Biothermodynamics

ON THE THERMODYNAMICS OF PROTON DISSOCIATION IN WEAK BIOLOGICAL POLYACIDS
An analysis of theoretical and experimental results

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A theoretical description of the proton dissociation process of weak polyacids is given. Incorporation of conformational variability in the free energy of a polyelectrolyte system provides quantitative fitting of experimental data. In addition, it extends the validity of the theory to cases in which a cooperative order-disorder transition takes place. Biopolymers considered are: poly(L-aspartic acid), poly(L-glutamic acid), samples of poly(uronic acid) and some carboxylic derivatives of a gelling bacterial polysaccharide.

Introduction

Ionic equilibria in simple acids (either mono- or oligoprotic) have long been understood on a quantitative basis. On the contrary, the proton dissociation of weak polyacids has only recently been investigated theoretically in detail, and many experimental data continue to be reported in the literature without optimal theoretical analysis. Scientific interest in polyacid dissociation derives from the fact that this process is the most effective way of tuning the charge density on the polymeric chain, and thus of studying the effects of charge density on the conformational and biological properties of the biopolymer. The abundant experimental literature on the apparent pK_a of various polyacids, and our own interest in the theoretical evaluation of the solution behaviour of ionic biopolymers (especially polysaccharides) has led us to develop methods to describe the acid dissociation of polyacids which may undergo conformational transitions and/or disaggregation processes [1–9].

Subject to the constraints imposed by the chemical structure of the chain, the distribution of charged groups and their degree of ionization contributes to determining the equilibrium chain conformation: both the coulombic inter-action among the charged groups and the distribution and concentration of the screening counterions are important. Most of the physico-chemical properties of the system result from a non-linear combination of these parameters. A general consequence of the presence of charged groups on a chain is a favourable contribution to the solubility of polymer in water.

However, one has not to forget that the variability of conformation alter the distances between charged groups on the polymeric chain and that the equilibrium is statistically defined by the free-energy minimum of the system.

As an important consequence of this free-energy balance, changes in temperature, ionic strength, pH, etc., can provoke changes in polyelectrolyte conformation, often cooperatively in the case of biopolymers, between states with different values of the formal charge density. These states may be char-acterized by different structural order (e.g. helix → extended chain transi-tion), by different degrees of flexibility of the chain (globular coil → expanded chain) or by different extent of aggregation (monomeric → dimeric or multimeric chains).

If the ionizable groups are rather weak (as, e.g. carboxylic groups), then the behaviour of the polymer will be a function of pH (i.e. of the degree of ionization α). For these reasons, study of the protonation deprotonation process of a carboxylic polymer is an efficient tool for understanding the various contributions and for revealing, through the change of charge density, variations occurring in the intramolecular and intermolecular interactions. For interpretation of experimental data and for understanding of the correla-tion between properties and structure, a theoretical approach based on a molecular model is necessary. The central problem, into which we will not enter in detail here, is that of quantifying the interactions among charges on the polymer and among these and their respective counterions. The most common approach is one which describes the linear polyelectrolyte using a model with cylindrical geometry.

Thermodynamics of proton dissociation of weak polyacids

Definition of the system

Let us define an experiment in which the pH of a solution containing a weak polyacid (typically a polycarboxylic acid) of known concentration. This value C is measured as a function of the volume of titrant (base) of known

concentration. Here we define the polymer concentration as the moles of ionizable groups per liter. The relationship between the two experimentally available data is governed by the (apparent) equilibrium constant, pK_a, defined in terms of the experimental parameters by

$$pK_a = pH - \log[\ (\alpha / (1 - \alpha)\ )]. \tag{1}$$

Here, α is the degree of ionization of the free acid form, which is related to the directly observable degree of neutralization, α_n, by the relation:

$$\alpha = \alpha_n + [\ H^+\ ] / C = \alpha_n + 10^{-pH} / C$$

When $\alpha_n = 0$ (the initial point in the titration of the polymeric acid), $\alpha \neq 0$ because of self-dissociation of the polyacid [8]. The apparent dissociation constant, pK_a, clearly depends on the charge density (i.e. conformation) of the chain, and, hence, on α. It is commonly expressed as

$$pK_a = pK_o + [\ 1 / (2.303RT)\ ]\ (\ \partial G^{el} / \partial \alpha\ ) \tag{2}$$

where G^{el} is the electrostatic free energy of the polymer and pK_o is the 'intrinsic' pK_a associated with the removal of a proton from the uncharged chain [10].

The aim of this paper is to provide an outline of the procedures used to evaluate the term:

$$\Delta pK_a = pK_a - pK_o = [\ 1 / (2.303RT)\ ]\ (\ \partial G^{el} / \partial \alpha\ ) = G_{diss}^{ion}(\alpha) \tag{3}$$

that, is the functional dependence of

$$\Delta pK_a = F(\ \alpha, \xi, C, C_s, T\ ) \tag{4}$$

where the charge density parameter, ξ, is fully defined below, and C_s is the concentration of low molecular weight salt.

A derivation of the electrostatic free energy of dissociation as a function of α for a polyelectrolyte in aqueous solution in the presence of an excess univalent salt was given by Manning [11] and for the case of the absence of salt by Paoletti et al. [2]. Reconsideration of the original approach [2, 11] has produced slightly different equations [4]. From these studies it has been unequivocally found that the electrostatic contribution to both the free energy and the enthalpy of dissociation are strongly dependent upon the charge density of the polymer, especially in the absence of added salt. In particular, the predicted values of the enthalpy of dissociation were in all cases exo-thermic with pronounced maximum (in the absolute value) at the α value correspond-

ing to $\xi = 1$ [2, 4, 6]. More recently, the problem of the conformational semi-flexibility of the polymer chain has been taken into account., showing that the calculated thermodynamic properties (properly averaged over a sample of conformations) are in better agreement with many experimental data [6]. While a few attempts have been made to obtain theoretical fit to experimental data [5–7], the above mentioned theoretical results have been used until today principally as a qualitative guideline for interpretation of the reported experimental behaviour.

A short summary of the theoretical approach

A number of treatments have been reported to account for the perturbation of the thermodynamic functions of polyelectrolyte solutions by the electrostatic potential. Reference can be made to the work by Manning [11, 12], Cleland [13] and Cesàro *et al.* [4–9]. The fundamental idea of Manning's approach is that the (linear) polymer carrying charged groups can be seen as an infinite line characterized by the linear charge density parameter, ξ.

With the above mentioned preliminary statements on the system out of the way, the derivation of the thermodynamic functions can be made within the framework of the statistical mechanical method. Thus, the canonical partition function for the system consisting of the polyelectrolyte molecules and the other low molecular weight components is written

$$Q = \int...\int \exp[-\beta H (\{p\}, \{q\})] \, \mathrm{d}\{p\} \, \mathrm{d}\{p\} \qquad (5)$$

where $\beta = (k_\beta T)^{-1}$ and $H ()$ is the canonial Hamiltonian and the integral is over the momenta p and the coordinates q of all atoms. We limit the consideration to the electrostatic (excess) free energy at equilibrium of a system containing a total of charges on the polymer chain $N_p = N_+ - N_-$, N_+ counterions and N_- cooions. The number of charges on each chain is related to the number average molecular weight, M_n, by $N_p/N = (\alpha \, M_n/M_o)/N$, where M_o is the molecular weight of the repeat unit and N is the total number of chains. By electrostatic (excess) free energy we mean $A - A_o$, where A is the free energy of the polyelectrolyte solution and A_o is the free energy of the same system but with all charges on the polymer set equal to zero, while the total number of mobile charges is set equal to $N_{\text{mobile}} = N_+ + N_p + N_-$.

If U_N is the potential energy of the charged polymer system and U_N^o is that of the uncharged one, then, following integration over the momenta, one obtains

$$\exp\left[-\beta(G-G_0)\right] = \exp\left[-\beta(A-A_0)\right] = \frac{\int...\int \exp\left[-\beta U_N\right] d\{q\}}{\int...\int \exp\left[-\beta U_N^o\right] d\{q\}}. \quad (6)$$

We have identified the Helmotz and the Gibbs free energy values, an approximation that is readily verified for condensed phases. The above condition can be taken in order to derive the excess thermodynamic functions.

Provided that no conformational change of the reference state occurs, and that the concentration of the polyelectrolyte vanishes (limiting law), the equations derived by G. S. Manning can be safely used. However, few (if any) polymeric chains are as rigid as the theory requires.

In these cases, one has only to redefine the meaning of A (or G) as the energy of a polyelectrolyte system which incorporates also all conformational variability. Then, to extend the validity of the polyelectrolyte theory, the conformational energies of the chain have to be evaluated.

This extension of the theoretical approach has already been presented in detail elsewhere to describe the behaviour of some semiflexible polyacids, like an amylose derivative [6], and polyglutamic acid [5]. The model schematically represented in Fig. 1 can account for the experimental behaviour described by one of a combination of the three following cases:

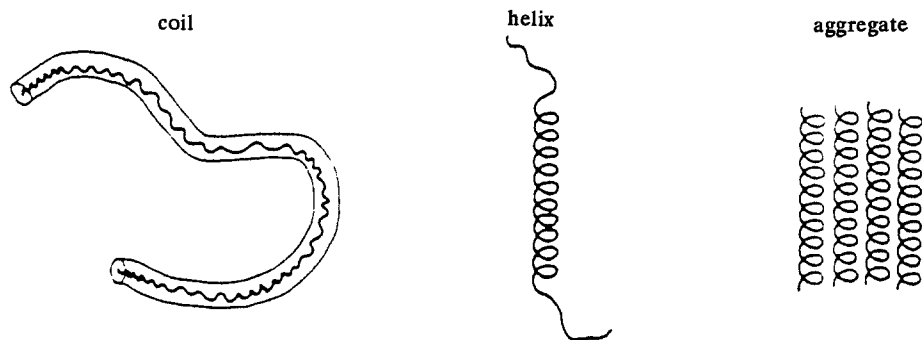coil   helix   aggregate

Fig. 1 Schematic picture of polymeric chain topologies

In the first case, the polymeric chains process locally an idealized structure which allows accordian-like motions. The chain geometry is defined, therefore, by the length b of the monomeric projection along the chain trajectory. At any given instant the system is assumed to consist of a population of suitable representative segment units (RSU) with a gaussian distribution $W(d)$ of the end-to-end distance of the segments, with d or b. The probability distribution, $W(b)$, is defined by the difference in the conformational energy

E_C of the segments having monomer distance b with respect to energy $E^0 = 0$ of the segment at the equilibrium distance, b^0:

$$E_c = k (b - b^0)^2, \qquad W(b) = \exp[-E_c(b)/RT]. \qquad (7)$$

The constant k depends on the molecular structure of the polymer, and it is defined here, simply on an empirical basis, as a chain stiffness constant. When charged groups are present on the chain, than the actual distribution function $W'(b)$ depends, indeed, on the other ionic components and can be expressed as

$$W'(b) = \exp[-A(b, Cs, ...)/RT] = \exp[-((A-A_o)+E_c(b))/RT]. \qquad (8)$$

In the second case, the polymeric chains may change conformation from that defined by an average monomeric distance b_1 to another with distance b_2, less expanded and possibly characterized by a different value of the stiffness constant k. The transition from one state to the other is driven by the balance of the free energy values of the two forms:

$$\Delta A = \Delta A_{(2<-1)} = <A_2(\alpha)> - <A_1(\alpha)> \qquad (9)$$

where the free energy of each species is given by the sum of the non-ionic, A^n, and the electrostatic, A_{el}, contributions:

$$A_i(\alpha) = A_i^n + A^{el}(\alpha). \qquad (10)$$

Provided that the ΔA contributions are either experimentally available or theoretically predicted, then the conformational transition may be described by using a proper cooperativity factor.

Finally, consider the case in which chain segments possessing the same average structure may associate to form aggregates. Here we suppose that chain aggregation is driven by a non-ionic energy which is eventually balanced by the electrostatic repulsion as the polymer undergoes ionization of the carboxylic groups. From the electrostatic point of view, the only important parameter affecting the energy of the aggregated chains is the resulting effective charge density. Pushing to its limit the validity of the infinite linear model., we assume that the axial dimension will always be larger than the cross-sectional dimension.

The stable aggregated structure is defined by the number of chains in the cluster, the free energy of aggregation, and the cooperativity of the aggregation process (that is, the average number of monomers taking part cooperatively in the process). The model does not involve two major questions of

debate in the literature, i.e. the topology of the chain aggregation (side-by-side or double-, triple-helix...) and the participation of the same chain in more than one cluster. The only important point for the model is the local situation in terms of number of monomers projected on the axis which defines the average charge distance b, and the distribution function of this distance.

The equations for the theoretical model of lienear polyelectrolyte

In the original treatment, proposed by Manning [10, 11], with the modifications reported to obtain the equations for the pK_a [6, 7], 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 it has been written (for the case $\xi < 1$) as:

$$g^{el} = -\xi \ln[1 - \exp(-Kb)] \tag{11}$$

where K is the Debye-Hückel parameter [7], b the intercharge distance (in Angstrom units), and ξ the charge density parameter defined as

$$\xi = e^2 / (\varepsilon k_B Tb) \tag{12}$$

with e the electronic charge and ε the bulk dielectric constant. Whenever the parameter $\xi > 1$, the theory predicts that the system is unstable. In water, 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 minimisation 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, have to be considered [2].

Using Eqs (2) and (3), and according to the value of the charge density parameter, we have [4, 7]:

$\xi < 1$

$$\Delta pK_a(\alpha, b) = -\frac{\alpha \xi^{\circ}}{2.303}\left\{2\ln\left(1-e^{-Kb}\right) + \frac{Kb}{e^{Kb}-1}\left[\frac{1}{2}\cdot\frac{\alpha}{(2R'+\alpha)}-1\right]\right\} \tag{13}$$

$\xi > 1$

$$\Delta pK_a(\alpha, b) = -\frac{1}{2.303\alpha \xi^{\circ}}\left\{2\ln\left(1-e^{-Kb}\right) + \frac{Kb}{e^{Kb}-1}\left[\frac{1}{2}\cdot\frac{1}{(2R'\xi^{\circ}+1)}-1\right]\right\}$$

$$\cdot \left\{ \left(1-\frac{1}{\alpha\,\xi^\circ}\right) \cdot \ln\frac{\left(\alpha-\frac{1}{\xi^\circ}\right)}{V_p C_p(R'+\alpha)} + \frac{1}{\alpha\,\xi^\circ} \cdot \ln\frac{R'+\frac{1}{\xi^\circ}}{(R'+\alpha)(1-V_p C_p)} + 1 - \frac{1}{\alpha\,\xi^\circ} \right\} \quad (14)$$

$$V_p C_p = \frac{\left(1-e^{-Kb}\right)^2 \cdot \left(1-\frac{1}{\xi}\right)}{\left(R'+\frac{1}{\xi}\right)} \cdot \exp\left[1+\frac{1}{2} \cdot \frac{Kb}{(e^{Kb}-1)(2R'\xi+1)}\right]$$

The semi-flexible model

Following the assumption made above, the actual polymer chain is replaced by an equivalent number of segment unites, RSU. The distribution of end-to-end distances of the segments, $W(d)$, is the quantity of interest, since it is related to the intercharge distance and to its distribution, $W(b)$; $b = d/n$, n being the number of charges on a polymer segment of end-to-end distance d. Thus, provided that some assumption is made regarding the conformational statistics of the segments into which the whole polymer is sectioned, any experimental thermodynamic property f may be 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. [6]), then

$$<f(\alpha)> \frac{1}{Z}\int f(\alpha, \{b\}) \exp[-\beta U(\{b\})]\, \mathrm{d}\{b\} \approx \int_0^\infty f(\alpha, b)\, W(b)\, \mathrm{d}b \quad (15)$$

The problem of relaxing the rigidity of the polyelectrolytic model was treated in the previous paper [6], studying an ionic derivative of amylose, a polymer possessing segments with a quasi-helical spring-like conformation in solution. In this case, because the charged group were located on the chain backbone, evaluation of the distribution of the charge density has been computed from a knowledge of the distribution function of the segment end-to-end distance, calculated using the Monte Carlo method.

Similar averaging procedures can be applied to any polymer for which the distribution function of segment lengths is known. If that information is not available, or if charged groups are subject to side-chain motion, the calculation of the average property cab 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 the term is introduced here for segmental motions on a distance scale longer than the value of K^{-1} (the Debye length), but shorter than the polymer persistence length. This can be defined as the average projection of the end-to-end distance vector on the first bond of the chain, in the limit of infinite chain length [10].

Titration curves of polycarboxylic polymers

In addition to being an increasing function of the degree of ionization α, the potentiometric behaviour of weak polyacids under various conditions of polymer concentration, C, and added ionic strength, C_S, shows the usual dependence of pK_a on C and C_S (at a fixed value of α), i.e. , pK_a is lowered by an increase in either C or C_S. A sharp increase in experimental pK_a is shown at high α values ($\alpha > 0.9$) by all weak acids. This is due mainly to the breakdown of the validity of the analytical expression of pK_a in this region.

Theoretical curves have been calculated with models of different stiffness in aqueous solution. The theoretical results reported in Fig. 2 refer to a rigid polymer (curve a) and to flexible polymers with a stiffness constant $k = 1.6$ (curve b) and 0.4 kcal/mol 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, since it shows an abrupt change of slope at $\alpha = 1/ \xi_0$ ($\xi = 1$), as already discussed elsewhere [4, 6], where ξ_0 is the numerical value of ξ at $\alpha = 1$.

Besides the more realistic trend of the pK_a (α) curves, confidence in the present formalism comes also from the fact that a correct prediction is given for the ionic strength dependence of the titration curves. In particular, the experimental data of McDiarmid and Doty [14] on the titration curves of poly(L-aspartic acid), PLAA, have been plotted in Fig. 3, which contains the theoretical curves obtained by using a stiffness constant, $k = 0.4$ kcal/mol Å^2, only slightly larger than that used for poly(D, L-glutamic acid), PDLGA [15]. This result is easily understood since the experimental pK_a curves for PDLGA in 0.01 M NaCl and for PLAA in 0.01 M NaClO$_4$ are quite comparable, 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 amino acids of about 0.42 ±0.11 is suggested (in NaCl 0.1 M) by Nozaki and Tanford [16] which compares well with the ΔpK_0 of 0.5 found from our fitting of the pK_a curves of the two polyacids. Quantitative agreement between theoretical and

experimental curves is obtained by using pK_0 values slightly, but systematical-
ly, different from those proposed by 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 ex-
trapolation as well as from the inadequancy of the theoretical treatment of a
polymer at a very low charge density. Notwithstanding these uncertainties,
our results are consistent with a dependence of pK_0 on ionic strength, in ad-
dition to its expected dependence on the structural features of the polyacids.
A detailed discussion of this aspect given, for instance, by Olander and
Holtzer [17] and by Nagasawa and Holtzer [18], is beyond the scope of the
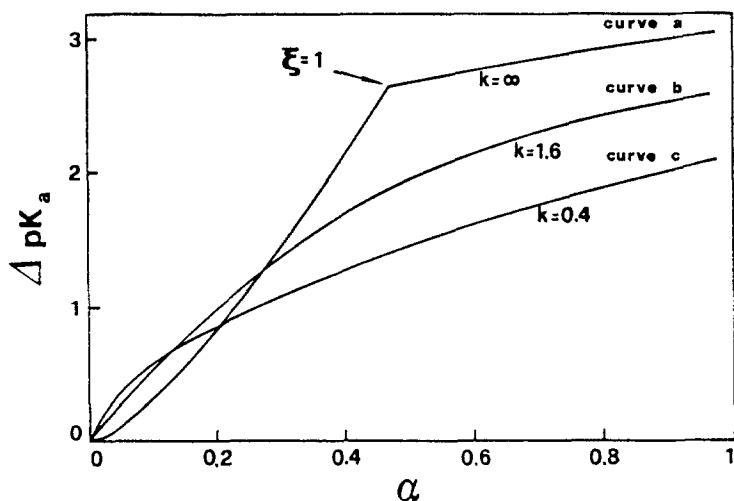present work.

Fig. 2 Theoretical pK_a curves calculated for three polyacids (same value of ξ and different
stiffness constant k)

Theoretical fitting of the experimental data provide information on the
polyelectrolytic dissociation process and on the actual dependence of the ap-
parent 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 inter-
related and are not always easily obtained from the experiments. In fact, ex-
perimental uncertainties have imposed some ambiguity in the analysis of
titration curves in particular when conformational transitions take place, as
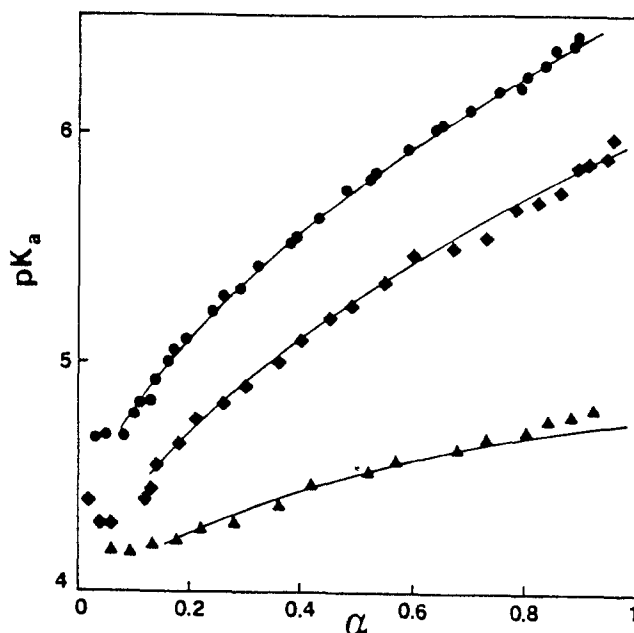in the case of poly(L-glutamic acid).

Fig. 3 Theoretical ΔpK_a curves (k = 1.674 kJ/mol $Å^2$) and experimental data of poly(L-aspartic acid) from ref. [14] in water (●), in 0.01 M (♦) and 0.2 M NaCl (▲)

Theory for the pH-induced conformational transition

Poly(L-glutamic acid), PLGA, is known to take on a helical conformation at low pH, when the carboxylic groups are uncharged. Upon ionization it undergoes a cooperative transition to a disordered conformation, characterized by sequences of conformations predominantly in the 'extended' form. Electrostatic interactions (of the Debye -Hückel type) strongly influence the conformational stability and trigger the transition that occurs upon proton dissociation. Theoretical approaches to the transition usually assume two definite conformational states, one α-helical and the other conventionally considered as a single disordered thermodynamic state and referred to as a coil. The process can be treated, indeed, as a transition between two distinct populations of conformational states, each described by a suitable conformational flexibility with average 'monomeric structural distance', b_1 = 1.5 Å (helix) and b_2 = 3.4 Å (coil). Thus, the free energy change that determines the state of the system is given by (Fig. 4):

$$\Delta G = <G_h^{ion}(\alpha)> - [<G_c^{ion}(\alpha)> + \Delta G^{conf}] \qquad (16)$$

where $<G_{h,c}^{ion}(\alpha)>$ is the ionic free energy of the helix (or coil) state averaged over the conformational population. The nonionic term ΔG^{conf} (indicated as Δ in Fig. 4) is the overall conformational energy difference between the two states (helix and coil) of the polymer in the zero-charge limit and can be computed directly from the experiment by a suitable integration of the pK_a curves (see for example ref. [18]).
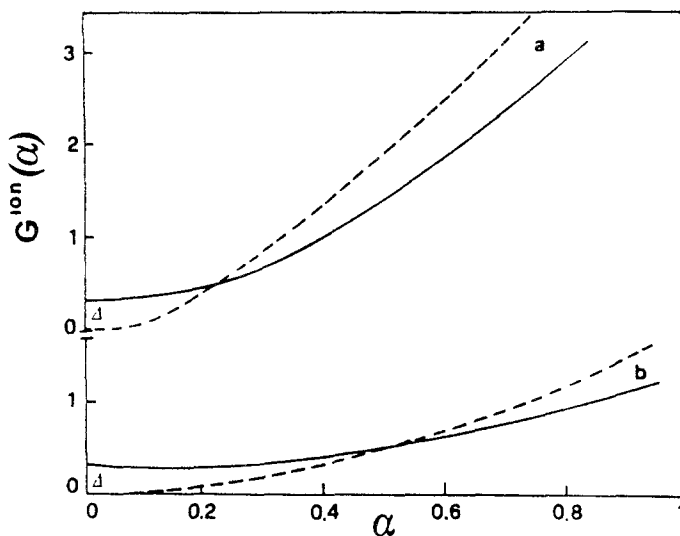

Fig. 4 Dependence of G^{ion} as a function of α for the helix (-- --) and the coil (——), in water (a) and in 0.2 M NaCl (b)

Following the metodology previously outlined, the thermodynamic averaging is done on a polymer-segment population for which the end-to-end distribution function is obtained by assuming a Hookean conformational free energy. The fitting of the experimental data is obtained with a suitable stiffness constant for each of the two conformations (helix and coil). The individual terms of the ionic contribution may then be calculated following the procedure used to describe the titration curves of poly(L-aspartic acid) and poly(D, L-glutamic acid) [15]. At any given point of the titration curve, the system contains a fraction f_h of the polymer in the helical conformation and $(1-f_h)$ as coil. Assuming the additivity rule, any property of the system is obtained by summing the contributions of both conformational states. For instance:

$$G^{ion} = f_h G_h^{ion} + (1-f_h) G_c^{ion} \tag{17}$$

where the ionic free energy, or better, the pK_a can be calculated for each conformation by the Eqs (13) and (14). Imposing the same reference state for the uncharged helix and the uncharged coil, we write

$$\Delta pK_a = f_h \, \Delta pK_{a_h} + (1 - f_h) \, \Delta pK_{a_c} \tag{18}$$

In order to evaluate the actual fraction of polymer in each state, the cooperativity of the process has to be taken into account. According to the theory of cooperative transitions, it suffices to define a cooperativity parameter σ, associated with the excess free energy of the initiation of a given (helical) state. For infinitely long chains, the value of σ is correlated with that of $\bigcap$ ($\sigma \approx \bigcap^{-\frac{1}{2}}$), operationally defined as the number of monomers in the cooperative unit. Following this approximation, the fraction of helical states can therefore be defined by a Boltzmann equation which contains N-times the free energy changes between the two states:

$$f_h (\alpha) = \frac{1}{Z} \exp - \frac{\bigcap \Delta G (\alpha)}{RT} \tag{19}$$

Z being the partition function of the two states system.

As a consequence, the fitting of the experimental titration curve(s) of PLGA is achieved by considering the proper flexibility and the cooperativity of the transition (Fig. 5) to describe not only the titration curve of PLGA at 25°, but also its dependence on ionic strength and temperature . Literature data unambiguously show that the transition point shifts to higher α values (i.e. pH) upon increasing the salt concentration, and that the cooperativity apparently decreases. The cooperativity of the transition is a function of temperature, as measured by the factor $\bigcap$ which multiplies the free energy change per residue. We find that $\bigcap$ is 14, 11 and 8 for 0.6, 25 and 48°, respectively, in agreement with the calculation of the van der Waals interaction energy for a α-helical polypeptide, which reaches 90% of the asymptotic value for a chain of ca. 10 residues. A major contribution to the stabilizing energy comes from the dipole-dipole interactions, as firstly outlined by Brant [19]. From the temperature dependence of the length of the cooperative unit one may calculate an enthalpic contribution of $\Delta H_\sigma \cong 3$ kcal/mol to the statistical weight σ of the Zimm and Bragg theory. This is contrary to the common assumption that the statistical weight σ is of merely entropic origin, but is consistent with conformational calculations which assign an important enthalpic contribution to σ (see for instance [19]).

Ionic polysaccharides

Polysaccharides containing carboxylic groups are very common in nature and can also be easily prepared from non-ionic polysaccharides by chemical modification. Poly(uronic acids), i.e. those containing glycuronic residues have been studied in detail in our laboratory. Among these polymers a large range of behaviour, including strong aggregation phenomena, have been observed. Some experimental pK_a *vs.* α plots show, as in the case of poly(mannuronic acid), a smooth increase with increasing α, typical of the dissociation curve of freely soluble polyacids [9]. The pK_a plots of poly(L-guluronic acid) [9] show a non-monotonic dependence on α, which depends, furthermore, on the procedure used to prepare the solution. The pronounced s-shaped curve is typical of other homo[polymers undergoing a cooperative conformational transition, but from the polyelectrolytic point of view it reflects solely a departure from the regular increase of the charge density on the polyacid with increasing α. The same, although less marked, phenomenon was encountered in the titration curves of poly(galacturonic acid), both in water and more evidently in salt solution [1, 9]. Finally, alginic acids (copolymers of mannuronic and guluronic acids) exhibit a behaviour which, although not completely regular, can be traced back, at least qualitatively, to the polymer composition of co-monomer blocks [9].
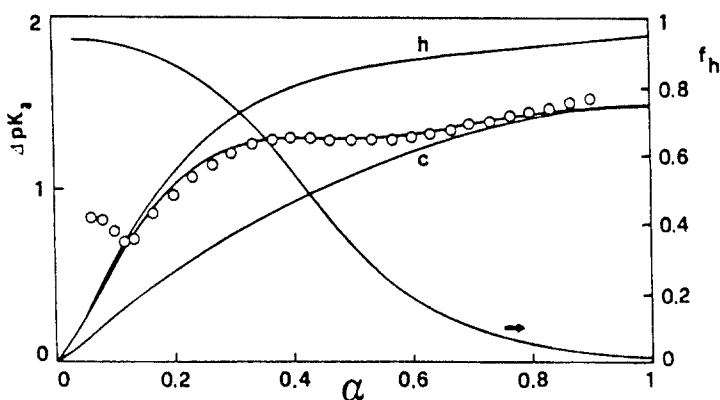

Fig. 5 Calculated ΔpK_a curve of PLGA in 0.01 M NaCl (data from ref. [18]). Curves h and c refer to full helix and coil conformation, respectively. The helix fraction f_h is also shown

Another complication arises from preparation of the solution of the homopolymeric acid form at high polymer concentration and/or in the presence of added salt, as it can easily induce chain aggregation. In fact, solu-

tions of samples prepared by percolation of the concentrated salt form through a cation-exchange column were shown to favor aggregation of the polymer, as indicated by the decrease with increasing α of the initial portion of the pK_a curves. This behaviour has been consistently reproduced, unless very low polymer concentration and removal of the aggregated material is used [9].

The question of aggregation can be addressed by studying the effect of introducing an increasing amount of chargeable carboxylic group on a neutral gelling polysaccharide (Fig. 6). The polymer in question is a capsular polysaccharide from a strain of Rhizobium (CPS) [20]. The CPS solutions form a firm gel at very low polymer concentration (down to 0.1 g/L): the gel is destroyed by increasing the temperature above ca. $50°$ and is thermoreversible. The neutral polymer can be easily modified to introduce (by periodate oxidation and subsequent chlorite oxidation) up to six carboxylic groups per repeat unit (only the three sugar units in the side chains are modified). Titration curves of the ionic derivatives clearly show the progressive decrease of the aggregation phenomena at low pH and the increasing electrostatic potential of the chain at a given α for the highly modifies samples [20].

As a theoretical interpretation of the effect of the aggregation process, we report experimental data for a cellulosic derivative, a polymer possessing a quite rigid and elongated chain structure. The pK_a curves, under various conditions of polymer and salt concentration, show unambiguously the progressive reduction of the apparent pK_a as a function of the increasing α [4]. Undoubtedly, the effective charge density on the polymeric aggregate at given α value is the controlling parameter between the interchain electrostatic repulsion and the attractive (non-ionic) energy of interaction.

We use the simplest hypothesis that a disaggregation occurs steadily upon ionization (Fig. 1, aggregate $\rightarrow$ helix), so that the average distance between charges on each aggregate is constantly equal to the onset of the counterion condensation phenomenon (that is $\xi = 1$). Although very crude, especially because no energetic insight is given for the aggregation process, this simple model correctly gives the dependence of pk_a upon the three system variables, i.e. α, C and C_s, without adjustable parameters (except the condition that ξ is constant).

Some comment on the enthalpy of dissociation

Given the theory for evaluating the excess free energy for proton dissociation processes, it is straightforward to obtain all other thermodynamic functions by proper derivation. Limiting our comments to the enthalpy of

dissociation, the intrinsic contribution of an individual carboxylic acid cannot be predicted, since in the literature values between -0.5 and $+0.5$ kcal/mol are reported [21]. Nevertheless, because $|\Delta H_{diss}|$ is always small for carboxylic groups, it can be assumed that the intrinsic ΔH_{diss} of the carboxyl group is zero and that the trend of experimental ΔH_{diss} of poly(uronic acids) is entirely due to the polyelectrolytic nature of the chain. At least two terms may be envisaged to contribute to ΔH_{diss} of which only the strictly electrostatic one can be subjected to more or less accurate predictions. The other is a conformational one (non-electrostatic) and arises from changes in the intra- and inter-molecular interactions accompanying the polyion expansion. A 'cooperative' conformational transition is one such source of non-ionic contribution to the enthalpy of dissociation. Elaboration of current polyelectrolyte theories predicts for the first contribution an exo-thermic non-linear dependence of the ΔH_{diss} values on b, the average distance between the fixed charges ($b = b^{\circ}/\alpha$, where b° is the structural distance between the ionizable groups in the fully dissociated form of the polyacid) [4]. The polyelectrolytes investigated qualitatively confirmed a dependence of ΔH_{diss} on b, which in a few cases, however, is smaller than the predicted one.

Poly(guluronic acid) and poly(mannuronic acid) exhibit practically the same ΔH_{diss} values, despite their structural differences. In particular, the
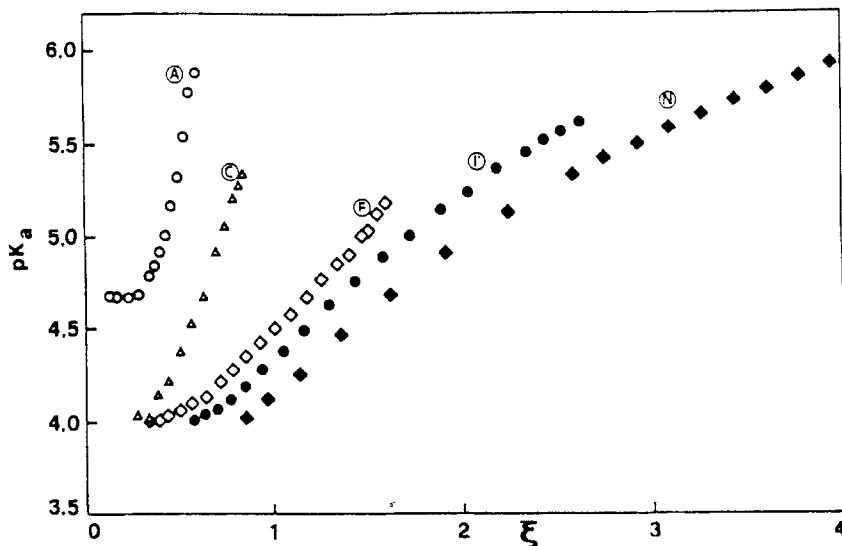

Fig. 6 pK_a of carboxylic derivatives of CPS [20] as a function of $\xi = \alpha \xi^{\circ}$, where ξ° varies from 1 (sample A) to 4 (sample N)

smooth trend of ΔH_{diss} data for poly(guluronic acid) in the whole range of α may appear surprising. Not only are the data almost constant around -2 kcal/mol for the sample prepared under non-aggregating conditions but it also seems uneffected by the aggregation. In addition, no evidence can be drawn for the transition occurring at high α values, which is at the limits of experimental accessibility. The constancy of ΔH_{diss} could also imply that the energetic contribution of the proton-mediated aggregation process is not very different from that of the polymer-solvent interaction, in contrast to the enthalpic effects observed for other polysaccharide-ion interactions, where major conformational changes are the prerequisite for the aggregation process. This interpretation is consistent with the previous conclusions [1] that the bell-shaped curve of the ΔH_{diss} of poly(galacturonic acid) reflects mainly a pH-induced conformational transition and not a significant aggregation which, however, may occur at very low pH and high C values. A final comment is necessary on the trend of ΔH_{diss} with α for alginic acids. Although the existence of a conformational transition was proposed, as suggested by the anomalous behaviour of many physico-chemical properties, it is clear that only for the acetylated sample is the enthalpic behaviour completely anomalous [22]. Indeed, particularly significant is the trend of the dilution enthalpy for the acetylated alginate in water [22]. A possible explanation is that changes occurring at the local level, related with the presence of the hydrophobic acetyl groups, add some non-linear contributions to the heat of dissociation (or of dilution).

On the basis of the data so far reported, it is evident that the calorimetric response (and the ΔH_{diss} values, in particular) is very sensitive to changes in the conformational state of polyelectrolytes in aqueous solution as a function of a suitable perturbative variable, like C or α °1, 2, 4, 6, 22é.

Conclusions

The results presented in this work give an interpretation of the dissociation behaviour of weak polyacids on the basis of simple equations from the theoretical treatment of polyelectrolyte solutions. We do not enter here into the question of whether other theoretical approaches could provide a better approximation. The aim of this work, in fact, is to emphasize that a theoretical assessment of the dissociation process is necessary, since experimentally most weak polyacids as a function of pH show a behaviour which discloses changes in conformation and/or aggregational phenomena. All these proces-

ses can be better understood and evaluated within a proper theoretical framework.

More work is in progress both ion the theoretical description of aggregated polyacids and on the collection of proper experimental data on ionic (carboxylated) polysaccharides.

<p style="text-align:center">*   *   *</p>

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Zusammenfassung — Eine theoretische Betrachtung der Protonendissoziation schwacher Polysäuren wird dargelegt. Die Berücksichtigung konformativer Änderungen auf die Grösse der freien Energie eines polyelektrolytischen Sytems ermöglicht eine theoretische Berechnung der Titrationskurven von Poly-L-Asparaginsäure als auch der Konformationsumwandlungen einiger Biopolymeren wie Poly-L-Glutaminsäure und carboxylierten Derivative von Polisacchariden.