Importance of Coulombic End Effects on Cation Accumulation Near Oligoelectrolyte B-DNA: A Demonstration using ^{23}Na NMR

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ABSTRACT The local cation concentration at the surface of oligomeric or polymeric B-DNA is expected, on the basis of MC simulations (Olmsted, M. C., C. F. Anderson, and M. T. Record, Jr. 1989. *Proc. Natl. Acad. Sci. USA.* 86:7766–7770), to decrease sharply as either end of the molecule is approached. In this paper we report ^{23}Na NMR measurements indicating the importance of this "coulombic" end effect on the average extent of association of Na^+ with oligomeric duplex DNA. In solutions containing either 20-bp synthetic DNA or 160-bp mononucleosomal calf thymus DNA at phosphate monomer concentrations [P] of 4–10 mM, measurements were made over the range of ratios $1 \leq [Na]/[P] \leq 20$, corresponding to Na^+ concentrations of 4–200 mM. The longitudinal ^{23}Na NMR relaxation rates measured in these NaDNA solutions, R_{obs}, are interpreted as population-weighted averages of contributions from "bound" (R_B) and "free" (R_F) ^{23}Na relaxation rates. The observed enhancements of R_{obs} indicate that R_B significantly exceeds R_F, which is approximately equal to the ^{23}Na relaxation rate in an aqueous solution containing only NaCl. Under salt-free conditions ([Na]/[P] = 1), where the enhancement in R_{obs} is maximal, we find that $R_{obs} - R_F$ in the solution containing 160-bp DNA is ~1.8 times that observed for the 20-bp DNA. For the 160-bp oligomer (which theoretical calculations predict to be effectively polyion-like), we find that a plot of R_{obs} vs. [P]/[Na] is linear, as observed previously for sonicated (~700 bp) DNA samples. For the 20-bp oligonucleotide this plot exhibits a marked departure from linearity that can be fitted to a quadratic function of [P]/[Na]. Monte Carlo simulations based on a simplified model are capable of reproducing the qualitative trends in the ^{23}Na NMR measurements analyzed here. In particular, the dependences of $R_{obs} - R_F$ on DNA charge $|Z|$ (320 vs. 38 phosphates) and (for the 20-bp oligomer) on [Na]/[P] are well correlated with the calculated average surface concentration of Na^+. Thus, effects of sodium concentration on R_B appear to be of secondary importance. We conclude that ^{23}Na NMR relaxation measurements are a sensitive probe of the effects of oligomer charge on the extent of ion accumulation near B-DNA oligonucleotides, as a function of [Na] and [P].

INTRODUCTION

This study was undertaken to determine whether ^{23}Na NMR spectroscopic measurements are sensitive to the coulombic end effect on the extent of sodium ion accumulation in the vicinity of B-DNA oligoelectrolytes, which is predicted to be significant on the basis of previous theoretical investigations. For a homologous series of cylindrical oligoions of fixed high axial charge density, such as B-DNA, the oligomer charge $|Z|$ is expected to be an important variable for the molecular and thermodynamic properties that are determined primarily by coulombic interactions with electrolyte ions. Grand canonical Monte Carlo (GCMC) simulations have been used to predict radial distributions of small univalent ions in the vicinity of DNA oligoanions, as well as preferential interaction coefficients that characterize thermodynamic effects of salt-oligoelectrolyte interactions (Mills et al., 1985, 1986; Olmsted et al., 1989, 1991, 1995; Olmsted, 1992). To focus on the essentially coulombic origin of end effects manifested by oligomeric and polymeric DNA, these "$|Z|$-mers" have been modeled as rigid, impenetrable cylinders with a uniform continuous or discrete axial charge distribution. For all $|Z|$ ($|Z| \geq 8$) and all salt activities examined (2–200 mM), cations are strongly accumulated (and anions excluded) from the surface of the DNA $|Z|$-mer. As $|Z|$ becomes sufficiently large (for either a long oligomer or polymeric DNA) the local concentration of cations at its surface is predicted by GCMC simulations to exhibit a trapezoidal profile with characteristic terminal and interior regions. In the terminal regions, the surface cation concentration is predicted to increase strongly with increasing distance from either end of the $|Z|$-mer. The length of each terminal region of B-DNA is predicted to be ~20 structural charges (phosphates) at relatively low salt (C_3 = 1.8 mM) and oligoion concentration (C_u = 2.5 mM), Olmsted et al., 1989). Sufficiently long $|Z|$-mers (of length exceeding that of the two terminal regions) are predicted to exhibit an interior region within which the local surface cation concentration is uniformly equal to that characteristic of the corresponding polyion. The two lengths of DNA investigated in the present study were selected both for practical convenience and on the basis of our previous theoretical calculations, which predict that the average (per-residue) thermodynamic and molecular properties of 160-bp DNA are close to the polyelectrolyte ($|Z| \rightarrow \infty$) limit, whereas the properties of the 20-bp fragment are dominated by the two terminal regions. The two $|Z|$-mers investigated here are

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(virtually) monodisperse in chain length (i.e. in number of phosphates). For the longer DNA, we purified and investigated mononucleosomal calf thymus DNA, which exhibits a narrow distribution of chain lengths centered at 160 ± 5 bp. The average local concentration of Na^+ near the 160-bp DNA ($|Z| \cong 320$) is expected to be determined largely (~95%) by its polyelectrolyte-like interior region. For the short DNA, we synthesized a 20-bp oligonucleotide ($|Z| =$ 38 phosphates). GCMC simulations predict that the average local concentration of Na^+ near the 20-bp DNA is smaller and varies more strongly with [NaCl] than for the polymeric case. This system allows us to investigate the effects of oligomeric length and salt concentration on ^{23}Na NMR relaxation measurements. Our investigation provides a direct experimental test of the magnitude and significance of the coulombic end effect on Na^+ accumulation in the vicinity of a B-DNA oligoelectrolyte, in the range of Na^+ concentrations from 4 to 200 mM and concentration ratios $1 \leq [Na]/[P] < 20$.

NMR BACKGROUND

NMR relaxation rate measurements of the ^{23}Na nucleus have provided both qualitative and quantitative information about the extent and nature of interactions of Na^+ with polymeric double-helical DNA, as well as the dynamic processes that govern the quadrupolar relaxation of Na^+ ions in the vicinity of B-DNA (Anderson et al., 1978; Bleam et al., 1980; Nordenskiöld et al., 1984; Braunlin et al., 1986; Padmanabhan et al., 1988, 1991). Numerous NMR studies of cation-DNA interactions have reported ^{23}Na NMR relaxation rates in solutions containing sonicated (polydisperse) polymeric DNA (typically of average size ~700 bp) (Reuben et al., 1975; Anderson et al., 1978; Bleam et al., 1980, 1983; Delville et al., 1986; Braunlin et al., 1987; Casu et al., 1987; van Dijk et al., 1987; Padmanabhan et al., 1988). There have also been ^{23}Na NMR investigations of monodisperse mononucleosomal DNA from calf thymus (147 bp with a distribution from 125 to 165 bp) (Strzelecka and Rill, 1990, 1992) and from chicken erythrocyte (146 bp) (van Dijk et al., 1987). Most of these studies were performed at lower magnetic field strengths than that employed here.

The field-dependent ^{23}Na NMR relaxation rates (longitudinal and transverse) observed for aqueous salt-free solutions of polymeric Na-DNA are significantly greater than that observed in a solution containing only NaCl at a comparable concentration and temperature (cf. Braunlin, 1995; Stein et al., 1995 for reviews). Such relaxation rate data have been analyzed in terms of a two state model, by assuming the Na^+ cations to be either "free" or "bound." The "bound" state is operationally defined to include that portion of mobile, freely exchanging sodium ions that are located close enough to the surface of DNA so that their relaxation rates are affected. The enhancement in the bound relaxation rate is attributable to increases in the mean square amplitude of the local field gradient and/or to longer effective correlation time(s) of ions in the near vicinity of a $|Z|$-mer. The "free" state encompasses all other environments of Na^+, generally assumed to be equivalent to those in a NaCl solution of comparable concentration containing no DNA.

Under the condition of fast exchange (Bleam et al., 1983; Nordenskiöld et al., 1984; van Dijk et al., 1987), the observed longitudinal or transverse relaxation rate (here designated simply R_{obs}) can be represented as a population-weighted average of the "free" (R_F) and "bound" (R_B) relaxation rates

$$R_{obs} = p_F R_F + p_B R_B = R_F + r_{Na}(R_B - R_F)[P]/[Na] \quad (1)$$

where p_F and p_B are the fractions of the total population of Na^+ in the "free" and "bound" environments, respectively ($p_F + p_B = 1$), $r_{Na} \equiv [Na]_B/[P]$ is the Na^+ binding density (fraction of a "bound" sodium ion per DNA phosphate) and [P] and [Na] are total concentrations of DNA phosphate and of Na^+, respectively. Under the conditions of interest here an equation analogous to Eq. 1 pertains to any spectral component of either the longitudinal or the transverse NMR relaxation process. For this study, longitudinal relaxation rates of $^{23}Na^+$ in NaDNA/NaCl solutions were measured. Spectroscopic methods and sample preparation are described in the following section.

Application of Eq. 1 to analyze titrations of polymeric (~700 bp) NaDNA with NaCl, typically performed at a relatively constant DNA phosphate concentration [P], indicates that R_{obs} is a linear function of $[P]/[Na^+]$ over the range of concentration ratios $\sim 1 \leq [Na]/[P] \leq \sim 20$, with an intercept R_F which is equal within uncertainty to the relaxation rate of ^{23}Na in NaCl under the experimental conditions. Linearity of the plot indicates that, for polymeric DNA, $r_{Na}(R_B - R_F)$ is independent of [Na], within uncertainty. However, this result does not suffice to prove that r_{Na}, R_B, and R_F are all independent of changes in [Na]/[P] in solutions of polymeric Na-DNA. Information about each of these three two-state parameters for polymeric DNA is now summarized.

r_{Na}

Rigorous Monte Carlo calculations of the counterion radial distribution surrounding a uniform cylindrical model of DNA (Mills et al., 1985) and also more detailed structural models (Reddy et al., 1987) predict that the average local concentration of Na^+ at the surface of polymeric DNA (designated $\bar{C}^+_\infty(a)$) should increase gradually as the bulk NaCl concentration increases. Since the experimental quantity r_{Na} obtained from Eq. 1 is expected to increase with increasing $\bar{C}^+_\infty(a)$, the invariance of r_{Na} ($R_B - R_F$) is a striking result. Analysis of competitive cation titrations yields estimates of r^o_{Na}, the value of r_{Na} pertaining to a salt-free polymeric Na-DNA solution, in the range 0.5–0.8 (Padmanabhan et al., 1988, 1990). Originally we interpreted this behavior in terms of the approximate counterion condensation theory, which predicts that $\bar{C}^+_\infty(a)$ for polymeric Na-DNA/NaCl solutions should be relatively invariant to [NaCl] up to at least 0.1–0.2 M and (if r_{Na} is assumed to correspond to the "condensed" counterions) predicts that $r_{Na} = 0.76$ (Manning, 1978). Since less approximate theoretical analyses (GCMC, PB) of the

same molecular model (the "standard" model) used by CC theory indicate that $\bar{C}_\infty^+(a)$ should increase detectably with increasing [NaCl], alternative explanations of the invariance of the product $r_{Na}(R_B - R_F)$ have also been proposed, including the possibility that the standard model is inadequate to predict r_{Na} (Jayaram et al., 1989) and/or the possibility that R_B decreases as r_{Na} increases (Reddy et al., 1987).

R_F

So long as the concentration of the oligonucleotide is in the range of the experiments considered here (or more dilute), the short range of the effects exerted by the $|Z|$-mer on the relaxation process of quadrupolar nuclei in its vicinity guarantees that R_F cannot be much different from the quadrupolar relaxation rate observed for sodium ions in a solution containing only salt. Although this relaxation rate does exhibit some concentration dependence, these "bulk solution" effects are insignificant over the experimental concentration range of interest here (Eisenstadt and Friedman, 1967).

R_B

In principle bulk solution effects could be manifested in a slight concentration dependence of R_B as well, most probably via the contribution of hydration to the local field gradient acting on the quadrupolar nucleus (van Dijk et al., 1987). A second potential source of concentration dependence of R_B is heterogeneity in the relaxation processes experienced by different subclasses of bound sodium nuclei. Although Eq.1 is written explicitly for two states (bound, free) of the exchanging nucleus, it can be construed more generally to pertain to nuclei exchanging among any number of magnetically distinct "bound" states. Since quadrupolar interactions govern the relaxation mechanism in this case, these states would be distinguished by local variations in the mean-square amplitude of the electric field gradient acting on the nucleus, and/or in the correlation time(s) characterizing the local diffusional motions that modulate the relaxation process on a molecular time scale. Thus, provided only that the exchange rates among all sites accessible to the sodium ions are rapid with respect to their relaxation rates in each of the bound sites, Eq. 1 is applicable to an arbitrary number of "bound states," with the identification $p_B R_B \equiv \Sigma p_{Bi} R_{Bi}$ and $p_B = \Sigma p_{Bi}$ and, as in the case of a single bound state, $r_{Na} \equiv [Na]p_B/[P]$. In this situation, even though the individual R_{Bi} may be concentration-independent, changes in [Na] which produce shifts in the relative populations of Na^+ at the magnetically distinct bound "sites" (or regions) could cause R_B to vary.

The recent work of Rossky and co-workers (Reddy et al., 1987; Chen and Rossky, 1993) has established that the use of molecular dynamics to simulate the observable relaxation rate(s), including all factors capable of affecting both the static and dynamic features of the quadrupolar relaxation process, will require a very detailed structural model of the system, including explicit consideration of interactions involving solvent molecules. Despite the considerable recent progress in this area, a generally reliable method of calculating a priori predictions of the NMR relaxation rate(s) characteristic of quadrupolar nuclei in the vicinity of a polymeric (or oligomeric) DNA molecule is not yet available. Moreover there exists no practical direct means of separately measuring R_B, the (mean) relaxation rate characteristic exclusively of nuclei sufficiently close to DNA that their relaxation processes are affected. Thus the factors comprising the product $r_{Na}(R_B - R_F)$ cannot be *directly* evaluated. An indirect method of measuring r_{Na} has been applied to analyze NaCl titrations of polymeric DNA with a competing multivalent cation such as Mg^{2+} or putrescine (see for example, Bleam et al., 1983). This approach is currently under investigation as a means of gaining analogous information about oligonucleotides.

MATERIALS AND METHODS

Salts and buffers

Anhydrous $MgCl_2$ (99.95% pure; Aldrich) and NaCl (Fisher Scientific) were used without further purification. Putrescine (Sigma) was dried under vacuum and also used without further purification. Two buffers were used: Tris/EDTA (10 mM tris-hydroxymethyl-aminomethane (Aldrich); 10 mM trisodium ethylene-diaminetetraacetic acid (Na_3-EDTA; Fisher Scientific, Aldrich), and HEPES (10 mM sodium HEPES (Sigma), 0.2 mM Na_3-EDTA) at a specified salt concentration (NaCl) and pH (obtained by titration with HCl). Deionized water purified by a Barnstead "E-pure" system was used in all experiments. According to the manufacturer, water purified in this way contains less than 0.2 pmol Mg^{2+} and 1.3 pmol Ca^{2+}.

Preparation and purification of 160-bp and 20-bp DNA

The 160 ± 5 bp mononucleosomal calf thymus DNA was purified from thymus glands as described by Wang et al. (1990). Its size was verified by gel electrophoresis in a 7.5% polyacrylamide gel, using 38 × 18 cm gel electrophoresis plates with size markers obtained from a *Hpa*II and *Eco*RI restriction digest of pBR322.

The 20-bp DNA oligomer was prepared from 2 complementary (but not self-complementary) strands, made by multiple 1-μmol scale syntheses at the University of Wisconsin Biotechnology Center and the Biochemistry facility. Sequences of the two strands were designed to avoid intrastrand base pairing (e.g., hairpins) and unusual interstrand secondary structures (e.g., palindromes, A-tracts). The sequences synthesized were

$^{5'}$ CGACT AGTGG CCTGA GATGC $^{3'}$

$^{3'}$ GCTGA TCACC GGACT CTACG $^{5'}$

This oligomer is 60% GC and contains 38 phosphates. Its termini consist of 2 GC pairs to reduce end-fraying at the temperature of the NMR experiments (23°C). Only one synthetic oligomer was examined in this initial study because of the large financial and preparatory investment involved in synthesizing and purifying the quantities of sample required for accurate NMR measurements.

Both 20 base single-stranded (ss) oligonucleotides were purified in three steps, the first and last of which were gradient reverse-phase HPLC on a Hamilton polystyrene-divinylbenzene copolymer (PRP-1) column using a solvent system consisting of 0.1 M triethylammonium acetate, pH 6.6 (Pierce) and acetonitrile (Fisher Scientific). First, the tritylate-protected ss-oligomer was separated by HPLC from deprotected strands and strands shorter than 20 bases. The purified tritylated ss-oligomer was deprotected by treatment with 80% acetic acid for 30 min at 23°C. Subsequently any

remaining tritylated oligomer was removed from the detritylated product by HPLC as above. After each step in the purification process, the ss-oligomers were evaporated to dryness using a concentrator/evaporator (Savant). Further details of the purification are provided by Stein (1994).

After HPLC purification, the dried ss-oligomers were dissolved into ~2.5 ml of HEPES buffer (100 mM NaCl, pH 7.7). The concentration of each strand was determined by absorbance measurements on a Cary 210 UV spectrophotometer by assuming the same extinction coefficient for each strand (see below). Equal molar concentrations (3.5–5.8 mM) of the two complementary single strands in the above HEPES buffer were mixed together and heated to 80°C for 5 min to denature any intramolecular structure. To anneal, the oligomer mixture was cooled slowly to ~55°C, equilibrated for 1 h and then cooled slowly to 22°C (usually overnight) before being stored at 4°C.

NMR sample preparation

Stock solutions of 160-bp and 20-bp duplex DNA were carefully dialyzed to obtain a salt-free state without denaturation. Dialysis tubing with molecular weight cutoffs of 10^4 and 10^3 g/mol were used for the 160-bp and 20-bp samples, respectively. The volume ratio of dialysis buffer to DNA solution was ~10^3/1. Each DNA solution was dialyzed at 4°C, first against a Tris or HEPES buffer containing EDTA to remove polyvalent metal ion impurities, then against unbuffered 0.1 M NaCl, and finally against 3 changes with intervals of 3 h of an unbuffered NaCl solution that was 3–4 times lower in concentration than the DNA phosphate concentration [P]. This procedure yielded an undenatured Na-DNA solution at a concentration of 4–10 mM in DNA phosphate containing essentially no NaCl ("salt-free"). The pH of the dialysis solution and DNA solution always exceeded 5.4.

After dialysis, DNA nucleotide phosphate concentrations [P] were determined from the absorbance at 260 nm on a Cary 210 spectrophotometer. The extinction coefficients used for 160 bp (ϵ = 6400/mol P) and for 20 bp (ϵ = 6840/mol P) DNA were calculated from nearest neighbor frequencies (Allen et al., 1972) using an end-effect correction to the extinction coefficient of the 20-bp oligomer (Scheffler et al., 1970). The propagated error in [P] is estimated to be ±4%.

The initial sodium concentration in the DNA solutions was determined by neutron activation analysis at the Nuclear Reactor Laboratory at the University of Wisconsin, and also from a standard curve method based on the integrated ^{23}Na-NMR peak intensities using NaCl standards in the range of 1 to 20 mM (Skoog, 1985; Braunlin and Xu, 1992). Random errors in determination of [Na] were typically 5–10% for neutron activation analysis and ~5% for the standard curve method; concentrations estimated by these two methods never differed by more than 9%. By propagating the errors in [Na] and [P], the uncertainty calculated for the initial [Na]/[P] ratio is approximately ±10%. The initial [Na]/[P] ratio ranged from 1.0–1.2 for the 20-bp DNA and from 1.0–1.1 for the 160-bp DNA.

NMR experiments

The ^{23}Na NMR experiments were performed on a Bruker AM-400 instrument operating at a sodium Larmor frequency of 105.84 MHz in the Biochemistry facility (NMRFAM). The temperature was maintained at 23 ± 1°C with a refrigerator cooling unit. Thermal melting studies on salt-free NMR samples, performed on a Cary 210 UV spectrophotometer at a wavelength of 290 nm, demonstrated that the 20-bp duplex was at least 96% native at the temperature (23°C) and DNA phosphate concentration (3.8 mM) of the NMR experiment (Stein, 1994). ^{23}Na longitudinal relaxation rates were determined by inversion recovery pulse sequence (180-τ-90) experiments. Peak heights and integrated intensities were determined using Bruker software on the AM-400 spectrometer. Analyses of peak heights and of integrated intensities by nonlinear regression analysis (using the program NONLIN; Johnson and Frasier, 1985) on our VAXstation 3100 were found to yield similar results for R_{obs}. Peak heights were used for simplicity of measurement.

The 160-bp and 20-bp Na-DNA samples (2.6–3.0 ml), containing 10% ^2H_2O (99.9% purity), were prepared in 10-mm NMR tubes that had been cleaned with nitric acid, washed with EDTA, and rinsed with doubly-distilled H_2O. These samples were titrated with known microliter amounts of a concentrated NaCl solution (1.5–2.0 M). At the end of each titration, the final volume exceeded the initial by no more than 8%. A total of 6–7 points were taken in NaCl titrations.

After a set of titration experiments, the respective DNA samples were combined and reduced in volume by 50% using a concentrator/evaporator. A series of dialyses (as above) was used to remove salts from these concentrated DNA solutions in preparation for the next series of NMR experiments. For the 160-bp DNA, the data pertain to three stock solutions individually made up from a single preparation of mononucleosomal calf thymus DNA. For the 20-bp DNA, data were pooled from four stock solutions, using material purified from two separate sets of oligomer syntheses.

Some variation was observed in the initial relaxation rates of the different samples of each DNA, presumably because the initial [Na]/[P] ratios differed slightly. (R_1 varied from 45–48 s^{-1} for the 20-bp DNA and from 67–70 s^{-1} for the 160-bp DNA.) To compare titrations performed on samples drawn from different stock solutions, the observed relaxation rates were normalized by subtracting the relaxation rate of Na^+ in a comparable NaCl solution (R_F), and then multiplying this difference by [Na]/[P] (cf. Eq. 1 above). Although the normalized relaxation rate $(R_{obs} - R_F)$[Na]/[P] should be the same at the initial condition for the various stock solutions of the same DNA length, we observe some variability (within 10%) between stock solutions, which we attribute to random experimental uncertainty in the determinations of [P] and [Na]. To compensate for this variation, the initial $([Na]/[P])_o$ was adjusted by no more than 10% to bring the normalized initial relaxation rates of the different stock solutions into agreement. Each adjustment is within the overall propagated error of [Na]/[P]. Use of an "adjusted" $[Na^+]$/[P] scales the observed relaxation rates throughout the entire titration curve.

Monte Carlo simulations: model

We adopt here the standard primitive model for a three component solution consisting of water, univalent cations and anions, and an oligoanionic nucleic acid. The univalent cations and anions are modeled as uniformly charged impenetrable spheres, both with a radius of 3 Å (taken to represent hydrated ionic radii; Mills et al., 1985). The entire solution is modeled as a dielectric continuum with dielectric constant ϵ = 78.7, the value characteristic of pure water at 25°C. The charge distribution on a B-DNA oligomer with $|Z|$ phosphates ($|Z|$ = 22, 38, 160, 320) is modeled as $|Z|$ discrete axial unit charges separated by a uniform spacing of 1.7 Å, the distance between adjacent phosphate charges projected onto the helical axis of B-DNA. The impenetrable model oligomer is assumed to exclude a cylindrical volume to the small ion centers, so that the distance of closest approach to the cylinder axis in the radial direction (a) is 13 Å and the axial distance of closest approach to a terminal charge on the oligomer is 6 Å.

Monte Carlo simulations: method

We give only the technical details of the Monte Carlo (MC) simulations. General descriptions of the MC method are available (Allen and Tildesley, 1987; Valleau and Whittington, 1977). For the simulations reported here, the MC cell is a rectangular prism (in some cases a cube) containing a single fixed oligomer, the center of which coincides with the center of the simulation cell and the axis of which coincides with the long axis of the simulation cell, and a fixed number of mobile cations and anions, chosen in such a way that the cell is electroneutral. The side lengths of the cell are $2R_{xy}$ (perpendicular to the oligoion axis) and $2R_z$ (parallel to the oligoion axis), with $R_z = R_{xy} - a + 1.7|Z|/2$, so that the concentration of oligoion charges $C_u = N10^{27}/8R_{xy}^2R_zN_A$, where R_{xy} and R_z are in Å, and where N_A is Avogadro's number. Fully periodic boundary conditions and minimum image interactions between all charges were included.

Here we designate by $\bar{C}^+_{|Z|}(a)$ the axially averaged surface counterion concentration associated with a $|Z|$-mer. More precisely, $\bar{C}^+_{|Z|}(a)$ is the average concentration of counterions within a small annular volume extending radially from a to $(a + 1)$ Å and axially over $1.7|Z|$ Å (centered at the $|Z|$mer center). Because the NMR effect is very local (expected to decay with distance, r, from the DNA surface as r^{-6}) we consider only a

"surface" layer of counterions (defined to be consistent with those reported in previous simulation studies; Olmsted et al., 1989). Equilibration of the simulation consisted of 1–5 × 10^5 MC steps, and $\bar{C}^+_{|Z|}(a)$ was evaluated from averages over 1–10 × 10^6 subsequent MC steps. Reported uncertainties are the standard error of the mean of averages of $\bar{C}^+_{|Z|}(a)$ over blocks of 2–20 × 10^4 consecutive MC steps (Allen and Tildesley, 1987). To avoid the necessity of considering the large number of ions that would be required for simulations on the 320-mer at large [Na]/[P], $\bar{C}^+_{320}(a)$ was determined at [Na]/[P] = 4 and 5 by extrapolations based on the linear dependence of $\bar{C}^+_{|Z|}(a)$ on $1/|Z|$.

RESULTS AND DISCUSSION

Differences in ^{23}Na longitudinal relaxation rates R_{obs} between salt-free 160-bp and 20-bp DNA

For salt-free solutions of 160-bp and 20-bp duplex DNA containing approximately one Na^+ per DNA phosphate, peak heights are plotted versus delay time (τ) for typical longitudinal relaxation rate experiments in Fig. 1. These figures (representative of relaxation rate experiments performed on 3 separate stock solutions of 160-bp DNA and 4 separate stock solutions of 20-bp DNA) demonstrate the quality of the data used to determine the observed longitudinal relaxation rate R_{obs} from a three parameter exponential fitting (Fukushima and Roeder, 1981). The signal-to-noise ratio for the peaks in the inversion recovery experiments was typically more than 20:1. Nonlinear least squares analysis of the exponential fitting yields $R_{obs} = 69 \pm 1\ s^{-1}$ for the 160-bp DNA and $R_{obs} = 47 \pm 1\ s^{-1}$ for the 20-bp DNA in the absence of added salt. We conclude that this very significant reduction in R_{obs} results from the reduction in length of the oligoelectrolyte. The only other conceivable origin of the observed difference in R_{obs} would be the somewhat different base compositions of the 20-bp DNA (60% GC) and the calf thymus 160-bp DNA (~45% GC). No precedent for such an extreme dependence of ^{23}Na NMR relaxation rates on base composition exists, as judged from comparative experiments with polymeric naturally occurring DNA of widely different base compositions (Stein, 1994). Interpreted in the context of Eq.1, the reduction in R_{obs} must result from reductions in the fraction of "bound" Na^+ per DNA phosphate under salt-free conditions, r^o_{Na}, and/or in the "bound" relaxation rate, R_B. A quantitative basis for deciding between these alternatives is provided by Monte Carlo simulations, reported in a subsequent section.

For both the 160-bp and 20-bp "salt-free" DNA solutions, R_{obs} is substantially larger than the corresponding ^{23}Na relaxation rate in the absence of DNA, which is 18.1 ± 0.4 s^{-1} for a 10 mM aqueous solution of NaCl in 10% D_2O at 23 ± 1°C. For the 160-bp DNA, R_{obs} exceeds R_F by a factor of 4; for the 20-bp DNA, R_{obs} exceeds R_F by a factor of 2.5. By contrast, for a 20-base *single-stranded* oligonucleotide, our preliminary results indicate that R_{obs} exceeds R_F under salt-free conditions by a factor of only 1.3. (Single stranded oligomers were not investigated in detail because of the larger uncertainties in the two-state parameters determined from R_{obs} by Eq. 1.)

Titrations of initially "salt-free" 160-bp and 20-bp DNA monitored by ^{23}Na R_{obs}

In Fig. 2, ^{23}Na longitudinal relaxation rates are plotted as a function of the ratio [Na]/[P] for titrations of initially "salt-free" 160-bp and 20-bp Na-DNA with NaCl. Addition of NaCl to the Na-DNA solution causes a monotonic reduction in the ^{23}Na relaxation rate. At high enough [Na] ([Na]/[P] > 20), R_{obs} approaches the relaxation rate of Na^+ in a comparable solution containing only aqueous NaCl, as observed previously in titrations of sonicated calf thymus DNA (~700 bp) (Anderson et al., 1978; Bleam et al., 1983; Padmanabhan et al., 1988). In Fig. 3, the data of Fig. 2 are plotted according to Eq. 1 as ($R_{obs} - R_F$) vs. [P]/[Na].

For the 160-bp DNA, ($R_{obs} - R_F$) is a linear function of [P]/[Na]. Linearity of this plot indicates that the quantity $r_{Na}(R_B - R_F)$ is independent of [Na] (within uncertainty). This observation would be most simply interpreted to imply that both r_{Na} and R_B are independent of [Na], as inferred previously from NaCl titrations of sonicated calf thymus

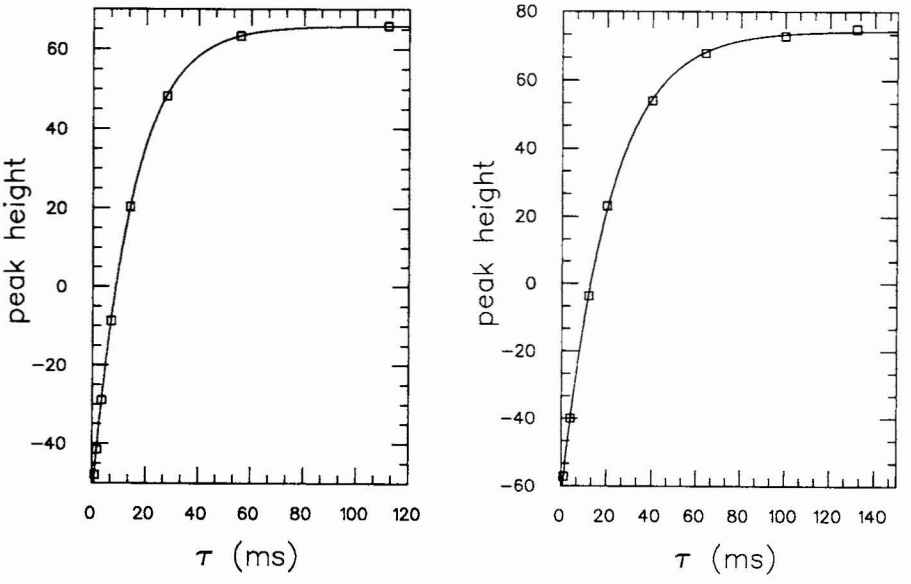

FIGURE 1 Determination of longitudinal relaxation rates from inverse recovery experiments. ^{23}Na peak height (arbitrary units) versus delay time (τ) for an inverse recovery experiment measuring the longitudinal relaxation rates at [Na]/[P] ≈ 1 for 160-bp DNA ([P] = 9.2 mM) (*left*) and 20-bp DNA ([P] = 4.3 mM) (*right*).

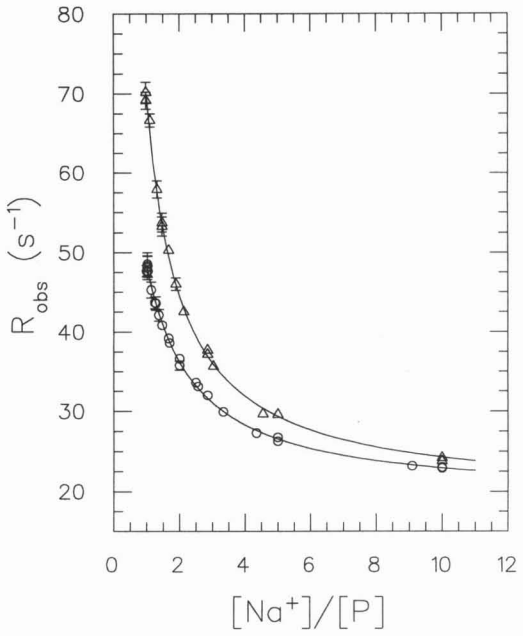

FIGURE 2 ^{23}Na longitudinal relaxation rates in NaDNA/NaCl solutions. Longitudinal relaxation rate, R_{obs} (in s^{-1}), are plotted vs. the ratio of sodium ion to DNA phosphate concentration, [Na]/[P], for NaCl titrations of 160-bp DNA (△) and 20-bp DNA (○). Relaxation rates were measured at ω_{Na} = 105.843 MHz and T = 23°C. (160 bp: [P] = 9.4 mM, [Na]/[P] = 1.1; [P] = 9.5 mM, [Na]/[P] = 1.0: 20 bp, [P] = 9.0 mM, [Na]/[P] = 1.0; [P] = 4.3 mM, [Na]/[P] = 1.0; [P] = 4.5 mM, [Na]/[P] = 1.0). Fitted curves through these data are nonlinear least squares fits to Eq. 2.

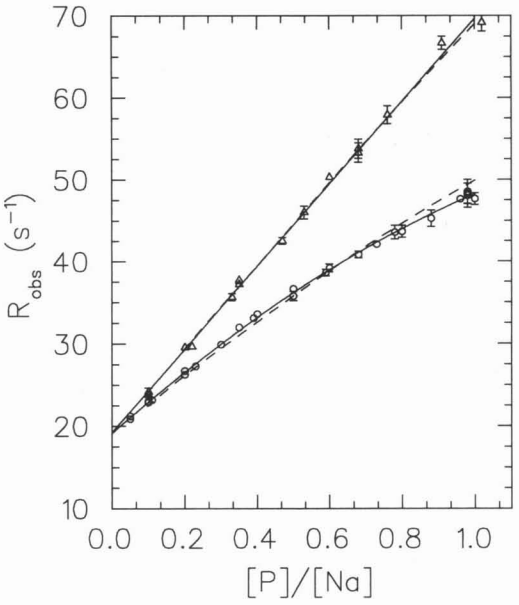

FIGURE 3 Two-state analysis of ^{23}Na longitudinal relaxation rates for 160-bp and 20-bp NaDNA in NaCl solutions. Longitudinal relaxation rates, R_{obs} (in s^{-1}) are plotted as a function of the ratio of DNA phosphate to sodium ion concentration, [P]/[Na], for NaCl titrations of 160-bp DNA (△) and 20-bp DNA (○). The solid lines represent the fitting of the data to a linear (160 bp) or quadratic (20 bp, Eq. 2) function of [P]/[Na]. The dashed lines represent the fitting of the data using Eq. 4, with α = 28 (for both 20 and 160-bp DNA) and $\bar{C}^{+}_{|z|}(a)$ obtained from MC simulations.

DNA having chain lengths in the polymeric range (≥700 bp) (Anderson et al., 1978; Bleam et al., 1980, 1983; Braunlin et al., 1986; Casu et al., 1987; Padmanabhan, 1988; Padmanabhan et al., 1988, 1991; Hald and Jacobsen, 1992). Analogous results have been obtained from sodium titrations of 146-bp chicken erythrocyte DNA (van Dijk et al., 1987) and of various sodium polyuronates (at 25,000 and 670,000 MW) (Grasdalen and Kvam, 1986). The best-fitted parameters obtained by linear least squares fitting of the titration curve of the 160-bp DNA to the two state model (Eq. 1) are $R_F = 19.3 \pm 0.6\,s^{-1}$ and $r_{Na}(R_B - R_F) = 50.4 \pm 0.9\,s^{-1}$. These best-fitted results are shown as the dashed line in Fig. 3. For the 160-bp DNA, R_F is at most slightly larger than the relaxation rate of Na^+ in a NaCl solution at the same temperature in the absence of DNA (R_F = 18.1 ± 0.4 s^{-1}) and is similar to the value of R_F determined by extrapolating to [P]/[Na] = 0 an analogous plot for the titration of ~700-bp DNA. The linear dependence on [P]/[Na] of the relaxation rate exhibited by the titration of the 160-bp DNA is qualitatively similar to that observed for ~700-bp DNA in previous studies (Padmanabhan, 1988; Padmanabhan et al., 1988, 1991), but in the present study the slope of the plot is smaller, because the applied field is higher. This field dependence is attributable to R_B exclusively, because R_F pertains to nuclei under the condition of extreme narrowing. (Explicit equations indicating the origin of the field dependence of R_B may be found, for example, in van Dijk et al., 1987.)

The corresponding plot of ($R_{obs} - R_F$) vs. [P]/[Na] shown in Fig. 3 for the 20-bp DNA exhibits curvature throughout the range examined. Curvature is most pronounced at lower [Na] ($1.0 \leq [Na]/[P] \leq 2.0$). Therefore Eq. 1 implies that $r_{Na}(R_B - R_F)$ varies with [Na] throughout the titration with NaCl. This behavior of R_{obs} differs qualitatively from that of 160-bp DNA, which by this criterion is equivalent to polymeric DNA. We found that the NaCl titration curve for the 20-bp DNA can be well-fitted by a quadratic in [P]/[Na]:

$$R_{obs} = R_F + S_1 \frac{[P]}{[Na]} + S_2 \left(\frac{[P]}{[Na]}\right)^2 \qquad (2)$$

Over the range $0.05 \leq [P]/[Na] \leq 1$, this empirical form suffices to account for the curvature (most prominent at higher [P]/[Na]) exhibited by the relaxation rates of 20-bp DNA in terms of the three constant parameters R_F, S_1 (positive) and S_2 (negative). Braunlin (1995) has independently observed that the functional form of Eq. 2 fits ^{23}Na NMR relaxation data on a 12-bp oligomer investigated under conditions substantially different from those of the work reported here.

The best-fitted quadratic pertaining to the titration data shown in Fig. 2 for 20-bp DNA is shown in Fig. 3 by a solid line. The best-fitted values of the coefficients calculated using Eq. 2 are R_F = 19.1 (± 0.2) s^{-1}; S_1 = 39.0 (± 0.9) s^{-1}; S_2 = −9.9 (± 0.8) s^{-1}. At sufficiently high salt concentrations Eq. 2 approaches the linear dependence on [P]/[Na] characteristic of Eq. 1. Comparison of the best-fitted value

of R_F (obtained by nonlinear least squares fitting of the entire titration curve of the 20 bp using Eq. 2) with the intercept of the linear plot of R_{obs} vs. [P]/[Na] for the titration of 160-bp DNA ($R_F = 19.3 \pm 0.6\ s^{-1}$) shows that these values are at most slightly larger than the relaxation rate of Na^+ in dilute NaCl solutions at this temperature in the absence of DNA ($18.1 \pm 0.4\ s^{-1}$).

Two-state analysis of R_{obs} as a function of $|Z|$ and [Na]/[P]

Fig. 3 presents the first comparative analysis of the concentration dependence of R_{obs} for two monodisperse DNA oligonucleotides (160 bp and 20 bp). Interpreted in the context of Eq. 1, the results shown in Fig. 3 clearly indicate that the product $r_{Na}(R_B - R_F)$ depends on $|Z|$ and, for the 20-bp DNA, also on the composition of the solution, and that $r_{Na}(R_B - R_F)$ increases with increasing $|Z|$ at any value of [P]/[Na] investigated ($1 > [P]/[Na] > 0.05$). For the 160-bp DNA, $r_{Na}(R_B - R_F)$ is (within uncertainty) independent of [Na]/[P]. However, for the 20-bp oligonucleotide, $r_{Na}(R_B - R_F)$ increases strongly with increasing [Na]/[P] (cf. Eq. 2):

$$r_{Na}(R_B - R_F) = S_1 + S_2[P]/[Na] \quad (3)$$

where S_1 is positive and S_2 is negative.

Especially for a short oligomer, a significant increase in r_{Na} is expected with increasing [Na^+] and/or increasing [P] from the MC simulations reported below. Theoretical predictions of the effect of varying $|Z|$ on the bound relaxation rate R_B are not available and will be difficult to obtain (for reasons indicated in the section on NMR background). Nevertheless, it is reasonable to infer from the short range of the interactions governing the magnitude of R_B that it is not sensitive to the length of the oligomer considered in this study. As an extreme case, we assume: 1) that R_B is independent of $|Z|$, and 2) that R_B is independent of [P]/[Na], and explore the consequences of these assumptions. According to Eq. 1, if R_B is independent of $|Z|$ and of [Na]/[P], any change in the product $r_{Na}(R_B\text{-}R_F)$ with these variables is attributable entirely to changes in r_{Na}, and therefore ratios of r_{Na} for different values of $|Z|$ or [Na]/[P] can be obtained from the experimental measurements. For the 160-bp DNA, the observation that the product $r_{Na}(R_B\text{-}R_F)$ is constant, interpreted according to the second assumption, indicates that the amount of Na^+ "bound" per phosphate does not change with [Na]/[P] over the range investigated. For the 20-bp DNA, however, the assumption that R_B is independent of [Na]/[P] leads to the finding that $r^{20bp}_{Na,20:1}/r^{20bp}_{Na,1:1} \cong 1.3$, which indicates that the number of "bound" Na^+ per phosphate of the 20-bp DNA increases by ~30% as [Na]/[P] increases from 1:1 to 20:1. (The superscript on r_{Na} gives the number of bp, and the second subscript gives [Na]/[P].) If R_B is also independent of $|Z|$, then for the salt-free case if follows that $r^{160bp}_{Na,1:1}/r^{20bp}_{Na,1:1} = 1.8$, which indicates that there is 80% more Na^+ "bound" per phosphate to the 160-bp DNA than to the 20-bp DNA under salt-free conditions investigated here. At [Na]/[P] = 20, this analysis of the NMR data indicates that there is ~40% more Na^+ bound per phosphate to the 160-bp DNA than to the 20-bp DNA. For the single-stranded 19-phosphate oligomer, not only r°_{Na} but also R_B must be significantly smaller than for the 38-phosphate double stranded oligomer (data not shown).

MC predictions of the surface concentration of Na^+ as a function of $|Z|$ and [Na]/[P]: comparison with ^{23}Na NMR results

We employ MC simulations to calculate $\bar{C}^+_{|Z|}(a)$, the axial average of the local counterion concentration in the vicinity of the $|Z|$-mer surface, at different $|Z|$, C_u, and [Na]/[P]. In particular, we test the hypothesis that r_{Na} is directly proportional to $\bar{C}^+_{|Z|}(a)$. As a starting point, we assume the standard cylindrical model for DNA, although we recognize that the details of short range interactions of Na^+ with the DNA atomic groups and/or water "bound" to either the DNA or the Na^+ are expected to vary significantly with position near the oligomer. The NMR measurement, being an average over all loci in the vicinity of an oligomer, cannot yield a detailed axial (or angular) profile of surface counterion concentration. We expect that the ion distributions predicted using the standard model, despite the absence of any axial or angular spatial variation, nevertheless can reflect the average accumulation of Na^+ near the actual surface of DNA. Although a quantitative test of this expectation has apparently not been performed, Jayaram et al. (1989) compared the mean electrostatic potential in the reference frame of a polyion modelled using explicit atom coordinates with that of the standard cylindrical model of DNA and found that the potentials were in quantitative agreement at a distance of 20 Å from the polyion. Since the electrostatic potential far from the polyion reflects the extent of charge neutralization by more closely associated counterions, we expect that the average local concentrations of counterions predicted using an explicit atom model would be similar to that reported here for the cylindrical model.

Assuming that R_B is independent of [Na] and $|Z|$, the hypothesis that r_{Na} is proportional to $\bar{C}^+_{|Z|}(a)$ implies that Eq. 1 can be rewritten as

$$R_{obs} = R_F + \alpha\bar{C}^+_{|Z|}(a)[P]/[Na] \quad (4)$$

where $\alpha \equiv r_{Na}(R_B - R_F)/\bar{C}^+_{|Z|}(a)$. To test the ability of Eq. 4 to fit the experimental data, we first calculated $\bar{C}^+_{|Z|}(a)$ using MC simulations at the same T, C_3 and (initial) C_u as in the experiment (Fig. 4). (The decrease of $\leq 8\%$ in C_u as a result of sample dilution during the course of a titration was determined to have a minimal effect on the predicted variation in $\bar{C}^+_{|Z|}(a)$ with [Na].) We find, in agreement with Olmsted et al. (1989) that $\bar{C}^+_{|Z|}(a)$ increases with $|Z|$ at fixed [Na]/[P] and that $\bar{C}^+_{|Z|}(a)$ increases with increasing [Na]/[P] at fixed [P], as was predicted by GCMC simulations on polymeric DNA (Mills et al., 1985).

MC determinations of $\bar{C}^+_{|Z|}(a)$ as a function of [P]/[Na] for both 160-bp and 20-bp DNA were fitted to a smooth functional form (spline) and the resulting fittings were used

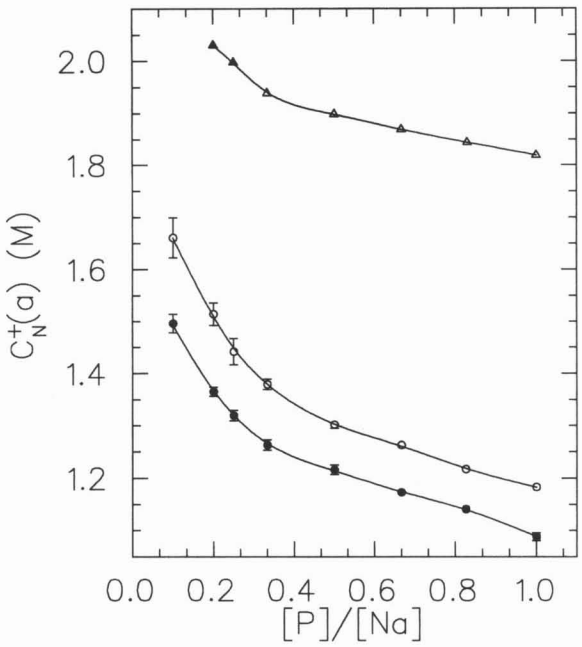

FIGURE 4 Predicted surface Na^+ concentrations for 160, 20-bp Na-DNA. Surface concentrations $\bar{C}^+_{|Z|}(a)$ of Na^+ were calculated from MC simulations for two values of $|Z|$ (160 bp: $|Z| = 320$, [P] = 9.5 mM (△, ▲): 20 bp, $|Z| = 38$, [P] = 9.5 mM (●), [P] = 4.5 mM (○) at some or all of 8 values of [Na]/[P] (1, 1.2, 1.5, 2, 3, 4, 5, 10). Values of $\bar{C}^+_{|Z|}(a)$ for 160 bp DNA were obtained directly from MC simulations ([Na]/[P] =1–3; △) and by extrapolation of the results of simulations of smaller oligomers based on the linear dependence of $\bar{C}^+_{|Z|}(a)$ on $1/|Z|$ at constant C_u (9.5 mM), ([Na]/[P] = 4, 5; ▲). The solid lines are spline fittings of the simulation results.

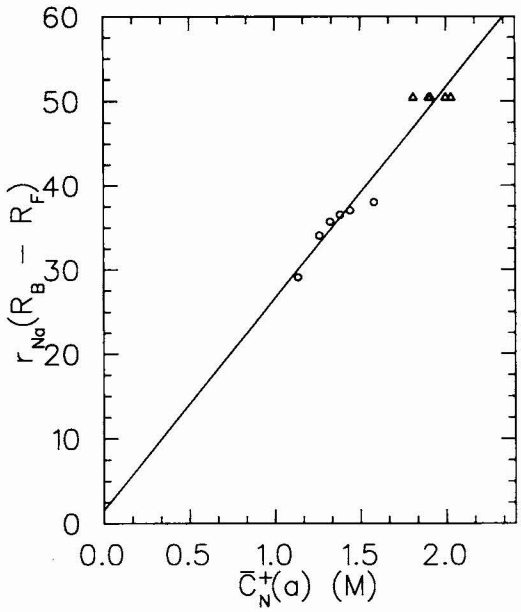

FIGURE 5 Comparison of GCMC predictions of $\bar{C}^+_{|Z|}(a)$ with NMR data for 20-bp, 160-bp NaDNA in NaCl. The two-state quantity $r_{Na}(R_B - R_F)$ was calculated from experimental values of R_{obs} by interpolation based on a linear (160 bp) or quadratic (20 bp) function of [P]/[Na]. $\bar{C}^+_{|Z|}(a)$ as a function of [Na]/[P] was obtained by MC simulations (Fig. 4). (△) 160 bp; (○) 20 bp. The line was obtained by linear regression of $r_{Na}(R_B - R_F)$ on $\bar{C}^+_{|Z|}(a)$ (intercept = 2 ± 3; slope = 25 ± 2).

with $R_F = 18.1\ s^{-1}$ in Eq. 4 to obtain a fitting of the experimental data by adjusting α (dashed lines in Fig. 3). (Our predicted values of $\bar{C}^+_{|Z|}(a)$ depend detectably on C_u (Fig. 4), so for the 20-bp DNA we averaged values of $\bar{C}^+_{|Z|}(a)$ obtained for the two experimental values of C_u.) The fact that a single value of α is capable of fitting the data for both DNA lengths supports our hypothesis that R_B is not sensitive to variations in $|Z|$.

The fittings of Eq. 4 to the data are illustrated by plotting the experimental quantity $(R_{obs} - R_F)$[Na]/[P], versus the corresponding values of $\bar{C}^+_{|Z|}(a)$ predicted by MC simulations on the standard model. The value of $r_{Na}(R_B - R_F)$ corresponding to $\bar{C}^+_{|Z|}(a)$ was obtained for values of $|Z|$ and [P]/[Na] corresponding to the MC simulations using the linear (160 bp) or quadratic (20 bp; Eq. 2) fitting of the experimental determinations of R_{obs} as a function of [P]/[Na] (Fig. 3). For each $|Z|$, the points at larger $\bar{C}^+_{|Z|}(a)$ correspond to larger values of [Na]/[P]. A perfect correlation of $R_{obs} - R_F$ with $\bar{C}^+_{|Z|}(a)$ would imply that such a plot should be linear with an intercept of 0. When the data obtained in this study were fitted to the straight line shown in Fig. 5, the intercept was 2 ± 3.

The fittings obtained using Eq. 4 do exhibit small but detectable deviations from the experimental data (Fig. 3, dashed lines). The satisfactory fitting of the data by a linear (160 bp) or quadratic (20 bp) function (Eq. 2, Fig. 3) suggests that $\bar{C}^+_{|Z|}(a)$ should be a constant (160 bp) or linear (20 bp) function of [P]/[Na]. However, Fig. 4 indicates that the $\bar{C}^+_{|Z|}(a)$ exhibit systematic deviations from these functional forms. Thus the real situation is somewhat more complicated than is indicated by Eq. 4. The two possible sources of complexity that might result in systematic deviation of R_{obs} from those given by Eq. 4 are: 1) variation of R_B with [Na]/[P], which includes either actual variation of R_B for a single bound state or variation of the "apparent" R_B associated with a more general multi-state model (described at the end of the section on NMR background); and 2) inadequacy of the model assumed for calculating r_{Na} (which could include oversimplification of the model assumed for the system and/or inadequacy of the assumption that $\bar{C}^+_{|Z|}(a)$ is proportional to r_{Na}). Further quantitative studies on oligonucleotides over a range of $|Z|$ and of concentrations of oligomer and of salt will address the origins of these systematic deviations.

In a number of recent studies (Olmsted et al., 1991, 1995; Zhang et al., unpublished observations) we have demonstrated that the thermodynamic consequences of coulombic end effects on various measurable thermodynamic properties of oligoelectrolyte B-DNA under typical experimental conditions can be analyzed accurately by calculating preferential interaction coefficients from the results of grand canonical Monte Carlo simulations on a model system whose properties are minimally parameterized. In contrast, the short range and complicated character of the interactions giving rise to the quadrupolar relaxation of sodium nuclei in the vicinity of an oligonucleotide, and the requirement that the local con-

centrations of these nuclei be accurately known within a relatively small volume, imply that accurate theoretical predictions of the magnitudes of the observable NMR properties can only be expected from a model of the system that is significantly more detailed than that which suffices for the accurate prediction of observable thermodynamic properties. Nevertheless, qualitative trends in both the $|Z|$ dependence and the dependence on [P]/[Na] of R_{obs} can be captured by simulations based on the same model that has proved successful for thermodynamic calculations.

CONCLUSIONS

The experimental results reported here are the first quantitative comparison of the extents of association of univalent cations with oligomeric (20 bp) and polymeric (160 bp) DNA. The enhancement of the ^{23}Na longitudinal relaxation rate ($R_{obs} - R_F$) at $[Na^+]/[P] \approx 1$ is 51 s^{-1} for the 160-bp DNA and 29 s^{-1} for the 20 bp, as compared to $R_F \cong 18$ s^{-1}. The titration experiments show that, in the solution containing 20-bp DNA, R_{obs} exhibits a nonlinear dependence on [P]/[Na] as [Na] increases, which differs from that observed here for 160-bp DNA (and that reported previously for polymeric length B-DNA). The NMR spectroscopic results presented here provide compelling evidence for the existence of a coulombic end effect, i.e., the nonuniformity of the axial profile of cation concentration near the ends of cylindrical $|Z|$-mers. Thermodynamic parallels of this molecular phenomenon are exhibited by the effects of oligonucleotide length on the salt-dependences manifested by the conformational transitions (Olmsted et al., 1991) and ligand binding equilibria (Olmsted et al., 1995; Bond et al., manuscript in preparation) of oligonucleotides.

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