Sundquist, W. I., Lippard, S. J., & Stollar, B. D. (1986) *Biochemistry 25*, 1520–1524.
Ushay, H. M., Tullius, T. D., & Lippard, S. J. (1981) *Biochemistry 20*, 3744–3748.
van der Veer, J. L., van den Elst, H., den Hartog, J. H. J., Fichtinger-Schepman, A. M. J., & Reedijk, J. (1986) *Inorg. Chem. 25*, 4657–4663.
van Hemelryck, B., Guittet, E., Chottard, G., Girault, J. P., Huynh-Dinh, T., Lallemand, J. Y., Igolen, J., & Chottard, J. C. (1984) *J. Am. Chem. Soc. 106*, 3037–3039.
Wing, R. M., Pjura, P., Drew, H. R., & Dickerson, R. E. (1984) *EMBO J. 3*, 1201–1206.

Interaction of an N-Methylated Polyamine Analogue, Hexamethonium(2+), with NaDNA: Quantitative ^{14}N and ^{23}Na NMR Relaxation Rate Studies of the Cation-Exchange Process†

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ABSTRACT: The interactions of the divalent hexamethonium (Hex^{2+}) cation with double-helical calf thymus DNA are investigated by means of ^{14}N NMR and, indirectly, by means of ^{23}Na NMR. During a titration of NaDNA with $HexBr_2$, the displacement of Na^+ from DNA by Hex^{2+} is monitored by concurrent measurements of the Lorentzian ^{14}N signals and the bi-Lorentzian ^{23}Na signals. The variations in the quadrupolar relaxation rates of ^{14}N and ^{23}Na are analyzed according to a simple two-state model for the competition between Hex^{2+} and Na^+ associated with DNA. From this analysis parameters characterizing the exchange process are evaluated, and the following conclusions are drawn: (1) The association of one Hex^{2+} displaces 1.7–2.0 sodium ions from the vicinity of the DNA. (2) Cation accumulation near DNA neutralizes approximately half of the phosphate charge at all points in the titration. (3) The exchange coefficient characterizing the displacement of Na^+ by Hex^{2+} is of the same order of magnitude as the exchange coefficients determined by NMR for other divalent cations such as Mg^{2+} and putrescine. These findings imply that the interaction of Hex^{2+} with DNA is primarily electrostatic in character. The transverse and longitudinal relaxation rates observed for ^{14}N are analyzed under the assumption that the quadrupolar relaxation processes of ^{14}N in Hex^{2+} associated with DNA can be characterized by a single-exponential correlation function with correlation time τ_{NB}. The resulting value of τ_{NB}, 7.8 ± 0.8 ns, is 3 orders of magnitude greater than that estimated for Hex^{2+} in the absence of DNA and is only 3–4 times greater than correlation times reported for ^{23}Na and other quadrupolar cations near DNA. These comparisons indicate that the observed enhancements in the relaxation rates of ^{14}N are due mainly to slowing of the motions that modulate its quadrupolar interactions in Hex^{2+} near DNA. The magnitudes of τ_{NB} and of the quadrupolar coupling constant of Hex^{2+} associated with DNA are consistent with the conclusion that this association is primarily electrostatic.

The polyamines putrescine(2+), cadaverine(2+), spermidine(3+), spermine(4+), etc. are important biosynthetic oligocations, which generally are found at high total concentrations (in the millimolar range) in both prokaryotic and eukaryotic cells. Though polyamines are necessary for normal cell growth, little is known about their roles in gene expression and other cellular processes (Cohen, 1971; Tabor & Tabor, 1976, 1984, 1985; Jänne et al., 1978; Morris & Marton, 1981). Electrostatic interactions of these oligocations with the highly charged biological polyanions DNA and RNA must play a major role in the stabilization of native structures and in processes such as DNA collapse/condensation. In addition, competitive ion-exchange processes involving associated polyamines must modulate the affinities of all other ligands, including proteins, that bind to DNA or RNA. The present study focuses on the competitive electrostatic interactions of a divalent polyamine and Na^+ with DNA and provides quantitative information about the competitive function of these oligocations in modulating ligand–nucleic acid interactions.

Equilibrium dialysis studies indicate no specificity in the association of polyamines with double-helical DNA (Hirschman et al., 1967; Shapiro et al., 1969). Furthermore, the magnitudes and salt dependences of the apparent DNA-binding constants are consistent with those expected for predominantly electrostatic interactions (Braunlin et al., 1982). Measurements of the ^1H nuclear Overhauser enhancements of spermine and its complex with DNA indicate that this tetravalent polyamine retains significant motional freedom when associated with DNA (Wemmer et al., 1985). This finding is consistent with delocalized, electrostatic association rather than the formation of a specific complex of the type

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observed by X-ray analysis of single crystals (Drew & Dickerson, 1981).

In the present study we have used ^{14}N NMR to monitor the interactions of the divalent cation hexamethonium [*N,N,N,N′,N′,N′*-hexamethyl-1,6-hexanediaminium or hexamethylenebis(trimethylammonium), hereafter abbreviated Hex^{2+}] with B-DNA in aqueous solution. This cationic polyamine analogue has two quaternary ammonium centers [$-N(CH_3)_3^+$] separated by six methylene (CH_2) groups. Since the nitrogens in Hex^{2+} are completely methylated, their NMR relaxation rates are not pH dependent, as are those of the naturally occurring polyamines (because of amino proton exchange). Experimental and theoretical aspects of ^{14}N NMR have been extensively reviewed by Witanowski and co-workers (Witanowski & Webb, 1973; Witanowski et al., 1972, 1977, 1981). The NMR sensitivity of ^{14}N, though less than that of ^{23}Na or ^{87}Rb, is still considerably greater than that of most of the other quadrupolar nuclei that have provided useful information about DNA–cation interactions, including $^{39}K^+$ (Braunlin & Nordenskiöld, 1984), $^{25}Mg^{2+}$ (Rose et al., 1980, 1982), and $^{43}Ca^{2+}$ (Reimarsson et al., 1979; Braunlin et al., 1987b). Since the nuclear spin quantum number $I = 1$ for ^{14}N, its quadrupolar relaxation processes in liquid systems decay as single exponentials, even at the high magnetic fields employed in the present study. (Explicit equations are given in the Appendix.) Consequently, measurements of ^{14}N NMR relaxation rates may be analyzed more accurately in comparison to analogous measurements on other quadrupolar nuclei having $I > 1$, e.g., ^{23}Na and ^{39}K, whose relaxation processes are biexponential at sufficiently high applied fields.

When NaDNA is titrated with Hex^{2+}, the resulting changes in the ^{14}N NMR relaxation rates of this ligand can be analyzed to quantify its interactions with DNA. As Hex^{2+} displaces sodium from the vicinity of DNA, changes in the ^{23}Na NMR relaxation rates provide complementary information about the ion-exchange process. The research reported here utilizes concurrent NMR measurements of ^{14}N in Hex^{2+} and $^{23}Na^+$ in order to characterize the competitive electrostatic interactions of Hex^{2+} and Na^+ with DNA.

Experimental Procedures

Materials. Calf thymus DNA (Sigma) was dissolved in 0.1 M NaCl/0.01 M tris(hydroxymethyl)aminomethane (Tris) (pH 7.6) and purified by phenol extraction. It was then precipitated with ethanol, redissolved in 0.1 M NaCl and 0.01 M Tris (pH 7.6), and dialyzed, first against two changes of ethylenediaminetetraacetic acid (EDTA) (0.01 M) in 0.1 M NaCl and 0.01 M Tris (pH 7.6) to remove polyvalent metal ion impurities and then against five changes of NaCl (0.1–0.01 M) containing no buffer (Bleam et al., 1980, 1983). The pH of the DNA stock solutions was approximately 6.0. The same procedure was followed to prepare HexDNA, except that $HexBr_2$ instead of NaCl was used in the final steps of the dialyses. $HexBr_2$ was obtained from Sigma and used without further purification. DNA concentrations (on a nucleotide basis) were determined from the UV absorbance at 260 nm by using an extinction coefficient of 6.62×10^3 M^{-1} cm^{-1} for calf thymus DNA. The percent hyperchromicity upon alkaline denaturation was 26.1 ± 0.3%. The ratio of absorbances at 260 and 280 nm was 1.78 ± 0.01. Sodium concentrations in solutions of NaDNA were determined by neutron activation analysis. These concentrations were also determined by calibrating integrated ^{23}Na NMR intensities measured during a titration of NaDNA with NaCl. The concentrations determined by the two methods differ by no more than 8%, which probably lies within the range of uncertainties associated with the different methods. The concentration of Hex^{2+} in the sample of HexDNA prepared by equilibrium dialysis was determined by calibrating the integrated ^{14}N NMR intensities measured during a titration of this solution with $HexBr_2$.

NMR Experiments. Samples for NMR experiments were prepared in well-cleaned 10-mm NMR tubes. Sample volumes typically were made up of ~2.7 mL of aqueous DNA solution and ~0.3 mL of D_2O to provide a lock signal. Sample pH was approximately 6.0. The NMR titrations were carried out in Bruker AM-500 and AM-360 spectrometers, in which the Larmor frequencies (ω_N) of ^{14}N are 36.092 and 26.016 MHz, respectively, and those of ^{23}Na are 132.196 and 95.263 MHz. The temperature was controlled at 297 ± 0.5 K unless otherwise stated. For all NMR spectra, acquisition times of at least 10 T_1 were used. Generally spectra consisted of 4096 data points, and the digital resolution was improved by zero filling. Titrations were carried out by adding microliter quantities of stock solutions containing NaCl or $HexBr_2$ to samples of aqueous DNA solutions contained in NMR tubes. These titrations were monitored by determining changes in the relaxation rates of ^{14}N and/or ^{23}Na as functions of solution composition.

The ^{14}N spectra were acquired with broad band decoupling. The decoupler power and offset were optimized so that the values of T_2^{-1} (as obtained from the line widths) and T_1^{-1} (as obtained from the (180°–τ–90°) inversion recovery method) were identical in aqueous solutions containing no DNA. No discernible chemical shifts were observed for the ^{14}N NMR signal of Hex^{2+} in DNA solutions during the course of the titration. The same values of decoupler power and offset were used in DNA solutions. In NaDNA solutions broad band decoupling decreased only those values of $\Delta\nu_{1/2}^N$ ($\lesssim$7 Hz) measured in the later stages of the titrations. Transverse relaxation rates (T_2^{-1}) for ^{14}N were obtained from the line widths at half-height $\Delta\nu_{1/2}$ ($T_2^{-1} = \pi\Delta\nu_{1/2}$). For the initial points of titrations, corresponding to $[Hex^{2+}]/[P] < 0.1$, an acceptable ^{14}N signal-to-noise ratio was obtained after about 60 000 scans (with acquisition times of 10–12 h). As Hex^{2+} was added during the course of the titration, this acquisition time decreased ultimately to about 45 min (at $[Hex^{2+}]/[P] \sim 1.5$).

To acquire ^{23}Na NMR spectra, about 800 scans were taken for total acquisition times of about 20 min. The transverse relaxation rates of ^{23}Na in DNA solution were evaluated by deconvoluting the observed non-Lorentzian ^{23}Na spectra into two Lorentzian components. Specifically, the line widths measured at one-half, one-sixth, and one-eighth of the peak heights were analyzed according to an extension of the method of Delville et al. (1979), using a nonlinear least-squares procedure based on the Marquardt algorithm (Bevington, 1969). To check the accuracy of this deconvolution procedure, the values of the relaxation rates calculated for the broad and narrow components (cf. Appendix) were used to generate simulated spectra. The line widths of the broad and slow components obtained by analyzing the simulated spectra lie within the ranges of uncertainty of the corresponding values obtained from the original experimental spectra.

Proton spectra for Hex^{2+} were obtained at 500.132 and 360.136 MHz at 297 K. The Redfield 2–1–4 pulse was used to suppress the strong water signals. The ^1H NMR spectrum of Hex^{2+} is identical with that reported previously (Dufourcq et al., 1972). The ^1H resonances of Hex^{2+} in DNA solutions are broadened somewhat in comparison to those obtained in the absence of DNA, but no changes in the ^1H chemical shifts were observed between the two spectra. At 300 K ^{13}C spectra

for a solution of Hex^{2+} (at ~0.25 M) were obtained at a resonance frequency of 90.557 MHz. Composite pulse decoupling was employed to enhance the ^{13}C signals (at natural abundance) in order to expedite the determination of longitudinal relaxation rates (T_1^{-1}) by the inversion recovery method.

Results and Analysis

Characteristics of the ^{14}N NMR of Hex^{2+} in the Absence of DNA. Since the two nitrogen atoms in the hexamethonium ion are magnetically equivalent, only one ^{14}N peak is observed in the NMR spectrum of an aqueous solution of $HexBr_2$. The line width of this peak is broadened by unresolved scalar coupling, which we eliminated by the application of low-power broad band decoupling, in order to isolate the purely quadrupolar contribution to the transverse relaxation. The undecoupled ^{14}N peak appears to have a Lorentzian line shape, with a line width at half-height, $\Delta\nu_{1/2}^N$, equal to 3.3 ± 0.1 Hz at both nitrogen Larmor frequencies employed in this study (ω_N = 26.016 and 36.129 MHz). At each of these frequencies low-power broad band decoupling reduced $\Delta\nu_{1/2}^N$ to 1.17 ± 0.05 Hz without affecting the Lorentzian form of the line shapes. This value corresponds to a transverse relaxation rate of 3.68 ± 0.15 s^{-1} and agrees well with the value of the longitudinal relaxation rate of 3.69 ± 0.1 s^{-1} obtained from our inversion recovery measurements. The equality of the two relaxation rates (within experimental error) and their independence of the field strength indicate that in aqueous solutions of $HexBr_2$ the quadrupolar relaxation processes of ^{14}N NMR in Hex^{2+} are in the extreme narrowing limit (cf. Appendix). Therefore, the quadrupolar relaxation rates measured for ^{14}N in Hex^{2+} cannot be analyzed directly in order to determine χ_N, the quadrupolar coupling constant, and τ_N, the correlation time that characterizes the time scale of the diffusional motions modulating the quadrupolar interaction. (Explicit equations relating the transverse relaxation rate of ^{14}N to the quadrupolar coupling constant and the correlation time are given in the Appendix.)

The value of the longitudinal relaxation rate measured by us for ^{14}N in Hex^{2+} is very close to 3.85 s^{-1}, the value reported previously for the single ^{14}N signal in the *n*-hexyltrimethylammonium ion (HTA^+) (Henriksson et al., 1977). The local environment of the nitrogen nucleus in HTA^+ is nearly identical with that of Hex^{2+}, and the sizes of the two ions are similar. Thus, it is plausible that the fluctuating local quadrupolar interactions experienced by ^{14}N in Hex^{2+} and HTA^+ are similar in magnitude and frequency. Both the correlation time τ_N and the coupling constant χ_N characteristic of HTA^+ in aqueous solution were estimated from ^{14}N relaxation rate measurements on HTA^+ and ^{13}C measurements on the *p*-C–H carbon of hexylpyridinium bromide. (For this carbon the correlation time of the dipolar relaxation was assumed to be the same as that of the quadrupolar relaxation of ^{14}N in HTA^+.) On the basis of these results, values of $\tau_N = 2.1 \times 10^{-11}$ s and χ_N = 111 kHz have been estimated for HTA^+ by Henriksson et al. (1977). Solid-state ^{14}N NMR measurements give values for χ_N of 116 kHz for decyltrimethylammonium bromide and 98 kHz for hexadecyltrimethylammonium bromide (Pratum & Klein, 1983). The magnitudes of these coupling constants include contributions from the crystalline field present in the solid state as well as the intramolecular contributions that are important in liquid solution.

For Hex^{2+} free in aqueous solution it may be possible to estimate τ_N and χ_N from the correlation time τ_c characteristic of the dipolar relaxation of the N–C_α methylene carbon directly bonded to nitrogen. For this carbon our inversion recovery measurements of the longitudinal relaxation rate give $\tau_c = 9.8 \times 10^{-12}$ s. (This value was calculated from the expression $r_{CH}^6/2T_{1C}\hbar^2\gamma_H^2\gamma_C^2$, where $\hbar$ is Planck's constant, γ_H and γ_C are the magnetogyric ratios of ^1H and ^{13}C, and r_{CH} = 0.107 nm is the distance of each directly bonded proton to the N–C_α methylene carbon.) Since the dipolar relaxation of the N–C_α methylene carbon in Hex^{2+} may be modulated significantly by internal motions other than those that modulate the quadrupolar interactions at ^{14}N, τ_c may be only a lower bound on τ_N. If $\tau_N \geq 9.8 \times 10^{-12}$, then $\chi_N = (2R_F^N/3\pi^2\tau_c)^{1/2} \leq 160$ kHz. In summary, direct evaluations of τ_N and χ_N from only the ^{14}N quadrupolar relaxation rates measured for Hex^{2+} in the absence of DNA are not possible because these rates are in the extreme narrowing limit even at the highest magnetic field strength used in the present study (500-MHz ^1H). However, the foregoing indirect comparisons permit order of magnitude estimates: $\tau_N = 10^{-11}$ s; χ_N = 100 kHz.

Effects of DNA on the Quadrupolar Relaxation Rates of ^{14}N in Hex^{2+}. In solutions containing DNA and Hex^{2+}, a single Lorentzian ^{14}N NMR peak is observed. Signals due to the nitrogens in the DNA bases are broadened beyond detectability, since their relaxation rates are extremely rapid compared to those of ^{14}N in a low molecular weight species such as Hex^{2+}. The relaxation rates of the base nitrogens of DNA are rapid because their asymmetric local environments give rise to large quadrupole moments (Moseley & Stilbs, 1978) and because the relatively slow motions of the DNA macromolecule result in long correlation times (on the order of microseconds). It follows that the ^{14}N resonances in nitrogen-containing ligands that form complexes with DNA having exchange lifetimes on the order of microseconds would be too broad to be detectable. The extent of formation of such complexes can therefore be determined by monitoring the integrated intensity of the ^{14}N resonance of the ligand. None of our ^{14}N spectra for Hex^{2+} in the presence of DNA showed any detectable loss of signal intensity. Thus, there is no indication that Hex^{2+} forms any long-lived (i.e., site-bound) complex with DNA.

In a DNA solution for which $[Hex^{2+}]/[P] = 0.1$ and $[Na]/[P] = 2.0$, the line width $\Delta\nu_{1/2}^N$ is ~15 Hz, more than an order of magnitude greater than the ^{14}N line width in the absence of DNA. This increase is much larger than the increase in the bulk viscosity of the solution attributable to the presence of DNA at a concentration [P] = 13 mM. (In a water–glycerol solution of equal viscosity, $\Delta\nu_{1/2}^N$ for Hex^{2+} is 2.3 Hz.) The broadening of $\Delta\nu_{1/2}^N$ observed in DNA solutions can be attributed to the short-range effect(s) of DNA on the quadrupolar relaxation processes of ^{14}N in Hex^{2+} ions that are sufficiently close to DNA, provided that these ions exchange rapidly with all the others in solution. Then, according to the two-state model, the observed line width, $\Delta\nu_{1/2}^N$ (Hz), can be described as the weighted sum of contributions from "bound" and "free" states of Hex^{2+}

$$\pi\Delta\nu_{1/2}^N \equiv R_2^N = p_F^N R_F^N + p_B^N R_{2B}^N \qquad (1)$$

where $p_F^N + p_B^N = 1$; R_{2B}^N (s^{-1}) characterizes those ions close enough to DNA to experience a significant enhancement in their transverse relaxation rates, whereas R_F^N (s^{-1}) characterizes the remainder of the ions, which are free in bulk solution. Equation 1 provides an operational definition of the "binding" of Hex^{2+} to DNA, which need *not* imply specific interactions with definite sites.

The condition of fast exchange requires that the rate at which Hex^{2+} exchanges between the bound and free environments must be faster than the relaxation rate in either

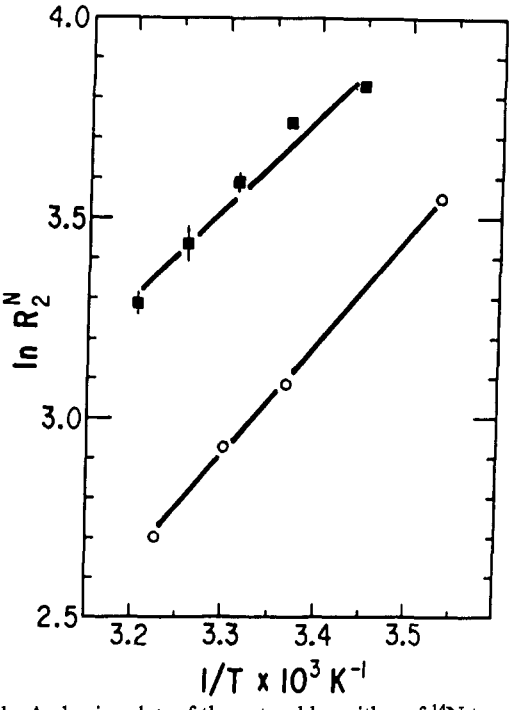

FIGURE 1: Arrhenius plots of the natural logarithm of ^{14}N transverse relaxation rates (in s^{-1}) vs reciprocal absolute temperature for two samples: (○) [P] = 8.5 mM, [Hex^{2+}] = 5.2 mM, measured at ω_N = 36.092 MHz; (■) [P] = 13.5 mM, [Hex^{2+}] = 1.41 mM, [Na^+] = 17.9 mM, measured at ω_N = 26.016 MHz.

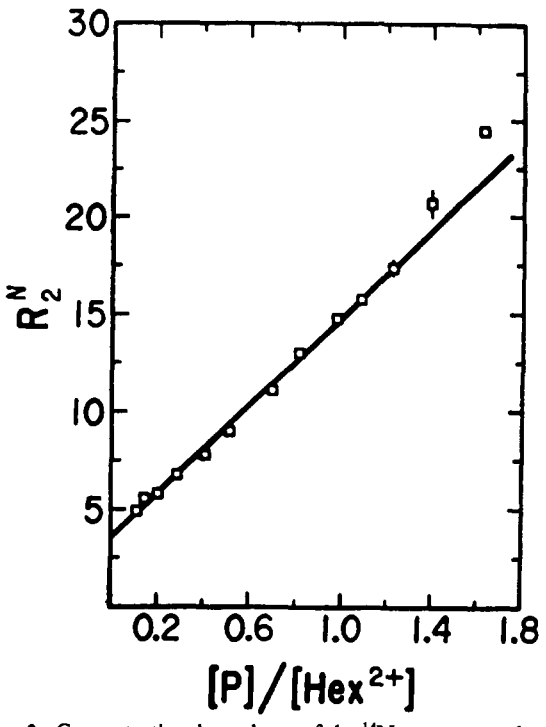

FIGURE 2: Concentration dependence of the ^{14}N transverse relaxation rate (in s^{-1}) at ω_N = 36.092 MHz and T = 297 K for a titration of HexDNA ([P] = 8.5 mM) with $HexBr_2$.

environment. For counterions in polyelectrolyte solutions, the existence of fast exchange can be tested qualitatively by determining the temperature dependence of the NMR line widths characteristic of the small ions. In Figure 1 are shown plots of ln R_2^N vs $1/T$, where T is the absolute temperature, for DNA solutions containing either relatively large or relatively small amounts of Hex^{2+} (in the latter case virtually all the divalent ions are associated with the DNA). At each of the field strengths investigated, the transverse relaxation process is outside the limit of extreme narrowing. However, the positive slopes of the Arrhenius plots in Figure 1 demonstrate that the temperature dependence of the observed relaxation rate R_2^N is directly proportional to that of the correlation time τ_{NB} governing the relaxation of nuclei associated with DNA. As noted below, an analysis of the quadrupolar relaxation processes of Hex^{2+} in the vicinity of DNA indicates that the magnitude of the quadrupolar coupling constant is due to intramolecular field gradients. Over the temperature range considered here, such field gradients are expected to be essentially constant. Hence, the temperature dependence of R_{2B}^N is governed by that of τ_{NB}, so long as the departure from extreme narrowing is not too large. It follows that the condition of rapid exchange pertains to the ^{14}N relaxation of Hex^{2+} in DNA solutions. (Further details on this point can be found in the Appendix.)

To investigate the interactions of Hex^{2+} with DNA, a titration of HexDNA with $HexBr_2$ was followed by determining the concentration dependence of $\Delta\nu_{1/2}^N$ at 36.129 MHz. Equation (1) can be used in analyzing this titration, provided that R_{2B}^N and R_F^N do not vary with the composition of the solution. This condition can be regarded as the essential assumption underlying the two-state model. If R_{2B}^N and R_F^N are constant, then the observable relaxation rate R_2^N (s^{-1}) = $\pi\Delta\nu_{1/2}^N$ (Hz) is a simple linear function of p_B^N

$$R_2^N = R_F^N + p_B^N(R_{2B}^N - R_F^N) = R_F^N + r_N^0(R_{2B}^N - R_F^N)[P]/[Hex^{2+}] \quad (2)$$

where r_N^0 is the number of Hex^{2+} ions bound per DNA phosphate. (The superscript "0" denotes the absence of any other low molecular weight counterion in the system.) The titration of HexDNA with $HexBr_2$ is represented in Figure 2 by a plot of R_2^N vs [P]/[Hex^{2+}]. According to eq 2 the slope of this plot corresponds to $r_N^0(R_{2B}^N - R_F^N)$, and the intercept, extrapolated to [P]/[Hex^{2+}] = 0, corresponds to R_F^N. The straight line shown in Figure 2 is a linear least-squares fitting of all of the data except the earliest two points in the titration (at the highest values of [P]/[Hex^{2+}]). If these points are included, the resulting best-fitted value of R_F^N is less than that of Hex^{2+} in the absence of DNA and hence is not consistent with the two-state model (see eq 1). Since the two points at the beginning of the titration are subject to the greatest uncertainty, they were excluded from the fitting. The resulting best-fitted values of $r_N^0(R_{2B}^N - R_F^N)$ and R_F^N are, respectively, 11.28 ± 0.2 and 3.52 ± 0.15 s^{-1}. The latter value is in good agreement with 3.54 ± 0.21 s^{-1}, the relaxation rate of Hex^{2+} in aqueous solution containing no DNA.

Effects of DNA on the Quadrupolar Relaxation Rates of ^{23}Na. Previous ^{23}Na NMR studies of the interactions of sodium with DNA (Anderson et al., 1978; Bleam et al., 1983; Nordenskiöld et al., 1984; Van Dijk et al., 1987) were conducted at magnetic field strengths lower than that employed here, for which ω_{Na} = 95.263 MHz. At this field strength the bi-Lorentzian ^{23}Na spectra can be deconvoluted accurately over a wide range of concentrations. Then the relaxation rates characteristic of the individual components can be analyzed according to the two-state model for quadrupolar relaxation. By analogy with eq 2, the following expression holds for the case of rapid exchange of sodium nuclei between bound and free states

$$R_{2f,s}^{Na} = R_F^{Na} + p_B^{Na}(R_{Bf,s}^{Na} - R_F^{Na}) = R_F^{Na} + r_{Na}^0(R_{Bf,s}^{Na} - R_F^{Na})[P]/[Na] \quad (3)$$

where f or s denotes either the fast or slow component of the transverse relaxation process of ^{23}Na (cf. Appendix). The subscript 2 has been omitted from $R_{Bf,s}^{Na}$ to simplify the no-

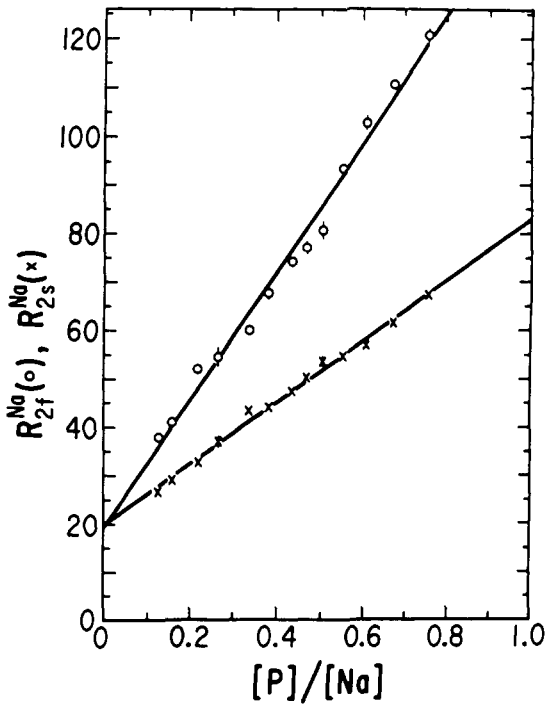

FIGURE 3: Concentration dependences of the deconvoluted ^{23}Na transverse relaxation rates (R_{2s}^{Na}, X; R_{2f}^{Na}, O) (in s^{-1}) measured at ω_{Na} = 95.263 MHz and T = 297 K for a titration of NaDNA ([P] = 13.5 mM) with NaCl.

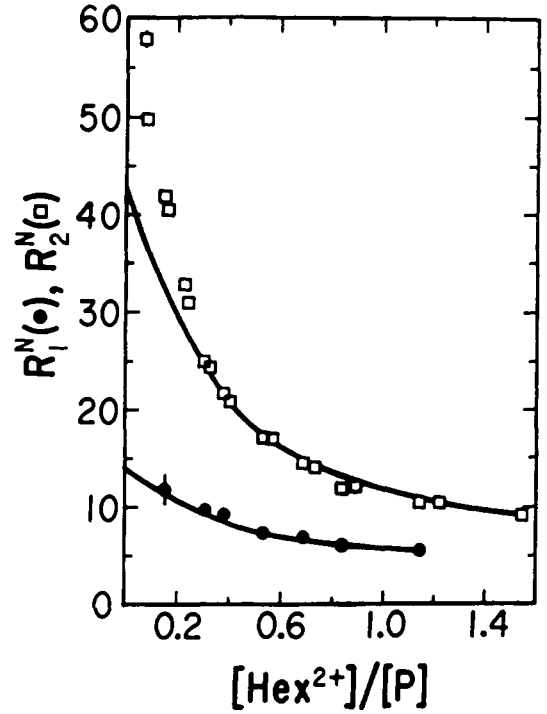

FIGURE 4: Concentration dependences of the transverse (R_2^N, □) and longitudinal (R_1^N, ●) relaxation rates (in s^{-1}) of ^{14}N measured at ω_N = 26.016 MHz and T = 297 K for titrations of NaDNA ([P] = 13.45 mM, [Na^+] = 17.8 mM) with $HexBr_2$. The solid line represents the best theoretical fitting of all the data to the ion-exchange model using parameters given in the last column of Table I.

tation, since for ^{23}Na only transverse relaxation rates are considered in this paper. For solutions containing NaCl and NaDNA in the concentration range investigated here, the condition of rapid exchange for ^{23}Na has been verified by determining the temperature dependence of the ^{23}Na relaxation rates (data not shown).

For a titration of NaDNA with NaCl plots of R_{2f}^{Na} and R_{2s}^{Na} vs [P]/[Na] are given in Figure 3. As in the case of the ^{14}N results shown in Figure 2, each of the sets of deconvoluted ^{23}Na relaxation rates can be fitted well to a straight line. According to eq 3, the different slopes of the two lines reflect the different magnitudes of R_{Bf}^{Na} and R_{Bs}^{Na}, and the extrapolated intercepts intersect, within uncertainty, at R_F^{Na} = 19.30 ± 0.41 s^{-1}, in agreement with the relaxation rate measured for ^{23}Na in NaCl solutions containing no DNA. Thus, it appears that both R_{Bf}^{Na} and R_{Bs}^{Na} are constant during the titration and hence that the two-state model may be used to analyze each component of the ^{23}Na transverse relaxation process, at least over the range of values of [P]/[Na] < 0.7. This inference is supported by previous ^{23}Na NMR results at lower magnetic fields (Anderson et al., 1978; Bleam et al., 1983) and by analogous NMR results for other quadrupolar nuclei such as ^{39}K (Braunlin & Nordenskiöld, 1984) in solutions containing DNA.

Use of Competitive Titrations To Investigate the Association of Hex^{2+} with DNA. The most probable interpretation of the linearity of the data shown in Figure 2 is that r_N^0 and R_B^N remain constant during the course of the titration. The binding density of Hex^{2+} apparently does not change as the ratio [Hex^{2+}]/[P] is varied from 0.6 (the value attained by dialysis against low concentrations of $HexBr_2$ in the absence of another counterion) to 5.0. It follows from eq 2 that the data in Figure 2 cannot yield separate values of r_N^0 and R_B^N. To arrive at a more complete description of the association of Hex^{2+} with DNA, its binding density (r_N) must be varied. This variation can be accomplished only if a second type of counterion is present. A univalent cation, such as sodium, is preferable as the competing species because its affinity for DNA is significantly lower than that of Hex^{2+}. The competitive ion exchange of Hex^{2+} and Na^+ in a DNA solution can be studied by two basic kinds of titrations: HexDNA can be titrated with NaCl or NaDNA can be titrated with $HexBr_2$. Only for the latter type of titration is it possible to achieve conditions under which nearly all the divalent ions are associated with DNA, and therefore (according to eq 1) the observed ^{14}N relaxation rate is maximally enhanced. Over the course of the titration the relative variation in $\Delta\nu_{1/2}^N$ is much larger than that of $\Delta\nu_{1/2}^{Na}$, but the NMR sensitivity of ^{23}Na is far greater than that of ^{14}N. Thus, ^{23}Na NMR relaxation rates can provide an alternative means of monitoring the ion exchange of Hex^{2+} for Na^+ as NaDNA is titrated with $HexBr_2$.

The results of two complete titrations of NaDNA with $HexBr_2$ are represented in Figures 4 and 5. Figure 4 shows the transverse relaxation rate of ^{14}N, obtained directly from $\Delta\nu_{1/2}^N$, and the longitudinal relaxation rate, determined from inversion–recovery experiments, plotted as functions of [Hex^{2+}]/[P]. Only one measurement of R_1^N was made in the early stage of the titration (at [Hex^{2+}]/[P] ~ 0.15), because at such low concentrations of ^{14}N nuclei extremely long data acquisition times are required to ensure adequate signal-to-noise ratios. Figure 5 shows the variation with [Hex^{2+}]/[P] of each of the relaxation rates calculated by deconvoluting components of the bi-Lorentzian ^{23}Na signal. The theoretical curves shown in Figure 4 and 5 were calculated by assuming a simple model of the competitive association of Hex^{2+} and Na^+ with DNA. The basic assumptions underlying this model are summarized next.

Two-State Model of Hex^{2+}–Na^+ Ion Exchange in DNA Solutions. The binding densities of Hex^{2+} and Na^+ can be related by the parameter n:

$$n \equiv (r_{Na}^0[P] - [Na]p_B^{Na})/[Hex^{2+}]p_B^N \quad (4)$$

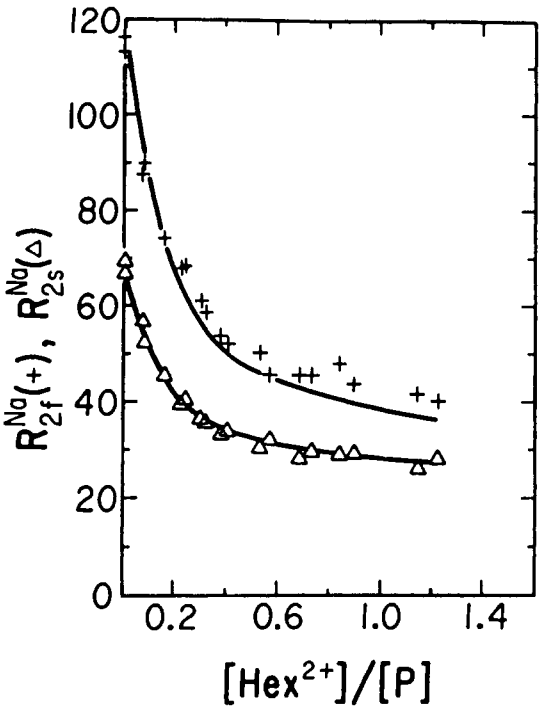

FIGURE 5: Concentration dependences of the slow (R_{2s}^{Na}, Δ) and fast (R_{2f}^{Na}, +) deconvoluted relaxation rates of ^{23}Na (in s^{-1}) measured at ω_{Na} = 95.263 MHz for titrations of NaDNA with $HexBr_2$ under the conditions specified for Figure 4. The solid line represents the best theoretical fitting of all the data to the ion-exchange model using parameters given in the last column of Table I.

Here r_{Na}^0 is the number of Na^+ ions bound per DNA phosphate in the absence of the competing Hex^{2+} ion; p_B^N and p_B^{Na} denote the fractions of ions (Hex^{2+} or Na^+, respectively) that are associated with DNA. These fractions are determined from NMR measurements by a two-state analysis using eq 2 for p_B^N or eq 3 for p_B^{Na}. According to eq 4, n is the ratio of the number of Na^+ ions that have been displaced from DNA to the number of Hex^{2+} ions that have become associated with DNA at a given point in the titration of NaDNA with $HexBr_2$. Hence, n may be interpreted as a stoichiometric coefficient for the ion-exchange process:

$$Hex_F^{2+} + nNa_B^+ \rightleftarrows Hex_B^{2+} + nNa_F^+$$

This exchange can be characterized by the relative affinity parameter D, defined as

$$(p_B^{Na})^n/(p_F^{Na})^n = Dp_B^N/p_F^N \quad (5)$$

If n and D remain constant as the binding densities of Hex^{2+} and Na^+ vary, then, for a given specification of these parameters, eq 4 and 5 can be solved simultaneously by numerical methods to determine the dependence of p_B^N or p_B^{Na} on $[Hex^{2+}]/[P]$, the independent variable in a titration of NaDNA with $HexBr_2$. The relaxation rate of ^{14}N (or ^{23}Na) at each point of the titration can then be calculated from eq 2 (or eq 3) for given specifications of R_{2B}^N and R_F^N (or $R_{Bf,s}^{Na}$ and R_F^{Na}). The results of applying the two-state ion-exchange model to analyze our ^{14}N and ^{23}Na NMR measurements are presented in the following section.

Use of the Ion-Exchange Model To Analyze the Concentration Dependences of the ^{14}N and ^{23}Na Relaxation Rates. The ultimate objective of this analysis is to find the set of parameter values that provide the best fitting of eq 2–5 to all of the ^{14}N and ^{23}Na relaxation rate data shown in Figures 4 and 5. A total of six relaxation rates (R_{2B}^N, R_{1B}^N, R_F^N, R_{Bf}^{Na}, R_{Bs}^{Na}, R_F^{Na}) are needed in addition to the three parameters that characterize the ion-exchange process (r_{Na}^0, n, D). To determine the parameter values that minimize the differences between calculated and observed relaxation rates, we employed a nonlinear least-squares fitting routine based on a modification of the Marquardt algorithm (Bevington, 1969). Reasonable levels of uncertainty (±10–20%) were attained for the best-fitted values of the parameters of greatest interest (r_{Na}^0 and D) by reducing the number of floated parameters in the fittings. The relaxation rates characteristic of the free ions, R_F^N and R_F^{Na}, were fixed at 3.54 and 19.30 s^{-1}, respectively. These values were obtained from linear least-squares fittings of eq 2 to the ^{14}N relaxation rates shown in Figure 2 and of eq 3 to the ^{23}Na relaxation rates shown in Figure 3. Measurements made in the absence of DNA indicate that the relaxation rates of $^{23}Na^+$ and ^{14}N in Hex^{2+} are unaltered in solutions where both ions are present at the concentration levels investigated in our competitive titrations of NaDNA with $HexBr_2$. When n is floated, in fitting the relaxation rates shown in Figures 4 and 5, its best-fitted value is close to 2. Fixing n at 2 does not alter the best-fitted values of the other parameters significantly but does reduce the uncertainties computed for these values by as much as 50%. Consequently, for all of the fittings whose results are reported here, n was fixed at 2, the value which ensures that the number of positive charges in the vicinity of DNA is conserved as Na^+ is displaced by Hex^{2+}.

Table I: Best-Fitted Parameters for the Ion-Exchange Model[a]

parameter	^{14}N[b]	^{23}Na[c]	^{14}N and ^{23}Na[d]
r_{Na}^0	(0.29 ± 0.01)	0.53 ± 0.06	0.52 ± 0.05
D (×10²)	(0.32 ± 0.19)	2.45 ± 0.29	2.39 ± 0.24
R_{2B}^N (s^{-1})	(61.7 ± 4.9)		42.7 ± 3.7
R_{1B}^N (s^{-1})	(17.3 ± 1.7)		13.9 ± 1.4
R_{Bf}^{Na} (s^{-1})		270 ± 31	276 ± 27
R_{Bs}^{Na} (s^{-1})		138 ± 14	141 ± 13

[a] Parameters obtained by nonlinear least-squares fitting of eq 2–5 with n = 2.00, R_F^N = 3.54 s^{-1}, and R_F^{Na} = 19.30 s^{-1}. [b] All of the ^{14}N data (R_2^N and R_1^N) shown in Figure 4 (cf. text). [c] All of the deconvoluted ^{23}Na relaxation rates (R_{2s}^{Na} and R_{2f}^{Na}) shown in Figure 5. [d] All of the ^{23}Na rates shown in Figure 5 together with only the ^{14}N data shown in Figure 4 for which $[Hex^{2+}]/[P] \geq 0.30$.

If R_F^N, R_F^{Na}, and n are fixed, fitting each of the four sets of relaxation rate data shown in Figures 4 and 5 requires the evaluation of only three parameters: r_{Na}^0; D; and one of the following, R_{2B}^N, R_{1B}^N, R_{Bf}^{Na}, R_{Bs}^{Na}. According to the two-state model for NMR relaxation, R_2^N and R_1^N must provide equivalent information about the ion-exchange process, as must R_{2f}^{Na} and R_{2s}^{Na}. On the basis of this assumption composite four-parameter fittings were obtained for all of the ^{14}N data in Figure 4 and, separately, for all of the ^{23}Na data in Figure 5. The resulting best-fitted values of the requisite parameters are presented in the first and second columns of Table I. The value of r_{Na}^0 obtained by fitting the ^{14}N data alone is at least 35% lower than that obtained by fitting all of the ^{23}Na data, and the best-fitted values of D obtained for the different nuclei differ by a factor of at least 4. These discrepancies far exceed the uncertainties assigned to these parameters by the nonlinear fitting routine. However, a composite fitting using only those ^{14}N data for $[Hex^{2+}]/[P] > 0.3$ together with all of the ^{23}Na data gives parameters (shown in the last column of Table I) that are in good agreement with those obtained by fitting only the ^{23}Na data. Since the ^{14}N relaxation rates for $[Hex^{2+}]/[P] < 0.3$ exhibit a breakdown in the two-state model (see the next section), the entries in the first column of Table I are shown in parentheses to indicate that our two-state analysis of the ^{14}N data entails some degree of inconsistency.

Analysis of the Quadrupolar Relaxation Rate of ^{14}N in Hex^{2+} Associated with DNA. When Hex^{2+} is associated with DNA, the time scale of the diffusional motions that modulate

Table II: ^{14}N Correlation Times and Quadrupolar Coupling Constants[a]

$[Hex^{2+}]/[P]$	R_1^N (s^{-1})	R_2^N (s^{-1})	$p_F^N R_F^N$ (s^{-1})	τ_{NB}[b] (ns)	χ_{NB} (kHz)	τ_{NB}'[c] (ns)
0.305	9.61 ± 0.28	24.91 ± 0.16	1.62	8.0 ± 0.3	26.6 ± 0.03	9.6 ± 0.5
0.382	9.17 ± 0.20	21.65 ± 0.04	1.90	7.5 ± 0.2	27.0 ± 0.2	8.8 ± 0.3
0.534	7.26 ± 0.16	17.11 ± 0.07	2.27	8.2 ± 0.3	26.0 ± 0.2	9.9 ± 0.4
0.687	6.85 ± 0.28	14.43 ± 0.09	2.51	7.6 ± 0.6	26.4 ± 0.5	9.0 ± 0.8
0.839	6.03 ± 0.25	11.88 ± 0.02	2.67	7.6 ± 0.6	25.2 ± 0.5	9.2 ± 0.9
1.145	5.43 ± 0.10	10.34 ± 0.09	2.88	8.1 ± 0.4	25.7 ± 0.3	9.8 ± 0.6

[a] Data for R_1^N and R_2^N taken from one of the titrations shown in Figure 4, over the range $0.30 \leq [Hex^{2+}]/[P] \leq 1.15$. [b] Correlation times calculated with eq A-3 by using values of p_F^N determined from the best-fitted values of D and r^0 given in third column of Table I. [c] Correlation times calculated with eq A-3, setting $p_F^N = 1$.

the quadrupolar relaxation of ^{14}N is characterized by the correlation time, τ_{NB}. With eq A-3, τ_{NB} can be calculated from measurements of both the longitudinal (R_1^N) and the transverse (R_2^N) relaxation rates of ^{14}N, provided that the difference between each of these relaxation rates and $p_F^N R_F^N$ sufficiently exceeds their associated uncertainties. This requirement is fulfilled over the range $0.30 \leq [Hex^{2+}]/[P] \leq 1.15$ for the ^{14}N relaxation rates measured during one of the titrations represented in Figure 4. For each pair of values R_1^N and R_2^N given in Table II the corresponding value of $p_F^N R_F^N$ was calculated by using eq 4 and 5 with $n = 2$ and the best-fitted values of r_{Na}^0 and D shown in the third column of Table I. Then for each set of R_1^N, R_2^N and $p_F^N R_F^N$, eq A-3 was solved for τ_{NB}.

The values of τ_{NB} given in Table II have an average of 7.8 ± 0.8 ns and exhibit no systematic trend. The corresponding values of χ_{NB}, calculated by using eq 1 and A-2, cluster around the average 26.3 ± 1.3 kHz. Both the correlation time and the quadrupolar coupling constant characteristic of ^{14}N in Hex^{2+} associated with DNA appear to be independent of the extent of titration, at least over the range of concentrations covered in Table II. At lower values of $[Hex^{2+}]/[P]$ (where the acquisition times necessary for the inversion recovery experiment are very long), only one measurement of R_1^N was made. The corresponding measurement of R_2^N implies a breakdown of the two-state model, as demonstrated by the plot of $R_1^N - R_F$ vs $R_2^N - R_F$ shown in Figure 6. It is therefore not anticipated that values of τ_{NB} or χ_{NB} calculated for $[Hex^{2+}]/[P] \lesssim 0.3$ would agree with those shown in Table II. For $[Hex^{2+}]/[P] > 1.15$ the calculation of τ_{NB} from eq A-3 is expected to grow increasingly sensitive to the model chosen to evaluate p_F^N, because $p_F^N R_F^N$ becomes an increasingly large fraction of R_2^N and (particularly) of R_1^N.

Over the range of concentrations covered in Table II, the sensitivity of our calculated correlation times to the accuracy of our description of the ion-exchange process was tested by calculating the set of correlation times τ_{NB}' that results from holding p_F^N fixed at the extreme value of unity. This condition could arise, for example, if the observed effect of DNA on ^{14}N relaxation rates were actually due to a very small (saturated) fraction of tightly bound Hex^{2+} ions, in which the ^{14}N relaxation rates are enhanced to a very large extent. Alternatively, setting $p_F = 1$ in eq A-3 could be justified by assuming that R_{1B}^N and R_{2B}^N actually are comprised of two contributions, one having a field dependence governed by eq A-1 (or A-2) and the other being a constant term equal in magnitude to R_F^N. A physical rationale for this model has been given recently (Halle & Wennerström, 1981; Halle et al., 1984). The values of τ_{NB}' shown in Table II exhibit no trend over the course of the titration, and their average 9.4 ± 1.2 ns, is only ~20% larger than the average value of τ_{NB} calculated on the basis of our ion-exchange model. This comparison indicates that the method used here to evaluate τ_{NB} may not be sensitive to the model assumptions made in describing either the concentration dependence of the association of Hex^{2+} with DNA or the form of the correlation function. Thus, the constancy of τ_{NB} demonstrated in Table II is not simply a necessary consequence of the a priori assumption that R_{2B}^N and R_{1B}^N are constant (which entered into the determination of the values of p_F^N used in calculating τ_{NB}).

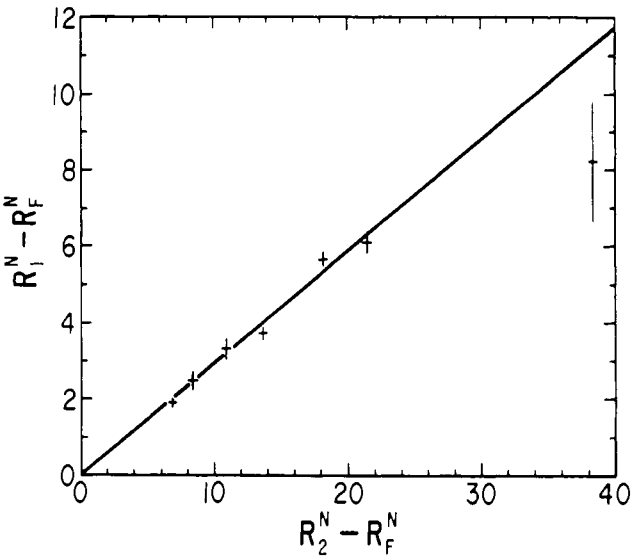

FIGURE 6: Relationship between the longitudinal ($R_1^N - R_F^N$) and transverse ($R_2^N - R_F^N$) relaxation rates of ^{14}N measured at ω_N = 26.016 MHz for a titration of NaDNA vs $HexBr_2$ under the conditions specified for Figure 4.

As a final check on the self-consistency of our approach, τ_{NB} was evaluated by introducing the best-fitted values of R_{1B}^N and R_{2B}^N given in the third column of Table I into eq A-1 and A-2. The resulting value, $\tau_{NB} = 8.4 \pm 1.6$, lies within the range of uncertainty of the values of τ_{NB} given in Table II. In summary, the dynamic time scale characteristic of the quadrupolar relaxation of ^{14}N in Hex^{2+} associated with DNA can be quantified without much reliance on the accuracy of the assumptions made in modeling the ion-exchange process. In contrast, estimates of χ_{NB} are strongly affected by any uncertainty in p_B^N, since eq A-1 or A-2 in combination with eq 1 gives the product $p_B^N \chi_{NB}^2$.

Discussion

Ion-Exchange Processes in DNA Solutions. Numerous important processes involving DNA, including conformational transitions, interactions with proteins, and intracellular packaging, are accompanied by changes in the axial charge density of DNA and hence changes in the concentrations of cations near this highly charged polyanion. Quantitative interpretations of DNA conformational and ligand-binding equilibria require information about the ion-exchange processes of the small cations in the vicinity of DNA (Anderson & Record, 1982; Record et al., 1981, 1985). To study the interactions of polyamine oligocations with DNA, we have de-

termined the concentration dependence of the ^{14}N NMR relaxation rate of nitrogen in a polyamine analogue, Hex^{2+}. As Hex^{2+} displaces Na^+ from the vicinity of DNA when NaDNA is titrated with $HexBr_2$, the ^{23}Na NMR relaxation rates provide complementary information about the interactions of Hex^{2+} with DNA.

According to the simple two-state model used here to describe the interactions of Na^+ and Hex^{2+} with DNA, three parameters (n, r_{Na}^0, and D) suffice to quantify the ion-exchange process. At the molecular level the stoichiometry of the ion-exchange process is given by n, specifically the number of Na^+ ions displaced from the vicinity of DNA by the association of each Hex^{2+} ion. The constancy of n can be tested by analyzing the relaxation rates of ^{23}Na and ^{14}N in terms of the following expression, derived by combining eq 1, 3, and 4

$$(R_{2i}^{Na} - R_F^{Na})\frac{[Na]}{[P]} = -n\frac{(R_{Bi}^{Na} - R_F^{Na})}{(R_B^N - R_F^N)}(R_{obsd}^N - R_F^N)\frac{[Hex^{2+}]}{[P]} + r_{Na}^0(R_{Bi}^{Na} - R_F^{Na})$$

where i represents either the fast or slow component of the ^{23}Na transverse relaxation process. Over the range of concentrations ($[Hex^{2+}]/[P] \geq 0.3$) where this equation can be applied to both sets of NMR data, plots of $(R_{2s}^{Na} - R_F^{Na})$ or $(R_{2f}^{Na} - R_F^{Na})$ versus $(R_2^N - R_F^N)[Hex^{2+}]/[P]$ are linear. Thus, it appears that n is constant, at least over the range of conditions for which the two-state model can be used to describe the relaxation rates of both ^{14}N and ^{23}Na. When the model parameter n is floated, its best-fitted value is 1.90 ± 0.35 if only the deconvoluted relaxation rates of ^{23}Na are fitted. A composite fitting that includes all ^{23}Na relaxation rates and those ^{14}N rates for $[Hex^{2+}]/[P] > 0.30$ gives $n = 2.01 \pm 0.29$. The maximum physically reasonable value of n for a divalent ion is 2. If $n > 2$, a net reduction of counterion charge in the near vicinity of DNA would result as the concentration of divalent cations is increased. For this system some theoretical approaches predict that n is less than 2 and varies significantly with salt concentration (Paulsen et al., 1988). However, our NMR results indicate that the stoichiometry of the Na^+–Hex^{2+} exchange is described adequately by the constant value $n = 2$.

If n has the constant value of 2, the extent of DNA charge neutralization does not vary as Na^+ displaces Hex^{2+}. For solutions containing an excess of either Na^+ or Hex^{2+}, the invariance of r_{Na}^0 and r_N^0 is indicated by the linearity of the plots given in Figures 2 and 3. The value of r_{Na}^0 reported in Table I is in the range of those inferred from previous NMR studies (Bleam et al., 1983; Braunlin et al., 1986). If only electrostatic interactions are considered to determine the distributions of small ions around a cylindrical polyion, the value of r_{Na}^0 is predicted to lie between 0.4 and 0.8 by various theoretical approaches, including Manning's counterion condensation (CC) model, the Poisson–Boltzmann (PB) cell model, and Monte Carlo (MC) simulations. The predictions of these theories for DNA solutions containing univalent ions have been discussed in a number of recent studies [see, for example, Mills et al. (1985) and references cited therein]. The constancy of r_{Na}^0 and n that has been inferred from the analysis presented here is at variance with the predictions of the PB cell model and MC simulations (Paulsen et al., 1988) but not with the CC model (Manning, 1978). The source of this discrepancy remains an object of active research.

The ion-exchange process is further characterized by the parameter D. The value of D reported in Table II [$(2.39 \pm 0.24) \times 10^{-2}$] was obtained by fitting all the ^{23}Na data and that part of the ^{14}N data for which $[Hex^{2+}]/[P] \geq 0.30$. Values of D have not been reported previously for other divalent cations. We have analyzed previously published ^{23}Na NMR relaxation rate data obtained in titrations of NaDNA with Mg^{2+} (Bleam et al., 1983) or putrescine (Put^{2+}) (Braunlin et al., 1986) according to the ion-exchange model utilized here for Hex^{2+}. Under the assumption that $n = 2$, estimates of D of 0.3×10^{-2} and 1×10^{-2} are obtained for Mg^{2+} and Put^{2+}, respectively (at 293.5 K). Thus, the exchange coefficients, D, of these divalent ions fall in the order $Mg^{2+} < Put^{2+} < Hex^{2+}$, which is inversely correlated with the ionic size. For competitive interactions of univalent ions with DNA, an analogous ordering was reported previously (Bleam et al., 1980). At present there is no independent experimental evidence to support the assumption that D is independent of the concentration of Hex^{2+} and/or Na^+. However, for a model system representing DNA solutions containing uni- and divalent counterions, both Monte Carlo and PB calculations predict only slight variations in D over the range of cation concentrations covered in our titrations. The predicted values of D (Paulsen et al., 1988) are of the same order of magnitude (10^{-2}) as that obtained here experimentally.

Over the initial portion of the titration curve illustrated in Figure 4, there is a significant discrepancy between the observed transverse relaxation data and the curve calculated by using the parameters given in Table I. This discrepancy indicates that there is at least one class of associated Hex^{2+} ions that is characterized by a significantly faster relaxation rate than the average rate manifested at ratios of $[Hex^{2+}]/[P]$ exceeding 0.30. Although the data at lower ratios of $[Hex^{2+}]/[P]$ are insufficient to permit quantification of this special class of associated Hex^{2+}, the population is evidently saturated before $[Hex^{2+}]/[P] = 0.3$. The apparent heterogeneity in ^{14}N NMR relaxation rates of Hex^{2+} need not imply specific binding "sites" for Hex^{2+}. At low ratios of cation to DNA phosphate, similar heterogeneous NMR effects have been inferred for some other divalent and higher valent ions including Ca^{2+} (Braunlin et al., 1987a,b), Mg^{2+} (Rose et al., 1980), $Co(NH_3)_6^{3+}$ (Braunlin et al., 1987a,b), and Mn^{2+} (Kennedy & Bryant, 1986).

There is no clear experimental evidence for the site binding of a ligand like Hex^{2+} with DNA in aqueous solution. Studies of the effects of a series of polyamines and their analogues on helix–coil melting temperatures have suggested the possibility of specific differences in the affinities of these ligands for DNA (Thomas & Bloomfield, 1984). Differential effects also were inferred in a previous ^{23}Na NMR study of the interactions of a series of polyamines and related ligands with DNA (Burton et al., 1980). Base-specific effects have been reported in equilibrium dialysis studies (Shapiro et al., 1969) and in melting studies of DNA in the presence of very high concentrations of the monovalent tetramethyl- and tetraethylammonium ions (Melchior & von Hippel, 1973). It is possible that the two positively charged ends of Hex^{2+} (which are analogous to the tetramethylammonium ion) show some preference for dA-dT pairs of the type observed for the tetramethyl- and tetraethylammonium ions. If the value of D implies that the local concentration of Hex^{2+} is high enough to drive a significant extent of site binding, then this effect could account for the contribution to the (average) observable ^{14}N relaxation rate that becomes relatively negligible (owing to saturation) at moderate total ligand concentrations. (To explore the possibility that binding of this type does exist, we are currently investigating the competitive association of Hex^{2+}

and Na^+ with DNAs of different base composition.)

The extent of association of sodium with DNA does not vary with base composition (Nordenskiöld et al., 1984), and there is no evidence that the association of Na^+ with DNA involves binding to specific sites. It is therefore reasonable to expect that displacement of Na^+ by Hex^{2+}, as reflected by changes in the ^{23}Na relaxation rate, is determined principally by the amount of associated Hex^{2+}. The detailed nature of the association of Hex^{2+} with DNA may not affect the applicability of the two-state ion-exchange model to Na^+ if the association of Na^+ with DNA is affected only by the compensation of phosphate charges brought about by the presence of bound Hex^{2+}. Table II shows that the values of the correlation time (and the quadrupolar coupling constant) are essentially constant over the range $[Hex^{2+}]/[P] \geq 0.3$. Moreover, the ratio of the correlation time of ^{14}N in Hex^{2+} in a DNA solution to that in the absence of DNA is of the same magnitude as the corresponding ratio for ^{23}Na (calculated by assuming a single correlation time for the dynamic processes modulating the NMR relaxation). This correspondence is consistent with the absence of any specificity in the interactions of Na^+ and Hex^{2+} with DNA.

In the present study we have demonstrated that ^{14}N NMR can be used to probe the interactions of a divalent ion, Hex^{2+}, with DNA and, in conjunction with ^{23}Na NMR, to quantify the ion-exchange process near DNA. Unlike the monatomic cations whose interactions with DNA have been studied previously, cations with positively charged ^{14}N centers afford the opportunity of testing whether their interactions with DNA are affected by variations in the charge, size, and chemical characteristics of the ligand. Studies along these lines are currently being pursued.

Acknowledgments

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Appendix

^{14}N NMR Relaxation. ^{14}N is a quadrupolar nucleus with spin $I = 1$. The NMR of ^{14}N in liquids is characterized by single-exponential relaxation under all conditions outside the slow-motion limit, where static quadrupolar splitting is manifested (Abragam, 1961; Lehn & Kintzinger, 1974). If the motions that modulate the quadrupolar interaction can be described by a single-exponential correlation function having the characteristic correlation time τ_{Ni}, then the longitudinal and transverse relaxation rate constants for ^{14}N in a particular environment i are, respectively

$$R_{1i}^{N} = \frac{3\pi^2}{10}\chi_{Ni}^2\tau_{Ni}\left(\frac{1}{1+\omega_N^2\tau_{Ni}^2} + \frac{4}{1+4\omega_N^2\tau_{Ni}^2}\right) \quad \text{(A-1)}$$

$$R_{2i}^{N} = \frac{3\pi^2}{20}\chi_{Ni}^2\tau_{Ni}\left(3 + \frac{5}{1+\omega_N^2\tau_{Ni}^2} + \frac{2}{1+4\omega_N^2\tau_{Ni}^2}\right) \quad \text{(A-2)}$$

where ω_N is the Larmor frequency and χ_{Ni} is the effective quadrupolar coupling constant of ^{14}N in the environment i (including the "asymmetry" factor, which cannot be evaluated separately from measurements on liquid samples). For ^{14}N in Hex^{2+}, the magnitude of χ_{Ni} is determined primarily by intramolecular contributions to the field gradient, and the magnitude of τ_{Ni} is determined by the time scale of the diffusional motions acting to modulate this intramolecular field gradient.

In the limit of "extreme narrowing", $\omega_N\tau_{Ni} \ll 1$, $R_{1i}^N = R_{2i}^N$, and each of these rates is proportional to $\chi_{Ni}^2\tau_{Ni}$. Outside this limit τ_{Ni} can be separated from χ_{Ni} by analyzing measurements of the transverse and longitudinal relaxation rates, provided that the relaxation processes conform to the two-state model under conditions of rapid exchange. Specifically, at each point in a titration of DNA with Hex^{2+}, the ratio R_{2B}^N/R_{1B}^N can be expressed either in terms of eq 1 and the analogous expression for R_1^N or in terms of eq A-1 and A-2 as

$$(R_2^N - p_F^N R_F^N)/(R_1^N - p_F^N R_F^N) = [10 + 37(\omega_N\tau_{NB})^2 + 12(\omega_N\tau_{NB})^4]/[10 + 16(\omega_N\tau_{NB})^2] \quad \text{(A-3)}$$

If p_F^N is evaluated on the basis of the ion-exchange model, then τ_{NB} can be calculated from measurements of R_2^N, R_1^N, and R_F^N by solving eq A-3 as a quadratic in $(\omega_N\tau_{NB})^2$. For this value of τ_{NB}, the corresponding value of χ_{NB} can then be calculated by using eq 1 and A-2.

^{23}Na NMR Relaxation. Measurements of ^{23}Na relaxation rates have provided useful information about the interactions of small cations with polyions in a variety of systems (Braunlin et al., 1986, and references cited therein). ^{23}Na has a nuclear spin $I = {}^3/_2$, and the NMR relaxation of the hydrated sodium ion in aqueous solution is completely dominated by the quadrupolar mechanism (Eisenstadt & Friedman, 1967; Forsen & Lindman, 1981). Thus, relaxation of the magnetic dipole on the sodium nucleus is due to the interactions of its nuclear electric quadrupole moment with local electric field gradients, which are modulated by various kinds of molecular motions. These field gradients arise from the water dipoles solvating the sodium ion and from other charged species in solution that are near enough to contribute. (In contrast, the field gradients acting on ^{14}N in Hex^{2+} appear to be primarily intramolecular in origin.) The transverse magnetization (M_{xy}) for a spin ${}^3/_2$ nucleus decays as the weighted sum of two exponentials (Hubbard, 1970)

$$M_{xy}(t) = M_{xy}(0)[0.6\exp(-R_{2f}t) + 0.4\exp(-R_{2s}t)] \quad \text{(A-4)}$$

where $M_{xy}(0)$ is the equilibrium magnetization and R_{2f} and R_{2s} are the fast (f) and slow (s) rate constants. Explicit expressions for R_{2f}^{Na} and R_{2s}^{Na} can be derived by assuming the two-state model with rapid exchange and a single-exponential correlation function for the quadrupolar interaction (Hubbard, 1970; Bull, 1972). It follows from eq A-4 that the Fourier transformed ^{23}Na NMR line shape is a superposition of two Lorentzian components. The broad component, whose integrated intensity is 60% of the total, has a line width at half-height of R_{2f}^{Na}/π (Hz). For the narrow component, comprising the other 40% of the signal, the line width at half-height is R_{2s}^{Na}/π (Hz). If the second-order (dynamic) frequency shift can be neglected, then each component peaks at the same Larmor frequency, ω_{Na} (Werbelow, 1979; Fouques & Werbelow, 1979; Werbelow & Marshall, 1981).

In the extreme narrowing limit, $R_{2f}^{Na} = R_{2s}^{Na}$ and the ^{23}Na NMR line shape is observed to be a single Lorentzian peak, as in an aqueous solution of NaCl. In a salt solution containing DNA the ^{23}Na NMR relaxation processes of nuclei associated with the polyion are outside the limit of extreme narrowing at the field strengths employed in the present study. Consequently, bi-Lorentzian line shapes are observed for ^{23}Na during titrations of NaDNA with $HexBr_2$. Deconvolution of these line shapes, according to an extension of the method of Delville et al. (1979), yields values of R_{2f}^{Na} and R_{2s}^{Na} for which the

concentration dependences can be analyzed according to the ion-exchange model described in the text.

REFERENCES

Abragam, A. (1961) *The Principles of Nuclear Magnetism*, Oxford University, Oxford, U.K.

Anderson, C. F., & Record, M. T., Jr. (1982) *Annu Rev. Phys. Chem. 33*, 191–222.

Anderson, C. F., Record, M. T., Jr., & Hart, P. A. (1978) *Biophys. Chem. 7*, 301–306.

Bevington, P. R. (1969) *Data Reduction and Error Analysis for the Physical Sciences*, pp 235–236, McGraw-Hill, New York.

Bleam, M. L., Anderson, C. F., & Record, M. T., Jr. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 3085–3091.

Bleam, M. L., Anderson, C. F., & Record, M. T., Jr. (1983) *Biochemistry 22*, 5418–5424.

Braunlin, W. H., & Nordenskiöld, L. (1984) *Eur. J. Biochem 142*, 133–137.

Braunlin, W. H., Strick, T. J., & Record, M. T., Jr. (1982) *Biopolymers 21*, 1301–1314.

Braunlin, W. H., Anderson, C. F., & Record, M. T., Jr. (1986) *Biopolymers 25*, 205–214.

Braunlin, W. H., Anderson, C. F., & Record, M. T., Jr. (1987a) *Biochemistry 26*, 7724–7731.

Braunlin, W. H., Drakenberg, T., & Nordenskiöld, L. (1987b) *Biopolymers 26*, 1047–1062.

Bull, T. E. (1972) *J. Magn. Reson. 8*, 344–353.

Burton, D. R., Forsen, S., & Reimarsson, P. (1981) *Nucleic Acids Res. 9*, 1219–1227.

Cohen, S. S. (1971) *Introduction to the Polyamines*, Prentice-Hall, Englewood Cliffs, NJ.

Delville, A., Detellier, C., & Laszlo, P. (1979) *J. Magn. Reson. 34*, 301–315.

Delville, A., Laszlo, P., & Schyns, R. (1986) *Biophys. Chem. 24*, 121–133.

Drew, H. R., & Dickerson, R. E. (1981) *J. Mol. Biol. 151*, 535–556.

Dufourcq, J., Clin, B., & Lemanceau, B. (1972) *FEBS Lett. 22*, 205–209.

Eisenstadt, M., & Friedman, H. L. (1967) *J. Chem. Phys. 46*, 2182–2187.

Forsen, S., & Lindman, B. (1981) *Methods Biochem. Anal. 27*, 289–486.

Fouques, C. E. M., & Werbelow, L. (1979) *Can. J. Chem. 57*, 2329–2332.

Halle, B., & Wennerstrom, H. (1981) *J. Magn. Reson. 44*, 89–100.

Halle, B., Wennerstrom, H., & Picullel, L. (1984) *J. Phys. Chem. 88*, 2482–2494.

Henriksson, U., Öldberg, L., Eriksson, J. C., & Westman, L. (1977) *J. Phys. Chem. 81*, 676–82.

Hirschman, S. Z., Leng, M., & Felsenfeld, G. (1968) *Biopolymers 5*, 227–233.

Hubbard, P. O. (1970) *J. Chem. Phys. 53*, 985–987.

Jänne, J., Poso, H., & Raina, A. (1978) *Biochim. Biophys. Acta 473*, 241–293.

Kennedy, S. D., & Bryant, R. G. (1986) *Biophys. J. 50*, 669–676.

Larsen, D. W. (1971) *J. Phys. Chem. 75*, 509–513.

Lehn, J. M., & Kintzinger, J. P. (1973) *Nitrogen NMR* (Witanowski, M., & Webb, G. A., Eds.) pp 79–161, Plenum, London.

Manning, G. S. (1978) *Q. Rev. Biophys. 11*, 179–246.

Melchior, W. B., Jr., & von Hippel, P. H. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 298–302.

Mills, P., Anderson, C. F., & Record, M. T., Jr. (1985) *J. Phys. Chem. 89*, 3984–3994.

Morris, D. R., & Marton, L. J. (1981) *Polyamines in Biology and Medicine*, Marcel Dekker, New York.

Moseley, M. E., & Stilbs, P. (1978) *Can. J. Chem. 59*, 1302–1305.

Nordenskiöld, L., Chang, D. K., Anderson, C. F., & Record, M. T., Jr. (1984) *Biochemistry 23*, 4309–4317.

Paulsen, M. D., Anderson, C. F., & Record, M. T., Jr. (1988) *Biopolymers* (in press).

Pratum, T. K., & Klein, M. P. (1983) *J. Magn. Reson. 53*, 473–485.

Record, M. T., Jr., Mazur, S. J., Melancon, P., Roe, J.-H., Shaner, S. L., & Unger, L. (1981) *Annu. Rev. Biochem. 50*, 997–1024.

Record, M. T., Jr., Anderson, C. F., Mills, P., Mossing, M., & Roe, J.-H. (1985) *Adv. Biophys. 20*, 109–135.

Reimarsson, P., Parello, J., Drakenberg, T., Gustavsson, H., & Lindman, B. (1979) *FEBS Lett. 108*, 439–442.

Rose, D. M., Bleam, M. L., Record, M. T., Jr., & Bryant, R. G. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 6289–6292.

Rose, D. M., Polnaszek, C. F., & Bryant, R. G. (1982) *Biopolymers 21*, 653–664.

Shapiro, J. T., Stannard, B. S., & Felsenfeld, G. F. (1969) *Biochemistry 8*, 3233–3239.

Tabor, C. W., & Tabor, H. (1976) *Annu. Rev. Biochem. 45*, 285–306.

Tabor, C. W., & Tabor, H. (1984) *Annu. Rev. Biochem. 53*, 749–790.

Tabor, C. W., & Tabor, H. (1985) *Microbiol. Rev. 49*, 81–99.

Thomas, T. J., & Bloomfield, V. A. (1984) *Biopolymers 21*, 1301–1314.

Van Dijk, L., Gruwel, M. L. H., Jesse, W., deBleijser, J., & Leyte, J. C. (1987) *Biopolymers 26*, 261–284.

Wemmer, D. E., Srivenugopal, K. S., Reid, B. R., & Morris, D. R. (1985) *J. Mol. Biol. 185*, 457–459.

Werbelow, L. G. (1979) *J. Chem. Phys. 70*, 5381–5383.

Werbelow, L. G., & Marshall, A. G. (1981) *J. Magn. Reson. 43*, 443–448.

Witanowski, M., & Webb, G. A., Eds. (1973) *Nitrogen NMR*, Plenum, London.

Witanowski, M., Stefaniak, L., & Webb, G. A. (1972) *Annu. Rep. NMR Spectrosc. 5A*, 395–464.

Witanowski, M., Stefaniak, L., & Webb, G. A. (1977) *Annu. Rep. NMR Spectrosc. 7*, 117–224.

Witanowski, M., Stefaniak, L., & Webb, G. A. (1981) *Annu. Rep. NMR Spectrosc. 11B.*