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A FIELD-DISSOCIATION RELATION FOR POLYELECTROLYTES WITH AN APPLICATION TO FIELD-INDUCED CONFORMATIONAL CHANGES OF POLYNUCLEOTIDES

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An extension to polyelectrolyte solutions of Onsager's field-dissociation relation for weak electrolytes can be derived in a simple way. It is found that, except in the limit of zero ionic strength, a strong applied electric field prevents counterion condensation from proceeding to completion. The extent of incompleteness initially varies linearly with the applied field. The field-dissociation relation can easily be incorporated into the theory of ionic effects on the stability of ordered polynucleotide structures, whereupon a dependence of the stability on field strength emerges. An explicit calculation for a cooperative transition of the DNA melting type is presented, and it is concluded that for sufficiently low ionic strengths, a field of the order of 10 kV/cm may be able to induce melting by lowering the T_m by a few degrees. The threshold effect found experimentally by Pörschke, and particularly the observed linear dependence of the threshold field on the logarithm of the ionic strength, appears here as a simple consequence of the linear increase of the stabilization free energy with the logarithm of ionic strength.

1. Introduction

It is known that application of strong electric fields can induce conformational changes in biopolyelectrolytes [1–3]. The simplest explanation [3] is that the field pulls some of the associated counterions away from the immediate neighborhood of the polyion, thereby destabilizing its structure. A quantitative theory, heretofore unavailable, is required for proper assessment of this hypothesis, and, as Pörschke [3] has suggested, a proper starting point would be the development of a polyelectrolyte analog to Onsager's analysis [4,5] for ordinary weak electrolytes. However, polyelectrolyte behavior is distinctive, and it should not be expected that the proper analogy will be a more or less obvious generalization of Onsager's result to the case for which the valence of one of the ions is very large. The most striking difference in the two systems, of course, is that it is *association* that is complete in polyelectrolyte solutions of vanishing ionic strength [6–8] rather than dissociation, so that a mass-action formulation is excluded. It will turn out for the polyelectrolyte case that the influence of both the ionic strength and the polyelectrolyte "valence" (i.e., charge density) on the field-dissociation relation could not have been guessed from the theory of weak electrolytes.

The effect of a strong field on the conformation of a biopolyelectrolyte cannot be inferred simply from knowledge of the degree of counterion dissociation as a function of field-strength. In addition, the dependence of the conformational stability (free energy associated with the conformational change) on the number of associated counterions is required. Thus, the problem is of some physical complexity, and the simplicity both of the results and their derivation is remarkable.

2. The field-dissociation relation

In a recent paper [8] a formula was derived that relates the degree r of counterion association (fraction of polyion charge compensated by counterions) with a polyion of axial charge spacing b to the molarity c_s of excess ordinary salt and to the free volume V_p

(cm^3) available to the associated counterions per mole of univalent charged groups fixed to the polyion,

$$r = 10^{-3} \gamma_s e^{-\phi_s} \nu |z| V_p c_s (1 - e^{-\kappa_s b})^{-2|z|\xi(1-r)} . \tag{1}$$

Other symbols are the Debye screening parameter κ_s, the counterion activity coefficient γ_s, the osmotic coefficient ϕ_s – all referring to an ordinary salt solution of molarity c_s for which the electrolyte formula contains ν counterions of valence z – and the dimensionless polyelectrolyte charge density parameter ξ, equal to $q^2/\epsilon kTb$, with q the protonic charge, ϵ the solvent dielectric constant, k Boltzmann's constant, and T the absolute temperature.

Both r and V_p may be regarded as functions of an externally applied field X, since the derivation of eq. (1), although conceived for the usual zero-field case, can be carried through for an arbitrary field strength if the distortion of the Debye screening atmosphere is neglected. If $r(0)$ denotes the value of r for zero field, then from [8],

$$r(0) = 1 - |z|^{-1} \xi^{-1} \tag{2}$$

and the degree of dissociation α will be defined by

$$r(X)/r(0) = 1 - \alpha . \tag{3}$$

The value of $V_p(0)$ is given by eq. (16) of ref. [8] but is not needed here; $V_p(X)$ has a generalized significance which will be discussed in due course. If eq. (1) is written both for arbitrary X and $X = 0$ and the ratio taken, the result is

$$V_p(X)/V_p(0) = (1 - \alpha)(1 - e^{-\kappa_s b})^{2\alpha(|z|\xi - 1)} \tag{4}$$

where eqs. (2) and (3) have also been used.

For zero field, V_p enters the theory [8] through an assumed form for the chemical potential of associated counterions,

$$\mu = \mu^* + RT \ln[|z|^{-1} r/V_p(0)] , \tag{5}$$

where the standard state chemical potential μ^* is the same as for free counterions. If $V_p(0)$ is interpreted as a volume (per polyelectrolyte equivalent), the argument of the logarithm becomes the local concentration of associated counterions [8]. If, further, $V_p(0)$ has an assumed cylindrical form axially centered on the polyion, then a radial distance a may be defined [8] by

$$V_p(0) = \pi a^2 b L , \tag{6}$$

where L is Avogadro's number. It has been demonstrated that $V_p(0)$, hence a, when determined self-consistently (i.e., by minimization of the total free energy), is uniquely determined by the charge spacing b and the ionic strength, and numerical values of a have been tabulated [8].

The functional dependence of V_p on X is crucial. It will be taken as

$$V_p(X) = V_p(0) \exp(-\bar{X}) , \tag{7}$$

with

$$\bar{X} = (\pi/4)(|z| q a X/kT) . \tag{8}$$

The argument proceeds from (and essentially ends at) recognition of the work that a counterion of charge zq must perform against the field if it is to remain associated [i.e., within the volume $V_p(0)$ of eq. (7)]. Since $V_p(0)$ is symmetrically positioned about the polyion axis, the average position of an associated ion is on the axis. If the axis is orthogonal to the applied field, the work is obviously given by the numerator of the second factor in eq. (8) if a is defined by eq. (6). The factor $(\pi/4)$ arises from an average over all orientations of the axis with respect to the field. The excess chemical potential of the associated counterions due to the field is, then, $L(\pi/4)|z|qaX$, where L is Avogadro's number. This term must be added to the zero-field chemical potential in eq. (5), an operation which is equivalent to the replacement of $V_p(0)$ in eq. (5) by $V_p(X)$ from eq. (7).

Substitution of eq. (7) into eq. (4) yields the desired field-dissociation relation for polyelectrolytes,

$$\bar{X} = -\ln(1 - \alpha) - 2\alpha(|z|\xi - 1) \ln(1 - e^{-\kappa_s b}) . \tag{9}$$

Note that in the limit of zero ionic strength ($\kappa_s b \to 0$), the field strength measured by $\bar{X}$ must become infinite to produce any degree of dissociation α greater than zero, i.e., counterion condensation remains complete in this limit for any finite field, no matter how large. For most cases of practical interest, the conditions $\alpha \ll 1$ and $\kappa_s b \ll 1$ are satisfied, and eq. (9) then reduces to a linear relation between α and X mediated by a logarithmic dependence on ionic strength (to which κ_s^2 is proportional),

$$\bar{X} = \alpha[1 - (|z|\xi - 1) \ln(\kappa_s b)^2] . \tag{10}$$

For an aqueous polyelectrolyte solution at 25°C with excess 1 : 1 salt of ionic strength 10^{-2} M, an applied field of 402 kV/cm produces 10% dissociation ($\alpha = 0.1$) if $\xi = 4$ (the approximate value for native DNA); while if $\xi = 2$ (the approximate value for a single-stranded polynucleotide), a lower field of 107 kV/cm produces the same extent of dissociation. At the higher ionic strength 10^{-1} M, dissociation requires less work because the dissociated ions enter a more effective screening atmosphere: the fields corresponding to $\alpha = 0.1$ are 262 kV/cm for $\xi = 4$ and 72 kV/cm for $\xi = 2$. The values of a required to arrive at these numbers were taken from table 3 in ref. [8]; a is in the neighborhood of 15 Å for all cases. Use of the approximate eq. (10) leads to a 12% error in the worst case above, which is comparable to the estimated 10% modeling inconsistency for this case discussed in [8].

Before ending this section, I must caution against oversimplified attempts to apply these results to strong-field effects on the conductivity of polyelectrolyte solutions. Although I have not yet had the opportunity to think through the problems involved, it strikes me as unlikely that the direct applicability of Onsager's formula to the conductivity of ordinary weak electrolytes will be paralleled here.

3. Field-induced melting of DNA

In the previous section it was determined that a field of about 70 kV/cm would cause 10% dissociation from a single-stranded polynucleotide at an ionic strength of 0.1 M. The field-induced conformational changes which have thus far been reported occur under conditions (lower ionic strength, somewhat lower field strengths, multi-stranded polynucleotide structures in some cases) that would result in still less dissociation. Hence, the field-dissociation relation is effectively linear, as in eq. (10). The importance of this observation is that, on its basis, a quite general argument can be given which leads to the relation,

$$\bar{g}(X) - \bar{g}(0) = |z|^{-1}(1 - |z|^{-1}\xi^{-1})\bar{X} , \qquad (11)$$

where $\bar{g} n_p RT$ is the free energy of a polyelectrolyte solution with polyions of reduced charge density ξ, counterions of valence z, and n_p moles of polyion charged groups (the polynucleotide phosphate groups).

The argument is simply that when the field is turned on, each mole of associated counterions experiences an increment in energy equal to $RT\bar{X}$, as explained in the preceding section. Also, from eqs. (2) and (3), there are $(1-\alpha)|z|^{-1}(1 - |z|^{-1}\xi^{-1})$ moles of associated counterions per mole of polyion charged groups. Since $\bar{X}$ is linear in α, it follows that eq. (11) describes the effect of the field to first order in α, and, since α is small, that first-order accuracy is sufficient. There is also a contribution to the free energy increment due to redistribution of associated and free counterions, that is, to advancement of the "reaction" (associated ion) → (free ion); but because this reaction is initially in equilibrium (at a free energy minimum), the corresponding free energy of advancement is second-order in α and does not contribute to the first-order effect. The explicit and exact calculation given in the Appendix confirms eq. (11) as the correct first-order expression and shows also that retention of second-order terms is equivalent to multiplying the right hand size of eq. (11) by $1 - \frac{1}{2}\alpha$, so that eq. (11) may be used if a loss of accuracy of less than 5% can be tolerated.

Consider now two possible conformations 1 and 2 of the same polyelectrolyte, such as the ordered and denatured forms of DNA. Assume that $\xi_1 > \xi_2$, and let the Δ operation signify "2 minus 1." Note also that from its definition, eq. (8), $\bar{X}$ may differ for forms 1 and 2 because the length a depends on ξ [8]. Then, from eq. (11),

$$\Delta\bar{g}(X) - \Delta\bar{g}(0) = |z|^{-1}\,\Delta[(1 - |z|^{-1}\xi^{-1})\bar{X}] . \qquad (12)$$

For the DNA transition in 1 : 1 salt, letting 1 be the double helix and 2 the separated coils, the appropriate numerical values for ξ [9] and a [8] yield, at 25°C,

$$\Delta\bar{g}(X) - \Delta\bar{g}(0) = -(0.94 \times 10^{-3})X , \qquad (13)$$

where X is in kV/cm. The small dependence of a on ionic strength has been neglected. Eq. (13) says that, although the free energy of both conformations is increased by application of the field, that of the double helix is increased more and, consequently, it is destabilized. The primary effect is due to the greater charge density of the double helix, which enables it to hold more associated counterions per phosphate group than the random coil; each associated counterion possessing the same incremental field energy (except for small differences caused by the inequality of a_1 and a_2), the double helix therefore suffers the higher energy increase.

Numerically, the destabilization free energy is small. In a field of 50 kV/cm, of the order of those utilized experimentally, eq. (13) indicates that $\Delta\bar{g}$ is decreased by only about 0.05 units, or about 0.03 kcal per mole phosphate (i.e., per mole base). Nevertheless, a mere 0.5 kcal per mole base results in a decrease of T_m by as much as 40 degrees [10], so eq. (13) is consistent with a lowering of the T_m by a few degrees at typical fields. Available data present difficulties of interpretation, and I am unable at present to make quantitative comparisons between theory and experiment.

The qualitative phenomenon of a threshold field found by Pörschke [3] may be an obvious consequence of eq. (12), since, even though $\Delta\bar{g}$ may be decreased by any non-zero field, it is only at the point for which the decrease is sufficiently large to lower the T_m to the ambient temperature that the conformational change will occur. At increased ionic strengths the T_m is higher, and a correspondingly larger field is required to lower the T_m to ambient; the threshold field therefore increases with ionic strength, in line with Pörschke's observations. The quantitative relation between the threshold field X_{th} and ionic strength c_s (excess 1 : 1 salt) may be found from the known dependence of $\Delta\bar{g}(0)$ on c_s for DNA melting [11] (use of the correct value for ξ_2 [9] leads to the value $\eta = 0.30$ in eq. (20) of ref. [11],

$$\Delta\bar{g}(0) = \Delta\bar{g}^{\circ} + 0.35 \log c_s \ . \tag{14}$$

Let now eq. (13) be written for $X = X_{th}$, with $\Delta\bar{g}(X_{th}) = 0$ and with eq. (14) for $\Delta\bar{g}(0)$. The result, at 25°C, is

$$\mathrm{d}X_{th}/\mathrm{d}\log c_s = 372 \text{ kV/cm}. \tag{15}$$

The linear dependence of X_{th} on log c_s agrees with Pörschke's data. The slope indicated in eq. (15) is much higher than that found by Pörschke, whose experiments, however, were not on induced melting of DNA but on the noncooperative conformational shifts of poly C and poly A. Eq. (15) predicts that if melting of DNA is induced at $c_s = 10^{-4}$ M, say, by a field of 20 kV/cm, then the fields needed for melting at 10^{-3} M should approach the highest obtainable in practice (several hundred kV/cm). This estimate is consistent with the known increase of T_m by about 20° per decade ionic strength increase and the lowering of T_m by only a degree or two for X of order 10 kV/cm.

I am grateful to Dietmar Pörschke for calling my attention to this problem.

Appendix

This section contains an exact result which confirms the accuracy of eq. (11). The quantity $\bar{g}(0)$ may be obtained in explicit form by summing its component contributions, all of which are listed in [8]; $\bar{g}(X)$ is then the direct result of replacing $r(0)$ and $V_p(0)$ in $\bar{g}(0)$ by $r(X)$ and $V_p(X)$ from eqs. (3) and (7). The result for $\bar{g}(X)$ has the form

$$\bar{g}(X) = \bar{g}(0) + |z|^{-1}\, r(0)\,\bar{X}(1-\alpha) + A_1\alpha + A_2\alpha^2 + A_3(\alpha), \tag{16}$$

where A_1 and A_2 are independent of both $\bar{X}$ and α and $A_3(\alpha) = O(\alpha^3)$. Use of eq. (2) with $r = r(0)$ and $V_p = V_p(0)$ directly yields the result $A_1 = 0$. Eq. (9) shows that

$$[|z|/r(0)]\, A_2\, \alpha^2 = \tfrac{1}{2}\alpha\bar{X} + \tfrac{1}{2}\alpha[\alpha + \ln(1-\alpha)] \ . \tag{17}$$

Substitution of eq. (17) into eq. (16), together with the explicit form of $A_3(\alpha)$, gives

$$\bar{g}(X) - \bar{g}(0) = |z|^{-1}\, r(0)\, [\bar{X}(1 - \tfrac{1}{2}\alpha) + A(\alpha)] \ , \tag{18}$$

where $A(\alpha) = O(\alpha^3)$, or, explicitly,

$$A(\alpha) = \tfrac{1}{2}\alpha^2 + (1 - \tfrac{1}{2}\alpha)[\alpha + \ln(1-\alpha)] = -\tfrac{1}{12}\alpha^3 + \ldots \tag{19}$$

With use of eq. (2), eqs. (11) and (18) may now be compared for values of α, which are, in practice, small.

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