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Polyelectrolytic aspects of the titration curve

The semi-flexible model *

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An analysis is given of the theoretical approach to the quantitative description of proton dissociation curves for weak polyacids. The basic model of the counterion condensation theory has been used, with the modification reported in the preceding study (S. Paoletti, A. Cesàro, C. Arce Samper and J.C. Benegas, Biophys. Chem. 34 (1989) 301). In this paper we demonstrate the effect of relaxing the hypothesis of a rigid conformation on the polyelectrolytic properties of weak polyacids. As an application of the present approach, a description is given of the titration curves for two weak polyacids, poly(DL-glutamic acid) (PDLGA) and poly(L-aspartic acid) (PLAA).

1. Introduction

The process of proton dissociation of a weak polyacid has attracted continuous experimental interest in the unusual effect of long-range electrostatic interactions on the dissociation of ionizable groups. For a long time, synthetic polyacids have also been studied as 'simple' models for the 'more complex' behavior of biological polyelectrolytes. Concurrent phenomena have been disclosed since then; in particular, the abrupt change in conformation (globular coil to expanded coil or helical to expanded coil) which takes place when electrostatic repulsive interactions overcome the other non-ionic attractive ones among chain segments (for a review see, for example, ref. 1). More recently, Strauss [2] has presented an analysis of titration curves in terms of successive ionization constants to obtain, through a small number of empirical parameters, the partition function as related to the ionization of the polyelectrolyte and, therefore, to describe the experimental behavior.

On the other hand, the theoretical description of proton dissociation of weak polyacids has been the subject of many investigations and is currently attracting renewed interest, since non-empirical approaches for the evaluation of electrostatic interactions have been proposed [3,4]. In the field of ionic polysaccharides, in particular, a number of experimental results have been collected in our laboratory [5–7] and refined theoretical predictions have been made [8–10]. In this field, however, the fit of the experimental data to the theoretical models still requires to be improved in order to determine unambiguously the molecular confor-

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mation in solution, as has been verified with other biopolymers.

In a previous paper [9], we adopted the concept of semiflexibility of polyelectrolytes in solution. In such a case, we were concerned only with the flexibility associated with the accessible conformational space of the macromolecule under consideration. Naturally, a polyelectrolyte adopts not just one allowed conformation (rigid rod-like chain), but, rather, a whole population of conformations. Therefore, the real chain was assumed to be replaceable by a sequence of segments, each of which is composed of a certain number of monomeric repeating units. The hypothesis implied that the conformational monomeric flexibility would give rise to a distribution of end-to-end distances for a given segment.

In the present paper, we deal with the verification of assumptions previously made, in particular as to the size of the segment and to other possible sources of flexibility (statistical distribution of charges, solvent and side chain effect, etc.). The approach is developed in the following sections, firstly as regards the effect of conformational energy flexibility, and then in relation to the total flexibility by using a fitting procedure for experimental titration curves. In the last case, for computational simplicity, we make the assumption that (at least around the equilibrium position) the polymer segments behave elastically, so that the energy is considered to be of the Hookian type. As an application of the present approach, a description is given of the titration curves for two weak polyacids, poly(DL-glutamic acid) (PDLGA) and poly(L-aspartic acid) (PLAA).

2. Results and discussion

2.1. Theoretical model of a rigid linear polyelectrolyte

The polyelectrolyte theory used here to describe the process of proton ionization has been extensively reported elsewhere [5,8,9]. Let us briefly reintroduce the starting equation of the original treatment presented by Manning [3,11], and the final modified equations for the $\mathrm{p}K_\mathrm{a}$ reported in refs 5 and 10.

The excess electrostatic free energy, G^{el}, is evaluated by summing the electrostatic interactions among all the charges on the infinitely long and rigid polymer, assuming only pairwise interactions of the Debye-Hückel type. For univalent mobile ions and fixed charges, this may be expressed as:

$$g^{\mathrm{el}} = \frac{G^{\mathrm{el}}}{n_\mathrm{p} RT} = -\xi \ln[1 - \exp(-kb)] \tag{1}$$

where $k^2 = 4\pi q^2 N I/(\epsilon k_\mathrm{B} T \times 10^{27})$ is the Debye-Hückel parameter, q the unit charge, T the absolute temperature, k_B Boltzmann's constant, ϵ the dielectric constant of the bulk media, I the ionic strength, b the intercharge distance (in Å), n_p the number of charges on the polymer, R the gas constant, and ξ the charge density parameter defined as

$$\xi = q^2/(\epsilon k_\mathrm{B} T b) \tag{2}$$

Whenever the parameter $\xi > 1$, the theory predicts that the system is unstable. This instability is removed by the condensation of a fraction r of counterions to compensate an equal fraction of polymer charges and reduce the effective value of the charge density parameter to unity (for univalent polymer ionizable groups and univalent counterions). The requirement of free energy minimization in the limit of infinite dilution results in the condition $r = 1 - \xi^{-1}$. In this case, changes in the free energy of mixing, arising from entropic contributions, must be considered [8].

The apparent dissociation constant, $\mathrm{p}K_\mathrm{a}$ is related to the electrostatic free energy contribution by the relation:

$$\Delta \mathrm{p}K_\mathrm{a} = \mathrm{p}K_\mathrm{a} - \mathrm{p}K_\mathrm{o} = \frac{1}{2.303RT} \frac{\partial (G^{\mathrm{el}}/n_\mathrm{m})}{\partial \alpha} \tag{3}$$

where n_m is the total number of ionizable groups. Using eqs 2 and 3 and according to the value of the charge density parameter, (i) for $\xi < 1$:

$$\Delta \mathrm{p}K_\mathrm{a}(\alpha, b) = -\frac{\alpha \xi^0}{2.303} \left\{ 2 \ln(1 - e^{-kb}) + \frac{kb}{e^{kb} - 1} \left[\frac{1}{2} \frac{\alpha}{2R' + \alpha} - 1 \right] \right\} \tag{4}$$

and
(ii) for $\xi > 1$:

$$\begin{aligned}\Delta \mathrm{p}K_{\mathrm{a}}(\alpha,b) = &-\frac{1}{2.303\alpha\xi^{0}}\left\{2\ln(1-e^{-kb})\right.\\ &+\frac{kb}{e^{kb}-1}\\ &\left.\times\left[\frac{1}{2}\cdot\frac{1}{2R'\xi^{0}+1}-1\right]\right\}\\ &+\frac{1}{2.303}\left\{\left(1-\frac{1}{\alpha\xi^{0}}\right)\right.\\ &\times\ln\left[\frac{(\alpha-(1/\xi^{0}))}{V_{\mathrm{p}}C_{\mathrm{p}}(R'+\alpha)}\right]\\ &+\frac{1}{\alpha\xi^{0}}\ln\left[\frac{(R'+(1/\xi^{0}))}{(R'+\alpha)(1-V_{\mathrm{p}}C_{\mathrm{p}})}\right]\\ &\left.+1-\frac{1}{\alpha\xi^{0}}\right\} \qquad (5)\end{aligned}$$

where all the parameters have their usual meanings [9,10].

2.2. The semi-flexible model

Following the assumptions of ref. 9, the actual polymer chain (of degree of polymerization DP) is replaced by an equivalent number of polymer segments, each of which is supposed to be made up of a given number of polymer repeating units, x. The total number of segments in a chain will be given by DP/x. This quantity only concerns the definition of charge concentration. The distribution of the end-to-end distance of the segments, $W_x(h)$, is the quantity of interest, since it is related to the intercharge distance and to its distribution, $W(b)$, where $b = h/N$, N being the number of charges on a polymer segment of end-to-end distance h and formed by x repeating units.

Thus, provided that some assumption is made regarding the statistics of the segments into which the whole polymer is sectioned, any experimental thermodynamic property f is evaluated as the proper average of that property over a population of segments having variable charge densities.

Formally, if the property under consideration is a function of the degree of ionization α and the fluctuations arise only from conformational flexibility (as in ref. 9), then

$$\langle f(\alpha)\rangle = \int_0^{\infty} f(\alpha,b)W'(b)\,\mathrm{d}b \qquad (6)$$

where $W'(b)$ is the distribution function of b, containing the electrostatic and the conformational energy contributions.

This problem was treated in the previous paper [9], where the polyelectrolytic properties of an ionic derivative of amylose, a polymer possessing segments with a quasi-helical spring-like conformation in solution was considered. In the case of the parent amylose, accurate Monte Carlo calculations of the end-to-end distance for different degrees of polymerization were available [12]. In addition, since charged groups were located on the main chain of the derivative, the evaluation of the charge density has been made from the knowledge of the segment expansion calculated using the Monte Carlo method.

Similar averaging procedure can be applied to any polymer for which the distribution function of the segment lengths is known. If that information is not available or if charged groups are subjected to side-chain motion, the calculation of the average property can still be performed by matching the data with an appropriate value of the 'elastic' conformational energy, which is treated as an adjustable parameter and determines the segment length distribution. This procedure can be used for different polymers or for different structures of a given polymer. It has to be pointed out that the 'flexibility' arises from thermal (entropic) fluctuations and that the term is introduced here for segmental motions on a distance range longer than the value of K^{-1} (the Debye length), but not greater than the polymer persistence length.

2.3. Monte Carlo calculation of the distribution function of end-to-end segment lengths

The distribution of segment lengths using Monte Carlo techniques is typically determined by generating a population of polymer segments of a given number of repeating units x. The controlling factors are the a priori probability of each state which is based on the conformational energy map calculated for the dimeric unit. Detailed studies of conformational energy calculation including various form of potentials have been carried out over many years [13–16]. For our application to PDLGA and to PLAA we referred to the calculations of Hesselink [17] on a model L-polypeptide, containing a Cβ side chain, and to those of Premilat and Hermans [18] on poly(L-alanine).

In Hesselink's work [17] the comparison of the Gaussian distribution with the actual distribution of the chain vector for short polypeptide chains indicates a large deviation from statistical-coil behavior. For chains comprising up to 10 peptide units, the actual distribution displays a steep peak around the most probable value for the end-to-end distance, a value which is not much shorter than the length of the fully extended polypeptide chain. As a gross estimate, more than 90% of the chains with 10 peptide units extend for more than 20 Å, having a most probable end-to-end distance of about 30 Å. For longer chains the distributions calculated by the Monte Carlo method and from the Gaussian distribution become increasingly closer to each other; nevertheless, systematic deviations are still observed.

If the end-to-end distance of a segment in the chain obeys a conformational free energy behavior of the hookian type, then

$$G(h)=k'(h-h_0)^2 \tag{7}$$

giving the constant k' the significance of a Hooke's constant, while h_0 is the average unperturbed value of the end-to-end segment length. The free energy, $G(h)$, is then identified with the non-ionic conformational energy which provides for the flexibility of the chain segment around its equilibrium position. By assuming Boltzmann statistics, the Gaussian distribution of end-to-end segment lengths is obtained:

$$W(h)\approx\exp(-G(h)/RT) \tag{8}$$

Premilat and Hermans [18] reported the average conformational energy as a function of the end-to-end distance for poly(L-alanine) oligomers (with $x=5$, 10, 15). A difference of about 2.5 kcal/mol chains is quoted by the authors between the energy of chains with the shortest end-to-end distance and the energy minimum corresponding to conformations near the most probable one, independently of the number of units in the chain. In fact, they commented that, while it is easy to change slightly the end-to-end distance of short chains, the "free energy change for large changes of the end-to-end distance is very large". This intrinsic stiffness is reflected in the distribution of the end-to-end distance of short chains, which is markedly different from that of the statistical coil. Indeed, when normalized in terms of residue units, the free energy change of the 15-unit chain spans over values of about 0.2 kcal/residue and increases with decreasing chain length, in agreement with the greater rigidity of shorter chains. This observation requires that the modelling of hookian behavior of a chain is limited to a proper value of the number of repeat units.

2.4. *Dependence of the distribution functions on the degree of polymerization of the segment*

In a previous paper [9], the application of the present semi-flexible model to an amylose derivative was carried out with the approximation that the conformational fluctuations that contribute to generate $W_x(h)$ were the essentially spring-like motions of pseudo-helical amylose [12]. One of the referees correctly called our attention to the fact that the shape of $W_x(h)$ is always a function of x and, therefore, the results might depend on x, especially for quite flexible chains. This comment is pertinent here in the attempt to generalize the semi-flexible polyelectrolytic model.

The first point to mention is that long-range ionic interactions of charged groups on a chain are screened above a given distance. Therefore, for the proper application of polyelectrolyte theories to chains differing from the rigid-line, one has only to be aware that the radius of curvature must be as large as possible in comparison with the Debye length. Compelling, though intuitive, arguments support this assertion, which is also based on the above-quoted results on the Monte Carlo generation of random-coil polypeptide chains. Limitations exist only for the generalization of the approach to freely jointed or freely rotating chains.

Concerning the chain length dependence, examination of the dependence of the characteristic ratio, c_n, as a function of the degree of polymerization, n, may clarify this point. In most real polymers, potential barriers to internal rotations hinder formation of closed loops for short segments. For short chains of an ordinary semi-flexible polymer, the consequence is the observed deviation from the 'Gaussian' distribution of the end-to-end distance and an approximately linear increase of c_n with n. The rigid rod-like chain, as modelled in Manning's theory, shows a linear increase in c_n with increasing n for any value of n. What is required for the semi-flexible chain to be compatible with the polyelectrolyte theory is that the end-to-end distance be linearly dependent upon n up to a value larger than, or at least comparable with, k^{-1}.

The above assertion, which may be considered only an approximation for low-charge polymers, becomes valid upon increasing the number of charges on the polymer. This is well accepted by the common phrase 'polyelectrolytes are extended random coils'. Consequently, we may redefine the stiffness constant $k^0 = k'x^2$, so as to be approximately independent of the number of units contained in the chain segment, with the understatement that x must be smaller than the number of repeat units formally contained in the persistence length. Therefore, eqs 7 and 8 can be rewritten, properly normalized by the number of repeat units.

2.5. *Distribution functions obtained by fitting experimental data*

With the formalism described above it is possible to take into account average polyelectrolyte properties for those polymers whose conformational flexibility results from the calculated end-to-end distribution functions of a suitable polymeric segment. When charge distance is subjected only to backbone conformational changes, as in the case of amylose derivatives [9], we have been able to interpret the experimental behavior (pK, ΔH_{diss} and viscosity) without using fitting parameters.

However, chain conformational flexibility is not the only factor which determines the distribution of intercharge distances. Indeed, charges positioned on a side chain are subjected to an 'arm-waving' like motion, whose amplitude could, in principle, be calculated. In addition, it is known that protons or other site-screening charges can readily jump from one site to another along the polymeric chain [19], giving rise to another kind of 'charge density fluctuation'. Strauss [2] has formulated a simple empirical analysis, from which the equilibrium constants and the partition function were obtained. No theoretical model was used to describe the titration curve; rather, accurate experimental data are needed to extract the distribution function. This work clearly shows that a distribution of charged groups is not unlikely to be present. The most simple combinatorial approach shows that, along the titration curve of a polyacid, the broadest distribution occurs at $\alpha = 0.5$, while the narrowest distribution of charges is at $\alpha = 1$. The energetics of the individual distribution redefine the merely combinatorial distribution.

To overcome the difficulty of taking into account all the sources of charge density fluctuations, it is sufficient to consider Hooke's constant k^0 as a fitting parameter to match the averaged thermodynamic function of eq 6 with the corresponding experimental data. In this case, the fitting procedure may serve to discriminate between the relative importance of non-conformational flexibility and that of backbone conformational flexibility.

2.6. *Titration curves of poly(DL-glutamic acid)*

As an application of the present formalism, the titration of poly(DL-glutamic acid) (P-DL-GA) has been considered. In fact, there are reasonably good experimental data [20] on this polymer which is free from cooperative conformational transitions and has already been considered by Manning [3] for comparison with the theoretical predictions of the counterion condensation theory.

The theoretical fitting of the experimental data is expected to provide information on the polyelectrolytic dissociation process and on the actual dependence of the apparent dissociation constant pK_a (and of pK_0) on ionic strength, temperature and degree of ionization α. All these features of the titration curves are interrelated and are not always easily obtained from experiments. These experimental uncertainties have imposed some ambiguity in the analysis of titration curves, in particular, when conformational transitions take place, as in the case of poly(L-glutamic acid).

Theoretical curves have been calculated with models of different stiffness in aqueous solution. The theoretical results reported in fig. 1 refer to a rigid polymer (curve a) and to flexible polymers with a stiffness constant $k^0 = 1.6$ (curve b) and 0.4 kcal mol^{-1} Å^{-2} (curve c). The calculation for a rigid polymer (curve a) follows the general trend of the experimental data, but does not show a good quantitative agreement. In addition, it shows an abrupt change of slope at $\alpha = 1/\xi^0$ ($\xi = 1$), as already discussed elsewhere [5,9].

The experimental potentiometric titration curves of Olander and Holtzer [20] are reported in fig. 2. These curves can be adequately fitted with the present model, assuming a hookian energy function which approximates that given by Premilat and Hermans [18]. Agreement with both the trend and the numerical values of the experimental data is shown by curves calculated with the value of $k^0 = 0.34$ kcal mol^{-1} Å^{-2}. Indeed, the best fit of the experimental data is obtained also by choosing p$K_0 = 4.7$, instead of 4.58, for the data at $C_s = 0.01$ M. As discussed by Olander and Holtzer [20] and others [21,22], a variation in the pK_0 value of 0.1 pK units is well within the uncertainty of both experimental data and extrapolation procedures. The theoretical curves also fit the other data, at different salt concentrations, since the addition of salts is explicitly taken into account by the polyelectrolyte theory as a screening effect of the fixed charges which counterbalances the increasing charge density on the polymer upon ionization. The titration curves of fig. 2 refer to $C_s = 0.01$ M, $C_s = 0.05$ M and $C_s = 0.1$ M, for the same value of $C_p = 10^{-3}$ equiv./l, according to the experimental conditions for the data of ref. 20.

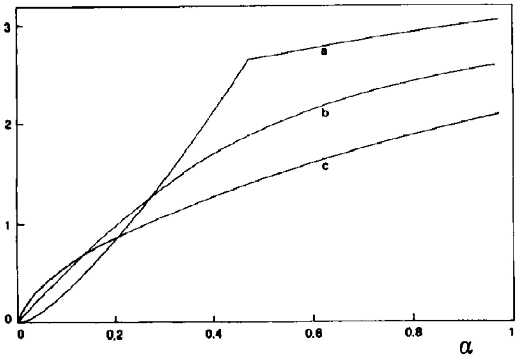

Fig. 1. Electrostatic pK_a for model weak polyacids with $b_0 = 3.57$ Å. Rigid polymer, according to eqs 4 and 5 of ref. 4 (curve a). Flexible polymers: $k^0 = 1.6$ (curve b) and 0.4 kcal mol^{-1} Å^{-2} (curve c), respectively ($C_m = 10^{-3}$ equiv./l in water at 298 K).

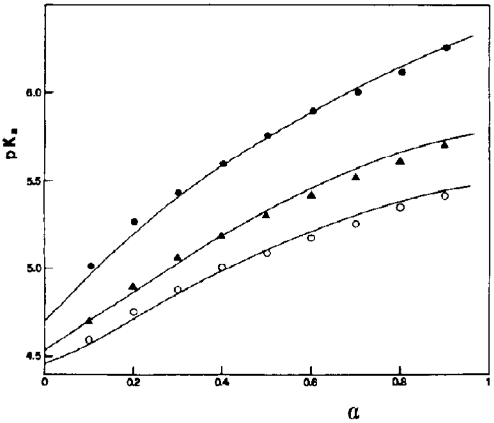

Fig. 2. Experimental data of poly(DL-glutamic acid) (from ref. 19) and calculated ΔpK_a curves (with $k^0 = 0.34$ kcal mol^{-1} Å^{-2}). Polymer concentration $C_m = 0.001$ equiv./l. Salt concentration, $C_s = 0.01$ (●), 0.05 (▲) and 0.1 M (○), respectively.

Confidence in the present formalism comes not only from the correct prediction of the ionic strength dependence of the titration curves of PDLGA, but also from the fitting of similar curves for PLAA. The experimental data of McDiarmid and Doty [23] on the titration curves of PLAA have been plotted in fig. 3, which also contains the theoretical curves obtained by using a stiffness constant, $k^0 = 0.4$ kcal/mol, which is only slightly larger than that used for PDLGA. This result is easily understood, since the experimental pK_a

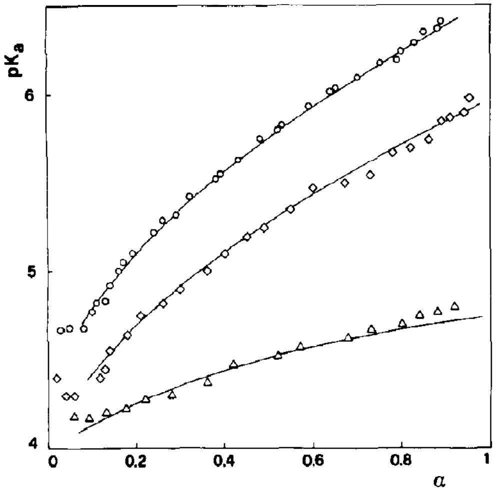

Fig. 3. Experimental data of poly(L-aspartic acid) (from ref. 22) and calculated ΔpK_a curves (with $k^0 = 0.4$ kcal mol^{-1} Å^{-2}). Polymer concentration, $C_m = 0.001$ equiv./l. Salt concentration, $C_s = 0$ (○), 0.01 (◇) and 0.2 M (△), respectively.

curves of PDLGA in 0.01 M NaCl and of PLAA in 0.01 M $NaClO_4$ are almost completely coincident, provided that the difference in pK_0 of the two polyacids is properly taken into account. A difference for the intrinsic pK_0 of the two aminoacids of about 0.42 ± 0.11 is suggested (in 0.1 M NaCl) by Nozaki and Tanford [21] which compares well with the ΔpK_0 of 0.5 found from the fitting in our case.

It is necessary to comment upon the fact that the quantitative agreement between theoretical and experimental curves is obtained by using pK_0 values slightly and systematically different than those proposed for the extrapolation of the experimental data. In all cases, the difference seems to be within the experimental error (less than 0.1 pK units), and could equally well derive from the empirical extrapolation as well as from the inadequacy of the theoretical treatment of a polymer at very low charge density. Notwithstanding these uncertainties, our results are consistent with a dependence of pK_0 on ionic strength, besides being a function of the structural features of the polyacid. A detailed discussion of this aspect, as given, for instance, by Olander and Holtzer [20] and by Nagasawa and Holtzer [24], is beyond the scope of the present work.

3. Conclusions

By adopting a statistical approach for the description of the proton dissociation process of weak polyacids, Manning's model for polyelectrolytes has been extended to non-rigid chains. It has been shown that real polymers conform to the prerequisites of the polyelectrolyte theory provided that the analysis of the conformational freedom of short segments supports the deviations from the statistical-coil behavior and, therefore, local chain stiffness.

Application of the theoretical treatment to a model case reveals that other sources of flexibility must be included in order to fit phenomenologically the experimental data. This further problem does not have an analytical solution as yet and is therefore treated with a fitting parameter. Motivation for using the mixed approach resides in the attempt, formalized in the subsequent paper [25], to deal with the case of the poly(L-glutamic acid) which undergoes the helix-to-coil conformational transition at an intermediate α value, already analysed in another paper [10]. In fact, the treatment of the extended coil poly(L-glutamic acid) will closely follow that already used for PDLGA, while the helical polypeptide has to be considered more rigid than the extended coil.

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