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(25) Valles and Macosko's[18] assertion that the reduced force is sensibly independent of strain (i.e., that the Mooney-Rivlin C_2 is negligible) is at variance with the behavior of PDMS networks universally observed by other investigators. In comparing their results with recent theory,[26] they incorrectly infer that displacement of junctions should be fully affine at small strains. In effect, they ascribe values of 3 and 2 to $1 + f_c/f_{ph}$ for functionalities $\varphi = 3$ and 4, respectively. These are upper bounds only. Actual values should fall substantially below these limits, even at small strains.[26]
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Electric Dipole Moments of DNA in Aqueous Solutions as Studied by the Reversing-Pulse Electric Birefringence

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ABSTRACT: Reversing-pulse electric birefringence (RPEB) study shows that the electric field orientation of native calf thymus DNA ($M_r = 4.4 \times 10^6$) and sonicated fragments ($M_r = 1.24 \times 10^5$) is accounted for by induced dipole moments originating from the interaction of the counterions (Na^+ and Mg^{2+}), which redistribute on the DNA surface, with the externally applied field. The moments are induced much faster than the overall molecular rotation of DNA with no extremum in the RPEB signals, except for the high molecular weight Na-DNA, which did not obey the Kerr law. The RPEB behavior of DNA in aqueous solutions is discussed with theoretical calculations.

The current consensus is in support of the notion that electric dipole moments are responsible for the orientation of DNA in aqueous solutions by an externally applied electric field.[1,2] However, the nature of the electric moments of DNA has long been controversial and is unresolved as yet. The orientation of an un-ionized rigid rodlike polymer in an electric field may be ascribed to the torque exerted by the field on its permanent dipole moment (μ) and the polarizability anisotropy ($\Delta\alpha = \alpha_{33} - \alpha_{11}$).[3] By virtue of its nearly palindromic structure, DNA should have only a negligible μ, if any. Nevertheless, there have been numerous reports on the apparent permanent dipole moment of DNA, deduced from measurements of dielectric[4,5] and electrooptical properties.[6-8]

Experimental results have been interpreted with three mechanisms: (1) counterion fluctuation,[9] (2) asymmetric counterion flow,[10] and (3) ionic atmosphere polarization.[11] The last of these seems to explain the orientation behavior of DNA adequately, since the electric birefringence (or dichroism) signal (Δn) of DNA in strong fields fits an orientation function based on the variables μ and $\Delta\alpha$.[12,13] In order to verify the orientation by counterion-induced dipole moments, the transient mobility of the counterion condensed on the DNA surface should be measured.

One of the direct physical methods is reversing-pulse electric birefringence (RPEB), introduced by O'Konski and Pytkowicz[14] and put on a theoretical basis by Tinoco and Yamaoka.[15] Theoretical calculations of RPEB demonstrated that the signal Δn of a DNA solution should, after rapid reversal of a square pulse, show an extremum Δ_m after time t_m if it possesses either a permanent dipole moment (Figure 1 of ref 15) or a slow-induced dipole moment (Figure 4 of ref 15). The signal remains without an extremum if DNA is oriented by either $\Delta\alpha$ or a fast-induced dipole moment. These induced moments may result from the redistribution of counterions along the long axis of the DNA chain with a relaxation time of τ_3. The overall rotational relaxation time of DNA around its short

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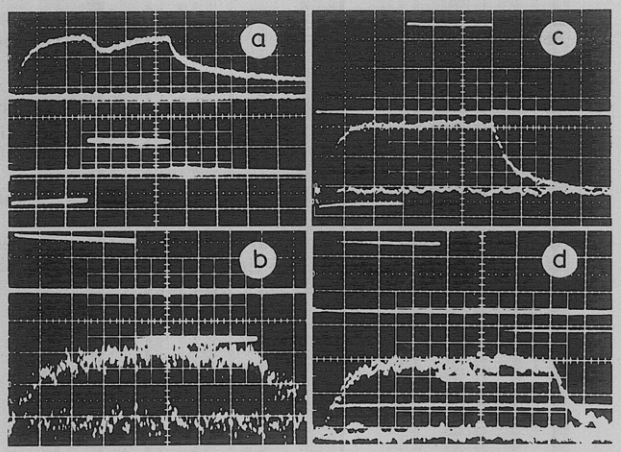

Figure 1. Oscillograms of RPEB signals and applied pulse forms for hDNA (left) and sDNA (right) in aqueous solutions at 7 °C and at 535 nm. The sign of Δn is always negative. The counterions of DNA are Na^+ (upper) and Mg^{2+} (lower). The concentrations of sDNA and hDNA are 0.17 mM in 1 mM NaCl solution, respectively, while they are 0.21 mM in 0.33 mM $MgCl_2$ solution, respectively. The field strengths in kV/cm are (a) 2.65, (b) 2.18, (c) 4.11, and (d) 5.39. The sweep times in μs/division are (a) 200, (b) 100, (c) 20, and (d) 20.

axis (τ_1) may be measured from the decay curve of the signal Δn. The height Δ_m and the time t_m depend on the ratio τ_3/τ_1;[15] thus, they are a direct measure of the counterion mobility on the DNA surface. Yet, the extensive use of RPEB has been hampered by instrumental difficulties.

With a newly built RPEB apparatus,[16] we are studying the electric moments of DNA of various molecular weights (M_r) and the effect of counterions on the electric orientation. We report some preliminary results of two calf thymus DNA samples: a high-M_r (ca. 4.4×10^6) wormlike DNA (hDNA) and its sonicated low-M_r (ca. 1.2×10^5) rigid rodlike fragment (sDNA).[17] Figure 1 shows typical oscillograms of the RPEB signal and the applied pulse field for hDNA (left) and sDNA (right) in aqueous solutions, in which the counterions are Na^+ (upper) and Mg^{2+} (lower), at a neutral pH of 6.4. When the square pulse is reversed, no extremum is observed at field strengths of 0–6 kV/cm; i.e., the depth $1 - \Delta_m$ is less than the 0.04 detectable limit, except for the Na-hDNA solution.[18–20] It should be noted that the quadratic dependence of the steady-state signals (the Kerr law) was observed at low fields for both Na- and Mg-sDNA and Mg-hDNA solutions. The hDNA behaves differently from sDNA in that the Na-hDNA shows an extremum at any field strength (0–5 kV/cm), while the Mg-hDNA shows no extremum at all.[21,22]

In Table I, the RPEB results are given at a fixed field strength of 5 kV/cm, since the τ_1 value depends on it, due to the polydispersity of M_r of DNA. These data clearly indicate that the time for the redistribution of *bound but mobile*[9] counterions Na^+ and Mg^{2+} (τ_3) is much faster than the time for the overall molecular rotation of sDNA (τ_1) and that the predicted depths are less than the experimental limit. The τ_1 value for Mg-hDNA is about one-third of that for Na-hDNA, indicating that Mg-hDNA is more shrunk in the molecular configuration than Na-hDNA, probably because of the stronger binding of Mg^{2+}.[23] Yet, interestingly, the Mg^{2+} seems to move on the DNA surface much faster than Na^+. The extremum observed for Na-hDNA cannot be explained by a simple theory, since the predicted height Δ_m (=0.98) differs greatly from the observed value ($\Delta_m = 0.78$ at $t_m = 87$ μs). This result may be related to the non-Kerr behavior of Na-hDNA.

Table I
Relaxation Times for Molecular Rotation τ_1 and Counterion Redistribution τ_3 and the Predicted Extremum Height Δ_m of DNA at 7 °C and at an Ionic Strength of 0.001

	Na-sDNA[a]	Mg-sDNA[a]	Na-hDNA[b]	Mg-hDNA[c]
$(\tau_1/\mu s)^d$	16.5	15.6	910	320
$(\tau_3/\mu s)^e$	≤ 0.75	≤ 0.72	≤ 360	≤ 15
$(\Delta_m)^f$	≥ 0.99	≥ 0.99	≥ 0.98	≈ 1

[a-c] The Kerr law nearly holds at $E = 5$ kV/cm for *a*, in a lower range ($E < 2.8$ kV/cm) for *c*, but only at the limiting low field for *b*. [d] These were observed values. τ_1 was obtained by the area method (Yoshioka, K.; Watanabe, H. *Nippon Kagaku Zasshi* **1963**, *84*, 626). [e] τ_3 was evaluated with the observed τ_1 by using eq 20 of ref 15 under the condition $1 - \Delta_m \leq 0.04$. [f] Δ_m was evaluated by using eq 20 with the observed τ_1 and the τ_3 which was estimated from eq 19 of ref 15 under the condition $t_m \leq 0.5$ μs. The time resolution of our RPEB apparatus is ca. 0.5 μs. The values estimated for Na-hDNA should be considered only approximate, since the field-strength dependence of its signals is almost linear rather than quadratic.

Addition of glycerol up to 20% did not alter the RPEB signals appreciably. The extremum of Na-hDNA bound by the dye 9-aminoacridinium cation completely disappeared as the mixing ratio of DNA-phosphate to dye was decreased to unity. Work is now in progress on the concentration effect of various counterions and the nature of buffer salts.

In conclusion, the electric field orientation of both rigid rodlike sDNA and wormlike hDNA originates predominantly from the electric dipole moment induced by the redistribution of counterions on the DNA surface. The mobility is much faster than the rotational diffusion of the whole DNA molecule. The extremum observed in the Na-hDNA solution should not be construed as evidence for the permanent dipole moment, since it disappears completely in Mg-hDNA; it probably results from a slow-induced dipole moment. If DNA should have the intrinsic permanent dipole moment, it must be negligibly small when compared with the induced moments. We believe that the RPEB method is useful for studying the role of the counterion in the electric field orientation of DNA.

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Matrix Treatment of Configuration-Dependent Physical Properties for Simple Chains Perturbed by Long-Range Interactions

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ABSTRACT: Matrix methods, widely used to relate configuration-dependent physical properties of unperturbed polymers to chain geometry and short-range contributions to potentials affecting rotation about bonds, have been adapted so that they reproduce several properties of certain chains perturbed by long-range interactions. This objective is achieved through a modification in the significance of certain elements in the statistical weight matrix. Matrices used can be of the same dimensions as those used to successfully treat the unperturbed chain. Illustrative calculations are performed for polymethylene chains containing various numbers of bonds, n, and with different extent of perturbation by long-range interactions. The model yields perturbed chains with the following characteristics: (1) Bond lengths and bond angles are the same as those for the unperturbed chain. (2) $(\alpha_s^5 - \alpha_s^3)/n^{1/2}$ reaches a nonzero asymptotic limit at large n ($\alpha_s^2 = \langle s^2\rangle/\langle s^2\rangle_0$, where $\langle s^2\rangle$ and $\langle s^2\rangle_0$ are the mean-square radius of gyration for perturbed and unperturbed chains, respectively). (3) Large expansions are achieved without alteration in the a priori probability for a trans placement in a long chain. (4) The effect of the perturbation on the ith bond increases as n increases. (5) Long-range interactions exert perturbations preferentially in the middle of the chain. (6) Perturbations are independent of the direction selected for indexing bonds in the chain. The magnitude of the perturbation for a polymer of specified n in a particular polymer-solvent system depends on an adjustable parameter denoted by K. In good approximation, K for polymethylene at 25 °C is found to be $[10C_M\psi_1(1 - \Theta/T)M_0^{1/2}]^{2/5}$. Therefore the model incorporates the thermodynamic character of polymer-solvent interaction as expressed by $\psi_1(1 - \Theta/T)$.

Complete understanding of the behavior of a macromolecule must include the ability to successfully relate configuration-dependent physical properties of the chain to its covalent structure. An impressive variety of configuration-dependent properties exhibited by long chain molecules unperturbed by long-range inateractions has been quantitatively related to the local covalent structure of the polymer through application of matrix methods.[1,2] This approach takes account of the actual structural geometry (bond lengths and bond angles) as well as short-range contributions to potentials affecting rotation about bonds in the chain. Accessible physical properties include size and shape of the unperturbed chain, as reflected by moments of the chain vector and radius of gyration and also by related tensors.[1-6] Several optical properties become accessible when contributions to the dipole moment and anisotropic part of the molecular polarizability tensor are assigned to each rigid group in the macromolecule.[1,2,7-13] Applicability of these matrix methods has been extended to encompass treatment of branched macromolecules unperturbed by long-range interactions.[14-17]

Much effort has been directed toward investigation of the manner in which perturbations produced by long-range interactions alter configuration-dependent properties. A common objective is evaluation of modifications in extension of the chain, as reflected by its mean-square end-to-end distance, $\langle r^2\rangle$, or mean-square radius of gyration, $\langle s^2\rangle$. Much less attention has been given to the actual chain geometry and rotational potentials than is possible in matrix treatments of configuration-dependent properties of unperturbed chains. Indeed, important consequences of perturbations produced by long-range interactions have been obtained from treatments which ignore connectivity of chain atoms and use instead a smoothed density of segments.[18-21] Chain atom connectivity may be recognized by using a pearl necklace model.[22,23] Perturbation of macromolecular dimensions is found to increase without limit as the number, n, of bonds in the chain increases. At sufficiently large n, $\langle s^2\rangle/\langle s^2\rangle_0$ becomes proportional to $n^{1/5}$.[18] Here $\langle s^2\rangle_0$ denotes the mean-square radius of gyration for a chain unperturbed by long-range interactions. Another approach to characterization of configuration-dependent properties of perturbed chain molecules is study of self-avoiding random walks on various types of lattices. Several such studies find representative samples of perturbed chains for which $\langle r^2\rangle/\langle r^2\rangle_0$ becomes proportional to $n^{1/5}$ at sufficiently large n.[24-26]

The power and broad applicability of matrix methods used to treat configuration-dependent properties of unperturbed chains makes it highly desirable to extend their

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