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Conformational transition of poly(α-L-glutamic acid)

A polyelectrolytic approach *

S. Paoletti [a], A. Cesàro [a], C. Arce Samper [b] and J.C. Benegas [b]

[a] *Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, Università di Trieste, 34127 Trieste, Italy and* [b] *Escuela de Fisica, Universidad Nacional de San Luis, 5700 San Luis, Argentina and International Center for Theoretical Physics, Trieste, Italy*

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The charge-induced conformational transition of poly(α-L-glutamic acid) (PLGA) is considered in this paper from the point of view of proton dissociation. Equations for the excess electrostatic Gibbs energy of dissociation (i.e., ΔpK_a) are derived as a function of the degree of ionization, α. These analytical equations are used to describe some experimental dissociation curves at different polymer and salt concentrations. The dependence of the calculated ΔpK_a with respect to the ionic strength for the two conformational states, α-helical and extended coil, respectively, is rather satisfactorily explained. Even more interesting are the predictions which are derived from this approach for the transition point, α_{tr} which is found to be ionic-strength dependent, in full agreement with the experimental results.

1. Introduction

The conformational stability of biopolymers is determined, besides intramolecular short-range interactions, by a wide number of physico-chemical parameter such as temperature, ionic strength and pH. In the case of weak polyacids, a change in pH may strongly influence the charge density of the (linear) polyion with dramatic alterations in the ensuing electrostatic interactions. As a final consequence, major conformational changes can take place, which are often very cooperative. Although in most physiological cases, the pH and ionic strength are well controlled, situations in which these parameters may reach extreme values cannot be considered as irrelevant.

The aim of this paper is to provide a quantitative description of the conformational transition in aqueous solution of a model biopolymer, viz., poly(α-L-glutamic acid) (PLGA), induced by changes in the pH value over the range from about pH 3.5 to neutrality [1–5]. The solution properties of PLGA have been studied quite extensively for several decades and accurate experimental data on its conformational behaviour are available to test theoretical predictions. The latter will be derived from application of the counterion condensation theory of linear polyelectrolytes [6–8] which has recently been attracting increasing interest in the field of molecular biophysics in view of its successful predictions of biopolyelectrolyte behaviour. The approach will be shown to be quite effective in the case of PLGA, leading one to optimism regarding further application to more 'real' systems like the weak polyacids of the extracellular matrix of both animal and vegetal tissues.

Correspondence address: S. Paoletti, Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, Università di Trieste, 34127 Trieste, Italy.

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2. Theory

2.1. Ionic free energy of a linear polyelectrolyte

The counterion condensation theory [6–8] provides the expression for the (total) ionic free energy of an infinite linear array of charges in a solvent (continuum) of dielectric constant ϵ. Central to the theory is the role of the linear charge density parameter, ξ (vide infra), and its relation to the instability condition

$$|Z_pZ_c|\xi > 1 \tag{1}$$

where Z_p and Z_c denote the numerical values of the charges on the ionic group on the polymer and the counterion, respectively. For the simplest case in which $Z_p = -Z_c = -1$ (to which the whole of the following discussion will be limited), a fraction r of counterions, per mole of fixed charge, will 'condense' around the polyanion whenever $\xi > 1$. The condensation volume, expressed per mole of fixed charge, is V_p. The condensation condition is reached when $b < l_B$, where b is the distance between the (evenly distributed) charges on the polymer axis and l_B the Bjerrum length:

$$l_B = \frac{q^2}{\epsilon k_B T} \tag{2}$$

with q being the value of the elementary charge, k_B Boltzmann's constant and T the absolute temperature. For water at 298 K, $l_B = 7.134$ Å.

It then follows that:

$$\xi = l_B/b \tag{3}$$

It is convenient therefore to separate the two cases:

(i) $\xi \leqslant 1$,

the no-condensation condition, for which the still classical derivation [9] of the electrostatic free energy upon summation of the screened Coulomb potentials on a linear array gives:

$$G^{\text{ion}} = G^{\text{el}} = -n_p \boldsymbol{R} T \xi (q_{\text{st}})^2 \ln(1 - e^{-kb}) \tag{4}$$

where n_p represents the number of polyion charges, q_{st} the number of electron charges which pertain to the structure of the polymer's charged groups (in the present case, $q_{\text{st}} = 1$), $\boldsymbol{R} = Nk_B$ the gas constant, N Avogadro's number, and k^{-1} (Å) the Debye length defined by:

$$k^2 = 4\pi(q^2/\epsilon k_B T)N(C_+ + C_-)/10^{27}$$

where C_+ and C_- denote the (molar) concentrations of counterions and coions, respectively. For practical reasons it is convenient to define:

$$k^2 = \lambda C_p(1 + 2R') \tag{5}$$

where $\lambda = 4\pi l_B N/10^{27}$, C_p represents the equivalent concentration of polymer in the volume V ($C_p = n_p/V$), C_s the molar concentration of supporting 1 : 1 electrolyte and $R' = C_s/C_p$.

(ii) $\xi > 1$

in which counterion condensation takes place to an extent given by

$$r = 1 - \xi^{-1} \tag{6}$$

which derives from a free energy minimisation condition [10]. For this case:

$$G^{\text{ion}} = G^{\text{el}} + G^{\text{mix}}$$

The electrostatic term is

$$G^{\text{el}} = -n_p \boldsymbol{R} T (q_{\text{eff}})^2 \xi \ln(1 - e^{-kb}) \tag{7}$$

where

$$q_{\text{eff}} = q_{\text{st}}(1 - r) \tag{8}$$

is the 'effective' number of electron charges on each structural polymer charged group.

G^{mix} includes the (de)mixing contributions of the condensed ('bound', b) and uncondensed ('free', f) counterions, coions (c) and solvent (solv)

$$G^{\text{mix}} = G^{\text{c}} + G^{\text{f}} + G^{\text{s}} + G^{\text{solv}} \tag{9}$$

$$G^{\text{b}} = n_p \boldsymbol{R} T r \ln\{r/V_pC_p(1 + R')\} \tag{9a}$$

$$G^{\text{f}} = n_p \boldsymbol{R} T (R' + 1 - r) \times \ln\{(1 - r + R')/(1 - V_pC_p)(1 + R')\} \tag{9b}$$

$$G^{\text{c}} = n_p \boldsymbol{R} T R' \ln\{1/(1 - V_pC_p)\} \tag{9c}$$

$$G^{\text{solv}} = n_p \boldsymbol{R} T r \tag{9d}$$

Upon insertion of the minimisation condition (eq. 6) into eqs. 7 and 9, one obtains:

$$\begin{aligned} G^{\mathrm{ion}} = n_{\mathrm{p}}RT\big[& -1/\xi \ln(1-e^{-kb}) \\ & +(1-1/\xi)\ln\{(1-1/\xi)/V_{\mathrm{p}}C_{\mathrm{p}}(1+R')\} \\ & +(R'+1/\xi) \\ & \times\ln\{(R'+1/\xi)/(1-V_{\mathrm{p}}C_{\mathrm{p}})(R'+1)\} \\ & +R'\ln\{1/(1-V_{\mathrm{p}}C_{\mathrm{p}})\}+(1-1/\xi)\big] \end{aligned} \tag{10}$$

Moreover,

$$V_{\mathrm{p}}C_{\mathrm{p}} = \frac{(1-e^{-kb})^2(1-1/\xi)\,e^{\left(1+\frac{kb}{2(1-e^{-kb})}\frac{1}{(2R'\xi+1)}\right)}}{R'+1/\xi} \tag{11}$$

In the case of a (weak) polyacid at a molar concentration of repeating unit of C_{m}, eqs. 4 and 10 can be rewritten for $\xi \leqslant 1$ as

$$G^{\mathrm{ion}} = G^{\mathrm{el}} = -\alpha n_{\mathrm{p}}RT\xi \ln(1-e^{-kb}) \tag{12}$$

and for $\xi > 1$ as

$$\begin{aligned} G^{\mathrm{ion}} = RT\Bigg[& -1/\xi \ln(1-e^{-kb}) \\ & +(\alpha-1/\xi)\ln\frac{\alpha-1/\xi}{V_{\mathrm{p}}C_{\mathrm{p}}(\alpha+R)} \\ & +(R+1/\xi)\ln\frac{R+1/\xi}{(1-V_{\mathrm{p}}C_{\mathrm{p}})(R+\alpha)} \\ & +R'\ln\left(\frac{1}{1-V_{\mathrm{p}}C_{\mathrm{p}}}\right)+\alpha-1/\xi\Bigg] \end{aligned} \tag{13}$$

where α denotes the degree of dissociation of the acidic groups and obviously $C_{\mathrm{p}} = \alpha C_{\mathrm{m}}$; $R = C_{\mathrm{s}}/C_{\mathrm{m}} = R'/\alpha$.

2.2. *Ionic free energy of dissociation of a (weak) polyacid*

Let us now consider the process of dissociation of a linear polyelectrolyte made of structurally identical weak acidic groups, having an 'intrinsic' dissociation constant K^{o}. In the absence of abrupt conformational changes of the chain, the apparent pK, pK_{a}, of the polyacid is a monotonically increasing function of α:

$$\mathrm{p}K_{\mathrm{a}}(\alpha) = \mathrm{pH} + \log(1-\alpha)/\alpha = \mathrm{p}K^{\mathrm{o}} + \Delta \mathrm{p}K^{\mathrm{ion}}(\alpha) \tag{14}$$

where $\Delta \mathrm{p}K^{\mathrm{ion}}(\alpha)$ is the increase in pK_{a} due to the rise in polyelectrolytic electrostatic potential on increasing α.

The counterion condensation theory can give an expression for $\Delta \mathrm{p}K^{\mathrm{ion}}(\alpha)$

$$2.303RT\Delta \mathrm{p}K^{\mathrm{ion}}(\alpha) = \frac{\partial(G^{\mathrm{ion}}/n_{\mathrm{m}})}{\partial\alpha} = G^{\mathrm{ion}}_{\mathrm{diss}}(\alpha)$$

recalling that

$$\xi(\alpha) = \alpha\bar{\xi} = l_{\mathrm{B}}/b(\alpha) = l_{\mathrm{B}}/\alpha\bar{b} \tag{15}$$

where, in the case of the fully ionised polyacid ($\alpha = 1$), $\bar{\xi}$ and $\bar{b}$ designate the dimensionless charge density parameter and the distance between charges, respectively.

For $\xi < 1$, the α derivative of eq. 4 gives:

$$G^{\mathrm{ion}}_{\mathrm{diss}}(\alpha) = -RT\alpha\bar{\xi}\left[2\ln(1-e^{-kb}) + \frac{kb}{e^{kb}-1}\left\{\frac{\alpha}{2(2R+\alpha)}-1\right\}\right] \tag{16}$$

The derivation of $G^{\mathrm{ion}}_{\mathrm{diss}}$ for $\xi > 1$ requires some comment. First, one must recognise r as being a variable which is independent of α, then one should obtain the α derivative of both eqs. 7 and 9, and eventually substitute for r the minimisation condition (eq. 6). This procedure led to consistent results, in both the original derivation of G^{ion} [11], and those of the temperature derivative, ΔH^{ion} [12], and pressure derivative, ΔV^{ion} [13]. Second, V_{p} must be regarded as a physical parameter of the system, functionally independent of α (as well as of r, T, P...), although its numerical value at equilibrium will depend on α (as well as on r, T, P...). Therefore, eqs. 6 and 11 on insertion into the α derivatives of eqs. 7 and 9a–d yield:

$$\begin{aligned} G_{\mathrm{diss}}^{\mathrm{ion}} &= G_{\mathrm{diss}}^{\mathrm{el}} - G_{\mathrm{diss}}^{\mathrm{mix}} \\ &= -\boldsymbol{RT}(1/\alpha\xi)\left[2\ln(1-e^{-kb}) \right. \\ &\quad \left. + \frac{kb}{e^{kb}-1}\left\{\frac{1}{2(2R\bar{\xi}+1)}-1\right\}\right] \\ &\quad +\boldsymbol{RT}\left[(1-1/\alpha\bar{\xi})\ln\frac{\alpha-1/\bar{\xi}}{V_{\mathrm{p}}C_{\mathrm{p}}(R+\alpha)} \right. \\ &\quad \left. +\frac{1}{\alpha\bar{\xi}}\ln\frac{R+1/\bar{\xi}}{(1-V_{\mathrm{p}}C_{\mathrm{p}})(R+\alpha)}+1-1/\bar{\alpha}\bar{\xi}\right] \end{aligned} \quad (17)$$

Analytical results obtained through using eqs. 16 and 17 have been recently reported (without discussion) for the case of polyuronates (carboxylic polysaccharides) [11], although in the study concerned more attention was given to the enthalpic aspect of proton dissociation. The numerical results from application of eqs. 16 and 17 in the cases of two polyacids (fig. 1) with corresponding values of $b = 1.5$ and 3.4 Å are reported in fig. 2, for $C_s = 0$ and $C_s = 0.2$ M, respectively. A general feature of the curves is that they are markedly influenced by the salt concentration and show a rapid increment in their initial part.

All curves are continuous functions of α, however, a discontinuity is shown in the slope for critical values (α_c) corresponding to $\xi = 1$. Beyond α_c the change in slope of the $G_{\mathrm{diss}}^{\mathrm{ion}}(\alpha)$ curve is less marked; the behaviour is reminiscent of that of the $\Delta \mathrm{p}K_a(\alpha)$ functions given in ref. 14. In that study, a thorough analysis was reported for the titration curve of the weak-acid polyelectrolytes based on the counterion condensation theory. Different expressions were given for the two α intervals, i.e. $\alpha < \alpha_c$ and $\alpha > \alpha_c$, respectively, and for the various degrees of sophistication of the model. In all cases, the slope of the $\Delta \mathrm{p}K(\alpha)$ plot was much gentler for $\alpha > \alpha_c$ than that for $\alpha < \alpha_c$.

The complexity of the analytical form of eqs. 16 and 17 can be reduced at first if one concentrates on the case of excess salt (i.e., $R \gg 1$), for which one obtains for $\xi \leqslant 1$:

$$\lim_{C_s/C_m \to \infty} G_{\mathrm{diss}}^{\mathrm{ion}} = -\boldsymbol{RT}\alpha\bar{\xi}\left[2\ln(1-e^{-kb}) - \frac{kb}{e^{-kb}-1}\right] \quad (18)$$

with

$$\lim_{C_s/C_m \to \infty} k^2 = 2\lambda R C_m = 2\lambda C_s \quad (19)$$

and for $\xi > 1$:

$$\begin{aligned} \lim_{C_s/C_m \to \infty} G_{\mathrm{diss}}^{\mathrm{ion}} &= \boldsymbol{RT}\left[1/\alpha\bar{\xi}\ln(1-e^{-kb})^2 + kb/(e^{kb}-1) \right. \\ &\quad \left. +(1-1/\alpha\bar{\xi})\ln\{1/(1-e^{-kb})^2\} + 1 - 1/\alpha\bar{\xi}\right] \\ &= \boldsymbol{RT}\left[-\ln(1-e^{-kb})^2 + kb/(e^{-kb}-1)\right] \end{aligned} \quad (20)$$

where $k^2 = 2\lambda C_s$ and

$$\lim_{C_s/C_m \to \infty} V_{\mathrm{p}}C_{\mathrm{p}} = (1-e^{-kb})^2(\alpha - 1/\xi)/R' \quad (21)$$

For large ξ values, $kb \ll 1$ and linearization of the exponentials in eq. 20 leads further to:

$$\lim_{kb \to 0} G_{\mathrm{diss}}^{\mathrm{ion}} = \boldsymbol{RT}(-\ln k^2b^2 + 1) \quad (22)$$

Eqs. 18 and 22 compare well with eqs. 63 and 60 of ref. 14. In that paper, a detailed theory of the titration curve is described which models protons as site-bound counterions, and metal counterions as territorially bound. The realistic description of the pH-titration curve, free of adjustable parameters [14], has in fact prompted us to extend the approach to the changes in enthalpy of proton dissociation [12]. Eq. 18 of the present paper is identical to eq. 63 of ref. 14, when one takes into account the conversion from $G_{\mathrm{diss}}^{\mathrm{ion}}$ to $(\mathrm{p}K_a - \mathrm{p}K_0)$, with the definition of A being recalled [14]

$$A^2 = 2\lambda(l_B)^2 \quad (23)$$

However, a finite difference, albeit small, between eq. 22 and eq. 60 of ref. 14 is evident. The latter, in the present notation, would read:

$$G_{\mathrm{diss}}^{\mathrm{ion}} = \boldsymbol{RT}[-\ln k^2b^2 + 1 - 1/\alpha\xi] \quad (24)$$

The difference is also evident on consideration of the fact that the common value of eq. 18 and eq. 63 of ref. 14 for $\bar{\xi} = 1$ ($\alpha_c = 1/\bar{\xi}$) reads:

$$\lim_{kb\to 0} G^{\text{ion}}_{\text{diss}} = RT(-\alpha\bar{\xi}\ln k^2b^2+1) = RT(-\ln k^2b_c^2+1) \qquad (25)$$

for $kb \ll 1$ and $b_c = \bar{b}/\alpha_c$, which is also the value of eq. 22 for $b = b_c$. The source of the difference in the values of $G^{\text{ion}}_{\text{diss}}$ between the predictions of eq. 22 and eq. 60 of ref. 14, which is equal to $(-1/\alpha\bar{\xi})RT$ units, remains to be elucidated. A possible cause of this discrepancy might be tentatively traced back to the differentiation of eq. 55 of ref. 14, which implied differentiation of $V'_A(\theta_H)$ with respect to θ_H, however using for $V'_A(\theta_H)$ the value stemming from the (still-to-be-made) minimisation procedure. In the present notation, $V'_A(\theta_H)$ has been simply called V_p, and θ_H is the subfraction of r pertaining to site-bound protons. As already stated, V_p is regarded here as being a physical parameter and therefore has not been subjected to differentiation.

3. Results and discussion

3.1. Biphasic potentiometric titration curves of poly(α-L-glutamic acid)

PLGA is one of the most extensively studied examples [15] of weak polyacids undergoing a pH-induced conformational transition. Evidence has consistently been accumulated to suggest that, at low pH, PLGA adopts an α-helical conformation (fig. 1), for which the average distance between the projections of COOH groups on the helical axis is [16,17]

$b_h = 1.5$ Å

and at higher pH the conformation is that of an extended coil (fig. 1), in which the projected distance between COOH groups is

$b_c = 3.4$ Å

The above values have been used to calculate numerically the data pertaining to the curves of fig. 2, in which the continuous lines and broken lines describe the counterion condensation theoretical $\Delta pK(\alpha)$ behaviour of an all-helical and an all-extended conformation of PLGA, respectively.

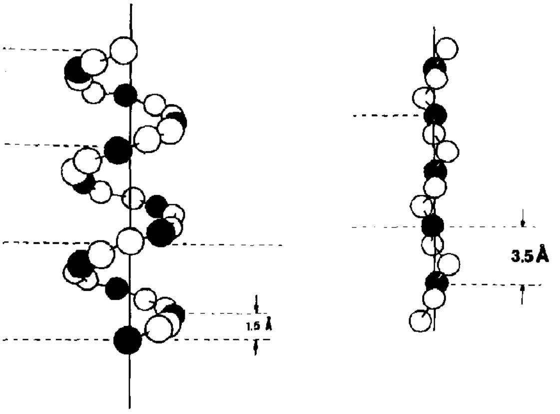

Fig. 1. Schematic structure of the α-helical and 'random' extended conformations of poly(α-L-glutamic acid).

Experimental titration curves for PLGA have been reported by many authors [1–5], with minor differences probably being due to the different experimental conditions. All experimental $pK_a(\alpha)$ curves are distinctively biphasic, i.e., they show two quasi-linear parts on both the low and high

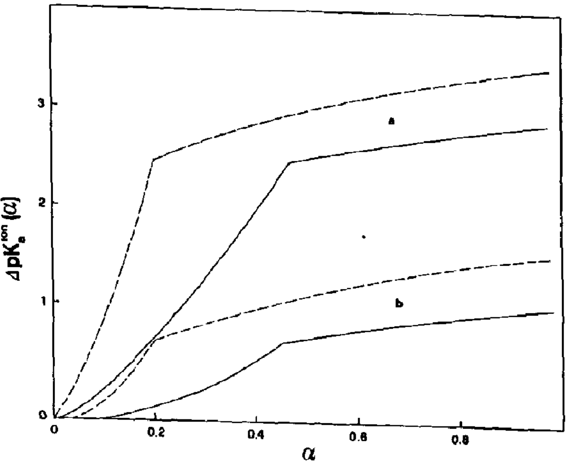

Fig. 2. Dependence of the electrostatic part of the dissociation constant as a function of the degree of ionization α, calculated for the two limiting conformations ((h(- - - - - -) and c(———)) drawn in fig. 1: (a) water; (b) 0.2 M aqueous salt solution, polymer concentration 0.0001 M.

sides, connected by a flat region which expands about over 0.2 α units. Increasing polymer concentration and/or ionic strength reduces both the absolute values of $pK_a(\alpha)$ and the slopes of the $pK_a(\alpha)$ curves: moreover, they widen the flat intermediate portion of the curves and shift it toward higher α values.

All of the available experimental results, except for those of McDiarmid and Doty [3], show at very low α ($\alpha \leqslant 0.2$) the typical pK_a trend due to pH-induced aggregation of the partially protonated α-helical form. This phenomenon has prevented the unambiguous extrapolation of $pK_a(\alpha)$ curves to $\alpha = 0$ in order to obtain the intrinsic pK° value. Many authors have dealt with this calculation [2], particularly in relation to the scope of obtaining the transition Gibbs energy ΔG_{tr}, which will be discussed below.

From the literature results, pK° can be regarded as being modestly dependent on ionic strength and biopolymer conformation. For the present comparison of theoretical results and experimental data, the pK° value has been taken as an adjustable parameter. Fig. 2a reports the full theoretical titration curves along with the experimental data for the sample case of $C_m = 0.0001$ and $C_s = 0.02$ M. A difference in pK° values between the helix and coil forms, ΔpK°, of about 0.24 units results in optimisation of the fit between theory and experiment. Similar operations were carried out for the respective cases $C_s = 0.005$ and $C_s = 0.2$ M, with $C_m = 0.0001$ M in every case. The fitted values of pK° are listed in table 1; from inspection of the data, one can conclude that the pK° may well be dependent on both C_s and conformation in agreement with literature findings but that the degree of dependence is not marked. It is not possible, at present, to attribute this either to a real change in the pK° of the uncharged chain (e.g., by improper calibration procedures neglecting effects of C_s on the activity coefficient of simple electrolytes) or to a change in the value of the non-ionic free energy of transition or both. Fig. 3b depicts an overall comparison between theory and experiments for the three cases.

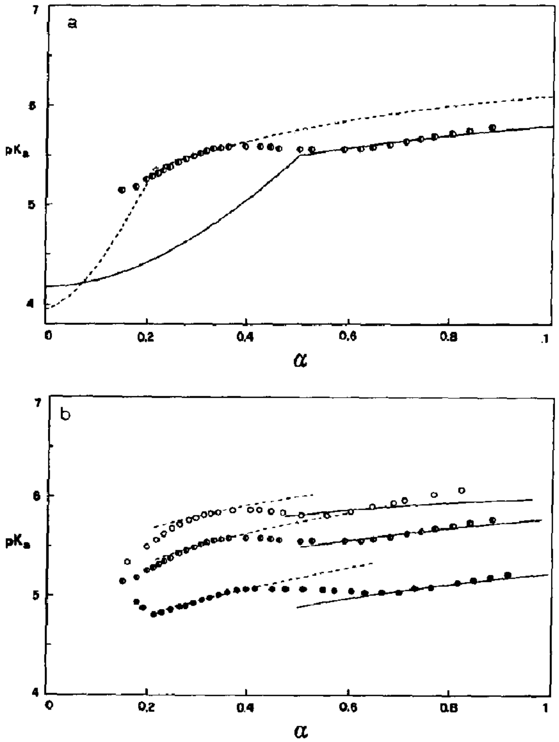

Fig. 3. Comparison of experimental and theoretical data of pK_a of poly(α-L-glutamic acid): (a) full theoretical curves and experimental data ($C_s = 0.02$ M); (b) in NaCl at 0.005 M (○), 0.02 M (◐) and 0.2 M (●). Experimental data taken from ref. 2. Theoretical lines were calculated for the α-helical (- - - - - -) and extended (———) conformations, respectively.

Table 1

Intrinsic pK° values for the helix and coil forms

	pK°		
	$I = 0.005$ M	$I = 0.02$ M	$I = 0.2$ M
Helix	3.90	3.96	4.20
Coil	4.20	4.20	4.30

The agreement between theory and experiment appears rather encouraging, especially as concerns the influence of the conformational state and ionic strength on the pK_a values. A very interesting feature which emerges from inspection of fig. 3b is that the flat intermediate portion of the titration plot, which is currently believed to correspond to the α-helix → coil transition, ranges over α values corresponding to $\xi > 1$, for both the helical form

($\alpha_c = 1/\bar{\xi} = 0.21$) and the extended coil ($\alpha_c = 1/\bar{\xi} = 0.5$).

3.2. Electrostatic contribution to the free energy of transition of poly(α-L-glutamic acid)

So far we have only dealt with a procedure for merely describing the experimental $pK_a(\alpha)$ curve of PLGA in the regions of either single conformation. A further step can be made by considering the total free energy difference (ΔG^t) of the two different conformations of PLGA:

$$\Delta G^{t}_{h\rightarrow c}(\alpha) = \Delta G^{\text{ion}}_{h\rightarrow c}(\alpha) + \Delta G^{\text{non-ionic}}_{h\rightarrow c} \tag{26}$$

where

$$\Delta G^{\text{ion}}_{h\rightarrow c} = G^{\text{ion}}_{c}(\alpha) - G^{\text{ion}}_{h}(\alpha) \tag{27a}$$

$$\Delta G^{\text{non-ionic}}_{h\rightarrow c} = G_c(\alpha = 0) - G_h(\alpha = 0) \tag{27b}$$

The values of $G^{\text{ion}}_c(\alpha)$ and $G^{\text{ion}}_h(\alpha)$ are given by the counterion condensation theory through eqs. 11 and 12 for $\xi \leqslant 1$ and $\xi > 1$, respectively, expressed per mol repeating unit:

$$G^{\text{ion}}(\alpha) = G^{\text{ion}}/n_m \tag{28}$$

$\Delta G^{\text{non-ionic}}_{h\rightarrow c}$ is the contribution to the free energy difference between the two conformations which includes all sources of interactions different from those that are purely electrostatic. By virtue of the manner in which it is computed from the experimental pH-titration data, it is in fact pertinent only to the uncharged state ($\alpha = 0$). At least as a first approximation, however, the reasonable assumption can be made that $\Delta G^{\text{non-ionic}}_{h\rightarrow c}$ is not dependent on α, so that eq. 26, although not rigorous, can be used over the whole range of α. The experimental $\Delta G^{\text{non-ionic}}_{h\rightarrow c}$ values show a slight or moderate dependence on the ionic strength, I, which indicates that the above assumption is indeed an oversimplification, however,the magnitude of their dependence is small [1,2]. In fig. 4 the dependence on α of G^{ion}_h and G^{ion}_c is demonstrated for PLGA at two different values of I using the above procedure and an average value of $\Delta G^{\text{non-ionic}}_{h\rightarrow c} = 320$ cal/mol [2]. An interesting observation from the data in fig. 4 is that the value of α at which the two free-energy curves intersect, α_{tr}, is uniquely determined by the α dependence of the G^{ion} functions and by the experimentally determined values of b_h, b_c and $\Delta G^{\text{non-ionic}}_{h\rightarrow c}$. No adjustable parameter, therefore, is needed for the analytical description of the transition of PLGA induced by a change in α. The initial result of the procedure is that the dependence of α_{tr} on I is then predicted in a straightforward manner, and the trend in its behaviour is in good agreement with that observed experimentally.

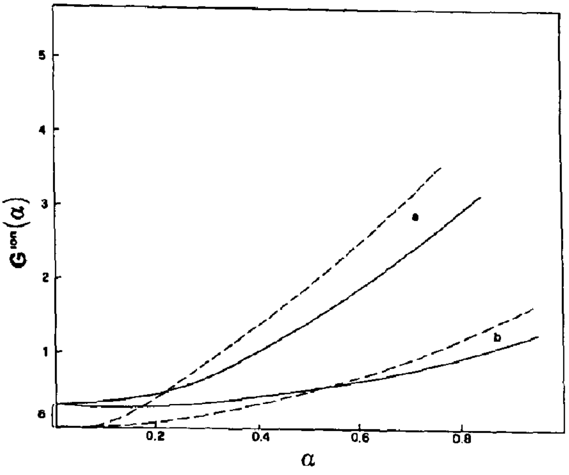

Fig. 4. Theoretical electrostatic free-energy change ($G^{\text{ion}}(\alpha)$) as a function of the degree of ionization α for the two limiting conformations ((h(- - - - - -) and c(———)) drawn in fig. 1: (a) water, (b) 0.2 M aqueous salt solution. (δ) $\Delta G^{\text{non-ionic}}_{h\rightarrow c}$ (see text).

The α dependence of the G^{ion} functions for the two conformational forms clearly justifies the common assertion that the transition from the α-helical to coil form occurs when electrostatic repulsion overcomes attractive interactions. The counterion condensation theory thus enables one to perform a simple calculation of the excess Gibbs energy of electrostatic nature which renders, at a given charge density, the compact α-helical form unstable with respect to the more extended conformation.

Moreover, if one again resorts to the literature for a description of two-state cooperative transitions [15], then one can express the fractions of

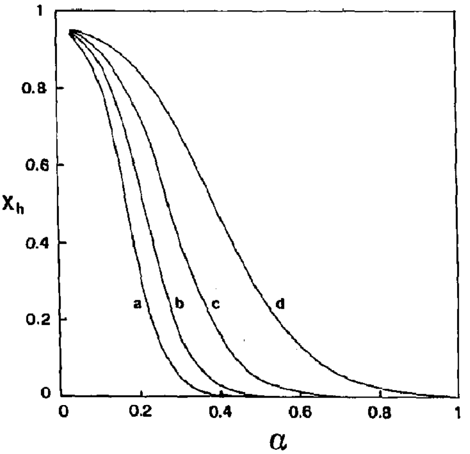

Fig. 5. Dependence of the fraction of helical conformation, X_h, as a function of the degree of ionization in aqueous salt solution. Data calculated according to eq. 29 with the experimental conditions of fig. 3 (b, 0.005 M; c, 0.02 M; d, 0.2 M), and in water (a).

α-helical and extended conformations within the Boltzmann statistics:

$$\frac{1 - X_h(\alpha)}{X_h(\alpha)} = \frac{X_c(\alpha)}{1 - X_c(\alpha)} = e^{(-N\Delta G^{t}_{h\to c}(\alpha)/RT)} \tag{29}$$

where N is the size of the cooperative unit. The results obtained for the calculation of $X_h(\alpha)$ are reported in fig. 5 for various values of I, using a value of $N = 20$ [18].

The incremental change in α_{tr} with respect to the ionic strength is in agreement with the experimental results, showing that the effects of the ionic strength on the electrostatic contribution to the transition process are satisfactorily described. Furthermore, from the data of fig. 5, it is interesting to note that the apparently increasing broadness of the transition curves (i.e., X_h vs. α) with rising ionic strength is a direct result of the polyelectrolytic approach.

4. Conclusions

A rather complete description of the pH-induced transition of PLGA has been achieved. In the first instance, a reasonable description of the $pK_a(\alpha)$ curve for the two conformational states of PLGA has been given. This ensures that both the theoretical description of the α-dependent electrostatic free energy (from counterion condensation theory) and the choice of structural parameters b (from experimental data) are fairly correct. Furthermore, it has been shown that, in addition to describing the experimental $pK_a(\alpha)$ curves, one can predict the effect of ionic strength on the pH-induced transition, with the aid of the counterion condensation theory and of independent experimental parameters.

However, one should bear in mind the limitations of this approach. To mention a few examples, one might start questioning the use of pK^0 as an adjustable parameter, and then argue as to the validity of a rigid conformational model being assumed for both helical and extended coil forms upon change in α. The independence of $\Delta G^{\text{non-ionic}}_{h\to c}$ and that of the size of the cooperative unit, N, from both α and I are also certainly oversimplifications. In fact, the final results indicate that improvements to the approach are not only possible, but also desirable. In particular, one can resort to theoretical treatments which describe the pK_a curves of the weakly ionizable polyacids more closely. Attempts in this direction have been undoubtedly successful but with enormous sacrifice in simplicity and/or independence of adjustable parameters [19,20].

Nonetheless, a more realistic view of the polyelectrolyte model described in this paper is worthy of consideration. One concerns the rather drastic assumption made in the present model of the absence of any statistical distribution of charged groups along the real chain [21]. On the other hand, one might even partially sacrifice the dogma of 'experiment first', recalling that the so-called 'experimental parameters', such as b, $\Delta G^{\text{non-ionic}}_{h\to c}$ or N, do not originate solely from the experiment, but result from a model interpretation as well. If so, one might even improve its ability to describe the system by fitting the true experimental data (i.e., pH) with the analytical expression derived from the counterion condensation theory, with modest adjustments to those parameters.

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References

1 O. Olander and A. Holtzer, J. Am. Chem. Soc. 90 (1968) 4549.
2 M. Nagasawa and A. Holtzer, J. Am. Chem. Soc. 86 (1964) 538.
3 R. McDiarmid and P. Doty, J. Phys Chem. 70 (1966) 2620.
4 M. Rinaudo and A. Domard, Biopolymers 12 (1973) 2211.
5 K. Nitta, M. Yoneyama and N. Ohno, Biophys. Chem. 3 (1975) 323.
6 G.S. Manning, J. Chem. Phys. 51 (1969) 924.
7 G.S. Manning, Q. Rev. Biophys. 11 (1978) 179.
8 G.S. Manning, Annu. Rev. Phys. Chem. 23 (1972) 117.
9 G.S. Manning, in: Polyelectrolytes, eds. E. Sélégny, M. Mandel and U.P. Strauss (Reidel, Dordrecht, 1973) vol. 1, p. 9.
10 G.S. Manning, Biophys. Chem. 7 (1977) 95.
11 A. Cesàro, F. Delben, A. Flaibani and S. Paoletti, Carbohydr. Res. 161 (1986) 355.
12 S. Paoletti, A. Cesàro, F. Delben, V. Crescenzi and R. Rizzo, in: Microdomains in polymer solutions, ed. P. Dubin (Plenum, New York, 1985) p. 159.
13 S. Paoletti, F. Delben, A. Cesàro and H. Grasdalen, Macromolecules 18 (1985) 1834.
14 G.S. Manning, J. Phys. Chem. 85 (1981) 870.
15 D. Poland and H. Scheraga, Theory of helix-coil transitions in biopolymers (Academic Press, New York, 1970).
16 S. Krimm and J.E. Mark, Proc. Natl. Acad. Sci. U.S.A. 60 (1969) 1122.
17 W.A. Hiltner, A.J. Hopfinger and A.G. Walton, J. Am. Chem. Soc. 94 (1972) 4324–4327.
18 R.L. Snipp, W.G. Miller and R.E. Nylund, J. Am. Chem. Soc. 87 (1965) 3547.
19 R. Liem, D. Poland and H. Scheraga, J. Am. Chem. Soc. 92 (1970) 5717.
20 J. Vila, J. Chem. Phys. 84 (1986) 6421.
21 A. Cesàro, S. Paoletti, R. Urbani and J.C. Benegas, Int. J. Biol. Macromol. 11 (1989) 66.

Glossary

Symbol	Meaning
b	average distance between charges projected onto the polymer axis
$\bar{b}$	average distance between structural groups
C_+	molar concentration of counterions
C_-	molar concentration of coions
C_m	molar concentration of polymer repeat units
C_p	molar concentration of polymer charged groups
C_s	molar concentration of univalent added salt
l_B	Bjerrum length
k	Debye length
k_B	Boltzmann's constant
n_p	number of polyion charges
N	Avogadro's number
pK_a	apparent dissociation constant
pK°	intrinsic dissociation constant
r	fraction of condensed counterions
$\mathbf{R}$	gas constant
R	salt/polymer charge ratio ($= C_s/C_m = R'/\alpha$)
R'	salt/polymer concentration ratio ($= C_s/C_p$)
T	temperature
V_p	counterion condensation volume
α	degree of dissociation
α	degree of neutralization
α_c	critical value of α for $\xi = 1$
α_{tr}	value of α at the midpoint of the helix-coil transition
θ_H	fraction of site-bound protons
ξ	charge density parameter
ξ	structural charge density parameter