Interactions of Small Ions with Sodium Deoxyribonucleic Acid by Self-Diffusion Studies

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ABSTRACT: Self-diffusion coefficients of Na^+, SO_4^{2-}, and Ca^{2+} ions in aqueous salt solutions containing salmon sperm NaDNA have been determined at 25 °C as a function of X, the ratio of DNA normality to added simple salt molarity. The results are expressed as the ratio D_i/D^0_i, the ion i self-diffusion coefficient in polyelectrolyte solution to that in infinitely dilute salt solution. The fraction of polyion charge sites compensated with counterion in aqueous Na_2SO_4 and $CaCl_2$ solution is calculated, and all results are correlated with the "limiting laws" of Manning.

It has been known for some time that the physical and chemical properties of deoxyribonucleic acid are generally influenced by ionic environment.[1] For example, the helix–coil transition temperature of DNA is known to be linearly related to the logarithm of the counterion activity.[2] It is presently believed[3] that a release of bound small counterions makes a significant contribution to the overall free-energy change in the binding of more complex cationic substances to DNA. It is clear that a good understanding of DNA–small ion interactions is fundamental to a quantitative understanding of the interaction of larger biomolecules with DNA.

Small metal ions may bind to the phosphate moieties or nitrogenous bases in DNA or at both sites.[4] Alkali metal and alkaline earth cations such as sodium[5] and magnesium[6] are known to interact purely at the phosphate moiety and presumably largely electrostatically. As DNA is a polyelectrolyte with backbone phosphate groups as the charge bearers, the Manning theory of polyelectrolyte solutions[7-10] provides a basis for theoretical interpretation of equilibrium and transport property data of small ions in solution containing DNA when the interaction of these small ions with DNA appears to be largely electrostatic in nature. As double-stranded DNA possesses one of the highest charge densities of all known polyelectrolytes, a study of equilibrium and transport of small ions in the presence of DNA provides an excellent test of Manning's theory in the limit of high polyion charge density. One such small ion transport property, the self-diffusion coefficient, should be a good indicator of small ion–DNA interactions, as may be inferred from measurements of small ion self-diffusion coefficients in the presence of other polyelectrolytes such as sodium carboxymethylcellulose,[11] sodium poly(styrene sulfonate),[12] sodium, calcium, and strontium chondroitin sulfate,[13,14] sodium alginate,[15] sodium ι-carrageenan,[15] sodium dextran sulfate,[16] and sodium heparin.[16] With this in mind, self-diffusion coefficients of Na^+, Ca^{2+}, and SO_4^{2-} in aqueous solution containing salmon sperm NaDNA have been determined at 25 °C over a wide range of composition by a radiotracer technique.

DNA is a heteropolynucleotide of composition fairly characteristic of the biological source from which it is obtained. The structural features of DNA have been very thoroughly investigated.[1] Only one structural parameter is necessary, however, for the theoretical calculations herein, this being the distance between successive phosphate groups as projected along the axis of the helix. It is generally accepted that the DNA double helix exists in aqueous solution in the B form[17,18] and therefore possesses a well-defined secondary structure which is not significantly altered by solvation or the ionic composition of the medium (except in systems highly dilute in DNA[19] or highly concentrated in small ions). In the B form, NaDNA has a phosphate–phosphate distance of 1.7 Å measured along the helix axis, corresponding to two intertwined chains each having phosphate–phosphate repeat distances of 3.4 Å, as indicated from X-ray diffraction studies;[20] this value will be adopted throughout this study.

Experimental Section

Salmon sperm NaDNA was furnished by Worthington Biochemical Co. (Lots No. 4355 and 4376). The DNA samples used had a protein content of less than 0.1%, as determined by the supplier. The DNA was dissolved at 4 °C in 200 mL of 0.01 N Na_2SO_4, NaCl, or $CaCl_2$, depending on the small ion to be studied. The dissolution was always accomplished within 24 h. The samples were then placed in dialysis bags formed of "Spectrapor 2" membrane tubing (Spectrum Medical Industries) having a reported molecular weight cutoff of 12000–14000. The filled bags were placed in 2-L test tubes filled with a 0.010 N solution of the appropriate salt at 4 °C for at least three replacements of the solution exterior to the dialysis bag.

The dialyzed samples were stored at 4 °C and were analyzed spectrophotometrically within 2 weeks. One milliliter of each of the samples was diluted with the appropriate salt solution to give a volume of 100 mL and equibrated at room temperature. These diluted samples were analyzed. The optical density of each sample at 260 nm was determined against a DNA-free salt solution reference on an Acta CIII spectrophotometer (Beckman, Inc.) by using the value (at 260 nm) of $\epsilon = 6350$ (in units of L cm^{-1} per mol of base pairs, and hence, L cm^{-1} per mol of charge sites). A minimum of three readings were obtained for each sample, and the average optical density at 260 nm was used to determine the DNA normality in each of the undiluted samples from Beer's law. The normalities of DNA solutions so determined were plotted against the grams of DNA used to prepare the solution, and linear behavior was always observed, indicating adherence to Beer's law, sample homogeneity, and strong evidence for the absence of denaturation in all samples in 0.01 N salt solution. The absence of denaturation in DNA over a wide range of ionic strengths has been reported;[21] the absence of denatured DNA was confirmed here, using a modification of the procedure described by Shack.[21] No denatured DNA could be detected in any of the ionic environments to be considered in this study, and a phosphate–phosphate spacing of 1.7 Å was assumed in all ionic environments under consideration here.

Radioactive salts $^{22}NaCl$, $Na_2{}^{35}SO_4$, and $^{45}CaCl_2$ were supplied by New England Nuclear Co. Stock solutions of radioactively labeled salt were prepared by diluting 0.1 mCi of $^{22}NaCl$ to 100 mL, 1 mCi of $Na_2{}^{35}SO_4$ to 250 mL, and 1.0 mCi of $^{45}CaCl_2$ to 100 mL. Tracer self-diffusion coefficients of Na^+ in aqueous Na_2SO_4 and NaCl, SO_4^{2-} in aqueous Na_2SO_4 and Ca^{2+} in aqueous $CaCl^2$, respectively, were determined as a function of X, a composition parameter defined as the ratio of the *normality* of the polyelectrolyte (here DNA) to the *molarity* of the simple salt. One or two milliliters of the appropriate radioactive salt stock solution were placed in a 10-mL volumetric flask and evaporated in a vacuum oven. The volumetric flasks were then filled to the mark with radioactively inert solutions of a given X value, and the

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radioactive solution was equilibrated to room temperature. The amount of labeled salt was always negligible in comparison to the amount of inert salt added.

All self-diffusion coefficient measurements were performed in a constant temperature bath at 25.00 ± 0.01 °C by the open-end capillary method. Precision bore capillaries of 1.00 ± 0.005 mm diameter and 2.82 ± 0.005 mm length were filled with the solution containing a small amount of radioactively labeled salt. Upon wiping the exterior surfaces of the filled capillaries, each was individually placed into 13 mm × 100 mm test tubes which were subsequently filled with a radioactively inert solution of identical composition. The only gradient present was that for the labeled simple salt, which tends to diffuse out of the capillary.

By measuring the radioactive activity of the capillary contents after a known measured time t, denoted as C_t, and the radioactive activity at the start of diffusion, C_0, the self-diffusion coefficients were determined. The relevant expression relating C_t/C_0 to D under the boundary conditions of this experiment[22-24] is

$$C_t/C_0 = 8\pi^{-2} \sum_{n=0}^{\infty} [\exp(-2n + 1)^2\pi^2 Dt/4L^2]/(2n + 1) \quad (1)$$

where D is the phenomenological self-diffusion coefficient for the labeled species, and L is the capillary length. Equation 1 was inverted, with the aid of a computer, to obtain D for a given C_t/C_0 measurement. For $C_t/C_0 > \sim 0.5$, a simple approximate inversion[22] of eq 1 is possible, which is accurate to three significant figures

$$D = \pi 4^{-1}[1 - (C_t/C_0)]^2 L^2/t \qquad C_t/C_0 > 0.5 \quad (2)$$

Equation 2 was utilized in calculations if the condition on C_t/C_0 was satisfied.

After a sufficient time was allowed for diffusion (~24 h for $^{22}Na^+$, 48 h for $^{35}SO_4^{2-}$, and 72 h for $^{45}Ca^{2+}$), the solutions surrounding each of the capillaries were withdrawn, the capillaries removed, and their exteriors wiped clean. The capillaries were placed open-end down into a vial containing 5 mL of scintillation fluid (insta Gel, Packard, Inc.). A series of capillaries were filled with the radioactively labeled solution of a given X value and inverted without diffusion having taken place into the scintillation fluid as above; this permitted determination of C_0. All vials were centrifuged at 5000 rpm for 5 min and then shaken for 30 s on a Vortex Genie (Scientific Industries, Inc.) to ensure complete dispensing of the capillary contents into the scintillation fluid. The radioactive activity of each vial was determined using a Packard Tri-Carb liquid scintillation spectrometer, Model 3385. Each vial was counted to a minimum of 15 000 counts, resulting in a standard deviation of <0.8% in the cpm values. Linearity between the radioactive concentration in the capillaries and cpm values was demonstrated.

Each self-diffusion coefficient reported is an average value based on at least six individual determinations of C_t and at least five values of C_0. In general, the standard error in each diffusion coefficient reported did not exceed ±3%.

Results and Discussion

Tracer self-diffusion coefficients of Na^+, Ca^{2+}, and SO_4^{2-} in the presence of salmon sperm NaDNA were determined at 25 °C as a function of X, defined as the ratio of the polyion (DNA) *normality* to the molarity of the added simple salt. This definition of X differs from Manning's[7,8] but follows that of several workers[13,16] and is convenient for discussing polyion–small ion systems with two counterion species. Adopted values of D^0_i (in cm^2 s^{-1}) are 1.33 × 10^{-5} for Na^+, 0.792 × 10^{-5} for Ca^{2+}, and 1.06 × 10^{-5} for SO_4^{2-}.

Theoretical curves indicated in all figures (except Figure 3) are predicted from Manning's theory[8,13] based on a model of the polyelectrolyte as an infinite line charge of uniform charge density, with the dimensionless charge density parameter ξ

$$\xi = e^2/\epsilon kTb \quad (3)$$

where e is the electronic charge, ϵ is the dielectric constant of the *pure* solvent, k is the Boltzmann constant, T is the absolute temperature, and b is the mean distance between successive charge-bearing groups as measured along the polyion axis. Manning's theory is capable of treating polyion–small ion interactions in terms of the two parameters ξ and X, but Manning's formulation neglects polyion–polyion interactions.

Perhaps the most significant result of the theory is the natural emergence of a "condensation rule" which states that if $\xi > |z_i z_p|^{-1}$, where z_p is the valence of a single polyion site and z_i the valence of small counterion i, sufficient amounts of ion i will condense onto the polyion until the effective value of ξ, i.e., the ξ value *after* counterion condensation, becomes $\xi_c \equiv |z_i z_p|^{-1}$. Through this rule, one may speak of a "bound" and an "unbound" counterion fraction. The bound counterions may have a mobility approaching that of the polyion ("localized condensation") or may retain much of the mobility of the free ion in solution for flow parallel to the polyion axis ("delocalized condensation"). A recent NMR study[25] and a relaxation kinetic study[26] have respectively suggested that Na^+ and Ca^{2+} bind to DNA in a "delocalized" fashion. Delocalized condensation has recently been placed on a firm theoretical basis by Yoshida.[27] It should be understood that in this study the *bulk* mobility of the small counterion is probed. Regardless of the details of the binding of the small ion, so long as it is truly *bound*, it will possess a bulk mobility approximately equal to that of the polyion. It was assumed that the increased viscosity of the medium, conferred by the high concentration of DNA, had little effect on the diffusion coefficient of small ions. We demonstrated that the chloride ion diffusion coefficients in aqueous NaCl solutions containing sodium poly(styrene sulfonate) of molecular weight 70 000 and 3 000 000 differed only a few percent when there was a large excess of polyelectrolyte to simple salt, i.e., $4 < X < 16$.[12] Further substantiation of this point is discussed in ref 12.

Manning's formulation treats all *uncondensed* counterions and all coions in the Debye–Huckel approximation, and his treatment is said to yield "limiting laws". In order to be correlated to Manning's theory, data ought to be extrapolated to zero concentration of all ionic species. As $\xi_c = 1$ for Na^+, $\xi_c = 0.5$ for Ca^{2+}, and $\xi = 4.19$ for DNA, only the $\xi > \xi_c$ case need be considered here. If one denotes z_p as the valence of a charge site on the polyion, $(z_1)_p$ as the valence of a charge site on the polyion, $(z_1)_p$ as the valence of the counterion originally present with the polyion *prior to* the addition of salt, and $(z_1)_s$ and $(z_2)_s$ as the valences of the counterions and coions provided by the added salt, respectively, then in the *common counterion* case, where the added counterion and counterion originally present are the same, the Manning theory yields

$$D_i/D^0_i = f_i[1 - z_i^2 A(\xi_c, \xi^{-1}X)] \quad (4)$$

where f_i is a condensation term which is unity for coions and for counterions it is

$$f_i = (\xi_c\xi^{-1}X + 1)/(X + 1) \quad \text{for} \quad \xi > \xi_c \quad (5)$$

and the Debye–Huckel interaction term $A_1(\xi,X)$ is given by

$$A_1(\xi,X) = \sum_{m=-\infty}^{\infty} \sum_{\substack{n=-\infty \\ m^2+n^2\neq 0}}^{\infty} [\pi(\xi z_p)^{-1}(m^2 + n^2) + |z_1|_p + (|z_1|_s + |z_2|_s)X^{-1}]^{-2} \quad (6)$$

For Na^+ diffusion in NaDNA, $\xi_c = 1$, and eq 4, 5, and 6 give

$$D_{Na^+}/D^0_{Na^+} = [(\xi^{-1}X + 1)/(X + 1)][1 - (A_1(1,\xi^{-1}X)/3)] \quad (7)$$

For SO_4^{2-} diffusion in NaDNA, when SO_4^{2-} is derived from Na_2SO_4, $\xi_c = 1$, $(z_2)_s = 2$, and eq 4 becomes

$$D_{SO_4^{2-}}/D_{SO_4^{2-}} = 1 - [4A_1(1,\xi^{-1}X)/3] \qquad (8)$$

When considering Ca^{2+} diffusion in DNA solution, the situation is complicated by the presence of two counterions, Na^+ and Ca^{2+}. This case has been discussed by Manning[9] for salt-free solutions and by Magdelenat et al.[13] for systems with added bivalent salt. Understanding that $X_{Ca^{2+}}$ is the ratio of DNA normality to the molarity of Ca^{2+} *added*, we find it clear that lower $X_{Ca^{2+}}$ values represent systems increasingly rich in Ca^{2+}. In calcium-free solution, $X_{Ca^{2+}} = \infty$ and $\xi_{eff} = 1$, as NaDNA is used. Upon addition of a small amount of Ca^{2+}, the added Ca^{2+} completely condenses and begins to replace the previously condensed Na^+. As a critical value $(X_{Ca^{2+}})_{2c}$

$$(X_{Ca^{2+}})_{2c} = 2\xi/(\xi - 1) \qquad (9)$$

all Na^+ ions will be replaced by Ca^{2+} ions; all Ca^{2+} ions in the system are condensed and all Na^+ ions uncondensed at this point according to theory. Further addition of Ca^{2+} results in continued condensation of Ca^{2+} ions until a second critical value $(X_{Ca^{2+}})_{1c}$ is reached where

$$(X_{Ca^{2+}})_{1c} = 4\xi/(2\xi - 1) \qquad (10)$$

At $X_{Ca^{2+}} = (X_{Ca^{2+}})_{1c}$, $\xi_{eff} = {}^1/_2$, and the polyion is "saturated" with Ca^{2+} ions. In systems for which $X_{Ca^{2+}} < (X_{Ca^{2+}})_{1c}$, all coions, Na^+, and *some* Ca^{2+} are predicted to be uncondensed. One has, in accord with the previous discussion concerning bound counterions

$$D_{Ca^{2+}}/D^0_{Ca^{2+}} = D_p/D^0_{Ca^{2+}} \quad \text{for} \quad X_{Ca^{2+}} > (X_{Ca^{2+}})_{1c} \qquad (11)$$

$$D_{Ca^{2+}}/D^0_{Ca^{2+}} = [(X_{Ca^{2+}})_{1c} - X_{Ca^{2+}}/(X_{Ca^{2+}})_{1c}] \times [1 - (4A_2(1/2,\xi^{-1}X/2)/3)] \quad \text{for} \quad [X_{Ca^{2+}} < (X_{Ca^{2+}})_{1c}] \qquad (12)$$

where D_p is the *polyion* self-diffusion coefficient and

$$A_2(1/2,\xi^{-1}X/2) = \sum_{\substack{m=-\infty \\ m^2+n^2\neq 0}}^{\infty} \sum_{n=-\infty}^{\infty} [2\pi(m^2 + n^2) + 2 - 2\xi + 12\xi X^{-1}{}_{Ca^{2+}}]^{-2} \qquad (13)$$

It should be noted that eq 11 and 12 are not strictly continuous in the neighborhood of $(X_{Ca^{2+}})_{1c}$, but the discontinuity is not appreciable if $D_p << D^0_{Ca^{2+}}$, which is the case here. For computational purposes, it should be noted that $A_2({}^1/_2,\xi^{-1}X/2) << 1$ except when $X_{Ca^{2+}}$ approaches $(X_{Ca^{2+}})_{1c}$. As a result, eq 12 simplifies if $(X_{Ca^{2+}})_{1c} > X_{Ca^{2+}}$

$$D_{Ca^{2+}}/D^0_{Ca^{2+}} \sim 1 - [X_{Ca^{2+}}/(X_{Ca^{2+}})_{1c}] \qquad (14)$$

Consequently, a plot of $D_{Ca^{2+}}/D^0_{Ca^{2+}}$ vs. $X_{Ca^{2+}}$ should be linear at low $X_{Ca^{2+}}$ with a slope of $[1/(X_{Ca^{2+}})_{1c}]$ according to Manning's theory. The linearity can easily be shown to be derived from the important theoretical result that a *constant* fraction of polyion sites is compensated with bound Ca^{2+}.

Equations 6 and 7 were used to generate theoretical lines for Na^+ self-diffusion coefficient ratios as functions of $X_{Ca^{2+}}$; eq 6 and 8 were used to generate theoretical lines for SO_4^{2-} self-diffusion and eq 11, 12, 13, and 14 to generate theoretical lines for Ca^{2+} self-diffusion.

Results for Na^+. Results of tracer self-diffusion studies of Na^+ in aqueous salt solutions of NaDNA are shown in Figures 1 to 4. In Figure 1, results for the case when SO_4^{2-} acts as the coion are shown. As Figure 1 indicates, there is good qualitative agreement at all salt concentrations: $D_{Na^+}/D^0_{Na^+}$ decreases rapidly below $X = 2$ and stabilizes considerably above $X = 2$. The same type of qualitative behavior of $D_{Na^+}/D^0_{Na^+}$ is seen in Figure 2, which summarizes the results when Cl^- acts as the coion.

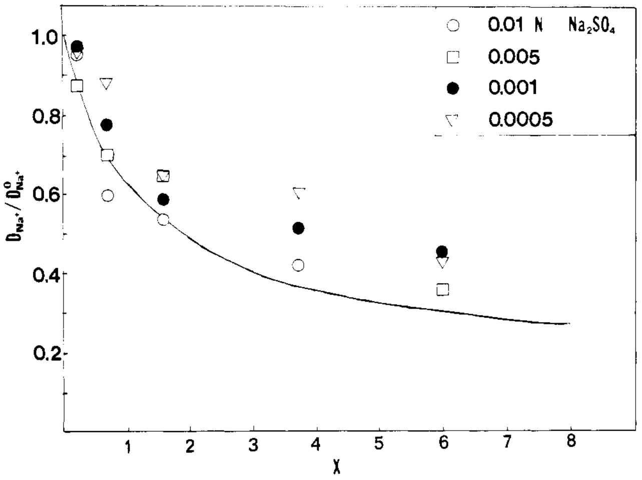

Figure 1. The dependence of the self-diffusion ratio of Na^+ on X in aqueous Na_2SO_4 solutions containing NaDNA. The solid line is predicted from Manning's theory.

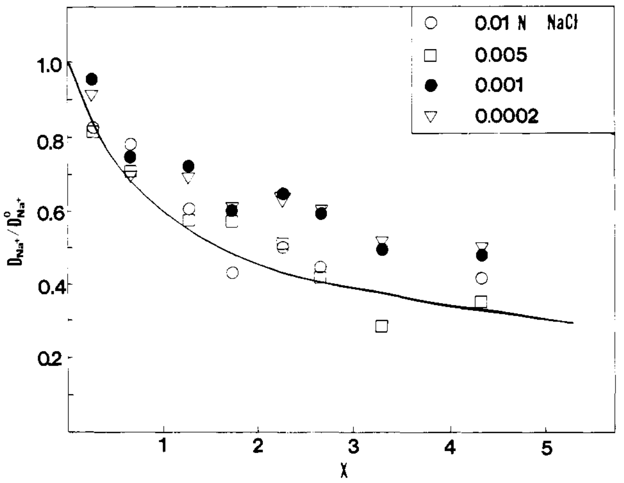

Figure 2. The dependence of the self-diffusion ratio of Na^+ on X in aqueous NaCl solutions containing NaDNA. The solid line is predicted from Manning's theory.

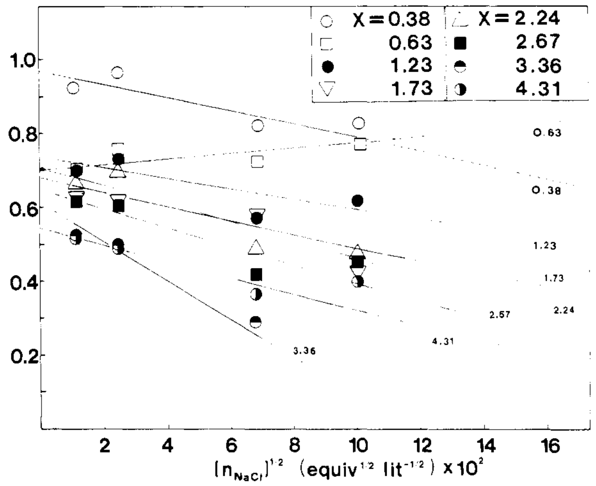

Figure 3. Plot of the self-diffusion ratio of Na^+ vs. $n_{NaCl}{}^{1/2}$ for aqueous NaCl solutions containing NaDNA used in extrapolation to zero ionic strength. The solid lines are the best fit straight line for the data of the X value indicated.

In order to permit a more direct comparison of the results obtained for Na^+ self-diffusion to Manning's theo-

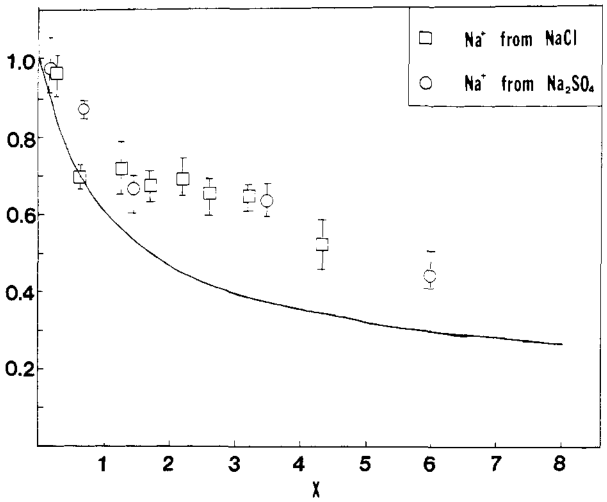

Figure 4. The dependence of the self-diffusion ratio of Na^+ extrapolated to zero ionic strength on X in aqueous NaCl or Na_2SO_4 solutions containing NaDNA. The solid line is predicted from Manning's theory.

retical eq 7, the values of $D_{Na^+}/D^0_{Na^+}$ were extrapolated to zero ionic strength of all ionic species. In making this extrapolation, one would like to plot $D_{Na^+}/D^0_{Na^+}$ vs. some function of the normality of added salt so that the resulting plot is reasonably linear. This leads one to consider the simple *salt concentration* dependence of $D_{Na^+}/D^0_{Na^+}$ (or, in general, any small ion equilibrium or transport property) at a *given* X value. This dependence has not been extensively investigated, and at present all theories of polyelectrolyte solutions are "limiting laws".[7,8,28] A reasonable approach which has proven successful for several workers[12,29] would be to plot $D_{Na^+}/D^0_{Na^+}$ vs. $n_s^{1/2}$ at a fixed X value and extrapolate to zero the added simple salt molarity. It is well known[30] that the limiting form of D_{Na^+} in a *simple* electrolytic solution is

$$D_{Na^+} = D^0_{Na^+} + S_D(n_{Na^+})^{1/2} \qquad (15)$$

where S_D is a proportionality constant, and eq 15 provides the real motivation behind the extrapolation procedure. For the NaDNA–NaCl system, $D_{Na^+}/D^0_{Na^+}$ is plotted vs. $n_{NaCl}^{1/2}$ at each X value considered in Figure 3. Although the data are rather limited, it is clear that a reasonably good correlation to linearity is obtained. Notice from Figure 3 that, except for X = 0.63 and 3.36, the slope of such a plot appears to be nearly independent of X. Excluding X = 0.63 and 3.36, and below $n_s^{1/2}$ = 0.1 equiv$^{1/2}$ L$^{-1/2}$, the Na^+ diffusion results fit an equation of the form

$$(D_{Na^+}/D^0_{Na^+})_{x=x,n_s=n_s} = (D_{Na^+}/D^0_{Na^+})_{x=x,n_s\to 0} + S_D(n_{NaCl})^{1/2} \qquad (16)$$

where $S_D = 1.8 \pm 0.8$ equiv$^{-1/2}$ L$^{1/2}$. The corresponding limiting form of $D_{Na^+}/D^0_{Na^+}$ at 25 °C may be calculated from Onsager's theory and conductance data for Na^+ diffusion in NaCl[31]

$$D_{Na^+}/D^0_{Na^+} = 1 - 0.201(n_{NaCl})^{1/2} \qquad (17)$$

The value of S_D for Na^+ diffusion in systems containing NaCl *and* NaDNA thus appears to be about an order of magnitude larger than in systems containing NaCl only. This suggests that the simple salt concentration dependence of Na^+ diffusion in NaDNA at a given X value should be at least partially explicable in terms of features not present in simple ionic systems. The fact that $D_{Na^+}/D^0_{Na^+}$ *increases* at a given X value with *increasing* salt concentration in the aqueous system sodium dextran sulfate–NaCl[16] further supports this conclusion. The results obtained here, of course, merely suggest the plausibility of eq 16 and do not conclusively demonstrate its validity.

Table I
Comparison between Experimental Values of $D_{Na^+}/D^0_{Na^+}$ Extrapolated to Zero Ionic Strength and Values Predicted from Manning's Theory in Aqueous NaDNA Solution Containing Na_2SO_4 and NaCl at 25 °C

salt	X	$(D_{Na^+}/D^0_{Na^+})_{n_s=0}$ exptl	$D_{Na^+}/D^0_{Na^+}$ theor
Na_2SO_4	0.37	0.98 ± 0.06	0.79
	0.68	0.87 ± 0.03	0.68
	1.50	0.66 ± 0.05	0.53
	3.58	0.63 ± 0.05	0.38
	6.01	0.47 ± 0.04	0.32
NaCl	0.38	0.96 ± 0.05	0.78
	0.63	0.70 ± 0.03	0.69
	1.23	0.73 ± 0.06	0.56
	1.73	0.68 ± 0.04	0.49
	2.24	0.71 ± 0.04	0.45
	2.67	0.65 ± 0.05	0.42
	3.36	0.62 ± 0.04	0.39
	4.31	0.53 ± 0.05	0.35

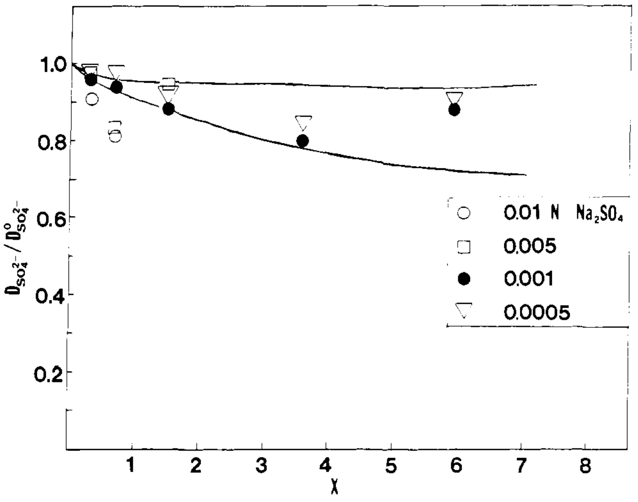

Figure 5. The dependence of the self-diffusion ratio of SO_4^{2-} on X in aqueous Na_2SO_4 solutions containing NaDNA. The solid line is predicted from Manning's theory.

Manning's theory predicts only the X dependence of the ordinate intercepts in Figure 3. These intercepts, denoted as $(D_{Na^+}/D^0_{Na^+})_{n_s=0}$, are plotted versus X for the NaDNA–NaCl system and the NaDNA–Na_2SO_4 system (the latter obtained from a plot analagous to Figure 3) in Figure 4[32] and tabulated in Table I. It is seen that the Manning eq 7 gives the correct qualitative behavior of all X values but otherwise significantly underestimates $(D_{Na^+}/D^0_{Na^+})_{n_s=0}$ for both the NaDNA–NaCl and NaDNA–Na_2SO_4 systems, particularly at high X values. Notice that $(D_{Na^+}/D^0_{Na^+})_{n_s=0}$ does not appear to depend strongly on the coion valence; the best fit curves through the data for Na^+ diffusion where Na^+ is derived from NaCl or Na_2SO_4 are superimposable within experimental error. Notice that Manning's eq 7 does depend on the coion valence by virtue of the appearance of $|z_2|_s$ in $A_1(\xi,X)$. It is easily shown by direct computation, however, that this dependence is a very weak one, amounting to a difference of less than 3% in $D_{Na}/D^0_{Na^+}$ when $|z_2|_s$ is varied from 1 to 2. This result is in accord with the results of studies on tracer diffusion of Na^+ ion in sodium polystyrene sulfonate solution with monovalent, divalent, and tetravalent coions.[12]

Results for SO_4^{2-}. The results of self-diffusion studies of SO_4^{2-} are displayed in Figure 5, the SO_4^{2-} being derived

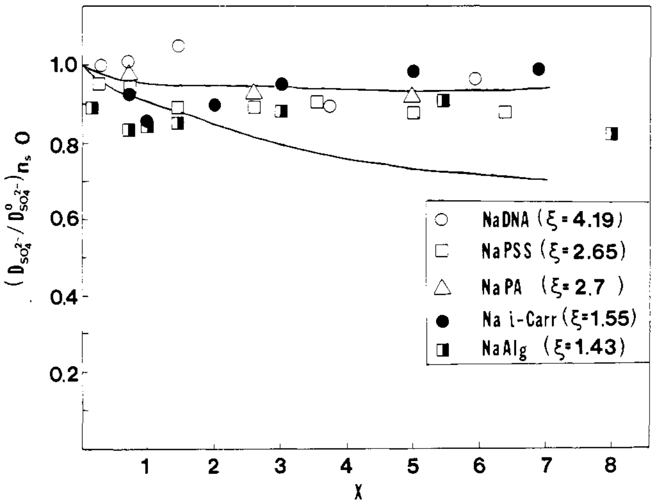

Figure 6. The dependence of the self-diffusion ratio of SO_4^{2-} extrapolated to zero ionic strength on X in aqueous Na_2SO_4 solutions containing NaDNA, NaPSS, NaPA, Na-ι-Carr, or NaAlg. The solid lines are predicted from Manning's theory for NaDNA for monovalent and divalent coions.

Table II
Comparison between Experimental Values of $D_{SO_4^{2-}}/D^0_{SO_4^{2-}}$ Extrapolated to Zero Ionic Strength and Values Predicted from Manning's Theory in Aqueous Na_2SO_4 Solution at 25 °C

X	$(D_{SO_4^{2-}}/D^0_{SO_4^{2-}})_{n_s=0}$ expt	$D_{SO_4^{2-}}/D^0_{SO_4^{2-}}$ theor
0.37	1.00 ± 0.02	0.97
0.68	1.01 ± 0.03	0.94
1.50	1.04 ± 0.05	0.88
3.58	0.90 ± 0.03	0.77
6.01	0.96 ± 0.03	0.70

from Na_2SO_4. The sulfate self-diffusion coefficients were extrapolated to zero ionic strength as was done previously, and $(D_{SO_4^{2-}}/D_{SO_4^{2-}})_{n_s=0}$ was plotted vs. X in Figure 6. Results are tabulated in Table II. Also shown in Figure 6 are values of $(D_{SO_4^{2-}}/D_{SO_4^{2-}})_{n_s=0}$ from studies on systems containing sodium poly(styrene sulfonate),[12] sodium polyacrylate,[12] sodium ι-carrageenan,[15] and sodium alginate[15] along with the Manning lines for a monovalent and a divalent coion. As may be inferred from Figure 6, $(D_{SO_4^{2-}}/D_{SO_4^{2-}})_{n_s=0}$ is very close to unity over a wide range of X values for the NaDNA–Na_2SO_4 system; the same holds true for systems containing other polyions of widely varying linear charge densities. Figure 6 suggests that the interaction of SO_4^{2-} with NaDNA is small if not completely nonexistent. Indeed, the experimental data correlate better to Manning theory predictions for a *univalent* coion, as was reported previously.[12]

Manning's theory treats all uncondensed counterions and all coions in the Debye–Huckel approximation. A divalent coion and an uncondensed divalent counterion are equivalent in Manning's formulation. This is best seen from eq 4 if one interprets the term in brackets as an "interaction term"; this term is clearly symmetrical in z. It has been shown by Iwasa[33] et al. that the formal equivalence between an uncondensed counterion and a coion of the same valence type can be eliminated by the inclusion of higher-order terms in the cluster expansion approach to equilibrium properties of polyelectrolyte solutions. The alternative "limiting laws" thereby obtained appear to be in better accord with experimental results for coion activity coefficients than Manning's theory.[34]

It is possible to combine the results of Na^+ and SO_4^{2-} diffusion in the NaDNA–Na_2SO_4 system to deduce the fraction r of sites on the polyion which are compensated by sodium ion. According to Manning's theory

$$(D_{Na^+}/D^0_{Na^+})_{n_s=0} = f_{Na^+}{}^{(u)}[1 - A_1(1,\xi^{-1}X)/3] \quad (18)$$

and

$$(D_{SO_4^{2-}}/D_{SO_4^{2-}})_{n_s=0} = 1 - 4A_1(1,\xi^{-1}X)/3 \quad (19)$$

where $f_{NA^+}{}^{(u)}$ is the fraction of sodium ions which are uncondensed. The fraction of sodium ions which are condensed is $f_{Na^+}{}^{(c)} = 1 - f_{Na^+}{}^{(u)}$, and rearranging eq 18 and 19 one obtains

$$f_{Na^+}{}^{(c)} = 1 - \frac{4(D_{Na^+}/D^0_{Na^+})_{n_s=0}}{3 + (D_{SO_4^{2-}}/D_{SO_4^{2-}})_{n_s=0}} \quad (20)$$

where it is understood that both diffusion ratios appearing are to be determined at the *same* X value. As rn_p equivalents of Na^+ of the total $(n_p + n_s)$ equivalents are presumed bound, one has

$$f_{Na^+}{}^{(c)} = rn_p/(n_p + n_s) = rX/(X + 1) \quad (21)$$

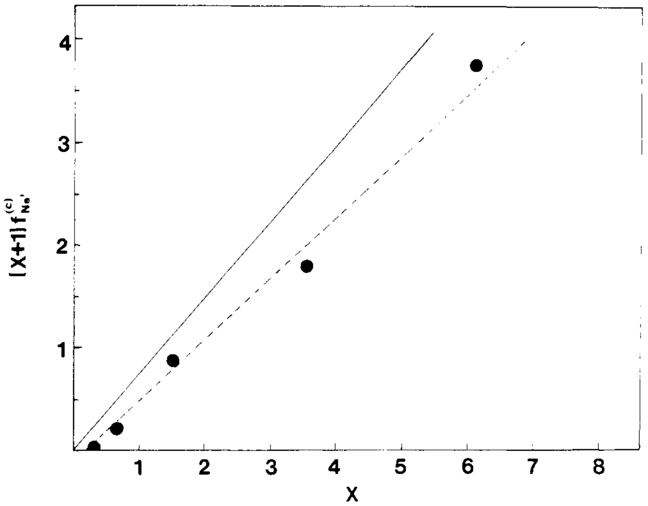

Figure 7. Plot of $(X + 1)f_{Na^+}{}^{(c)}$ vs. X for aqueous Na_2SO_4 solutions containing NaDNA. The solid line is predicted from Manning's theory. The broken line is the best fit straight line through the experimental data.

Counterion condensation onto polyelectrolytes has been operationally defined[10] as association such that the total fraction of polyion sites compensated for with counterion remains *invariant* over a wide range of X values. If the interaction of Na^+ with NaDNA is properly described as "counterion condensation", then a plot of $f_{Na^+}{}^{(c)}(X + 1)$ vs. X should be linear with slope r. Such a plot was made and is displayed in Figure 7 by using eq 21. Very good correlation to linearity is obtained with $r = 0.61 \pm 0.05$. For a monovalent counterion, Manning's theory yields the simple relation between r and ξ

$$r = 1 - \xi^{-1} \quad (22)$$

Using the determined value for r, one computes the apparent ξ value of 2.6 ± 0.4, which is somewhat lower than the ξ value of 4.2 computed from the structure. These results suggest that the interaction of Na^+ can be usefully described as "counterion condensation". Anderson et al.[35] have studied calf thymus NaDNA–Na^+ interactions (the Na^+ being derived from NaCl) by NMR and have also concluded that the fraction of sites on DNA compensated with Na^+ is invariant to variations of X. These studies have indicated that $r = 0.75 \pm 0.1$, which is somewhat higher than the value of 0.61 ± 0.05 determined here. The analysis of Anderson is based on a two-state ("bound" and

Table III
Calculated Values of the Fraction of Polyion Sites Compensated with Na^+ for Salmon Testes NaDNA in Aqueous Na_2SO_4 Solutions at 25 °C

$n_{Na_2SO_4}$, equiv L^{-1}	r	$n_{Na_2SO_4}$, equiv L^{-1}	r
0[a]	0.61 ± 0.05	0.0050	0.72 ± 0.03
0.00050	0.68 ± 0.02	0.010	0.70 ± 0.03
0.0010	0.61 ± 0.02		

[a] Obtained from diffusion ratios extrapolated to zero ionic strength.

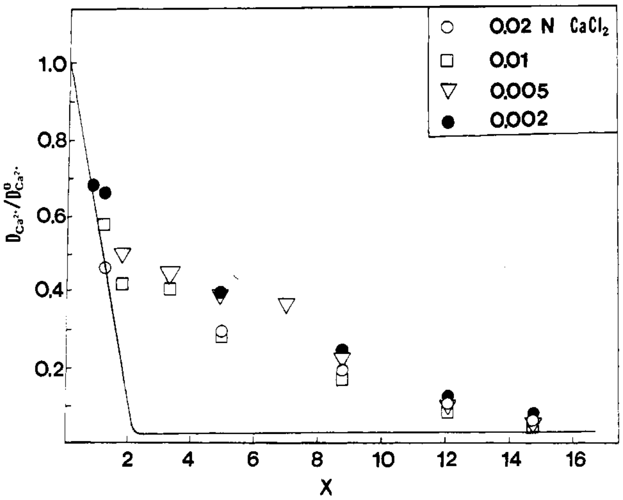

Figure 8. The dependence of the self-diffusion ratio of Ca^{2+} on X for $X > 1$ in aqueous $CaCl_2$ solutions containing NaDNA. The solid line is predicted from Manning's theory.

"free") model for the counterions; the diffusion method used here may be detecting different degrees of counterion binding, which might account for the lower value of r determined here. Examination of $(D_{Na^+}/D^0_{Na^+})_{n_s=0}$ and $(D_{SO_4^{2-}}/D^0_{SO_4^{2-}})_{n_s=0}$ in quasi-salt free ($X \to \infty$) systems might lend support to this.

The procedure discussed here for the determination of r can be extended to finite salt concentrations by simply using $D_{Na^+}/D^0_{Na^+}$ and $D_{SO_4^{2-}}/D^0_{SO_4^{2-}}$ at *finite* salt concentrations and eq 21 to determine $f_{Na^+}^{(c)}$ then plotting $(X + 1)f_{Na^+}^{(c)}$ vs. X. Such plots were made for 0.010, 0.0050, 0.0010, and 0.00050 N Na_2SO_4 and were always found to correlate strongly to linearity. The determined values of r at each salt concentration are displayed in Table III. From Table III one finds that the fraction of sites (presumably phosphate moieties) compensated with Na^+ remains nearly constant at 0.6–0.7 over the salt concentration range 0–10^{-2} N Na_2SO_4. Anderson et al.[35] have noted the invariance of r for calf thymus NaDNA in aqueous NaCl for salt concentrations up to 0.5 N, which compares favorably to the results obtained here. The actual values of r also compare favorably with the theoretical value of 0.76 obtained using eq 22, particularly at high salt concentrations. Also, recall eq 19 was used in the determination of r. From what has been found here concerning the DNA-SO_4^{2-} interaction, it appears this expression *may not* be applicable. If one repeats the calculation of r assuming $D_{SO_4^{2-}}/D^0_{SO_4^{2-}} \sim 1$ for all X, as experimental results seem to indicate, one obtains slightly higher values for r, thereby giving slightly better agreement with theory.

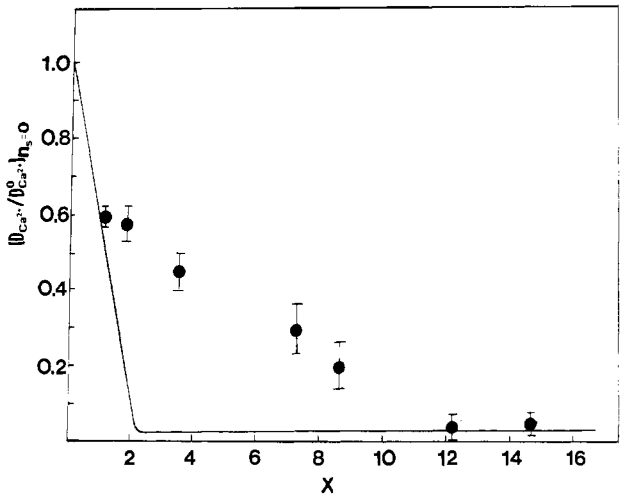

Figure 9. The dependence of the self-diffusion ratio of Ca^{2+} ion extrapolated to zero ionic strength on X for $X > 1$ in aqueous $CaCl_2$ solution containing NaDNA. The solid line is predicted from Manning's theory.

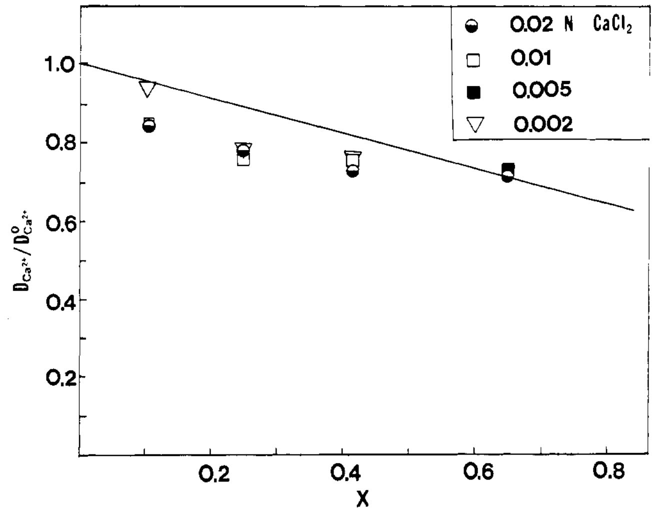

Figure 10. The dependence of the self-diffusion ratio of Ca^{2+} ion on X for $X < 1$ in aqueous $CaCl_2$ solution containing NaDNA. The solid line is predicted from Manning's theory.

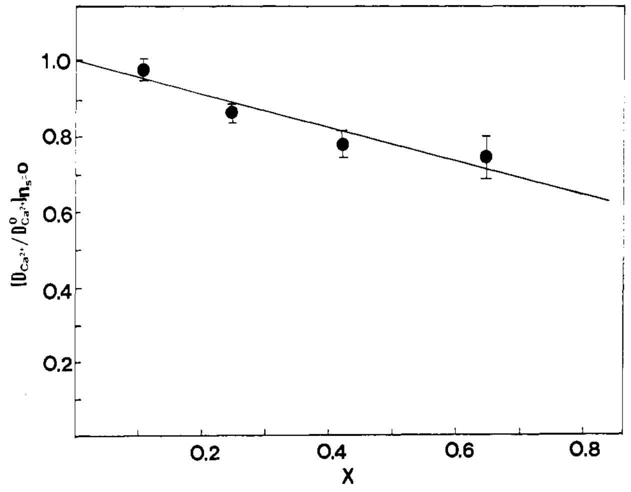

Figure 11. The dependence of the self-diffusion ratio of Ca^{2+} ion extrapolated to zero ionic strength on X for $X < 1$ in aqueous $CaCl_2$ solution containing NaDNA. The solid line is predicted from Manning's theory.

Results for Ca^{2+}. Results of studies of Ca^{2+} self-diffusion in NaDNA are displayed in Figures 8 to 11; Figures 8 and 9 indicate results for $X_{Ca^{2+}} > 1$, while Figures 10 and 11 give results for $X_{Ca^{2+}} < 1$. Observe from comparison of Figures 9 and 11 with Figure 4 that the self-diffusion coefficient of Ca^{2+} is depressed to a much greater extent (at a corresponding X value) than Na^+ in aqueous solution

Table IV
Comparison between Experimental Values of $D_{Ca^{2+}}/D^0{}_{Ca^{2+}}$ Extrapolated to Zero Ionic Strength and Values Predicted from Manning's Theory in Aqueous NaDNA-$CaCl_2$ Solutions at 25 °C

X	$(D_{Ca^{2+}}/D^0{}_{Ca^{2+}})_{n_s=0}$ expt	$D_{Ca^{2+}}/D^0{}_{Ca^{2+}}$ theor
0.116	0.98 ± 0.03	0.95
0.246	0.86 ± 0.04	0.89
0.416	0.78 ± 0.02	0.82
0.642	0.76 ± 0.06	0.72
1.37	0.60 ± 0.02	0.40
1.97	0.57 ± 0.05	0.13
3.52	0.46 ± 0.05	[a]
5.04	0.36 ± 0.05	[a]
7.18	0.30 ± 0.06	[a]
8.52	0.21 ± 0.05	[a]
12.06	0.06 ± 0.05	[a]
14.68	0.06 ± 0.04	[a]
17.0	0.06 ± 0.04	[a]

[a] For $X > (X_{Ca^{2+}})_{1c} = 2.27$, $D_{Ca^{2+}}/D^0{}_{Ca^{2+}} = D^0{}_p/D^0{}_{Ca^{2+}}$, where $D^0{}_p$ is the polyion self-diffusion coefficient at infinite dilution.

containing NaDNA. Values of $D_{Ca^+}/D^0{}_{Ca^{2+}}$ extrapolated to zero ionic strength as was done previously are tabulated in Table IV. Notice that at low X (below $X_{Ca^{2+}} = 1$) there is quantitative agreement with theory. Figure 11, in particular, shows that $(D_{Ca^{2+}}/D^0{}_{Ca^{2+}})_{n_s=0}$ is linearly depressed with increasing $X_{Ca^{2+}}$ at low $X_{Ca^{2+}}$, as is theoretically predicted. Positive deviations from linearity probably become significant near $X_{Ca^{2+}} \simeq 1$, and above $X_{Ca^{2+}} = 1$ the agreement with theory is not good. According to theoretical predictions $(D_{Ca^{2+}}/D^0{}_{Ca^{2+}})_{n_s=0} = (D^0{}_p/D^0{}_{Ca^{2+}})$ for $X_{Ca^{2+}} > 2.3$, the value of $(X_{Ca^{2+}})_{1c}$ for NaDNA. As our DNA samples have high molecular weights, reported to be several million by the supplier, one expects $D^0{}_p \sim 10^{-7}$ cm^2, so that $D_p/D^0{}_{Ca^{2+}}$ is on the order of 0.01. Such marked depression of $D_{Ca^{2+}}$ appears to be attained, but only at $X_{Ca^{2+}} > \sim 10$. In the approximate composition domain $1 < X_{Ca^{2+}} < 10$, there appears to be a *gradual* depression of $D_{Ca^{2+}}$ with increasing $X_{Ca^{2+}}$. This depression is much less precipitous than that for $X_{Ca^{2+}} < 1$. There may be a change in the self-diffusion coefficient depressing power of the polyion near $(X_{Ca^{2+}})_{1c}$, but it is not as sharp as theory predicts. The most likely cause of this discrepancy is that the Manning formulation assumes all counterion condensation (whether "localized" or "delocalized" in nature) is *complete*. Apparently Ca^{2+} can effectively "reduce ξ" while retaining some radial mobility, i.e., bulk mobility; only for $X_{Ca^{2+}} > \sim 10$ is the system sufficiently poor in Ca^{2+} that all Ca^{2+} present is completely condensed, or at least nearly so. At low $X_{Ca^{2+}}$, all Ca^{2+} present is condensed, so that the completeness of condensation is relatively unimportant, and good agreement with theory is observed.

An important feature of the theoretical lines which is experimentally verified is the linearity at low $X_{Ca^{2+}}$. As $A_2\,(^1/_2,\xi^{-1}X/2) << 1$ at low $X_{Ca^{2+}}$, only the condensation term contributes significantly, and one has from eq 12

$$D_{Ca^{2+}}/D^0{}_{Ca^{2+}} = [(X_{Ca^{2+}})_{1c} - X_{Ca^{2+}}]/(X_{Ca^{2+}})_{1c} \qquad (23)$$

Of the n_p polyion "sites" available, let r be compensated with calcium, then

$$f_{Ca^{2+}}{}^{(c)} = rn_p/n_{Ca^{2+}} = rX_{Ca^{2+}}/2 \qquad (24)$$

but from eq 24 one has

$$f_{Ca^{2+}}{}^{(c)} = X_{Ca^{2+}}/(X_{Ca^{2+}})_{1c} \qquad (25)$$

Upon comparing eq 25, 24, and 23, one notes that at low $X_{Ca^{2+}}$ the absolute value of the slope of the plot $D_{Ca^{2+}}/D^0{}_{Ca^{2+}}$ vs. $X_{Ca^{2+}}$ is equal to $^1/_2$, half the fraction of sites compensated with calcium. The linearity of Figure 11 means that r is essentially independent of $X_{Ca^{2+}}$ at low $X_{Ca^{2+}}$, in the limit of infinite dilution. One finds a slope of −0.43 ± 0.04, which gives $r = 0.84 \pm 0.08$. The theoretical value of r is $1 - (2\xi)^{-1} = 0.88$, which compares favorably to the value determined here. At low $X_{Ca^{2+}}$, the interaction of Ca^{2+} with NaDNA appears to be well-modeled as a "counterion condensation" as operationally defined by Manning.[10]

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