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IONIC STRENGTH AND COUNTERION REPULSION AS FACTORS IN THE BEHAVIOR OF POLYIONS IN ORIENTING ELECTRIC FIELDS

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The effect of counterion–counterion repulsion on the orientation of DNA, a polyion of high charge density is examined by electric-field orientation experiments. The charge species of the counterion and the ionic strength effect the orientation in a manner consistent with a theoretical treatment of the polarization of high charge density polyelectrolytes in terms of the effect of the applied field on the equilibrium distribution of condensed counterions on the polyion.

1. Theoretical

DNA and the polynucleotides which have been studied to help understand the physical properties of that ubiquitous genetic material, are polyelectrolytes with a high charge density. Typically, in their double helical or other multiple stranded forms at or near neutral pH, the projection of the formal charges contributed by their phosphate groups on the principal axis results in a value of the linear charge density of about 0.5/Å. For B-DNA, the value of b, the linear separation of the projected charges, is 1.7Å, which corresponds to a density of 0.59/Å. For polyelectrolytes with this charge density in aqueous salt solution in which the concentration of salt is in excess of that of the polyion, counterions will "condense" to form a cylindrical sheath of more or less hydrated positive charges, some 4 to 10 Ångströms thick [1]. In low ionic strength solutions, the condensed charges are, therefore, much closer to the polyelectrolyte molecule than to the remaining free charges in solution which are typically 30 to 100 Ångströms apart, depending on their concentration. The extent of the condensation of counterions to be expected under a variety of conditions of salt concentrations and charge has been discussed by Manning [2] and by Record [3] and their collaborators. Some consequences of their model of linear polyelectrolytes relating to the orientation of the nucleic acids and polynucleotides by electric fields have been discussed in three recent papers [4–6].

The proximity of the counterion charges to the helix is a consequence, and a demonstration, of the attractive force between them. Each may be considered, therefore, to be polarizable in an electric field: the helix, in the usual fashion by the relative distortion of the nuclei and electrons of the covalently bonded atoms which gives rise to the classical polarizability; and the condensed ions by the distortion of their distribution relative to the rest of the helical molecule, which results in an additional polarization. We will try to show here that experimental electric-field induced dichroism data are in accord with a model which includes the attraction of the densely negatively charged helix for the positive counterions as well as the effect of repulsion between the positively charged counterions.

The orientation of a linear polyelectrolyte molecule in an electric-field may be ascribed to the torque exerted by the field on its permanent and induced moments. DNA, by virtue of its nearly palindromic structure, can have only a negligible permanent dipole moment, if any. Despite that, there have been numerous reports of the dipole moment of DNA obtained from measurements of its dielectric properties [7] and from electro-optic measurements [8]. There does not appear to be an unambiguous interpretation of the observations that lead to this result [9]. The preferred explanations fall into three principal categories. One involves a mean square deviation from centro-symmetry due to ion-fluctuations on the molecular surface [10] the second is a field-induced assymetry in the counterion flow in the vicinity of the polyion [11], and the third postulates a saturation of the distortion from centro-symmetry of the condensed counterion atmosphere by the applied fields even at very low field strengths [12]. The model on which the latter is based has its origin in a theory of the polarization of the ion atmosphere which considers the attractive forces between the polyion and the counterions but does not consider the repulsion between counterions [13]. It is also consistent with observations that despite the lack of a permanent dipole moment, the orientations behavior of DNA in strong electric fields fits orientation functions derived for systems with large values of field invariant dipole moments [8]. This model has been shown to be in qualitative accord with data that compares the orientation of DNA to that of poly(A) in an electric field, but it was quantitative limitations which may be lie in the failure to include the effect of the repulsion between the counterions. This problem has not been ignored in investigations of polyelectrolyte properties [14–18], but has not yet been adequately attacked with respect to its effect on the orientation of polyelectrolytes in electric fields. Recently, Manning proposed an explanation for the axial polarizability of a polyion in which the counterion repulsion is a function of the ionic strength of the solution, the charge of the counterion, and the linear charge density of the polyion [19]. The resulting expression,

$$\alpha_{\parallel} = \frac{Z^2e^2nL^2/12kT}{1-2(Z\xi-1)\ln\kappa b}, \qquad (1)$$

predicts values of the polarizability anisotropy, $(\alpha_{\parallel}-\alpha_{\perp}) \approx \alpha_{\parallel}$, for double stranded helical DNA and poly(A) which are consistent with those derived from electric dichroism orientation data [4].

In this paper, measurements of electric-field induced dichroism of DNA in $MgCl_2$ and NaCl solutions at low field strengths are also shown to be consistent with the predictions of eq. (1). In addition to the comparison between the di- and uni-valent ions, Mg^{2+} and Na^+, other observations on ionic strength dependence are reported. Explicit theoretical application to orientation effects has not yet been made but some functional dependencies are implied by the ad hoc treatment and the experimental observations discussed here. In eq. (1), k, T, and e have their usual meaning; Z is the number of formal charges on the counterions; κ is the Debye-Hückel screening parameter and is equal to $[8 \times 10^{-3}\,\pi N_0(e^2/\epsilon kT)I]^{1/2}$ [where N_0 is Avrogado's number, ϵ is the bulk dielectric constant of the solution, and I is the ionic strength of the solution]; L is the contour length of the polyion and $n = iN$, is the number of condensed counterions on the polyion molecule with N charged groups (phosphates in the case of DNA at neutral pH). ξ is a charge density parameter given by

$$\xi = e^2/\epsilon kTb. \qquad (2)$$

The parameter, ξ, is derived from an extension of Debye-Hückel–Bjerrum theories of simple electrolytes to these densely charged substances, which requires that in the limit of low ionic strengths, counterion condensation is extraordinarily stable for linear polyions with a projected axial charge separation of less than 7.1 Å [20].

On the basis of calssical considerations, the assumption that at low or moderate field strengths, the polarization of this highly stable counterion sheath relative to the polyion induces a moment parallel to helical axis which is proportional to the field strength, was utilized in the work on poly(A) referred to above [4]. The same assumption is utilized here but the polarization is now specified by eq. (1), which includes the effect of counterion repulsion.

2. Experimental

Measurements of the electric dichroism were made as specified in ref. [21], except that in place of photographic recording of signals the output of the photomultiplier is stored on a Nicholet model 1090AR oscilloscope with 0.5 μs resolution, interfaced with a Hewlett-Packard model 9825A programable calculator for immediate data processing.

Three samples of DNA were utilized in these experiments: (i) Calf-thymus DNA (Worthington Biochemicals Corp.) of mean molecular weight, 500 000 Daltons, prepared by sonication in short bursts in a Bronson sonicator at temperatures near 0°C under helium. (ii)

The same as (i), but with a mean molecular weight of 260 000 Daltons. (iii) Duck reticulocyte nuclei DNA containing a mixture of 140 and 150 base pair fragments prepared by restriction enzyme digestion. (We wish to thank Dr. Gary Felsenfeld for a gift of the latter sample.) Concentrations of DNA (on a phosphate basis) used in the electro-optic experiments were 0.1 to 0.5 of the lowest counterion or buffer concentration for which data are presented in the figures. In all cases, the solutions for use were prepared by partial dilution from the original concentrated preparations in 0.1 molar NaCl followed by exhaustive dialysis in the cold against the buffer or salt solution specified. Samples were maintained at or below 7°C at all times, except during transfer to the electro-optic cell when they may have warmed to 10° to 15°C momentarily. All samples were tested for hypochromocity which was always better than 32% and produced optical rotatory dispersion curves characteristic of native DNA. The molecular weights were determined by ultracentrifugation by Ms. Georgiana Sandeen, whom we also wish to thank. 2-(N-Morpholino)-ethanesulfonic acid (MES) buffers where utilized were prepared just before dialysis.

3. Results

The field strength dependence of the electric dichroism of the 260 nm band of both calf-thymus DNA samples in NaCl solutions and of the 500 000 Dalton sample also in $MgCl_2$, were measured in unbuffered solutions and will be reported elsewhere. From these, the low-field data which are quadratic in the field strength E to about 2000 V/cm were extrapolated linearly in E^2 to 6325 V/cm in order to compare these results with those obtained on lower molecular weight fragments of Duck reticulocyte DNA which require a higher voltage to achieve similar degrees of orientation. These data are plotted against ionic strength of the solvent in figs. 1 and 2. In fig. 1, a comparison is made between the dependence of the parallel (to the electric field direction) dichroism of the calf-thymus DNA is unbuffered solutions and in solutions buffered with the zwitterionic buffer, MES, as well as with Duck reticulocyte nuclei DNA in MES buffered solution. It will be observed that in the solution buffered with MES, the dichroism is almost independent of the ionic strength of the solution. In fig. 2, where the dichroism is plotted against the nat-

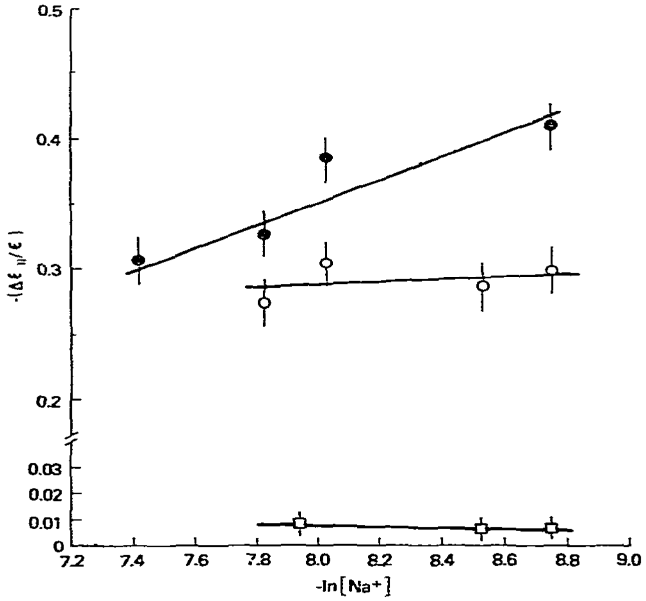

Fig. 1. Ionic strength dependence of the electric-field induced linear dichroism of DNA. ● = sonicated DNA, M.W. ≈ 260 000 in NaCl solutions. ○ = sonicated DNA, M.W. ≈ 260 000 in solutions buffered with MES. □ = Duck reticulocyte DNA, M.W. ≈ 86 000 in solutions buffered with MES; measurements at λ = 260 nm, Temp. = 5°C, E = 6325 V/cm.

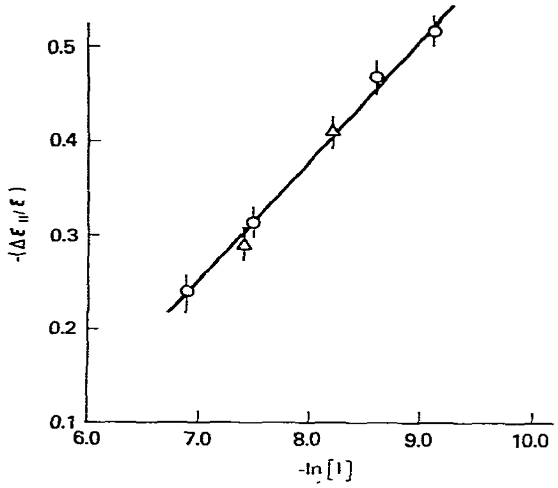

Fig. 2 Electric dichroism of DNA in the presence of mono- and di-valent salts. Sonicated DNA, M.W. ≈ 500 000, λ = 260 nm, E = 6325 V/cm. ○ = in NaCl solutions. △ = $MgCl_2$ solutions.

ural logarithm of the ionic strength in order to compare the data in NaCl and $MgCl_2$, it is clear that to within the precision of the experimental results, the dichroism is the same with Mg^{2+} and Na^+ counterions when the ionic strength of solution is the basis for comparison. This result while more sensitive, is not very different from that of Hogan, Dattagupta and Crothers [11] whose data plotted against the reciprocal of the square-root of ionic strength show a slight difference between Na^+, Ca^{2+}, and Mg^{2+} counterions.

4. Discussion

4.1. Orientation function

The orientation function of a linear polyion in low and moderate electric fields was given by Kikuchi and Yoshioka [12] as:

$$\Phi_K(n,L,E) = nK^2E^2/45 + \text{smaller terms when } n, K \text{ are not large.} \tag{3}$$

Here K is defined as:

$$K = KE = ZeLE/2kT \tag{4}$$

and all the symbols are as defined in the introduction. The axial polarizability on which equations (3) and (4) are based is

$$\alpha_{\parallel} = nZ^2e^2L^2/12kT. \tag{5}$$

If by analogy, eq. (1) is substituted for the polarizability in order to include the effects of counterion repulsion, the degree of orientation at moderate field strengths can be written as

$$\Phi_M(n,L,E) = nM^2E^2/45 + \ldots, \tag{6}$$

where

$$M = ME = \frac{ZeLE/2kT}{[1 - 2(Z\xi - 1)\ln\kappa b]^{1/2}}. \tag{7}$$

It is to be understood that in eqs. (6) and (7), as well as in (3) and (4), only the contribution of the condensed counterions to the orientation is included (see the discussion in ref. [4]). In addition to the explicit Z dependence of eqs. (6) and (7), the number n of condensed ions/polyion is also dependent on the charge type. Manning has shown [2] that this Z dependence is given by

$$i = Z^{-1}(1 - Z^{-1}\xi^{-1}). \tag{8}$$

Incorporating (8) in (6) and (7), the orientation function for low or moderate values of n and M becomes

$$\Phi_M(n,L,E) = \frac{(Z - \xi^{-1})}{1 - 2(Z\xi - 1)\ln\kappa b}\,\frac{Ne^2L^2E^2}{180k^2T^2}. \tag{9}$$

The corresponding expression using (3) and (4) is:

$$\Phi_K(n,L,E) = (Z - \xi^{-1})\frac{Ne^2L^2E^2}{180k^2T^2}\,. \tag{10}$$

An immediate implication of the comparison of these two expressions is that the predicted dependence on the charge species is quite different. For DNA, for whic $b = 1.7$Å, $\xi = 4.2$, eq. (10) predicts that in $MgCl_2$ solution, at 280 K, the orientation of DNA should be abou two-and-a-half times larger than in NaCl solution under the same conditions, independently of ionic strength. Eq. (9), however, predicts that the orientation will be dependent on ionic strength through the Debye-Hückel screening parameter, and that over a very wide range of ionic strengths there should be very little difference between the degree of orientation in the presence of Mg^{2+} or Na^+ counterions. In fig. 3, a plot of the charge dependent factor of eq. (9) over a wide range of ionic strengths shows that while the orientation of Mg^{2+} is expected to be slightly higher than in Na^+ solutions, the difference is only about 5%. Since the experimental precision in these experiments is just about this magnitude and since, in addition, only a part of the orientation results from counterion polarization, the implicatio to be drawn from eq. (9) is that the degree of orientation in Mg^{2+} and Na^+ solution of the same *ionic strengt* should be indistinguishable. The results shown in fig. 2 are in exact agreement with this prediction. It has been pointed out to us that the Oosawa [14] Schurr [15] treatment of the dielectric properties of linear polyelectrolytes, where counterion-counterion repulsion is examined, leads to a limiting expression for the polarizability [17]:

$$\alpha_{\parallel} \approx \frac{L^3E}{24\ln(R/a)} \tag{11}$$

that implies a similar independence of the orientation on the charge species. This appears to be a result of the fact that increased repulsion between divalent counter-

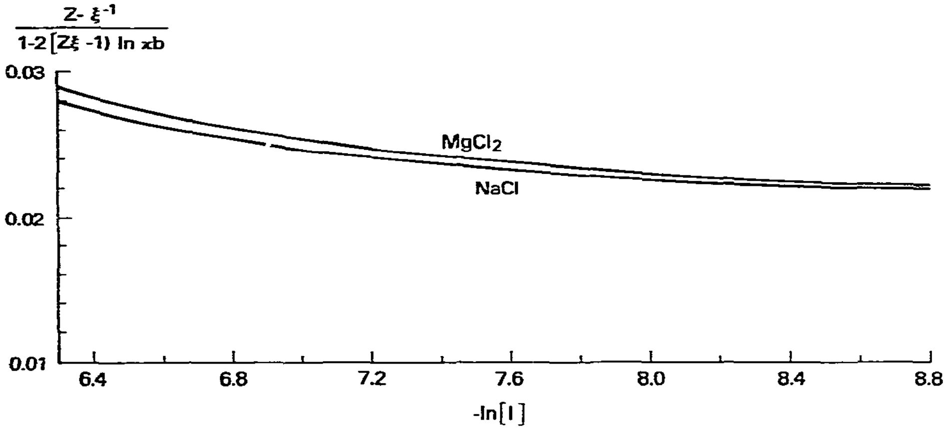

Fig. 3. Dependence of the factor $(Z-\xi^{-1})/[1-2(Z\xi-1)\ln\kappa b]$ of eq. (9) on the ionic strength for mono- and di-valent counterions.

ions, counterbalance the increase in polarizability implied by Mandel's [13] expression, eq. (5). It is difficult to test this prediction because of the unknown dependence on the charge species of the parameter R/a which is the ratio of the radius of the cylindrical free volume offered to a single molecule to the radius of the cylindrical phase of bound counterions. Moreover, the approximation which leads to eq. (11) may be inadequate because the range of its validity is limited to values of R/a which are generally larger than those appropriate to these polyelectrolytes. It is also important to note that the difference in the thermodynamic activity of the counterions is not included in any of these analyses. At the low, but nevertheless excess counterion concentrations used in these experiments, this is probably a valid approximation so far as the activity of the free ions in solution is concerned, but at the surface concentration of the counterions on the helix, some differences are to be expected.

4.2. Ionic strength dependence

The assumption implicit in eq. (9), which derives from eq. (8) and the concept of the high stability of the charge compensation for polyions with $\xi > 1$, is that ξ and therefore the fractional charge compensation i, are independent of ionic strength. Because of the dependence of the pK of the basic nitrogens in DNA and the polynucleotides on the ionic strength, ξ is invariant only when, as we will see below, an electrically neutral zwitterion provides the source of positive charge for association with the basic nitrogen atoms. In this case, the concept of charge stability is not violated by the peculiarity of the polyion species. There remains the dependence of the Debye screening constant on the ionic strength; eq. (9) predicts that because of this, even with a constant charge density, the electric-field orientation of polyons should be dependent on the ionic strength of the solution in which it is measured, albeit a weak dependence over the range of ionic strengths of interest, as the upper line in fig. 4 shows. Both our

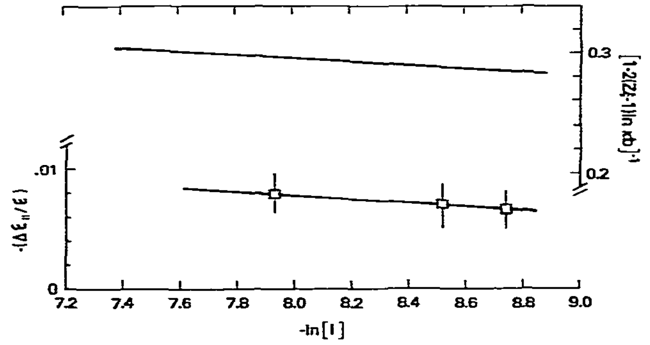

Fig. 4. A comparison of the ionic strength dependence under conditions of constant charge density predicted by eq. (9) for DNA in neutral solutions with the observed electric dichroism of Duck reticulocyte DNA in NaCl solutions buffered with MES. Values of the parameters for the plot of the factor $[1-2(Z\xi-1)\ln b]$ are $Z=1$, $\xi=4.2$, $b=1.7$Å; κ calculated as described in text.

data (fig. 1 and ref. [6] and those of Hogan et al. [11] show a rather strong dependence (in a direction inverse to that predicted by eq. (9)) of the dichroism of DNA on ionic strength measured, respectively, in unbuffered and in phosphate buffered solutions. We see, however, that in solutions buffered with the zwitterionic buffer MES, both the 260 000 Dalton calf-thymus DNA and 86 700 – 92 800 Dalton Duck reticulocyte DNA exhibit only a weak dependence, if any, on the ionic strength (fig. 1). A comparison of the ionic strength dependence of $-\Delta\epsilon_{\|}/\epsilon$ for the Duck reticulocyte DNA with that of the ionic strength parameters in eq. (9) in fig. 4, shows the two to be in reasonable agreement both as to direction and magnitude. The difference in ionic strength dependence of the orientation in the presence of the zwitterion as compared to that in phosphate buffered and in unbuffered solutions this appears to be related to the fact that the electrically neutral zwitterions are bound in response to changes in pK [22]. While it is not possible to extrapolate their results on tetralkylammonium salts to MES, it is interesting to note that Anderson, Record and Hart [23] have recently shown that at low Na^+/PO_4^- ratios, such as those used in the experiments with MES buffer, the tetralkylammonium salts effectively compete with Na^+ as a counterion for DNA. The positive charge of MES is assumed to reside at or near the tertiary alkyl, monohydrogen substituted nitrogen atom. The orientation of poly(A) in MES buffered solutions has also been shown to be independent of ionic strength [4]. The response to variations in the ionic strength dependence of the pK of the basic nitrogens of these polyelectrolytes is, however, dependent on details of the structural parameters as is evident from differences in the behavior of poly(A) and poly(C) [6]. Further work on this is now underway.

In summary, the condensed counterions on a highly charged linear polyion appear to be polarizable in an electric field wich accounts for, at least in part, the orienting torque. Electric-field orientation is thus dependent on the parameters which affect the polarization, the charge species of the counterion and the ionic strength of the counterion salt. The explicit functional dependence does appear to be consistent with the Kikuchi and Yoshioka model [12] when the polarization includes the effect of counterion repulsion as developed by Manning [19].

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