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

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ABSTRACT: The dependences on salt concentration and pH of the helix-coil transition of aqueous poly(glutamic acid) have been studied experimentally and theoretically. In the theoretical model the different free energy contributions (electrostatic free energy and free energy of binding protons) to the propagation parameter in the Zimm-Bragg model of helix-coil transitions are calculated from the full Poisson-Boltzmann equation, taking the degree of protonation and electrostatic interactions into account in a self-consistent manner resulting in qualitative agreement with experimental data. For the general case of an equilibrium between two titrating polyelectrolytes the model predicts that the salt concentration dependence may become very weak in a large pH range. In the specific case of poly(glutamic acid) it is found that the salt dependence can only be accounted for by assuming that the intrinsic dissociation constants differ for the helix and the coil conformations.

I. Introduction

The helix-coil transitions of polypeptides have been extensively studied, and the general nature of this phenomenon is well understood.[1] Nevertheless, for charged polypeptides unresolved questions still remain. The problem is taking into account the electrostatic interactions, the degree of protonation (which regulates the charge density), and the corresponding free energy of binding in a self-consistent manner.

In the frequently used approach of Zimm and Rice[2] including a later refinement[3] a direct evaluation of the above free energy contributions is avoided and the variation in the propagation parameter, which determines the helix-coil equilibrium (see below), is obtained directly as a composite quantity through an integration of the titration curves with respect to pH. The Zimm-Rice approach rests on the assumption that the fully charged polypeptide must be in the coil conformation and that the uncharged polypeptide must be in the helix conformation (this latter assumption was removed in the refinement referred to above). Both of these assumptions should become unnecessary in a more complete treatment. There appears, however, to be few attempts in this direction, probably because the model of Zimm and Rice has been very successful in giving almost quantitative agreement with experimental data,[3-6] thus discouraging alternative attempts. Previous studies, which had not adopted the Zimm-Rice model,[2] have used various approximations such as the linearized Poisson-Boltzmann equation[7] or Manning's limiting laws,[8,9] sometimes in combination with degrees of protonation obtained empirically.[8]

In a series of papers[10-12] we have studied the helix-coil transitions of nontitrating polyelectrolytes, in which case the Zimm-Rice model is not applicable, utilizing the full Poisson-Boltzmann equation in the analysis of the electrostatic interactions. In all cases the results were in good agreement with experiment and, especially, it was found that the model could provide valuable insights into general and sometimes unsuspected, electrostatic effects.[10,11] Therefore we believed it to be of interest to extend our previous studies to the case of titrating polyelectrolytes since it is obviously desirable to be able to treat the electrostatic free energy contribution to conformational transitions in the same theoretical model regardless of whether the polyion charges titrate or not. Also, this approach does not involve the approximations of the Zimm-Rice model[2,3] mentioned above. The related problem of charge-regulated protein denaturation has been studied by Stigter and Dill,[13] utilizing an electrostatic approach based on the Poisson-Boltzmann equation in the limit of infinite dilution of the protein.

II. Experimental Section

II.1. Materials. The sodium salt of poly(L-glutamic acid) (PLGA) was obtained from Sigma and used without further purification. The average viscosimetric molecular weight, as reported by Sigma, was 77 800.

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

$$f = \frac{A - A_{coil}}{A_{helix} - A_{coil}} \tag{1}$$

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 helical content of a high molecular weight polypeptide is given by the Zimm-Bragg model as[14]

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

where σ_{ZB} is the initiation parameter, which reflects the extra difficulty of initiating a helical region, and s is the propagation term, which corresponds to the free energy of adding a helical unit to an existing helical region. In our model the free energy contributions to the propagation parameter are divided into three parts

$$-kT \ln s = \Delta\mu_{el} + \Delta\mu_{binding} + \Delta\mu_{nonel} \tag{3}$$

where $\Delta\mu_{el}$ is the electrostatic contribution per repeating unit as calculated from the Poisson-Boltzmann equation, $\Delta\mu_{binding}$ is the free energy contribution arising from the different degrees of protonation of the coil and helix conformations, and $\Delta\mu_{nonel}$ is the nonelectrostatic contribution, which is assumed independent of pH and salt

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concentration but will be a function of temperature. The first two terms, $\Delta\mu_{el}$ and $\Delta\mu_{binding}$, are interdependent quantities as is described below.

III.2. Electrostatic Model. In the Poisson–Boltzmann cell model,[15-18] the free energy of each conformation is calculated from the Poisson–Boltzmann equation in a cylindrical cell model. Here the solution is divided into uniform cylindrical cells, containing water and mobile ions, with the polymer chain centered in the cell and approximated as a cylindrical rod of radius a having a uniform surface charge density σ. The radius of the cell, b, is given by the polymer concentration and, for systems containing both coils and helices, by eqs 26–28. In cylindrical symmetry the Poisson–Boltzmann equation becomes

$$\frac{1}{r}\frac{d}{dr}\left(r\frac{d\phi}{dr}\right) = -\frac{eN_A}{\epsilon_0\epsilon_r}\sum_i c_{i0}z_i \exp(-e\phi z_i/kT), \quad a < r \le b \tag{4}$$

to be solved with the boundary conditions $d\phi/dr(b) = 0$ and $d\phi/dr(a) = -\sigma/\epsilon_0\epsilon_r$ ($\phi(b)$ is taken to be zero by convention). Here c_{i0} is the concentration of mobile ions of charge z_i at $r = b$ (the other symbols carry their usual meanings). The charge density, σ, is given by the degree of protonation as

$$\sigma = \sigma_0(1 - x) \tag{5}$$

where σ_0 is the surface charge density of the fully ionized polypeptide and x is the degree of protonation for the conformation (helix, coil) under consideration. The value of x is given by the law of mass action

$$x = \frac{K_0' \exp(-e\phi(a)/kT)c_{H,0}}{1 + K_0' \exp(-e\phi(a)/kT)c_{H,0}} \tag{6}$$

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, and $c_{H,0}$ is the hydrogen ion activity ($c_{H,0} = 10^{-pH}$). The eqs 4–6 have to be solved by an iterative procedure until the Poisson–Boltzmann equation and the law of mass action are both satisfied.

From the solution of the Poisson–Boltzmann equation the electrostatic free energy of the cell, G_{el}, may be evaluated[17] as

$$G_{el} = E_{el} - TS_{mix} \tag{7}$$

(neglecting volume changes), where

$$E_{el} = (\epsilon_r\epsilon_0/2)\int (\nabla\phi)^2\, dV \tag{8}$$

and

$$TS_{mix} = -N_A kT\sum_i \int c_i[\ln(c_i/c_{solvent}) - 1]\, dV \tag{9}$$

Here E_{el} is the electrostatic interaction energy (per repeating unit), S_{mix} the entropy of mixing due to the distribution of mobile ions in the cell, c_i and $c_{solvent}$ is the concentration of mobile ionic species i and solvent (water), respectively. By differentiating the free energy, one obtains the chemical potentials as[18]

$$\mu_i = \mu_i^0 + kT \ln(c_{i0}/c_{solvent}) \tag{10}$$

(single-ion potential of mobile ionic species i), and

$$\mu_{P,ion} = \mu_{P,ion}^0 - e\phi(a) - E_{el} + kTV_{solvent}N_A\sum(c_{i,av} - c_{i0}) + kT\ln(1 - x) \tag{11}$$

$$\mu_{PH} = \mu_{PH}^0 - E_{el} + kTV_{solvent}N_A\sum(c_{i,av} - c_{i0}) + kT\ln x \tag{12}$$

Here $\mu_{P,ion}$ is the polyion chemical potential for an ionized repeating unit and μ_{PH} is the chemical potential for an uncharged protonated unit, $V_{solvent}$ is the volume of solvent (per repeating unit) in the cell, and $c_{i,av}$ is the cell average concentration of mobile ionic species i in the cell. In the above expressions we have added to the electrostatic terms derived in ref 18 the ideal entropy of mixing between protonated and unprotonated units as given by the logarithmic terms and also the nonelectrostatic standard potentials, μ_i^0, etc. Since the homogeneous surface charge approximation is introduced before the Poisson–Boltzmann equation is solved, the polyion standard potential, $\mu_{P,ion}^0$, will also contain the difference in electrostatic self-energy (or Born energy) between a surface charge and a discrete charge.

The polyelectrolyte chemical potential for a charged unit is then given by

$$\mu_P = \mu_{P,ion} + \mu_H \tag{13}$$

where μ_H is the chemical potential of the protons (the chemical potentials of protons are introduced since the chemical potential of the polymer is taken relative to the fully protonated state, see μ_{nonel} below). Recalling that the protonated and unprotonated units are in equilibrium with each other gives

$$\mu_{PH} = \mu_{P,ion} + \mu_H \tag{14}$$

which leads to the binding isotherm defined in eq 6 with the binding constant defined as

$$kT\ln(K_0') = \mu_{P,ion}^0 + \mu_H^{0\prime} - \mu_{PH}^0 \tag{15}$$

where

$$\mu_H^{0\prime} = \mu_H^0 - kT\ln c_{solvent} \tag{16}$$

The chemical potential of the entire polymer chain is now given by a summation over all repeating units

$$\mu_{polymer} = n_p\mu_P + n_{PH}\mu_{PH} \tag{17}$$

(n_p and n_{PH} are the number of unprotonated and protonated repeating units, respectively) or, expressed per repeating unit

$$\mu_{rep} = (1 - x)\mu_P + x\mu_{PH} \tag{18}$$

For reasons that will emerge in the following we have chosen to divide μ_{rep} into different parts by a rearrangement of the terms in μ_{rep} as

$$\mu_{rep} = (1 - x)\mu_P + x\mu_{PH} = \mu_{el} + \mu_{binding} + \mu_{nonel} \tag{19}$$

with the different terms defined as

$$\mu_{el} = -e\phi(a)(1 - x) - E_{el} + kTV_{solvent}N_A\sum(c_{i,av} - c_{i0}) \tag{20}$$

$$\mu_{binding} = kT\{x\ln x + (1 - x)\ln(1 - x) + (1 - x)\ln K_0' + (1 - x)\ln c_{H,0}\} \tag{21}$$

and

$$\mu_{nonel} = \mu_{PH}^0 \tag{22}$$

The standard chemical potentials in μ_{rep} are now taken

care of by μ_{nonel} and by the binding constant in eq 21 (see also eq 15). Expressions corresponding to $\mu_{el} + \mu_{binding}$ have also been derived in the limit of infinite dilution of the polyion in refs 19 and 20 and applied to the problem of charge-regulated protein denaturation.[13] The above equations can be given rather simple physical interpretations. Starting from left to right in eq 21, the two first terms within the brackets represent the ideal entropy of mixing between occupied and unoccupied sites, the third term represents (the negative of) the intrinsic free energy of binding, and the last term is the entropy of taking one proton from a site to the bulk. Furthermore, in the absence of electrostatic interactions, eq 21 reduces to the free energy of binding derived by Schellman[21] for uncharged components, with the trivial difference that the reference state in Schellman's derivation was $x = 0$ instead of $x = 1$ as in our case (this follows from the definition of μ_{nonel} in eq 22). Equation 20 represents the purely electrostatic part. To us this serves as a motivation for the unconventional step of separating the free energy contributions into different parts.

The chemical potential difference between the two conformations is then given by

$$\Delta\mu_{el} = \mu_{el}(\text{helix}) - \mu_{el}(\text{coil}) \tag{23}$$

$$\Delta\mu_{binding} = \mu_{binding}(\text{helix}) - \mu_{binding}(\text{coil}) \tag{24}$$

and

$$\Delta\mu_{nonel} = \mu_{PH}^{0}(\text{helix}) - \mu_{PH}^{0}(\text{coil}) \tag{25}$$

$\Delta\mu_{nonel}$ thus represents the free energy difference between an uncharged helix and an uncharged coil.

The equilibrium condition specifies that the chemical potentials of all mobile species are the same for coil and helix cells, or equivalently[10]

$$c_{i0}(\text{coil}) = c_{i0}(\text{helix}) \tag{26}$$

For the polyion the difference in the electrostatic and binding chemical potential between the coil and the helix conformations will be finite also at equilibrium ($\Delta\mu_{el} + \Delta\mu_{binding} \neq 0$). However, the total chemical potential difference between the helix and the coil conformations must, of course, be zero at equilibrium; $\Delta\mu_{el} + \Delta\mu_{binding}$ is then exactly balanced by $\Delta\mu_{nonel}$ and by the entropy of mixing helix and coil units as given by the Zimm–Bragg theory.[14]

For a complete specification of the system, two additional conditions are needed to give the correct overall amounts of salt and solvent in the system, i.e.

$$c_{i,av}(\text{coil})\,(1 - f) + c_{i,av}(\text{helix})\,f = \langle c_i \rangle \tag{27}$$

and

$$V_{solvent}(\text{coil})\,(1 - f) + V_{solvent}(\text{helix})\,f = \langle V_{solvent} \rangle \tag{28}$$

where the brackets designate global averaging. If the salt concentration is significantly higher than the polyelectrolyte concentration, the above conditions (eqs 27 and 28) can be neglected since c_{i0} will approach $\langle c_i \rangle$ and the cell model is then equivalent to the Poisson–Boltzmann equation for an isolated rod in solution. An iterative scheme for the inclusion of eqs 27 and 28 has been given in ref 10 for the case of nontitrating polyelectrolytes (note that the sign convention for $\Delta\mu$ is inversed in ref 10).

III.3. Model Parameters. The parameters needed to specify the rod dimensions are 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 the radius a and one value of the intrinsic dissociation constant (see below) have been taken from previous analyses of titration data using the Poisson–Boltzmann equation where the titration curves are well reproduced.[22] Maxfield et al.[23] have shown that the intrinsic dissociation constants for the helix and coil conformations are significantly different ($\Delta pK_0 = pK_0(\text{helix}) - pK_0(\text{coil}) = 0.13 \pm 0.06$), and we will here assume one constant value for the coil conformation (from ref 22) and two different values for the helix conformation corresponding to $\Delta pK_0 = 0.2$ and $\Delta pK_0 = 0$. These two values have been chosen since $\Delta pK_0 = 0.2$ gives a maximum estimate, in view of the relative uncertainty, of the effect of a difference in the intrinsic dissociation constants while $\Delta pK_0 = 0$ has the merit of being a simple and instructive model case. The parameters are listed in Table I.

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

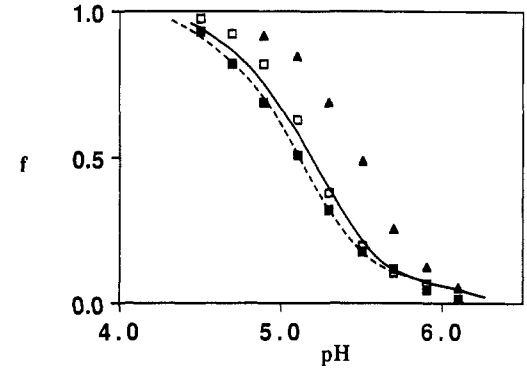

Figure 1. Helical content as a function of pH at different salt concentrations. Experimental conditions: 13 mM PLGA at 25 °C. Salt concentrations: 0.04 M NaCl (▲), 0.1 M NaCl (□), 0.2 M NaCl (■). The theoretical curves were calculated with $\Delta pK_0 = 0.2$ as described in the text. Salt concentrations: solid line, 0.04 M; dashed line, 0.2 M.

As for the source of the difference in pK_0 one likely reason, as suggested by Maxfield et al.,[23] is that the effect is due to a difference between the two conformations in the interaction between an ionized unit and the polymer backbone. In our terminology this would mean that $\mu_{P,ion}^{0}(\text{helix}) - \mu_{P,ion}^{0}(\text{coil}) > 0$, probably due to an image charge interaction[27] between the charge and the polymer backbone, which would reflect that the helix conformation is slightly more hydrophobic than the coil conformation. As was pointed out in the theoretical section, the self-energy of a discrete charge cannot be explicitly dealt with in a homogeneous surface charge approximation, and a difference in the self-energy for discrete charges between the two conformations must therefore be introduced empirically as a difference in pK_0.

IV. Results and Discussion

Our experimental results (Figure 1) on the dependences of the PLGA helix–coil transition on pH and salt concentrations agree with those of previous studies.[3-6,8,24,25] We have here chosen to represent all data, experimental as well as theoretical, as a function of pH rather than, as seems to be more common, a function of the degree of ionization.[3,5,8,24,25] The reason is that in general the degree of ionization will be different for the helix and the coil conformations.

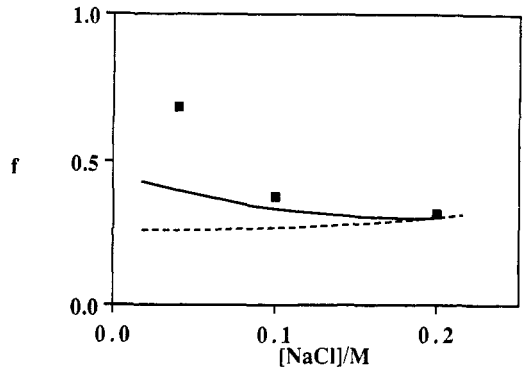

Figure 2. Helical content as a function of salt concentration at a constant pH (pH = 5.3). Filled boxes indicate experimental data, and the lines were calculated with $\Delta pK_0 = 0$ (dashed line) and with $\Delta pK_0 = 0.2$ (solid line).

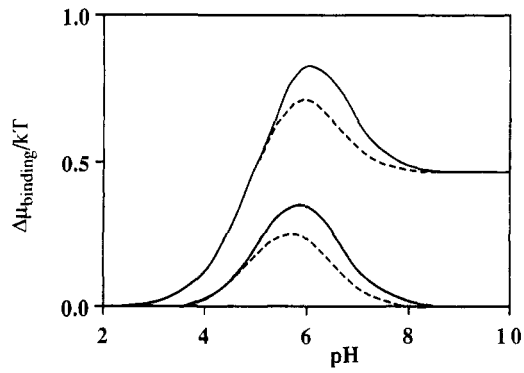

Figure 3. Theoretical pH dependence of $\Delta\mu_{binding}/kT$ at 0.1 M (solid line) and 0.2 M (dashed line) NaCl. The two upper curves are calculated for $\Delta pK_0 = 0.2$ and the two lower for $\Delta pK_0 = 0$.

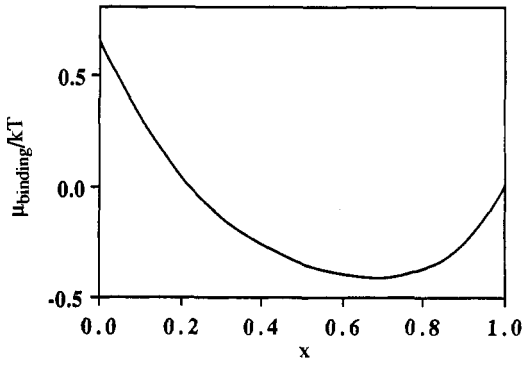

Figure 4. $\mu_{binding}/kT$ calculated as a function of the degree of binding for fixed values of K_0' and $c_{H,0}$ ($K_0'c_{H,0} = 2$ was used).

The helix conformation is stabilized by low pH, and addition of salt shifts the transition to lower pH (in the range 0–0.2 M salt where the identity of the salt is of little importance).[26] The pH dependence is expected since the increased protonation of the polypeptide gives a lower charge density at low pH and the electrostatic repulsion on going from coil to helix is thereby reduced. The salt concentration dependence, on the other hand, is more surprising. As the addition of salts also reduces the electrostatic interactions one would by a similar reasoning expect the helix to be stabilized by added salt (as has been observed for other helix-forming polyelectrolytes), making the helix formation possible at higher charge densities. One would therefore expect addition of salt to shift the transition to higher pH. Instead, the reverse dependence on salt concentration is observed.

The salt dependence is made more clear in Figure 2 where the helix fraction is plotted as a function of the salt concentration at a constant pH (pH = 5.3). Also included in the same diagram are the theoretical predictions from the model (with fitted values for σ_{ZB} and $\Delta\mu_{nonel}$, see below) for the two different values of ΔpK_0. What is noteworthy in Figure 2 is that the salt concentration dependence is very weak for $\Delta pK_0 = 0$ and also opposite in direction compared to experimental data. These features remain throughout the pH interval relevant for the conformational transition. With $\Delta pK_0 = 0.2$, on the other hand, a qualitatively correct behavior is predicted by the model. The theoretical pH dependence for this case has therefore been included in Figure 1, resulting in a qualitative agreement with experimental data (only two curves are shown for clarity).

The adjustable parameters in the model were obtained by a fit to the experimental curve with 0.2 M NaCl (Figure 1), giving $\sigma_{ZB} = 4.5 \times 10^{-3}$ and $\Delta\mu_{nonel} = -0.2569\ kT$ for $\Delta pK_0 = 0$ and $\sigma_{ZB} = 1 \times 10^{-2}$ and $\Delta\mu_{nonel} = -0.4678\ kT$ for $\Delta pK_0 = 0.2$. The fitted values will depend somewhat on the value of pK_0(coil), but the pH and salt concentration dependencies in the helix content are insensitive to small variations in pK_0(coil). The values obtained agree well with an earlier estimate,[23] utilizing the host–guest method (see ref 23 and references cited therein) with PLGA as the guest component and N^5-(4-hydroxybutyl)-L-glutamine as the host, except $\Delta\mu_{nonel}$ for $\Delta pK_0 = 0.2$, which is about 50% too high. This suggests that the difference in the intrinsic dissociation constants is overestimated. The value of the cooperativity parameter indicates an average length for helical regions of about 10–15 repeating units (corresponding to 15–22 Å). Ideally the length of a helical region should be long compared to the Debye length since both helix and coil regions are assumed to be of infinite length in solving the Poisson–Boltzmann equation. The Debye length of a 0.1 M 1:1 electrolyte solution is about 9.6 Å, and the cooperativity is therefore lower than what would have been desirable. However, the length dependence in the electrostatic free energy is not expected to introduce any significant changes. The approach can therefore be expected to be qualitatively correct also when the cooperativity is low.

The qualitative trends in the different contributions to the propagation parameter are relatively easy to understand. Starting with the binding free energy contribution (Figure 3), the dependencies on pH and salt concentration are given by the difference in protonation between the helix and the coil conformations. For $\Delta pK_0 = 0$ (Figure 3) this contribution vanishes at low and high pH when both conformations of the polypeptide are fully protonated and fully ionized, respectively. At intermediate pH, however, the two conformations are protonated to different extents, since the same degree of ionizations would have given rise to different charge densities, and $\Delta\mu_{binding}$ favors the coil conformation. The result that the binding free energy favors the coil conformation can be understood from Figure 4 where $\mu_{binding}$ has been plotted as a function of x for fixed values of K_0' and $c_{H,0}$. The minimum of the free energy curve in Figure 4 occurs at $x = x_0$, which is given by

$$x_0 = K_0'c_{H,0}/(1 + K_0'c_{H,0}) \qquad (29)$$

which is the degree of binding in the absence of electrostatic interactions. Any deviation from this degree of binding increases the binding free energy monotonically, and since $x_{helix} > x_{coil} > x_0$ (due to electrostatic interactions), the binding free energy will always favor the coil conformation. The difference when $\Delta pK_0 \neq 0$ (Figure 3) is that $\Delta\mu_{binding}$ at high pH levels out at a value corresponding to 2.303 ΔpK_0 and, depending on the sign and magnitude of ΔpK_0, the binding free energy difference need not necessarily

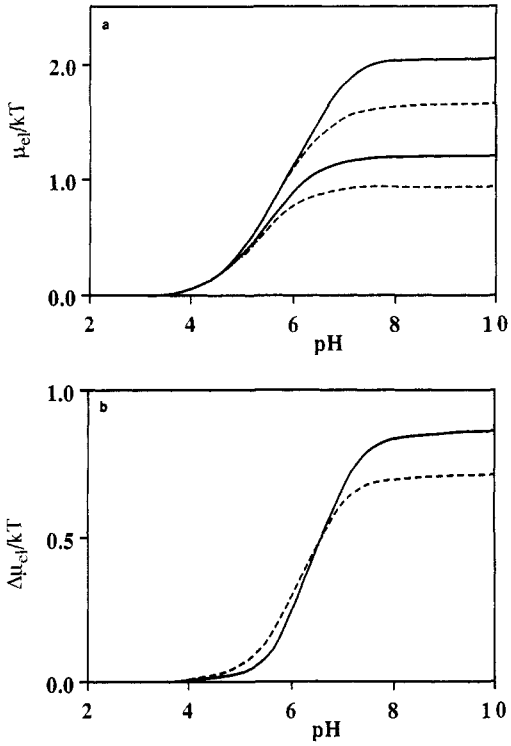

Figure 5. Calculated pH dependence for $\Delta pK_0 = 0$ of (a) μ_{el}/kT and (b) $\Delta\mu_{el}/kT$ at 0.1 M (solid line) and 0.2 M (dashed line) NaCl. The two upper curves in (a) are helix curves and the two lower are coil curves.

favor the coil conformation anymore. On addition of salt $\Delta\mu_{binding}$ decreases (Figure 3), thus decreasing the coil stabilization, since the difference in protonation between helix and coil decreases. Again, this is not necessarily true for $\Delta pK_0 \neq 0$.

The electrostatic free energy for $\Delta pK_0 = 0$ as a function of pH for the two polymer conformations is given in Figure 5a at two salt concentrations. The electrostatic free energy increases monotonically as the ionization increases at increasing pH, and the salt concentration dependence is in the expected direction; the electrostatic interactions are reduced by the addition of salt. The plateau values at low and high pH correspond to the case when both conformations are either fully protonated or fully ionized, as above. The electrostatic contribution to the helix–coil equilibrium is then given by taking the difference between the two conformations (Figure 5b). The pH dependence is still monotonic and in the expected direction, but the salt concentration dependence is slightly more complex. At high pH the polypeptide in both of its conformations is fully ionized regardless of the salt concentration (practically) and the salt dependence is large and in the expected direction (stabilizing the helix on increasing the salt concentration). At pH $\approx$ 6.5, however, there is a crossover and the salt concentration dependence is shifted and also becomes very weak due to two counteracting effects. The electrostatic interactions are reduced by salt as above, but the charge density is not independent of the salt concentration anymore. Addition of salt at intermediate pH increases the degree of ionization significantly. This increase in charge density is most pronounced for the helix, making the electrostatic free energy difference almost independent of the salt concentration and opposite in direction to what is usually expected in the calculated range. At high enough salt concentrations the electrostatic free energy difference must, of course, approach zero at any pH.

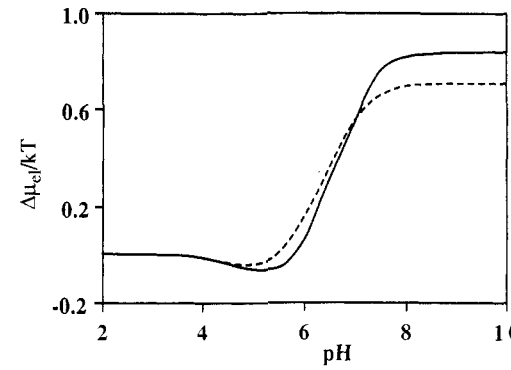

Figure 6. As for Figure 5b, but $\Delta pK_0 = 0.2$.

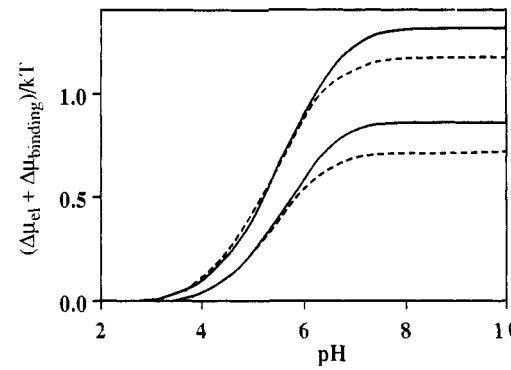

Figure 7. Theoretical pH dependence of $(\Delta\mu_{el} + \Delta\mu_{binding})/kT$ at 0.1 M (solid line) and 0.2 M (dashed line) NaCl. The two upper curves are calculated for $\Delta pK_0 = 0.2$ and the two lower for $\Delta pK_0 = 0$.

The pH and salt concentration dependencies in the electrostatic free energy difference for $\Delta pK_0 = 0.2$ (Figure 6) are similar to those for $\Delta pK_0 = 0$, but the inverse salt concentration dependence at low pH (pH < 6.5) is more pronounced and there is also a weak minimum at low pH. According to our analysis, it is this inversed salt dependence that is responsible for the decrease in the helix content in the experimental data on addition of salt. The reason for this effect can be understood with the aid of Figure 5a. The data in Figure 5a were calculated with $\Delta pK_0 = 0$, and introducing $\Delta pK_0 = 0.2$ corresponds to shifting of the helix curves 0.2 pH units to the right. The part of the helix curves where the salt concentration dependence is weak becomes shifted to a pH where the salt concentration dependence for the coil conformation is higher and addition of salt will therefore stabilize the coil conformation ($\Delta\mu_{el}$ increases). As above, the electrostatic free energy for both conformations must approach zero at salt concentrations that are high enough and $\Delta\mu_{el}$ must therefore start to decrease again. In our calculations this will happen at approximately 0.3 M of salt. This model prediction can, however, not be tested against experimental data since ion specific salt effects start to become important at concentrations higher than 0.3 M and the helix content can both increase and decrease depending on the identity of the salt.[26]

The variation in the sum of the two free energy contributions, $\Delta\mu_{el} + \Delta\mu_{binding}$, with salt concentration and pH (Figure 7) is in qualitative agreement with experimentally accessible data for the PLGA–water system (Figure 1) for $\Delta pK_0 \neq 0$. It may be noted that the minimum in Figure 6 disappears when the binding free energy difference is added.

V. Summary

In this work, we have studied the helix–coil transition of a titrating polyelectrolyte with a theoretical model, which

gives explicit predictions of the effects of salt concentration and pH on the different free energy contributions. One interesting feature is the result that for an equilibrium between two titrating polyelectrolytes, with pK_0 values that are not too different, there may appear a region where the equilibrium becomes almost independent of the salt concentration over quite large ranges in pH and in salt concentration despite the fact that both conformations are charged.

For the specific case of PLGA the model gives a qualitative agreement with experimental data and predicts that the dependence in the conformational transition of the salt concentration is due to a difference in K_0 for the helix and coil conformations in agreement with the experimental finding by Maxfield et al.[23] The model furthermore predicts that, if the intrinsic dissociation constants were the same for both conformations, the salt concentration dependence would have been very weak and in the opposite direction.

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