Interactions of Polyelectrolytes with Simple Electrolytes. III. The Binding of Magnesium Ion by Deoxyribonucleic Acid[1]

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Abstract: The interaction between magnesium ion and DNA in aqueous solution was studied by dialysis equilibrium, viscosity, and dilatometry measurements. In systems containing both MgDNA and $MgCl_2$, but no other simple electrolyte, the Donnan equilibrium results which extended over $MgCl_2$ concentrations ranging from 0.003 to above 7 N could be ascribed almost entirely to the volume excluded by the DNA to the $MgCl_2$. In systems containing, in addition, sufficient tetramethylammonium chloride to maintain either the chloride ion normality or the ionic strength of the "external" solution at 0.2, the dialysis equilibrium studies yielded binding isotherms which indicated that the association of magnesium ion is three orders of magnitude weaker with DNA than with polyphosphates. Yet, over a more than 1000-fold variation of the magnesium ion activity, the results followed the mass action law if account was taken of the effects of the electrostatic potential at the surface of the DNA molecule. The association constant was of the same order as those previously obtained for the alkali metal ions. The intrinsic viscosity of the sonified DNA decreased only slightly with magnesium ion binding, indicating a rather rigid structure of the DNA. For the reaction, $Mg^{2+} + -PO_4^- \longrightarrow -PO_4Mg^+$, the observed volume increase ranged from 6.4 to 8.7 ml/mole. These low values indicate that the desolvation accompanying the binding reaction is significantly less than for polyphosphate and poly(vinyl phosphonate). This result, as well as the relative weakness of the binding, is ascribed to the special structure of DNA which, in contrast to the other polyelectrolytes, prevents close contact by magnesium ion with more than one phospate group.

It has become apparent in recent years that the interactions between polyelectrolytes and their counterions may be much more complex than had been originally supposed. In many instances the long-range coulomb interactions which are common to all such systems have masked the more specific short-range interactions which are characterized by changes in the solvation shells of the participating species. Utilizing dilatometry, equilibrium dialysis, viscosity, and electrophoresis, there has been uncovered a remarkable diversity in these short-range interactions between different macroion–counterion combinations.[3–7] In order to obtain a better understanding of such interactions, it was decided to supplement the original exploratory investigations with more comprehensive and quantitative studies of selected macroion–counterion systems. Among these the deoxyribonucleic acid (DNA)–magnesium ion system appeared to us especially attractive for a number of reasons. There is ample evidence for the existence of strong interactions between DNA and Mg^{2+}.[8–12] Furthermore, it appears that the interaction occurs between the phosphate groups of DNA and Mg^{2+} and that the base groups of DNA are not directly involved,[13,14] a situation which makes possible comparison with structurally simpler polyelectrolytes, *e.g.*, the long-chain polyphosphates whose interactions with Mg^{2+} have been investigated previously.[15] Yet there are also significant differences from other polyelectrolyte systems. For example, the native DNA structure is more rigid, and its phosphate groups are more widely spaced. Furthermore, unlike magnesium salts of other polyacids, undenatured MgDNA is soluble in water both in the absence and in the presence of simple magnesium salts; this property allows us to carry out dialysis equilibrium studies with such systems in the absence of other cations and thus to extend to a divalent cation similar studies reported previously for univalent cations only.[16]

In this paper we shall present results of such dialysis equilibrium studies of the MgDNA–$MgCl_2$ system over a wide range of $MgCl_2$ concentrations (from 0.003 to about 7 N $MgCl_2$) and shall evaluate these results with the help of a slightly modified version of a previously developed theoretical treatment.[17] We shall also present results of membrane equilibrium, viscosity, and dilatometry studies of the aqueous DNA–$MgCl_2$–TMACl[18] system, designed to give information about the changes in the solvation and in the molecular dimensions accompanying the binding of magnesium ion by DNA.

Experimental Section

Materials. Two DNA salmon sperm (SDNA) samples obtained in the sodium salt form from the Worthington Biochemical Corp. were used. For membrane equilibrium determinations we used the same sample (Lot No. 6118) as in our previous work.[16] Its N/P ratio was 1.42. As the sample had been stored in a refrigerator for several months, we redetermined the extent of denaturation and also $\epsilon(P)_{259}$, the absorptivity per gram-atom of phosphorus per liter.

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(3) U. P. Strauss and Y. P. Leung, *J. Am. Chem. Soc.*, **87**, 1476 (1965).
(4) U. P. Strauss and P. Ander, *ibid.*, **80**, 6494 (1958).
(5) U. P. Strauss, D. Woodside, and P. Wineman, *J. Phys. Chem.*, **61**, 1353 (1957).
(6) U. P. Strauss and P. D. Ross, *J. Am. Chem. Soc.*, **81**, 5295 (1959).
(7) U. P. Strauss and P. D. Ross, *ibid.*, **81**, 5299 (1959).
(8) J. Shack, R. J. Jenkins, and J. M. Thompsett, *J. Biol. Chem.*, **203**, 373 (1953).
(9) G. Zubay and P. Doty, *Biochim. Biophys. Acta*, **29**, 47 (1958).
(10) K. C. Banerjee and D. J. Perkins, *ibid.*, **61**, 1 (1962).
(11) J. W. Lyons and L. Kotin, *J. Am. Chem. Soc.*, **86**, 3634 (1964).
(12) J. W. Lyons and L. Kotin, *ibid.*, **87**, 1670 (1965).
(13) G. G. Hammes, G. E. Maciel, and J. S. Waugh, *ibid.*, **83**, 2394 (1961).
(14) J. W. Lyons and L. Kotin, *ibid.*, **87**, 1781 (1965).
(15) U. P. Strauss and A. Siegel, *J. Phys. Chem.*, **67**, 2683 (1963).
(16) U. P. Strauss, C. Helfgott, and H. Pink, *ibid.*, **71**, 2550 (1967).
(17) L. M. Gross and U. P. Strauss in "Chemical Physics of Ionic Solutions," B. E. Conway and R. G. Barradas, Ed., John Wiley and Sons, Inc., New York, N. Y., 1966, p 361.
(18) TMACl = tetramethylammonium chloride.

A denaturation of about 5% was detected by the method of Hotchkiss,[19] and $\epsilon(P)_{259}$ was found to be 6550 ± 30. For viscosity and dilatometric measurements the other SDNA sample (Lot No. 6HA), which had an N/P ratio of 1.58, was used. The latter sample was found to have the same $\epsilon(P)_{259}$ and to give the same membrane equilibrium results as the former.

The molecular weight of both samples was reduced by means of sonification under conditions described previously.[16] From the sonified solutions NaDNA was isolated by alcoholic precipitation followed by exhaustive washing with a 3:1 alcohol–water mixture and finally with 95% alcohol. The resulting NaDNA was redissolved in 0.2 N TMACl and dialyzed at about 5° against several changes of 0.2 N $MgCl_2$ or 0.2 N TMACl in order to remove sodium ion. Aliquots of these stock solutions were then dialyzed first at 5° and in the last two changes at 25.0 ± 0.1° against appropriate solutions containing either $MgCl_2$ alone or both $MgCl_2$ and TMACl.

All the inorganic chemicals used were reagent grade.

Membrane Equilibrium. Membrane equilibrium was determined at 25° by the method described previously.[4,16] The DNA concentration ranged from 0.008 to 0.04 N in phosphorus. The chloride concentration was determined by potentiometric titration in 60% acetic acid with silver nitrate using a combination of silver–silver chloride and glass electrodes. Titrations were carried out to near the end point by adding about 95% of the titrant (in most cases 0.2 N $AgNO_3$) by weight and delivering the remainder with a microburet containing approximately 0.01 N silver nitrate. A precision of better than one part per thousand was obtained. The analysis for magnesium ion concentration was carried out with a Techtron Model AA-4 atomic absorption spectrophotometer. All samples to be analyzed were diluted with 0.2 N TMACl to a magnesium concentration of about 1×10^{-4} to 2×10^{-4} N. Dilution with water, rather than with 0.2 N TMACl tended to yield erroneously low values of the magnesium ion concentration in the presence of DNA. The instrument was calibrated with standard solutions of magnesium chloride in 0.2 N TMACl. It was established that DNA did not interfere with the analysis. The DNA concentration was determined from the measurements of the optical density at 259 mμ as described previously.[16] It was ascertained that the extinction coefficient did not change during equilibration in the dialysis experiments and that the analysis was not influenced by the presence of magnesium. In all cases, the pH was maintained between 6 and 7.

Viscosity. Viscosities were measured at 25.0° in a Zimm–Crothers Couette-type viscometer[20] at an average shear stress of approximately 0.013 dyne/cm² (0.56 rps in 0.2 N TMACl). Since we worked with a sonified low molecular weight material, we did not consider it necessary to repeat the measurements at lower shear rates, and the results may be considered as having been obtained in the limit of zero rate of shear.

In preparing the solutions which consisted of DNA, $MgCl_2$, and TMACl in water, precautions were taken to ensure that all samples were dialyzed in exactly the same manner. As in the case of membrane equilibrium measurements, the total chloride normality or the ionic strength in the external solutions was maintained at 0.2. To obtain an intrinsic viscosity, dilutions were made of the most concentrated DNA solution with the external solution so as to keep the chemical potentials of the diffusible components constant.[21] The DNA concentration ranged from 0.0004 to 0.006 equiv of P/l.

Particular care, necessary especially in the presence of magnesium ion, was taken to avoid degradation of the DNA by microorganisms. Not only the DNA solutions, but also all solutions used for dilution were prepared in sterile water and passed through 0.22-μ pore size Millipore filters prior to dialysis. Likewise all bottles, pipets, and the viscometer were sterilized.

According to Scruggs and Ross,[22] the intrinsic viscosities of DNA in 0.2 N NaCl and 0.2 N TMACl solutions are equal. Using the experimental value of $[\eta]$ (in l./equiv of P) in 0.2 N TMACl and a modified equation of Doty, McGill, and Rice[23]

$$[\eta] = (1.45 \times 10^{-7})M_0^{2.12}P_w^{1.12} \quad (1)$$

(19) R. D. Hotchkiss in "Methods of Enzymology," Vol. 3, S. P. Colowich and N. O. Kaplan, Ed., Academic Press Inc., New York, N. Y., 1957, p 710.

(20) B. H. Zimm and D. M. Crothers, *Proc. Natl. Acad. Sci. U. S.*, **48**, 905 (1962).

(21) U. P. Strauss, *J. Polymer Sci.*, **33**, 291 (1958).

(22) R. L. Scruggs and P. D. Ross, *Biopolymers*, **2**, 593 (1964).

(23) P. Doty, B. B. McGill, and S. A. Rice, *Proc. Natl. Acad. Sci. U. S.*, **44**, 432 (1958).

which was obtained from studies on fragments of sonified NaDNA in 0.2 N NaCl, it was possible to estimate an average degree of polymerization P_w = 1300. The average monomer molecular weight of NaDNA, M_0, was assumed to be 330.

Dilatometry. Volume changes were measured at 30° using a Linderstrom–Lang dilatometer according to the method of Rasper and Kauzmann.[24] In all cases the dilatometer, containing the initial solutions covered with purified kerosene, was left in the thermostat overnight before the solutions were mixed. The bath temperature was controlled within 0.001°.

In all runs corrections for the volume change resulting from dilution of the DNA, $MgCl_2$, and TMACl on mixing were necessary; in some cases these amounted to almost one-third of the total observed volume change. The procedure for making such corrections has been discussed previously.[3] The validity of the procedure was tested by comparing two experiments in which widely differing volumes and concentrations of the initial $MgCl_2$ solutions were chosen in such a manner that the final solutions were identical. The relevant data are contained in the fourth and fifth rows of Table IV below, and it is seen that the resulting (corrected) volume changes are unaffected by the large differences between the initial $MgCl_2$ solutions.

Results and Discussion

Membrane Equilibrium Parameter. The results of membrane equilibrium distribution measurements are characterized by the parameter Γ, defined by the relation

$$\Gamma = \lim_{n_p = 0} (n'_s - n_s)/n_p \quad (2)$$

where n_p and n_s are normalities of polyelectrolyte and simple electrolyte, respectively; primed symbols refer to the polyelectrolyte free "external" solution and unprimed to the polyelectrolyte-containing "internal" solutions. For reasons discussed previously,[16] values of Γ are obtained from the slopes of plots of n_s against n_p.

The dependence of Γ on the magnesium chloride normality in the external solution for the MgDNA–$MgCl_2$ system at 25° is shown by the right-hand curve in Figure 1 (open hexagons). Perhaps the most striking aspect of these results is the sharp rise of Γ with increasing electrolyte concentration. The value of 1.90 which Γ attains when n'_{MgCl_2} = 7.36, is apparently much higher than any values of Γ reported previously, and also much higher than the "ideal" value, which is $^1/_3$, for macroions in 2:1 electrolyte solutions. However, the experimental results strikingly support recent theoretical treatments which take into account the volume excluded to the simple electrolyte by the macroion.[4,17] Because the small activity coefficients observed for Mg^{2+} in MgDNA systems[12] indicate very low values of the electrostatic potential near the macroion, the theoretical expression for Γ takes on an especially simple form; the Poisson–Boltzmann equation for the cylindrical rod model may be linearized, and eq 19 of ref 17 yields

$$\frac{\Gamma}{n_s} = \frac{N_A \pi a^2 b}{1000} + \frac{\alpha}{2I'} \quad (3)$$

where N_A is Avogadro's number, a the effective radius of the DNA molecule, b the average axial projection of the distance between the nearest phosphate groups, I' the ionic strength of the external solution, and α the charge fraction of the DNA. The values of Γ/n'_s obtained from our experimental data for the MgDNA–$MgCl_2$ system are given in Table I. Ignoring for the moment the result at n'_s = 7.36, we note that Γ/n'_s is constant within the limits of exper-

(24) J. Rasper and W. Kauzmann, *J. Am. Chem. Soc.*, **84**, 1771 (1962).

Table I. Membrane Equilibrium Results at 25° for the MgDNA–$MgCl_2$ System

n'_s	Γ	Γ/n'_s
0.0286	0.010 ± 0.004	0.35 ± 0.14
0.0319	0.017 ± 0.004	0.53 ± 0.12
0.0615	0.027 ± 0.005	0.44 ± 0.08
0.133[a]	0.055 ± 0.005	0.41 ± 0.04
0.198	0.080 ± 0.01	0.40 ± 0.05
1.04	0.47 ± 0.02	0.45 ± 0.02
7.36	1.90 ± 0.2	0.26 ± 0.03

[a] Obtained as a point of intersection of the curves in Figure 1.

imental error over a remarkably large range of $MgCl_2$ concentrations. This constancy would be predicted if Γ/n'_s were determined by the first term on the right-hand side of eq 3 alone. The magnitude of the first term may be calculated from currently accepted values of molecular dimensions. Taking b = 1.7 Å[25,26] and a = 11.5 Å (the sum of the radii of the DNA and chloride ions *i.e.*, 9.7[25,26] and 1.8 Å,[27,28] respectively), we obtain 0.42 for the desired term. The close fit with the experimental values of Γ/n'_s strongly supports our supposition, which implies that, over the range of $MgCl_2$ concentrations given in Table I, the ionic contribution to Γ (represented by the last term of eq 3) is negligible compared to the contribution of the short-range repulsions between the macroion and the small ions (represented by the first term on the right-hand side of eq 3). The fit further suggests that the DNA helix behaves like a cylinder in excluding the salt, *i.e.*, that the water of hydration in the grooves of the helix is impermeable to simple electrolytes. It would not be unreasonable to expect deviations from this simple behavior at very high salt concentrations where the water structure may be altered sufficiently to permit penetration of the salt into the DNA grooves. This may be the explanation for the low value of Γ/n'_s in the 7.36 N $MgCl_2$ solution.

One might think that the low value of Γ in this solution could also be caused by binding of chloride ion to the base groups of DNA. The following considerations argue strongly against such binding. If chloride ion were bound to DNA at all, the more polarizable bromide ion should be bound to a greater extent. This order of anion binding is generally observed for colloids and polyelectrolytes.[29,30] Consequently, one would expect that substitution of bromide for chloride ion would lower Γ. In fact, our value of Γ obtained for TMADNA in 0.2 N TMACl (see Table II below) is identical with that obtained previously in 0.2 N TMABr.[16] Furthermore, the value of Γ determined in 0.4 N $MgBr_2$ and represented in Figure 1 by a filled-in hexagon lies slightly above the $MgCl_2$ curve.[31]

(25) F. H. C. Crick and J. C. Watson, *Proc. Roy. Soc.* (London), A223, 80 (1954).
(26) R. Langridge, D. A. Marvin, W. E. Seeds, H. R. Wilson, C. W. Hooper, M. M. F. Wilkins, and L. D. Hamilton, *J. Mol. Biol.*, 2, 38 (1960).
(27) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," 2nd ed, Academic Press Inc., New York, N. Y., 1959, Chapter 9.
(28) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolyte Solutions," 3rd ed, Reinhold Publishing Corp., New York, N. Y., 1958, pp 164, 510.
(29) H. R. Kruyt, Ed., "Colloid Science," Vol. II, Elsevier Publishing Co., New York, N. Y., 1949, p 691.
(30) D. L. Woodside, Ph.D. Thesis, Rutgers, 1961.
(31) Data in the literature presented as evidence for the existence of chloride ion binding to the base groups of DNA [J. Shack, R. J. Jenkins, and J. M. Thompsett, *J. Biol. Chem.*, 198, 85 (1952)] may also be interpreted otherwise.

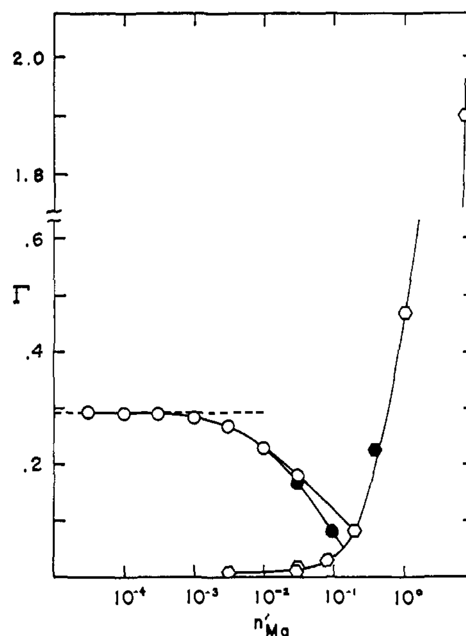

Figure 1. Membrane equilibrium parameter Γ as a function of the magnesium ion normality in the external solution at 25°: ○, MgDNA in $MgCl_2$; ●, MgDNA in $MgBr_2$; ○ and ●, DNA in $MgCl_2$–TMACl at n_{Cl}' = 0.2 and I' = 0.2, respectively. The dashed horizontal reference line represents Γ of TMADNA in 0.2 M TMACl in the absence of magnesium ion.

Magnesium Ion Binding. We have seen that for the three-component systems discussed in the preceding section the membrane equilibrium distribution is determined almost exclusively by nonelectrical factors. This behavior implies that there is sufficient binding of magnesium ion to bring the net charge of the DNA very close to zero. A similar conclusion was reached by Lyons and Kotin on the basis of emf measurements carried out at excess concentrations of $MgCl_2$ even lower than ours.[12] In view of the smallness of the charge contribution to Γ, a more quantitative determination of the magnesium ion binding appears to be unattainable with the membrane equilibrium data for our MgDNA–$MgCl_2$ systems.

This difficulty may be overcome by adding a swamping 1:1 electrolyte as a fourth component. The results reported below in this and the following sections were obtained in DNA–$MgCl_2$–TMACl systems, by carrying out some runs with n'_{Cl} and others with I' held constant at 0.2.

If the TMACl is present in sufficient excess, the fraction of phosphate groups neutralized by bound magnesium ion may be determined by dialysis equilibrium measurements, using the relation

$$\beta_{Mg} = (n_{Mg} - n'_{Mg})/n_p \qquad (4)$$

The values of β_{Mg} are given in the second column of Table II. The binding of magnesium ion by DNA is seen to be appreciable, but considerably weaker than with long-chain polyphosphates.[15] Thus, while for the latter the limit of β_{Mg}/n'_{Mg} as n'_{Mg} aproaches zero equals 3 × 10^5, for the DNA this limit is 2 × 10^2. We will defer discussion of this more than 1000-fold difference to a later section.

Table II. Membrane Equilibrium Results and Derived Binding Parameters for DNA-$MgCl_2$-TMACl System at 25°

n'_{Mg}	β_{Mg}	Γ^a	α	ϕ_E	K_{Mg}
		n'_{Cl} = 0.2			
0	0	0.296	0.60	1.85	(3.3)^b
3.03×10^{-5}	0.0050	0.295	0.595	1.84	3.4
1.03×10^{-4}	0.0150	0.293	0.58	1.81	3.3
3.04×10^{-4}	0.0400	0.290	0.555	1.74	3.5
1.00×10^{-3}	0.100	0.285	0.52	1.64	3.3
2.99×10^{-3}	0.198	0.270	0.44	1.41	3.7
0.0098	0.370	0.228	0.29	1.13	4.1
0.0300	0.557	0.180	0.20	0.80	3.9
		I' = 0.2			
0.0297	0.576	0.167	0.18	0.75	4.6
0.094	0.86	0.080	0.04	0.25	6.1

^{a} $\Gamma = \lim_{n_p=0} (n'_{Cl} - n_{Cl})/n_p$. ^{b} This value was obtained by extrapolating β_{Mg}/n'_{Mg} to β_{Mg} = 0.

It should be noted that, by definition, β_{Mg} is a measure of the excess of magnesium ions on the macroion side of the membrane and may therefore contain contributions both from specific "site-binding," associated with solvation changes of the participating ionic groups, and from "ionic atmosphere binding," arising from nonspecific long-range electrostatic interactions. We are persuaded that the latter make, at most, a minor contribution to β_{Mg} so that β_{Mg} is essentially a measure of site-binding.[32] It is of interest to see whether this interpretation of our data is consistent with the law of mass action. Following the procedure given in previous papers,[16,17] we may state this law in the following form suitable for polyelectrolytes

$$K_{Mg} = \frac{\beta_{Mg}/2}{(\alpha + \beta_{Mg}/2)n'_{Mg} \exp(2\phi_E)} \tag{5}$$

In view of our definitions of α and β_{Mg}, the numerator and the quantity in the first parentheses in the denominator denote the fractions of phosphate groups bound to magnesium and free, respectively. The quantity $n'_{Mg} \exp(2\phi_E)$ is the effective free magnesium ion concentration at a binding site, where ϕ, the absolute value of the reduced potential (*i.e.*, $\phi = |e\Psi/kT|$), has the value of ϕ_E.[17]

Values of both α and ϕ_E are needed to test the applicability of eq 5. Values of α were determined from experimental values of Γ, given in the third column of Table II and also shown in Figure 1, as follows. At low concentrations of free magnesium ion ($n'_{Mg} \leq 3 \times 10^{-3}$), where the environment of the macroion consists almost entirely of TMA^+ and Cl^- ions, α was obtained from graphs of Γ *vs.* α, constructed from the theoretical curves appropriate for DNA in TMACl given in Figure 2 of ref 16. At higher concentrations of $MgCl_2$, where the charge of DNA becomes low, α was calculated from eq 3, using the value of 0.42, derived above, for the first term on the right-hand side. Once α is known, ϕ_E is obtained directly by interpolation of the theoretical curves in Figure 6 of ref 17 using the same parameters for the molecular dimensions of DNA as used in the calculation of α. The values of α and ϕ_E are given in the fourth and fifth columns of Table II.

The values of K_{Mg} obtained from eq 5 and shown in the last column of Table II are seen to be remarkably constant over a 3000-fold range in the free magnesium concentration. In view of the uncertainties in some of the molecular parameters used in the calculation, the observed variation in the values of K_{Mg} is not signficant. The results support our conclusion that β_{Mg} is essentially a measure of site binding and confirm our previous finding that the mass action law is applicable to specific counterion binding by macroions. It should be noted that the magnitude of K_{Mg} is relatively low and of the same order as the association constants for the binding of the alkali metals with DNA.[16]

Viscosity. The viscosity results, which for DNA normalities below 5×10^{-3} equiv/l. were found to follow the equation

$$\eta_{sp}/n_p = [\eta] + k'[\eta]^2 n_p \tag{6}$$

are summarized in Table III. The first three columns give the normalities of TMA^+ and Mg^{2+} and the ionic strength of each external solution used for dilution. The fourth column contains β_{Mg} obtained from the dialysis equilibrium results. The fifth column contains the intrinsic viscosity [η], expressed in l./equiv. The intrinsic viscosity is seen to decrease with increasing magnesium ion binding, but to a much smaller extent than has been observed for unsonified DNA.[11,12] For instance, at an ionic strength of 0.2 *M*, the intrinsic viscosities of both T_4phage DNA[22] and calf thymus DNA[11] were about 40% lower in a Mg^{2+} than in a TMA^+ or Na^+ environment. Since it is unlikely that the salmon sperm DNA is significantly more rigid than the DNA from the other two sources, the results appear to indicate that during the sonification process the DNA molecule is broken preferentially at its flexible points, and that the fragments, with an average molecular weight about 15 times as small as the original material, behave like nearly rigid polyelectrolyte molecules.

Table III. Intrinsic Viscosities and Huggins' Constants of DNA in $MgCl_2$-TMACl Solutions at 25°

n'_{TMA}	n'_{Mg}	I'	β_{Mg}	[η]	k'
0.2000	0	0.20	0	101	0.52
0.1935	0.0065	0.20	0.30	94	0.52
0.1600	0.0400	0.22	0.61	92	0.71
0	0.2000	0.30	~1	85	0.94
0	0.1333	0.20	~1	88	0.75

It should be pointed out that the decrease in the viscosity is not necessarily caused solely by configuration changes of the macroion. It may also be the result of an increase in the mobility of the solvent molecules near the DNA surface as a consequence of the decreased electrical field.[33,34] However, in view of

(32) A generous overestimate of the contribution of the ionic atmosphere binding to β_{Mg} may be obtained as follows. We imagine the macroion surrounded by a cylindrical shell of thickness $1/\kappa$, the characteristic Debye-Hückel distance, and length $N_A b$, *i.e.*, a shell belonging to 1 equiv of phosphate groups and extending over the effective range of the electrostatic forces. The contribution of the ionic atmosphere binding to β_{Mg} closely corresponds to the number of equivalents of magnesium ion contained in the shell. In computing this number, we assume that the effective magnesium concentration equals $n'_{Mg} \exp(2\phi_E)$ throughout the shell (for the definition of ϕ_E, see text). This assumption will produce an artificially high value for the desired quantity since, in fact, the potential is not constant but rapidly falls off with increasing distance from the macroion surface. It is significant that even our overestimate is never higher than 20% of the value of β_{Mg}.

the small values of Δv observed in the dilatometry experiments (*vide infra*), this solvent effect is probably not large.

The values of the dimensionless Huggins' constant, k', shown in the last column of Table III, are seen to increase with both increasing binding of magnesium and increasing ionic strength. This effect very likely reflects a net increase of the attractive forces between polymer molecules as the electrical repulsions are diminished.

Volume Changes. The results of the dilatometry experiments are given in Table IV. Each initial solution I contained DNA and had been brought to membrane equilibrium against 0.2 M TMACl. Each initial

Table IV. Volume Changes for Magnesium Ion Binding by DNA

Solution I		Solution II		Final solution			Δv,
Vol, ml	$n_p \times 10^2$	Vol, ml	$n_{Mg} \times 10^2$	$n_p \times 10^2$	$n'_{Mg} \times 10^2$	β_{Mg}	ml/equiv
			$n'_{Cl} = 0.2$				
10.00	2.40	2.50	1.12	1.92	0.07	0.080	4.37
10.00	2.40	2.50	3.40	1.92	0.30	0.198	3.92
10.00	2.40	2.50	8.50	1.92	1.00	0.365	3.42
10.00	2.40	2.54	20.00	1.91	3.00	0.555	3.19
10.00	2.40	10.00	7.34	1.20	3.00	0.555	3.17
8.62	2.40	10.26	20.00	1.10	10.00	0.785	3.20
			$I' = 0.2$				
10.00	1.58	4.35	13.76	1.10	3.50	0.610	3.36
10.00	1.58	16.10	13.76	0.605	8.00	0.817	3.26

solution II contained $MgCl_2$ and TMACl with the concentrations adjusted so as to obtain the desired composition of the final solution. The volumes of solutions I and II, and their DNA and Mg^{2+} normalities, respectively, are given in the first four columns. The final solution, obtained by mixing solutions I and II, is described in the next three columns which give the DNA normality, the normality of free magnesium ion (actually, the normality of magnesium ion in the hypothetical DNA-free solution which would be in membrane equilibrium with the final solution), and β_{Mg}. The latter two quantities were obtained from the membrane equilibrium results described above, assuming that the effect on β_{Mg} of the 5° temperature difference between the two types of experiments may be neglected. The last column contains the volume change in milliliter per equivalent of bound magnesium ion, defined by the relation

$$\Delta v = 1000\Delta V/(V\beta_{Mg}n_p) \tag{7}$$

where ΔV and V are the observed volume change and the volume of the final solution, respectively (expressed in arbitrary, but identical units of volume). Two features of these results are noteworthy. First, Δv, although fairly constant at high degrees of binding, increases somewhat as the value of β_{Mg} becomes small. A similar effect has been observed for other polyelectrolytes[3,35] and has been ascribed to a decrease in the electrostriction of the solvent around the polyanion as the charge on the latter decreases.[35] The second and more significant feature is the relative smallness of our Δv values compared to those observed for other polymeric phosphates and phosphonates. Thus, under similar experimental conditions, Δv for long-chain polyphosphates ranged from 21 to 26 ml/equiv of bound magnesium ion, while for singly charged poly(vinyl phosphonate) a value close to 16 ml was obtained.[3] The smallness of Δv in the case of DNA is closely related to the low values of β_{Mg}/n'_{Mg} and K_{Mg} obtained in the dialysis equilibrium experiments and reflects a rather weak interaction between magnesium ion and DNA. Evidently, this interaction, even though it obeys the mass action law, is accompanied by relatively little desolvation of either ion.

The difference in the binding behavior of DNA on the one hand, and polyphosphates and polyvinylphosphonates on the other hand, seems to be due to the difference in their structures. In contrast to the DNA molecule with its wide spacing between neighboring phosphate groups and its rigid configuration, both of which serve to prevent close contact by magnesium ion with more than one phosphate group, the structure of the flexible polyelectrolytes permits the chelation of a given magnesium ion by several ligand groups. The observed contrast in binding behavior emphasizes the role of chelation in specific counterion binding by macroions.

(33) M. van der Waarden, *J. Colloid Sci.*, **9**, 215 (1954).
(34) G. J. Harmsen, J. Van Schooten, and J. T. G. Overbeek, *ibid.*, **10**, 315 (1955).
(35) W. Kauzmann, A. Bodansky, and J. Rasper, *J. Am. Chem. Soc.*, **84**, 1777 (1962).