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Representation of Polyacids by Subunits in the Analysis of Ionization Equilibria. Significance and Thermodynamic Applications

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ABSTRACT: A recently published treatment of potentiometric titration data obtained with polyacids in terms of ionization constants of oligomeric subunits has been further developed and analyzed. The treatment provides simple expressions for calculating ionization constants, partition functions, and free energies of ionization for the polyacid. The physical meaning of the oligomeric subunit has been reinterpreted, and the new interpretation has been used to elucidate the applicability of the treatment to the previously described analysis of conformational transitions, which may lead to bimodal population distributions among the various deprotonation states of the subunit. The application of the method to potentiometric titration data is shown to result in unimodal distributions for poly(acrylic acid) and (carboxymethyl)cellulose but in bimodal distributions for poly(L-glutamic acid) in the pH range where this acid is known to undergo a helix–coil transition. The generalization of the treatment to binding equilibria involving other ligands and macromolecules is discussed.

Introduction

It was recently shown that under suitable circumstances conformational transitions of polyacids could be characterized by a novel method.[1] This method involves the treatment of potentiometric titration curves in terms of successive ionization constants applied to oligomeric subunits of the polymer chain and thereby allows the resolution of the subunits into species differing in their states of deprotonation. Specifically when this technique was applied to a copolymer of maleic acid and butyl vinyl ether, two distinct peaks appeared in the population distribution functions of these species over the pH range where the copolymer is known to undergo a conformational transition. In contrast, only single-peaked distribution functions were obtained for the copolymer of maleic acid and methyl vinyl ether, for which no evidence for the occurrence of a conformational transition is known.

Further investigation has shown that the method has a number of additional features which can significantly facilitate the presentation, treatment, and interpretation of data describing binding equilibria of polyelectrolytes in general. Thus it is possible to obtain, in terms of a small number of empirical parameters, simple analytical expressions for equilibrium constants, free energy, and partition functions related to the ionization of the polyelectrolyte. It is significant that no molecular model is assumed in the calculation of the partition functions, which can, therefore, serve as means of comparison with theoretical partition functions based on such models.

In this paper we want to describe these features as well as give a revised interpretation of the meaning of the subunit which clarifies the application of the method to the treatment of conformational transitions.

Empirical Equation

We shall follow the previously described method, which is based on the potentiometric titration of polyacid being performed in excess supporting electrolyte so that activity coefficients may be considered constant.[1] The data are described by the equation

$$\alpha = \frac{\alpha_m}{N} \frac{\sum_{i=1}^{N} i\beta_i h^i}{1 + \sum_{i=1}^{N} \beta_i h^i} \tag{1}$$

where α is the degree of deprotonation defined, according to our previous usage, so that its value at complete deprotonation, α_m, equals the number of acidic groups per repeat unit of the polyacid. The quantity h is the antilogarithm of the pH and represents the reciprocal of the hydrogen ion activity. Equation 1 follows directly from eq 6 and 10 of ref 1, where α_m, for a maleic acid copolymer, was taken as equal to 2. Equation 6 of that reference was derived for an entity AH_N, considered as a polyacid subunit containing N acidic groups; the coefficients β_i were defined as overall ionization constants of these subunits. We deviate here from the previous presentation in that we shall defer this identification of eq 1 and the β_i's with the subunits. We shall instead start out by regarding eq 1 as an empirical equation which describes the experimental titration curve with N adjustable parameters β_i. Equation 1, after clearing of fractions, represents a set of linear equations in the β_i's, one for each data point, which may be solved by conventional methods, one of which has been described previously.[1] The value of N depends on the complexity of the titration curve and should be chosen large enough so that eq 1 represents the experimental data within their precision limits. To give a rough idea of the magnitude of N, we have found that with the currently attainable precision in potentiometric titrations, minimum values of N needed for poly(acrylic acid) and for the copolymer of maleic acid and butyl vinyl ether are 4 and 8, respectively.

Dissociation Constants, Partition Function, and Species Mole Fractions

If we define $\sum_N$ by the relation

$$\textstyle\sum_N = 1 + \sum_{i=1}^{N} \beta_i h^i \tag{2}$$

eq 1 can be rewritten in the form

$$\alpha = \frac{\alpha_m}{N} \frac{\mathrm{d} \ln \sum_N}{\mathrm{d} \ln h} \tag{3}$$

On integration, we obtain

$$\ln \textstyle\sum_N = \displaystyle\frac{N}{\alpha_m} \int_0^h \frac{\alpha}{h} \, \mathrm{d}h \tag{4}$$

It should be noted that in the limit of $h = 0$, $\ln \sum_N = 0$ while according to eq 1 the ratio α/h remains finite.

If both sides of eq 4 are divided by N, the right-hand side depends only on the experimental quantities α and

h. This leads to the important result that $(\ln \sum_N)/N$ or $(\sum_N)^{1/N}$ is invariant with N. Thus, if a satisfactory set of N parameters, β_i, has been found, this invariance makes it possible to construct other sets of parameters with larger N which will fit the data equally well. Specifically, this principle may be applied to N^*, the number of acidic groups in the actual polymer molecule (an asterisk will generally be used to annotate symbols pertaining to the whole polymer molecule), so that we can obtain $\sum^*$ by the equation

$$\ln \textstyle\sum^* = (N^*/N) \ln \sum_N \tag{5}$$

or, in an equivalent form

$$1 + \sum_{i=1}^{N^*} \beta^*_i h^i = \Big(1 + \sum_{i=1}^{N} \beta_i h^i\Big)^{N^*/N} \tag{6}$$

By expanding the right-hand side of eq 6 and equating coefficients of equal powers of h, we can obtain the N^* parameters β^*_i in terms of the N parameters β_i.[2] The relations for the first and last coefficients are especially simple; i.e.

$$\beta^*_1 = (N^*/N)\beta_1 \tag{7}$$

and

$$\beta^*_{N^*} = (\beta_N)^{N^*/N} \tag{8}$$

These equations indicate that β_1/N and $\mathrm{p}\beta_N/N$, where $\mathrm{p}\beta_N$ denotes the negative logarithm of β_N, are invariant with N.[3]

It is easy to verify that the β^*_i's are the equilibrium constants for the reactions

$$\mathrm{A}^*_0 = \mathrm{A}^*_i + i\mathrm{H}^+ \qquad i = 1, 2, ..., N^* \tag{9}$$

i.e.

$$\beta^*_i = [\mathrm{A}^*_i]/[\mathrm{A}^*_0]h^i \qquad i = 1, 2, ..., N^* \tag{10}$$

where A^*_0 and A^*_i denote the un-ionized and ionized polyacid molecular species[4] AH_{N^*} and $\mathrm{AH}_{N^*-i}{}^{i-}$, respectively, and the brackets denote concentrations. The parameters K^*_i, defined by

$$K^*_i = \beta^*_i/\beta^*_{i-1} \qquad i = 1, 2, ..., N^* \tag{11}$$

are the stepwise dissociation constants of the polyacid.

Since the β^*_i's and, by inference, the K^*_i's are defined in terms of concentrations of polyion species, they implicitly contain the activity coefficients of these species. They will, therefore, be constant in any one supporting electrolyte medium but may be expected to vary from one medium to another.

From eq 8 it is apparent that the quantity $\beta^*_i h^i$ is proportional to the concentration of species A^*_i. Therefore, $\sum^*$ may be viewed as a partition function of the polyacid, and

$$x^*_i = \beta^*_i h^i / \textstyle\sum^* \qquad i = 1, 2, ..., N^* \tag{12}$$

gives the mole fraction of species A^*_i relative to the total polyacid, while

$$x^*_0 = 1/\textstyle\sum^* \tag{13}$$

gives the mole fraction of undissociated species A^*_0.

The equations in this section have been derived chiefly for developing the relations contained in the following sections. Their use for obtaining numerical values of the β^*_i's is limited because of the amplification of experimental error estimates in the application of eq 6. It can be shown that the average error estimates of the $\mathrm{p}\beta^*_i$'s and $\ln \sum^*$ are approximately N^*/N times those of the $\mathrm{p}\beta_i^{(N)}$[3] and $\ln \sum_N$, respectively, and that only the $\mathrm{p}K_i$'s are not affected by this error magnification with increasing N. However, these limitations are of little practical consequence since any measurable property of the polyacid that might be described by the N^* β^*_i parameters can be characterized equally well and more efficiently by the N β_i parameters.

The error magnification in going from $\sum_N$ to $\sum^*$ does not affect the validity of the relations derived in the following sections. In these derivations eq 5 is used to go from the "true" $\sum^*$ to $\sum_N$. This procedure reverses and thereby cancels the error magnification.

Free Energy of Dissociation

Let us consider the deprotonation of 1 mol of polyacid A^*_0 according to eq 9. At any value of h and the corresponding value of α we will have a mixture of species A^*_i with mole fractions x^*_i. Following a method introduced by Schellman,[5] we will show in the Appendix that the free energy of this process is given by the expression

$$\Delta G^*_{\mathrm{p}} = -RT \ln \textstyle\sum^* \tag{14}$$

The above equation refers to the hydrogen ion at its ambient chemical potential in the equilibrium mixture. Another useful free energy function applies to a process which differs from the above only in that the hydrogen ion is in its standard state. It is shown in the Appendix that this free energy, also for 1 mol of polyacid, is expressed by the equation

$$\Delta G^{\circ *}_{\mathrm{p}} = -RT\left(\ln \textstyle\sum^* - \dfrac{N^*\alpha}{\alpha_{\mathrm{m}}} \ln h\right) \tag{15}$$

By the definitions of α_{m} and N^*, the quantity N^*/α_{m} gives the number of repeat units in the polyacid molecule. Dividing eq 14 and 15 by this number and applying eq 5, we obtain the free energies per mole of repeat unit, ΔG and ΔG°, referring to hydrogen ion at ambient and standard state chemical potentials, respectively.

$$\Delta G/RT = -(\alpha_{\mathrm{m}}/N) \ln \textstyle\sum_N \tag{16}$$

and

$$\Delta G^\circ/RT = -(\alpha_{\mathrm{m}}/N) \ln \textstyle\sum_N + \alpha \ln h \tag{17}$$

Applying eq 4, we obtain

$$\Delta G/RT = -\int_{h=0}^{h=h} \alpha \, \mathrm{d} \ln h \tag{18}$$

and

$$\Delta G^\circ/RT = \int_0^\alpha \ln h \, \mathrm{d}\alpha \tag{19}$$

The derivation of eq 19 involves integration by parts and the fact that the product $\alpha \ln h$ vanishes as α and h approach zero. Equation 2 may be expressed in the form

$$\textstyle\sum_N = \beta_N h^N \left(1 + \dfrac{\beta_{N-1}}{\beta_N h} + \dfrac{\beta_{N-2}}{\beta_N h^2} + ... + \dfrac{1}{\beta_N h^N}\right) \tag{20}$$

In the limit of $h = \infty$, this leads, after rearrangement, to

$$\ln \beta_N = \lim_{h\to\infty} (\ln \textstyle\sum_N - N \ln h) \tag{21}$$

which, together with eq 17 in the same limit, gives

$$\Delta G^\circ(\alpha = \alpha_{\mathrm{m}})/RT = -(\alpha_{\mathrm{m}}/N) \ln \beta_N \tag{22}$$

Applying eq 19, we obtain

$$\mathrm{p}\beta_N = (N/\alpha_{\mathrm{m}}) \int_0^{\alpha_{\mathrm{m}}} \mathrm{pH} \, \mathrm{d}\alpha \tag{23}$$

This equation provides an alternative method for calculating β_N from the experimental data. In view of the invariance relation expressed by eq 8, eq 23 may also be considered to give the overall equilibrium constant for the complete ionization of any N-protic polyacid. Equations 16, 17, and 22 permit the convenient calculation of ΔG and ΔG° from the β_i parameters.

Interpretation of $\sum_N$

According to eq 6 the partition function of the macromolecule, $\sum^*$, can be represented as a power of $\sum_N$. This is what would be expected if the polyacid molecule consisted of independent noninteracting subunits containing N ionizable groups and having $\sum_N$ as their partition function. However, the polyacids under consideration have no identifiable, well-separated subunits in which each ionizable group would interact solely with groups of the same subunits but not with those of other subunits. As has already been mentioned, N has no intrinsic molecular significance but may be any number large enough so that eq 1 represents the potentiometric titration curve within the attainable experimental precision.

Nevertheless, by introducing a different interpretation for the subunit, the identification of $\sum_N$ with the partition function of the subunit may be preserved. The subunit is defined to be any chain segment containing N ionizable groups. Since $\sum_N$ and $\sum^*$ contain exactly the same information, obtained from experimental data pertaining to the actual polyacid, the subunit may be viewed as representing and sampling the behavior of the polyacid molecule as a whole. We therefore denote it by the term "representative sample subunit" or RSSU. The values of its ionization constants, β_i, are the results of interactions with groups located both within and outside the subunit. A significant consequence of identifying $\sum_N$ with the partition function of the RSSU is that the quantity $\beta_i h^i / \sum_N$ denotes the probability that i of its protons are dissociated or, expressed alternately, that on selecting at random and independently a large number of RSSU's, the relation

$$x_i = \beta_i h^i / \textstyle\sum_N \tag{24}$$

gives the mole fraction of subunits with i dissociated protons.

Application to Analysis of Conformational Transitions

The foregoing analysis helps to elucidate the conditions necessary for observing a bimodal distribution in the species mole fractions, x_i, for a polyacid undergoing a conformational transition. At any pH in the transition region the polyacid solution may be viewed as a mixture of the two conformations which must differ significantly in their degrees of ionization for the effect to be noticeable at all. If now each polyacid molecule existed in its entirety in one or the other conformation, the mole fractions, x^*_i, of the whole polyacid molecule would be expected to have a bimodal distribution. However, it is more common for the cooperative unit to be much smaller than the macromolecule, which then consists of alternating stretches of the two conformations. The degree of ionization of each macromolecule would now be an average of the degrees of ionization of the conformations, and the x^*_i distribution would show a single peak only. Consider now a representative sample subunit (RSSU) which reports the ionization behavior of N consecutive ionizable groups on the macromolecule. We may imagine the observed distribution of the x_i's to result from a scan of the polyacid molecules by the RSSU. If in such a scan the frequency with which the RSSU overlaps predominantly one or the other conformation is large compared to the frequency with which is spans significant portions of both conformations, the x_i distribution will arise chiefly from the individual conformations and may be expected to be bimodal. This condition will be satisfied if the cooperative unit is large compared to the RSSU.

Table I
Stepwise Ionization Constants for Polyacids with $N = 8$

	$\mathrm{p}K_i$		
i	PAA[a]	CMC[b]	PGA[c]
1	3.60	2.85	4.12
2	4.10	3.20	4.06
3	4.51	3.48	4.32
4	4.90	3.73	5.43
5	5.29	4.00	4.51
6	5.72	4.29	5.24
7	6.19	4.64	5.44
8	6.88	5.15	6.32

[a] In 0.2 NaCl. From ref 6 (code B7). [b] In 0.1 M NaCl. From ref 7 (code 93D). [c] In 0.2 M NaCl. From ref 8.

Applications to Experimental Results

In addition to the two polyacids treated previously, three more polyacids have now been analyzed, namely, poly(acrylic acid) (PAA), (carboxymethyl)cellulose (CMC), and poly(L-glutamic acid) (PGA). It is generally believed that the first two of these do not and the last of these does undergo a conformational transition. In order to discuss the results, which are presented in Table I, it is convenient to develop first a simple criterion by which the existence and location of a minimum in the population distribution of the RSSU can be inferred directly from the ionization constants, without the need for the space-consuming tabulations or graphs of the x_i's.

For x_i to be a minimum at a particular pH, we must have, by application of eq 24, the inequalities

$$\beta_{i-1} h^{i-1} > \beta_i h^i < \beta_{i+1} h^{i+1} \tag{25}$$

Dividing by $\beta_i h^i$ and defining stepwise ionization constants by

$$K_i = \beta_i / \beta_{i-1} \tag{26}$$

we obtain

$$1/K_i h > 1 < K_{i+1} h \tag{27}$$

Since $K_i h$ is positive, this can be written as

$$K_i h < 1 < K_{i+1} h \tag{28}$$

On dividing by h and taking negative logarithms, this becomes

$$\mathrm{p}K_i > \mathrm{pH} > \mathrm{p}K_{i+1} \tag{29}$$

which is the desired criterion for x_i to have a minimum.

By the same method it can be shown that the condition for a maximum in x_i is

$$\mathrm{p}K_i < \mathrm{pH} < \mathrm{p}K_{i+1} \tag{30}$$

All the $\mathrm{p}K_i$ values in Table I were calculated by eq 1 and 26. Those for PAA and CMC were based on experimental results of Mandel[6,7] and those for PGA on the results of Wada.[8] It is clear that for PAA and CMC the $\mathrm{p}K_i$'s increase steadily with i, indicating the absence of minima in the population distribution.[9] At any pH the distribution has a single maximum according to criterion 30. In contrast, in the case of PGA the distribution functions are seen to be bimodal at every pH between 4.51 and 5.43. For example, for pH 5, x_4 is a minimum according to criterion 29 while x_3 and x_5 are maxima according to criterion 30. The occurrence of a helix–coil transition in this pH range has been directly demonstrated by specific rotation data.[8]

The use of the inequality 30 to diagnose minima in the population distribution also furnishes a simple means for determining whether a minimum is experimentally significant or whether it may be an artifact arising from experimental error. In our own experimental work with copolymers of maleic acid and alkyl vinyl ethers we have found that the reproducibility of pK_i values obtained from several independent titration experiments was within 0.2 unit, and therefore we consider decreases in successive pK_i's of greater than 0.4 unit as permitting the inference of a minimum in x_i. If we assume that the experimental accuracy of the PGA titrations was about the same as ours, we may conclude that the decrease from pK_4 to pK_5 is significant but that we may not attach significance to the decrease from pK_1 to pK_2.

Concluding Remarks

The method described in this paper is not limited to the deprotonation of polyacids but may be generalized to other polyelectrolyte equilibria. The extension to the dissociation of ligands other than protons is obvious. To treat association equilibria, the formulas developed here apply directly, with the modification that the reciprocal activity, h, must be replaced by the activity of the ligand and the β_i's must be regarded as association instead of dissociation constants.

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Appendix. Derivations of Eq 14 and 15

The chemical potential, μ, of a polyacid, partially deprotonated according to eq 9, is given by the expression

$$\mu = \sum_{i=0}^{N^*} x^*_i\mu_i + \sum_{i=0}^{N^*} ix^*_i\mu_{\mathrm{H}^+} \qquad \text{(A1)}$$

where μ_i and μ_{H^+} are the chemical potentials of species A^*_i and H^+, respectively. Subtracting the identity $\mu_0 = \mu_0\sum_{i=0}^{N^*} x_i^*$ from both sides of eq A1, we obtain

$$\mu - \mu_0 = \sum_{i=0}^{N^*} x^*_i(\mu_i + i\mu_{\mathrm{H}^+} - \mu_0) \qquad \text{(A2)}$$

Because the system is in equilibrium according to eq 9, each expression in parentheses on the right-hand side of eq A2 vanishes, and

$$\mu = \mu_0 \qquad \text{(A3)}$$

Thus the chemical potential of the polyacid is that of the un-ionized species, and the free energy of ionization per mole of polyacid is given by

$$\Delta G^*_{\mathrm{p}} = \mu_0(\text{after ionization}) - \mu_0(\text{before ionization}) = RT \ln \frac{[\mathrm{A}^*_0]_{\text{after}}\gamma_{0,\text{after}}}{[\mathrm{A}^*_0]_{\text{before}}\gamma_{0,\text{before}}} \qquad \text{(A4)}$$

Neglecting changes in the activity coefficient of the uncharged species and assuming no change in solution volume, we obtain

$$\Delta G^*_{\mathrm{p}} = RT \ln x^*_0 \qquad \text{(A5)}$$

which, by eq 13, becomes

$$\Delta G^*_{\mathrm{p}} = -RT \ln \Sigma^* \qquad \text{(A6)}$$

This is the desired eq 14, which gives the free energy of ionization per mole of polyacid at ambient chemical potential of hydrogen ion.[5]

To calculate the free energy change for the same process except that the dissociated hydrogen ion is in its standard state, we modify the chemical potential in eq A1 by adding the quantity $\sum_{i=0}^{N^*} ix^*_i\mu^\circ_{\mathrm{H}^+} - \sum_{i=0}^{N^*} ix^*_i\mu_{\mathrm{H}^+}$. This has the effect of adding this quantity to the right-hand sides of eq A2–A6, also. (The term persists through eq A4 because it is zero before ionization.) By applying eq 12 to eq 1, we obtain the identity

$$\sum_{i=0}^{N^*} ix^*_i = N^*\alpha/\alpha_{\mathrm{m}} \qquad \text{(A7)}$$

By the definition of h, we have

$$\mu_{\mathrm{H}^+} - \mu^\circ_{\mathrm{H}^+} = -RT \ln h \qquad \text{(A8)}$$

Then the desired free energy change is

$$\Delta G^{\circ *}_{\mathrm{p}} = -RT(\ln \Sigma^* - (N^*\alpha/\alpha_{\mathrm{m}}) \ln h) \qquad \text{(A9)}$$

which is eq 15.

It should be noted that the total concentration of the polyacid is assumed to be constant in these processes and small enough (in an absolute sense and also compared to the concentration of the supporting electrolyte) so that interactions between macromolecules may be neglected. Equations A1–A9 may consequently be considered to apply to the polyacid at infinite dilution in the electrolyte medium. By regarding the pH, like temperature and pressure, as a potential, one may therefore define the polyacid in a fixed medium of excess supporting electrolyte to be in its standard state at every pH, even though it consists of a large number of species differing in ionization states. This concept may be applied to obtain standard free energy differences between two conformations at any pH when the standard free energy difference at zero charge is known.[10]

References and Notes

(1) Strauss, U. P.; Barbieri, B. W.; Wong, G. *J. Phys. Chem.* **1979**, *83*, 2840.
(2) We assume that for a high polymer, N^*/N can be adequately approximated by an integer.
(3) It should be noted that for complete identification the β_i's belonging to a particular set should be labeled with N, i.e., $\beta_i^{(N)}$, to distinguish them from the β_i's belonging to a set with a different number of coeffients. To avoid excessive notation this superscript is omitted when the meaning is clear without it.
(4) It should be noted that what is denoted throughout this paper as the species with i dissociated protons consists of $N!/i!(N-i)!$ microscopic subspecies which may differ in energy and other thermodynamic properties. The legitimacy of lumping these subspecies into one species has been amply treated in the literature. See, for instance: King, E. J. "Acid-Base Equilibria"; Pergamon Press: Oxford, 1965; Chapter 9.
(5) Schellman, J. A. *Biopolymers* **1975**, *14*, 999.
(6) Mandel, M. *Eur. Polym. J.* **1970**, *6*, 807.
(7) Mandel, M. *J. Polym. Sci., Part A-2* **1970**, *8*, 1841.
(8) Wada, A. *Mol. Phys.* **1960**, *3*, 409.
(9) It should be noted that this finding remains the same if smaller values of N are used for PAA and CMC.
(10) Strauss, U. P.; Barbieri, B. W. *Macromolecules* **1982**, *15*, 1347.