Electrophoretic Light Scattering Study of Counterion Condensation on Polylysine

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ABSTRACT: Electrophoretic light scattering was used to study the electrophoretic mobility of polylysine as a function of pH in both NaCl and phosphate buffer solutions at an ionic strength of 0.01 M. Results are interpreted in terms of the counterion condensation theory. It is concluded that this theory may be qualitatively correct, but its application to the calculation of the electrophoretic mobility is not yet quantitatively satisfied.

Introduction

The counterion condensation theory[1-3] of Manning describes the association of electrolytes with polyelectrolytes in salt solution. One consequence of this theory is the prediction that the electrophoretic mobility of the polyelectrolyte is inversely proportional to the valence of the counterion. Therefore, electrophoretic mobility measurements can provide experimental proof of the theory. Polylysine in particular has been the subject of several previous studies, but the data do not yield an unambiguous interpretation.

Previous quasielastic light scattering (QELS) and electrophoretic light scattering (ELS) studies of polylysine have focused on the ionic strength dependence of the diffusivity or mobility in the low salt regime. Dynamic light scattering of polylysine in dilute univalent salt solutions[4-6] has established the existence of so-called ordinary and extraordinary phases under different salt concentrations. The apparent diffusion coefficient, D_{app}, was found to first rise and then dramatically drop by over one order of magnitude with decreasing salt concentration (C_s). The onset of this drop in D_{app} occurs at C_s on the order of several millimolar, below which the system is said to be in the "extraordinary phase". By using electrophoretic light scattering (ELS), Wilcoxon and Schurr[7] showed different behavior of the electrophoretic mobility for polylysine in the two different phases and a molecular weight dependence of the mobility in the range $0 < C_s < 0.1$ M. However, the electrophoretic mobility reported by Zero and Ware[8] showed no anomalous behavior through the extraordinary to ordinary phase transition. Wilcoxon and Schurr's results are consistent with counterion condensation theory at $C_s = 0.1$ M, but not at salt concentrations less than 0.1 M. Schurr's mobility data at $C_s < 0.1$ M are about 3 times larger than those of Ware.

In the present paper, we examine the pH dependence of the electrophoretic mobility of polylysine in both 0.01 M NaCl and phosphate buffer solutions with the same ionic strength (0.01 M). The experimental results are compared with Manning's counterion condensation theory.

Experimental Section

Materials. Two polylysine samples were from Sigma with weight average molecular weights (M_w) of 6.7×10^4 and 5.9×10^4, respectively. The polydispersity of each sample (M_w/M_n) was 1.1. NaCl, NaH_2PO_4, Na_2HPO_4, and Na_3PO_4 were all analytical grade from Sigma. All water was deionized and distilled. All solutions were prepared dust-free by filtering through Gelman 0.2-μm syringe filters. Phosphate buffer solutions were prepared by mixing monobasic and dibasic phosphate salts or dibasic and tribasic phosphate salts to the desired pH in such a way as to maintain constant $I = 0.01$ M.

Electrophoretic Light Scattering. ELS measurements were made at four scattering angles (8.6, 17.1, 25.6, and 34.2°), using a Coulter (Hialeah, Florida) DELSA 440 apparatus. The light source was a He-Ne laser ($\lambda = 632.8$ nm). The electric field was applied at a constant current of 1.5 mA. The temperature of the thermostated chamber was maintained at 25 °C. The sample chamber has a total volume of about 1 mL. A rectangular channel runs through a 5-mm thickness of the insert, connecting the hemispherical cavities in each electrode. Electroosmotic corrections were determined by measuring the spatial flow profile in the chamber and taking the mobility readings at a distance 16% of the rectangular length from the respective walls of the chamber. This procedure was verified by using the DELSA electrophoretic mobility standard.

In ELS, the photon-counting heterodyne correlation function for a solution with an electrophoretically monodisperse solute can be written as[9]

$$C(\tau) = \beta_0\delta(\tau) + \alpha_0 + \alpha_1 \exp(-2Dk^2\tau) + \alpha_2 \exp(-Dk^2\tau)\cos(\Delta\omega\tau) \quad (1)$$

where β_0, α_0, α_1, and α_2 are constants independent of correlation time, τ, and $\delta(\tau)$ is the delta function. k is the amplitude of the scattering vector, given by $k = (4\pi n/\lambda)\sin(\theta/2)$, where n is the refractive index of the medium, λ is the wavelength of the excitation light in a vacuum, and θ is the scattering angle. D is the diffusion coefficient. The cosine term is due to simultaneous electrophoresis and diffusion.

The Fourier transform of eq 1 with respect to time, as stipulated by the Weiner-Khinchine theorem,[10] gives the power spectrum:

$$S(\omega) = \beta_0 + \alpha\delta(\omega) + \frac{2(\alpha_1/\pi)Dk^2}{\omega^2 + (2Dk^2)^2} + \frac{\alpha_2 Dk^2}{2\pi} \times \left[\frac{1}{(\omega + \Delta\omega)^2 + (Dk^2)^2} + \frac{1}{(\omega - \Delta\omega)^2 + (Dk^2)^2}\right] \quad (2)$$

where α is a constant independent of ω.

In both eqs 1 and 2, $\Delta\omega$ is the difference between the angular frequency of the scattered light, ω_s, and that of the reference beam, ω_r, which is the same as that of the incident beam. Since the frequency of the incident beam is modulated in the scattered light by the amount of the Doppler shift frequency, $\Delta\omega$ is given by

$$\Delta\omega = \frac{2\pi n}{\lambda} EU \sin\theta \quad (3)$$

where E (V/cm) and U ((μm s^{-1})/(V cm^{-1})) are the applied electric

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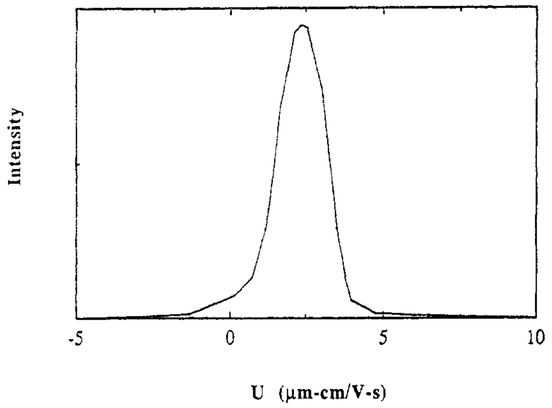

Figure 1. Electrophoretic light scattering spectrum of 0.1 g/L polylysine in 0.01 M NaCl obtained at pH 6.26 and scattering angle 8.6°.

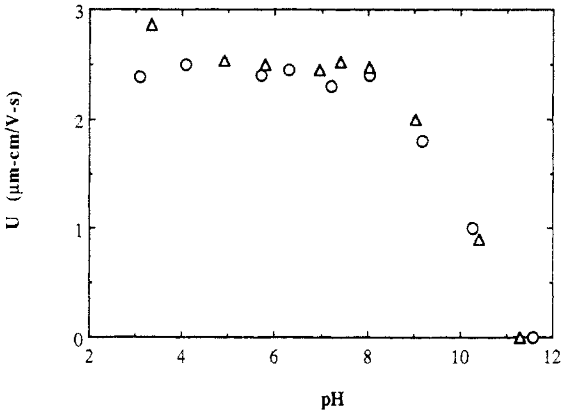

Figure 2. Electrophoretic mobility of 0.1 g/L polylysine in 0.01 M NaCl as a function of pH. Δ and O represent data for polylysine samples with MW 5.9 × 10^4 and 6.7 × 10^4, respectively.

field strength and electrophoretic mobility, respectively. Therefore, U can be directly evaluated from the power spectrum. The U values obtained in this work are repeatable within less than 10%.

Results and Discussion

The electrophoretic light scattering spectrum obtained at a scattering angle of 8.6° for polylysine in 0.01 M NaCl at pH 6.29 is shown in Figure 1. The average frequency of the spectrum gives a mobility of 2.4 (μm cm)/(V s). The mobility obtained at different pH's for polylysine samples of different MW's is plotted in Figure 2. The mobility of polylysine starts decreasing at pH > 8. For pH ≤ 8 the measured U values are constant (2.4 (μm cm)/(V s)) within experimental uncertainty. This mobility change with pH is a consequence of the protonation degree (γ) of polylysine. From potentiometric titration[11] γ is nearly equal to 1 when pH ≤ 8 and changes from 0.9 to 0.1 when pH changes from 10 to 11. Since the mobility of a polyion is identical to the ratio of the ion charge (q) to the ion friction factor (f), as given by

$$U = q/f \tag{4}$$

the proportionality of U to γ is expected. At pH ≤ 8 polylysine is fully protonated. The amply charged polymer retains an extended configuration due to charge repulsion; hence the friction factor remains constant for pH ≤ 8. Along with constant $\gamma \approx 1$, this leads to constant mobility at pH values ≤8. At pH 10, γ equals 0.9, but the mobility obtained is smaller than that at $\gamma \approx 1$ by more than a factor of 2. This suggests that f is a complex function of γ at pH > 8.

According to Hermans,[12] polyelectrolyte chains at high salt are electrophoretically free-draining and the electrophoretic mobility is given by

$$U = q_0/f_0 \tag{5}$$

where q_0 is the residue charge and f_0 is the residue frictional coefficient. Thus, the mobility of free-draining polyelectrolytes should be independent of molecular weight. In support of this, identical mobility is observed for the two different MW polylysine samples, as shown in Figure 2.

In counterion condensation theory a linear polyelectrolyte is modeled as an infinitely thin line of average charge spacing b.[1-3] Small electrolyte counterions are treated as point charges of valence Z. The ratio of the net charge Q (charge after counterion neutralization) to the formal charge of a polyelectrolyte is given by

$$F = \frac{Q}{qN} = |Z|^{-1}\xi^{-1} \qquad \xi > |Z|^{-1}$$

$$F = \frac{Q}{qN} = 1 \qquad \xi < |Z|^{-1} \tag{6}$$

where q is the repeat unit structural charge and N is the degree of polymerization. The linear charge density parameter, ξ, is defined as

$$\xi = \frac{q^2}{\epsilon k_b T b} \tag{7}$$

where ϵ is the dielectric constant of the solvent, k_b is the Boltzmann constant, b is the Bjerrum length, and T is the absolute temperature. The effective charge on the polyion is reduced by counterion condensation at $\xi > |Z|^{-1}$. The dependence of electrophoretic mobility on counterion valence is thus expected, since it is defined as the ratio of the effective charge to the friction coefficient. Manning[1] has developed an analytical form for the electrophoretic mobility, U, as given by

$$U = U^*\left(\frac{\alpha}{\beta}\right)$$

$$U^* = \frac{1}{300}\left\{\frac{Fq}{3\pi\eta b}|\ln(\kappa b)|\right\} \tag{8}$$

where U^* is the electrophoretic mobility calculated by considering screening effects only, η is the solvent viscosity, κ is the Debye–Hückel parameter, and α/β in eq 8 is a correction factor for the asymmetry field. For a univalent salt

$$\alpha = 1$$

$$\beta = 1 + 1.62 \times 10^4 U^*\left(\frac{1}{\lambda_1} + \frac{1}{\lambda_2}\right) \tag{9}$$

where λ_1 and λ_2 are the equivalent conductances of the cation and anion, respectively. Using eq 8 with a mean-spacing b = 3.6 Å between charges in polylysine,[13] we obtain U = 6.07 (μm cm)/(V s) for I = 0.01 M NaCl at T = 298 K. This value is much larger than the observed U = 2.4 (μm cm)/(V s). This discrepancy between experiment and theory for polyelectrolytes at low ionic strength was also reported by Ware et al.[8,14] The observed mobility of U = 2.4 (μm cm)(V s) is consistent with the values (2–3 (μm cm)/(V s)) reported by Zero and Ware,[8] but much lower than those reported by Wilcoxon and Schurr.[7]

Figure 3 shows the electrophoretic mobility of polylysine with MW = 5.9 × 10^4 in phosphate buffer with an ionic strength of 0.01 M, as a function of pH. In the present pH range the anions are a mixture of $H_2PO_4^-$ and HPO_4^{2-}. On the basis of the pH titration of phosphate,[15] the mobility

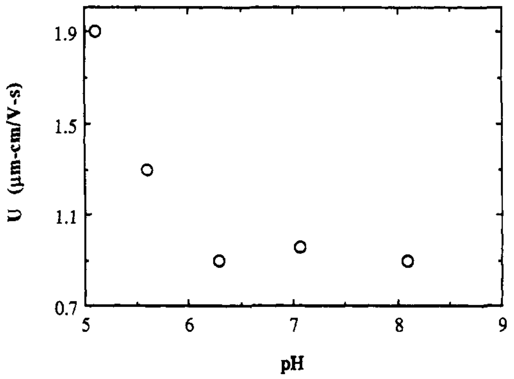

Figure 3. Electrophoretic mobility of 0.1 g/L polylysine (MW 5.9 × 10^4) in phosphate buffer with an ionic strength of 0.01 M as a function of pH.

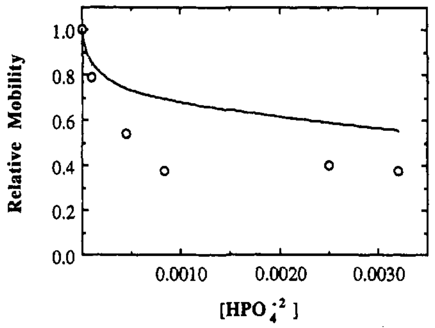

Figure 4. Relative electrophoretic mobility (ratio of U in the presence and absence of divalent HPO_4^{2-}) of polylysine (MW 5.9 × 10^4) in phosphate buffer with an ionic strength of 0.01 M as a function of HPO_4^{2-} mole concentration. The line is calculated from counterion condensation theory.

data in Figure 3 may be plotted as a function of the concentration of the divalent anion HPO_4^{2-}, as shown in Figure 4.

In Manning's theory, for the case of a solution containing a univalent counterion of concentration C_1 and Z-valent counterion of concentration C_Z, the fraction F of charges that remain uncompensated by screening counterions is given by

$$F = 1 - (\theta_1 + Z\theta_Z) \qquad (10)$$

where θ_1 and θ_Z are the relative fractional binding of the monovalent and Z-valent counterions, respectively. θ_1 and θ_Z are determined from the following equations:[2,14]

$$1 + \ln(10^3\theta_1/V_pC_1) = -2\xi(1 - \theta_1 - Z\theta_Z)\ln(1 - e^{-\kappa b}) \qquad (11)$$

$$\ln(\theta_Z/C_Z) = \ln(10^{-3}V_p/e) + Z\ln(10^3 e\theta_1/V_pC_1) \qquad (12)$$

where V_p is the volume about the polyion within which a counterion is considered to be territorially bound. In Figure 4 the dependence of U on [HPO_4^{2-}] is calculated by using eqs 8–12. For the ionic conductances λ_i we used the limiting values in pure water instead of those at the corresponding small-electrolyte concentration. The value of V_p was calculated by using the following equation for a 1:1 salt[2]

$$V_p = 41.1(\xi - 1)b^3 \qquad (13)$$

Clearly, the calculated relative mobility values are only consistent with the current experimental data at very low HPO_4^{2-} concentrations. The discrepancy between the theoretical curve and experimental data may arise from limitations in the model underlying eq 8; we also note that the assumption that the counterions hydration layers are unperturbed by binding, implicit in the calculation of f, might be more problematic for the present system than for, e.g., Na^+ or Mg^{2+} and DNA. The present discrepancy is also in accord with the conclusion reached by Ware et al., namely that the theory overestimates the electrophoretic mobility for DNA[14] and polylysine[8] in low salt solutions. The theory as summarized in eq 8 may be qualitatively correct, but its application to the calculation of the electrophoretic mobility is not yet quantitatively satisfied.

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