Articles

Rotational Dynamics of Hexaamminecobalt(III) Bound to Oligomeric DNA: Correlation with Cation-Induced Structural Transitions†

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ABSTRACT: Two common assumptions are that duplex DNA structure shows little sensitivity to ionic conditions and that simple cations bind to DNA in a nonspecific fashion. Here we examine these assumptions using as a model ligand the inorganic cation $Co(NH_3)_6^{3+}$. We find, upon titration with $Co(NH_3)_6^{3+}$, that certain DNA oligonucleotides show pronounced changes in circular dichroism spectra. For oligonucleotides such as d(GGCCGGCC), with contiguous, same-strand guanines, the transition is toward A-DNA characteristics. For those oligonucleotides that manifest such large $Co(NH_3)_6^{3+}$-induced changes in circular dichroism spectra, ^{59}Co NMR relaxation measurements demonstrate that $Co(NH_3)_6^{3+}$ tumbling motions are greatly inhibited, so that the effective NMR correlation time approaches that of overall tumbling of the DNA oligomer. Very large upfield ^{59}Co chemical shifts are observed for $Co(NH_3)_6^{3+}$ bound to such oligonucleotides. For another class of oligomers, which show no significant changes in the circular dichroism spectrum in the presence of $Co(NH_3)_6^{3+}$, the tumbling motions of bound $Co(NH_3)_6^{3+}$ are largely independent of those of the oligomer, and much more modest ^{59}Co chemical shifts are observed. Oligomers in a third class cause significant rotational inhibition of bound $Co(NH_3)_6^{3+}$, and only modest changes in ^{59}Co chemical shifts. Oligonucleotides in this class appear not to undergo large structural changes in the presence of $Co(NH_3)_6^{3+}$. Within these broad categories, a remarkable variability in ^{59}Co NMR parameters, and in structural perturbations, is apparent for the 13 oligonucleotides that we have examined.

DNA structure is exquisitely sensitive to environmental influences. These influences may be nonspecific, as when the B–A transition is induced upon lowering the water activity. They may also be quite specific, as when a protein or a drug binds to a target sequence and induces DNA structural alterations to accommodate the bound ligand.

Although $Co(NH_3)_6^{3+}$ does not occur naturally in biological systems, it has proven a very useful model cation, and its interactions with DNA have been well studied. Compared to the trivalent polyamine spermidine, $Co(NH_3)_6^{3+}$ is very effective at inducing the B–Z transition (Behe & Felsenfeld, 1981) and in promoting DNA compactions (Widom & Baldwin, 1980). $Co(NH_3)_6^{3+}$ likewise dramatically affects the kinetics of cruciform extrusion (Sullivan & Lilley, 1987). An additional consideration is that $Co(NH_3)_6^{3+}$ is widely used in crystallization solvents for oligonucleotides. It is worth noting that, under the low water activity conditions present in the crystals of such oligonucleotides, A-DNA is commonly observed. In previous work, we have demonstrated a pronounced, %GC-dependent, heterogeneity of $Co(NH_3)_6^{3+}$ local binding environments on bacterial DNAs (Braunlin & Xu, 1992). We have also shown that $Co(NH_3)_6^{3+}$ can induce a B–A transition for the oligonucleotide d(CCCCGGGG), in dilute aqueous solution (Xu et al., 1993). The model that we have proposed for the interaction involves specific recognition of N7 and O6 groups on neighboring guanine residues in the major groove of A-DNA. Exactly this sort of recognition has been observed in the crystal structure of tRNA, crystallized in the presence of $Co(NH_3)_6^{3+}$ (Hingerty et al., 1982). Guanidinium side chains of arginines show similar specific recognition through major groove interactions with guanine (Steitz, 1990). Hence, studying the interaction of $Co(NH_3)_6^{3+}$ and DNA oligomers may help clarify the essential features of DNA recognition by hydrogen bonding.

It has been argued that major groove recognition of A-DNA or of RNA is improbable given the narrowness of the major groove for such species. However, this argument is based on "canonical" A-DNA features. In fact, the major groove width of A-DNA is highly variable, and is quite wide in DNA molecules with runs of same-strand guanine residues (McCall et al., 1986).

It is well-known that the Zn finger protein TFIIIA recognizes the same target sequence on the rRNA gene as it does on the rRNA product. Since B-RNA is much less energetically feasible than A-DNA, this led to the suggestion that TFIIIA might recognize A-form DNA as well as RNA (McCall et al., 1986; Rhodes & Klug, 1986; Fairall et al., 1989). As pointed out by McCall et al. (1986), a putative DNA and RNA recognition sequence of TFIIIA contains a succession of guanine runs. On the basis of their crystal structure determination of d(GGATGGGAG), these workers argued that specific recognition of A-DNA or RNA in the major groove could provide a plausible picture of the TFIIIA interaction. Nuclease digestion patterns also suggested an A-DNA pattern (Rhodes & Klug, 1986). Interpretations based on other experimental evidence (circular dichroism,

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NMR, and the effect on binding to RNA and DNA of single nucleotide changes) contest the notion that TFIIIA recognizes similar determinants on A-DNA and RNA (Gottesfeld et al., 1987; Aboul-ela et al., 1988; You et al., 1991).

Although our work does not resolve this issue, we have demonstrated that even a very simple ligand such as $Co(NH_3)_6^{3+}$ can induce dramatic structural transitions, including the B–A transition, in dilute solution, most likely via a major groove interaction. Given the simplicity of the $Co(NH_3)_6^{3+}$ interaction, it is quite likely that similar major groove recognition of A-DNA and RNA will be observed for protein–nuclei acid complexes.

Here we examine the ^{59}Co NMR chemical shifts and relaxtion behavior of $Co(NH_3)_6^{3+}$ when bound to 13 different oligomeric DNAs, and correlate changes in these parameters with structural perturbations, as monitored by circular dichroism. The results obtained demonstrate a striking variability in the DNA binding behavior of this very simple cationic ligand.

MATERIALS AND METHODS

Experimental Procedures. All oligonucleotides were synthesized on an Applied Biosystems PCR-Mate DNA synthesizer, on a 10-μM scale. Following synthesis, the oligonucleotides were dissolved in buffer, filtered to remove organic salts, and dialyzed using Spectrapore 1000 MW cutoff dialysis tubing. Dialysis changes were performed after at least 4 h. The first change was against 0.1 M Tris, pH 7.6, followed by two changes of 0.1 M NaCl and then three changes of 0.001 M NaCl. The oligonucleotide concentrations were determined from UV absorbance measurements, performed under denaturing conditions, using extinction coefficients calculated by the nearest-neighbor method (Cantor et al., 1970). The initial Na^+ concentrations of the DNA samples were determined by comparison of the integrated ^{23}Na signal intensities with intensities from a standard curve of NaCl in D_2O.

The oligoribonucleotide r(GGCCGGCC) was synthesized, purified by reverse-phase HPLC, and dialyzed into low-salt solution as previously described (Xu et al., 1993).

All DNA samples for the NMR measurements were lyophilized 3 times into 99.8% D_2O. A stock sample of 0.14 M $Co(NH_3)_6Cl_3$ was prepared by weighing out $Co(NH_3)_6Cl_3$ and lyophilizing 3 times into D_2O. The gravimetrically determined $Co(NH_3)_6^{3+}$ concentration agreed to within 1% with the concentration determined spectrophotometrically using an extinction coefficient at 473 nm of 56.2 M^{-1} cm^{-1}.

^{59}Co NMR measurements were performed at 119.8 MHz on an Omega 500 NMR spectrometer. The temperature was calibrated by measuring the difference between the proton resonances of methanol (Van Geet, 1968). ^{59}Co shifts are referenced with respect to the shift of a solution of 0.1 M $Co(NH_3)_6Cl_3$ at 21.4 °C. Consistent with convention, downfield shifts are taken to be positive.

Circular dichroism (CD) spectra were obtained with a Jasco 600 circular dichrometer. The sample temperature was ambient. CD data were transformed into molar ellipticity ($\Delta\epsilon$) in units of $M^{-1}\cdot cm^{-1}$ of monomer subunits. Base lines of the spectra were corrected by subtracting spectra of the same solutions in the presence and absence of DNA. The pH of the solution was monitored for all samples, and was always 6.0 ± 0.2.

Proton NMR chemical shifts in the DNA base region were monitored as a function of temperature on the Omega instrument in order to estimate the midpoint of the helix–coil transition of the oligonucleotides studied. On the basis of these measurements, it could be ascertained that, for the oligonucleotides studied, the transition midpoint is always in excess of 30 °C.

Data Analysis. The ^{59}Co transverse relaxation rate (R_2) of $Co(NH_3)_6^{3+}$ in aqueous solutions of simple salts has contributions from two types of relaxation processes (Rose & Bryant, 1979):

$$R_2 = R_2^{sc} + R_2^{Q} \tag{1}$$

Here R_2^{sc} represents the contribution to R_2 from scalar coupling of the ^{59}Co and ^{14}N nuclei. R_2^{Q} is the contribution to R_2 from interaction of the ^{59}Co nuclear quadrupole moment with fluctuating electric field gradients at the nucleus. The longitudinal relaxation of $^{59}Co(NH_3)_6^{3+}$ is determined by quadrupolar interactions, which gives, under the conditions of extreme narrowing prevalent in simple salt solutions, $R_1 = R_2^{Q}$.

For ^{59}Co coupled to six ^{14}N groups, the scalar contribution to the transverse relaxation is given by

$$R_2^{Sc} = 16\pi^2[J(Co\text{–}N)]^2 T_{1N} \tag{2}$$

where J(Co–N) is the cobalt–nitrogen coupling constant and $T_{1N} = R_{1N}^{-1}$ is the longitudinal relaxation time of ^{14}N. The longitudinal relaxation rates of ^{59}Co and ^{14}N in simple salt solutions of hexaamminecobalt(III) are described by

$$R_1 = R_2^{Q} = \frac{3\pi^2}{10}\frac{2I+3}{I^2(2I-1)}X^2\tau_c \tag{3}$$

where $I = 1$ for ^{14}N and $I = {}^7/_2$ for ^{59}Co. X is the quadrupole coupling constant, and τ_c is the correlation time for isotropic rotation of the $Co(NH_3)_6^{3+}$ complex. Note that combining eq 2 and 3 implies that R_2^{sc} and R_2^{Q} have opposite dependences on τ_c, and hence on temperature. As a further consequence, any process that lengthens τ_c, for example, association with a macromolecule, should increase the quadrupolar component of the relaxation and decrease the contribution from scalar coupling. Under conditions favoring association, in solution with high molecular weight DNA, the $^{59}Co(NH_3)_6^{3+}$ transverse relaxation rate decreases with increasing temperature, indicating a shortening of τ_c and dominance of the quadrupolar relaxation mechanism (Braunlin et al., 1987).

For nuclei such as ^{59}Co, with $I > 1$, if the quadrupolar relaxation mechanism dominates, and if $\omega_o\tau_c$ is close to unity, then single-exponential R_1 and R_2 relaxation rates will be observed, but R_1 will not equal R_2. Halle and Wennerström (1981a) have derived the following approximate analytical expressions for R_1 and R_2 under such "near extreme narrowing" conditions:

$$R_1 = \frac{3\pi^2}{10}\frac{2I+3}{I^2(2I-1)}X^2[0.2J(\omega_o) + 0.8J(2\omega_o)] \tag{4a}$$

$$R_2 = \frac{3\pi^2}{10}\frac{2I+3}{I^2(2I-1)}X^2[0.3J(0) + 0.5J(\omega_o) + 0.2J(2\omega_o)] \tag{4b}$$

where $J(\omega)$ is the reduced spectral density function, which describes the frequency dependence of the molecular motions modulating the quadrupolar interaction.

For the situation where isotropic rotational motions dominate the relaxation, the reduced spectral densities have the

familiar Lorentzian form:

$$J(\omega) = \frac{\tau_c}{1 + \omega^2\tau_c^2} \quad (5)$$

Given values for R_1 and R_2, eq 4 and 5 may be solved for τ_c and X. If isotropic motions do dominate the relaxation, then the error in determining τ_c by the ratio of R_1 to R_2 is less than 10% if $\omega_o\tau_c < 1.5$ (Halle & Wennerström, 1981a). For an ion that is tightly bound to a nearly spherically symmetric DNA octamer (see below), τ_c would equal the rotational correlation time for tumbling of the DNA.

If a partial averaging of the quadrupolar interaction occurs on a time scale that is rapid compared to the tumbling of the DNA, then a two-step spectral density function might be a more appropriate model (Halle & Wennerström, 1981b; Halle et al., 1984), and the effective reduced spectral density would be of the form:

$$J(\omega) = (1 - A)\tau_f + \frac{A\tau_s}{1 + \omega^2\tau_s^2} \quad (6)$$

where $AX = X_{eff}$ is the residual coupling constant remaining after the rapid averaging process (Halle et al., 1984). A is a squared order parameter between 0 (no immobilization) and 1 (complete immobilization). τ_f is a correlation time characterizing the fast motion, and τ_s is the correlation time for tumbling of the DNA molecule. For relaxation in the near extreme narrowing regime, if τ_f is much smaller than τ_s, and A is sufficiently close to 1, then the first term on the right-hand side of eq 6 is negligible. Under these conditions, if eq 4a,b are solved under the assumption of a Lorentzian spectral density, then the correlation time τ_s for tumbling of the DNA may still be determined, and the effective coupling constant will reflect the extent of partial averaging.

RESULTS

Oligonucleotides Can Be Classified Based on $^{59}Co(NH_3)_6^{3+}$ NMR Relaxation Behavior, and on Circular Dichroism Spectra. Thirteen different oligonucleotides were synthesized, purified, and titrated with $Co(NH_3)_6Cl_3$ to a ratio of approximately one $Co(NH_3)_6^{3+}$ per DNA duplex. The oligonucleotide designated as d(*CG*CG*CG*CG) is one for which all cytosines have been synthesized with a methyl group at the 5 position. Such methylation stabilizes Z-DNA relative to B-DNA (Behe & Felsenfeld, 1981; Fuji et al., 1982; Quadrifoglio & Manzini, 1984). Under the conditions of these experiments, as judged by circular dichroism, this oligomer is about 50% in Z-form at room temperature.

Under the low-salt conditions of these experiments, binding measurements indicate that essentially all $Co(NH_3)_6^{3+}$ in solution should be bound to the DNA (Braunlin et al., 1987; Plum & Bloomfield, 1988). For all of the oligonucleotide solutions, temperature-dependent determinations were made of the $^{59}Co(NH_3)_6^{3+}$ line widths ($\Delta\nu_{1/2}$) and longitudinal relaxation rates (R_1). The transverse relaxation rate (R_2) is estimated as $R_2 = \pi^*\Delta_{1/2}$. As shown in Table I, on the basis of the observed relaxation behavior, and on $Co(NH_3)_6^{3+}$-induced structural changes, as monitored by CD, oligonucleotides could be classified into one of these classes. The behavior of class 1 oligonucleotides is illustrated in Figure 1 for the representative oligonucleotide d(GGCCGGCC). As shown in Figure 1, $^{59}Co(NH_3)_6^{3+}$ relaxation in solution with oligonucleotides of this class shows the characteristic quadrupolar temperature dependence, indicating a significant inhibition of $Co(NH_3)_6^{3+}$ tumbling motions due to interaction with the DNA. Using eq 4 and 5, a comparison of R_1 and R_2 allows us to calculate effective correlations times and coupling constants for $^{59}Co(NH_3)_6^{3+}$ in the presence of these oligonucleotides. The values thus calculated are also given in Table I. It is noticeable that, although the coupling constants show some variation (see below), the correlation times thus calculated are all within 20% of the value of 1.8 ns that is calculated for d(GGCCGGCC).

Table I: Classification of Oligonucleotides on the Basis of ^{59}Co NMR and Circular Dichroism Spectra[a]

class	oligonucleotide	R_1 (s^{-1})	R_2 (s^{-1})[b]	σ (ppm)	χ_{eff} (MHz)	τ_{eff} (ns)
1	d(GGGGCCCC)	471.2	1394.1	−46.477	2.0	1.8
	d(GGCCGGCC)	473.8	1262.1	−42.515	1.9	1.6
	d(CCCCGGGG)	429.7	1333.2	−33.424	1.9	1.8
	d(AAGGCCTT)	313.9	921.9	−30.921	1.6	1.8
	d(*CG*CG*CG*CG)	226.9	730.3	−29.609	1.4	1.9
	d(CCGATCGG)	193.0	613.4	−22.258	1.3	1.9
2	d(GGAATTCC)	109.8	332.7	−19.073	2.4[b]	0.045
	d(CATATATG)	152.6	380.3	−18.714	2.4[b]	0.063
	d(GTATATATAC)	136.2	339.5	−17.847	2.4[b]	0.056
	d(AATTAATT)	105.9	332.6	−17.684	2.4[b]	0.044
	d(AAAATTTT)	89.1	308.5	−16.050	2.4[b]	0.037
3	d(CTCTAGAG)	159.2	414.2	−19.236	1.1	1.5
	d(GCGCGCGC)	152.0	479.1	−18.878	1.1	1.9
	$Co(NH_3)_6Cl_3$	24.2	383.0	0.000	2.4[b]	0.010[c]

[a] All solutions contain close to one $Co(NH_3)_6^{3+}$ per DNA duplex. The concentration of the DNA strand varies between 1.59 and 3.42 mM. The concentration of $Co(NH_3)_6^{3+}$ varies between 0.84 and 1.72 mM. The temperature is 21.4 °C. The concentration of the $Co(NH_3)_6Cl_3$ simple salt solution (final row) is 0.1 M. The ^{59}Co chemical shift of this solution at 21.4 °C is set to zero, and used as a reference for ^{59}Co chemical shifts in DNA solutions. [b] This value is calculated from eq 3, assuming a rotational correlation time of 10 ps for $Co(NH_3)_6^{3+}$ in simple salt solution. [c] This estimate is from Hartmann and Sillescu (1964).

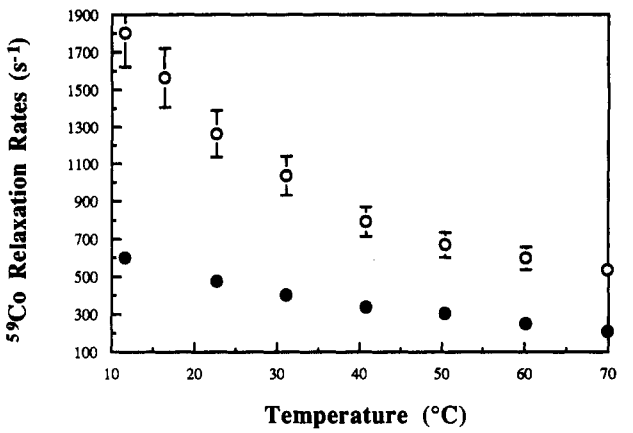

FIGURE 1: ^{59}Co longitudinal R_1 (●) and transverse R_2 (○) relaxation rates at 119.6 MHz versus temperature for a sample containing 2.4 mM d(GGCCGGCC) strands and 1.2 mM $Co(NH_3)_6Cl_3$. On the basis of proton NMR chemical shifts, the melting temperature is estimated to be greater than 60 °C.

A DNA octamer is roughly 27 Å long. Including a hydration layer, the diameter of a DNA helix is about 26 Å. Hence, to a good approximation, a DNA octamer is well described as a hydrodynamic sphere of radius 13 Å. For such a sphere, assuming a solvent viscosity $\eta_0 = 10^{-2}$ g·cm^{-1}·s^{-1}, and a temperature of 295 K, then a calculation using the Stokes–Einstein equation predicts a rotational correlation time of 2.2 ns. Upon comparison, it is clear that for class 1 oligonucleotides, the rotational motions dominating the $^{59}Co(NH_3)_6^{3+}$ relaxation are the overall tumbling motions of the DNA oligonucleotide. In other words, on the nanosecond time scale, the $Co(NH_3)_6^{3+}$ is site-bound and largely immobilized on the surface of the DNA.

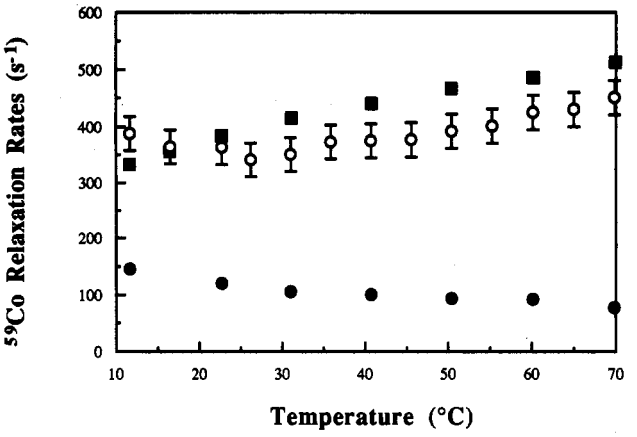

FIGURE 2: ^{59}Co longitudinal R_1 (●) and transverse R_2 (○) relaxation rates at 119.6 MHz versus temperature for a sample containing 1.8 mM d(GGAATTCC) strands and 1.2 mM $Co(NH_3)_6Cl_3$. On the basis of proton NMR chemical shifts, the melting temperature is estimated to be greater than 40 °C. The transverse relaxation rates for a sample of 0.1 M $Co(NH_3)_6Cl_3$ (■) are shown for reference.

This situation is to be contrasted to that of the class 2 oligonucleotides, illustrated in Figure 2 for the oligonucleotide d(GGAATTCC). For d(GGAATTCC), we have also monitored the assigned base-proton NMR spectrum (Broido et al., 1984, 1985) to determine a transition midpoint for the helix–coil transition of around 40 °C under the conditions of these experiments. Class 2 oligonucleotides show the temperature dependence characteristic of the scalar coupling mechanism. The dominance of the scalar coupling mechanism is consistent with the observation that, over most of the temperature range monitored, the $^{59}Co(NH_3)_6^{3+}$ line width is larger in simple salt solution than it is in the presence of class 2 oligonucleotides. By comparison to other cobalt complexes (Hartmann & Sillescu, 1964; Craighead et al., 1975), a reasonable estimate of the rotational correlation time of $^{59}Co(NH_3)_6^{3+}$ in simple salt solution would range from 10–30 ps. On the basis of Hartmann and Sillescu's estimate of 10 ps, the observed $^{59}Co(NH_3)_6^{3+}$ longitudinal relaxation rates in the presence of oligonucleotide allow estimates to be made of effective rotational correlation times for class 2 oligonucleotides. The values thus obtained are also given in Table I, and indicate only very modest inhibition of the $Co(NH_3)_6^{3+}$ tumbling motions when this cation is bound to class 2 oligonucleotides.

An additional distinguishing feature of class 1 oligonucleotides is that they all show large changes in circular dichroism spectra when titrated with $Co(NH_3)_6Cl_3$. In a previous study, we have discussed in detail the example of d(CCCCGGGG), which undergoes a B–A transition in the presence of $Co(NH_3)_6^{3+}$ (Xu et al., 1993). Two additional examples are shown in Figures 3 and 4. In Figure 3, d(*CG*CG*CG*CG) is titrated with $Co(NH_3)_6Cl_3$. As is apparent from the change in sign of the CD peaks, for this oligonucleotide, a B–Z transition is induced by $Co(NH_3)_6^{3+}$ binding. The changes observed in Figure 4, for the oligonucleotide d(GGCCGGCC), are also quite dramatic, and in this case show features of a $Co(NH_3)_6^{3+}$-induced transition to a structure intermediate between A-DNA and B-DNA: (1) The conservative π-π^* bands near 260 nm, that are evident in the absence of $Co(NH_3)_6^{3+}$, become nonconservative in the presence of $Co(NH_3)_6^{3+}$; i.e., the peak intensity ratio, of 264 nm versus 238 nm, increases. (2) Upon titration with $Co(NH_3)_6Cl_3$, the n–π^* band at 214 nm becomes more negative, and the band at 285 nm is shifted to 290 nm and decreases in intensity. These changes are indicative of base-tilting. (3) As a

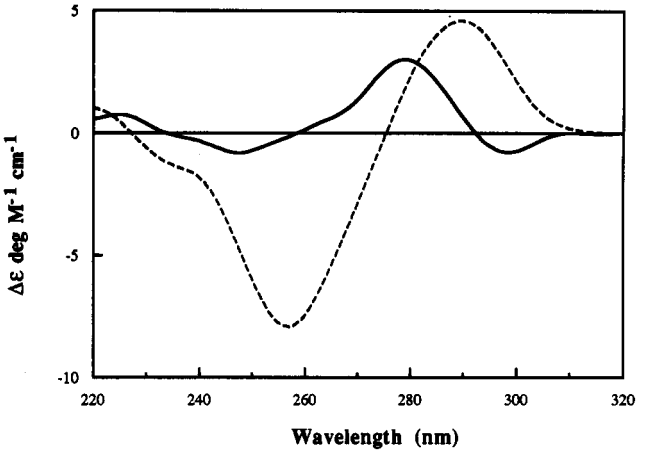

FIGURE 3: Circular dichroism spectra of 5.8 μM d(*CG*CG*CG*CG) strand. The dashed curve is for a sample with no added salt (Na^+ concentration roughly 50 μM). The solid curve is the same sample titrated with 12 μM $Co(NH_3)_6Cl_3$.

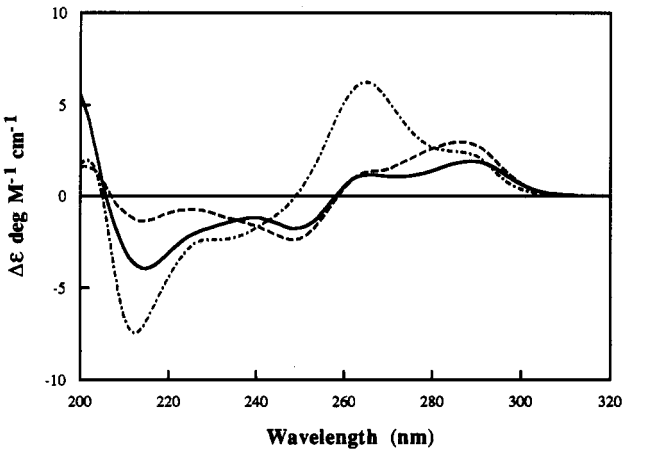

FIGURE 4: Circular dichroism spectra of 8.6 μM d(GGCCGGCC) strand. The dashed curve is for a sample in the presence of 0.1 M NaCl. The solid curve is for the same sample, with no added NaCl, but with 17.8 μM $Co(NH_3)_6Cl_3$. The total Na^+ concentration for this latter sample is estimated at 60 μM. For comparison, the dot–dash curve shows a sample of 5.9 μM r(GGCCGGCC) in 0.1 M NaCl.

consequence of these changes in the n–π^* regions, the CD spectrum of d(GGCCGGCC) in the presence of $Co(NH_3)_6^{3+}$ shows greater similarity to the spectrum of r(GGCCGGCC) (Figure 4). In addition, (4) the NMR chemical shifts of the GH8 protons on d(GGCCGGCC) move upfield of CH6 in the presence of $Co(NH_3)_6^{3+}$, a characteristic of A-DNA and RNA (unreported results). All class 1 oligonucleotides show noticeable changes in CD spectra upon titration with $Co(NH_3)_6Cl_3$. This situation is likewise to be contrasted with that of class 2 oligonucleotides, which show no large CD changes upon titration with $Co(NH_3)_6Cl_3$.

A third class of oligonucleotide can also be distinguished. As illustrated in Figure 5, oligomers such as d(GCGCGCGC) show the characteristic quadrupolar relaxation behavior, prior to the onset of melting. Effective correlation times are also in the range of 2 ns (Table I). However, class 3 oligonucleotides show no significant CD changes upon titration with $Co(NH_3)_6Cl_3$ (results not shown). The relatively small effective quadrupole coupling constants determined for class 3 compared to class 1 oligonucleotides are indicative of an increase in the rotational degrees of freedom (smaller A in eq 6) for $Co(NH_3)_6^{3+}$ bound to the former compared to the latter.

Intimate Association and Cation-Induced Structural Transitions Are Reflected in $^{59}Co(NH_3)_6^{3+}$ Chemical Shifts and

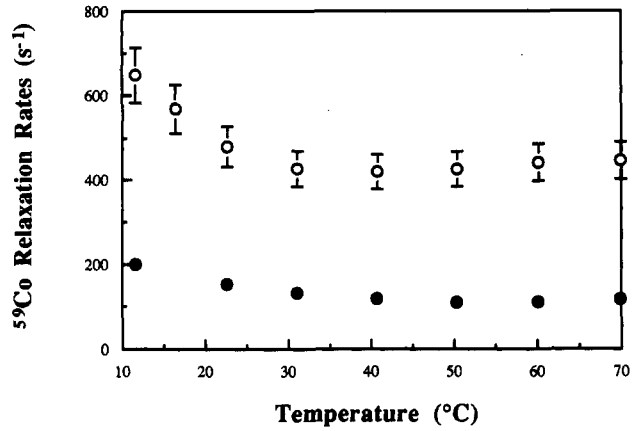

FIGURE 5: ^{59}Co longitudinal R_1 (●) and transverse R_2 (○) relaxation rates at 119.6 MHz versus temperature for a sample containing 2.1 mM d(GCGCGCGC) strands and 1.2 mM $Co(NH_3)_6Cl_3$. On the basis of proton NMR chemical shifts, the melting temperature is estimated to be greater than 60 °C.

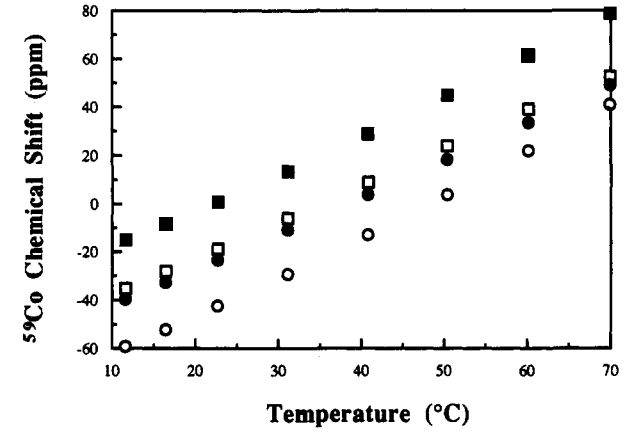

FIGURE 6: ^{59}Co chemical shifts vs temperature curves for $Co(NH_3)_6Cl_3$ in the presence of d(GGCCGGCC) (○). GGAATTCC (●), and GCGCGCGC (□). For comparison, the chemical shifts are also shown for a 0.1 M standard solution of $Co(NH_3)_6Cl_3$ (■). Linear least-squares fits to these data give slopes of between 1.5 and 1.6 ppm per degree centigrade.

Quadrupole Coupling Constants. $^{59}Co(NH_3)_6^{3+}$ chemical shifts are exquisitely sensitive to environmental influences. For all of the oligonucleotides examined, the $^{59}Co(NH_3)_6^{3+}$ chemical shift is nearly linear with temperature, with a slope of around 1.6 ppm/°C (Benedek et al., 1963). However, the magnitude of the shift varies significantly from oligomer to oligomer. The general trend is that the largest upfield shifts of the $^{59}Co(NH_3)_6^{3+}$ resonance are observed for class 1 oligonucleotides, amounting to nearly 50 ppm for the case of d(GGGGCCCC). Oligonucleotides in classes 2 and 3 all show bound $^{59}Co(NH_3)_6^{3+}$ chemical shifts of around −20 ppm with respect to 0.1 M $Co(NH_3)_6Cl_3$. To illustrate these points, representative chemical shift versus temperature curves are shown in Figure 6. It is also noteworthy that within class 1 oligonucleotides, the largest upfield chemical shifts appear to correlate with the most dramatic changes in CD spectra.

The effective quadrupole coupling constants given in Table I are all smaller than the value of 2.4 MHz that can be estimated on the basis of the work of Hartmann and Sillescu (1964). For a one-site model, in which isotropic rotation of the bound complex is assumed, a smaller quadrupole coupling constant would imply that bound $Co(NH_3)_6^{3+}$ experiences a significantly more symmetric environment than does free $Co(NH_3)_6^{3+}$. This is unlikely, and thus suggests a breakdown of the model. One possibility is that the observed coupling constants reflect some degree of partial averaging of the quadrupolar interaction by rapid local motions. As discussed above (eq 6), if the time scale of such local motions is sufficiently well separated from the time scale of oligomer tumbling, then the application of eq 4a,b will still give an accurate estimate of the correlation time for oligomer tumbling. The effective quadrupole coupling constant will, however, reflect the relative contributions of the rapid and slow processes to the decay of correlation of the principal component of the electric field gradient tensor. In terms of this model, a decrease in the effective quadrupole coupling constant would reflect an increase in the rotational degrees of freedom of the bound $Co(NH_3)_6^{3+}$. Another possible explanation for the decreased quadrupole coupling constant would be that tightly bound, rotationally immobilized $^{59}Co(NH_3)_6^{3+}$ is in rapid exchange with nonspecifically bound, rotationally mobile $^{59}Co(NH_3)_6^{3+}$. The experimental data reported here do not allow a distinction to be made between the two hypotheses. Both could give calculated effective correlation times on the time scale of DNA tumbling. Both hypotheses are also consistent with the observation that the magnitude of the effective quadrupole coupling constants in Table I always increases in parallel with the magnitude of the ^{59}Co chemical shift, and also with the most dramatic structural effects, as monitored by CD.

The correlation between large chemical shift changes, large quadrupole coupling constants, and large effects on CD is particularly clear when comparing class 1 oligonucleotides such as d(GGGGCCCC), which show large CD changes, and class 3 oligonucleotides such as d(GCGCGCGC), which show no significant changes in CD. Oligonucleotides such as d(CCGATCGG), with modest but significant CD changes, and modest chemical shifts and coupling constants, may represent the transition between classes 1 and 3.

DISCUSSION

In previous work, we have shown that there are at least three classes of cation binding environments on duplex polymeric DNA (Braunlin & Xu, 1992). A slowly exchanging class of bound $^{59}Co(NH_3)_6^{3+}$, which is only apparent for *Micrococcus lysodeikticus* DNA (72% GC), gives a very broad, upfield-shifted peak at 85 ppm upfield of the 0.1 M $Co(NH_3)_6Cl_3$ standard solution. Two additional classes are in rapid exchange. One of these rapidly exchanging classes corresponds to transiently localized DNA, whereas the other corresponds to nonspecifically bound, highly mobile $^{59}Co(NH_3)_6^{3+}$. The ratio of $^{59}Co(NH_3)_6^{3+}$ in each of these latter two classes decreases with GC content, and over the course of a $Co(NH_3)_6Cl_3$ titration.

A significant simplification in the current work is that the tumbling motions of the oligonucleotides studied are nearly isotropic. Tight binding of $^{59}Co(NH_3)_6^{3+}$ is characterized by a dominance of the quadrupolar relaxation mechanism over the scalar coupling mechanism, and moreover by an effective correlation time reflecting the overall tumbling of the DNA molecule. The rotational inhibition of bound $^{59}Co(NH_3)_6^{3+}$ is reflected not only in the relaxation behavior but also in the effective quadrupole coupling constant (with a decrease in the coupling constant corresponding to an increase in the rotational degrees of freedom).

It is clear from Table I that $Co(NH_3)_6^{3+}$ shows a remarkable range of binding behavior to oligonucleotide DNA. Nonetheless, on the basis of the results of the current study, we are also able to define three broad classes of bound $^{59}Co(NH_3)_6^{3+}$. As for the polymer case, the rotational mobility again shows a tendency to decrease with increasing GC content. However,

it is clear from Table I that GC content alone is not the decisive factor determining the rotational freedom of the $Co(NH_3)_6^{3+}$ cation in the bound state. On the basis of the results of Table I, rotational immobilization on right-handed duplex DNA does seem to correlate with runs of neighboring guanine residues; however, the position of d(GGAATTCC) in class 2 demonstrates that this is not the sole determining factor. Other important factors are undoubtedly end-effects on the local electrostatic potential, which would favor ion binding in the middle of the duplex (Record & Lohman, 1978; Olmsted et al., 1989; 1991; Mills et al., 1992), and end-effects on DNA conformational flexibility. It remains to be seen how $^{59}Co(NH_3)_6^{3+}$ rotational immobilization on d(*CG*CG*CG*CG) is related to the reduced mobility of spermine on d(*CG*CG*CG) (Banville et al., 1991) when compared to d(GGAATTCC) (Wemmer et al., 1985).

Class 2 DNA oligomers tend to be AT-rich. The rotational mobility of $Co(NH_3)_6^{3+}$, in a solution of these DNA oligomers, appears only 5 times slower than it is in simple salt solution. This indicates that $Co(NH_3)_6^{3+}$ does not bind tightly to these oligodeoxynucleotides. The slightly impeded mobility of $Co(NH_3)_6^{3+}$ leaves scalar relaxation as the dominant transverse relaxation process, as indicated by the characteristic temperature dependence of the ^{59}Co line width. The modest slowing of the rotational motions results in line widths that are smaller in DNA solution than they are in simple salt solution. This characteristic feature arises because a slight rotational inhibition of $Co(NH_3)_6^{3+}$ decreases T_{1N}. As the coupling constant J(Co–N) is relatively constant (Rose & Bryant, 1979; Braunlin, unreported experiments), the scalar coupling contribution to the transverse relaxation rate decreases. Therefore, evidence suggests that $Co(NH_3)_6^{3+}$ cations are delocalized and mobile when bound to DNA to the second class, and do not penetrate into DNA grooves.

The involvement of guanines, and of guanine runs, in $Co(NH_3)_6^{3+}$ binding is reasonable from a structural perspective. Pronounced regions of negative electrostatic potential are localized in the major groove on the N7 and O6 of guanine residues (Pullman & Pullman, 1981; Jayaram et al., 1989). These two residues can both act as hydrogen bond acceptors. Two neighboring (same-strand) guanine residues would thus provide four potential hydrogen bond acceptors for a hydrogen bond donating ligand such as $Co(NH_3)_6^{3+}$. Exactly this type of binding site has been found on tRNA, where $Co(NH_3)_6^{3+}$ has been localized in the major groove of a duplex region, spanning two neighboring guanine residues (Hingerty et al., 1982). It is worth noting that canonical A-DNA has a very narrow major groove that would prohibit penetration of a ligand as large as $Co(NH_3)_6^{3+}$ (~5-Å diameter). However, the major groove width of A-DNA (or RNA) containing stretches of contiguous guanines is anomalously wide and, as the work of Hingerty et al. (1982) demonstrates, does provide ample room for such penetration. In particular, the crystal structure of d(GGCCGGCC) shows a wide major groove, and other features of a structure intermediate between B-DNA and A-DNA (Wang et al., 1982).

Penetration of $Co(NH_3)_6^{3+}$ into the major groove also provides a plausible explanation for the observation that increased rotational immobilization of $Co(NH_3)_6^{3+}$ (i.e., larger correlation times and quadrupole coupling constants) correlates with a larger upfield ^{59}Co chemical shift. Such a large upfield shift indicates that tbe ^{59}Co nucleus is shielded from the magnetic field by electron currents, most likely from bases in DNA grooves. This indicates that for class 1 oligonucleotides, $Co(NH_3)_6^{3+}$ is probably inside the DNA grooves, and close to the DNA bases. The narrowing of the groove required to form a tight complex in turn provides an explanation for the ability of $Co(NH_3)_6^{3+}$ to induce transitions of these oligonucleotides in the direction of B–A.

In DNA solutions of class 1 oligonucleotides, it is tempting to speculate that larger upfield shifts of $^{59}Co(NH_3)_6^{3+}$ could indicate deeper penetration of $Co(NH_3)_6^{3+}$ into DNA grooves where the electric currents on DNA bases may influence the shielding of the ^{59}Co nucleus. A tentative order of penetration of $Co(NH_3)_6^{3+}$ into grooves of DNA of the first group might then be estimated from Table I: d(GGGGCCCC) ≈ d(GGCCGGCC) > d(CCCCGGGG) > d(AAGGCCTT) > d(CCGATCGG). The localization of $Co(NH_3)_6^{3+}$ bound to d(*CG*CG*CG*CG) is probably similar to that determined by Gessner et al. (1985) for $Co(NH_3)_6^{3+}$ bound to d(CGCGCG), because both of these sequences assume the Z-form in the presence of $Co(NH_3)_6^{3+}$.

Comparing the results of CD and ^{59}Co NMR, it is clear that rotational inhibition of $Co(NH_3)_6^{3+}$ when bound to DNA oligomers with runs of consecutive guanines correlates with severe perturbation of DNA conformation. The structures of such DNA oligomers are known to be very polymorphic (Haran et al., 1987). This conformational flexibility in turn helps to explain how putative major groove binding ligands such as $Co(NH_3)_6^{3+}$ can induce significant DNA structural perturbations. On the basis of the crystal structures now availalble, it seems that such "induced-fit" binding is often the rule when DNA binding proteins recognize specific DNA sequences (Steitz, 1990).

In vivo Z-DNA has been detected (Lafer et al., 1981), and A-DNA has been suggested to exist in the presence of TFIIIA (Klug & Rhodes, 1987). $Co(NH_3)_6^{3+}$ is well-known to induce the B–Z transition. Here we have also examined how DNA with A-form characteristics can be induced by a strong interaction of $Co(NH_3)_6^{3+}$ and DNA. DNA binding proteins are rich in arginines and lysines. These amino acids have side chains similar to the ligand groups on $Co(NH_3)_6^{3+}$. Therefore, it seems quite likely that A- and Z-DNA could be induced *in vivo* by the interaction of DNA with basic proteins.

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