Induction of B-A Transitions of Deoxyoligonucleotides by Multivalent Cations in Dilute Aqueous Solution

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ABSTRACT Circular dichroism (CD) spectra of d(CCCCGGGG) in the presence of $Co(NH_3)_6^{3+}$ are very similar to spectra of r(CCCCGGGG). In contrast, B-form characteristics are observed for d(CCCCGGGG) in the presence of Na^+ and Mg^{2+}, even at high salt concentrations. Spermidine induces modest changes of the CD of d(CCCCGGGG).

The NMR chemical shifts of the nonexchangeable protons of d(CCCCGGGG) in the absence and presence of $Co(NH_3)_6^{3+}$ were assigned by proton two-dimensional (2D) NOESY and COSY measurements. The chemical shifts of the GH8 protons of d(CCCCGGGG) move upfield upon titration with $Co(NH_3)_6Cl_3$. The sums of the sugar H1′ coupling constants decrease with added $Co(NH_3)_6Cl_3$. Cross peak intensities in the 2D proton NOESY spectra show a transformation from B-DNA to A-DNA characteristics upon the addition of $Co(NH_3)_6Cl_3$.

The temperature-dependent ^{59}Co transverse and longitudinal relaxation rates demonstrate that $Co(NH_3)_6^{3+}$ is site-bound to the oligomer. Such localization is not a general feature of $Co(NH_3)_6^{3+}$ binding to oligonucleotides.

^{59}Co NMR relaxation and CD measurements demonstrate chiral discrimination by d(CCCCGGGG) for the two stereoisomers of $Co(en)_3^{3+}$. Both stereoisomers bind tightly as judged by ^{59}Co NMR, and both cause large (but nonequivalent) changes in the CD of this oligomer.

INTRODUCTION

The structural polymorphism of DNA provides a wealth of possibilities for recognition by gene-regulatory proteins and other DNA binding ligands (Calladine, 1982; Steitz, 1990; Drew et al., 1990). This structural variability depends not only on sequence, but also on the environment of the DNA.

The environmental constraints imposed on DNA under conditions of x-ray crystal structure determinations are low water activity and generally high multivalent cation concentrations. Additional constraints are provided by steric "packing" effects. High-resolution NMR structure determinations are on the other hand generally performed in dilute aqueous solution in the absence of multivalent cations. As a consequence, x-ray and NMR studies are complementary in the sense that they provide information on the manner in which DNA molecules respond to the extremes of the environmental conditions that may modulate their structure. In this context we note that A-DNA is commonly observed in the crystal. In solution the B-form is more often evident. The middle ground between these two extremes has been less well studied. It is the subject of the current investigation. Here, we investigate the role that simple multivalent cations alone may play in inducing variations in the local structure of DNA.

Based on Raman spectroscopic evidence, the polymeric DNA poly(dG)·poly(dC) exists in A-form at DNA base concentrations approaching the molar range, and in B-form at millimolar concentrations (Nishimura et al., 1985; Benevides et al., 1986). The B-A transition of the oligomer d(CCCGGG) has been induced by reducing the water activity with trifluoroethanol (Wolk et al., 1989). Here we demonstrate that by addition of salts of multivalent cations it is possible to induce the B-A transition of deoxyoligonucleotides of the type $d(G_n)\cdot d(C_n)$ in dilute aqueous solution. The B-A transition thus induced does not result from the delocalized binding of cations, but requires cation binding to defined locations on the DNA oligomer.

Barton and coworkers have demonstrated that $Co(NH_3)_6^{3+}$ can mediate the specific cleavage of DNA at 5′G-pur-3′ sequences (Fleisher et al., 1988). Such sequences can thus provide specific binding sites for $Co(NH_3)_6^{3+}$. Our work supports this conclusion for $Co(NH_3)_6^{3+}$ binding to the subset of 5′G-G-3′ sequences. Moreover, our work demonstrates that $Co(NH_3)_6^{3+}$ does not just passively recognize existing structures. In order to facilitate specific binding to d(CCCCGGGG), $Co(NH_3)_6^{3+}$ induces a profound structural transformation from B-DNA to A-DNA. Induced-fit binding is also generally observed in crystal structures of specific DNA-protein complexes (Steitz, 1990). In all cases examined, such binding occurs through specific recognition of the bases that project into the major groove of B-DNA. This result is consistent with the prediction that hydrogen bonding surfaces in the major groove are better adapted to sequence discrimination than surfaces in the minor groove (Seeman et al., 1976). Our work provides additional support for the notion that induced-fit binding is a common phenomenon when ligands bind in DNA grooves. Moreover, it is tempting to speculate that the binding of simple cations or basic proteins to DNA and simultaneous induction of B-A transitions could inhibit specific protein binding and thus function as a gene control mechanism (*a*) by limiting

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access via narrowing the major groove, and (*b*) by specifically binding to and thus blocking access to groups in the major groove.

Although the biological significance of A-DNA has not been demonstrated, it is known that the zinc finger protein TFIIIA binds target sequences on both the 5 S RNA gene as well as the 5 S RNA itself (Klug and Rhodes, 1987). Since A-DNA is much more energetically favorable than B-RNA, this has led to the suggestion that the DNA binding site for TFIIIA might adopt the A-form (Rhodes and Klug, 1986). For canonical A-DNA or RNA, the major groove is too narrow to allow significant access by proteins or most other ligands. However, the target sequence for TFIIIA contains contiguous stretches of guanine residues. It has been argued that the relatively wide major groove that is adopted by such stretches in the A-form could allow for sequence-specific recognition of A-DNA or RNA (McCall et al., 1986). DNase digestion patterns, and some circular dichroism results further support the notion that TFIIIA binds to A-DNA, or A-like DNA (Rhodes and Klug, 1986; Fairall et al., 1989). However, the idea that TFIIIA recognizes A-DNA has been questioned by several workers (Gottesfeld et al., 1987; Aboul-ela et al., 1988; You et al., 1991). Others have argued that TFIIIA may recognize a form intermediate between A-DNA and B-DNA (Fairall et al., 1989; Huber et al., 1991). It appears that the evidence to date is not conclusive, and that, in general, the potential biological relevance of A-DNA remains an unresolved issue.

With respect to whether or not A-DNA is present in vivo, one issue that has not been systematically addressed is the role of ionic associations. In this context it is certainly worth noting that ionic interactions with histones stabilize DNA that is packaged in the nucleus, and that the environmental constraints on the DNA may indeed be more closely approximated in the crystal than in dilute monovalent salt solution.

In addition to TFIIIA, several other zinc finger proteins also recognize DNA sequences possessing stretches of guanines. The induction by cobalt ammine cations of structural transitions of duplex DNA containing such stretches may provide a useful model for the potential induction of similar transitions by zinc finger proteins such as TFIIIA (Rhodes and Klug, 1986), Sp1 (Kadonga et al., 1987), and MBP-1 (Sakaguchi et al., 1991). In this light it is known from crystal structures that hexaamminecobalt(III) cations (Hingerty et al., 1982) as well as guanidinium groups on arginine residues (Steitz, 1990) have proton donor ligands that can make simultaneous hydrogen bonding contact in the major groove with N7 and O6 groups on neighboring guanine residues.

In the crystal structure of the DNA complex with the three zinc fingers of the Zif268 protein (Pavletich and Pabo, 1991), five contacts are in fact made in the major groove between guanine residues and arginine side chains. The structure of the DNA in this crystal structure, while overall in the B-form, differs significantly from the canonical B-form conformation, possibly reflecting protein-induced structural perturbations.

Chiral discrimination by metal complexes for different DNA structures is another active area of research that has been pioneered by Barton and coworkers (Barton et al., 1984; Barton, 1986). Here we also explore the question of whether very simple chiral complexes can induce significant conformational transitions of duplex deoxyoligonucleotides, and the extent to which the conformations induced depend on the chirality of the complex.

MATERIALS AND METHODS

Sample preparation

The deoxyoligonucleotide, d(CCCCGGGG), was synthesized on an Applied Biosystems Model 391 DNA synthesizer using the standard cyanoethyl phosphoramidite chemistry. The deprotected oligomer was dissolved in water and dialyzed against 0.1 M NaCl solution once, and 1 mM NaCl solution three times. The oligomer solution was then filtered through a 0.45-μm Millipore filter and lyophilized. The DNA samples for the CD experiments were prepared by diluting the lyophilized octamer to the desired concentration corresponding to 0.5 UV absorbance units at 260 nm. The samples for NMR experiments were further lyophilized against 99.9% D_2O three times and then dissolved in 99.96% D_2O for H-1 COSY and NOESY experiments, or in 99.9% D_2O for Co-59 NMR experiments. The extinction coefficient, ϵ_{260} = 53.8 (mM cm)$^{-1}$, of d(CCCCGGGG) at 260 nm was calculated according to the nearest neighbor formula proposed by Cantor et al. (1970). The sodium ion concentrations of the low salt NMR samples were estimated from ^{23}Na NMR intensity measurements that were compared to standard curves obtained from ^{23}Na NMR spectra of variable concentration sodium chloride concentrations.

The RNA oligomer r(CCCCGGGG) was synthesized on an Applied Biosystems 392 DNA/RNA synthesizer. The 1-μmol columns for synthesis were obtained from Glen Research. A 3:1 mixture (v/v) of 30% aqueous ammonium hydroxide:absolute alcohol was used to cleave the oligoribonucleotides from the support. The cleaved oligoribonucleotides were collected in glass vials and incubated overnight at 55°C to remove the the base protecting groups. The ammonia/ethanol solution of RNA was transferred to polypropylene vials and vacuum-dried. 1 M tetra-*n*-butylammonium fluoride in tetrahydrofuran (THF) (Aldrich P/N 21614-3) was added (0.4 ml) to the crude RNA for deprotection of the 2′-OH protecting group. The solutions were vortexed thoroughly and left at ambient temperatures for 20–24 h. The tetra-*n*-butylammonium fluoride/THF solution was evaporated in vacuum. After drying, the RNA was dissolved in sterile water and transferred to a dialysis membrane (molecular weight cutoff 500) purchased from Spectrum. The RNA solution was dialyzed overnight in a solution of 0.1 M NaCl. The dialysis was repeated in 0.01 and 0.001 M NaCl solutions. After dialysis, the RNA solution was filtered using a 0.45-μm Millipore filter and lyopholized. The dry product was dissolved in 1 ml of sterile, deionized, and millipore-filtered water.

The sample was purified by high-performance liquid chromatography (HPLC) using a Zorbax Rx C-18 reversed-phase column. The mobile phase is a solvent buffer system composed of 0.1 M triethylammonium acetate (buffer A) and acetonitrile (buffer B). The HPLC was run for 60 min using a flow rate of 1.0 ml/min with a gradient of 100% buffer A initially and 17% buffer B at the end. The oligonucleotide was dialyzed again into 0.001 M NaCl, as described in the preceding paragraph. The final yield of RNA was about 0.3 μmol of RNA strand.

Hexaamminecobalt(III) chloride, tris(ethylenediamine)cobalt(III) chloride, spermidine, L(+)-tartaric acid, magnesium chloride, and sodium chloride were purchased from Aldrich. D(−)-Tartaric acid was purchased from Lancaster Synthesis. Hexaamminecobalt(III) chloride was purified by recrystallization (Douglas, 1978). D(+) and L(−) enantiomers of tris(ethylenediamine)cobalt(III) chloride were resolved with L(+)-tartaric acid and D(−)-tartaric acid, respectively. The chloride salts were obtained by displacing tartrate with a concentrated sodium chloride solution according to procedures described by Rochow (1960). The purity of the resolution was

checked by a polarimeter at 589 nm. The specific rotations $[\alpha]_D$ of D(+)- and L(−)-tris(ethylenediamine)cobalt(III) chloride were measured to be 161 and −161°, respectively. Reported specific rotations are ±154° (Werner, 1912). The chloride salt of spermidine was pH adjusted with HCl to pH 7.00 and used without further purification.

Circular dichroism experiments

CD spectra were obtained with a Jasco 600 circular dichrometer. The sample temperature was ambient. CD data were transformed into molar ellipticity $[\theta]$ in the units of degree cm²/dm of monomer subunits. Baselines 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.

NMR experiments

Two-dimensional proton NMR experiments were performed on the GE-Omega 500 NMR spectrometer in the chemistry department at the University of Nebraska. The carrier frequency was set at HDO (the residual water resonance frequency). The chemical shift scale was set with reference to 3-(trimethylsilyl)propanesulfonic acid sodium salt. Phase-sensitive double-quantum filtered COSY (DQF-COSY) was performed on d(CCCCGGGG) in 0.1 M NaCl solution. Due to sensitivity limitations, DQF-COSY experiments were unsuccessful for the more dilute solutions containing $Co(NH_3)_6Cl_3$, and unfiltered phase-sensitive COSY experiments were performed. For both types of COSY experiments, data sets (2K) were collected for 1024 evolution (t_1) values with 32 scans/t_1 increment and a 1.5-s delay, during which the HDO resonance was presaturated. The data sets were apodized with an optimized Lorentz window function in the acquisition dimension (t_2), with a 65°-shifted sine bell function in the evolution dimension, and were zero-filled to 4K by 4K. NOESY data sets (1K) were obtained with 512 t_1 values, mixing times of 250 and 100 ms, and a 1.5-s delay between pulses. The HDO signal was removed by presaturation during this delay with a DANTE pulse train of 5° pulses separated by 100-μs delays (Morris and Freeman, 1978). The data sets were also apodized with a Lorentz function in the t_2 dimension and a 65°-shifted sine bell function in the t_1 dimension. The evolution dimension was zero-filled to give a final data set size of 1K by 1K. In order to recover cross-peak intensities near the carrier frequency, the SCUBA technique was employed, using a 40-ms refocussing delay (Brown et al., 1988). The temperature was controlled at 21.4°C.

^{59}Co NMR experiments were performed on the GE-Omega 500 NMR. Chemical shifts for $Co(NH_3)_6Cl_3$ titrations were referenced to 0.1 M $Co(NH_3)_6Cl_3$ solution at 21.4°C. For the $Co(en)_3Cl_3$ titrations, the reference was 0.1 M $Co(en)_3Cl_3$ at 21.4°C. The ^{59}Co observeD frequency was 119.6 MHz for $Co(NH_3)_6Cl_3$ and 119.5 MHz for $Co(en)_3Cl_3$. For the variable temperature measurements, the NMR probe temperature was calibrated against methanol (Kaplan et al., 1975). The pH of all samples was monitored before and after titrations. The pH was always 6.0 ± 0.2.

RESULTS AND DISCUSSION

CD spectra

A- and B-DNA show distinctly different CD spectra. The base-pair tilting found in A-form DNA leads to a nonconservative appearance of the π-π^* bands at around 260 and at 235 nm. In addition, the significant base-pair tilting of A-DNA results in the characteristic appearance of n-π^* bands at 210 and 300 nm (Yang and Samejima, 1968; Maestre, 1970). As shown in Fig. 1, the CD spectrum of d(CCCCGGGG) in 0.1 M NaCl solution shows the conservative pattern characteristic of B-form DNA. This spectrum is very similar to that obtained for d(CCCGGG) under similar

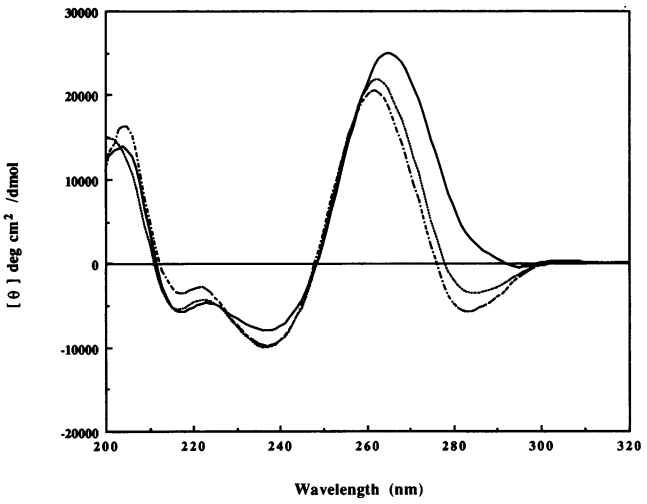

FIGURE 1 CD spectra of 9.0 μM $d(C_4G_4)$ in the presence of (*a*) 0.1 M NaCl (*dash-dot line*); (*b*) 86 μM spermidine (*dotted line*); (*c*) 18 μM $Co(NH_3)_6Cl_3$ (*solid line*). At 262 nm the molar ellipticity of $d(C_4G_4)$ is largest in $Co(NH_3)_6Cl_3$ solution, intermediate in spermidine solution, and weakest in NaCl solution. At 238 nm the molar ellipticity of $d(C_4G_4)$ decreases in the presence $Co(NH_3)_6Cl_3$. At 214 nm the molar ellipticity of $d(C_4G_4)$ increases for both the $Co(NH_3)_6Cl_3$ and the spermidine solutions. The pH of all solutions was constant at 6.0.

conditions (Wolk et al., 1989). In contrast, the titration of a low-salt solution of d(CCCCGGGG) with $Co(NH_3)_6Cl_3$ leads to significant CD spectral changes that level off at a ratio of about four $Co(NH_3)_6^{3+}$/oligomer duplex. As shown in Fig. 1, in the presence of $Co(NH_3)_6^{3+}$, the positive peak that is initially at 262-nm shifts to 265 nm and broadens. The peak intensity ratio of the bands at 265 and 237 nm increases from 2.08:−1.00 to 3.10:−1.0. The negative peak at 283 nm in the B form shifts to 295 nm. The intensity of the 214-nm band increases. In general, the appearance of intense negative peaks between 210 and 220 nm, and an increase in the intensity ratio of the bands at 265 and 237 nm are characteristics of both RNA and A-DNA (Gray and Ratliff, 1975; Gray et al., 1981; Saenger, 1983; Charney et al., 1991; Johnson et al., 1991). Since $Co(NH_3)_6^{3+}$ has no significant UV absorption above 230 nm, asymmetric perturbations of this cation could not lead to these CD spectral changes. In principle $Co(NH_3)_6^{3+}$ could, upon binding, alter DNA electronic transitions and hence influence the CD spectrum even in the absence of DNA structural changes. However, this is unlikely in the present case since the CD spectrum of d(CCCCGGGG) titrated with $Co(NH_3)_6Cl_3$ is very similar to the spectrum obtained in 80% trifluoroethanol (data not shown). Moreover, as shown in Fig. 2, the spectrum of d(CCCCGGGG) in the presence of $Co(NH_3)_6^{3+}$ is quite similar to the spectrum of r(CCCCGGGG).

These experiments were performed at pH 6.0. Control experiments performed in the presence of $Co(NH_3)_6^{3+}$ showed no significant perturbation of the CD spectra over the pH range from 5.0 to 8.6. As illustrated in Fig. 3, CD experiments at different DNA concentrations showed that the percentage of A-DNA in solution depends only on the cobalt-to-DNA

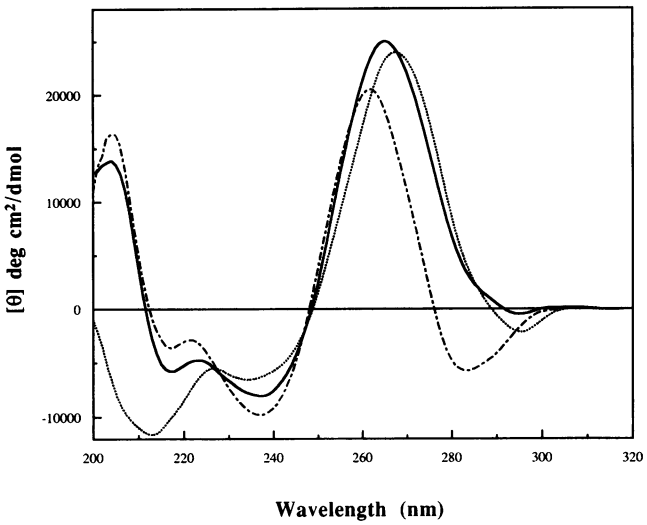

FIGURE 2 (*a*) CD spectrum of 7.04 μM r(C_4G_4) in 0.1 M NaCl (*dotted line*). For comparison, we show the CD spectra of 9.0 μM d(C_4G_4) in the presence of (*b*) 0.1 M NaCl (*dash-dot line*); (*c*) 18 μM $Co(NH_3)_6Cl_3$ (*solid line*). The pH of all solutions was constant at 6.0.

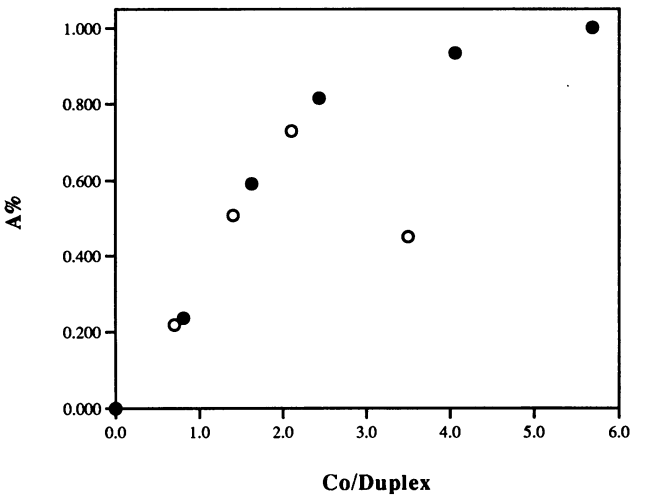

FIGURE 3 The percentage of duplex d(C_4G_4) in the A-form, determined from the CD intensity at 238 nm. The solid circles correspond to a titration of an aqueous solution of 8.7 μM d(C_4G_4) strand (4.4 μM duplex). The hollow circles correspond to a titration of 103 μM d(C_4G_4) strand (51 μM duplex). For the latter titration, precipitation occurred upon titrating from 2.1 to 3.5 Co/duplex.

phosphate ratio, but not on the DNA concentration. In this figure the %A-DNA was calculated assuming that the ellipticity at a particular wavelength is the weighted average of the ellipticity in the B and A forms:

$$f_A = \left(\frac{\theta - \theta_B}{\theta_A - \theta_B}\right)$$

where f_A is the fraction of oligonucleotide in the A-form, θ is the observed ellipticity, θ_B is the ellipticity in the absence of $Co(NH_3)_6^{3+}$, and θ_A is the limiting ellipticity at high cobalt to duplex (Co/D) ratios. The points shown in Fig. 3 were calculated at a wavelength of 238 nm. Indistinguishable results were obtained at wavelengths of 216 and 267 nm. At a DNA duplex concentration of 4.4 μM, the B-A transition is complete prior to the onset of precipitation. At the higher concentration of 51 μM duplex, precipitation occurs at a cobalt to duplex ratio of about 3. At this ratio, the transition is 80% complete. Precipitation is readily monitored both by visual inspection, and from a decrease in f_A following precipitation. At 51 μM duplex, precipitation occurred upon titrating from a Co/D ratio of 2.1 to a ratio of 3.5. f_A calculated following precipitation decreased from 0.73 to 0.45. The same f_A was calculated at wavelengths of 216, 238, and 267 nm. This result is expected if $Co(NH_3)_6^{3+}$ binds preferentially to the A-form, and if the A-form is favored in the precipitate. Additional precipitation experiments were performed at higher DNA concentrations. As judged by visual inspection, and confirmed by a decrease in the A-form as determined by base proton chemical shifts (see below), at a $Co(NH_3)_6^{3+}$-to-duplex ratio of 1.0, precipitation occurs at a duplex concentration of between 2 and 2.5 mM. The NMR experiments described below were all performed at a Co/D ratios close to 1.0, and at duplex concentrations of less than 1.5 mM. In no cases was visible precipitation observed in any of the samples for which NMR spectra are reported here, over the time course of these experiments.

The CD spectra of d(CCCCGGGG) in the presence of $MgCl_2$, spermidine and D(+)- and L(−)-tris(ethylenediamine)cobalt(III) chloride were also measured in order to investigate whether A-form DNA can be stabilized by other multivalent cations. The CD spectrum of d(CCCCGGGG) in the presence of $MgCl_2$ is nearly identical to that obtained in the presence of 0.1 M NaCl (data not shown). As shown in Fig. 1, spermidine causes a slight red-shift of the 262-nm band, and the intensity of 214-nm band increases. The most dramatic effect of spermidine is on the band at 219 nm, which is sensitive to n-π^* transitions. Consequently, it seems that spermidine may push d(CCCCGGGG) to a structure intermediate between A and B forms.

Compared to the 0.1 M NaCl spectrum, D(+)-tris(ethylenediamine)cobalt(III) chloride causes the 262-nm band to shift to 267 nm, and the peak intensity ratio at 267 and 235 nm to increase to 3.16:−1.00 (Fig. 4). L(−)-Tris(ethylenediamine)cobalt(III) chloride shifts the 262-nm band to 269 nm, and the peak intensity ratio 269:237 nm increases to 2.40:−1.00. Both enantiomers of $Co(en)_3^{3+}$ thus induce structures with A-form characteristics. These structures are probably not identical. The L(−) enantiomer appears to cause the most severe base tilting as reflected in the magnitude of the 219-nm peak.

^{59}Co NMR experiments

^{59}Co chemical shifts, linewidths ($\Delta\nu_{1/2}$), and longitudinal (R_1) relaxation rates were measured as functions of temperature for a low salt sample containing one $Co(NH_3)_6^{3+}$/d(CCCCGGGG) duplex. Under the conditions of these experiments, previous nmr (Braunlin et al., 1987)

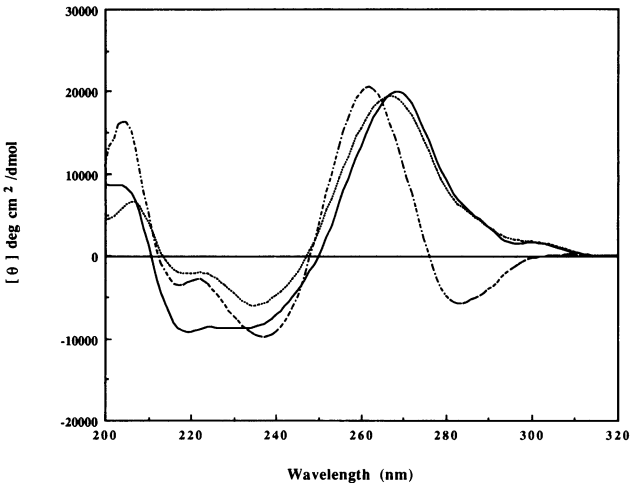

FIGURE 4 CD spectra of 9.0 μM $d(C_4G_4)$ in the presence of (*a*) 0.1 M NaCl (*dash-dot line*); (*b*) 14 μM L(−)$Co(en)_3Cl_3$ (*solid line*); (*c*) 14 μM D(+)$Co(en)_3Cl_3$ (*dotted line*). The positive band of $d(C_4G_4)$ shifts to 269 nm in the presence of L(−)$Co(en)_3Cl_3$, and to 267 nm in D(+)$Co(en)_3Cl_3$ solution. The negative peak intensity at 237 nm is strongest for D(+)$Co(en)_3Cl_3$, and intermediate for L(−)$Co(en)_3Cl_3$. The negative peak intensity at 219 nm is strongest for L(−)$Co(en)_3Cl_3$, and intermediate for D(+)$Co(en)_3Cl_3$. The pH of all solutions was constant at 6.0.

and equilibrium dialysis binding measurements (Plum and Bloomfield, 1988) on polymeric DNA suggest that essentially all of the $Co(NH_3)_6^{3+}$ in solution should be bound to the DNA. This idea is confirmed by salt-dependent equilibrium dialysis measurements of $Co(NH_3)_6^{3+}$ binding to octameric DNA, which predict an apparent equilibrium binding constant of 1×10^4 M^{-1} under the conditions of the present measurements (unpublished results). Consequently, the nmr parameters measured under these conditions are those of the bound $Co(NH_3)_6^{3+}$.

The ^{59}Co chemical shifts for the sample are nearly 40 ppm upfield of the shift for a standard solution of 0.1 M $Co(NH_3)_6Cl_3$. This shift is nearly 20 ppm upfield of shifts that are characteristic of $Co(NH_3)_6^{3+}$ nonspecifically associated with polymeric (Braunlin and Xu, 1992) and oligomeric (Xu and Braunlin, 1992) DNA. The large ^{59}Co chemical shifts in d(CCCCGGGG) solution suggest a strong shielding of the ^{59}Co nucleus that might for example result from ring currents on base-pairs inside DNA grooves. These chemical shift results thus *suggest* an intimate interaction between $Co(NH_3)_6^{3+}$ and d(CCCCGGGG).

In the absence of oligomer, $^{59}Co(NH_3)_6^{3+}$ longitudinal relaxation is completely controlled by the quadrupolar relaxation mechanism, which results in a characteristic decrease in R_1 with increasing temperature. In contrast, the ^{59}Co transverse relaxation ($R_2 = \pi_*\Delta\nu_{1/2}$) is determined primarily by a scalar interaction with the ^{14}N nucleus, with a minor contribution from the quadrupolar mechanism (Rose and Bryant, 1978). The relevant correlation time for the scalar interaction is the ^{14}N T_1, and hence an inverse quadrupolar temperature dependence is observed, i.e., in $Co(NH_3)_6Cl_3$ solution, the ^{59}Co linewidth increases with increasing temperature. Any interaction resulting in a slowing of the molecular reorientation of the ^{59}Co nucleus should decrease the ^{14}N T_1, and thus decrease the magnitude of the scalar contribution to the ^{59}Co R_2. Such an interaction should on the other hand increase the quadrupolar contribution to the relaxation.

We have found that for certain oligonucleotides, interaction with oligomer results in relatively modest changes in the $^{59}Co(NH_3)_6^{3+}$ relaxation behavior (Xu and Braunlin, 1992). Oligomers in this class include the oligomers d(GGAATTCC), d(AATTAATT), d(CATATATG), and d(GTATATATAC). In the presence of such oligomers, the $^{59}Co(NH_3)_6^{3+}$ R_1 increases by a factor of about five, and the linewidths actually decrease compared to that observed in 0.1 M $Co(NH_3)_6Cl_3$ solution. Moreover, the scalar relaxation still dominates the linewidth. For such oligomers the rotational motions dominating the ^{59}Co relaxation are thus nearly independent of the overall rotation of the oligomer molecule. Titration with $Co(NH_3)_6Cl_3$ results in modest effects on the CD spectra and proton nmr of such oligomers.

As shown in Fig. 5, this situation is to be contrasted to that observed for $Co(NH_3)_6^{3+}$ bound to d(CCCCGGGG). Here, the scalar coupling mechanism contributes an insignificant amount to the observed relaxation behavior, and the observed relaxation shows the characteristic quadrupolar temperature dependence. Hence, the tumbling motions providing a pathway for relaxation are significantly slower than are observed in simple salt solution. Under the conditions of Fig. 5, temperature-dependent proton nmr measurements allow the melting temperature to be estimated at around 60°C. Using the near extreme narrowing equations of Halle and Wennerström (1981), from the ratio of R_1 to R_2, an effective correlation time (τ_c) for these tumbling motions was calculated to be 1.8 ns at 21.4°C. From the Stokes-Einstein equation for a sphere of radius 13 Å (an octamer is of nearly equal length and width), the rotational correlation time (τ_r) of the whole piece of d(CCCCGGGG) is calculated to be 2.2 ns. The near

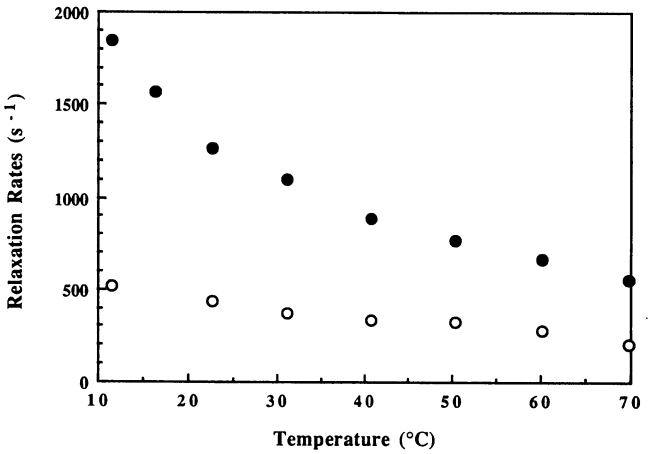

FIGURE 5 Co-59 longitudinal R_1 (○) and transverse R_2 (●) relaxation rates at 119.6 MHz versus temperature for a sample containing 2.9 mM $d(C_4G_4)$ strands and 1.43 mM $Co(NH_3)_6Cl_3$. The sodium ion concentration is estimated at 20 mM. The pH of the solution was 6.0. Based on proton nmr chemical shifts, the melting temperature is approximately 60°C.

equality of τ_c and τ_r implies that $Co(NH_3)_6^{3+}$ is tightly bound to d(CCCCGGGG) on the nanosecond time scale of oligomer rotation.

For the oligomers d(GCGCGCGC) and d(CTCTAGAG), which show no significant changes in their CD spectra upon titration with $Co(NH_3)_6Cl_3$, ^{59}Co relaxation behavior is observed that is intermediate between that found for oligomers such as d(GGAATTCC) and that for d(CCCCGGGG). As a consequence, it is clear that the results reported here depend on more than GC content, but rather reflect sequence-specific binding.

^{59}Co nmr experiments were also performed for a racemic mixture, and for separated enantiomers of tris(ethylenediamine)cobalt(III) cations in the presence of d(CCCCGGGG). As shown in Fig. 6, for the racemic mixture in d(CCCCGGGG) solution, two overlapping ^{59}Co peaks are perceived at low cation binding density. As the temperature is raised, these peaks shift as a unit until melting occurs, at which point only a single peak is observed. Stereospecific assignment of these two peaks was made by comparison with ^{59}Co nmr experiments on the two separated enantiomers in d(CCCCGGGG) solutions. From these experiments it is evident that the broad upfield peak, which has a shorter T_1, corresponds to L(−)-tris(ethylenediamine)cobalt(III). The downfield peak, with a relatively narrow linewidth, and a longer T_1, corresponds to D(+)-tris(ethylenediamine)cobalt(III). From a comparison of the linewidths and the T_1 values of these separated enantiomers in d(CCCCGGGG) solutions, the effective correlation times of each of these two enantiomers are found to be close to the rotational correlation time of d(CCCCGGGG). This indicates that both of the enantiomers bind tightly to d(CCCCGGGG). However, the binding environments clearly differ for these two enantiomers. The ^{59}Co relaxation rates depend on two factors: the rotational dynamics (described by τ_c) and the asymmetry of the electric field gradient at the position of the ^{59}Co nucleus (included in the quadrupolar coupling constant). Since in the present context, nearly identical τ_c values are obtained for the two stereoisomers, the larger longitudinal and transverse relaxation rates that are obtained for the L(−)-tris(ethylenediamine)cobalt(III) cation could indicate either or both of 1) a less symmetric nuclear electric field gradient for this isomer or 2) the involvement of certain types of nonisotropic motions (i.e., motions not describable by a single correlation time, see, e.g., Halle et al. (1984)). The primary cause for a static decrease in symmetry could be attributed to distortions in the electron cloud surrounding the ^{59}Co nucleus. Such distortions could for example reflect the geometry of the cation with respect to the conjugated electron clouds of the nucleic acid bases or the geometry of hydrogen bond donations by the ethylenediamine ligands. Regarding the second, dynamic effect, if the relaxation behavior of the ^{59}Co nucleus were to reflect a rapid partial averaging of the quadrupolar interaction, followed by a slow loss of correlation due to DNA tumbling, then if relaxation were analyzed assuming isotropic rotation, one might still obtain the correct correlation time for DNA tumbling, and an apparent coupling constant that would be reduced by an amount reflecting the degree of partial averaging (Halle et al., 1984). In either of these two cases, stereospecific differences in the cation binding environments, as monitored by ^{59}Co NMR, correlate with apparent differences in the structural consequences of binding as reflected in the CD spectra of Fig. 4. We note for the record that similar stereoselectivity is observed for $Co(en)_3^{3+}$ binding to other oligonucleotides possessing two or more contiguous guanine residues (Xu and Braunlin, 1992).

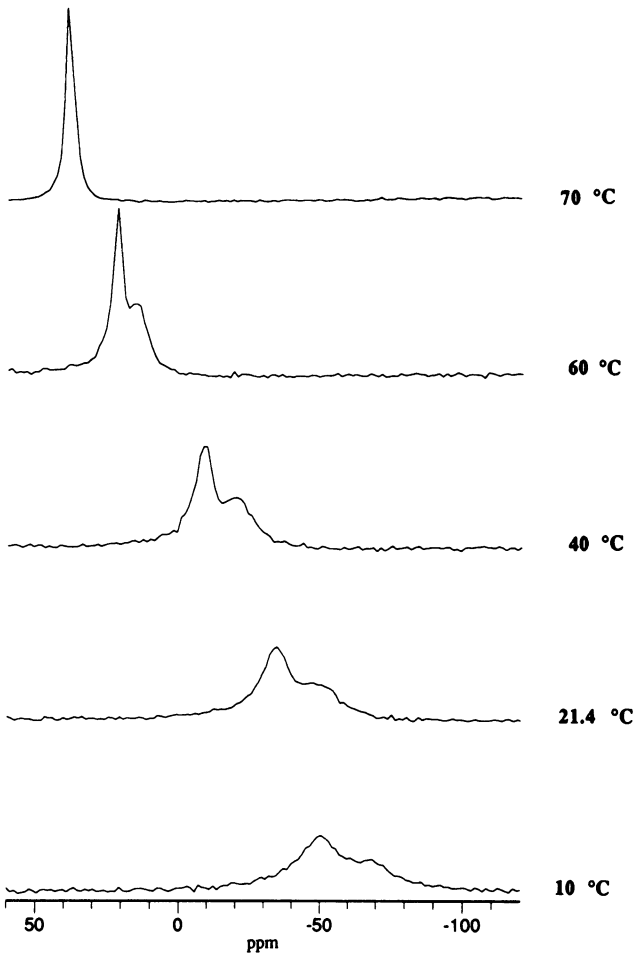

FIGURE 6 Co-59 nmr spectra as a function of temperature for a 750 μM racemic mixture of D(+) and L(−) $Co(en)_3Cl_3$, in the presence of 2.75 mM $d(C_4G_4)$ strands. The chemical shifts are referenced with respect to the shift of a 0.1 M solution of $Co(en)_3Cl_3$ at 21.4°C, which was set equal to zero. The spectral width was 40,000 Hz, the acquisition time was 6.4 ms, and the pulse recycle time was 40 ms (shorter recycle times are not allowed by the nmr software). 100,000 transients were collected for each spectrum.

H-1 2D NMR experiments

The CD results demonstrate unequivocally that $Co(NH_3)_6^{3+}$ induces a B-A transition of d(CCCCGGGG). H-1 nmr experiments support this conclusion and allow additional structural and dynamic information to be obtained.

Assignments

Characteristic differences between A and B form DNA are also reflected in proton chemical shifts, sugar puckering, and

base proton to sugar proton distances. However, because the distances of GH8/CH6 to its own H1′ and its preceding H1′ are within 4.0 Å for both B- and A-DNA, the commonly used H6/H8(i) ↔ H1′(i) ↔ H6/H8(i+1) connective pathway for the assignment of nonexchangeable protons of B-DNA can also be used for A-DNA (Reid, 1987; Patel et al., 1987).

The assignments were made as follows. COSY spectra were collected, from which the CH5 and CH6 resonances were identified. By default, the remaining base resonances were identified as originating from GH8 protons. From these experiments, the sugar ring connectivities (e.g., H1′ ↔ H2′/H2″) were also established by spin system. Following these COSY experiments, we then collected NOESY spectra at long mixing times (250 ms). In these NOESY spectra, there is a unique CH6 cross peak to an H1′ resonance that in turn gives a cross peak to a GH8 resonance (Fig. 7, *a* and *b*). This allowed us to identify the C4H6, G5H8, and G5H1′ residues. The remaining H1′ and CH6/GH8 resonances were then assigned by the H6/H8(i) ↔ H1′(i) ↔ H6/H8(i+1) pathway (Fig. 7, *a* and *b*). From comparison with the COSY spectra, the CH5 resonances could then be assigned. The peak at (5.72, 7.84) in Fig. 7 *a* is the cross peak of C1H6 and C2H5.

For the NOESY spectrum at 0.1 M NaCl (Fig. 7 *a*) a potential ambiguity arises for G6H1′ and G7H1′. Given the cross peak positions, an assignment "walk-through" involves traversing the vertices of a rectangle. As a consequence, the peak positions for these resonances could be switched (i.e., G6H6 could resonate at 5.76 rather than 5.62 ppm, and G7H6 could resonate at 5.62 rather than 5.76 ppm). This ambiguity does not arise for the spectrum obtained in the presence of $Co(NH_3)_6^{3+}$ (Fig. 7 *b*). These peaks can however be followed as the $Co(NH_3)_6^{3+}$ titration proceeds (Fig. 8). It is on this basis that the assignments are made for these two resonances. For the NOESY spectrum in the presence of $Co(NH_3)_6^{3+}$ (Fig. 7 *b*) another potential ambiguity arises since the C2H1′ ↔ C3H6 and the C3H1′ ↔ C4H6 cross peaks are quite weak. However, these assignments are readily confirmed by examination of the H1′ ↔ H2′/H2″ scalar connectivities, and the H6/H8 ↔ H2′/H2″ NOESY connectivities. Moreover, the C2H1′ ↔ C3H6 and the C3H1′ ↔ C4H6 cross peaks are quite clear in the NOESY spectra performed in the absence of $Co(NH_3)_6^{3+}$ (Fig. 7 *a*). Thus we are also quite confident of these assignments. As we discuss below, the base protons show noticeable exchange broadening in the presence of $Co(NH_3)_6^{3+}$. Chemical exchange and cross-relaxation could both influence the intensities of these peaks. In order to clarify this issue, NOESY buildup curves at several mixing times would need to be collected, and compared to theoretical spectra generated from a relaxation matrix analysis (Gorenstein et al., 1990; Borgias and James, 1991). Such an analysis is beyond the scope of the current work.

Based on the NOESY intensity patterns, the H2′ and H2″ resonances could also be distinguished. For example, the H1′ to H2″ distance is usually significantly shorter than the H1′ to H2′ distance. Also, as we discuss in more detail below, at short mixing times, A-DNA and B-DNA show characteristic H6/H8 ↔ H2′/H2″ NOESY intensity patterns. As expected, the H2′ protons generally resonate upfield of the H2″ protons. Although less useful in the current context, the H6/H8(i) ↔ H2′/H2″(i) ↔ H6/H8(i+1) connectivities give results that are consistent with our assignments. The COSY connectivities allowed us to identify the H3′ and H4′ resonances. The H5′ and H5″ resonances could not be resolved. The nonexchangeable resonances of d(CCCCGGGG) were separately assigned in the presence of 0.1 M NaCl, and of 1.46 mM $Co(NH_3)_6Cl_3$, and are listed in Tables 1 and 2. The assignments in 0.1 M NaCl correspond well to the assignments for the hexanucleotide d(CCCGGG) (Wolk et al., 1989).

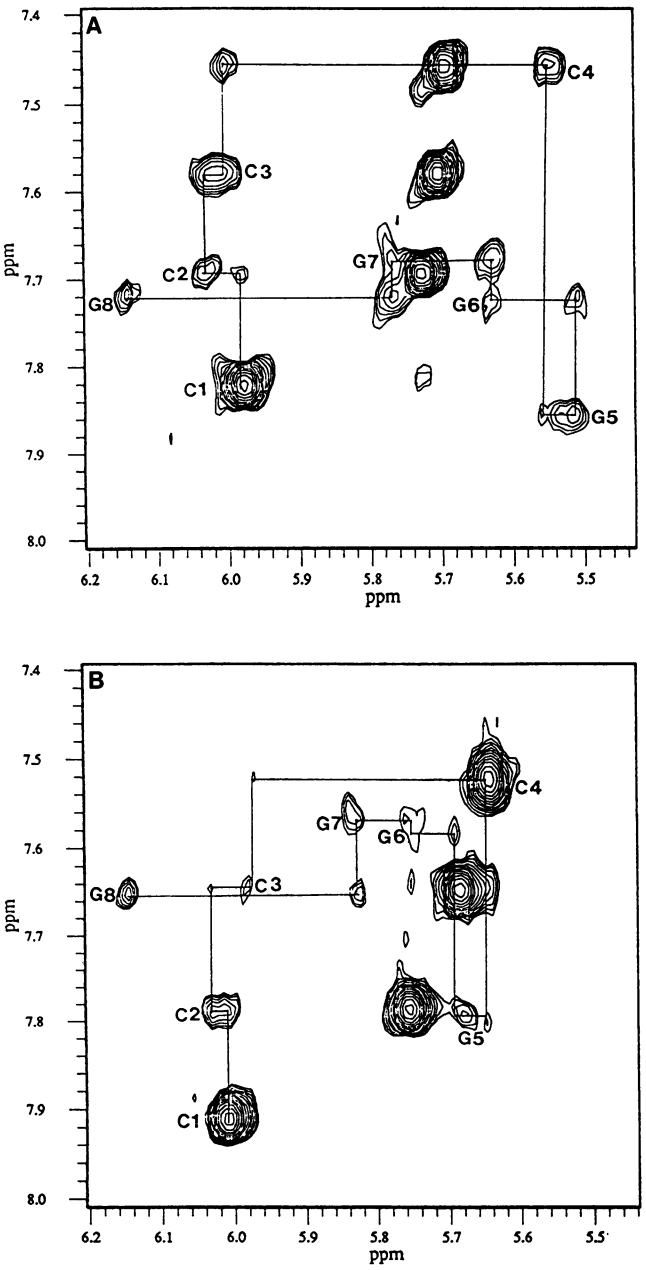

FIGURE 7 (*a*) GH8/CH6-H1′ region of the NOESY spectrum for a sample containing 4.62 mM strand concentration of $d(C_4G_4)$ and 0.1 M NaCl. (*b*) GH8/CH6-H1′ region of the NOESY spectrum for a sample containing 2.75 mM strand concentration of $d(C_4G_4)$ and 1.46 mM $Co(NH_3)_6Cl_3$. For both experiments, the temperature was 21.4°C and the mixing time was 250 ms.

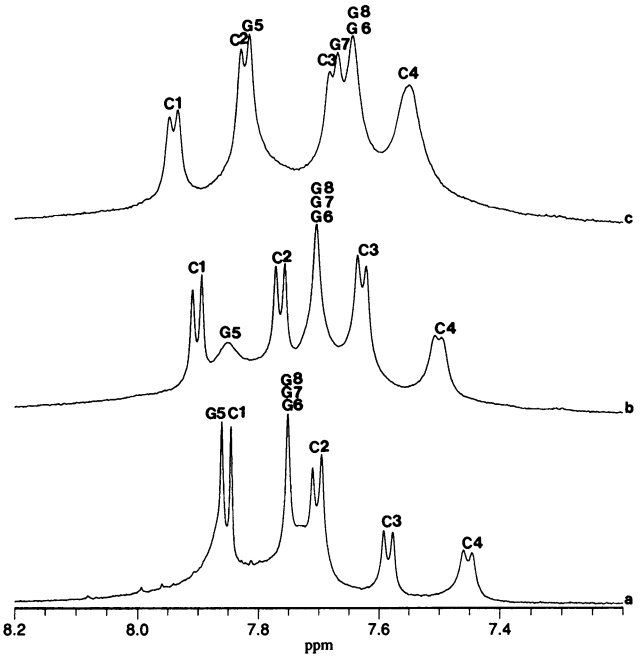

FIGURE 8 One-dimensional nmr spectra of the base proton region of $d(C_4G_4)$ at 21.4°C. The sample contained 2.9 mM octamer strands and no $Co(NH_3)_6Cl_3$ (*bottom*), 0.85 mM $Co(NH_3)_6Cl_3$ (*middle*), 1.99 mM $Co(NH_3)_6Cl_3$ (*upper*).

Proton NMR characterization of the B-A transition

It is apparent that the chemical shifts of GH8 move upfield and the chemical shifts of CH6 move downfield upon the addition of $Co(NH_3)_6^{3+}$. To illustrate this point, one-dimensional spectra of the base proton region are shown in Fig. 8. Although the transition to A-form is not complete, the chemical shift changes of GH8 and CH6 are large (approximately 50 Hz, or 0.1 ppm). Similar upfield shifts of GH8 relative to CH6 are observed for r(CCCGGG) compared to d(CCCGGG) (Wolk et al., 1989). Based on the CD measurements, at one $Co(NH_3)_6^{3+}$/duplex, d(CCCCGGGG) is 30% in the A-form. As discussed above, at much higher $Co(NH_3)_6^{3+}$ to duplex ratios precipitation occurs for the oligonucleotide concentrations of the nmr experiments. Based on a two-state exchange model, the observed chemical shifts shown in Table 2 can be extrapolated to obtain limiting shifts for A-form d(CCCCGGGG). The results of this extrapolation are given in Table 2 for the base protons. A comparison with the results of Wolk et al., 1989 demonstrates a very good match with protons of r(CCCGGG). In particular, as is observed for the RNA oligomer, all CH6's are shifted downfield of the GH8's.

Peak broadening is observed as the titration proceeds. This broadening is most severe for the peaks that show the largest shifts over the course of the titration. Such selective broadening is characteristic of an exchange process. Based on the broadening of the C4H6 peak, and using the method of Piette and Anderson (1959) an exchange lifetime for going between the B and A forms can be estimated at 0.6 ms. The lifetime for the reverse transition is estimated at 0.5 ms. In this calculation for the C4H6 peak in the upper spectrum in Fig. 8, we use an estimated chemical shift change of 117 Hz (Table 2) ongoing between the A and B forms, an excess linewidth of 35.7 Hz, and $f_A = 10.42$, determined from the CD results of Fig. 3. A more detailed interpretation of the observed line broadening to obtain dynamic information regarding the B-A transition will require additional measurements as a function of temperature and $Co(NH_3)_6^{3+}$, as well as a careful theoretical analysis, and is in progress.

The sums of vicinal proton-proton coupling constants, i.e., $\sum H1'(=J_{1'2'} + J_{1'2''})$, $\sum H2'(=J_{1'2'} + J_{2'3'} + J_{2'2''})$, $\sum H2''(=J_{1'2''} + J_{2''3'} + J_{2'2''})$, $\sum H3'(=J_{2'3'} + J_{2''3'} + J_{3'4'})$, of the deoxyribose ring are very sensitive to the sugar pucker. Using the method of Rinkel and Altona (1987) these sums can be used to estimate the fraction of sugar 3'-endo or 2'-endo based on a model of a fast equilibrium between these two conformations. The absence of extra proton-proton cross peaks in our NOESY spectra indicates there is fast or near fast exchange among sugar conformations. The isochronous (or near isochronous) H2' and H2'' resonances of d(CCCCGGGG) leave $\sum H1'$ and $\sum H3'$ as proper indicators of the fractions of sugar 3'-endo or 2'-endo pucker. In the presence of $Co(NH_3)_6^{3+}$, due to inadequate resolution of proton-proton cross peak slices from phase-sensitive COSY spectra, only the sum of the H1' coupling constants, $\sum H1' = J_{1'2'} + J_{1'2''}$ could be determined. In the absence of $Co(NH_3)_6^{3+}$ the individual sugar coupling constants could be read from phase-sensitive DQF COSY spectra, and the data are consistent with those of Wolk et al. (1989). The fractions pN of 3'-endo (N-form) and pS of 2'-endo were calculated with the formulae $pS = (\sum H1' - 9.8)/5.9$ and $pS + pN = 1.0$ (Rinkel and Altona, 1987). The values obtained for the $\sum H1'$, together with the results of such calculations of pN in the absence and presence of $Co(NH_3)_6^{3+}$ are also presented in Tables 1 and 2. A comparison of the results as tabulated in Tables 1 and 2 suggests a dramatic increase in the 3'-endo (pN) sugar conformation upon the addition of $Co(NH_3)_6Cl_3$. Nevertheless, the fractions of 3'-endo (pN) appear to remain low at C3 and G5 in the presence of $Co(NH_3)_6^{3+}$. In other words, the oligonucleotide conformation shows the pattern A-A-B-A-B-A-A-A. Benevides et al. (1986) also observed this pattern in crystal A-form d(CCCCGGGG) and d(GGCCGGCC) with Raman spectroscopy. It appears that the alternating middle segment B-A-B-A may be a unique characteristic of A-form d(CCGG) existing in both crystals and aqueous solutions.

Under the conditions of Table 2, the CD measurements predict that the DNA should be 30% in the A-form. In contrast, as tabulated in Table 2, the calculated %N as determined by the $\sum H1'$ coupling constants, ranges from 24 to 61%, and averages 53%. Hence, the $\sum H1'$ coupling constant measurements appear to overestimate the fraction of DNA in the A-form. In part this discrepancy could reflect the different dependencies of the two techniques on geometry. Whereas the CD spectrum is sensitive primarily to base transition dipole orientation, the nmr measurements are directly sensitive to sugar pucker. Another more fundamental problem is that

TABLE 1 Proton chemical shifts, ∑H1′ and pN of d(CCCCGGGG) in 0.1 M NaCl

	C1	C2	C3	C4	G5	G6	G7	G8
H6	7.82	7.70	7.59	7.46				
H5	5.98	5.72	5.70	5.68				
H8					7.86	7.73	7.68	7.74
H1′	5.98	6.02	6.00	5.56	5.51	5.62	5.76	6.14
H2′	2.21	2.29	2.22	2.01	2.69	2.56	2.59	2.47
H2″	2.55	2.52	2.51	2.33	2.69	2.71	2.69	2.36
H3′	4.68	4.89	4.87	4.85	4.97	4.96	4.98	4.63
H4′	4.11	4.23	4.22	4.11	4.33	4.36	4.14	4.21
∑H1′(Hz)*	13.4	15.0	15.0	15.1	15.0	15.1	15.5	15.0
pN	0.38	0.13	0.11	0.11	0.13	0.11	0.03	0.14

The sample contained 4.62 m*M* NaCl at 21.4°C.
*The error of reading the coupling constant ∑H1′ is ±1 Hz.

TABLE 2 Proton chemical shifts, ∑H1′ and pN of d(CCCCGGGG) in the presence of $Co(NH_3)_6Cl_3$

	C1	C2	C3	C4	G5	G6	G7	G8
H6	7.92 (8.15)*	7.79 (7.99)*	7.65 (7.78)*	7.53 (7.68)*				
H5	6.01	5.75	5.68	5.64				
H8					7.80 (7.67)*	7.59 (7.28)*	7.57 (7.32)*	7.66 (7.48)*
H1′	6.02	6.03	6.00	5.64	5.68	5.74	5.82	6.14
H2′	2.34	2.35	2.29	2.15	2.67	2.56	2.56	2.67
H2″	2.60	2.57	2.54	2.38	2.67	2.67	2.66	2.37
H3′	4.67	4.85	4.84	4.83	4.94	4.84	4.91	4.59
H4′	4.09	4.23	4.22	4.11	4.32	4.34	4.13	4.14
∑H1′(Hz)‡	12.1	12.1	13.3	12.3	14.3	12.4	12.1	12.1
pN	0.61	0.61	0.41	0.58	0.24	0.57	0.61	0.60

The sample contained 2.75 mM d(CCCCGGGG), 1.46 mM $Co(NH_3)_6Cl_3$ at 21.4°C, pH 6.0.
*The chemical shifts extrapolated to 100% A-DNA based on CD measurements.
‡The error of reading the coupling constant ∑H′ is ±1 Hz.

differential cross relaxation of two coupled protons can result in apparent coupling constants that are smaller than actual coupling constants (Harbison, 1992). In the present case, this would result in an overestimation of the %N.

For NOESY experiments performed at short mixing times, the characteristic distance constraints of base-sugar protons for A- versus B-DNA are evident in the cross peak region of GH8/CH6 versus H2′/H2″ (Neuhaus and Williamson, 1989). For the relatively short (100 ms) mixing times of these experiments, larger cross peak integrals correspond to shorter proton-proton distances (this correspondence no longer holds for the 250-ms mixing times of Fig. 7). For B-DNA the intraresidue H2′ to H8/H6 distances and inter-residue H2″ to H8/H6 distances are close to 2.0 Å, and hence give rise to intense cross peaks. For A-DNA only inter-residue H2′ to H8/H6 distances are near 2.0 Å. The chemical shifts of H2′ and H2″ fall between 2.0 and 2.8 ppm, with the upfield half of this chemical shift region occupied by H2′, and the downfield half by H2″. Consequently, characteristic NOESY cross peak intensity patterns are observed in the GH8/CH6 versus H2′/H2″ region for A-DNA compared with B-DNA. For B-DNA the cross peaks have nearly the same intensities across this region. This pattern is observed for a mixing time of 100 ms for d(CCCCGGGG) in the absence of $Co(NH_3)_6Cl_3$. For A-DNA the upfield half of this region shows a significantly greater integrated intensity than does the downfield half. This A-DNA pattern is apparent for d(CCCCGGGG) in the presence of $Co(NH_3)_6Cl_3$.

CONCLUDING REMARKS

The appearance of nonconservative π-π^* transition bands at 265 nm and 237 nm, and the increase in intensity of n-π^* bands at 214 nm in the CD of d(CCCCGGGG) in the presence of cobalt(III) ammine cations indicate increased base-pair tilting, a characteristic of A-DNA. Comparisons with spectra obtained in 80% trifluoroethanol, and with the RNA oligomer r(CCCCGGGG) demonstrate that $Co(NH_3)_6^{3+}$ induces a B-A transition.

A combination of 2D proton NOESY and COSY experiments allowed us to assign all nonexchangeable base and sugar protons of d(CCCCGGGG). In the presence of $Co(NH_3)_6Cl_3$, the following observations further demonstrate that d(CCCCGGGG) undergoes a transition to A-form DNA: 1) As observed for r(CCCGGG) compared to d(CCCGGG), upfield shifts are found for GH8 relative to CH6. Extrapolated limiting shifts for the A-form oligomer are very similar to those observed for the hexamer r(CCCGGG) (Wolk et al., 1989). 2) Coupling constants determined from two-dimensional phase-sensitive DQF COSY indicate a predominantly 3′-endo sugar pucker. 3) For short mixing times, 2D proton NOESY spectra clearly show base to sugar cross-peak intensities that are characteristic of A-form geometry.

CD and nmr measurements demonstrate conclusively that d(CCCCGGGG) in an aqueous solution can be induced into an A-form DNA structure by cobalt(III) ammine cations. In contrast, this oligomer remains in B-form at molar concentrations of NaCl or tenth molar concentrations of $MgCl_2$. ^{59}Co nmr reveals rotational immobilization of $Co(NH_3)_6^{3+}$ in the presence of d(CCCCGGGG).

The two enantiomers of tris(ethylenediamine)cobalt(III) cations both bind tightly to d(CCCCGGGG) as judged by ^{59}Co nmr. As monitored by circular dichroism, both enantiomers appear to induce structural perturbations in the direction of B-A transitions. However, the structural perturbations that are induced for this oligomer appear to differ significantly for the two enantiomers. It is tempting to speculate that our results reflect a deeper penetration of the L(−)--tris(ethylenediamine)cobalt(III) cation into the grooves of d(CCCCGGGG) compared with D(+)-tris(ethylenediamine)cobalt(III). This idea is consistent with the stereoselectivity of right-handed DNA for cations of complementary handedness in the groove binding mode (Barton et al., 1984; Barton, 1986).

In recent work, we have also found that cobalt(III) ammine cations, but not Mg^{2+} or spermidine, perturb the structure of not only d(CCCCGGGG) but also other deoxyoligonucleotides possessing contiguous stretches of adjacent guanine residues. In all cases, large structural perturbations monitored by CD correlate with specific localization of $Co(NH_3)_6^{3+}$ as monitored by ^{59}Co nmr. In contrast, in those cases where $Co(NH_3)_6^{3+}$ binding failed to induce significant changes in the CD, the ^{59}Co nmr indicated loose, nonspecific binding. For the two enantiomers of the tris(ethylenediamine)cobalt(III) cation, circular dichroism, equilibrium dialysis, ^{59}Co nmr, and proton nmr measurements all indicate specific binding, stereoselective recognition, and structural perturbation of these oligonucleotides.

According to x-ray crystallography the A-form d(CCCCGGGG) displays an 8.6-Å width of the major groove (Haran et al., 1987) which is nearly twice the average value for the A family. The diameter of $Co(NH_3)_6^{3+}$ is about 5 Å, and thus this cation should fit readily into the major groove of this A-form oligomer, with potential stabilization by hydrogen bonding to N7 and O6 groups on neighboring guanine residues. Such binding has been determined by x-ray analysis for $Co(NH_3)_6^{3+}$ binding to duplex (A-form) regions on tRNA (Hingerty et al., 1982). Such a favorable geometric arrangement could provide a mechanism for the induction of A-form structure by Co(III) ammine cations. The results of the present study support the notion that induced-fit mechanisms provide a common means for ligands, including proteins, to recognize specific DNA sequences in biological systems. The ability of a simple cation such as $Co(NH_3)_6^{3+}$ to induce the B-A transition, and thereby dramatically limit protein access to the major groove, supports the conjecture that the interactions with DNA of simple cations or basic proteins such as histones or protamines could provide control mechanisms for gene expression.

Zinc finger proteins such as TFIIIA bind and recognize contiguous guanine stretches on both DNA and RNA. For example, the sequence GGATGGGAG appears particularly important for TFIIIA binding (McCall et al., 1986). It will be of some interest to (*a*) determine how readily such sequences adopt the A conformation in the presence of Co(III) ammine cations and (*b*) compare the structural consequences of the binding of such proteins to their target sequences with the results of such model studies.

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