Biophysical Chemistry 11 (1980) 157–166

THE ROLE OF THE IONIC ENVIRONMENT IN THE ORIENTATION OF NUCLEIC ACIDS IN ELECTRIC FIELDS

Elliot CHARNEY
Laboratory of Chemical Physics, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014, USA

Received 4 May 1979
Revised manuscript received 4 October 1979

The relationship between polyelectrolyte theories based on linear charge density models and the electric-field induced orientation of the polyelectrolytes, poly(A), poly(C) and DNA is examined by varying their ionic environment with respect to ionic strength and acidity. The degree of counterion condensation on the polyelectrolytes predicted by the theories of Manning and Record is shown to be related linearly to the orientation as measured by their dichroism in the field. Microstructural differences between poly(A) and poly(C) account for the differences in their dependence on the pH of the medium which affects the counterion condensation and thus the polarization in the orienting electric fields. The results consequently support recent treatments of linear polyelectrolytes having a high charge density which model them as smoothly charged linear polyions, but indicate that these models are insufficient to account for some of the effects of microstructural variations.

1. Theoretical

From the results of electro-optic measurements on certain polynucleotides and DNA over the past several years, it has become increasingly clear that the orientation of these molecules in electric fields up to 20 or 30 kV/cm cannot be explained by the presence of a permanent dipole moment, nor by any mechanism which does not account for the very strong influence of the high charge density of the polyelectrolytes. While attention has been called to the effect of the charges [1,2], the specific relationship of the moments induced by the fields, or of the orienting torques, to the molecular parameters have remained obscure and confusing, in part because the many reported measurements have been made under widely different conditions of molecular size, solution ionic strength, temperature and pH, and in part, because of fundamental differences in experimental and theoretical approaches. During this same period, substantial progress has been made in polyelectrolyte theory. We report here some results of our attempt to determine the extent to which the electric field orientation behavior is consistent with predictions which can be drawn from these theories. Our approach has been to perturb the ionic environment by varying the ionic strength or acidity in order to produce predictable changes (from other experimental observations and models of polyelectrolyte behavior) in the counterion density on the macromolecules and to observe the resulting changes in the dichroism produced by the application of pulsed electric fields to solutions containing them [3–5]. In particular, for the experiments reported here, we looked at the dichroism of DNA [6–8], polyriboadenylic acid [9], and polyribocytidylic acid [10] under conditions of ionic strength or H^+ ion concentration designed to determine the relation of the responses to the predictions of the polyelectrolyte models of Manning [11], Record [12], Mandel [13,14], and McTague and Gibbs [15] and their respective colleagues; all of which are based on an extension of Debye-Hückel-Bjerrum theory of simple electrolytes. We have previously reported results [9] of one such perturbation in a more extensive paper on the electric dichroism of polyriboadenylic acid. While the results reported here are quantitative, we hold in abeyance proposing a quantitative relationship to orientation theory for reasons which will be discussed below. The results will demonstrate, however, that the perturbations produce effects on the

orientation which are consistent with some aspects of polyelectrolyte theory.

These models and theoretical treatments have much in common and, in general, are based on the picture of a linear polyelectrolyte with a uniform distribution of charge which condenses counterions to a greater or lesser extent, depending on the polyion charge density. For monovalent charges this density is given parametrically by $\xi = e^2/\epsilon kTb$. In this expression, ϵ is the bulk dielectric constant of the solvent, e is the electronic charge in absolute esu, k and T are, respectively, the Boltzmann constant and the temperature in degrees Kelvin, and b is the average spacing in Ångströms of the projection of the charged groups on the axis of the fully extended polyion. For DNA, the value of b in solution near neutral pH is 1.7 Å (half of the 3.4 Å axial translation between adjacent phosphates of the assumed B structure because these are two intertwined strands) and the resulting value of ξ is 4.2. Manning [11] and others [16,17] have shown that for values of $\xi > 1$, counterions should condense on the linear array to reduce ξ to 1. The fraction $i = (1 - \xi^{-1}) = 0.76$ of the N phosphate groups are neutralized by associated or "condensed" counterions. The theory requires that these counterions form a (partially hydrated) sheath which has been calculated at 14 Å in radius [18] and since the radius of the DNA helix without the counterions is approximately 10 Å, the associated counterions cannot be more than about 4 Å from the negatively charged phosphate groups, essentially in the range of van der Waal's contact. The field of an isolated negative charge at 4 Å is approximately 10^8 V/cm. The condensed positive counterions are also in the field of the negative and positive free ions in solution, which form the Debye atmosphere, as well as those of the adjacent phosphates, but the mean distance between the free ions and the polyelectrolyte in the most concentrated solution (1 mM) used in these experiments is 55 Å calculated on the basis of the volume concentration of ions. Thus in the absence of an applied field, the condensed counterions are attracted to the polyelectrolyte by a force resulting from a field of the order of 10^8 V/cm or more, and attracted away with fields of the order of 5×10^5 V/cm. These are, of course, also repulsive forces between neighboring condensed counterions of the same sign but these are at least partially compensated by neighboring phosphate charges of the polyion of opposite sign. The foregoing considerations are a great over-simplification as any perusal of the cited papers will show. The "condensed" ion atmosphere for example, cannot be a precise sheath with a discrete boundary but at the very least must go over smoothly into a Poisson-Boltzmann distribution as the distance from the polyion increases. Nevertheless, the effective attractive force on the positive counterions is orders of magnitude greater than the ionic forces trying to remove them and provides a mechanism for their polarization in an electric field. [Note that in this discussion and throughout this communication we avoid any discussion of free energy and therefore of the real activity coefficients of the ionic substituents. Obviously, quantitative relations would be modified by their inclusion.] The applied field in these experiments, which may range up to 2.5×10^4 V/cm is far too low compared to the attracting field of 10^8 V/cm to substantially redistribute the counterions along the polyelectrolyte, in the sense of causing large variations in the charge density, until a high degree of orientation is achieved. Instead the polyion and its closely associated or "condensed" counterions are polarized in the applied field in much the same way that the bound valence electrons and nuclei are polarized by the field [19]. There is some difference between the polarization of the valence electrons and of the counterions with respect to the polyion. The induced moment at low fields, $na\epsilon E$, (for the same number, n, of charges involved) may be somewhat larger for the counterions than for valence electrons because the dielectric constants, ϵ, are larger in the aqueous environment of the counterions. Models which predict that the polyion-counterion polarization will saturate at very low fields to produce a large equivalent dipole moment require that the number of counterions and therefore the molecular length be very large and are based on a continuous line charge, not a discrete array [11,20,21]. For a true line charge there should be no component of the field of the polyion parallel to the axis. However, because the charge array actually consists of a non-linear (helical) array of discrete charges, 6.5 Å apart in B-DNA, components of the field will exerts strong attractive forces on the counterions axially as well as radially. If the applied field strength is sufficient to cause substantially complete orientation of the macromolecules, translation of the positive counterions along the polyion may occur but replacement by free ions from the solution should keep the charge density stationary. At all moderate values of the field strength greater than zero, however, a distortion of the counterion-polyion configuration is pro-

duced which results in an induced moment proportional to the field strength. [It is possible to argue that the axial attraction is not very large on the basis of the linear continuous charge array of the theoretical models, and this must be approximately true for the exact axial direction. However, our results on poly(C) (vide infra) imply that while the linear approximation is qualitatively a good one, there are properties which demonstratably reflect the microscopic charge distribution of the polymers. Note, for example, that Manning [22] even while developing the linear model, has concluded from the data of Skerjavic and Strauss [23] that the charged sites in DNA are too far from each other to cooperate appreciably in providing effectively multivalent sites for cations.] Recent treatments by Soumpasis [24] and by Schellman and Stigter [25] have a bearing on this problem. Soumpasis concludes that a helical charge distribution such as that of the DNA backbone can be treated by the continuous straight line charge on which the considerations of the present paper are based, at least as far as thermodynamic properties are concerned, in the limit of infinite dilution. He points out, however, that in processes where the polyion potential enters directly, as it does in the electric-field orientation phenomena described here, the specific structure of the polyion must be considered.

Schellman and Stigter [25] have also concluded that corrections for the periodicity and the discreteness and size of the ionic charge should enter in first order in the electrical free energy associated with the polyion. The fact that the electrical free energy enters directly into the orientation function (the $\mu E/kT$ parameter where μ is the total moment in the field E) indicates, moreover, that the sensitivity of electric-field orientation to the charge density may have components of the real helical periodicity as well as components based on the smoothed axial projection of the charges. Finally, we report here experiments on samples low enough in molecular weight so that the additional contribution of the polarization of valence electrons is not negligible [9].

In the foregoing discussion, the basic assumption has been that the "condensation" model is a physical reality. While a substantial amount of evidence is accumulating to support this, there are theoretical proposals for the orientation mechanism which do not depend on counterion "condensation" and which, in fact, imply that the entire charge distribution can be described by a continuous Debye atmosphere obtained by a careful application of the Poisson-Boltzmann equation and variants thereof [26]. It is clear that at this point the "condensation" models cannot be said to have been proven. In this paper, we examine aspects of the behaviour of the nucleic acids in orienting electric fields which have some bearing on their validity.

In the experiments to be described, the solutions of the nucleic acids are subjected to electric field pulses just long enough to allow the molecules to reach a stationary state. The magnitude of the resulting dichroism is the ratio of the difference in the measured absorbance of the solution for plane polarized light in two perpendicular directions, in this case, parallel and perpendicular to the electric field directions, to the isotropic absorbance of the same solution:

$$\Delta\epsilon/\epsilon = (\epsilon_{\parallel} - \epsilon_{\perp})/\epsilon. \quad (1)$$

It is related to molecular parameters through an optical factor, $G(\theta)$, which is dependent on the polarization direction of an optical transition moment at the wavelength of measurements with respect to fixed axes in the molecule and, to the orientation factor, $\Phi(E)$; the orientation factor is, as indicated, dependent on the field strength, E, and on the dielectric properties of the macromolecules:

$$\Delta\epsilon/\epsilon = \Phi(E) \cdot G(\theta). \quad (2)$$

Unfortunately these factors appear as the product, so that the direct determination of either requires that the other be unity or be a known function calculable from known molecular parameters. The optical factor at any given wavelength in an absorption band can only be unity by an accident of nature. For a stiff cylindrical rod or prolate elipsoid of revolution, $G(\theta)$ is given by $(3/2)(3\cos^2\theta - 1)$, where θ is the angle which the optical transition moment makes with the rod axis. This factor is unity only for the special circumstance that $\theta = 41.81°$. For the optical transitions of the nucleic acid bases in the 2600 Å region of the ultraviolet spectrum, the principal transition moment is expected to be almost in the plane of the nucleic acid bases which make angles close to perpendicular to the helix axis; in this case, the value of $G(\theta)$ should be close to -1.5. In fact, values from about -0.6 to -1.4 have been measured by applying sufficiently strong fields to achieve substantially complete orientation for which $\Phi(E) = 1$, or obtained by extrapolation from measurements at lower field strength [8]. Unfortunately, nearly complete orienta-

tion can only be achieved for molecules with a high enough molecular weight to be flexible. Because the field dependence of the optical factor of flexible chains has not been definitively treated, the interpretation of these values is open to some question. Ideally, therefore, the measurements are made on short stiff rod-like segments of the polymeric molecules, but then complete orientation is not usually achieved because the electrical polarization which varies approximately as the square of the molecular length of the polyelectrolyte rod is insufficient. [For double-standed poly(A), it has been shown that orientation saturation may just barely be achieved with a sample low enough in molecular weight to be rigid and that the dichroism (and this the optical factor) is the same at high field strength as that of higher molecular weight poly(A), [9] .] One of the objects of these investigations then is to deduce the functionsl dependence of $\Phi(E)$ on the field strength and on the molecular charge distribution. For electrically neutral molecules, these functions are well known [4,27,28] . At moderately low field strengths they reduce to a linear dependence on the differential electric polarizability parallel and perpendicular to the rod axis, and to a quadratic dependence on the field strength and on the projection of the permanent dipole moment along the rod axis. Because of the quadratic dependence on E, this region of field strengths is sometimes known as the Kerr region. [Note that in reference [9] , the notation for the anisotropy of the electric polarizability parameter was δ. We revert here to our earlier notation, γ, [4] and regret any inconvenience this may cause.] In the Kerr region

$$\Phi(\beta,\delta,E) = (\beta^2 + 2\gamma)\,(E^2/15),$$

$$\beta = \mu/3kT; \qquad \gamma = (\alpha_{\parallel} - \alpha_{\perp})/2kT. \tag{3}$$

DNA, poly(A), and poly(C) are not neutral molecules however, except under special conditions of H^+ ion concentration. Various attempts to explain the field-induced orientation of charged polyelectrolytes have been made, the most recent for DNA by Hogan, Dattagupta and Crothers [29] , but we shall be principally comparing our results to the theory of Kikychi and Yoshioka [30] , who have developed a model of the orientation which at low field strengths is based solely on a parameter nK^2, related to the charge density parameter of Manning, Mandel and others:

$$\Phi(n,L,E) = nK^2E^2/45 + \text{smaller terms when } n, K \text{ or both are not large,}$$

$$K = ZeL/2kT, \qquad n = iN. \tag{4}$$

The parameter n is the number of condensed counterions on the polymer of length L having N charged grou in a uniform linear array along the polymer and K is defined by $K = KE$.

On the basis of a comparison of the orientation of DNA and poly(A), we have recently concluded that the orientation, and therefore, the dichroism when the orientation is not complete, is, in fact, determined by the additive effects of the neutral dielectric properties and of the polyion-counterion polarization [9] :

$$\frac{\Delta\epsilon/\epsilon}{E^2} = \frac{1}{15}\,[\beta^2 + 2\gamma + (nK^2)/3]\,G(\theta), \tag{5}$$

an expression obtained by adding the ionic polarization result of Kikuchi and Yoshioka [30] to the expression for the orientation arising from the neutral valence stru ture [4] . Because DNA and the polynucleotides have substantially no dipole moment, $\beta = 0$ for these molecules, but we see no reason to conclude that neutral valence dielectroc anisotropy, γ, disappears when the molecule carries a charge. There are questions of the relative importance of these effects a charge. There are questions of the relative importance of these effects and, of the extent to which the Kikuchi and Yoshioka model is a good representation of the polyelectrolyte charge contribution.

The results presented here do not yet give quantitative answers to these questions, but they do accord witl the expected behavior of the electric dichroism. For example, the model requires that the orientation be lincarly dependent on i, the effective concentration of condensed counterions on the polyion [see eq. (4)] . The titration of the basic nitrogen atoms on a nucleic acid base in a charged polynucleotide tends to reduce the number of condensed alkali metal counterions associated with the negatively charged phosphate groups. The exact way in which the condensed counterion concentration varies with the H^+ concentration is calculabl from the polyelectrolyte theories of Manning [31] and of Record [12] , and from acid-base tritration data [32] Combining this with the model of Kikuchi and Yoshiok we predict that the dichroism of an acid titratable poly-

nucleotide will increase (in absolute value) with an increase in pH in a way which is linearly dependent on the resulting increase in the concentration of condensed alkali counterions, presumably because the counterions are polarizable with respect to the axial direction of the polyion: Record [12] has concluded that the fractional counterion charge on these polyelectrolytes is given by:

$$i = (1 - \xi^{-1} - \omega), \tag{6}$$

where ω is the degree of protonation of the polyelectrolyte. For a given polyelectrolyte, let

$$(1 - \xi^{-1}) = \psi \tag{7}$$

then from $n = iN$ [eq. (4)],

$$n = (\psi - \omega)N. \tag{8}$$

For a monodispersed sample of fixed N (or a polydispersed sample with a mean value $\bar{N}$), the substitution of eq. (8) in eq. (5) yeilds,

$$\frac{\Delta\epsilon/\epsilon}{E^2} = \frac{1}{15}\,[\beta^2 + 2\gamma + (\psi - \omega)N\bar{K}^2/3] \cdot G(\theta). \tag{9}$$

Thus under conditions where $G(\theta)$ is independent of E, the dichroism is predicted to be linearly proportional to $(\psi - \omega)$ and therefore to the fractional concentration of condensed alkali counterion of a (partially) titrated polyion, as calculated from eq. (6). The degree of protonation, ω, may be obtained from acid-base titration data. While the optical factor $G(\theta)$ may be dependent on E for long flexible polyelectrolytes, for the relatively low molecular weight, relatively rigid samples used in these experiments, no detectable dependence of the optical factor $G(\theta)$ on the field strength is expected [9].

2. Experimental

Samples of DNA were calf-thymus from Worthington Biochemical Corp., carefully sonicated in the cold under helium in 0.1 or 0.2 M NaCl. Samples of poly(A) and poly(C) were from P.L. Biochemicals. The sedimentation coefficient of the single-stranded poly(C) sample was $\langle S_{20,w}\rangle = 7.2$ corresponding to a mean molecular weight for the double-stranded species of 4.4×10^5. We wish to thank Georgianna Sandeen for this measurement.

Table 1
Electric dichroism [a] dependence on pH

pH	$\Delta\epsilon/\epsilon$ poly(A) [b]	poly(C) [c]
4.66	−0.4296	−0.2279
5.65	−0.4774	−0.2777
6.50	−0.5590	−0.2773

a) The noise level in these experiments is ± 0.005 in the value of $\Delta\epsilon/\epsilon$. Each value is the average of at least twenty separate measurements.
b) E = 9300 V/cm. c) E = 7600 V/cm.

The sedimentation coefficient of the poly(A) sample used to obtain the results of table 1 and fig. 1 was reported by the supplier to have the same coefficient $\langle S_{20,w}\rangle = 7.2$ All solutions were prepared for use by exhaustive dialysis in the cold to the final solvent concentrations reported in the figure captions. The concentrations of poly(A), poly(C) and DNA were generally 0.25 to 0.5 of the lowest Na^+ ion or buffer concentrations reported in the figure captions. The electric di-

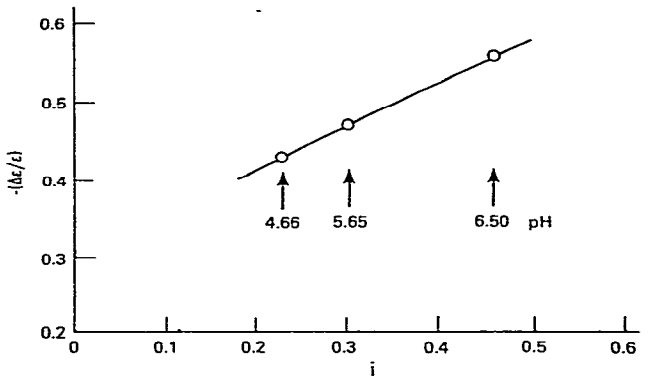

Fig. 1. The electric dichroism of double-stranded poly(A) as a function of the fractional charge density neutralized by condensed counterions in solutions of different pH. The calculation of i proceeds from the expression $i = (1 - \xi^{-1} - \omega)$ where ω is the degree of protonation of the polyelectrolyte. Values of ω were obtained from the data of Holcomb and Timasheff [31], extrapolated to an ionic strength of 0.8×10^{-4} M K^+ from data at 0.15, 0.01, and 0.001 M KCl. The data are for poly(A) in 2×10^{-4} M buffers of 2-(N-Morpholino)ethanesulfonic acid; γ = 2520 Å, E = 9280 kV/cm.

chroism method and procedures are described in ref. [6]. All measurements were made at or below 5°C.

3. Results and discussion

3.1. The pH dependence of the orientation of poly(A)

At salt concentrations below 0.001 M in the region between pH = 6.5 nad pH = 4.5 poly(A) maintains a double helical structure which is half-protonated at the higher pH and is subject to additional protonation as the concentration of H^+ is increased with little or no structural change. An increase in protonation reduces the fractional charge i in accordance with the prediction of eq. (6) and consequently should decrease the dichroism [eq. (9)]. The observations recorded in table 1 and fig. 1 demonstrate the predicted linear dependence on i.

3.2. Ionic strength dependence

A further prediction of the theory, which arises from the concept of the thermodynamic stability of a linear charge distribution, is an extraordinary stability of the polyion charge distribution in solutions of varying ionic strength [18,31]. For these electro-optic measurements, this means that at least at low field strength, if no structural change affecting the optical factor $G(\theta)$ occurs as a result of the variation of ionic strength, the dichroism should be independent of ionic strength of the solvent. This independence of ionic strength has been observed in buffered solution of double-stranded poly(A) buffered with 2-(morpholino)-ethanesulphonic acid (MES) at field strengths up to about 13 kV/cm [9]. In unbuffered solutions, however, and in solutions buffered with phosphate buffers, the electric dichroism fo DNA even at low field strength has a logarithmic (base e) dependence on ionic strength. Both our measurements of the dichroism at ionic strength from 0.09 mM to 1.0 mM NaCl with and without phosphate buffer and data derived from the recent publication of Hogan, Dattagupta and Crothers [29] from 1.4 mM to 10 mM salt in phosphate buffered solutions are shown in fig. 2. Deviation from the linear response to $\ln[Na^+]$ begins to appear in the latter only at the highest ionic strength. We may ask, in view of the very high stability attributed to the charge distribution of polyelectrolytes with

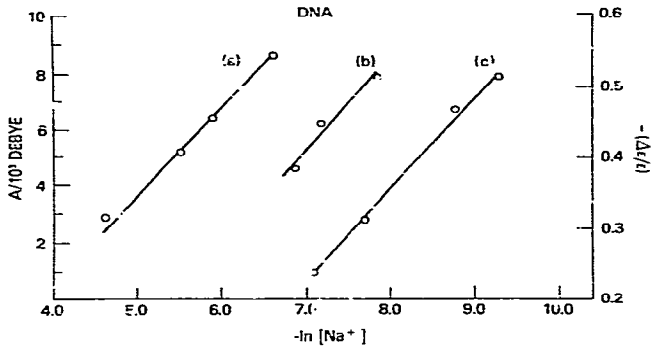

Fig. 2. Dependence of the orientation of DNA on the $\ln[Na^+]$ over the range from 9×10^{-5} to 10^{-2} molar, as measured by [a] the calculated induced dipole moment (left ordinate) from electric dichroism measurements [28], [b] and [c], directly from the electric dichroism (right ordinate) [41]. The data for [a] are adapted from fig. 6 of ref. [26]. The data for [b] were obtained in solutions buffered with phosphate at pH = 7.2. The data for [c] are in unbuffered solutions containing NaCl.

$\xi < 1$, why the charge density of DNA and with it the dichroism should vary with ionic strength. There appea to be a relatively simple explanation. The high stability of the counterion condensation and therefore of the charge fraction of a polyelectrolyte is dependent not only on the high density of charge but also on the absence of new sources of electrostatic charge on the polyion when the ionic environment is perturbed. This is simply not the case for the polynucleotides and DNA The presence of basic nitrogens and possibly also other sites whose pK is strongly dependent on the ionic stren of the environment, results in the introduction (or removal) of charged sites in addition to those of the phosphate backbone. The pK of these sites increases with decreasing ionic strength resulting in increase in the degree of protonation [33]. Eq. (8) predicts that in this situation, n increases with decreasing ionic stren The data plotted in fig. 2 demonstrates that the dichroi increases with decreasing ionic strength as expected fro the ionic strength on the pK's of the basic sites. The effect of buffer is, to some extent, to offset the tendency of the basic sites to protonate and so a small difference exists between the ionic strength dependence in phosphate buffered and in unbuffered solution. This situation is further complicated by the effect of the ionic strength on the buffer dissociation and on the fact that each of the four nucleotides of DNA behave differently

The relative independence from ionic strength effects in poly(A) [9], and also in DNA in MES buffers [34] appears to be related to the fact that MES exists as a zwitterion at the pH of the experiments although the evidence that these zwitterions satisfy the "protonation" requirements arising from the variations in pK is not conclusive.

3.3. Microstructural differences

The foregoing results are consistent with the basic concept of the linear charge density models which take no account of microstructural differences between polyelectrolytes with the same linear charge density, and are in accord with the hypothesis that counterion polarization is a significant but not exclusive factor in electric-field orientation of these polyelectrolytes. From an examination of the electric dichroism of poly(C) we can demonstrate similar polyelectrolyte behavior, but in addition by comparison with the poly(A) results, we can show that microstructural differences occur; in this case, because of the difference between the polarization of protons used to make hydrogen bonds which are located approximately at the position of the helix axis, and of protons which displace or interact with the surface alkali counterions.

Referring to table 1, the results show that unlike poly(A), in this pH region, the dichroism of poly(C) is not monotonic, linear or otherwise, with respect to pH. Instead, there is no change at all in the dichroism between pH 5.5 and 6.5. This appears to be related to the structural differences of poly(A) and poly(C) and is consistent with their expected polyelectrolyte properties. In fig. 3, the data of Chou and Thomas [35] for the acid titration of poly(C) using a Raman spectral band near 1390 cm^{-1}, are plotted. These authors conclude that there is no structural change over a pH range, which when extrapolated to the solution ionic strength of our measurements, includes the measurements at pH 5.65 and 6.50. The pronounced structural change revealed by the Raman spectrum just below pH 5 has been reasonably interpreted as the disruption of the double-stranded structure. Despite the fact that is is questionable [36] whether the structure originally proposed for double-stranded poly(C) was in fact present in the fibres used in the X-ray study [37], a considerable amount of other data indicates that it is highly likely to be the correct double-stranded structure (fig. 4).

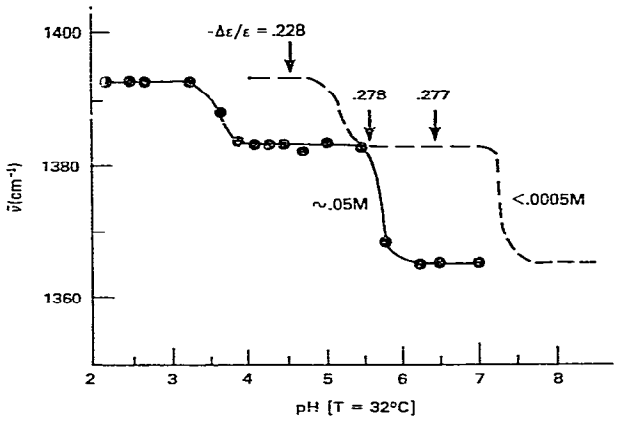

Fig. 3. The Raman titration of the 1390 cm^{-1} band of poly(C). The dashed line is an extrapolation of the observations of Chou and Thomas [33] to an ionic strength of less than 0.0005 M based on acid titration data. The solid line is drawn through their experimental points taken at 0.15 M.

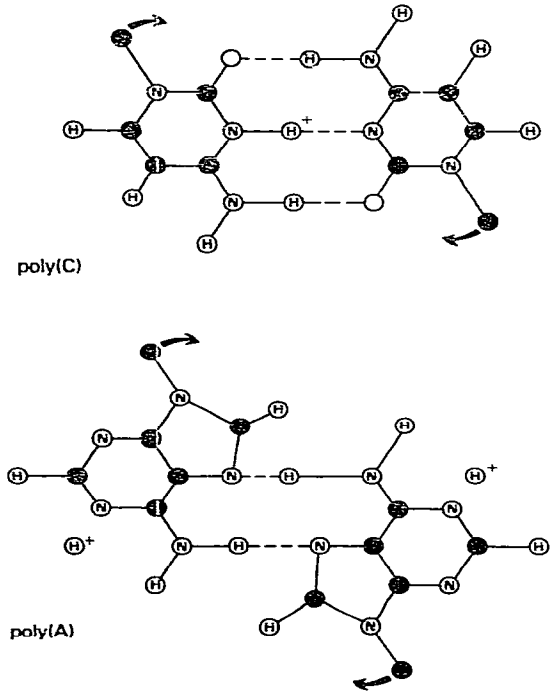

Fig. 4. The hydrogen bonding schemes of the double helical forms of poly(A) and poly(C). See text.

In this poly(C) structure, a proton is required to make a third hydrogen bond between the two strands. This proton, which is the one provided between pH 7 and 5, lies virtually along the helix axis more than 6 Å from the nearest phosphate groups, out of range of providing significant electrostatic shielding of the negative phosphates, and largely shielded from the solvent. Consequently, it exerts little or no influence on the counterion condensation. This reveals itself in the electric dichroism by its independence of pH. In poly(A) on the other hand, the proton binds to the N1 nitrogen, within the van der Wall's radius of the phosphate groups, and the surrounding solvent. The polyelectrolyte models cited here do not take into account the effect of these microstructural differences in the polyelectrolyte. For example, the protons of poly(C) and poly(A) are not distinguishable in a theory which projects all counterion charges on the linear axis; in this respect the theoretical treatments are deficient, as the data on the dependence of the electric dichroism of these two polynucleotides on the pH demonstrates. The fact is that the protons in double helical poly(C) are in an environment of low dielectric constant compared to the protons of poly(A), the latter of which are in a largely aqueous environment having a dielectric constant of about 80. The effective fields which induce an orienting moment by the polarization of the poly(C) protons may be smaller in magnitude by a ratio of 20 to 40 than those acting on the poly(A) protons. The effect of the microstructure thus manifests itself both in degree of alkali counterion condensation and in the relative importance of the contributions of the protons to the induced polarization and to the orienting torque. We should find that at low and moderate fields, the degree of orientation of poly(C) under the same environmental conditions will be smaller than that of the same molecular weight poly(A), a fact which is born out experimentally [10]. These microstructural differences do not in any way vitiate the general conclusion of the present observations, that the orientation behavior of the polynucleotide is consistent with current theories of the counterion condensation effects of high charge densities of rod-like polyelectrolytes.

Recently, Hogan et al. [29] have concluded that the orientation of DNA results from the torque exerted by an anisotropic field created by the field induced counterion flow past the polyelectrolyte. We have no criteria with which to assess that model but do wish to comment briefly that the flow of uncondensed ions past the polyion as a result of the normal conduction process is bidirectional and must result in very small anisotropies in the field on the polyion. It is not clear whether ther is any appreciable flow of condensed positive counterions except perhaps at the highest fields. Expressions have been proposed for the magnitude of the counterion polarizability by Mandel and by Manning [13,38]. It is significant that this polarizability can only result if there is a distinct distortion of the counterion atmosphere from an equilibrium distribution. Manning's [38] equation:

$$\alpha = \frac{(Z^2e^2/12kT)nL^2}{\frac{1}{3} - 2(Z\xi - 1)\ln(\kappa b)}$$

partially accounts for counterion repulsion through the use of the Debye screening constant, κ, for monovalent ions ($Z = 1$); κ should be about 10^{-2}/Å to 10^{-3}/Å in these solutions, and the calculated counterion polarizability anisotropy $(\alpha_{\parallel} - \alpha_{\perp}) \approx \alpha$ for molecules in the 100 000 to 200 000 Dalton range has values in the range of 0.5×10^{-16} to 5×10^{-16} cm^3; this is just the order of magnitude calculated to result in the observed orientation of poly(A) and DNA [9]. We have noted that in the earlier paper [9], the difference between the electric-field orientation of poly(A) and DNA was analyzed in terms of the additive induced polarizations of the counterions and the valence electrons of these molecul The results presented here lend additional support to the orientation of the counterion polarization and are consistent with that interpretation. In the previous paper, the effective number of condensed counterions calculated on the basis of the assumed anisotropic polarizability of the valence electrons and dichroism data was shown to be smaller than that predicted by the Kikuchi and Yoshioka model, but it was pointed out that these authors recognized that this would occur because of their failure to include the effects of counterion-to-counterion repulsion. Further work on this aspect is required. However, even without the inclusion of the counterion repulsion, if the dichroism expected from the classical dielectric polarization of the neutral molecule is substracted from the observed dichroism [fig. 3 of ref. [9]), then the ratio of the slopes of the lines for DNA to poly(A) is 2.7. This ratio should be proportional to the ratio of the fractional concentrations of condensed counterions and this compares very

favorably with the theoretical ratio of 3.5 within the limits imposed by the experimental uncertainties and the assumptions. The value 3.5 is obtained from the ratio of the values of $i = (1 - \xi^{-1} - \omega)$. For neutral DNA, $\omega = 0$ and the value of i is 0.76 as noted above. For double-stranded poly(A), $\xi^{-1} = 0.27$ and for pH = 6.2 extrapolation of the data of Holcomb and Timasheff [31] to the conditions of the experiment yields $\omega = 0.54$. The resultant value of i is 0.19 from which $[i(\text{DNA})/i(\text{poly(A)})] = 3.5$.

It is no surprise that nucleic acids and polynucleotides should exhibit polyelectrolyte behavior [2,39,40]. However, the orientation mechanism of these macromolecules in moderately strong electric fields is still a subject of much uncertainty. Because of microstructural effects which are not described by any current theory, it is still not possible to provide generalized analytical orientation functions from theoretical considerations, but the results of this investigation demonstrate that predictable relationships exist between the polyelectrolyte properties and the orientation.

I would like to thank Che-Hung Lee for his help in some of the experimental preparations; Michel Mandel, Philip Ross and Michio Shirai, for useful comments on an early verion of this manuscript; and Gerald S. Manning for a discussion of the expression for the effect of counterion repulsion on the polyelectrolyte polarizability.

Note added in proof: A recent paper by Sokorov and Weill [42], which appeared after this paper was submitted for publication contains an extensive discussion of the origin of the orienting moment in linear polyelectrolytes.

References

[1] M. Tricot and C. Houssier, in: Polyelectrolytes (Technomics Publishing Company, Inc., 1976) pp. 43–90.
[2] C.T. O'Konski and S. Krause, J. Phys. Chem. 74 (1970) 3243.
[3] W. Liptay and Z. Czekalla, Naturforsch A. 15 (1960) 1072.
[4] K. Yamaoka and E. Charney, J. Amer. Chem. Soc. 94 (1972) 8963.
[5] E. Fredericq and C. Houssier, Electric dichroism and electric birefringence (Oxford University Press, London 1973).
[6] K. Yamaoka and E. Charney, Macromolecules 6 (1972) 66.
[7] E. Charney and K. Yamaoka, Macromolecular Preprint, Vol. 1, XXIII International Congress of Pure and Applied Chemistry (1971), pp. 252-255.
[8] J. Hofrichter and W.A. Eaton, in: Annual review of biophysics and bioengineering, Vol. 5 (1976) pp. 511–560.
[9] E. Charney and J.B. Milstien, Biopolymers 17 (1978) 1629.
[10] Holly Ho Chen and E. Chanrey, in preparation.
[11] G.S. Manning, J. Chem. Phys. 51 (1969) 924.
[12] M.T. Record, Jr., C.P. Woodbury and T.M. Lohman, Biopolymers 15 (1976) 893.
[13] M. Mandel, Mol. Phys. 4 (1961) 489;
see also the discussion by G. Weilland and C. Hornick, in: Polyelectrolytes, ed. E. Selegny (R. Deidel Publishing Company, Dordrecht-Holland, 1974).
[14] F. van der Touw and M. Mandel, Biophys. Chem. 2 (1974) 218.
[15] J.P. McTague and J.H. Gibbs, J. Chem. Phys. 44 (1965) 4295.
[16] F. Oosawa, Polyelectrolytes (Marcel Dekker, New York (1971);
see also G.M. Gross and U.P. Strauss, in: Chemical physics of ionic solutions, eds. B.E. Conway and R.G. Barnada (John Wiley and Sons, Inc., New York, 1966) p. 347.
[17] A.D. MacGillivary, J. Chem. Phys. 45 (1966) 2184.
[18] G.S. Manning, Biophys. Chem. 7 (1977) 95.
From calculations of the volume available to the hydrated counterios, the outer radious could be as much as 17Å and the inner radius of about 10Å for polyelectrolytes with the charge density of the helical polynucleic acids. 14Å is a reasonable mean of these values – personal communication from G.S. Manning.
[19] See for example, the discussion by E. Neumann, in: Colston Papers, Vol. 29, in Colston Conference: The behavior of ions in macromolecular and biological systems. ed. D.H. Everett (Scientifica Publ. Ltd., Bristol, U.K. 1977).
[20] G.T. O'Konski and M. Shirai, in preparation.
We wish to thank both for the opportunity to examine a draft of this communication.
[21] G.Z. Schwarz, Phys. Chem., Neue Folge 19 (1959) 286.
[22] G.S. Manning, Quart. Rev. Biophys. 11 (1978) 179.
[23] J. Skerjanic and U.P. Strauss, J. Amer. Chem. Soc. 90 (1968) 3081.
[24] D. Soumpasis, J. Chem. Phys. 69 (1978) 3190.
[25] J.A. Schellman and D. Stigter, Biopolymers 16 (1977) 1415.
[26] J. Skolnick and M. Fixman, Macromolecules 11 (1978) 867, and personal communications from M. Fixman.
[27] C.T. O'Konski, K. Yoshioka and W.H. Orttung, J. Phys. Chem. 63 (1959) 1558.
[28] M.J. Shah, IBM Technical Report, TB-02-250 (1963); J. Phys. Chem. 67 2215.
[29] M. Hogan, N. Dattagupta and D.M. Crothers, Proc. Natl. Acad. Sci. USA 75 (1978) 195.
[30] K. Kikuchi and K. Yoshioka, Biopolymers 15 (1976) 583.
[31] G.S. Manning and A. Holtzer, J. Phys. Chem. 77 (1973) 2206.
[32] D.N. Holcomb and S.N. Timascheff, Biopolymers 6 (1968) 513.

[33] H. Morawtz, Macromolecules in solution (Interscience Publishers, John Wiley and Sons, New York, 1965) Chap. VII.
[34] E. Charney, K. Yamaoka and G.S. Manning, Biophys. Chem. 11 (1980) 167.
[35] C.H. Chou and G.J. Thomas Jr., Biopolymers 16 (1977) 765..
[36] S. Arnott, R. Chandraskaran and A.G.W. Leslie, J. Mol. Biol. 106 (1976) 734.
[37] R. Langride and A. Rich, Nature 198 (1963) 725.
[38] G.S. Manning, Biophys. Chem. 9 (1978) 65.
[39] R.H. Cole, Ann. N. Y. Acad. Sci. 203 (1977) 59.
[40] M. Mandel, Ann. N. Y. Acad. Sci. 203 (1977) 74.
[41] These data are from measurements by K. Yamaoka in our laboratory in 1975; manuscript in preparation.
[42] S. Sokorov and G. Weill, Biophys. Chem. 10 (1979) 161.