DIELECTRIC BEHAVIOR OF DNA SOLUTION AT RADIO AND MICROWAVE FREQUENCIES (AT 20°C)

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ABSTRACT The dielectric constant and conductivity of calf thymus DNA were investigated at frequencies between 0.1 MHz and 70 GHz. This work is to investigate the dielectric properties of DNA in low gigahertz region and also to study whether the dielectric behavior of the water is affected by the presence of highly charged DNA. The results of these measurements indicate the presence of two anomalous dispersions, the one between 1 MHz and 1 GHz and the second one above 1 GHZ. The dispersion at low frequencies is likely to arise from polar groups in the DNA molecule. The relaxation behavior of unbound water in DNA solution is only slightly affected by the presence of DNA at concentrations below 1%.

INTRODUCTION

Research by several investigators has shown that DNA molecule has a very large dipole moment along the axis of double helix (1–6). Because of the long contour length of DNA along which counter ions migrate to produce an induced dipole moment (7, 8), the relaxation frequency of DNA is found at ultra-low frequencies. Thus far, the interest in the dielectric behavior of DNA has been limited to frequencies below 1 kHz except for a paper by Mandel that dealt with measurements between 10 kHz and 100 MHz (9). The results obtained by Mandel demonstrate a dispersion in a range between 1 and 50 MHz.

Recently, it was suggested that DNA may exhibit a resonance absorption in gigahertz region due to a coherent vibration excitation of the double helix (10). Swicord and Davis (11) carried out dielectric measurements using mammalian as well as bacterial DNA between 8 and 12 GHz and reported the attenuation coefficient of DNA solution as significantly higher than that of water, indicating a substantial absorption in this frequency range. More recently, Foster et al. (12) investigated the dielectric behavior of calf thymus DNA between 0.3 and 12 GHz and reported that DNA solution exhibited a dielectric behavior that is not significantly different from that of pure water.

These conflicting views seem to arise from the insufficient frequency coverage of these experiments. We decided to carry out dielectric measurements using four different instruments to cover a frequency range between 0.01 MHz and 70 GHz. The research was intended to establish experimentally the frequency profile of the dielectric constant of DNA solution, in an attempt to interpret correctly its dielectric behavior and the possible implications of that behavior.

MATERIALS AND METHODS

DNA sample was purchased from Sigma Chemical Co. Ltd. (London) and used for measurements without further purification unless otherwise stated. DNA was dissolved in water (the pH was adjusted to 7.0 using Tris buffer) at the concentration of 0.3 to 1.0%. Measurements were carried out with unheated DNA solutions as well as with the sample, which was heated for 30 min at 90°–95°C.

Four measuring systems were used for these measurements. (*a*) Hewlett-Packard (Hewlett-Packard Co., Palo Alto, CA) impedance analyzer 4192A (10 Hz to 12 MHz), (*b*) Hewlett-Packard Boonton RX-meter (500 kHz to 100 MHz), (*c*) Hewlett-Packard time domain spectrometer (TDS) (10 MHz to 10 GHz), and (*d*) frequency domain wave-guide system (70 GHz). Of these, the measurements using a Hewlett-Packard impedance analyzer and also those using a RX meter were carried out at the Department of Bioengineering, University of Pennsylvania. The highly computerized TDS system that was used for these measurements was described in detail in a previous publication (13). The 70 GHz wave-guide system was discussed by Szwarnowski and Sheppard, and the full description of this system is found in their article (14).

RESULTS

Fig. 1 illustrates the frequency profile of the dielectric constant of DNA solution as well as that of pure water between 10 MHz and 70 GHz. Fig. 2 shows a portion between 100 kHz and 10 GHz with enlarged ordinates. The portions designated by letters *A*, *B*, and *C* are measured using an impedance analyzer, RX meter, and TDS system. As shown, these instruments cover a wide frequency range with sufficient overlap to warrant continuity. These two figures clearly demonstrate two dispersions with DNA solution, i.e., the one between 1 and 500 MHz and the second above 1 GHz. Curve 3 in Fig. 1 shows the dielectric loss (ϵ'') of DNA solution. This curve, in agreement with ϵ' curve, clearly demonstrates the presence

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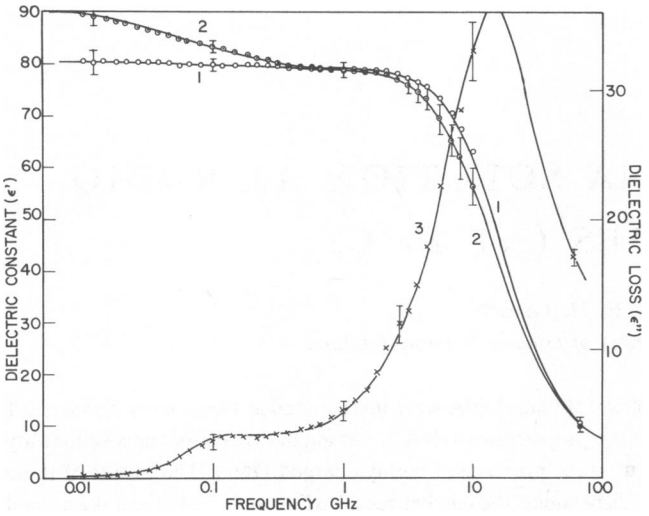

FIGURE 1 Frequency dependence of the dielectric constant of DNA solution (curve *2*) and pure water (curve *1*). Vertical bars on curve *2* are standard deviations. Curve *3* shows the dielectric loss (ϵ'') of DNA solution. Note two absorption peaks. Concentration, 1%; temperature, 20°C. pH, 7.0.

of two absorption peaks. The dispersion between 1 MHz and 1 GHz is similar to the one observed by Mandel (9) and the second dispersion above 1 GHz is obviously due to the relaxation of water. The plateau between 500 MHz and 1 GHz (ϵ_w) is actually the low frequency end of the dispersion of water, which is more or less unbound to DNA. The value of ϵ_w is often used to calculate the effective volume of polymers including bound water layer. Therefore, if DNA is surrounded by a large hydration shell having a small dielectric constant, the value of ϵ_w will be considerably smaller than that of pure water. The observation that the value of ϵ_w of DNA solution is similar to the

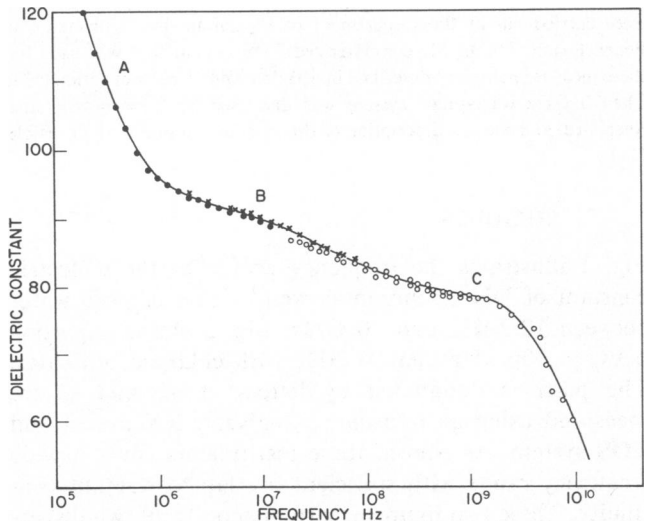

FIGURE 2 Frequency profile of the dielectric constant of DNA solution (1%). *A* (●) was obtained with a Hewlett-Packard impedance analyzer, *B* (+) with a RX-meter, and *C* (○) was obtained with the Hewlett-Packard TDS system.

dielectric constant of pure water suggests that the amount of irrotationally bound water around DNA molecule is relatively small. To calculate the actual amount of hydrated water, the value of ϵ_w must be determined accurately. At the concentration of DNA used for these measurements, i.e., 1% or less, the difference between the dielectric constants of DNA solution and that of water is too small to detect with sufficient accuracy.

The dielectric dispersion at low frequencies (below 1 GHz) is dependent on the concentration of DNA (Fig. 3), and, therefore, must be due to DNA molecules. The dielectric increment of DNA is unaffected by heating the sample (See the cross in Fig. 3). Bearing in mind that heating separates the double strands, we infer that the dispersion may arise from the rotational or vibrational motions of base molecules rather than the vibration of double helical structure of DNA.

So far, the results of the measurement of dielectric constant and conductivity or loss factor ϵ'' have been displayed against frequency. These plots are usually sufficient to calculate the amplitude of dielectric dispersion and relaxation frequency f_c. In many cases, however, the measurement over a wide frequency range is prevented by instrumental limitation. In our measurements, although a wide frequency range is covered, there is a wide gap between 10 and 70 GHz. Therefore, some manipulation of data was required to locate the center frequency of the dielectric dispersion of unbound water. Fuoss and Kirkwood (15) proposed the following equation to display the experimental results:

$$\epsilon'' = \epsilon''_m \operatorname{sech} [\beta \ln(f_c/f)], \quad (1)$$

where ϵ''_m and ϵ'' are, respectively, the dielectric loss at the relaxation frequency and at other frequencies. The value of ϵ''_m can be calculated using Eq. 2 (16):

$$\epsilon''_m = (\epsilon_s - \epsilon_\infty)/2, \quad (2)$$

where ϵ_s and ϵ_∞ are low- and high-frequency dielectric

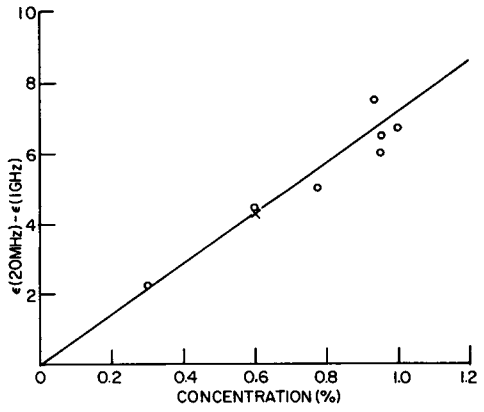

FIGURE 3 Concentration dependence of the dielectric increment of DNA solution. The cross (+) shown in this figure was obtained with heated DNA solution and circles (○) were obtained with native DNA.

constants. Because ϵ_s is found to be 80 (see Fig. 2) and ϵ_∞ is known to be 5.8 for pure water (assuming the ϵ_∞ of DNA solution is about the same), the value of ϵ''_m is most likely to be 37.1. The values of β other than unity indicate a distribution of relaxation times. The value β is 1.0 for a single time constant. In general $1 \geq \beta > 0$ depending on the distribution of relaxation times. Eq. 1 can be rearranged to obtain Eq. 3:

$$\cosh^{-1}\left(\frac{\epsilon''_m}{\epsilon''}\right) = \beta \ln(f_c/f). \tag{3}$$

Therefore, if $\cosh^{-1}(\epsilon''_m/\epsilon'')$ is plotted against $\ln(f/f_c)$, a straight line should result. Because, f_c is unknown, we can plot $\cosh^{-1}(\epsilon''_m/\epsilon'')$ against $\ln f$. This method enables the relaxation frequency to be determined graphically from the intersection of this plot and zero line. Fig. 4 shows the Fuoss-Kirkwood plot for pure water. As predicted, the plot is linear and the intersection between the plot and zero line is located at 18.6 GHz. This frequency is very close to the tabulated relaxation frequency for water at the same temperature, i.e., 17 GHz. Moreover, the slope of this plot is 1.0 within experimental error, i.e., $\beta = 1.0$. Fig. 5 illustrates the Fuoss-Kirkwood plot for DNA solution. Clearly, the plot is no longer linear at low frequencies where a small dispersion was found. The plot becomes linear at high frequencies where the free water dispersion dominates. Because the span of the linear portion is rather short, the determination of relaxation frequency and the slope of the plot is subject to some uncertainty. We found using this plot the center frequency around 16.6 GHz and a slope of 0.98. The value of center frequency is somewhat lower than that of pure water. The difference in the slope between DNA solution and water is so small, it may be considered within experimental error.

Fig. 6 shows the Cole-Cole plot (16) for pure water and

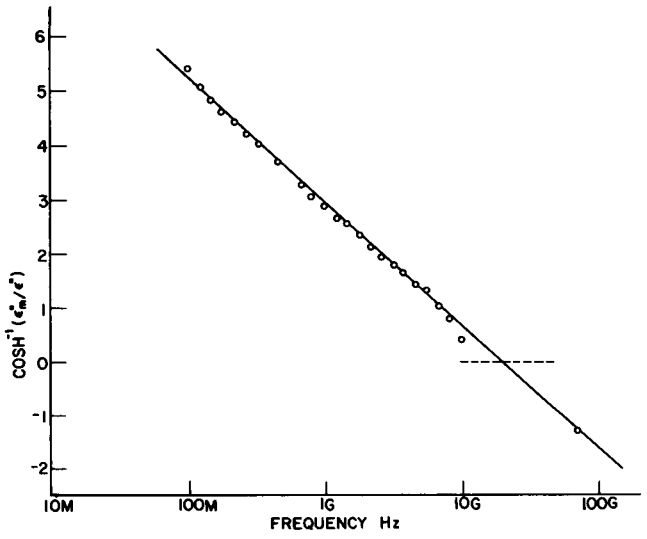

FIGURE 4 Fuoss-Kirkwood plot for pure water. Relaxation frequency is found to be 20 GHz and the slope is 1.0.

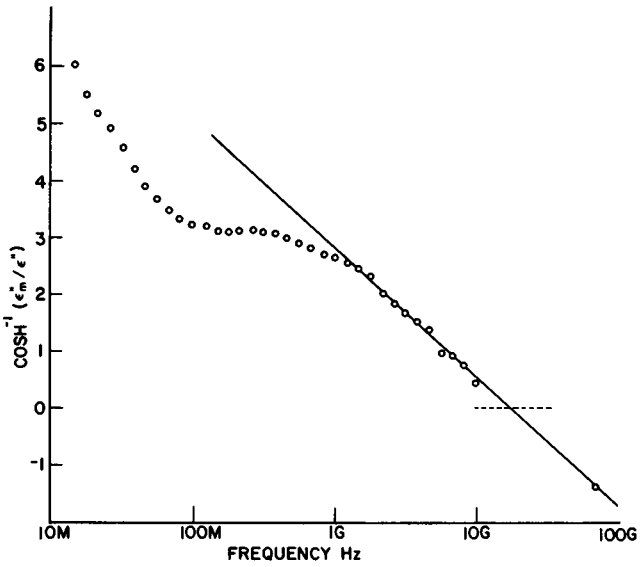

FIGURE 5 Fuoss-Kirkwood plot for DNA solution (1%). Note that the plot is no longer linear at low frequencies because of the presence of a dispersion. Relaxation frequency is ~17 GHz, and the limiting slope is slightly < 1.0.

for DNA solution. The distribution parameter α for pure water is 0.0 within the experimental error whereas the value for DNA solution is ~0.05. The α parameter defined by Cole and Cole is related to the Fuoss-Kirkwood β-parameter by the following equation:

$$\beta = \frac{1}{\sqrt{2}} \frac{n}{\cos\left(\ln \frac{\pi}{4}\right)} \tag{4}$$

where $n = 1 - \alpha$. Therefore, the values of α and β parameters are interchangeable. The value of 0.05 for α is equivalent to a value of 0.94 in β unit. Thus, the results obtained by Cole-Cole plot and Fuoss-Kirkwood plot are mutually consistent. In view of these, it seems reasonable to

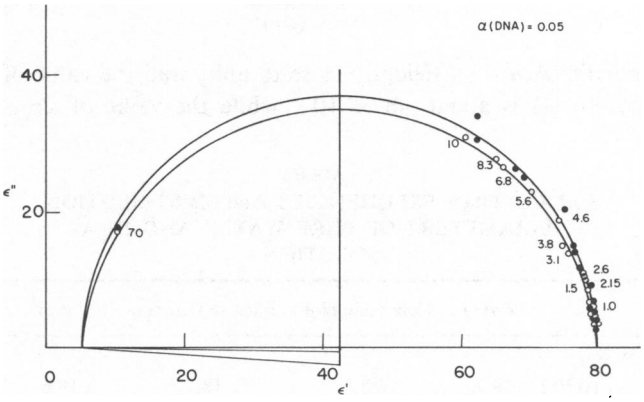

FIGURE 6 Cole-Cole plot of pure water and DNA solution (●) and DNA solution (○). Note the small depression of the arc for DNA solution giving rise to an α – parameter of 0.05, whereas that for pure water gives rise to a value of 0.0. Numbers are frequencies in gigahertz.

believe that the dielectric dispersion of water in the presence of DNA is slightly different from that of pure water.

Another way of investigating the possibility of a distribution of relaxation times is to determine the slope of the graph of ϵ' against $\log f$ using either the Debye theory (17) or the equation derived by Cole and Cole. As shown in Fig. 1, the dispersion of ϵ' for DNA solution is broader than that of pure water and the difference between these two curves is definitely beyond the experimental error as indicated by standard deviations. However, as shown, both curves gradually approach one another as frequency increases. Thus, the difference in dielectric constant near the center frequencies, i.e., 17–20 GHz, is quite small and the values at 70 GHz are nearly identical. Table I shows the values of dielectric constant of DNA and that of pure water at 70 GHz. The curve 2 in Fig. 1 can best be fitted with an α-parameter of 0.06 using the Cole-Cole equation (see Eq. 5) whereas that for curve 1 can be reproduced assuming α value of 0 or $n = 1.0$:

$$\epsilon' - \epsilon_\infty = (\epsilon_s - \epsilon_\infty)\frac{1 + (\omega\tau)^n \cos\left(n\frac{\pi}{2}\right)}{1 + 2(\omega\tau)^n \cos\left(n\frac{\pi}{2}\right) + (\omega\tau)^{2n}} \quad (5)$$

where ω is angular frequency and is equal to $2\pi f$. τ is relaxation time and is $1/2\pi f_c$.

Lastly, another way to determine relaxation time is to reduce conductivity data to a linear plot using the following method. The Cole-Cole equation for the imaginary part ϵ'' is

$$\epsilon'' = (\epsilon_s - \epsilon_\infty)\frac{\left\{1 + (\omega\tau)^n \cos\left(n\frac{\pi}{2}\right)\right\}\omega\tau}{1 + 2(\omega\tau)^n \cos\left(n\frac{\pi}{2}\right) + (\omega\tau)^{2n}} \quad (6)$$

where n is 1 for pure water and 0.96 for DNA. For water therefore Eq. 6 reduces to the Debye equation, i.e.,

$$\epsilon'' = \frac{(\epsilon_s - \epsilon_\infty)\,\omega\tau}{1 + (\omega\tau)^2}. \quad (7)$$

For DNA n is sufficiently close to unity and the value of $\cos(n\,\pi/2)$ is about $\sim 8 \times 10^{-3}$ while the value of $\omega\tau$ is between 10 and 0.1 in the frequency range of our interest. Thus the term involving $\cos(n\,\pi/2)$ can be ignored without serious error. Using the well-known relation $\epsilon''\epsilon_0 = k/\omega$ in Eq. 7, we obtain

$$k = \frac{2\pi(\epsilon_s - \epsilon_\infty)\epsilon_0(f^2/f_c)}{1 + (\omega\tau)^2}, \quad (8)$$

where ϵ_0 is the permittivity of free space. Inverting this equation and rearranging, we obtain

$$\frac{1}{k} = \frac{Bf_c}{f} + \frac{B}{f_c}, \quad (9)$$

where $B = 1/2\pi\epsilon_0(\epsilon_0 - \epsilon_\infty)$. In other words, the plot of $1/k$ against $1/f^2$ should be a straight line with a slope of $f_c/[2\epsilon_0\pi(\epsilon_s - \epsilon_\infty)]$. Fig. 7 shows the plots for pure water and for DNA solution. As shown, the plot of $1/k$ vs. $1/f^2$ is a straight line as expected for pure water and we obtain from the slope a relaxation frequency of 18.6 GHz. On the other hand, the same plot for DNA is linear only at high frequencies where the dispersion of water is dominant and becomes nonlinear at frequencies where the dispersion of DNA prevails. The high-frequency slope corresponds to a relaxation frequency of 16.6 GHz. Although Eq. 9 is valid only for a single time constant, the deviation of the dispersion of DNA from one time-constant behavior is so small that the use of this equation produces only a minute error in the value of relaxation frequency. In any event, the relaxation frequency of DNA solution is again slightly lower than that of pure water. Table I summarizes the relaxation frequencies of water and DNA solutions determined using the methods discussed above. Also the distribution parameters are listed in the same Table.

TABLE I
RELAXATION FREQUENCIES AND DISTRIBUTION PARAMETERS OF PURE WATER AND DNA SOLUTION

	ϵ' vs. f	Cole-Cole plot	Fuoss-Kirkwood	k^{-1} vs. f^{-2}
Water				
$f_c(GHz)$	18.7	16.8	18.7	18.6
α	0	0	$0(\beta = 1.0)$	—
DNA				
$f_c(GHz)$	17.4	14.9	15.9	16.6
α	0.05	0.045	$0.02(\beta = 0.98)$	—

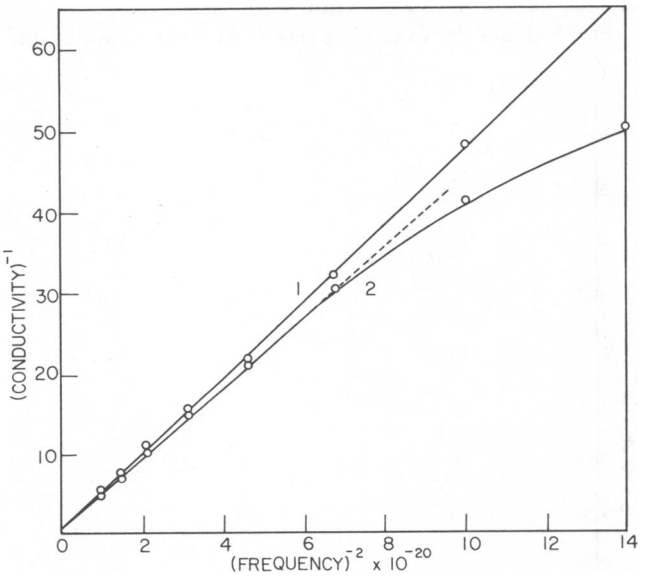

FIGURE 7 This plot is based on Eq. 7. The ordinate is k^{-1} and abscissa is f^{-2}. Curve *1* is for pure water and curve *2* is for DNA solution. The slope of these plots gives rise to relaxation frequencies of 19.9 GHz and 17 GHz, respectively, for water and DNA solution.

The foregoing analysis has provided information on the dielectric parameters of the γ-dispersion. Turning now to the smaller dispersion investigated in this work, a Cole-Cole plot of the data contained in the frequency range 10 MHz-1 GHz is shown in Fig. 8. Despite the interfering effect of the γ dispersion at the lower values of ϵ' the Cole-Cole circle appropriate to the smaller dispersion is clearly resolved and is consistent with a relaxation frequency of 81 ± 4 MHz at 20°C. A similar Cole-Cole plot drawn for the sonicated sample have a relaxation frequency of 82 ± 4 MHz.

DISCUSSION

The purpose of this work was to attempt to characterize the small dispersion in DNA solution reported by Mandel (9) in the 10 MHz to 10 GHz region. It was also hoped to provide information about how the presence of DNA might affect the free water dispersion.

First of all, by covering a wide frequency range between 10 KHz and 70 GHz (with an interruption between 10 and 70 GHz), we found a dielectric dispersion between 1 MHz and 1 GHz. The dispersion curve is broad and amplitude is small. Nevertheless, all three instruments used for these measurements revealed this anomalous dispersion. Based on the observation that the increment of this dispersion depended linearly on the concentration of DNA, it is highly likely that the origin is some polar groups in DNA molecule. In view of the similarity in the magnitude and frequency, this dispersion may be identified with the one observed by Mandel. At higher frequencies, particularly above 10 GHz, our measurements are still too coarse to detect the presence or absence of resonances which usually give rise to a very sharp absorption. The previous measurements done by Swicord and Davis (11) and by Foster et al. (12) likewise cover limited frequency ranges. Under these circumstances, it is difficult to draw a conclusion one way or the other.

The second question is whether or not the dielectric behavior of unbound water is affected by the presence of DNA molecules in the solution. There are two reasons to believe that the orientation of water molecules may be altered in DNA solution. The one is the very high macroscopic viscosity of the solution, which may be more than 10 times that of pure water even at a concentration of 1%. However, as has been shown, the dielectric behavior is only slightly affected by the presence of DNA, and there is no proportionality between the increase in macroscopic viscosity of the solution and the change in dielectric properties. The second reason to believe that DNA may affect the orientation of H_2O is the high density of negative charges due to phosphate groups in DNA. Since the dipole moment of water is well known (2.5 D), the free energy of charge-dipole interaction in the first hydration shell can be calculated using the following equation (see reference 18):

$$u = (-57.6\, n\, \mu_w \cos\theta)/r^2\epsilon, \qquad (10)$$

where n is the valence and is one for phosphate group. θ is the angle between the dipole vector and the line connecting the charge and the center of dipole. Assuming that dipole vectors are radially distributed around the charge, θ is nearly zero. ϵ is the dielectric constant and can be assumed to be 1. Using these numbers, we obtain a value of -11 kcal/mol assuming the distance between them to be 3 Å. Thus, the rotation of water in the primary hydration shell is severely restricted because of the charge-dipole interaction. However, the water under investigation is outside the primary layer. The calculation of interaction energy between a charge and a dipole with a layer of nonrotational water molecules between them can only be roughly estimated using Eq. 11. The distance between the charge and a dipole was assumed to be 8 Å, and dielectric constant was, because of ice-like structure in the primary hydration shell, assumed to be 5 at microwave frequencies. Further, assuming that water dipoles are more randomly oriented in the secondary hydration layer, the average angle is likely to be 60°. We arrive at an energy value of -0.432 kcal/mol. This value is considerably smaller than that in the first layer. Therefore, the charges of phosphate groups in DNA may not have a significant long range effect on the orientational behavior of water molecules outside the primary hydration layer.

To investigate the dielectric behavior of water in DNA solution we used four different methods to analyze experimental data: (*a*) ϵ' vs. frequency plot, (*b*) Cole-Cole plot, (*c*) Fuoss-Kirkwood plot, and (*d*) k^{-1} vs. f^{-2} plot. As shown in Table I, the relaxation times determined using these methods are slightly different from one another. However, all these analyses demonstrate that the relaxation frequency of water in DNA solution is lower than that of pure water by 1–3 GHz. In addition, the presence of charged DNA molecules causes a small distribution of relaxation times judged by the values of α and β parameters. Thus, after all, it seems that the charge of DNA has certain effects on the orientation of water molecules even outside the primary hydration shell. However, the effect is very

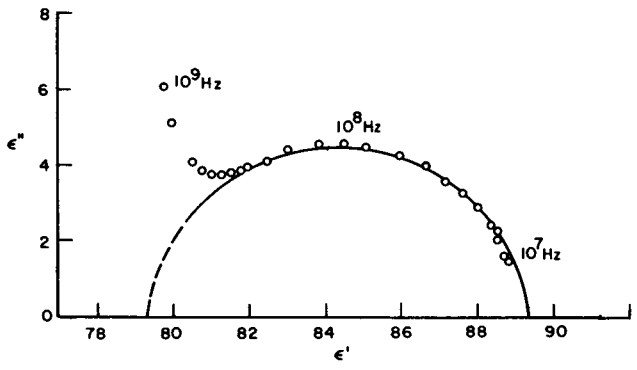

FIGURE 8 The Cole-Cole plot of DNA for the dispersion between 0.01 MHz and 1 GHz. The sudden increase in dielectric loss (ϵ'') is due to the dispersion of unbound water. Numbers are frequencies in hertz.

small and the biological implication of the effect is uncertain. No evidence was found for enhanced absorption in calf thymus DNA solutions in the γ dispersion region. The possibility of the existence of this phenomenon is being investigated currently by Nightingale and Grant (in preparation) using various DNA preparations, and the results will form a future publication.

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