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Polyelectrolyte Orientation in Electric Fields: The Effect of the Dielectric Constant

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ABSTRACT: The orienting torque on an asymmetric macromolecular polyion in an electric field is a function of the dielectric properties of the solvent in which it is embedded, but no current theory gives a totally adequate description of the phenomenon. In this paper the effect on the electric-field-induced orientation of DNA of variation of the dielectric constant in water–alcohol solutions is analyzed in terms of theories which describe the orientation as resulting substantially from the polarization of the polyion–counterion moiety of this high charge density polyion. The observations are shown to be compatible with this description. The observations also demonstrate clearly the transition between two conformations of DNA between 60 and 80 vol % ethanol in low salt solution and are quantitatively consistent with the optical and charge density changes resulting from the conformational transition between B (or C) form and A form structures.

The high sensitivity of the dielectric and electrooptical properties of polyelectrolytes of the ionic and dielectric environment in which the measurements are carried out has provided a classical target for theoreticians for many years. Specifically, it is over a decade now since McTague and Gibbs[1] and, a few years later, Hornick and Weill[2] discussed the problem of counterion–counterion repulsion as a factor in the dielectric properties of polyions in solution. In this paper we examine the explicit dependence of the electric-field-induced orientation of the linear polyion DNA on the bulk dielectric constant of the solvent. The dielectric constant does not enter explicity in the treatment by McTague and Gibbs, although an analogous "screening parameter" is introduced into the expression for the probability of counterion occupation of a site on the polyion immediately preceding an occupied site. The Hornick and Weill treatment, which results from the theory for the counterion repulsion energy derived by Oosawa[3] on the basis of a Poisson–Boltzman distribution of charge around the polyion, does result in an expression for the polarizability parallel to the DNA axis which is linear in the dielectric constant, ϵ. This expression has a mathematical similarity to the one derived more recently by Manning[4] which we have used to derive eq 1. However, an adequate comparison is difficult because of a parameter R/A which appears in the Hornick and Weill treatment whose explicit dependence on ϵ is unknown. R/A is the ratio of the radius of the cylindrical volume offered to a single molecule to the radius of the cylindrical phase of bound counterions. More recently, Tricot et al.[5] measured the electric-field-induced birefringence of aqueous solutions of poly(*N*-methyl-2-vinylpyridinium bromide) and examined the relationship of the Oosawa–Hornick and Weill treatment and of a treatment of Van der Touw and Mandel[6] to their results. They concluded that the former was inadequate to explain their results [although it should be noted that it is possible that the Oosawa treatment is dependent on the presence of excess counterions in the solutions and thus not entirely applicable to the poly(*N*-methyl-2-vinylpyridium bromide) experiments]. Van der Touw and Mandel's analysis does not account for counterion–counterion repulsion. It is, therefore, not strictly comparable either to the Hornick and Weill or to the present treatment. More importantly, from the point of view of the present work, the effect of the dielectric constant is not explicitly analyzed although it is implicit in a parameter which relates the polarization to the ratio of the effective field on the polyion to the static applied field. Recently, on the basis of an ad hoc hypothesis that is not, however, without considered theoretical foundation, we proposed[7] that the polyelectrolyte term in the orientation function for linear (prolate) polyelectrolytes at field strengths low enough to exhibit a quadratic dependence on E would take the form

$$\Phi(n,L,E) = \frac{(Z - \xi^{-1})}{1 - 2(Z\xi - 1)\ln \kappa b}\,\frac{Ne^2L^2E^2}{180k^2T^2} \qquad (1)$$

where the factor $[1 - 2(Z\xi - 1)\ln \kappa b]$ arises from considerations introduced by Manning[4] to account for the effect of the counterion–counterion repulsion on the polarizability of a high charge density polyelectrolyte. In this equation, Z is the charge on the counterion; $\xi = e^2/\epsilon kTb$ is a charge density parameter containing the electronic charge, e; b is the distance between the charges projected on the axis of the polyelectrolyte (1.7 Å in B form DNA); kT is the Boltzmann energy at the Kelvin temperature T; $\kappa = [8 \times 10^{-3}\pi N_0(e^2/\epsilon kT)I]^{1/2}$ is the Debye-Hückel screening parameter in which N_0 is Avogradro's number and I is the ionic strength of the solution; and N and L are respectively the number of charges on the polyion and its length L. In this equation, the dielectric constant appears explicitly in ξ and κ. In addition, it may be expected to exert its influence on one or both of the following: the effective field E and the structural parameter b.

An intense interest in the orientation of nucleic acids and polynucleotides has led us to examine the dependence of the orientation on the dielectric constant modeled for DNA by eq 1. The method chosen is to measure the electric dichroism in solutions where the dielectric constant is varied by the addition of ethanol to an aqueous solution at a constant concentration of NaCl and DNA. DNA is a polyion in which the charged phosphate groups projected on the axis of the B form double helix are 1.69 Å apart. While the DNA may exist in a superhelical form[8] in which the projection of the phosphates on the superhelical axis is somewhat less than 1.69 Å, we believe that in an electric field the polarization of DNA may be treated like that of a linear rod whose charge density is determined by the axial separation of the phosphates on the helical axis. Transformation to other linear structures can and does occur. B-DNA is transformed to A-DNA at high (60–80%) alcohol concentrations[8–11] and very likely to C-DNA at intermediate (10–60%) alcohol concentrations.[8,10,11] In using the terminology A, B, and C form in this paper, we refer specifically to the tilt and/or twist of the nucleotide bases and to the linear projection of the phosphate positions on the helical axis. This caveat is especially important with respect to the C form which is specifically known only for a species which exists in very high salt under conditions of substantial dehydration. The value of b is affected drastically by the transformation from the B to the A form in which it is 1.28 Å but only slightly in the transformation to the C form in which it is 1.66 Å. Both transformations have been included in the calculations of the relative orientations described below, and the concomittant effect on the optical factor in the electric dichroism measurements from which the relative orientations are extracted has been included as well. The method used for the electric dichroism measurements is described elsewhere.[7] Finally, the dielectric constant has an effect on the magnitude of the orienting field. There is no precise consensus on the description of this effect, but it is not large and has been discussed many times starting with Debye[12] and Onsager.[13] We have chosen to incorporate it in a form derivable from the expression given by Oosawa[14] which begs the question of whether it arises from a cavity field or a reaction field and is more or less independent of the molecular shapes

$$(\epsilon - \epsilon_0)/\epsilon_0 = 3(E'/E)(N\nu/V) \qquad (2)$$

where E' is the effective field in the medium of dielectric constant ϵ, and E is the applied field in the pure solvent (here the acqueous salt solution) with a dielectric constant ϵ_0. N is the number of macroions (polyions) in V mL of solution, and ν is the apparent volume of the macroion. The effective field and the applied field are related by the induced polarization

$$P = N\alpha E' \qquad (3)$$

through the dielectric corallary of Maxwell's equation

$$\epsilon E = E + 4\pi P = E + 4\pi N\alpha E' \qquad (4)$$

Combining this with the expression for the axial polarizability of the polyion derived by Manning[4]

$$\alpha = \frac{Z - \xi^{-1}}{[1 - 2(Z\xi - 1)\ln \kappa b]} \frac{Z^2e^2NL^2}{12kT} \qquad (5)$$

by algebraic manipulation, it can be shown that the relative values of the effective field, E'_i, in the alcohol solutions to that, E'_0, in the aqueous salt solutions taken as standard are given by

$$\frac{E'_i}{E'_0} = \frac{(\epsilon_i - 1)}{(\epsilon_0 - 1)} \frac{\Phi'(\epsilon_0)}{\Phi(\epsilon_i)} \qquad (6)$$

where the index i represents the volume percent alcohol in the solution and the $\Phi'(\epsilon)$ are orientation functions described by eq 1. As noted above, this effect is not large (see column 5, Table I). Because the field E enters as the square in the orientation functions, the dielectric effect due to the effective field enters as the ratio $(E'/E_0)^2$ which decreases by about 17% between 0 and 80% alcohol. A noteworthy feature, however, is that at low alcohol concentrations, it is as much as 5% higher than it is in the absence of alcohol. It is this increase which accounts for the inflection of the curves of Figure 1 near 10% ethanol. In the figure, the calculated values of the overall orientation function relative to those in 0% alcohol are plotted as a function of the alcohol concentration and are compared to the data obtained from measurements on sonicated calf-thymus DNA with a molecular weight of 170 000 daltons in 0.12 mM salt solutions of varying alcohol concentration. The agreement between the calculations and the measurements appears to be extraordinarily good. The results of experiments performed in 0.24 mM NaCl-water-alcohol solutions are the same within the accuracy of the experiments in accord with predictions. The precision of the measurements is given approximately by the vertical bar on the data points of Figure 1.

Table I

% of alcohol	ϵ^a	$\Phi(\epsilon)^b$	$\Phi(\epsilon,b)^b$	$(E'/E'_0)^2$ ^{c}	$f(\alpha)^d$
0	87.4	0.023	0.023	1.00	1.000
10	84.4	0.022	0.021	1.05	1.005
20	77.7	0.020	0.020	1.02	1.010
40	64.6	0.017	0.017	0.97	1.021
60	51.9	0.014	0.013	0.91	1.031
70	45.4	0.013	0.009	0.88	0.855
80	39.9	0.011	0.011	0.84	0.679

[a] Values of the dielectric constants are from G. Akerlof, *J. Am. Chem. Soc.*, **54**, 4128 (1932). See text for temperature specifications. [b] $\Phi(\epsilon)$ and $\Phi(\epsilon,b)$ are calculated with eq 1 respectively without and with the value of b adjusted for the B → C → A conformational transition of DNA, using values from the data in S. Zimmerman, *Biopolymers*, **15**, 1015 (1976). [c] Calculated from eq 4. The square of the ratio of E'/E'_0 is required to account for the quadratic E dependence of the orientation at low fields. [d] Calculated using the assumption that the 260-nm optical transitions of DNA are in the plane of the bases which make angles with the helix axis of approximately 83, 86, and 71°, respectively, for the B, C, and A conformations.

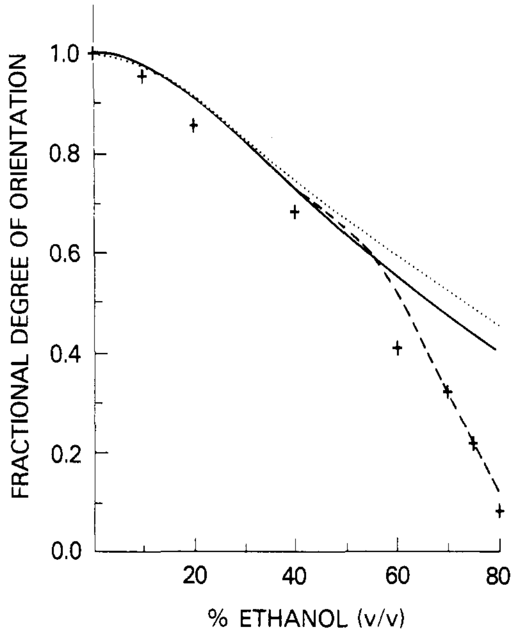

Figure 1. DNA orientation in ethanol-water solutions at a sodium ion concentration of 0.12 mM. The solid line is the calculated effect of variations of the dielectric constant on the polarizability and the effective field (columns 3 and 5 of Table I). The dashed line also includes the effect of changes in DNA conformation in ethanol-water solutions (columns 4, 5, and 6 of Table I). The dotted line is a calculation comparable to that of the solid line, based on a linear dependence of the orientation on the dielectric constant (see text). The crosses are data obtained from the field dependence of the electric dichroism of 170 000 daltons calf-thymus DNA.

Experimental *Section*

Calf-thymus DNA, Worthington Biochemicals Lot 35A614, dissolved in 0.1 N NaCl, sonicated in the cold (0 °C) under helium in short bursts was exhaustively dialyzed against 1.2 or 2.4 × 10^{-4} M NaCl. During and after dialysis, the samples were maintained below about 3 °C at all times, including during the transfer to the electrooptical cell. Measurements in aqueous salt solution were made at 0 °C and those in the alcohol solutions at -7 °C. The molecular weight of the DNA was determined by sedimentation velocity by Ms. Georgiana Sandeen, to whom we are indebted. Characterization of the sample by the hypochromism, CD, and A_{260}/A_{280} ratio showed it to be effectively native and deprotinated. The fractional degree of orientation at each alcohol concentration (based on the orientation of 0% as a standard) is obtained from the slopes of the curves of reduced electric dichroism plotted against the square of the field strength at low field strengths. Data and curves plotted from eq 1 and 4, as well as from the calculated effect of the DNA conformational transitions, are plotted in Figure 1 for experiments performed in 0.12 mM NaCl. At this scale, both the predicted and the measured effect of doubling the ionic strength to 0.24 mM are negligible.

Discussion

It is not our intention in this paper to discuss the basic validity of eq 1 as a model for the orientation of polyelectrolytes in electric fields or to compare this model with others which have been proposed.[15] In particular, the treatment on which this analysis is based does not include the possible effect on the orienting torque of an anisotropy in the field acting on the polyion arising from a phase lag between the flow of ions in the diffuse layers surrounding the polyion and in the bulk of the solvent. It is true, nevertheless, that the ability of the model to predict or explain the effect of various perturbations of the environment of the polyelectrolyte has a bearing on the degree of confidence which may be placed in it. Very specifically, we have attempted in this work to see whether the effect of variations of the bulk dielectric constant on the field-induced orientation of DNA is adequately given by eq 1, which is an ad hoc synthesis of Manning's expression for the polarizability of a linear polyion with the Kikuchi and Yoshioka expression[16,17] for the orientation.

These experiments were done in solvent mixtures of dilute aqueous salt solution and ethanol. Because the addition of ethanol produces a structural change in DNA, and because we have assumed in this treatment that the measured dichroism at low field strengths is a direct reflection of the orientation, it has been necessary to reflect on the effect of the structural changes in the calculated values of the relative orientation function. This effect is pronounced in the transition of the A form between 60 and 80% ethanol. In Table I, the factor $f(\alpha)$, derived from the square of the projection of the angle which the bases make with the helix axis in A, B, and C forms, is calculated on these assumptions: (1) that a smoothly linear transition occurs between the forms in going from 0 to 60% ethanol (B → C) and 60 to 80% ethanol (C → A), and (2) that the transition moments are in the base planes perpendicular to the tilt axis. Neither of these assumptions can be strictly true, but both are expected to be good approximations as the results plotted in Figure 1 indicate. In the figure, the dashed and solid curves represent the relative orientation functions predicted with and without accounting for the structural change. It is obvious that quantitative agreement with the observations is much better when the effect of ethanol on the structural parameters is included. It is equally clear, however, that the significant effect on the orientation is produced by variations in dielectric constant and that eq 1 describes very well the general features of this effect. The quantitative improvement resulting from the inclusion of changes in the structure brought about by the ethanol is such as to give substantial support to the conclusion that at least the structural change to the A form takes place in the range 60–80% ethanol concentration. The agreement between the observations and the predictions on the change from B → C form at lower alcohol concentration is somewhat more ambiguous and, in fact, may be slightly better if no transformation to C-DNA is included in the calculated values. The dotted curve of Figure 1 is a plot of the ratio of the dielectric constant of the solvent to that of the zero percent alcohol solvent and represents the explicit dielectric constant dependence of the Hornick and Weill[2] treatment. It was pointed out above that the dielectric constant dependence of the ratio R/A, which appears as a factor in the polyion polarizability, precludes an exact comparison with their analysis. Nevertheless, it is clear that it would be difficult to choose unambiguously between these treatments.

The question of the physical origin of the dielectric effect is a complex one. The field of a single ion at a distance r is given by $e/\epsilon r^2$, so that the repulsive force between two like ions due to their respective fields is increased by reducing the dielectric constant of the medium between them. One would expect, therefore, that the counterion–counterion repulsion on a polyion would increase with decreasing dielectric constant and that as a result somewhat fewer counterions are highly associated or condensed on the polyion as the dielectric constant decreases. If the degree of orientation at low fields is a function of the number of condensed counterions, then lowering the dielectric constant should decrease the orientation because of this assumption made in the derivation of eq 1.[7,16] The magnitude of the repulsive counterion interaction is modified, however, by the effect of the dielectric constant of the attractive forces between the polyion and the counterions. The sum of these interactions is evidently a tendency to increase the stability of the condensed counterion–polyion layer, thereby decreasing the tendency of the electric field to disturb it axially and effectively decreasing the axial polarizability on which the orientation depends. It would appear from these results that the Manning expression[4] for the axial polarizability of the polyion–counterion system gives a fairly good representation of this combined effect. It remains to be seen whether other, perhaps more stringent, tests prove to be equally confirmative of this analysis.

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