^{23}Na NMR Relaxation Study of the Effects of Conformation and Base Composition on the Interactions of Counterions with Double-Helical DNA†

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ABSTRACT: NMR relaxation rates (T_1^{-1} and T_2^{-1}) have been determined for ^{23}Na in aqueous salt solutions containing various types of helical double-stranded deoxyribonucleic acids. These measurements were performed on three synthetic polynucleotides having different overall conformations, poly(dA-dT)·poly(dA-dT) (alternating B-DNA), poly(dG-dC)·poly(dG-dC) at low salt (B-DNA), and Br-poly(dG-dC)·Br-poly(dG-dC) (left-handed Z-DNA), and on four types of natural DNA differing in base composition, *Clostridium perfringens* (26% GC), calf thymus (40% GC), *Escherichia coli* (50% GC), and *Micrococcus lysodeikticus* (72% GC). For all types of DNA investigated, except poly(dA-dT)·poly(dA-dT), the ^{23}Na NMR spectra measured at 21 °C and an applied field of 4.7 T are non-Lorentzian. These non-Lorentzian spectra were analyzed on the basis of the two-state model and the standard theory of nonexponential quadrupolar relaxation processes in order to obtain estimates of the correlation times (τ_c) characteristic of the sodium nuclei associated with the various nucleic acids. All of the correlation times estimated in this way are in the range of nanoseconds. The magnitudes of these correlation times show a significant dependence on the overall conformation of the nucleic acid (B vs. Z) but not on its base composition. To investigate the concentration dependence of τ_c, sodium or magnesium salts were added to solutions of Br-poly(dG-dC)·Br-poly(dG-dC) (Z-DNA). The pronounced salt dependences exhibited by the estimated correlation times can be accounted for by assuming that the dynamics of the ^{23}Na relaxation process are dominated by the radial translational diffusion of sodium ions away from the nucleic acid.

The overall conformation of a nucleic acid in solution is in part a function of the nature and the concentration of the electrolyte components. For example, the conversion of alternating purine–pyrimidine sequences from the right-handed (B) to the left-handed (Z) conformation is induced when the concentration of added salt exceeds a critical value depending on the valence and nature of the cation (Pohl & Jovin, 1972; Behe & Felsenfeld, 1981). The helix–coil transition temperatures (T_m) of natural DNAs are a function of the concentration(s) of added salt(s) as well as of the G-C content of the DNA. Moreover, the G-C dependence of T_m is a function of the salt concentration, and the salt dependence of T_m is a function of the G-C content [Blake & Haydock, 1979; see also Record et al. (1981)]. These interdependences may be interpreted on the basis of theoretical descriptions of the interactions between the polyanionic nucleic acid and the small mobile ions in solution (Manning, 1978; Record et al., 1978; Anderson & Record, 1982, 1983). It is therefore of interest to investigate experimentally how these ion–nucleic acid interactions are affected by changes in the overall conformation of the nucleic acid or by variations in its average base composition. For this purpose, cation NMR provides a relatively direct and sensitive probe.

Most monatomic cations have spin quantum number $I > 1$ and thus have quadrupole moments. NMR spectroscopy of nuclei such as ^{23}Na and ^{87}Rb (both with $I = {}^3/_2$) and ^{25}Mg ($I = {}^5/_2$) has provided much useful information about the interactions of small ions with charged biological macromolecules. In particular, ^{23}Na has frequently been utilized, because this nucleus has advantageous NMR properies from both technical and theoretical viewpoints. Numerous ^{23}Na NMR investigations of synthetic polyelectrolytes have been reported [van der Klink et al., 1974; Gustavsson et al., 1978; see references cited in Forsén & Lindman (1981)]. In several of these studies, non-Lorentzian sodium spectra were observed. Such spectra result from nonexponential quadrupolar relaxation processes and, in favorable cases, can be analyzed to obtain information at the molecular level about small ion–polyion interactions. Specifically, the analysis of non-Lorentzian spectra can provide estimates of correlation times, which reflect the motional processes affecting ^{23}Na relaxation, and of quadrupolar coupling constants, which reflect the local electrostatic environment of a sodium ion associated with a polyion.

Since previous ^{23}Na NMR studies of double-helical DNA were performed at magnetic fields lower than 2.5 T, the observed ^{23}Na spectra were Lorentzian, and therefore, separate estimates of correlation times and quadrupolar coupling constants could not be obtained. These earlier investigations were directed principally at determining the relative affinities of various univalent counterions for double-helical DNA (Bleam et al., 1980) or at evaluating the fraction of association univalent cations per DNA phosphate (Anderson et al., 1978; Bleam et al., 1983). From the latter study, it emerged that an unambiguous determination by ^{23}Na NMR of the extent to which counterions are associated with DNA requires a more detailed understanding of the quadrupolar relaxation process. The primary goals of the research reported here are to investigate whether the overall conformation and base composition of a nucleic acid can affect the electrostatic and dynamic aspects of ^{23}Na NMR relaxation and whether the relaxation rates of associated sodium nuclei depend on solution composition.

† From the Department of Chemistry, College of Letters and Science, and the Department of Biochemistry, College of Agriculture and Life Science, University of Wisconsin, Madison, Wisconsin 53706. *Received January 4, 1984.* This research was supported by grants from the National Science Foundation (PCM 81-20031) and the National Institutes of Health (GM 23467) to M.T.R. and by American Cancer Society Institutional Research Grant IN-35 to L.N. The stay of one of us (L.N.) at the University of Wisconsin was made possible in part by grants from the American Scandinavian Foundation and the Swedish Royal Academy of Science and by sabbatical leave from the University of Stockholm.

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0006-2960/84/0423-4309$01.50/0

Materials and Methods

Synthetic polynucleotides [poly(dG-dC)·poly(dG-dC), abbreviated $d(GC)_n$, and poly(dA-dT)·poly(dA-dT), abbreviated $d(AT)_n$] were obtained from P-L Biochemicals (Milwaukee, WI). The $d(GC)_n$ was dissolved in 20 mM sodium citrate, pH 7.2/1 mM ethylenediaminetetraacetic acid (EDTA)/3.5 M NaCl; the high salt lowers the pH to 6.4. The sample was split in two parts, one of which was brominated to yield Br-poly(dG-dC)·Br-poly(dG-dC), abbreviated Br-$d(GC)_n$ (Lafer et al., 1981). Br-$d(GC)_n$ was characterized by ultraviolet (UV) and circular dichroism (CD) spectroscopy. The circular dichroism spectra at low salt of $d(GC)_n$ and Br-$d(GC)_n$ demonstrated the B and Z conformation of the two polynucleotides; Br-$d(GC)_n$ gave rise to the familiar inverted CD spectrum characteristic of left-handed Z-DNA in solution. Poly(dA-dT)·poly(dA-dT) was dissolved in standard tris(hydroxymethyl)aminomethane (Tris) buffer (0.1 M NaCl/0.01 M Tris, pH 7.8/0.001 M EDTA). All three polynucleotides were then extensively dialyzed as described earlier (Anderson et al., 1978; Bleam, 1980).

Calf thymus DNA (Worthington) was purified by phenol extraction as previously described (Bleam, 1980; Bleam et al., 1983). Ethanol-precipitated calf thymus DNA and bacterial DNA (Sigma Chemical Co., used without further purification) were dissolved in standard Tris buffer and then sonicated as described by Bleam (Bleam et al., 1983). Dialyses were then performed for the synthetic polynucleotides. The native double-stranded form of all polynucleotides was checked by measuring the hyperchromicity at 260 nm. Average molecular weights of both the natural and synthetic polynucleotides were estimated by polyacrylamide gel electrophoresis (cf. Table I).

Phosphate concentrations were determined optically by using the following extinction coefficients: calf thymus, $\epsilon^{260} = 6.62 \times 10^3$ M^{-1} cm^{-1}; *Clostridium perfringens*, $\epsilon^{260} = 6.6 \times 10^3$ M^{-1} cm^{-1}; *Escherichia coli*, $\epsilon^{260} = 6.3 \times 10^3$ M^{-1} cm^{-1}; *Micrococcus lysodeikticus*, $\epsilon^{260} = 6.3 \times 10^3$ M^{-1} cm^{-1} (Wakelin & Waring, 1976); poly(dA-dT)·poly(dA-dT), $\epsilon^{262} = 6.6 \times 10^3$ M^{-1} cm^{-1} (Inman et al., 1962); poly(dG-dC)·poly(dG-dC), $\epsilon^{254} = 8.4 \times 10^3$ M^{-1} cm^{-1} (Wells et al., 1970); Br-poly(dG-dC)·Br-poly(dG-dC), $\epsilon^{255} = 6.72 \times 10^3$ M^{-1} cm^{-1} (A. Möller, personal communication and observation at this laboratory). Sodium ion concentrations were measured by neutron activation as previously described (Bleam et al., 1980). Magnesium titrations were carried out by adding small amounts of concentrated $MgCl_2$, prepared from the dried hexahydrate salt, to the NMR sample.

^{23}Na NMR measurements were performed on samples containing 40% D_2O (v/v) by use of a JEOL FX-200 NMR spectrometer (Chemistry Instrumentation Center) at a resonance frequency of 52.74 MHz. Temperature was controlled at 20.5 ± 0.5 °C unless otherwise stated. Longitudinal (T_1) relaxation time measurements were made by using the inversion recovery (180°–τ–90°) pulse sequence. Transverse (T_2) relaxation times were obtained from a Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence (Farrar & Becker, 1971). The T_1 and T_2 values were obtained by a linear least-squares fit of the logarithm of the magnetization vs. delay time. Reported values are averages of at least two measurements. The experimental errors in the individual relaxation measurements are estimated to be 10–15% for T_2 and 5–10% for T_1. Extraction of the two transverse relaxation rates T_{2f}^{-1} and T_{2s}^{-1} (defined under ^{23}Na Relaxation Theory) from non-Lorentzian spectra was accomplished by fitting the observed line shape to a sum of two Lorentzians with the appropriate amplitude weighting. The fitting was evaluated by visual comparison of theoretical and experimental spectra. Reported line widths, $\Delta\nu_{1/2}$, are full line widths at half the peak heights of the absorption signals with an estimated accuracy of 3%. The line width at half-height, $\Delta\nu_{1/2}$, for free sodium ion was determined to be 6.6 Hz at 20.5 °C for a 0.1 M NaCl sample containing 40% D_2O.

^{23}Na Relaxation Theory

^{23}Na is a quadrupolar nucleus with a spin quantum number $I = {}^3/_2$. For sodium ions in aqueous solution, the NMR relaxation process is due to the fluctuating interaction between the nuclear quadrupole moment and the electric field gradient at the site of the nucleus (Cohen & Reif, 1957). The mean square field gradient created by the fluctuating charge distribution surrounding the nucleus determines the magnitude of the quadrupolar interaction strength. In general, the quadrupolar relaxation of a spin ${}^3/_2$ nucleus decays as a weighted sum of two exponentials (Hubbard, 1970). The resulting transverse magnetization in a coordinate system rotating at the resonance frequency is

$$M_{xy}(t) = M(0)[0.6 \exp(-T_{2f}^{-1}t) + 0.4 \exp(-T_{2s}^{-1}t)] \quad (1)$$

where $M_{xy}(t)$ and $M(0)$ are the transverse magnetization at time t and the equilibrium magnetization, respectively, and T_{2f} and T_{2s} are the "fast" and "slow" time constants, respectively. The high-resolution spectrum is obtained by taking the Fourier transform of eq 1. The result is a superposition of two Lorentzians with an amplitude weighting of 1.5:1 and with different line widths at half-height, $(\pi T_{2f})^{-1}$ and $(\pi T_{2s})^{-1}$, respectively. The two Lorentzian components have the same Larmor frequency if the second-order (dynamic) frequency shift can be neglected (Webelow, 1979; Fouques & Werbelow, 1979; Werbelow & Marshall, 1981). An expression similar to eq 1 also holds for the longitudinal relaxation (Hubbard, 1970).

For the interpretation of ^{23}Na relaxation measurements in polyelectrolyte solutions, the two-state model is commonly assumed. Hence, the sodium-23 ions are considered to be either in the "free" state, equivalent to the aqueous bulk phase where the relaxation is characterized by a single rate constant (R_F), or in a "bound" state associated with the polyion, where (in general) the transverse relaxation is characterized by two rates, $(T_{2f}^B)^{-1}$ and $(T_{2s}^B)^{-1}$. If the exchange between the two states is fast on the NMR time scale (if the exchange rate is fast compared to the intrinsic relaxation rates in the two states), then the two rate constants T_{2f}^{-1} and T_{2s}^{-1} are given by (Bull, 1972)

$$T_{2f}^{-1} = p_F R_F + p_B (T_{2f}^B)^{-1} \quad (2a)$$

$$T_{2s}^{-1} = p_F R_F + p_B (T_{2s}^B)^{-1} \quad (2b)$$

where p_F and p_B are the average fractions of sodium ions in the free and the bound state, respectively. These free and bound states are defined from the point of view of the quadrupolar interaction experienced by the sodium ion. Thus, in the present context, the bound state refers to sodium ions in the vicinity of a nucleic acid which are subject to a different mean square electric field gradient and/or modulation of the quadrupolar interaction as compared to ions which are in the free reference state, defined as being the same as for ions in a dilute aqueous solution. This operationally defined bound state need not correspond to the extent of counterion association predicted on the basis of the counterion condensation theory (Manning, 1979) or estimated by integrating the Poisson–Boltzmann counterion radial distribution function (Katchalsky, 1971).

Additional assumptions about the relaxation in the bound state, characterized by T_{2f}^{B} and T_{2s}^{B}, must be introduced in order to get explicit expressions for the relaxation times T_{2s} and T_{2f}. It is common to assume that the molecular motion(s) modulating the quadrupolar interaction is (are) isotropic and that the relevant correlation function of the field gradient decays exponentially with an effective correlation time τ_c, giving for eq 2a,b (Abragam, 1961; Hubbard, 1970)

$$T_{2f}^{-1} = p_F R_F + p_B \frac{\pi^2}{5} \chi_B^2 \left(\tau_c + \frac{\tau_c}{1 + \omega_o^2 \tau_c^2} \right) \quad (3a)$$

$$T_{2s}^{-1} = p_F R_F + p_B \frac{\pi^2}{5} \chi_B^2 \left(\frac{\tau_c}{1 + \omega_o^2 \tau_c^2} + \frac{\tau_c}{1 + 4\omega_o^2 \tau_c^2} \right) \quad (3b)$$

where ω_o is the resonance frequency and χ_B the quadrupolar coupling constant (in hertz) in the bound state:

$$\chi_B \equiv \left| \frac{eQ}{h} \frac{\partial^2 V_z^F}{\partial z^2} \right| \quad (4)$$

eQ is the nuclear quadrupole moment, h is Planck's constant, and V_z^F is the z component of the mean electrostatic potential in the principal axis system of the field gradient tensor (F), assumed to have cylindrical symmetry. In the zero-field (extreme narrowing) limit, when $\omega_o \tau_c << 1$, the two transverse relaxation rates are equal to each other and to the longitudinal rate, T_1^{-1}. In this case, τ_c cannot be separated from the product $p_B \chi_B^2$. In the intermediate range, as long as $\omega_o \tau_c \lesssim 1.5$, the relaxation remains "nearly exponential", and the following equations are accurate first-order expressions (McLachlan, 1964; Bull, 1972; Halle & Wennerström, 1981):

$$T_1^{-1} = p_F R_F + \frac{p_B \pi^2 \chi_B^2}{5} \tau_c \left(\frac{0.4}{1 + \omega_o^2 \tau_c^2} + \frac{1.6}{1 + 4\omega_o^2 \tau_c^2} \right) \quad (5a)$$

$$T_2^{-1} = p_F R_F + \frac{p_B \pi^2 \chi_B^2}{5} \tau_c \left(0.6 + \frac{1}{1 + \omega_o^2 \tau_c^2} + \frac{0.4}{1 + 4\omega_o^2 \tau_c^2} \right) \quad (5b)$$

The applicability of these expressions for the evaluation of T_1 and T_2 from linear fits of the time-dependent magnetization in pulse experiments (inversion recovery and CPMG) depends on the magnitudes of p_B, χ_B, and τ_c (Halle & Wennerström, 1981).

When the quadrupolar relaxation is outside the limit of extreme narrowing, the correlation time τ_c can be obtained by a number of methods (Gustavsson et al., 1978). A fit of the Fourier-transformed spectrum to a sum of two Lorentzians gives the two relaxation rates T_{2f}^{-1} and T_{2s}^{-1} (eq 3a,b). The appropriate pulsed experiments give T_1^{-1} and T_2^{-1} (eq 5a,b). Because R_F is known and p_F can be estimated, for example, from the counterion condensation theory, subtracting the product $p_F R_F$ from each of the rate constants permits the separation of τ_c from $p_B \chi_B^2$. The values of τ_c calculated in this way are insensitive to the uncertainty in p_F. In contrast, χ_B cannot be evaluated accurately without an independent means of determining p_B.

The preceding theoretical development forms the basis for the analysis of the ^{23}Na NMR results discussed below. A number of similar ^{23}Na relaxation studies of polyelectrolyte systems have appeared in the literature [for a review of work on both synthetic and biological systems, see Forsén & Lindman (1981)]. It must be noted that the conventional assumption of a single exponential correlation function may not be valid for the systems under consideration here. In polyelectrolyte systems, the averaging of the quadrupolar relaxation is more generally described by a two-step model which leads to a superposition of two correlation functions (Wennerström et al., 1974; Halle & Wennerström, 1981). More recently, a mean field theoretical description of the dynamics of quadrupolar relaxation for counterions in polyelectrolyte solutions has been developed (B. Halle et al., private communication). However, the incorporation of a more detailed dynamic model into the analysis of the ^{23}Na NMR data reported here is not expected to alter the general conclusions of this work.

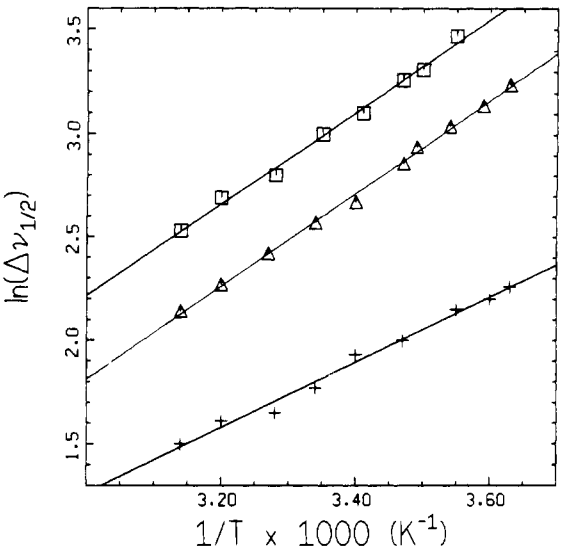

FIGURE 1: Temperature dependence of the ^{23}Na line width at half-height in solutions of d(GC)$_n$ (△) ([P] = 2.6 mM, [Na]/[P] = 1.5), Br-d(GC)$_n$ (□) ([P] = 3.2 mM, [Na]/[P] = 1.4), and in aqueous solution (+) ([Na] = 0.1 M).

Results

Line Shapes and Relaxation Times. (A) Synthetic Polynucleotides. To establish that the fast exchange limit is applicable to sodium ions in the synthetic polynucleotide solutions, the ^{23}Na line width at half-height, $\Delta\nu_{1/2}$, was measured as a function of temperature in the range 0–50 °C in solutions of d(GC)$_n$ and Br-d(GC)$_n$. In both cases, the ^{23}Na line shapes are non-Lorentzian, and $\Delta\nu_{1/2}$ is not a direct measure of the relaxation rate. However, the temperature dependence of $\Delta\nu_{1/2}$ remains an accurate qualitative indicator of the fast exchange condition. For solutions of NaDNA (calf thymus), it has been shown that p_B has a negligible temperature dependence (Bleam et al., 1983). It is therefore reasonable to assume that in the present case the observed temperature dependence of $\Delta\nu_{1/2}$ reflects the temperature dependence of the relaxation rate. Figure 1 presents plots of ln $\Delta\nu_{1/2}$ vs. $1/T$ (reciprocal absolute temperature) for solutions of d(GC)$_n$, Br-d(GC)$_n$, and, for comparison, an NaCl solution containing no polyions. The positive slopes of the lines for d(GC)$_n$ and Br-d(GC)$_n$ clearly demonstrate that fast exchange is valid for ^{23}Na relaxation in these solutions. Since the relationship between $\Delta\nu_{1/2}$ and τ_c is very complicated for non-Lorentzian line shapes, accurate activation parameters cannot, in general, be estimated simply from plots of ln $\Delta\nu_{1/2}$ vs. $1/T$.

In solutions of the two polynucleotides d(GC)$_n$ and Br-d(GC)$_n$, the ^{23}Na relaxation deviates to various extents from the extreme narrowing condition, as demonstrated by the non-Lorentzian line shapes and the inequality of T_1 and T_2.

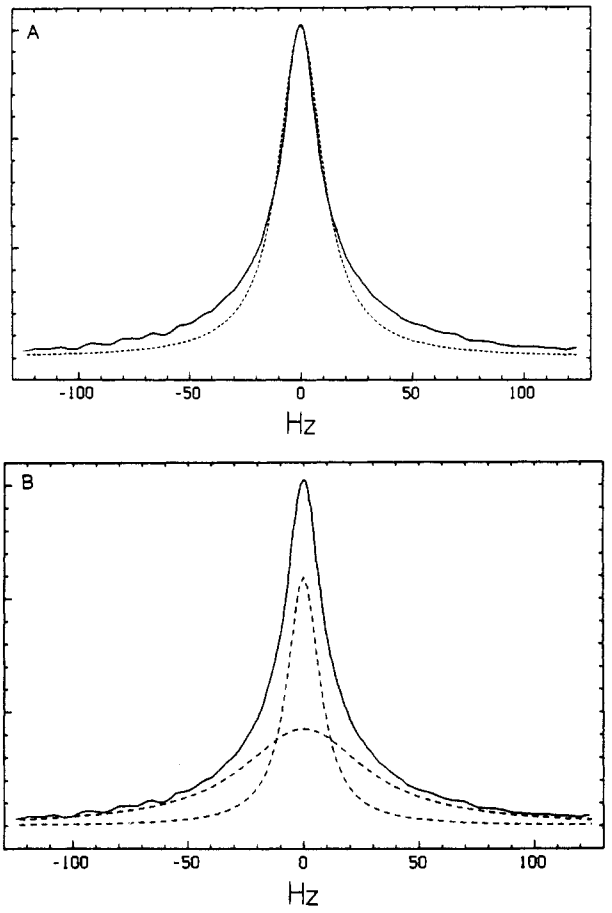

FIGURE 2: ^{23}Na line shape in Br-d(GC)$_n$ solution. (A) Experimental line shape (solid curve) and Lorentzian line shape (dashed curve) with the same amplitude and peak height as the experimental spectrum. (B) Experimental line shape (solid curve) and the fast and slow components (dashed curves) contributing to the theoretical line shape, calculated with T_{2f} = 4.8 ms and T_{2s} = 18.5 ms. The experimental conditions for the experimental line shape in these figures are given in Table I. The number of accumulated scans was 270 000, and the recycle time was 98 ms.

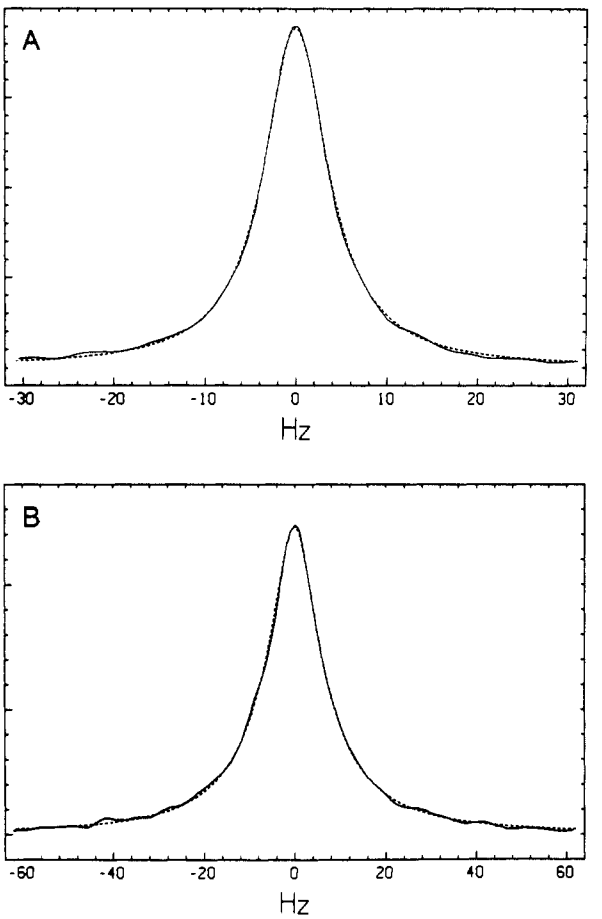

FIGURE 3: (A) Experimental (solid curve) and theoretical (dashed curve) ^{23}Na line shapes in d(GC)$_n$ solution. The theoretical curve was calculated with T_{2f} = 14.7 ms and T_{2s} = 29.4 ms. Experimental conditions are given in Table I. The number of accumulated scans was 73 000, and the recycle time was 146 ms. (B) Experimental (solid curve) and theoretical (dashed curve) ^{23}Na line shapes in d(AT)$_n$ solution. The theoretical curve was calculated with T_2 = 38 ms. Experimental conditions are given in Table I. The number of accumulated scans was 20 000, and the recycle time was 228 ms.

This effect is most clearly illustrated in Figure 2 for Br-d(GC)$_n$ (Z-DNA). Figure 2A shows a typical experimental ^{23}Na spectrum, together with a calculated Lorentzian curve with the same amplitude and line width at half-height. The non-Lorentzian character is quite obvious; the experimental spectrum is broader at the bottom and narrower at the top. Figure 2B shows the two Lorentzian components that provide the best fit to the experimental spectrum. These components, weighted according to eq 1, provide a fit that is indistinguishable from the experimental spectrum. The general relaxation theory for non-Lorentzian resonances of spin $^3/_2$ nuclei predicts a second-order (dynamic) shift between the two Lorentzian components which, if detectable, would cause the measured line shape to be asymmetric (Werbelow & Marshall, 1981). This effect may complicate the adjustment of the phase and thereby increase the uncertainty in the line shape. For the synthetic deoxyribopolynucleotides under consideration in this section, we have not been able to detect any definite asymmetry in the line shapes. Thus, no dynamic shift between the two Lorentzian components was assumed in fitting the experimental spectra.

In Figure 3, experimental and fitted spectra are shown for (a) d(AT)$_n$ and (b) d(GC)$_n$. For d(AT)$_n$, a single Lorentzian gives a good fit. The spectrum for d(GC)$_n$ can best be fitted to a sum of two Lorentzians. The experimental spectra in Figures 2 and 3 were all recorded under similar experimental conditions (see figure captions), and the line widths at half-height thus give a qualitative comparison of the relaxation enhancement in the bound state. The line width for d(AT)$_n$ (8.5 Hz) is surprisingly small (only 2 Hz broader than the free sodium ion line width). The line widths for d(GC)$_n$ and Br-d(GC)$_n$ (14.2 and 23.5 Hz, respectively) differ by nearly a factor of 2 but are both substantially smaller than those previously observed by Bleam et al. (1983) for calf thymus and T7 DNA under similar conditions (~40 Hz; see below). Estimates of the correlation time τ_c for sodium ions associated with d(GC)$_n$ and Br-d(GC)$_n$ have been obtained by deconvoluting the observed non-Lorentzian spectra and applying eq 3a,b. Correlation times can also be estimated by direct measurement of T_1 and T_2 and the application of eq 5a,b. Values of τ_c estimated from the ratio T_1/T_2 are systematically lower than those obtained from the deconvolution of line shapes. We have relied on the latter method because for solutions of Br-d(GC)$_n$ $\omega_0\tau_c$ is so large (>1.5) that it exceeds the range of accuracy of eq 5a,b. (It is possible that the systematic discrepancies between correlation times determined by the two methods are real and that they result from a breakdown of the assumption that the field gradient correlation function is a single exponential. Further investigation of this point is in progress.)

In Table I, the results of ^{23}Na relaxation rate measurements and of deconvolution of the non-Lorentzian ^{23}Na spectra to

Table I: ^{23}Na Relaxation Times and Results of Line-Shape Analysis in Solutions of Br-d(GC)$_n$, d(GC)$_n$, and d(AT)$_n$[a]

system	[P] (mM)	[Na]/[P]	M[b]	T_2 (ms)	T_1 (ms)	T_{2f} (ms)	T_{2s} (ms)	$\omega_0\tau_c$	τ_c (ns)	$(r^o)^{1/2}\chi$ (kHz)
Br-d(GC)$_n$	3.2	1.4	1×10^6	8.6	20.6	4.8	18.5	2.0	5.9 ± 0.4	100 ± 20
d(GC)$_n$	2.6	1.5	1×10^6	23.1	31.6	14.7	29.4	1.2	3.5 ± 0.3	66 ± 14
d(AT)$_n$	3.6	1.4	0.3×10^6	40.8	44.6					

[a] At a ^{23}Na resonance frequency of 52.7 MHz and at a temperature of 20.5 ± 0.5 °C. The correlation times were calculated by assuming r^o (=[Na]$_B$/[P]) equals 0.76 for d(GC)$_n$ and d(AT)$_n$ and 0.74 for Br-d(GC)$_n$, the values predicted by the counterion condensation theory (Manning, 1979). [b] Molecular weight estimated from gel electrophoresis.

Table II: ^{23}Na Relaxation Times and Results of Line-Shape Analysis for Four Natural DNAs[a]

system	[P] (mM)	[Na]/[P]	M[b]	T_2 (ms)	T_1 (ms)	T_{2f} (ms)	T_{2s} (ms)	$\omega_0\tau_c$	τ_c (ns)	$(r^o)^{1/2}\chi$ (kHz)
Cl. perfringens (26% GC)	3.2	1.8	0.4×10^6	12.4	19.0	9.8	17.5	0.9	2.7 ± 0.3	100 ± 20
calf thymus (40% GC)	4.5	1.6	3×10^6	5.9	10.8	4.8	9.8	1.0	3.0 ± 0.3	132 ± 25
E. coli (50% GC)	4.9	1.5	0.4×10^6	7.3	14.2	7.2	12.4	0.8	2.5 ± 0.3	110 ± 20
M. lysodeikticus (72% GC)	4.8	1.5	1×10^6	11.8	18.8	8.2	17.0	1.2	3.5 ± 0.4	93 ± 18

[a] At a ^{23}Na resonance frequency of 52.7 MHz and at a temperature of 20.5 ± 0.5 °C. The correlation times were calculated by assuming r^o (=[Na]$_B$/[P]) equals 0.76, the value predicted by the counterion condensation theory (Manning, 1979). [b] Molecular weight estimated from gel electrophoresis.

give T_{2f} and T_{2s} are shown for the three synthetic polynucleotides Br-d(GC)$_n$, d(GC)$_n$, and d(AT)$_n$. Also given are the values for $\omega_0\tau_c$, τ_c, and the product $(r^o)^{1/2}\chi_B$, calculated from T_{2f} and T_{2s} in combination with eq 3a,b. Here r^o is the fraction of sodium ions bound per DNA phosphate, so that $p_B = r^o[P]/[Na]$, where [P] and [Na] are the total phosphate and sodium concentrations, respectively. Within experimental error, there is no difference between T_1 and T_2 for d(AT)$_n$. Thus, we can obtain only an upper bound for the correlation time in this system ($\tau_c < 1$ ns). As expected from the line shapes, the difference between T_1 and T_2 is larger for Br-d(GC)$_n$ than for d(GC)$_n$ and gives a longer correlation time for Br-d(GC)$_n$ (5.9 ns) than for d(GC)$_n$ (3.5 ns). This effect is also seen in the difference between T_{2f} and T_{2s} for the fast and slow components. It should be pointed out that this pronounced difference is reproduced to within 5% with samples prepared from different batches of d(GC)$_n$ obtained from the manufacturer. The values of the product $(r^o)^{1/2}\chi_B$ in Table I are of the order 100 kHz; that of Br-d(GC)$_n$ (100 kHz) is larger than that of d(GC)$_n$ (66 kHz). This relatively small difference is probably outside the range of experimental uncertainty. If r^o for Br-d(GC)$_n$ is assigned the value 0.74 (obtained by identifying the bound state in the NMR two-state model with the state of condensed counterions in Manning's counterion condensation theory, and by using the value ξ = 3.8 for the *average* reduced axial charge density of Z-DNA), the quadrupolar coupling constant is calculated to be 116 kHz. This value is close to previous estimates of the ^{23}Na quadrupolar coupling constant in synthetic polyelectrolyte systems (Gustavsson et al., 1978).

(*B*) *Natural DNA.* For the four natural DNA samples that have been investigated, the ^{23}Na relaxation is nonexponential under the conditions employed. (Nonexponential relaxation results in non-Lorentzian spectra and the inequality of T_1^{-1} and T_2^{-1}.) [This effect has also been observed for ^{87}Rb in calf thymus DNA solution (D. K. Chang, personal communication).] For all four natural DNAs (in contrast to the synthetic polynucleotides), a slight asymmetry of the line shape, always to the high-field side of the spectrum, was observed. A dynamic shift would cause the broader (fast) component to be shifted upfield relative to the narrow one (Werbelow & Marshall, 1981). Including a shift between the two contributing Lorentzians improves the fitting considerably but does not change the ratio between the two time constants T_{2f} and T_{2s}, and consequently does not affect the evaluation of τ_c. The

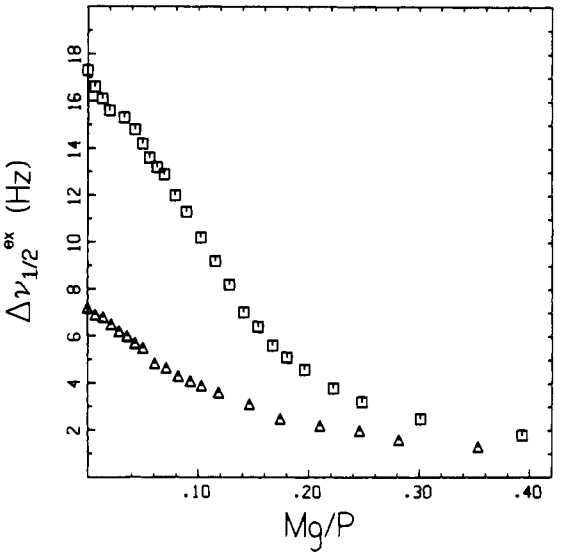

FIGURE 4: Excess ^{23}Na line widths at half-height, $\Delta\nu_{1/2}^{ex}$, as a function of [Mg]/[P] during magnesium titration of Br-d(GC)$_n$ (□) and d(GC)$_n$ (△). For Br-d(GC)$_n$, [P] = 2.8 mM and [Na]/[P] = 1.7. For d(GC)$_n$, [P] = 2.6 mM and [Na]/[P] = 1.9. The temperature was 20.5 ± 0.5 °C.

shifts in each case are small; the largest value, 3.4 Hz, is obtained for calf thymus DNA.

In Table II, relaxation times and the results of line-shape analysis, together with calculated values of τ_c and $(r^o)^{1/2}\chi_B$, are presented for the natural DNA samples: *M. lysodeikticus*, *E. coli*, calf thymus, and *Cl. perfringens*. The ratios of T_1/T_2 and T_{2f}/T_{2s} are rather similar for all systems and give correlation times that are all approximately 3 ns. The values of the product $(r^o)^{1/2}\chi_B$ are also similar (approximately 100 kHz), though the value in calf thymus solution (132 kHz) is slightly larger than those for the other samples.

Magnesium Titrations in Solutions of d(GC)$_n$ and Br-d(GC)$_n$. To obtain information on the extent of association of sodium ions with DNA, the ^{23}Na line width at half-height has been determined from titrations with a strong competitor such as magnesium (Bleam, 1980; Bleam et al., 1983). Experiments of this type also give information on the effectiveness of magnesium as a competitor with sodium ion for association with DNA. In Figure 4, the excess ^{23}Na line width at half-height, $\Delta\nu_{1/2}^{ex}$, in solutions of d(GC)$_n$ and Br-d(GC)$_n$ is plotted as a function of [Mg]/[P]. ([Mg] and [P] represent the total

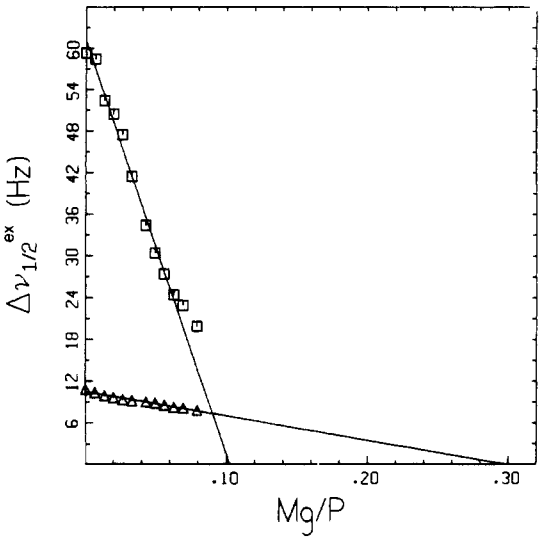

FIGURE 5: Fast (□) and slow (△) components of the ^{23}Na line shape, plotted as $\Delta\nu_{1/2}^{ex} = \Delta\nu_{1/2} - \Delta\nu_{1/2,F}$ as a function of [Mg]/[P] during magnesium titration of Br-d(GC)$_n$.

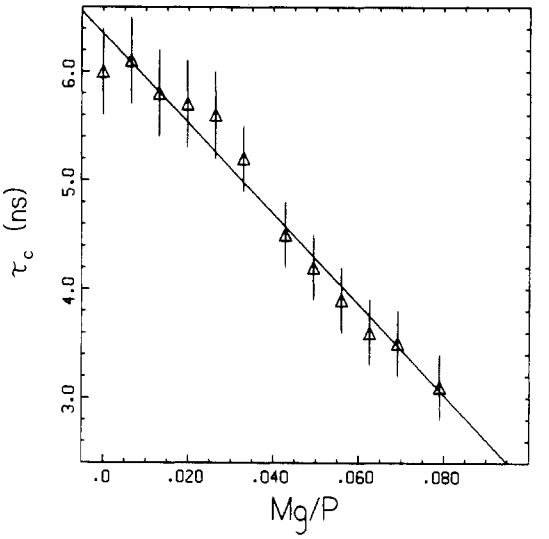

FIGURE 6: Correlation time τ_c as a function of [Mg]/[P] during magnesium titration of Br-d(GC)$_n$.

magnesium and phosphate concentrations, respectively; $\Delta\nu_{1/2}^{ex} = \Delta\nu_{1/2} - \Delta\nu_{1/2,F}$, where $\Delta\nu_{1/2,F}$ is the free sodium line width.) The strong preference of magnesium for DNA is clearly demonstrated for both d(GC)$_n$ and Br-d(GC)$_n$. This qualitative effect is expected from both the counterion condensation and the Poisson–Boltzmann theories of cylindrical polyelectrolytes. It can also be noted that both curves appear essentially linear over the range $0 \leq [Mg]/[P] < 0.1$.

Analogous magnesium titration curves have been determined for solutions of calf thymus DNA (Bleam, 1980; Bleam et al., 1983). Those experiments were conducted at a field strength of 2.35 T, where the ^{23}Na line shape is essentially Lorentzian, and the transverse relaxation rates can be obtained directly from the line widths at half-height: $\Delta\nu_{1/2} = (\pi T_2)^{-1}$. On the basis of the two-state model for rapidly exchanging nuclei, the excess line width $\Delta\nu_{1/2}^{ex}$ is related to [Mg]/[P] by the following equation (Bleam et al., 1983):

$$\Delta\nu_{1/2}^{ex} = (\Delta\nu_{1/2,B} - \Delta\nu_{1/2,F})([P]/[Na])(r^o - np_M[Mg]/[P]) \quad (6)$$

where the subscripts B and F refer to sodium ions associated with DNA and in the free state, respectively, n is the ratio of the total number of sodium ions that have been displaced by magnesium to the total number of associated magnesium ions at some point in the titration, and p_M is the fraction of associated magnesium ions, $[Mg]_B/[Mg]$. According to eq 6, the simplest interpretation of the observed linearity of the initial portion of a magnesium titration curve is that the quantities $\Delta\nu_{1/2,B} - \Delta\nu_{1/2,F}$ and np_M are constant. If they are, then the intercept at $\Delta\nu_{1/2}^{ex} = 0$ obtained by extrapolating the linear portion of the titration curve is predicted to be $r^o/(np_M)$.

Since the ^{23}Na resonance in solutions of d(GC)$_n$ does not exhibit large deviations from extreme narrowing ($\omega_o\tau_c \lesssim 1$), eq 6 could be applied directly to the analysis of magnesium titrations of d(GC)$_n$. However, for this system, the line-width enhancements were relatively small ($\Delta\nu_{1/2}^{ex} \lesssim 2\Delta\nu_{1/2,F}$). In solutions of Br-d(GC)$_n$, the observed $\Delta\nu_{1/2}^{ex}$ values are significantly larger, and the line shapes are distinctly non-Lorentzian ($\omega_o\tau_c \lesssim 2$). These line shapes can, however, be resolved into fast and slow components according to the preceding discussion. Then, on the basis of the two-state model, eq 6 can be applied to analyze the excess line widths at half-height for each component, $\Delta\nu_{1/2,f}^{ex}$ and $\Delta\nu_{1/2,s}^{ex}$. The results of this analysis of the ^{23}Na line shape for a magnesium titration of Br-d(GC)$_n$ are presented in Figure 5. The initial portion of the curve for the fast component is linear, with an extrapolated intercept (at $\Delta\nu_{1/2,f}^{ex} = 0$) of 0.10. For the slow component, which is approximately linear over an extended range, the intercept at $\Delta\nu_{1/2,s}^{ex} = 0$ is 0.30. The discrepancy between the two intercepts definitely exceeds the uncertainties arising from scatter in the data or errors in the Lorentzian fitting procedure. The inequality of the intercepts can only be explained in terms of eq 6 by concluding that at least one of the two bound line widths, $\Delta\nu_{1/2,f}^{B}$ and $\Delta\nu_{1/2,s}^{B}$, does vary with the addition of magnesium. To test this conclusion further, the correlation time at each point of the titration was evaluated.

To calculate τ_c from $\Delta\nu_{1/2,f}^{B}$ and $\Delta\nu_{1/2,s}^{B}$ at a particular point in the titration, it is necessary to know the extent of sodium ion association or, equivalently, r^o and np_M. The latter product is expected to be virtually constant during the initial stage of the titration from calculations based either on the Poisson–Boltzmann equation (Bleam, 1980) or on a modification of Manning's two variable theory of counterion condensation (C. F. Anderson, unpublished results). For the purpose of a representative calculation, np_M has been assigned the maximal value of 2, and r^o has been set equal to 0.74, the limiting law value predicted for left-handed Z-DNA by the counterion condensation theory. Resulting values for τ_c calculated with eq 3a,b and 6 from the fast and slow components of the ^{23}Na line shape are plotted in Figure 6 as a function of [Mg]/[P]. Alternative physically reasonable choices of r^o and np_M lead to plots that are qualitatively analogous to Figure 6. Clearly, the correlation time characterizing ^{23}Na relaxation is a monotonically decreasing, approximately linear function of the magnesium ion concentration. A dependence of τ_c and hence of $\Delta\nu_{1/2,f}^{B}$ and $\Delta\nu_{1/2,s}^{B}$ on [Mg] would account for the difference in the extrapolated initial slopes of the curves plotted in Figure 5 for the two components of the ^{23}Na resonance. The bound line width characteristic of the fast component, which is dominated by the first term (τ_c) in eq 3a under the conditions of these experiments, is expected from the results in Figure 6 to *decrease* with increasing magnesium additions. In contrast, the bound line width characteristic of the slow component under the conditions of these experiments is predicted to *increase* with increasing magnesium additions, because according to eq 3b $\Delta\nu_{1/2,s}^{B}$ has a predominantly inverse dependence on τ_c.

If the quadrupolar coupling constant of the associated sodium nuclei does not vary during the magnesium titration, then

Table III: Salt Dependence of ^{23}Na Transverse Relaxation Rates and Correlation Times in Solutions of Br-d(GC)$_n$ at a Constant Phosphate Concentration of 3.4 mM[a]

[Na] (mM)	T_{2s} (ms)	T_{2f} (ms)	τ_c (ns)
4.9	17.7	4.8	5.6 ± 0.4
5.2	18.3	5.6	5.2 ± 0.4
5.7	19.1	6.8	4.6 ± 0.3
6.2	19.8	7.7	4.3 ± 0.3
6.7	20.4	8.7	3.9 ± 0.3
7.4	22.6	11.4	3.4 ± 0.3
7.9	23.2	13.3	2.9 ± 0.3

[a] At a ^{23}Na resonance frequency of 52.7 MHz and at a temperature of 20.5 ± 0.5 °C. The content of D_2O in this sample was 24% (v/v). The line width at half-height for a sodium chloride solution (0.1 M), $\Delta\nu_{1/2,F}$, with 24% D_2O was determined to be 6.2 Hz. The correlation times were calculated by assuming r^o (=$[Na]_B/[P]$) equals 0.74, the value predicted by the counterion condensation theory (Manning, 1979).

it follows from the preceding analysis that the intercepts of the titration curves shown in Figure 5 for the fast and slow components of the ^{23}Na resonance provide lower and upper bounds, respectively, on the quantity $r^o/(np_M)$. The upper bound (0.30) is approximately equal to the value predicted for Z-DNA by the Poisson–Boltzmann equation but is significantly lower than the value predicted by the counterion condensation model (0.4). [These theoretical values for $r^o/(np_M)$ have been estimated from analogous calculations for the case of uni- and divalent ion association with B-DNA (Bleam et al., 1983).] Before drawing any inferences about the accuracy of either the Poisson–Boltzmann equation or the counterion condensation model, however, it will be necessary to perform additional titrations at different magnetic field strengths. These experiments (in progress) will serve to test the form of the correlation function characteristic of associated sodium ions, as well as the constancy of their quadrupolar coupling constant with respect to changes in the concentration(s) of added salt(s).

Sodium Titrations of Solutions of Br-d(GC)$_n$. The dependence of the correlation time on the addition of magnesium to Br-d(GC)$_n$ solutions could be due to a general salt effect on the modulation of the quadrupolar relaxation of ^{23}Na in DNA solutions. In order to test this hypothesis further, we have evaluated τ_c at various points in a titration of Br-d(GC)$_n$ with NaCl. In Table III, the result of line-shape analysis, giving the two transverse relaxation times T_{2s} and T_{2f}, is presented together with the correlation times obtained from eq 3a,b for seven different sodium concentrations at a constant polynucleotide concentration. In Figure 7, the correlation time τ_c is plotted as a function of total sodium concentration; τ_c decreases in an approximately linear manner with increasing sodium concentration. An explanation for the results in Figures 6 and 7 is proposed under Discussion.

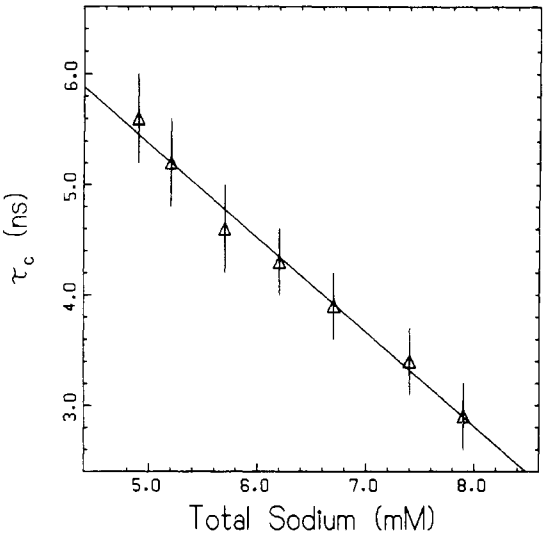

FIGURE 7: Correlation time τ_c as a function of total sodium concentration, [Na], during sodium titration of Br-d(GC)$_n$. Experimental conditions are given in Table III.

Discussion

Quadrupolar Coupling Constants. The values of the product $(r^o)^{1/2}\chi_B$ are similar in all six DNA systems for which we have obtained this quantity from the line-shape analysis. According to various theoretical predictions, the fraction of DNA phosphate neutralized by bound sodium, r^o, lies in the range 0.34–0.76. The value 0.76 is obtained from the counterion condensation theory of cylindrical polyelectrolytes as applied to B-DNA (Manning, 1979). The lower value, 0.34, can be obtained from a numerical solution of the Poisson–Boltzmann equation applied to a solution of B-DNA in which associated sodium is not site bound (Bleam, 1980). If r^o is given the value 0.76 for the calf thymus DNA system, then from the corresponding value of $(r^o)^{1/2}\chi_B$ (132 kHz) (Table II), the quadrupolar coupling constant is estimated to be 151 kHz. With r^o = 0.34, a quadrupolar coupling constant of 226 kHz is obtained. The value of χ_B for ^{23}Na associated with DNA is thus probably not larger than a few hundred kilohertz. This upper bound is unexpectedly small, because χ_F, the quadrupolar coupling constant of ^{23}Na in sodium solutions containing no polyions, lies in the range of 1–4 MHz according to various theoretical estimates [see Engström et al. (1982) and references cited therein]. It is unlikely that the time-averaged charge distribution surrounding the sodium nuclei that are associated with DNA could be more symmetrical than that around free sodium ions. As Forsén and Lindman have noted (Lindman, 1978; Forsén & Lindman, 1981), the surprisingly small magnitudes of the quadrupolar coupling constants that have been evaluated for ^{23}Na in polyelectrolyte systems suggest that the quadrupolar relaxation occurs as a two-step process (Wennerström et al., 1974; Halle & Wennerström, 1981).

In the two-step model, the quadrupolar interaction is first averaged by a fast, slightly anisotropic motion to a small but nonzero value. In the second step, this residual quadrupolar interaction is then averaged to zero by a much slower motion. If this model is adopted to explain the relaxation of ^{23}Na in the presence of DNA, the fast motion could be attributed to rapid molecular motions in the first hydration shell of the sodium ion, fast diffusion of the sodium ion around the polyion rod, or the anisotropic reorientation of a hydrated sodium ion that is slightly distorted from its mean spherical symmetry in the free state. The slow motion could be due to internal (segmental) motion within the DNA molecule or to radial translational diffusion of the sodium ion away from the influence of the polyion. Either of these possibilities could account for the relatively long (nanosecond) correlation times that have been calculated in the present study for ^{23}Na associated with DNA. Because the two-step model predicts that only the *residual* quadrupolar interaction is reflected in the magnitude of $(r^o)^{1/2}\chi_B$, it can provide an explanation for the surprising finding that χ_B is much less than χ_F. It should be recognized that this model is predicated on the basis of two correlation functions, whereas eq 3 was derived by assuming that the field gradient correlation function is a single exponential. Nevertheless, the relative magnitudes of the correlation times in Tables I and II that were evaluated by using eq 3a,b should provide a reliable basis for the present quali-

tative discussion (L. Nordenskiöld, unpublished results).

Correlation Times in the Different DNA Solutions. In the present NMR study, samples of the synthetic polynucleotides differed from those of the natural DNAs in that the DNA concentrations and in some cases also the average molecular weights of the synthetic polynucleotides were lower. However, these differences in conditions between the two types of systems were not very large. We therefore expect that for all systems under consideration here, the experimental conditions are sufficiently similar for the purpose of a qualitative comparison.

The four natural DNAs differ in base composition but can all be assumed to have the same overall B-DNA conformation (Zimmerman, 1982). The correlation times presented in Table II are all approximately 3 ns and may not differ significantly, in view of experimental uncertainties and differences in the molecular weights. Furthermore, there is no clear trend in correlation time as a function of base composition. Among the synthetic polynucleotides investigated, only $d(GC)_n$ is believed to have the B-DNA conformation (Chen et al., 1983); accordingly, the correlation time estimated for $d(GC)_n$, 3.5 ns, is most similar to the values found for the natural DNAs. The correlation times for the other two synthetic polynucleotides are substantially different. For $d(AT)_n$, the correlation time is shorter, whereas for Br-$d(GC)_n$ it is longer, than for the other systems. It is by now generally accepted that these two polynucleotides adopt solution conformations different from the ordinary B-DNA structure (Lafer et al., 1981; Chen et al., 1983). Br-$d(GC)_n$ adopts a left-handed Z-DNA conformation (Lafer et al., 1981). It has been proposed that $d(AT)_n$ has a so-called alternating B-DNA conformation with a dinucleotide repeating unit (Klug et al., 1979). The results summarized in Tables I and II indicate that the correlation time τ_c for the modulation of the quadrupolar interaction for sodium ions associated with DNA depends strongly on the overall DNA conformation but is rather insensitive to base composition. Furthermore, in Br-$d(GC)_n$ (Z-DNA) solutions, τ_c decreases when either sodium ions or magnesium ions are added to the system. If compared as a function of the amount of added salt, the extent of this linear decrease is much more pronounced for magnesium than for sodium. The data in Figure 7 for the sodium titration correspond to an approximately 2-fold decrease in correlation time for an addition of 0.9 equiv of sodium per phosphate. The data in Figure 6 for the magnesium titration correspond to an approximately 2-fold decrease in correlation time for an addition of 0.08 equiv of magnesium per phosphate. In the next section, we discuss the implications of these observations with respect to the possible molecular motions affecting the quadrupolar relaxation of ^{23}Na associated with a nucleic acid.

Origin of the Correlation Times. The preceding analysis indicates that the quadrupolar interactions of sodium ions associated with nucleic acids having different conformations and base compositions are modulated on the nanosecond time scale. In general, these correlation times could be due to several different types of molecular motions including (for example) macromolecular reorientation of the DNA molecule, diffusional motion of the sodium ion along the slightly curved rodlike DNA molecule, internal (segmental) motion within the DNA, and radial translational diffusion of sodium ions from the vicinity of the polyion (Wennerström et al., 1974; Gustavsson et al., 1978; Lindman, 1978). For DNA of the molecular weight investigated in the present study, DNA reorientation is definitely too slow a process (Hagerman, 1981).

For the Br-$d(GC)_n$ system, the sodium and magnesium titration results presented in the previous section show a (linear) decrease in correlation time with the addition of sodium or magnesium ions. These observations can most easily be explained by a model in which the translational diffusion of sodium ions away from the polyion provides a significant contribution to the correlation time characteristic of associated sodium nuclei. As a basis for interpreting the effect of salt on this diffusional process, it may be assumed that the electrostatic potential outside the polyion is determined by the cylindrical Poisson–Boltzmann (PB) equation (Katchalsky, 1971). Since the gradient of this potential (i.e., the field) will decrease with increasing salt concentration (Gross & Strauss, 1966), the radial translational motion of counterions under the influence of a polyion is expected to be faster, and the corresponding correlation time smaller, at higher salt concentrations (Lifson & Jackson, 1962; B. Halle et al., private communication). Calculations using the PB equation indicate that the efficacy of added salt in decreasing the field around the polyion is larger for divalent than for univalent ions (D. K. Chang, unpublished results). Furthermore, it is possible that some of the associated magnesium ions are bound to the closely spaced phosphate charges on the Z-DNA Br-$d(GC)_n$ (Crawford et al., 1980). This binding would cause a reduction of the mean polyion charge density, a decrease in its surrounding field, and thus a decrease in the correlation time for radial diffusion. On the basis of these qualitative electrostatic considerations, the relative effects of magnesium and sodium on τ_c, as demonstrated in Figures 7 and 8, can be rationalized.

The synthetic polynucleotides $d(AT)_n$ and $d(GC)_n$ have different overall conformations (Zimmerman, 1981) but the same average surface charge density, which is slightly larger than that of the Z conformation of Br-$d(GC)_n$. On the basis of the published structural data (Wang et al., 1979), the average axial charge density of Br-$d(GC)_n$ can be estimated to be slightly lower than that of B-DNA. At a given salt concentration, the PB equation predicts that the field near a cylindrical polyion is determined primarily by its average axial charge density. Consequently, the radial diffusion of counterions under the influence of the polyion field is expected to be almost the same for $d(GC)_n$ and Br-$d(GC)_n$. This expectation does not agree with the observed trend in correlation times (Tables I and II). Thus, if radial diffusion does make the dominant contribution to these correlation times, it appears that this motion cannot be simply described by use of the conventional PB equation. In order to arrive at an accurate evaluation of τ_c for radial diffusion, it may be necessary, for example, to consider the details of the local phosphate charge distribution, which differ considerably in the two DNA conformations (B and Z). Alternatively, the dependence of τ_c on the conformation of the nucleic acid may indicate that the quadrupolar interaction is significantly modulated by motions other than radial diffusion. In view of the difference in flexibility between $d(GC)_n$ and Br-$d(GC)_n$ (Thomas & Bloomfield, 1983), the longer correlation time for the latter could be due to slower segmental motions. It is not obvious, however, why these motions should exhibit the pronounced salt dependence demonstrated in Figures 6 and 7. Finally, the disparity in τ_c between $d(GC)_n$ and Br-$d(GC)_n$ could in part reflect the inadequacy of the assumption that the field gradient correlation function is a single exponential. Further studies utilizing quadrupolar NMR are in progress to probe the dynamic aspects of the interactions of small ions with nucleic acids.

Conclusions

At an applied field of 4.7 T, the ^{23}Na relaxation is nonexponential, and the corresponding line shape in the frequency

domain is non-Lorentzian for six different deoxyribonucleic acids of differing overall conformation and base composition. At high magnetic fields, nonexponential relaxation thus seems to be a general phenomenon for ^{23}Na in DNA solutions at moderate concentrations. Analysis of the non-Lorentzian line shapes in terms of a single exponential field gradient correlation function indicates that the correlation time for the modulation of the quadrupolar interaction is of the same magnitude in solutions for four different DNA samples having the same (B) DNA conformation but varying in base composition (26–100% GC content). Significantly different values for the correlation time have been estimated for Br-poly(dG-dC)·Br-poly(dG-dC), having the Z-DNA conformation, and poly(dA-dT)·poly(dA-dT), which has been suggested to have an alternating B-DNA conformation. Thus, for these nucleic acids, it appears that the dynamics of the process modulating the ^{23}Na quadrupolar interaction are sensitive to the overall DNA conformation but not to its base composition.

An analysis of the effect on the ^{23}Na line shape of titrating Br-d$(GC)_n$ (Z-DNA) solutions with sodium or magnesium salts shows that the correlation time characteristic of associated sodium nuclei decreases approximately linearly with the addition of salt. The process responsible for the slow correlation time averaging out the quadrupolar interaction can be identified with the radial translational diffusion of sodium ions out from the vicinity of the polyion. According to this model, the decrease in τ_c with increasing salt concentration is due to the concomitant decrease in the attractive electrostatic field surrounding the polyion.

Acknowledgments

Helpful instructions from Dr. William Braunlin in the initial stages of this project are gratefully acknowledged. We are indebted to Pamela Mills for running the sodium titration spectra and to Jung-Hye Roe for doing the gel electrophoresis experiments. One of us (L.N.) is very grateful to Professor Wennerström for enlightening discussions on the theoretical aspects of quadrupolar relaxation in polyelectrolyte systems. We thank B. Halle, H. Wennerström, and L. Piculell for communicating a preprint of their paper on correlation times in polyelectrolyte solutions.

Registry No. d$(AT)_n$, 26966-61-0; d$(GC)_n$, 36786-90-0; Na, 7440-23-5; Mg, 7439-95-4.

References

Abragam, A. (1961) *The Principles of Nuclear Magnetism*, Oxford University Press, London.

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

Anderson, C. F., & Record, M. T., Jr. (1983) *Structure and Dynamics: Nucleic Acids and Proteins* (Clementi, E., & Sarma, R. H., Eds.) p 301, Adenine Press, New York.

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

Behe, M., & Felsenfeld, G. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 1619.

Blake, R. D., & Haydock, P. V. (1979) *Biopolymers 18*, 3089.

Bleam, M. L. (1980) Ph.D. Thesis, University of Wisconsin, Madison, WI.

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

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

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

Chen, C. W., & Cohen, J. S. (1983) *Biopolymers 22*, 879.

Cohen, M. H., & Reif, F. (1957) *Solid State Phys. 5*, 321.

Crawford, J. L., Colpak, F. J., Wang, A. H.-J., Quigley, G. J., van Boom, J. H., van der Marel, G., & Rich, A. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 4016.

Engström, S., Jönsson, B., & Jönsson, B. (1982) *J. Magn. Reson. 50*, 1.

Farrar, T. C., & Becker, E. D. (1971) *Pulse and Fourier Transform NMR*, Academic Press, New York.

Forsén, S., & Lindman, B. (1981) *Methods Biochem. Anal. 27*, 289.

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

Gross, L. M., & Strauss, U. (1966) *Chemical Physics of Ionic Solutions* (Conway, B. E., & Barradas, R. G., Eds.) p 361, Wiley, New York.

Gustavsson, H., Lindman, B., & Bull, T. (1978) *J. Am. Chem. Soc. 100*, 4655.

Hagerman, P. J. (1981) *Biopolymers 20*, 1503.

Halle, B., & Wennerström, H. (1981) *J. Chem. Phys. 75*, 1928.

Halle, B., Andersson, T., Forsén, S., & Lindman, B. (1981) *J. Am. Chem. Soc. 103*, 500.

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

Inman, R. B., & Baldwin, R. L. (1962) *J. Mol. Biol. 5*, 172.

Katchalsky, A. (1971) *Pure Appl. Chem. 26*, 327.

Klug, A., Jack, A., Viswamitra, M. A., Kennard, O., Shakked, Z., & Steitz, T. A. (1979) *J. Mol. Biol. 131*, 669.

Lafer, E. M., Moller, A., Nordheim, A., Stollar, B. D., & Rich, A. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 3546.

Lifson, S., & Jackson, J. L. (1962) *J. Chem. Phys. 36*, 2410.

Lindman, B. (1978) *J. Magn. Reson. 32*, 39.

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

Manning, G. S. (1979) *Acc. Chem. Res. 12*, 443.

McLachlan, A. D. (1964) *Proc. R. Soc. London, Ser. A 280*, 271.

Pohl, F. M., & Jovin, T. M. (1972) *J. Mol. Biol. 67*, 375.

Record, M. T., Jr., Anderson, C. F., & Lohman, T. M. (1978) *Q. Rev. Biophys. 11*, 103.

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

Thomas, T. J., & Bloomfield, V. A. (1983) *Nucleic Acids Res. 11*, 1919.

van der Klink, J. J., Zuiderweg, L. H., & Leyte, J. C. (1974) *J. Chem. Phys. 60*, 2391.

Wakelin, L. P. G., & Waring, M. J. (1976) *Biochem. J. 157*, 721.

Wang, A. H. J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. L., van der Marel, G., & Rich, A. (1979) *Nature (London) 282*, 680.

Wells, R. D., Larson, J. E., Grant, R. C., Shortle, B. E., & Cantor, C. R. (1970) *J. Mol. Biol. 54*, 465.

Wennerström, H., Lindblom, G., & Lindman, B. (1974) *Chem. Scr. 6*, 97.

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

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

Zimmermann, S. B. (1982) *Annu. Rev. Biochem. 51*, 395.