the combined uncertainty in the four measurements required to calculate b is about 9%, the value of $b = 0.38$ nm for the double helix agrees reasonably well with the values previously reported, 0.41[14a,15,16] and 0.43[14b,17a] nm, from X-ray data. It is not unreasonable, however, that the value in solution would be somewhat less than that in the solid, due to dielectric screening of charge repulsion in solution. The value of $b = 0.89$ nm for the random coil is, again considering uncertainties, somewhat less than the values of 1.0[14a,15–17a] and 1.03[14b] nm obtained from fully extended molecular models and is more than twice the value of b obtained here for the double helix. This suggests that the transition in the range 10–25 °C corresponds to separation of the duplex with some retention of the helical shape.

The technique and method presented here require no special preparation of the sample, employ simple and inexpensive equipment, and can be carried out in a few hours. For a given solvent and temperature range, the measurements required to compensate for nonelectrostatic effects on diffusion coefficient need be done only once. The relationship between the measured value (the transport-limited current) and the value sought (the charge spacing), as given by eqs 1, 2, and 4, is simple and robust. Furthermore, solutions with some additional electrolyte can be handled readily by simple extensions of this treatment. Finally, the charge spacing is obtained for both conformers in the same solution, using the same technique and interpretation. This minimizes the effect on the derived charge spacing of uncertainties in either the measurements or their interpretation.

(12) Samples of κ-car were dialyzed against ultrapure water, filtered, lypholized, and stored at 5 °C to inhibit bacterial growth. Concentrations of polyelectrolyte are reported as equivalent concentrations, i.e., as concentration of sodium ion. For more details, see ref 3.

(13) Aspinall, G. O. *The Polysaccharides*; Academic Press: New York, 1985.

(14) (a) Nilsson, S.; Piculell, L. *Macromolecules* **1989**, *22*, 3011. (b) Nilsson, S.; Piculell, L.; Jonsson, B. *Macromolecules* **1989**, *22*, 2367.

(15) Anderson, N. S.; Campbell, J. W.; Harding, M. M.; Rees, D. A.; Samuel, J. W. B. *J. Mol. Biol.* **1969**, *45*, 85.

(16) Snoeren, T. H. M.; Payens, T.A. J. *Biochim. Biophys. Acta* **1976**, *437*, 264.

(17) (a) Yuryev, V. P.; Plashchina, I. G.; Braudo, E. E.; Tolstoguzov, V. B. *Carbohydr. Polym.* **1981**, *1*, 139. (b) Arnott, S.; Scott, W. E.; Rees, D. A.; McNab, C. G. A. *J. Mol. Biol.* **1974**, *90*, 253.

(18) (a) Piculell, L.; Rymden, R. *Macromolecules* **1989**, *22*, 2376. (b) Ridout, M. J.; Garza, S.; Brownsey, G. J.; Morris, V. J. *Int. J. Biol. Macromol.* **1996**, *18*, 5.

(19) (a) Rochas, C.; Rinaudo, M. *Biopolymers* **1980**, *19*, 1675. (b) Paoletti, S.; Delben, F.; Cesaro, A.; Grasdalen, H. *Macromolecules* **1985**, *18*, 1834. (c) Manning, G. S. *Macromolecules* **1990**, *23*, 5149.

(20) Electrochemical measurements were carried out with a three-electrode system in a jacketed glass cell enclosed in an aluminum Faraday cage. Mercury film silver-based disk microelectrodes, $r = 15\ \mu$m, were used as working electrodes. Temperature was controlled using a refrigerated circulator (Isotemp model 1016P, Fisher). Staircase voltammetry (step height, 5 mV; frequency, 1 Hz) was applied with a model 283 potentiostat (EG&G PARC) controlled via a personal computer. For more details, see ref 3.

(21) Circular dichroism spectra were obtained using a Jasco J-600 spectropolarimeter for the wavelength range 500–200 nm in a jacketed cell of 1-cm path length.