Helix-Coil Transition of a Titrating Polyelectrolyte Analyzed within the Poisson-Boltzmann Cell Model. Effects of pH and Counterion Valency

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ABSTRACT: The influence of counterion valency and concentration of salt on the pH-induced helix-coil transition of aqueous poly(glutamic acid) has been studied experimentally and theoretically. The conformational equilibrium is not influenced by the addition of divalent salt (2:1 electrolyte) despite both conformations being charged. Addition of 1:1 electrolyte to mixed systems, 1:1 and 2:1 electrolyte, produced a weak decrease in the helical content. These features were analyzed by the theoretical model based on the Poisson-Boltzmann cell model taking into account both the electrostatic free energy and the free energy of binding due to the protonation of carboxylate groups.

I. Introduction

The helix-coil transition of biological macromolecules such as polypeptides, DNA, and polysaccharides is a well-known phenomenon.[1] In a series of papers[2-6] we have studied electrostatic effects on the coil-helix equilibria for charged polymers by calculating the electrostatic contribution to the propagation parameter in the Zimm-Bragg model[7] of helix-coil transitions. In the case of specific binding of ions to the polymer, such as protons to carboxylate groups, it is also necessary to take into account the degree of binding, which regulates the charge density, as well as the corresponding free energy of binding. The electrostatic interactions and the binding are analyzed utilizing the full Poisson-Boltzmann equation in a cylindrical cell model (PBCM).[6,8,9] Previous investigators have either used electrostatic approximations such as the linearized Poisson-Boltzmann equation[10] or Manning's limiting laws[11,12] or else assumed that the fully charged polypeptide must be in the coil conformation (the Zimm-Rice model).[13]

In earlier studies on nontitrating helix-forming charged polymers it was found that divalent counterions and especially mixtures of mono- and divalent salt could have large and sometimes unexpected effects on the coil-helix equilibria. Furthermore, it was shown that the PBCM could handle these effects in a qualitatively correct and semiquantitatively accurate fashion.[2] It was also found that titrating and nontitrating helix-forming polymers behaved quite differently on addition of 1:1 electrolyte.[6] There appear to be few studies on the effect of counterion valency on the coil-helix equilibria of polypeptides in the literature.[14-16] We therefore believed it to be of interest to extend our previous study on salt effects on polypeptide conformational equilibria to the case of divalent salt and mixtures of mono- and divalent salt.

II. Experimental Section

II.1. Materials. The sodium salt of poly(L-glutamic acid) (PLGA) was obtained from Sigma. The sample was dialyzed against Millipore filtered water and ion-exchanged to the calcium form. The average viscosimetric molecular weight, as reported by Sigma, was 72 500.

II.2. Methods. The pH of the PLGA solutions was adjusted by adding 0.2 M HCl to alkaline solutions. The helical content was evaluated by UV absorbance at 235 nm, using

$$f = (A - A_{\text{coil}})/(A_{\text{helix}} - A_{\text{coil}}) \qquad (1)$$

where f is the fraction of helix conformation, A is the measured absorbance, and A_{coil} and A_{helix} are the absorbances for the all-coil and all-helix solutions, respectively.

III. Theoretical Model

III.1. General Considerations. The helix-coil transition of a high molecular weight polypeptide is well described by the Zimm-Bragg model.[13] In the model two parameters are needed to define the helical content f, i.e., the initiation parameter, σ_{ZB}, which reflects the entropy cost of initiating a helical region, and the propagation parameter, s, corresponding to the free energy of adding a helical unit to an already existing helical region. With the above parameters the helical content is obtained as

$$f = \frac{1}{2}\left(1 + \frac{s-1}{((s-1)^2 + 4\sigma_{\text{ZB}}s)^{1/2}}\right) \qquad (2)$$

In our model the free energy contributions to the propagation parameter are divided into three parts

$$-kT \ln s = \Delta\mu_{\text{el}} + \Delta\mu_{\text{binding}} + \Delta\mu_{\text{non-el}} \qquad (3)$$

where $\Delta\mu_{\text{el}}$ is the electrostatic contribution per repeating unit as calculated from the Poisson-Boltzmann equation, $\Delta\mu_{\text{binding}}$ is the free energy contribution arising from the different degrees of protonation of the coil and helix conformations, and $\Delta\mu_{\text{non-el}}$ is the nonelectrostatic contribution, which is assumed to be independent of pH and salt concentration but will be a function of temperature. The first two terms, $\Delta\mu_{\text{el}}$ and $\Delta\mu_{\text{binding}}$, are interdependent quantities as is described below.

III.2. Electrostatic Model. The theoretical model has been presented in detail earlier,[5] and apart from a small extension discussed below, only a very brief description is given here. In the model the polymer chain is approximated as a cylindrical rod with a radius a and a homogeneous surface charge density σ. The electrostatic interactions are then obtained by solving the Poisson-Boltzmann equation in a cylindrical cell.[8,9,17,18] Since the PLGA is a titrating polymer, the surface charge density will depend on the degree of protonation

$$\sigma = \sigma_0(1 - x) \qquad (4)$$

where σ_0 is the surface charge density of the fully ionized polypeptide and x is the degree of protonation. The degree

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Table I. PLGA Model Parameters

	l/Å	a/Å	pK_0
coil	3.5	11	4.375
helix	1.5	14	4.375, 4.575

of protonation is given by the law of mass action as

$$x = \frac{K'_0 \exp(-e\phi(a)/kT)c_{\mathrm{H},0}}{1 + K'_0 \exp(-e\phi(a)/kT)c_{\mathrm{H},0}} \qquad (5)$$

Here K'_0 is the intrinsic binding constant ($K'_0 = 1/K_0$, where K_0 is the intrinsic dissociation constant), $\phi(a)$ is the electrostatic surface potential as obtained from the Poisson–Boltzmann equation, and $c_{\mathrm{H},0}$ is the hydrogen ion activity ($c_{\mathrm{H},0} = 10^{-\mathrm{pH}}$). Note that the solution of the Poisson–Boltzmann equation depends on the charge density. The Poisson–Boltzmann equation and eqs 4 and 5 therefore have to be solved by an iterative procedure until self-consistency is obtained. Since σ_0 is different for the two conformations (helix, coil), the degrees of protonation for the coil and helical conformations will also differ in general. Given the solution of the Poisson–Boltzmann equation and the degree of protonation, the electrostatic and binding free energies for each conformation can be calculated.[5] The free energy contribution to the propagation parameter is then given by the difference between the coil and helix conformations

$$\Delta\mu_{\mathrm{el}} = \mu_{\mathrm{el}}(\mathrm{helix}) - \mu_{\mathrm{el}}(\mathrm{coil}) \qquad (6)$$

and

$$\Delta\mu_{\mathrm{binding}} = \mu_{\mathrm{binding}}(\mathrm{helix}) - \mu_{\mathrm{binding}}(\mathrm{coil}) \qquad (7)$$

The intrinsic binding constant in eq 5 can be related to the chemical potentials of the standard states as[5]

$$kT \ln (K'_0) = \mu_{\mathrm{P,ion}}{}^0 + \mu_{\mathrm{H}}{}^0 - kT \ln c_{\mathrm{solvent}} - \mu_{\mathrm{PH}}{}^0 \qquad (8)$$

where $\mu_{\mathrm{P,ion}}{}^0$ is the standard chemical potential of a ionized repeating unit, $\mu_{\mathrm{H}}{}^0$ is the standard chemical potential of protons, c_{solvent} is the concentration of the solvent (water), and $\mu_{\mathrm{PH}}{}^0$ is the standard chemical potential of an uncharged protonated repeating unit.

III.3. Model Parameters. The parameters used in this study are the same as were used in our earlier study on PLGA conformational equilibria.[5] Two parameters are needed to specify the rod dimensions, the length per repeating unit l and the radius a. Values of l have been chosen as the standard values for polypeptides in their respective conformations. The values of a and K_0 were taken from a Poisson–Boltzmann analysis of titration curves.[19] Here, as in our previous study, two different values of K_0(helix) have been used since it has been shown by Maxfield et al.[20] that the intrinsic dissociation constants are significantly different for the coil and helix conformations ($\Delta\mathrm{p}K_0 = \mathrm{p}K_0(\mathrm{helix}) - \mathrm{p}K_0(\mathrm{coil}) = 0.13 \pm 0.06$). We have therefore used two different values of the dissociation constant for the helix conformation corresponding to $\Delta\mathrm{p}K_0 = 0.2$ and $\Delta\mathrm{p}K_0 = 0$. These values were chosen since $\Delta\mathrm{p}K_0 = 0.2$ represents an upper limit for $\Delta\mathrm{p}K_0$ and $\Delta\mathrm{p}K_0 = 0$ is taken as an simple model case. In our earlier study[5] different values for $\Delta\mu_{\mathrm{non\text{-}el}}$ and σ_{ZB} were obtained for each value of $\Delta\mathrm{p}K_0$. These values were obtained by a fit to experimental data on the pH-induced conformational transition. The values are listed in Tables I and II. From eq 8 it follows that a higher value of K'_0 for the helical conformation can be related to a difference in the standard chemical potentials:

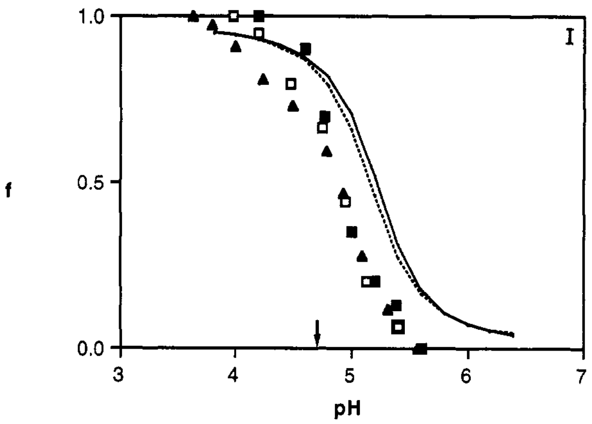

Figure 1. Helical content as a function of pH at different salt concentrations. Experimental conditions: 6.5 mM Ca–PLGA at 25 °C. Salt concentrations: 10 mM $\mathrm{CaCl_2}$ (▲), 20 mM $\mathrm{CaCl_2}$ (□), 30 mM $\mathrm{CaCl_2}$ (■). The theoretical curves were calculated with $\Delta\mathrm{p}K_0 = 0.2$. Salt concentrations: solid line, 10 mM; dashed line, 30 mM. The arrow indicates pH 4.7; below this pH some aggregation of PLGA occurs. The experimental uncertainty (for pH >4.7) is indicated by the error bar in the right corner.

Table II. Helix–Coil Transition Parameters

	$\Delta\mathrm{p}K_0$	
	0	0.2
$\Delta\mu_{\mathrm{non\text{-}el}}$	$-0.2569kT$	$-0.4678kT$
σ_{ZB}	0.0045	0.01

$$K'_0(\mathrm{helix}) > K'_0(\mathrm{coil}) \Rightarrow (\mu_{\mathrm{P,ion}}{}^0 - \mu_{\mathrm{PH}}{}^0)_{\mathrm{helix}} > (\mu_{\mathrm{P,ion}}{}^0 - \mu_{\mathrm{PH}}{}^0)_{\mathrm{coil}} \qquad (9)$$

It can be shown (see Appendix) from electrostatic considerations that if the polymer chain has a different dielectric response than the surrounding water, the difference in radius between the coil and helix conformations is enough to produce a value of $\Delta\mathrm{p}K_0 > 0$. By assuming that electrostatic interaction is the only mechanism for $\Delta\mathrm{p}K_0 > 0$, a simple model with a discrete charge imbedded at the center of a rod with a dielectric constant ϵ_{r} in the range 2–10 and a surrounding medium with $\epsilon_{\mathrm{r}} = 80$ (corresponding to water) provides a quite reasonable estimate of $\Delta\mathrm{p}K_0$.

IV. Results and Discussion

The effect of pH and concentration of divalent salt on the helix–coil equilibria is illustrated in Figure 1. As was found in many earlier studies[5,11,19,21-25] with monovalent salt, the helix conformation is stabilized by a low pH. The stabilization of the helix at low pH takes place because the electrostatic repulsion between charges on the helix is reduced by a high degree of protonation. The influence of the salt concentration on the conformational equilibria is, except at low pH, negligible. The effect of salt at the low pH side of the transition is probably caused by aggregation, which was found to occur at pH values less than 4.7 and does not therefore reflect the pure helix–coil equilibrium. Also for pH less than 4.7 the aggregation causes an increase in the turbidity, making the measurements less accurate. Applying the model described above with the parameters for $\Delta\mathrm{p}K_0 = 0.2$ produces theoretical curves in qualitative agreement with the experimental data (Figure 1). The theoretical curves are shifted to a somewhat higher pH compared to the experimental data, but the very weak effect of adding divalent salt is correctly reproduced. Calculations with $\Delta\mathrm{p}K_0 = 0$ give a similar set of curves as with $\Delta\mathrm{p}K_0 = 0.2$ but with the weak salt

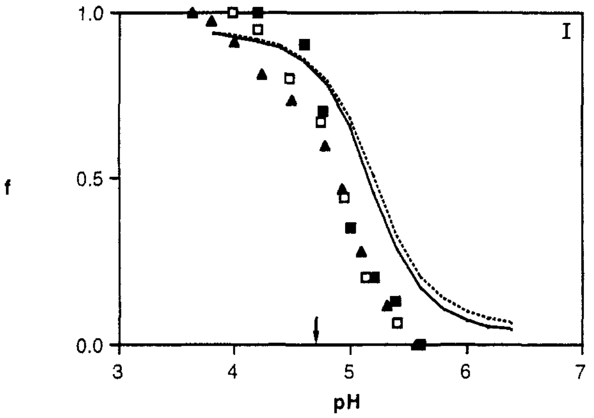

Figure 2. As in Figure 1 but with the theoretical curves calculated with $\Delta pK_0 = 0$.

concentration dependence in the opposite direction (Figure 2). The theoretical curves can be made to coincide in pH with the experimental results by a small adjustment of $\Delta\mu_{\text{non-el}}$ in Table II, $\Delta\mu_{\text{non-el}} = -0.33kT$ for $\Delta pK_0 = 0.2$ and $\Delta\mu_{\text{non-el}} = -0.17kT$ for $\Delta pK_0 = 0$.

The independence in the conformational transition on addition of salt is quite remarkable when compared with nontitrating helix-forming polymers. For nontitrating systems it has been found that the equilibria between two polyelectrolyte conformations depends very sensitively on the presence of divalent counterions.[2-4,26] A few millimolar of added divalent salt may shift the equilibria entirely from 0% to 100% helix, whereas to achieve the same effect by adding monovalent salt, concentrations orders of magnitudes higher may be needed. With PLGA the situation is the opposite; by comparing with our earlier study[5] it can be seen that addition of monovalent salt has a greater effect on the helix–coil equilibria than divalent salt. The reason for the difference between titrating and nontitrating systems lies of course in the ability of a titrating polymer to adjust the charge density on addition of salt since the degree of protonation depends on both pH and the salt concentration as well as the conformational state, helix or coil. As a consequence there are two compensating effects on addition of salt. There is the normal screening of electrostatic interactions on addition of salt, which favors the conformation with the highest charge density, in this case the helix. The screening of electrostatic interactions by adding salt also produces an increased degree of ionization. In the pH interval relevant for the conformational transition this increase in ionization or charge density is significantly more pronounced for the helical conformation, which counteracts the normal screening effect. The combined effect of the normal electrostatic screening and the increase in charge density makes the conformational equilibrium very weakly dependent on the salt concentration in the studied range. By comparing Figures 1 and 2 it can be seen that the theoretically calculated salt concentration dependences with $\Delta pK_0 = 0.2$ and 0, although very weak, are opposite in direction. The reason is that changing ΔpK_0 from 0 to 0.2 increases the binding constant of protons to the helix. The increased binding reduces the charge density of the helical conformation and therefore reduces the electrostatic free energy of the helical conformation. Roughly in the pH interval 4–6, addition of salt will therefore stabilize the coil conformation. The above analysis is rather schematic, *but the mechanism is qualitatively the same as in our* earlier study[5] on the conformational transition of PLGA, where the mechanism was analyzed in some detail in terms of the different free energy contributions for the case of added monovalent salt.

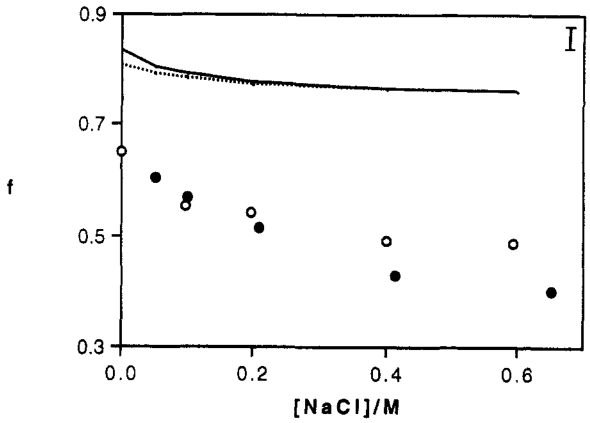

Figure 3. Helical content as function of salt concentration (NaCl) at a fixed pH (pH 4.8) at two different fixed concentrations of $CaCl_2$. Experimental conditions: 6.5 mM Ca-PLGA at 25 °C. Concentrations of $CaCl_2$: 5 mM (●), 20 mM $CaCl_2$ (○). Theoretical curves were calculated with $\Delta pK_0 = 0.2$. Concentrations of $CaCl_2$: solid line, 5 mM; dashed line, 20 mM.

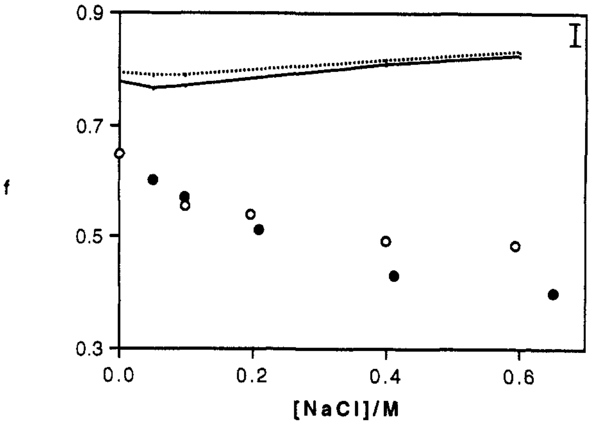

Figure 4. As in Figure 3 but with the theoretical curves calculated with $\Delta pK_0 = 0$.

Since it is known from earlier studies that mixed mono- and divalent salt can produce quite unexpected effects on conformational equilibria, we also studied the influence of mixed salt on PLGA (Figures 3 and 4). In Figures 3 and 4 the helical content is given as a function of added 1:1 electrolyte at two different fixed concentrations of 2:1 electrolyte and at a constant pH. As above, the effect of added salt is weak. The theoretical curve reproduces this result with both values of ΔpK_0. The offset between the experimental and theoretical curves appears since the theoretical curves are shifted to a slightly higher pH compared to the experimental curves (see Figures 1 and 2). Looking at the finer details there are some deviations between the experimental and the theoretical curves. Addition of 1:1 electrolytes decreases the helical content somewhat, and the theory predicts an even weaker salt dependence. With $\Delta pK_0 = 0$ (Figure 4) the theoretical curve predicts a slight increase in helical content, whereas $\Delta pK_0 = 0.2$ (Figure 3) predicts a weak decrease as 1:1 electrolyte is added.

V. Summary

In this work, the effect of salt concentration (2:1 electrolytes and mixtures of 1:1 and 2:1 electrolytes) on the pH-induced conformational transition of PLGA has been studied. It was found that the concentration of 2:1 electrolyte had almost no effect on the conformational transition despite the fact that both conformations, helix and coil, are charged. Also for mixed salt systems, 1:1 and 2:1 electrolyte, the effect of added salt was weak: addition of 1:1 electrolyte to a solution with a fixed concentration of 2:1 electrolyte decreased the helical content slightly; addition of 2:1 electrolyte at a fixed concentration of 1:1 electrolyte produced a weak stabilization of the helical conformation. The weak effect of adding salt is qualitatively correctly reproduced by the theoretical calculations using parameters obtained from systems with only 1:1 electrolyte being present. The drastic difference in sensitivity on addition of salt between titrating and nontitrating system is therefore correctly reproduced by the PBCM. A nonzero value of ΔpK_0 has been demonstrated by Maxfield et al.[20] In an earlier study with 1:1 electrolytes it was found that a nonzero value of ΔpK_0 was necessary to produce a qualitatively correct salt dependence.[5] In view of the results of Maxfield et al. and the results from our previous study on 1:1 electrolytes, a theoretical motivation is given to why there should be such a difference based on the electrostatic self-energy of an ionized unit in the coil and helical conformations, respectively.

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Appendix

To obtain a simple estimate of the difference in the intrinsic binding constant between the coil and helical conformations, we model an ionized unit in the polymer chain as a cylindrical rod with radius a (see Table I) with a dielectric constant ϵ_r and with one isolated point charge q located at the center, $r = 0$ and $z = 0$, r and z being the radial coordinate and the coordinate along the cylinder axis, respectively. The simplification of looking at only one isolated charge corresponds to a low pH since the surrounding repeating units in the polymer chain will then be protonated and uncharged. It can of course be objected against the model that it is unrealistic to represent an ionized repeating unit with a point charge located at the center of the polymer chain and that it would be more realistic to locate the charge closer to the dielectric interface. Changing the location of the charge would make the calculations considerably more involved and is qualitatively the same as changing the dielectric constant. Bringing the charge closer to the interface corresponds to an effective value of ϵ_r closer to the value of ϵ_{H_2O}. Since there is anyhow no rigorous method for determining the dielectric constant of a polymer chain or, for that matter, the radii of the two conformations, we prefer the above crude model as a way of demonstrating that changes in the electrostatic self-energy are enough to produce a value of ΔpK_0 with a correct sign and a reasonable order of magnitude. To further simplify the analysis, the two limiting cases of the salt concentration being zero and infinitely high are studied. Starting with zero salt concentration, the surroundings of the rod are taken to be a simple dielectric continuum ($\epsilon_{H_2O} = 80$). The electrostatic potentials can be calculated by solving the Laplace equation with cylindrical symmetry, the result being[27]

$$\phi_I = \int_0^\infty \left[A(k) I_0(kr) + \frac{q}{2\pi^2 \epsilon_0 \epsilon_r} K_0(kr) \right] \cos(kz)\, dk \tag{A1}$$

and

$$\phi_{II} = \int_0^\infty B(k) K_0(kr) \cos(kz)\, dk \tag{A2}$$

where ϕ_I and ϕ_{II} are the electrostatic potentials inside ($r \le a$) and outside the rod ($r > a$), respectively. I_0 and K_0 are modified Bessel functions of the first kind (K_0 in this context should not be confused with the intrinsic dissociation constant). $A(k)$ and $B(k)$ are response functions describing the effect of the dielectric interface. The second term in eq A1 is the source term describing the Coulomb potential of a point charge in cylindrical coordinates. The response functions can be determined from the ordinary boundary condition, i.e., that the electrostatic potential and the electric displacement should be continuous at the interface

$$\phi_I(r=a) = \phi_{II}(r=a) \tag{A3}$$

and

$$\epsilon_r \frac{d\phi_I}{dr}(r=a) = \epsilon_{H_2O} \frac{d\phi_{II}}{dr}(r=a) \tag{A4}$$

Applying the above conditions to eqs A1 and A2 leads to

$$A(k) I_0(ka) + \frac{q}{2\pi^2 \epsilon_0 \epsilon_r} K_0(ka) = B(k) K_0(ka) \tag{A5}$$

and

$$\epsilon_r A(k) I_1(ka) - \frac{q}{2\pi^2 \epsilon_0} K_1(ka) = -B(k) K_1(ka) \epsilon_{H_2O} \tag{A6}$$

where I_1 and K_1 are modified Bessel functions. Rearranging eqs A5 and A6 finally gives

$$A(k) = \frac{q}{2\pi^2 \epsilon_0} \left(\frac{1}{\epsilon_{H_2O}} - \frac{1}{\epsilon_r} \right) \frac{K_0(ka) K_1(ka)}{I_0(ka) K_1(ka) + \frac{\epsilon_r}{\epsilon_{H_2O}} K_0(ka) I_1(ka)} \tag{A7}$$

The self-energy of the charge due to the dielectric interface can now be calculated as

$$U_{\mathrm{self}} = {}^1/_2 q \phi_{\mathrm{pol}} \tag{A8}$$

where ϕ_{pol} is the polarization potential at the position of the charge due to the dielectric interface and is given by

$$\phi_{\mathrm{pol}} = \int_0^\infty A(k)\, dk \tag{A9}$$

The above equation can be obtained from eq A1 by removing the source term and letting $r = 0$ and $z = 0$. Numerical evaluation of the integral and substitution into eq A8 give the self-energy as

$$U_{\mathrm{self}} \approx \frac{q^2}{4\pi^2 \epsilon_0 a} \left(\frac{1}{\epsilon_{H_2O}} - \frac{1}{\epsilon_r} \right) \left(1.367 - 0.16 \frac{\epsilon_r}{\epsilon_{H_2O}} \right) \tag{A10}$$

for $\epsilon_r < 14$. The other limit of a very high salt concentration means that the electrostatic potential is zero outside the cylinder, which is obtained by letting $B(k) = 0$. The final result is the same as eq A10 after taking the limit $\epsilon_{H_2O} \to \infty$.

The difference in electrostatic self-energy, ΔU, is obtained by introducing the different radii for the coil and helical conformations

$$\Delta U = U_{\mathrm{self}}(a_{\mathrm{helix}}) - U_{\mathrm{self}}(a_{\mathrm{coil}}) \tag{A11}$$

The value of ΔU is an estimate of the electrostatic part of the difference in standard chemical potential (see eq 9) above.

$$\Delta U = (\mu_{\mathrm{P,ion}}{}^0 - \mu_{\mathrm{PH}}{}^0)_{\mathrm{helix,el}} - (\mu_{\mathrm{P,ion}}{}^0 - \mu_{\mathrm{PH}}{}^0)_{\mathrm{coil,el}} \tag{A12}$$

If it is assumed that the mechanism outlined above is the only reason for the inequality of eq 9, it follows by combining eqs 8 and A12 that

$$\Delta pK_0 = 0.4343(\Delta U/kT) \tag{A13}$$

To calculate ΔU, it is necessary to select a value of ϵ_r for the polymer chain. The polymer chain should be considerably less polar than water, and since the dielectric constant of hydrocarbons is about 2, this may be regarded as bounds for ϵ_r, $2 < \epsilon_r < \epsilon_{H_2O}$. In the limit of the salt concentration being zero, $\epsilon_r = 2$ gives $\Delta pK_0 = 1.0$, and $\epsilon_r = 10$ gives $\Delta pK_0 = 0.16$. Calculations in the limit of very high salt concentration give somewhat higher values of ΔpK_0, $\Delta pK_0 = 0.21$ with $\epsilon_r = 10$. With higher values of ϵ_r the value of ΔpK_0 will of course be lower, ΔpK_0 being roughly proportional to $1/\epsilon_r$. With the above result it is possible to test how valid it is to approximate the coil conformation at low charge densities as a rod. Using literature data, the persistence length of an uncharged polypeptide in the coil conformation can be estimated to about 17 Å.[29] Changing the upper integration limit in eq A9 from infinity to one persistence length gives the electrostatic self-energy which originates from within a distance of less than one persistence length along the cylinder axis. The result is that more than 95% of the electrostatic self-energy is generated by the dielectric response less than one persistence length away from the charge. The modeling of the coil conformation as a stiff rod should therefore not be critical in this case.

The estimate of the difference in intrinsic binding constant between the coil and helical conformations calculated above compares quite favorably with the experimental result of Maxfield et al.[20] ($\Delta pK_0 = 0.13 \pm 0.06$). However, not too much emphasis should be put on the precise numerical values, keeping in mind the crudeness of the model, but rather that electrostatic self-energies give a significant contribution to ΔpK_0.

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