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Dielectric dispersion for short double-strand DNA

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A complex dielectric constant for double-strand DNA molecules with a length of not greater than 120 base pairs in an aqueous solution containing 30 mM NaCl was systematically measured as a function of chain length in such a way that experimental uncertainties associated with the molecular-weight distribution of specimens were virtually excluded. In contrast to the past experimental and theoretical studies for much longer DNA molecules, both the molar specific dielectric increment and the relaxation time are proportional to the chain length. These scaling rules cannot be accounted for by any theory so far proposed that gives analytical expressions for those two quantities in the long-chain limit.

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The purpose of this communication is to provide the broad community of readers related to the dielectric properties of polyelectrolyte solutions with decisive results from well-controlled experiments about the length dependence of dielectric dispersion for rigid rodlike molecules, specifically double-strand DNA (dsDNA), as one of the most representative and important examples. The scaling rules as obtained are remarkably different from the ones for longer DNA molecules with the broad distribution of molecular weight. Namely, both the molar specific dielectric increment and the relaxation time are proportional to the chain length. Qualitative or at best semiquantitative interpretation of these results is proposed below. Our intended contribution, however, might rather be to inspire the interest of related theorists in more quantitative discussion.

The question to be discussed here can be formulated as follows. DNA with length L corresponding to N base pairs (bp) is dissolved in water with salt concentration C_s large enough for dsDNA not to be denatured, so that the weight concentration C_w corresponds to the molar concentration C_m. The specific dielectric increment is defined here as $\Delta\epsilon_m=\Delta\epsilon_0/C_m$, where $\Delta\epsilon_0$ is the limiting value of the dielectric increment of the DNA solution relative to the dielectric constant of the solvent as the frequency of the external electric field f approaches zero. Then, what are the exponents of L that characterize the dependence of $\Delta\epsilon_m$ and the relaxation time τ? To the best of our knowledge, the complex dielectric constants of DNA with $N\lesssim 100$ have never been systematically measured before to answer this question in spite of its relevance for understanding the dynamics of short DNA practically used for many applications such as DNA microarrays.

The remarks about this somewhat classical but important question are divergent as pointed out by two of the pioneers in this field [1]. On the basis of experimental studies for mainly calf thymus DNA with the molecular weight of more than 1×10^6 and its ultrasonically fragmented portions, it has been claimed that $\Delta\epsilon_m$ and τ are, respectively, proportional to L^2 and L [2], L^3 and L^2 [3,4], or L^2 and L^2 [5]. It should be noted that the different definitions of specific dielectric increment are used over these literatures. Thus, here the proposed scaling rules have been restated according to our definition described above.

An impedance analyzer (4294A, Agilent Technologies) was used for dielectric measurement. A capacitor-type cell made of acrylic resin has two parallel platinum electrodes with the spacing of 3.5 mm and the diameter of 8 mm, which were carefully plated with platinum black to reduce electrode-polarization effects. The temperature of the sample solution was maintained at 298 K by circulating water around the cell. Measurements were performed over the frequency range from 40 to 1.1×10^8 Hz with the applied peak-to-peak voltage of 0.3 V.

To exclude uncertainties associated with the chain-length distribution inherent to specimens with biological origin used for the past studies, synthetic DNA with lengths of 10, 30, 59, 90, and 120 bp (Tsukuba Oligo Service) was used. The sequence for 120 bp taken from the p53 gene is (TAA ACG CAC ACC TCA TAA ACC TAC TGT CTT TGT GAA AAG CTG TAT CAC ACC ACC ACG GGA TAC TCG GCG GAC TCC AAC CGA GAC TGA CAT GGT GGT AGG GAT GTT GAT GTA CAC ATT GTC) from 5′ to 3′ terminals. The other shorter sequences were taken from the 120-bp sequence with the same 3′ terminals. The high-performance liquid chromatography (Waters 600, Waters) was used to confirm that each specimen virtually has a unique molecular weight since a very sharp single peak without shoulder features was observed. Firstly, freeze-dried specimens as received were dissolved in 30 mM NaCl. Secondly, the initial concentrations were determined from the absorbance of ultraviolet light at a wavelength of 260 nm, and then they were diluted with the same solvent to the final concentrations of $C_w=1$, 2, 3, and 4 mg/ml.

Equal volumes of two solutions of single-strand DNA (ssDNA) with perfectly complementary sequences were mixed, heated at 368 K for 5 min, and slowly cooled to the

ambient temperature for 1 h to form dsDNA. It was confirmed that hybridization reactions proceeded completely except for 10 bp, because the absorbance at the wavelength of 260 nm as a function of temperature measured using the photospectrometer (DU800, Beckman Coulter) showed a melting curve characteristic for dsDNA. For 10 bp, on the other hand, the melting temperature was close to but slightly higher than 298 K, so that some portion of dsDNA is expected to be denatured into the complementary pairs of ssDNA. Nevertheless, the data was included to qualitatively assess the trend of the scaling rules in the limit of $L \rightarrow 0$.

The evaluation of the cell constant and the calibration of the residual inductance and the parasitic capacitance over the whole circuitry were performed according to the method described elsewhere [6]. The complex dielectric constants and dc conductivities were measured for solvents with $C_s = 10-50$ mM, and the complex dielectric constant for a hypothetical solvent that has the same dc conductivity as the sample DNA solution was derived by interpolation and subtracted from that of the sample solution to yield the complex dielectric increment $\Delta\epsilon^*$ with the correction of electrode-polarization effects automatically implemented.

The dielectric dispersions for DNA with all the five chain lengths ($C_w = 4$ mg/ml) are shown in Fig. 1. In Fig. 1(a), the symbols and solid lines represent experimental and fitted data, respectively. Fitting was performed for only the real part of $\Delta\epsilon^*$ with the assumption of an empirical Cole-Cole function,

$$\Delta\epsilon^*(\omega) = \frac{\Delta\epsilon_0}{1 + (i\omega\tau)^\beta}, \tag{1}$$

where $i = \sqrt{-1}$ and $\omega = 2\pi f$. The parameter β does not strongly depend on L and remains at 0.56 with the standard deviation of 0.02 over all the chain lengths. Even though this is indirect evidence, it was assumed that the broad dispersions as seen in Fig. 1(a) are not due to the overlap of more than two dispersions related to completely different relaxation mechanisms.

Dielectric losses were simulated using the parameters as obtained and compared with the experimental ones in Fig. 1(b). The simulated and experimental data are in quite good agreement, so that reliable data that satisfy the Kramers–Kronig relation were obtained in spite of high conductivity of solvents. Though it might not be easy to visually discriminate the closely overlapped data for 90 and 120 bp in Fig. 1(a), the difference of the critical frequencies can be seen as the peak shift of the dielectric losses in Fig. 1(b).

The experimental reproducibility was assessed by preparing two sample solutions of 59 bp DNA ($C_w = 4$ mg/ml) and performing measurements for them separately. The deviations of the two data sets were 6.6% and 3.2% for $\Delta\epsilon_0$ and τ, respectively. These errors are considered to be mainly attributable to sampling of a small volume of concentrated DNA solution for dilution. On the other hand, the standard errors of the parameters for fitting were estimated to be 1.5% and 1.4% for $\Delta\epsilon_0$ and τ, respectively. Therefore, the experimental errors as the sums of squares of these two factors do not affect our conclusions about the scaling rules at all.

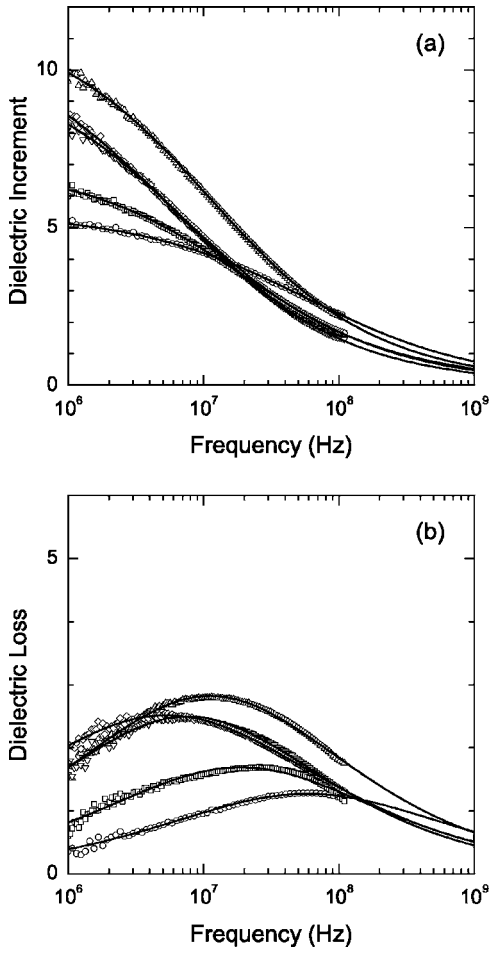

FIG. 1. The real (a) and imaginary (b) parts of the complex dielectric constants for DNA with the length of 10 (○), 30 (□), 59 (△), 90 (▽), and 120 (◇) bp at the weight concentration of 4 mg/ml. The solid curves for the real and imaginary parts are theoretical curves calculated from a single Cole-Cole function with the fitted parameters for the real parts. Only alternate data points are shown in the viewgraphs for easier visual inspection, though all points were used for fitting.

The dependence of $\Delta\epsilon_m$ on L is shown in Fig. 2. At a constant C_w, $\Delta\epsilon_m$ is approximately proportional to L. For $L \leqslant 120$, it is obvious that DNA molecules can be treated as rigid rodlike molecules, so that this dependence directly reflects the chain-length dependence of the induced dipole moments. It should be noted, however, that the deviation from the linear relationship is seen for 10 and 30 bp. For 10 bp, this is partially explained by denature of dsDNA as mentioned before. Since hybridization reactions proceeded completely for 30 bp as confirmed via melting curve measurement, further explanation is required to fully account for the data.

For a specific L, $\Delta\epsilon_m$ increases as C_w decreases, due to the weaker interaction between molecules. To assess the effects of intermolecular interaction, measurement for more dilute solution is necessary, but reliable data were obtainable only for $C_w \geqslant 1$ mg/ml using our experimental setup. It is seen that $\Delta\epsilon_m$ is nearly convergent at $C_w = 1$ mg/ml except for 120 bp. This can be reasonably understood, considering the

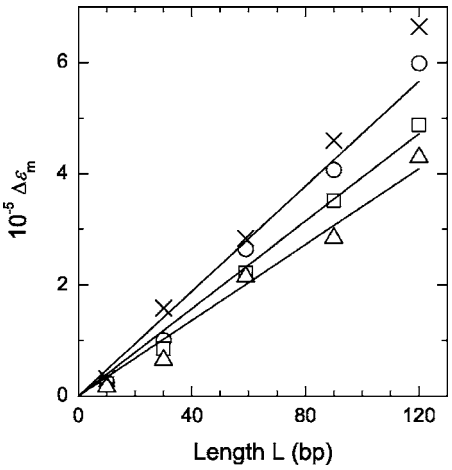

FIG. 2. The molar specific dielectric increments $\Delta\epsilon_m$ as a function of chain length for the weight concentrations of 1 (×), 2 (○), 3 (□), and 4 (△) mg/ml. The solid lines are the least-square fits to the experimental data. The fit for 1 mg/ml is not shown to avoid the overlap with that for 2 mg/ml.

overlap concentration of 3.5 mg/ml for 120 bp, simply estimated as $M_w/\frac{4}{3}\pi R_g^3 N_A$, where M_w is the molecular weight, N_A is the Avogadro's number, and $R_g = L/2$. Therefore, the data for shorter lengths represent the dielectric dispersions in the semidilute or dilute regime.

Next, the dependence of τ on L is shown in Fig. 3. For 10 and 30 bp at $C_w = 1$ mg/ml, however, β and τ were strongly correlated and could not be independently determined by fitting of Eq. (1), so that the corresponding data were excluded. For the determination of $\Delta\epsilon_m$, on the other hand, since the optimal values are not affected by the combination of β and τ in the course of fitting, the data for 10 and 30 bp were

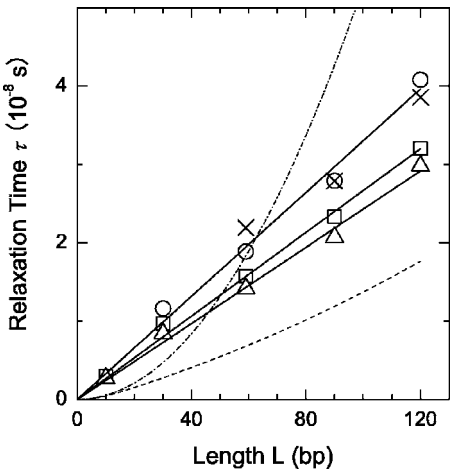

FIG. 3. The relaxation times as a function of chain length for the weight concentrations of 1 (×), 2 (○), 3 (□), and 4 (△) mg/ml. The solid lines are the least-square fits to the experimental data. The fit for 1 mg/ml is not shown to avoid the overlap with that for 2 mg/ml. The data for 10 and 30 bp at 1 mg/ml are not shown for the reason written in the text. The broken and dashed-dotted lines show the simulated results with and without counterion interaction effects, respectively. For the simulation, the friction constant of 7.4×10^{-12} kg/s was used as in Ref. [16].

included in Fig. 2. Unexpectedly, τ also exhibits a linear dependence on L. Similar to $\Delta\epsilon_m$, τ at a constant L also increases as C_w decreases. Again, it is seen that τ is nearly convergent at $C_w = 1$ mg/ml.

In the previous part, the linear dependence of $\Delta\epsilon_m$ and τ on L has been experimentally established for short dsDNA solutions with high salt in the semidilute or dilute regime. Next, dielectric theories so far proposed for polyelectrolyte solutions are revisited below in attempting to interpret the scaling rules. Prior to that, however, possible relaxation mechanisms should be identified.

Fortunately, with regard to short DNA, past electro-optical studies [7–9] can be referred to, where birefringence originating from the response of rodlike molecules to a pulsed electric field is measured. When a constant field is turned on, the dipoles are induced by counterion polarization and aligned along the field direction with the rotational dynamics being dependent upon the ratio of the rotational and counterion relaxation times. On the other hand, the relaxation of the induced dipoles is completely controlled by rotational diffusion when the field is abruptly turned off. Thus, on the basis of the theory about the coupled rotational diffusion and counterion polarization [8], the relaxation times for the two mechanisms can be determined.

Analysis of the transient electric birefringence data for 124 bp DNA [8] leads to the relaxation times of 1.3×10^{-6} s and 1.2×10^{-7} s for rotational diffusion and counterion polarization, respectively, though the experimental conditions are different from the ones for this study (the sample temperature is 293 K and $C_s = 1$ mM, but C_w is not denoted). The relaxation time for 120 bp DNA in this study is in the range of $3-4\times10^{-8}$ s over different C_w. Since it is well demonstrated that τ decreases with increasing C_s [10], the consistency between the two studies is fairly good, if the dominant process is attributed to counterion polarization. Therefore, counterion polarization is considered to be a faster process, and the effects of rotational diffusion can be excluded in the discussion below. In fact, since τ for rotational diffusion depends on the cube of L [8], the scaling rule observed in this study cannot be explained.

Therefore, only counterion polarization for rigid rodlike molecules spatially fixed along the electric-field direction is considered below. Relatively minor effects of counterion polarization perpendicular to the molecular axis, ion convection, and solvent flow are neglected for simpler discussion. In the limit of dilution, the static dielectric increment $\Delta\epsilon_0$ and the complex polarizability $\alpha^*(\omega) = \alpha'(\omega) + i\alpha''(\omega)$ can be connected as follows [11]:

$$\Delta\epsilon_0 = n\epsilon_0\alpha'(0) + \lim_{\omega\to 0} n\frac{\sigma_0}{\epsilon_\nu\omega}\alpha''(\omega), \tag{2}$$

where n is the number density of molecules, ϵ_0 and ϵ_ν are the dielectric constants for the solvent and the permittivity of free space, respectively, and σ_0 is the dc conductivity of the solvent. Many theories so far proposed can be classified into two categories that are different in the evaluation of relative importance of the two terms in Eq. (2).

The first category emphasizes the role of counterions

firmly bound to molecules [12–15]. The average of the square of the instantaneous dipole moment resulting from thermal fluctuation of counterion distribution in the absence of field is derived and related to α', namely the first term of Eq. (2). If the interaction between counterions is neglected, the scaling rules of $\Delta\epsilon_m \propto L^3$ and $\tau \propto L^2$ are derived from the analytical expressions (Eqs. (17) and (20) in Ref. [14]), but they are not consistent with our experimental results.

It has been pointed out that the counterion interaction is not as small as speculated in the original theory [16]. Thus, τ was calculated as a function of chain length using the analytical expression for the interaction term (Eq. (11) in Ref. [16]). The simulated results are shown in Fig. 3. Even though the relation is not completely linear, this simulation gives the scaling rule with the exponent close to unity if the interaction term is included. This suggests that rigorous theoretical treatment of counterion migration along the molecule can lead to a more accurate description of τ for short DNA. However, even with the reduction of the scaling exponent by one, $\Delta\epsilon_m$ is still not consistent with our experimental results.

As well recognized [1], it is not acceptable for quantitative discussion to discard the second term of Eq. (2) especially when the solution is highly conductive. The second category of theories takes into account the polarization of diffuse electric double layers [17–19], where the second term is dominant. Dynamical formulation for rodlike molecules has only recently proposed that explicitly discusses the dielectric relaxation of short DNA in high-salt solvent [19], even though it has not been experimentally supported yet. In that study, $\Delta\epsilon_0$ and τ are derived from the balance condition of the currents from the bulk electrolyte solution and along a molecule at the chain ends. The scaling law of $\Delta\epsilon_m \propto L$ is fairly consistent with our experiment, while that of $\tau \propto L^2$ is not (Eqs. (18) and (21) in Ref. [19]).

One possible reason of this contradiction is the oversimplification of counterion migration along the molecule, because the formula for τ in Ref. [19] is identical to the one in Ref. [14] if the counterion interaction term is neglected. Therefore, it is expected that an extension of this theory with more accurate treatment of counterion interaction can account for the scaling rules in this study, and such effort is required. As briefly suggested in Ref. [19], $\Delta\epsilon_m$ follows another higher-order scaling rule for dsDNA shorter than some critical length. The observed deviation from the linear relation for 10 and 30 bp as pointed out before could be manifestation of such a rule.

As discussed so far, a model that can provide satisfactory explanation of our experimental results was not found among the past theories. Such theories assume $L \gg a, r_D$, where a and r_D are the molecular diameter and the Debye length, respectively, and analytical expressions for $\Delta\epsilon_m$ and τ are only obtainable as limiting laws of long-chain approximation. In contrast, $L \approx 3-40$ nm and $a \approx r_D \approx 2$ nm in our experimental conditions, so that the assumptions might not be valid. In spite of sustained experimental and theoretical efforts, understanding of the dielectric properties of even the simplest rigid rodlike molecules is not complete yet. Therefore, the dielectric data for short DNA are still meaningful as a reference for a theory that can fully explain the anomalous behaviors as found in this study without long-chain approximation. Such a theory should be the firm basis to revisit the dielectric properties of more complex molecules that are longer and have a secondary structure.

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