

Relative binding affinities of monovalent cations for double-stranded DNA

(^{23}Na NMR/alkali metal ions/condensation theory)

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ABSTRACT The competition between sodium and various other monovalent cations that bind to helical DNA in aqueous solution has been studied by ^{23}Na NMR. Variations in the sodium linewidth with the concentration of the other ion have been analyzed with an equation that describes the competitive binding in terms of two parameters: r , the total extent of counterion binding, and D , a measure of the binding affinity of a cation relative to sodium. The concentration dependence of these parameters was found to be minimal. In the absence of a competing cation the constancy of r has been demonstrated over a range of DNA phosphate concentrations (0.0025–0.015 M) and NaCl concentrations (0.003–1.3 M). For the cations investigated the range in D values is small (0.5–0.9), and the relative binding affinities follow the order: $\text{NH}_4^+ > \text{Cs}^+ > \text{K}^+ > \text{Li}^+ > \text{Na}^+$.

The utility of ^{23}Na NMR as a means of studying the interactions of counterions with polyions in solution has been demonstrated in a wide variety of systems, including soluble RNA (1), polyacrylate (2–4), polyphosphate (5), polystyrenesulfonate (6), mucopolysaccharides (7), polymethacrylate (4), and phosphatidylserine vesicles (8). Previous studies (9, 10) of double-stranded DNA by using ^{23}Na relaxation rate measurements have been principally concerned with quantifying the extent to which monovalent counterions are bound in order to test the applicability of different theories of polyelectrolyte binding. The purpose of the research communicated here is to establish the relative binding affinities for helical DNA of a series of monovalent cations whose binding interactions are anticipated to be predominantly electrostatic. The effectiveness with which these cations compete with sodium is determined by analyzing measurements of the ^{23}Na linewidth as a function of solution composition. This analysis incorporates the assumption that r , the extent of counterion association with DNA, does not depend on the identities or concentrations of the competing monovalent cations. The results conform to this assumption, which is one of the basic features of Manning's model for counterion–polyion interactions (11, 12).

THEORETICAL BACKGROUND

The ^{23}Na NMR spectrum of an aqueous sodium chloride solution containing double-stranded DNA is a single peak whose lineshape, under the conditions of the present study, is Lorentzian. (The criterion is given in the next section.) Therefore, the condition of extreme narrowing applies to the quadrupolar relaxation rates of all nuclei contributing to the signal, and chemical exchange among all significantly populated magnetic environments accessible to the sodium ions is fast in comparison to the ^{23}Na relaxation rates in each of these environments (4, 13). It follows that the linewidth of the peak at half height,

$\Delta\nu_{1/2}$, is a direct measure of R , the transverse relaxation rate averaged over all distinct magnetic environments.

The interpretation of the competitive binding experiments reported here does not require a detailed consideration of the influence of a polyion on the relaxation rates of sodium nuclei in solution. The short-range character of this interaction (2) assures that it affects only those nuclei whose distance from the polyion surface lies within a few hydrated ionic radii. Thus, the radial extent of this effect corresponds to a physically reasonable operational definition of the boundary within which counterions may be considered associated with the polyion. Nuclei within this boundary probably sample a variety of local field gradients arising from the polyion charged groups. If the distribution of bound nuclei among these various "sites" is insensitive to changes in the total salt concentration, a constant mean relaxation rate, R_B , may be ascribed to all counterions whose relaxation rates are affected by association with the polyion. Previous NMR studies indicate that the value of R_B characteristic of a number of polyions (2, 5), including helical DNA (10), has no discernible salt dependence over a wide range of ionic strengths. Theoretical explanations for this observation may be based on either the condensation model (3) or the counterion distribution predicted by the Poisson–Boltzmann equation.

Sodium nuclei that are not close enough to the polyion to be affected by its field may be classified as "free" and assigned the mean relaxation rate R_F . At ionic strengths lower than 1 M, this quantity has a negligible salt dependence (10). Under the conditions of "rapid exchange" specified above, the observed relaxation rate R (which equals $\pi\Delta\nu_{1/2}$) can be written:

$$R = p_B R_B + p_F R_F, \quad [1]$$

in which p_B and p_F are the fractions of bound and free sodium, as defined by the preceding discussion ($p_B + p_F = 1$). In view of the constancy of R_B and R_F with respect to variations in ionic strength, the variation of the sodium linewidth during the course of a titration can be ascribed solely to changes in p_B .

Because interest centers on the extent to which counterions are bound to the polyion, the quantity r is defined as the number of bound counterions per structural charge. For titrations of NaDNA with NaCl, in which no other counterion is present, Eq. 1 may be rewritten:

$$R = R_F + r(R_B - R_F)P/\text{Na}, \quad [2]$$

in which P is the number of DNA phosphate charges and Na is the total amount of sodium present. From this equation it is clear that a plot of the sodium linewidth vs P/Na tests whether the extent of counterion binding is constant as ionic strength is varied. This result is anticipated on the basis of a previous ^{23}Na NMR study of counterion binding to double-stranded DNA (10). A theoretical basis for the constancy of r is provided

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by Manning's theory of counterion condensation (11, 12) (refer to *Discussion*).

If another type of monovalent ion, M, competes with sodium in binding to DNA, then the definition of τ becomes:

$$\tau P \equiv M_B + Na_B, \quad [3]$$

in which the subscript B , as above, denotes a species that is determined by the NMR measurement to be bound to the polyanion. In order to analyze the competition between sodium and the other counterion, it is convenient to define the parameter:

$$D \equiv Na_B M_F / Na_F M_B. \quad [4]$$

This quantity is effectively an equilibrium quotient (expressed in terms of mole ratios) for the reaction in which a free sodium ion enters into association with a polyanion and a counterion of the other type is released. Eqs. 1, 3, and 4 may be combined to give:

$$R = R_F + \frac{1}{2} (R_B - R_F) \left\{ \frac{D + M/Na}{D - 1} + \frac{\tau P}{Na} + \left[\left(\frac{D + M/Na}{D - 1} + \frac{\tau P}{Na} \right)^2 - \frac{4\tau DP/Na}{D - 1} \right]^{1/2} \right\}. \quad [5]$$

If each of the three parameters τ , D , and R_B is insensitive to variations in ionic strength, this equation can be used to analyze titrations of NaDNA with MCl and of MDNA with NaCl. The results of analysis based on this equation are presented below.

MATERIALS AND METHODS

The procedure for preparing the DNA samples, described in detail elsewhere (10), involved sonicating and dialyzing concentrated DNA solutions (0.017 M DNA phosphate). Two unbuffered stock solutions were used in this study: calf thymus NaDNA and phenol extracted phage T7 NaDNA. The DNA concentrations were determined after dialysis by diluting aliquots of the solutions and measuring the absorbance at 260 nm with a Gilford spectrophotometer. Neutron activation analysis, performed by the Nuclear Reactor Laboratory at the University of Wisconsin, was used to measure the sodium ion concentrations in the DNA solutions. Accurately weighed samples containing 0.4 ml of undiluted DNA were irradiated for 15–30 min and the resulting γ radiation was later counted at various wavelengths for 15–30 min. The initial phosphate-to-sodium ratios were approximately 0.63.

The titrations were carried out in 12-mm NMR tubes by adding small amounts of concentrated chloride salt solutions with lambda pipets. The volume of the solutions in the tubes was 3.4 ml, 0.4 ml of which was $^2\text{H}_2\text{O}$. The diluted samples were obtained by adding deionized water to the stock T7 DNA solution. The same stock calf thymus NaDNA solution was used in all of the competition titrations to allow a direct comparison of the results. The pH, measured after the titrations were completed, varied from 5.9 to 6.4. Each titration consisted of 16–23 data points.

The experiments were performed on a Varian XL-100/15 spectrometer with a Varian 620/L computer-based Fourier transform data system and a Sykes cassette. The ^{23}Na spectra were obtained by using the Varian Gyro Observe system. The $^2\text{H}_2\text{O}$ resonance was used for the lock signal. The temperature was controlled at approximately 25°C by passing a constant flow of nitrogen through the probe, limiting long-term drifts to $\pm 1^\circ\text{C}$. The linewidths were later discovered to have a significant temperature dependence and better temperature control has since been attained. The full linewidths were

measured at half the peak heights of the absorption signals with a precision of better than 2%. From the amplitude and linewidth at half height of the ^{23}Na spectrum obtained for a typical sample, a Lorentzian lineshape was generated by computer and found to coincide closely with the original.

RESULTS

The results of several titrations of NaDNA with NaCl are shown in Fig. 1, in which the linewidths of the ^{23}Na spectra are plotted versus the P/Na ratio. The linearity of the plots indicates that the extent of sodium ion association with DNA remains constant over an appreciable range of ionic strength (see Eq. 4). The small deviation in linearity seen in the calf thymus DNA titration (Fig. 1A) in the low-salt region (0.024–0.037 M Na^+) could be caused by the presence of protein, which can compete with sodium ions for binding to the DNA. When calf thymus DNA was treated with phenol to remove protein and subsequently titrated with NaCl, the deviation at the low salt end of the titration decreased.

Three T7 DNA titrations, each at a different DNA concentration, are presented in Fig. 1B. Dilution of the DNA concentration by 1:6 does not affect the linearity or the slope of the plot. Hence, τ remains constant for different DNA phosphate

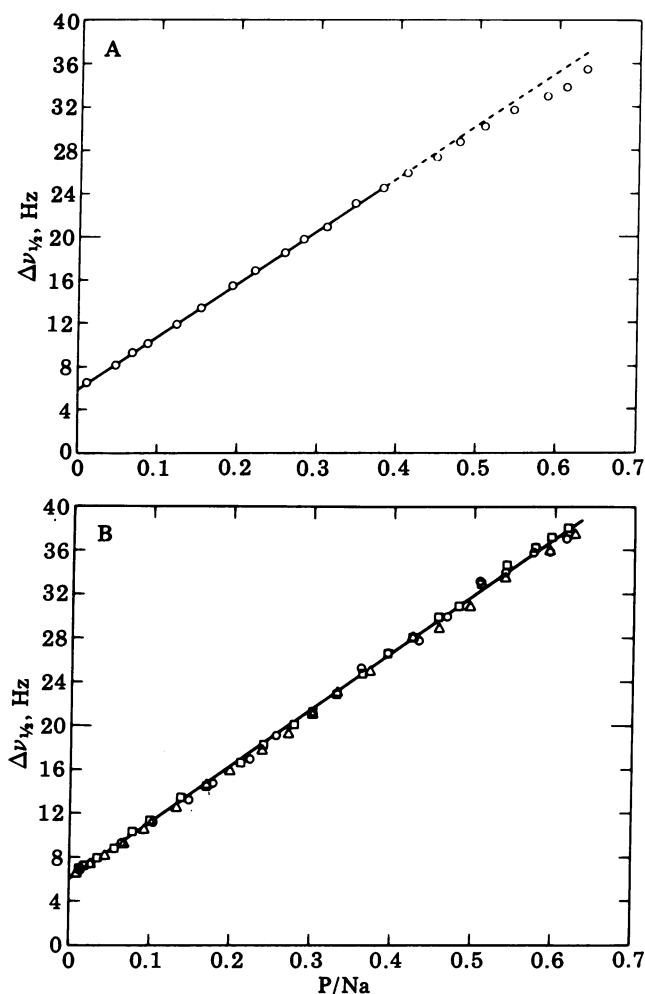


FIG. 1. (A) Linewidth measurements as a function of P/Na for 15.2 mM calf thymus NaDNA. The line (slope = 49.0 Hz, intercept = 5.9 Hz) represents a linear least squares fit to the data points for which $P/Na < 0.4$. (B) Linewidth measurements as a function of P/Na for T7 NaDNA at 15.1 mM (\square), 5.04 mM (Δ), and 2.55 mM (\circ). All the data points were used in the linear least squares fit (slope = 51.6 Hz, intercept = 6.0 Hz).

concentrations as well as over a wide range of salt concentrations (0.003–1.3 M Na⁺). The downward deviation seen in the titration with calf thymus DNA is not present in the titrations with T7 DNA. T7 DNA is expected to have a lower extent of protein contamination, because it is more easily separated from its viral coat protein than calf thymus DNA is from its tightly binding nuclear proteins.

A series of competition experiments was performed in which calf thymus NaDNA was titrated with various chloride salts. Fig. 2 shows the results of titrations with Bu₄NCl (tetrabutylammonium chloride), NaCl, NH₄Cl, and MgCl₂. The very gradual decrease in the ²³Na linewidth as Bu₄N⁺ is added illustrates the strong preference for Na⁺ binding to DNA over that of Bu₄N⁺. Similarly, the weakness of the Na⁺ binding compared to that of Mg²⁺ results in a sharp decrease in the ²³Na linewidth for the Mg²⁺ titration. Titration curves for Li⁺, K⁺, and Cs⁺ fall between those for Na⁺ and NH₄⁺. Even though the differences in binding affinities among these ions are small, the sensitivity of the linewidth measurements to the fraction of Na⁺ bound allows these differences to be detected.

In Fig. 3 the competition data are plotted as the reciprocal of the salt added and the preference for binding to DNA is seen to be NH₄⁺ > Cs⁺ > K⁺ > Li⁺ > Na⁺. The corresponding linewidths for the two K⁺ titrations differ by 0.3 Hz or less, demonstrating excellent reproducibility for titrations from a given stock DNA solution at a constant temperature. Values for the competition parameter *D*, defined in Eq. 2, were determined by a nonlinear least squares fit of the data to Eq. 5. Only those data points for which *P/M* < 1 were used in the fitting process, to avoid the low-salt region, where protein contaminants might become significant. A unique determination of *D*, *R_B*, and *r* could not be made because of the high correlation of these parameters. However, from the slope of the NaCl titration (Fig. 1A), one can calculate pairs of possible values for *r* and *R_B* and use these in fitting the competition data. If *r* is fixed at 0.76, the value predicted by the condensation theory, it follows from Eq. 2 that *R_B* = 221 sec⁻¹. With these values for *r* and *R_B*, the best fitted values for *D* and *R_F* are given in the legend to Fig. 3. The theoretical curves generated from Eq. 5 by using the parameter values determined in this way agree well with the experimental data (Fig. 3). Furthermore, if *R_B* is fixed at 221 sec⁻¹ and if *r* as well as *D* and *R_F* is allowed to vary in the fitting process, all the resulting *r* values for the different MCl

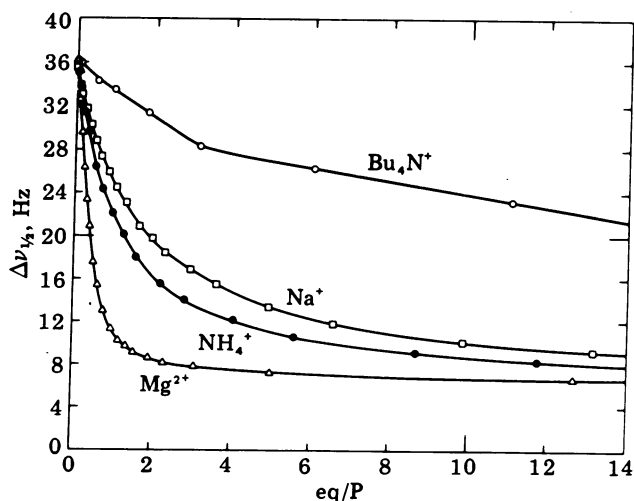


FIG. 2. Linewidth measurements of calf thymus NaDNA (0.015 M) as a function of the ratio of equivalents of added salt to DNA phosphate, eq/*P*.

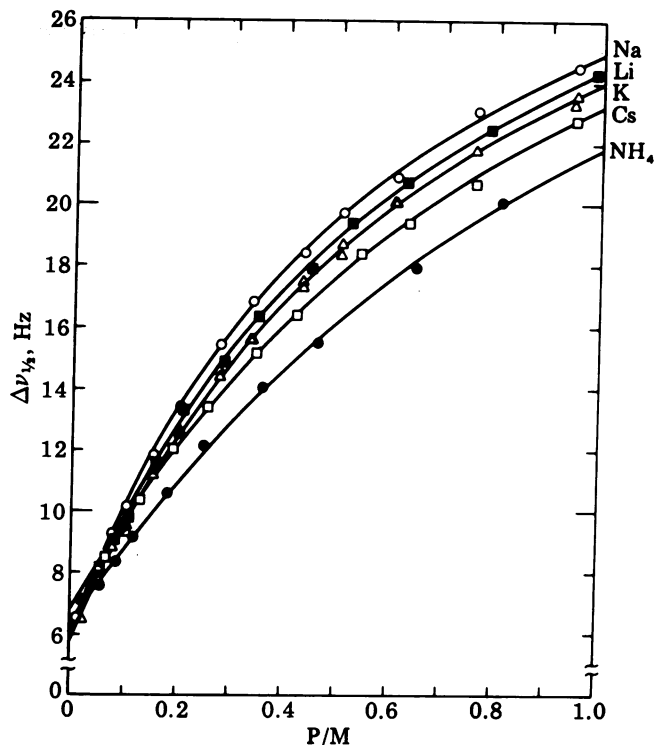


FIG. 3. Linewidth measurements as a function of the ratio of DNA phosphate to added salt, *P/M*, for 0.015 M calf thymus NaDNA. The curves represent nonlinear least squares fits of the data to Eq. 5, using *r* = 0.76 and *R_B* = 221 sec⁻¹. The values for the competition parameter *D* and the free linewidths are 0.89 ± 0.05 and 6.0 Hz for Li⁺, 0.84 ± 0.05 and 5.8 Hz for K⁺, 0.64 ± 0.1 and 6.7 Hz for Cs⁺, and 0.52 ± 0.1 and 6.3 Hz for NH₄⁺.

titrations are within 4% of 0.76. In addition to *r* = 0.76, *r* and *R_B* pairs corresponding to *r* = 0.9, 0.6, and 0.45 were also tried, with little difference in the *D* values obtained (see Table 1) or in the quality of the fit. At the low salt end of the competition titrations (*P/M* > 1) the data points fall slightly below the theoretical curves, as they did in the NaCl titration.

Deviations from the curves based on the model are noticeable at the high-salt end of the Cs⁺ and NH₄⁺ titrations. The intercepts for both these titrations are larger than expected in view of the NaCl titration, and the last points of the Cs⁺ titration merge with those for K⁺ and Li⁺. The larger intercepts might be interpreted as an actual increase in the ²³Na free linewidths with increasing salt concentration; however, when 0.02 M NaCl in the absence of DNA was titrated with CsCl up to 0.6 M, no significant change in the linewidth could be detected. Furthermore, *T*₁ measurements show that the ²³Na relaxation rate for a NaCl solution is less than for a solution of the same ionic strength that also contains CsCl (14). Therefore, a more probable explanation for the observed increase in intercepts is a slight variability in one or more of the parameters *r*, *R_B*, and *D*, which are assumed constant in the fitting procedure.

Table 1. Comparison of the competition parameter, *D*, for monovalent ions, using different values of *r* in the fitting procedure

<i>r</i>	<i>R_B</i> , sec ⁻¹	<i>D</i>			
		Li ⁺	K ⁺	Cs ⁺	NH ₄ ⁺
0.9	190	0.88	0.83	0.62	0.50
0.76	221	0.89	0.84	0.64	0.52
0.6	275	0.89	0.85	0.66	0.54
0.45	361	0.90	0.87	0.68	0.57

In the fitting procedure, some arbitrary decisions were made, such as which r , R_B pair to use, whether to fix the intercepts of all the titration curves at the same value, and how many points of the titrations to include in the analysis. The error limits given in the legend of Fig. 3 allow for variations due to judgments of this kind. Also considered is the error in the determination of the initial sodium ion concentration by neutron activation and the effect of possible temperature drifts.

DISCUSSION

Interactions of alkali metal ions with highly charged rodlike macromolecules in solution can be described theoretically in terms of a model for counterion "condensation" developed recently by Manning (11). Considering only long-range electrostatic interactions among point charges, the model accounts for the value and salt invariance of the charge fractions of a number of polyions that have been studied by various experimental methods (12, 15). Manning's model is sufficiently general that it would be possible to include the effects of specific, short-range interactions between the polyion and bound small ions (16). However, it is apparently unnecessary to consider such interactions in computing the total extent of counterion binding to rodlike polyions of sufficiently high structural charge density. For example, the extent of Mn(II) binding to polyphosphate, as determined by magnetic resonance (17), is in good agreement with the value predicted by the condensation model assuming only electrostatic interactions. However, there is evidence that a substantial fraction of the bound Mn(II) ions are partially dehydrated and immobilized on the polyphosphate backbone (17, 18). In polyion solutions containing more than one type of monovalent counterion, the *total* extent of ion binding appears to be insensitive to the chemical identities and relative amounts of the different counterions, provided that the ionic strength is sufficiently low (12, 19).

On the basis of these considerations [and others discussed by Anderson *et al.* (10)], it is expected that the charge fraction of helical DNA under the conditions investigated in the present study is a constant whose value is unaffected by the chemical identities of the competing ions. This expectation is fulfilled by the utility of Eq. 5 in fitting the data presented in Fig. 3. Both r and D are insensitive to variations in ionic strength (with the minor exceptions, at high ionic strength, of Cs^+ and NH_4^+). Although a single value of r can be used in fitting all the data, the value of D clearly depends on the type of ion competing with sodium. The relative binding affinities of these monovalent ions apparently reflect specific differences in their binding characteristics which, however, do not detectably affect the total extent of counterion binding to DNA.

A number of previous studies have employed various techniques to measure the competitive binding of monovalent counterions for rodlike polyions. The results of these studies, some of which are discussed below, indicate relatively small, but generally definite, differences in the relative binding affinities of alkali metal ions. The relative binding affinities for helical DNA of the monovalent ions investigated in the present study can be inferred by inspection of the data plotted in Fig. 3. Although the numerical values obtained for D depend somewhat on the approach taken to fitting the data, none of the approaches indicated above led to a different ordering of the binding affinities. It can be inferred from the order reported here that, in general, increasing binding affinity is correlated with decreasing hydrated radius of the ion (20). This trend can be attributed to a purely electrostatic interaction between the polyion and its hydrated counterions, if these are modeled as hard spheres of charge. This explanation cannot account for the placement of lithium and ammonium in the observed order of

relative binding affinities, but these ions might be expected to exhibit some degree of nonelectrostatic interaction with DNA. The possibility of forming hydrogen bonds to phosphate oxygens is present for ammonium, and the binding of lithium may have some covalent character.

The ordering of monovalent counterions observed in the present determination of relative binding affinity to DNA is closely paralleled by the effectiveness of these ions in altering the duplex rotation angle of the helix. Using band counting in agarose gel electrophoresis and buoyant banding in CsCl /propidium diiodide, Anderson and Bauer (21) measured the extent of DNA supercoiling, hence the duplex rotation angle, of closed circular viral PM2 DNA as a function of ionic strength and ion type. They found that the duplex rotation angle increases with increasing ionic strength and that the extent of this change varies with the counterion in the order: $\text{NH}_4^+ > \text{Cs}^+ > \text{Rb}^+ > \text{Li}^+ > \text{K}^+ > \text{Na}^+$. Precisely the same ordering has been observed by Hanlon and coworkers (22–24) from an analysis of the effects of these monovalent ions on the circular dichroism of calf thymus DNA in aqueous solution. An analogous study performed earlier by Ivanov *et al.* (25) in a mixed aqueous/methanol solvent led to the similar ordering: $\text{Cs}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+ > \text{Li}^+$. Except for the placement of lithium, both this order and that reported by Hanlon *et al.*, and by Anderson and Bauer (21) conform to the relative binding affinities of these ions for helical DNA that have been determined in the present study by ^{23}Na NMR linewidth measurements. Although it may be premature to construct a detailed molecular explanation for this striking similarity, it is clear that the effects exerted by these ions on certain structural changes of double-stranded DNA can be correlated with relatively slight differences in their specific binding interactions with DNA.

Binding interactions of small ions with polyions have frequently been studied by determining the ionic strength dependence of either the polyion electrophoretic mobility (a transport property) or the Donnan salt exclusion coefficient (a thermodynamic property). Applied to investigate the relative binding affinities of alkali metal ions for DNA, these methods have indicated orderings that differ (slightly) from each other and from that reported here. From measurements of DNA electrophoresis Ross and Scruggs (26) deduced the relative binding affinities: $\text{Li}^+ > \text{Na}^+ > \text{K}^+$. To draw inferences about ion binding from ionic effects on electrophoretic mobility it is necessary to make assumptions concerning, for example, the location of the hydrodynamic shear surface for DNA in the presence of the different cations and the significance of variations in local viscosity in determining the apparent extent of binding (12, 27). The salt dependence of the Donnan coefficients of helical DNA in the presence of several types of monovalent counterions has been determined by Strauss *et al.* (28, 29). If these coefficients are plotted directly as a function of ion concentration, comparison of the resulting curves indicates that the apparent relative binding affinities follow the order: $\text{Li}^+ > \text{K}^+ > \text{Na}^+ > \text{Me}_4\text{N}^+$ (tetramethylammonium). Somewhat different orderings can be obtained if the data are analyzed in terms of the Poisson–Boltzmann model, taking account of differences in the size of the small ions and including the possibility of site binding (which is assumed to lower the structural charge density of the polyion). For example, when Strauss *et al.* (29) followed this approach and incorporated a mass action treatment of specific ion binding, they arrived at the order: $\text{Li}^+ > \text{Na}^+ > \text{K}^+ > \text{Me}_4\text{N}^+$. Equilibrium dialysis and electrophoretic transport measurements are probably sensitive to kinds and extents of small ion–polyion interactions that are different from those that affect the relaxation rate of ^{23}Na . On the basis of the discussion presented above (*Theoretical Background*) it can

be concluded that the contribution of the polyion field to the electric field gradient acting on mobile nuclei is very short ranged. It follows that the enhancement of the observed sodium linewidth is a sensitive measure of the fraction of sodium ions that are in the immediate vicinity of the DNA surface.

The modest range in value of the D parameters presented in Table 1 indicates that these monovalent ions exhibit relatively slight differences in binding affinity for double-stranded DNA. Therefore, it is likely that the nature of the monovalent counterion need not be taken into account in analyzing the effects of changing ionic strength on equilibria such as the nonspecific binding of proteins to helical DNA. [Details of such analyses may be found in the review by Record *et al.* (30).] The results of the present study provide a determination of the binding affinity for DNA of some monovalent ions relative to that of sodium, but do not permit the total extent of ion binding to be determined. Analysis of the data, based on Eq. 5, indicates the validity of the assumption that r remains constant, regardless of the type or concentration of monovalent ions present (for ionic strengths in the range 0.04 to 1 M). To evaluate r by means of ^{23}Na NMR spectroscopy, it is necessary to determine the extent of sodium binding in the presence of an ion whose binding affinity is either very different from that of sodium or known independently by other means. An earlier study (10) involved the titration of a tetrabutylammonium salt of DNA with sodium, whose binding affinity is substantially greater. Another logical choice for the competing ion is magnesium, whose affinity for DNA is much greater than that of sodium. A determination of the charge fraction of DNA based on analyses of ^{23}Na NMR linewidths from titrations of NaDNA with MgCl_2 will be reported elsewhere.

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