EFFECT OF IONS ON COUNTERION FLUCTUATION IN LOW-MOLECULAR WEIGHT DNA DIELECTRIC DISPERSIONS

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ABSTRACT   The dielectric permittivity of aqueous solutions of low-molecular weight DNA ($M_r = 3.2 \times 10^5$) in the presence of $MgCl_2$ and $AgNO_3$ has been measured in the frequency range from 5 kHz to 30 MHz, at a temperature of 25°C. The DNA concentration was 3.5×10^{-4} M in terms of phosphate and the salt concentration was varied from 1×10^{-5} to 2×10^{-4} M. The dielectric results have been analyzed in terms of two contiguous dielectric dispersions, and characteristic parameters have been discussed on the basis of polyelectrolyte theories which deal with counterion fluctuation. Some molecular parameters of the DNA molecule in electrolyte solutions are estimated.

INTRODUCTION

The dielectric behavior of DNA has received considerable attention in the past decade (1) and it has been established that DNA aqueous solutions, like other highly charged polyelectrolytes, display two low-frequency dielectric dispersions.

The first dispersion (α dispersion), due to counterion fluctuations along the polyion under the external field, appears in the frequency range between 10 and 10^4 Hz, depending essentially on the molecular weight of DNA; both the dielectric increment and the relaxation time are approximately proportional to the square of the length of the rodlike molecules. The second dispersion (β dispersion), ranging from ~10 kHz to a few MHz), has been generally explained with the Maxwell-Wagner type effect, where the dielectric properties of the solution are expressed as consisting of polymer domain and the outer medium, with different conductivities and dielectric constants.

To clarify the specific effect of different ions on the DNA dielectric properties, we have chosen as counterions Mg^{++} and Ag^+, whose behavior towards DNA molecules is well documented in the literature. In fact, many studies have been performed on the interation of metal ions with DNA (for a comprehensive review, see reference 2) using a variety of experimental techniques ranging from optical measurements and sedimentation coefficient to hydrodynamic analysis, etc. It is well known (3) that Mg^{++} ions strongly interact with the phosphate groups of DNA, thus forming a counterion cloud around it; Ag^+ ions, on the contrary, are buried in the interior of the DNA molecule, tightly bound to the bases.

The dielectric properties of high-molecular weight DNA in the presence of monovalent and bivalent ions were extensively examined by Takashima (4), but the experi-

mental investigation was limited to the dispersion at lower frequencies. With low-molecular weight DNA, such as that used in this work, both dispersions are evident, and therefore the effect of ions on the characteristic parameters of the dielectric dispersions may be studied.

The DNA concentration was maintained at the value of 3.5×10^{-4} M. At this concentration, it may be assumed (5) that intermolecular interactions are negligible. The counterion concentrations were varied from 10^{-5} to 2×10^{-4} M, yielding a molar counterion to DNA molar ratio in a range where saturation of the charged sites was not reached. More elevated concentrations of counterions could not be examined with our experimental setup, owing to the high conductivity values of the solutions.

EXPERIMENTAL

Materials and Methods

200 mg of calf thymus DNA (Worthinton, Freehold, NJ, grade 1, sodium salt) were dissolved in 100 ml of 50 mM Tris-HCl, pH 9.0, NaCl 0.5 M and deproteinized with two phenol extractions, followed by one chloroform-isoamylic alcohol (24:1) treatment. Two volumes of ethanol were added to the aqueous layer. The precipitated DNA, after standing overnight at −20°C, was collected by centrifugation at 15,000 rpm in a Sorvall SS 34 rotor (Sorvall, Newton, CT) for 30 min at 4°C. The DNA was dried under vacuum and dissolved in 100 ml of distilled deionized water ($\sigma = 10^{-6} \ \Omega^{-1} \ cm^{-1}$). Protein analysis according to the Lowry method (6) yielded a value of <0.1% wt/wt of contamination. The DNA solution, kept in a water ice bath was sonified in a MSE sonifier (Mullard, Cincinnati, OH) (30 mm probe at 20,000 cycles/s) for 10 min with cooling intervals. After exhaustive dialysis against water at 4°C, a slight turbidity of insoluble materials was removed by centrifugation in a Beckman ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA) with a SW 41 Ti rotor at 50,000 g for 60 min at 4°C. The molecular weight analysis of the DNA was performed by agarose gel electrophoresis (7) with a gel concentration of 1.4% using as size markers the DNA fragments of FX 174 RF DNA generated by treatment with the restriction enzyme Hae III. The DNA in the gel was intercalated with

ethidium bromide at 0.5 μg/ml, visualized by a short wave UV light and photographed using an orange Wratten filter. After recording the patterns of the sonicated DNA and the size markers with a microdensitometric system (Beckman Instruments, Inc., ACTA III model), the molecular weights distribution was normalized in a linear plot shown in Fig. 1.

The DNA solution (1.5 mg/ml) was divided in two 50-ml aliquots one of which was added with $MgCl_2$, and the other with $AgNO_3$ from concentrated stock solutions to a final concentration of counterions of 0.1 M. The DNA was precipitated with ethanol and, overnight at $-20°C$, collected by centrifugation. The samples were dissolved in 40 ml of 0.01 M Na_2 EDTA solution and separately dialyzed against 2 liters of the same solution with two changes. Subsequently the two aliquots were dialyzed to equilibrium against 10^{-4} M $MgCl_2$ and $AgNO_3$, respectively.

The DNA concentration was determined by phosphate analysis (8), and the molar absorption coefficients, in terms of phosphate concentration, were determined by optical absorbance determination at 2,600 Å with an ACTA III (Beckman Instruments, Inc.) spectrophotometer. Values of 6,580 and 6,470 ± 100 $M^{-1}cm^{-1}$ were obtained for Ag-DNA and Mg-DNA, respectively, in good agreement with the literature values. The higher value for the extinction coefficient in Ag-DNA sample is due to a slight denaturation following the intercalating of the Ag^+ ions between the bases of DNA.

The stock solutions were stored at $-20°C$. Appropriate dilutions were made to the desired values of the DNA and electrolyte concentrations, i.e., DNA concentration Cp = 3.5×10^{-4} M and electrolyte concentration ranging from 10^{-5} to 2×10^{-4} M. Before and after each dielectric measurement, optical absorbance of the samples was measured to determine the extent of possible denaturation. We found an increase of the extinction coefficient of ~1% and 5% for Mg- and Ag-DNA respectively, at the lowest salt concentration.

Dielectric Measurements

Measurement of the permittivity and the total dielectric loss of DNA in aqueous $MgCl_2$ and $AgNO_3$ solutions were carried out in the frequency range from 5 kHz to 30 MHz at a temperature of 25.0°C, using two different bridge techniques. In the low-frequency range (5 kHz to 1 MHz), an automatic capacitance bridge (Hewlett-Packard Co., Palo Alto, CA, model 4270A), modified to allow continuous frequency measurements, was used. Between 1 and 30 MHz the investigations were performed with a vector impedance meter (Hewlett-Packard Co., model 4815A). A source of experimental error in measuring the complex dielectric constant of highly conductive solutions was electrode polariza-
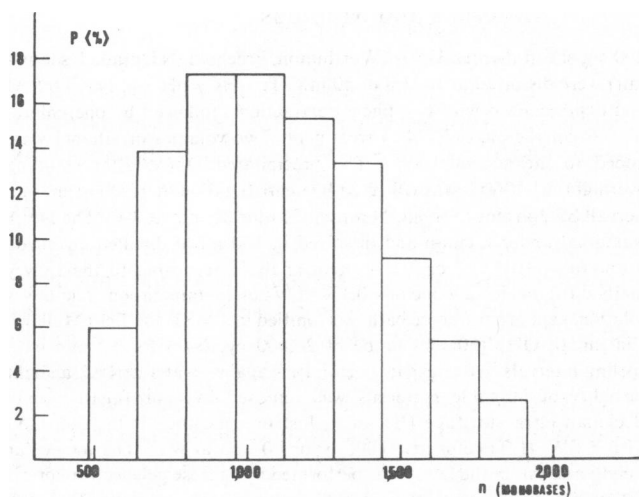

FIGURE 1 Histogram for the distribution of the number N of monobases in the sonicated DNA as determined from agarose-gel electrophoresis.

tion effects. The correction of the dielectric data for this effect was generally difficult and, in some cases, misleading, owing to the contribution of the bulk conductivity of the solute polyions. For this reason, substitution methods (9) were avoided, and we adopted the procedure recently described by Blom (10) for the determination of the complex dielectric constant of conducting solutions.

The cell used in connection with the bridges was a temperature-controlled parallel plane capacitor with variable electrode spacings. The platinum electrodes were carefully plated with platinum black, according to the procedure suggested by Maczuk and Schwan (11). The validity of the polarization correction method was ascertained by measuring the permittivity and total dielectric loss of $MgCl_2$ and $AgNO_3$ aqueous solutions with the same conductivity as the DNA solutions used in this investigation. As can be seen in Figs. 2 and 3, a constant value of the permittivity over the whole frequency range was obtained.

The dielectric loss ϵ_d'' can be determined subtracting the losses due to the ionic conductivity σ from the measured total loss ϵ'' according to the equation $\epsilon_d'' = \epsilon'' - \sigma/\epsilon_0\omega$ where ω is the angular frequency and ϵ_0 the absolute dielectric constant of free space.

As shown in this equation, a small error in conductivity measurements, also due to the drift of DC conductivity with time, may cause a substantial error in the dielectric loss in the low frequency interval and for high value of σ. Consequently, only the permittivity ϵ' could be determined reliably as a function of frequency.

For liquids with conductivity of $\sim 10^{-4}$ Ω^{-1} cm^{-1}, the errors in ϵ' were within 1–1.5% and in ϵ_d'' within 10%, owing to the subtraction of the DC conductivity term. These estimates were based mainly on the reproducibility of results in repeated trials over a long period of time.

Figs. 2 and 3 show the experimental results obtained for Ag-DNA at a salt concentration of 10^{-5} M and 1.5×10^{-4} M and for Mg-DNA at a salt concentration of 10^{-5} M and 2×10^{-4} M, respectively.

RESULTS

In agreement with what is generally found for polyelectrolytes, we attempted to fit the data to a linear combination of two relaxation processes on the basis of contributions from two overlapping Cole-Cole (12) relaxation mechanisms.

The equation for the permittivity ϵ' is as follows:

$$\epsilon' = \epsilon_x + 1/2 (\epsilon_s - \epsilon_{sx})$$

$$\left\{1 - \frac{\sinh[(1 - \beta_1) \ln \omega\tau]}{\cosh[(1 - \beta_1) \ln \omega\tau_1] + \sin\pi \beta_1/2}\right\} + 1/2 (\epsilon_{sx} - \epsilon_x)$$

$$\cdot \left\{1 - \frac{\sinh[(1 - \beta_2) \ln \omega\tau_2]}{\cosh[(1 - \beta_2) \ln \omega\tau_2] + \sin\pi \beta_2/2}\right\}, \quad (1)$$

where $\Delta\epsilon_1 = \epsilon_s - \epsilon_{sx}$ and $\Delta\epsilon_2 = \epsilon_{sx} - \epsilon_x$ are the dielectric increments of the two dispersions and ϵ_s and ϵ_x are the extrapolated permittivities at low and high frequency, respectively. ϵ_{sx} is the infinite frequency dielectric constant for the low-frequency dispersion and the low-frequency dielectric constant for the high frequency dispersion. τ_1 and τ_2 are the relaxation times and ω the angular frequency, and β_1 and β_2 are the characteristic parameters for the distributions of the relaxation times.

As can be seen in Figs. 2 and 3, the dispersion curves in the high-frequency range tended towards a constant value that was equal, within the limits of the measuring accuracy, to that of water. On the other hand, owing to the moderate values of the salt concentration, the permittivity
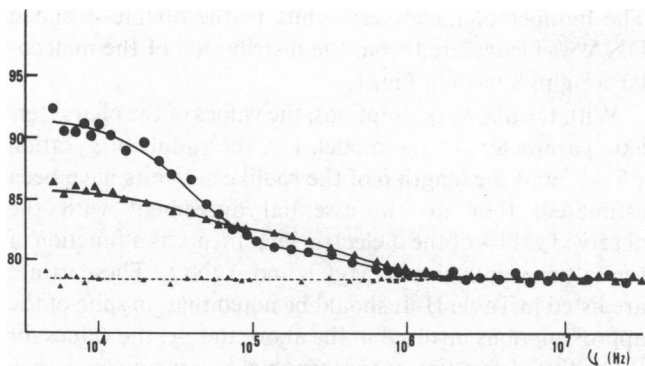
FIGURE 2 Permittivity ϵ' of DNA in $MgCl_2$ aqueous solution as a function of frequency at a temperature of 25°C and at two salt concentrations: (▲) 10^{-5} M; (●) 2×10^{-4} M. △, permittivity of 2×10^{-4} M $MgCl_2$ electrolyte solution. The DNA concentration 3.5×10^{-4} M. For clarity of presentation, the measurements at intermediate salt concentrations have been omitted.

of the aqueous $MgCl_2$ and $AgNO_3$ was almost equal to the dielectric constant of pure water. Therefore, the fitting procedure was performed assuming $\epsilon_\infty = 78.5$, the permittivity of pure water at 25°C. The parameters τ_1, τ_2, $\Delta\epsilon_1$, $\Delta\epsilon_2$, β_1, and β_2 were obtained by fitting the data to Eq. 1.

In this procedure, we observed that the parameter β_2 assumed values of ~0.01–0.005 for each set of data. For this reason, we chose to decrease the number of free parameters in the fitting procedure, and consequently we assumed $\beta_2 = 0$ for all our results. The results of this analysis are shown in Table I. The solid curves in Figs. 2 and 3 were calculated from the parameters of the least-square fits.

As can be seen from an inspection of Table I, the total dielectric increment decreased as a function of ionic concentration for Ag-DNA in $AgNO_3$ aqueous solution, in accordance with the dielectric behavior of polyelectrolytes. On the other hand, the dielectric increment for Mg-DNA in $MgCl_2$ aqueous solution showed a small increase with
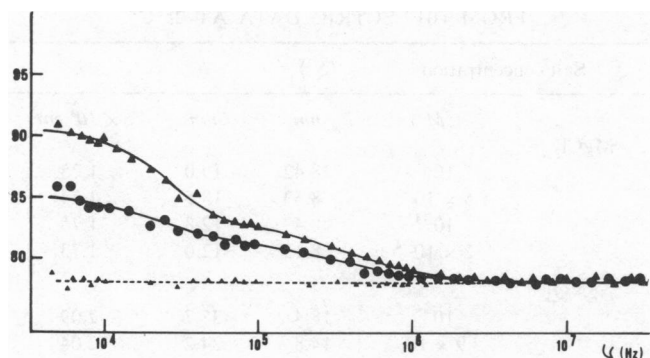

FIGURE 3 Permittivity ϵ' of DNA in $AgNO_3$ aqueous solution as a function of frequency at a temperature of 25°C and at two $AgNO_3$ concentrations (Cs): (▲) 10^{-5} M; (●) 1.5×10^{-4} M. △ permittivity of 1.5×10^{-4} M $AgNO_3$ solution. The DNA concentration was 3.5×10^{-4} M. For clarity of presentation, the measurements at intermediate salt concentrations have been omitted.

TABLE I
DISPERSION PARAMETERS FOR
DNA SOLUTIONS*

Salt concentration		ϵ_s	$\Delta\epsilon_1$	$\Delta\epsilon_2$	β_1	τ_1	τ_2
	M					$\times 10^6$ s	$\times 10^7$ s
$MgCl_2$	10^{-5}	89.6	6.4	4.7	0.13	21.5	3.9
	5×10^{-5}	90.0	7.0	4.5	0.13	12.7	3.5
	10^{-4}	90.2	9.0	2.7	0.13	6.2	3.4
	2×10^{-4}	90.7	9.8	2.4	0.10	3.9	3.2
$AgNO_3$							
	10^{-5}	91.0	9.0	3.4	0.17	7.9	2.9
	3.9×10^{-5}	87.5	7.0	2.0	0.30	5.3	2.6
	10^{-4}	86.1	5.5	2.1	0.15	3.8	2.4
	$1.5 \times 10^{+4}$	84.2	4.5	1.2	0.20	3.5	2.4

*Solutions contained 3.5×10^{-4} M DNA.

increasing the ionic concentration. As pointed out by Minakata (13), at relatively low salt concentrations, the increase in dielectric increment caused by divalent ions and the tendency of the dielectric increment to decrease because of increased ionic strength may cancel each other.

In addition, we have tried to study the effect of DNA on the dielectric properties of divalent cations like M_n^{++}, which is known to interact with both phosphate groups and bases (14). Results of preliminary measurements carried out on Mn-DNA in $MnCl_2$ are in qualitative agreement with the results reported in this paper, but the dielectric increments assumed values that were too low to be accurately measured with the experimental setup available to us.

DISCUSSION

A heterogeneous dielectric system consisting of macromolecular particles embedded in a medium of different dielectric properties generally exhibits dielectric dispersions often interpreted either in terms of a Maxwell-Wagner relaxation combined with a high surface conductivity (15) or in terms of counterion fluctuations (16).

The Maxwell-Wagner effect has been extensively studied and experimentally observed for the most part in colloidal suspensions where the dispersed phase shows regular and known shapes. Moreover, it is difficult to take this effect into account in polyelectrolyte solutions, mainly for two reasons. First, assuming a two-phase approximation (17), the whole solution must be considered to consist of two regions, and it is difficult to assign appropriate values for the dielectric constant and conductivity of a single-polymer domain. Second, it is generally not sufficient to analyze the dielectric behavior using only the bulk properties of the constituents, since experiments make clear the existence of a polarization mechanism that is due to interfaces, and it is difficult to define a boundary in the neighborhood of the polyion to distinguish internal and external conductive regions. For these considerations we have limited our discussion to the counterion fluctuation

theories, particularly those models that have been proposed to account for the two observed dispersions of DNA solutions.

Comparison with Continuum Counterion Distribution Model

In this model (18), the polyion is represented by a sequence of rigid, charged subunits, and the two dielectric relaxations observed are justified by taking into account a local counterion fluctuation within the subunits of the polyion (responsible for the "high-frequency" dispersion) and the counterion fluctuation over the whole polyion (responsible for the "low-frequency" dispersion). Recently, this model has been applied (19) to low-molecular weight DNA in water and in 3×10^{-4} M NaCl aqueous solution, and a reasonable agreement was obtained. In this theory, neglecting counterion-counterion interactions, for a dilute solution of monodisperse polyions, the following expressions for the two dielectric increments are assumed:

$$\Delta\epsilon_1 = \frac{BfN(Ze)^2Cp}{3\epsilon_0KT}(\langle S^2 \rangle - b^2/12) \qquad (2)$$

$$\Delta\epsilon_2 = \frac{BfN(Ze)^2Cpb^2}{36\epsilon_0KT} \qquad (3)$$

where B is the internal field correction factor, f is the average fraction of associated counterions, N the total number of charged sites per polyion, ze the charge of the counterions, Cp the concentration of polyions per unit volume, ϵ_0 the absolute permittivity of vacuum, K the Boltzmann constant, and T the absolute temperature; b is the length of the rigid rodlike subunit, and $\langle S^2 \rangle^{1/2}$ is the radius of gyration.

Although Mandel and co-workers (19) have shown the existence of intermolecular interactions, even in the concentration range up to 3×10^{-3} M, it must be noted that at these concentrations the effect of interations are almost negligible, as experimentally confirmed by Sakamoto et al. (20).

For a comparison of our results with this model, the quantities appearing in Eqs. 2 and 3 were estimated as follows: the f parameter (which is also obviously related to the effective degree of dissociation, α, of DNA, $f = 1 - \alpha$ has been estimated from conductivity measurements as described in detail by Sakamoto et al. (20). The values for DNA in aqueous $MgCl_2$ and $AgNO_3$ were calculated assuming the ions' bulk values for their mobilities and an estimated value of 3.4×10^{-4} cm^2/V·s for the electrophoretic mobility of DNA according to the determinations from electrophoresis by Olivera et al. (21) and, more recently, by Schellman et al. (22). The calculations were based on the assumption that at these low ionic strengths, the electrophoretic charge of DNA is almost independent of the salt concentration. The field correction factor B was assumed to be close to unity, as usual for a polar solvent.

The number of monomeric units in the double-stranded DNA was calculated from the distribution of the molecular weights shown in Fig. 1.

With the above assumptions, the values of the characteristic parameters of the model, i.e., the radius of gyration $\langle S^2 \rangle^{1/2}$ and the length b of the rodlike subunits have been estimated; they are in essential agreement with the observed values of the dielectric increments as a function of the salt concentration of $MgCl_2$ and $AgNO_3$. These values are listed in Table II. It should be noted that, in spite of the approximations involved in the above theory, the values for the radius of gyration are reasonable; but, in any case, they should only be considered as showing trends of change with the concentration of the electrolyte. The length b of the subunits should be regarded as rough estimates, as well. Nevertheless, the b parameter was found to decrease with increasing the electrolyte concentration as the repulsive electrostatic forces became smaller, also in agreement with the indication of Vrengdenhil et al. (19); in this context the length of the polyion should be regarded as the end-to-end length, and consequently is should be affected by the solvent ionic strength.

The values of $\langle S^2 \rangle^{1/2}$ for Mg-DNA in $MgCl_2$ electrolyte solutions seem to be constant over the whole electrolyte concentration range; presumably this is due to the different interactions that Mg^{++} exert on the DNA structure in comparison with univalent cations as Ag^+. The values of the relaxation times, particularly those determined by the diffusion of counterions along the rodlike subunit, calculated with the parameters estimated from the two dielectric increments, seem to be within the one order of magnitude smaller than those determined from the experimental data. In spite of this discrepancy, in this model, the charge fluctuation within the subunit of the polyion has to be considered an acceptable attempt to justify the high frequency dielectric dispersion, as also pointed out by Odijk

TABLE II
MOLECULAR PARAMETERS OF DNA DETERMINED
FROM DIELECTRIC DATA AT 25°C*

Salt concentration	$\langle S^2 \rangle^{1/2}$	b	h
	M	nm	nm	$\times 10^8$ cm
MgCl$_2$				
	10^{-5}	8.42	19.0	1.75
	5×10^{-5}	8.53	18.5	1.74
	10^{-4}	8.49	12.8	1.75
	2×10^{-4}	8.13	12.0	1.73
AgNO$_3$				
	10^{-5}	18.4	33.7	2.09
	3.9×10^{-5}	14.8	24.2	2.04
	10^{-4}	13.7	24.8	1.89
	1.5×10^{-4}	11.7	18.7	1.77

$\langle S^2 \rangle^{1/2}$ and b are the radius of gyration and length of subunits in the continuum model, respectively. h is the distance of the charged groups in the discrete model.
*The solutions contained 3.4×10^{-4} M DNA.

(23), also in terms of a longitudinal polarization. On the other hand, some approximations are involved, as pointed out by Mandel, particularly in the absence of a factor that accounts for the effect of saturation of binding ion, and the counterion-counterion interaction energy is omitted.

Comparison with Discrete Charged Site Models

The discrete charged site model of the counterion fluctuation can explain several dielectric properties of polyelectrolyte solutions, for instance, the very large dielectric increment for polyion of high molecular weight and the broadness of the relaxation time distribution.

Minakata has recently derived a modification of the theory of the dielectric dispersion of a rodlike polyelectrolyte based on the thermal fluctuation of the counterion concentration previously proposed by Oosawa (24). The model proposed by Minakata (25) is based on an expansion in a Fourier series of the thermal fluctuation of the counterion concentration considered for one-dimensional linear lattice having discrete charged sites corresponding to the ionic groups of the polyion. This model takes into account the interaction of the counterions with the external medium, so that the second dielectric dispersion at higher frequencies could be justified.

In this model, the total dielectric increment as a function of frequency is given by

$$\Delta\epsilon(\omega) = \frac{4\pi BN\ (Ze)^2\ l^2 n_o\ (1\ -\ n_o)}{3nKT} \sum_q$$

$$\cdot \left\{ \left[1\ +\ nn_o(1\ -\ n_o) \frac{\Phi(q)}{KT} \right] (1\ -\ \cos q)(1\ +\ \omega^2\tau_q^2) \right\}^{-1} \quad (4)$$

where

$$\Phi(q) = -\frac{(ze)^2}{\epsilon_o l} \ln\ [1\ -\ 2 \cos q \exp\ (-K_Dh)\ +\ \exp\ (-2K_Dh)]$$

is the counterion interaction energy in the Debye-Hückel approximations and $\tau_q^{-1} = [2W(1\ -\ \cos q)\ +\ C/n_0]\ [1\ +\ nn_0(1\ -\ n_0)\ \Phi(q)/KT]$ is the relaxation time of mode q.

Here n is the number of charged sites, n_0 the average number of counterions at a site, $l = nh$ is the length of the linear polyion in solution; h is the average charge spacing along the axis of the real polyion chain, i.e., contour length divided by number of charged groups; ze is the charge of the counterions,

$$K_D = \left(\frac{4\pi e^2}{\epsilon KT} \sum_i v_i z_i^3 \right)^{1/2}$$

is the inverse of the Debye thickness layer, where v_i is the number of ions of valence z_i per unit volume and q stands for the wave mode number given by $q = 2\pi m/n$ ($m = 1, 2, \ldots, n/2$). ϵ_0 is the dielectric constant of free space, K the Boltzmann constant, and T the absolute temperature. N is the concentration of polyions per unit volume. W is the

average value of the rate constant of ion transfer of the adjacent lattice points, and C is the rate constant which takes into account the perpendicular diffusion of ions.

In this model, the relaxation times of the two observed dispersions are governed by the values assumed by the parameters W and C, which seem very difficult to estimate independently. This circumstance leaves them as the pivotal unknowns of the system. A difficulty in relating observed relaxation times with the ones calculated on the basis of this model is that values of W and C are not directly obtainable from experimental data. Although the range of possible values for these parameters is very wide, the results are very sensitive to small variations in these parameters.

In this model, the rate constant W is assumed to be directly proportional to the total conductivity increment; unfortunately, this quantity cannot be directly measured owing to the present limitation of our experimental setup. For these reasons, we have compared our results only with the static dielectric increment, which is obviously independent of time. The static dielectric expression has been slightly modified to take into account the distribution in the number of the ionic groups in the polyion molecules as shown in Fig. 1, according to the relation

$$\Delta\epsilon(0) = \frac{4\pi BN\ (ze)^2 n_o\ (1\ -\ n_o)}{3KT} \sum_j h^2 p_j n_j \sum_q$$

$$\cdot \left\{ \left[1\ +\ nn_o\ (1\ -\ n_o) \frac{\Phi(q)}{KT} \right] (1\ -\ \cos q) \right\}^{-1} \quad (5)$$

where p_j is the percentage of the number of monobases n_j in the polyion molecules. Also in this case, for comparison with experimental results, the field correction factor B is assumed equal to unity.

The extension of this model, derived for a rodlike polyelectrolyte, to DNA may appear doubtful, owing to the flexible structure of this molecule. Nevertheless, as pointed out by Mandel (26), the model may be valid when the negatively charged phosphate groups in nucleotide chains are considered distributed, on the average, regularly along the major axis of the wormlike cylinder representing the DNA molecule.

The characteristic parameter of this model, the average distance between two charged groups, h, has been estimated to obtain agreement with the total observed dielectric increment, as a function of the salt concentration, for the two electrolytes used. The average number of counterions at a site, n_0, has been estimated, as previously pointed out, from conductivity measurements, also in accordance with the Manning theory (27-29) for the conductance of solutions of polyelectrolyte salts. These assumptions are supported, as will be shown, by the agreement between the predicted results and the experimental data.

More recently, Vaughan and co-workers (14, 30) have derived a model for the dielectric behavior of linear polyelectrolytes based on the calculation of the dipole

correlation function of counterions diffusing along the macroion surface. This model is characterized by a more detailed mechanism for the dissociation and reassociation of the counterions influenced by screened coulombic repulsions, in comparison with the Minakata model. However, it must be noted that, as pointed out by Vaughan, the static dielectric increments are roughly equal to those valued with the Minakata model for the same sample, and consequently only this latter model will be considered.

Numerical evaluation of Eq. 5 gives the observed dielectric increments with parameters estimated as follows. For the DNA in $MgCl_2$ solution, the values of h, as listed in the Table II, exhibit an almost constant value over the ionic strength range 10^{-5} to $2 \cdot 10^{-4}$ M. As can be seen in Fig. 4, the dependence of the static dielectric constant as a function of the electrolyte concentration is in good agreement with the Minakata model, assuming a value of $\sim 1.75 \times 10^{-8}$ cm for the h parameter, when the spacing between charged groups is taken along the axis of the polyion chain. With an helical spring of 3.4 Å, the projection of the charged groups of the double-stranded DNA on the major axis of the molecule yields an apparent distance between the lattice points in good agreement with the above value.

This evidence seems to suggest that the length of the polyion in the Minakata model has to be considered as the extended length of the polyion in its wormlike state, and therefore independent of the ionic strengh. This result provides a valuable check in testing counterion fluctuation and furthermore supports the view that phosphate groups alone are the most likely sites of interaction with divalent cations as Mg^{++}.

For the Ag-DNA in $AgNO_3$ a quite different situation emerges from an inspection of the results listed in the Table II. In this case, as pointed out by Duane et al. (31), Ag^+ ions formed complexes by interactions with the purine bases of the DNA chain, rather than by interactions with the phosphates. If the dielectric increment as a function of the salt concentration must be accounted for only on the
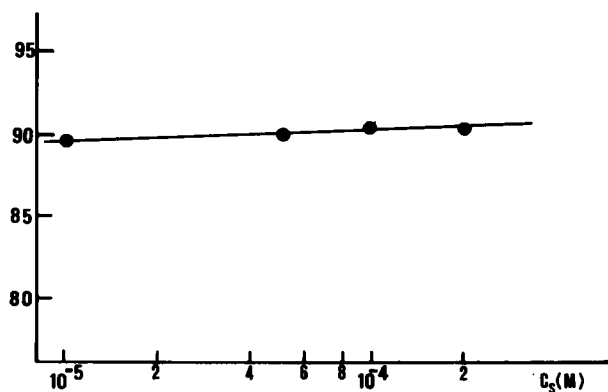
basis of the Minakata model, then a decrease in the h values as the ionic strength is increased must be considered, as shown in Table II. This implies that the charged groups projected along the major axis of the polyion in the wormlike state approach each other.

This fact suggests that the polyion in solution of monovalent cations takes a conformation of a flexible chain rather than a rigid rod, resulting in qualitative agreement with the binding behavior of $Ag+$ ion, which preferentially complexes between succeeding bases, as pointed out by Daune (32).

Fig. 5 shows the dielectric increment $\Delta\epsilon_1$ of the low-frequency and $\Delta\epsilon_2$ of the high-frequency dielectric dispersions, respectively, for the DNA in $MgCL_2$ and in $AgNO_3$ electrolyte solutions, resulting from the fit procedure used. As can be seen, the high-frequency dielectric increments for the two salts are almost equal, while notable differences occur in the low-frequency increments, as a function of the salt concentration. This fact further supports the concept that the second dielectric dispersion is due to the interaction of the bound counterions with the outer medium, rather than to a counterion fluctuation in the subunit, as in the contiuum counterion distribution model. In fact, although interactions of Ag^+ and Mg^{++} ions with the DNA molecule are very different, when interactions with free counterions of the bulk medium are considered, these ions give rise to quite similar dielectric increments.

In conclusion, the dielectric measurements on highly charged polyelectrolyte solutions, like DNA, carried out at various counterion concentrations seem to confirm that the low-frequency dielectric dispersion arises from the counterion fluctuation along the polyion. Moreover, further studies are required to elucidate the type of interactions
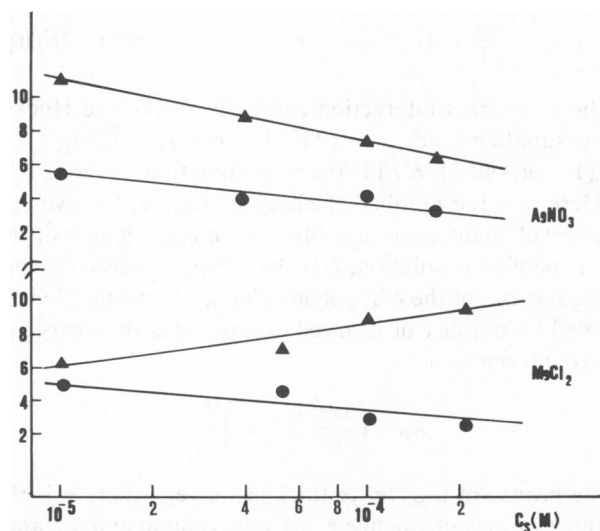
FIGURE 5 The dielectric increment ($\Delta\epsilon$) as a function of the salt concentration (C_s) for the two observed dispersions for DNA in $MgCl_2$ and in $AgNO_3$. ▲, increment of the low-frequency dispersion; ●, increment of the high-frequency dispersion.

FIGURE 4 Static dielectric constant (ϵ_s) of DNA in $MgCl_2$ as a function of the salt concentration. Solid line, values calculated from the Minakata model assuming $b = 1.75 \times 10^{-8}$ cm.

that occur between the counterion and polyion and the bounded counterions and the outer medium.

REFERENCES

1. Charney, E., and C. H. Lee. 1980. Polyelectrolyte orientation in electric fields: the effect of the dielectric constant. *Macromolecules.* 13:66–68.
2. Tung, M. S., R. J. Molinari, R. H. Cole, and J. H. Gibbs. 1977. Influence of temperature and ionic strength on the low frequency dielectric dispersion of DNA solutions. *Biopolymers.* 16:2653–2669.
3. Ross, L. D., and R. L. Scruggs. 1964. Electrophoresis of DNA. II. Specific interactions of univalent and divalent cations with DNA. *Biopolymers.* 2:79–89.
4. Takashima, S. 1967. Effect of ions on the dielectric relaxation of DNA. *Biopolymers.* 5:899–913.
5. Takashima, S. 1963. Dielectric dispersion of DNA. *J. Mol. Biol.* 7:455–467.
6. Lowry, O. M., N. J. Rosebrougn, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folinphenol reagent. *J. Biol. Chem.* 193:265–279.
7. Sugden, J., B. de Troy, R. J. Roberts, and J. Sambrook. 1975. Agarose slabgel electrophoresis equipment. *Anal. Biochem.* 68:36–46.
8. Knight, C. A., and B. R. Woody. 1958. Phosphorous content of tobacco mosaic virus. *Arch. Biochem. Biophys.* 78:460–468.
9. Ferry, J. D., and J. L. Oncley. 1941. Studies of the dielectrical properties of protein solutions. III. Lactoglobulin. *J. Am. Chem. Soc.* 63:272–278.
10. Blom, J. 1979. Measurements of dielectric relaxation of conducting solutions at low frequencies. *J. Phys. E. Sci. Instrum.* 12:889–893.
11. Schwan, H. P. 1963. *Phys. Tech. Biol. Res.* Vol. 6, Chpt. 6.
12. Cole, K. S., and R. H. Cole. 1941. Dispersion and absorption in dielectric. *J. Chem. Phys.* 9:341–351.
13. Minakata, M. 1972. Dielectric properties of polyelectrolytes. III. Effect of divalent cations of dielectric increment of polyacid. *Biopolymers.* 11:1567–1582.
14. Meyer, P. I., and W. E. Vaughan. 1980. Dielectric behaviour of linear polyelectrolytes. *Biophys. Chem.* 12:329–339.
15. Sachs, S. B., A. Raziel, H. Eisenberg, and A. Katchalsky. 1969. Dielectric dispersion properties in aqueous polyelectrolyte solutions. *Trans. Faraday Soc.* 65:77–90.
16. Oosawa, F. 1971. Polyelectrolytes. Marcel Dekker, Inc., New York.
17. Oosawa, F. 1957. A simple theory of thermodynamic properties of polyelectrolyte solutions. *J. Polym. Sci.* 23:421–430.
18. van der Touw, F., and M. Mandel. 1974. Dielectric increment and dielectric dispersion of solutions containing simple charged linear macromolecules. *Biophys. Chem.* 2:218–230.
19. Vreugdenhil, Th., F. van del Touw, and M. Mandel. 1979. Electric permittivity and dielectric dispersion of low-molecular weight DNA at low ionic strength. *Biophys. Chem.* 10:67–80.
20. Sakamoto, M., R. Hayakawa, and Y. Wada. 1979. Dielectric relaxation of DNA solution. III. Effect of DNA concentration, protein contamination and mixed solvents. *Biopolymers.* 18:2769–2782.
21. Olivera, B. M., P. Baime, and N. Davidson. 1964. Electrophoresis of the nucleic acids. *Biopolymers.* 2:245–257.
22. Schellman, J. A., and D. Stigter. 1977. Electrical double layer, zeta potential and electrophoretic charge of double-stranded DNA. *Biopolymers.* 16:1415–1434.
23. Odijk, T. 1979. Possible scaling relations for semidilute polyelectrolyte solution. *Macromolecules.* 12:688–693.
24. Oosawa, F. 1970. Counterion fluctuation and dielectric dispersion in linear polyelectrolyte. *Biopolymers.* 9:677–688.
25. Minakata, A. 1977. Dielectric dispersion of polyelectrolytes due to ion fluctuation. *Ann. NY Acad. Sci.* 303:107–120.
26. Mandel, M. 1961. The electric polarization of rod-like charged macromolecules. *Mol. Phys.* 4:489–496.
27. Manning, G. S. 1970. On the interpretation of conductance measurements in salt free polyelectrolyte solutions with an application to the helix-coil transitions of poly (D-glutamic acid). *Biopolymers.* 9:1543–1546.
28. Manning, G. S. 1975. A limiting law for the conductance of the rod model of a salt free polyelectrolyte solution. *J. Phys. Chem.* 79:262–265.
29. Manning, G. S. 1972. Polyelectrolytes. *Annu. Rev. Phys. Chem.* 23:117–140.
30. Meyer, P. I., G. E. Weseberg, and W. E. Vaughan. 1981. Dielectric behaviour of polyelectrolytes. II. The cylinder. *Biophys. Chem.* 13:265–273.
31. Daune, M., C. A. Dekker, and H. K. Schachman. 1966. Complexes of silver ion with natural and synthetic polynucleotides. *Biopolymers.* 4:51–68.
32. Daune, M. 1969. Interaction of metal ions with DNA. I. Behaviour of a polyelectrolyte and binding of ions. *Biopolymers.* 7:659–670.