Chiral Recognition of Deoxyoligonucleotides by Δ- and Λ-Tris(ethylenediamine)cobalt(III)†

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ABSTRACT: ^{59}Co NMR and CD measurements show for both stereoisomers of $Co(en)_3^{3+}$ a similar trend in the sequence dependence of DNA recognition, as was reported previously for $Co(NH_3)_6^{3+}$. In particular, specific binding is evident to DNA molecules possessing runs of two or more same-strand guanine residues. The binding of either isomer to such sequences induces structural transitions toward A-DNA characteristics. Such measurements also show significant differences between the two stereoisomers in terms of how they recognize specific duplex DNA sequences. Δ-$Co(en)_3^{3+}$ binds more tightly than Λ-$Co(en)_3^{3+}$ to right-handed, guanine-rich DNA, whereas Λ-$Co(en)_3^{3+}$ binds more tightly than Δ-$Co(en)_3^{3+}$ to left-handed DNA. The two stereoisomers bind in an indistinguishable manner to AT-rich DNA.

On a time average, cations that are trapped near the surface of DNA should tend to accumulate near favorable binding environments. As a consequence, even though electrostatic association may provide the dominant source of binding free energy, specific recognition may occur. Contacts with the functional groups involved in such specific recognition may be favorable for some DNA conformations and unfavorable for others. As a consequence, by binding to a particular conformation, even simple cations can modulate DNA structure. Hence, the $Co(NH_3)_6^{3+}$ cation, through localized binding, induces a B-A transition in the oligonucleotide d(CCCCGGGG) (Xu et al., 1993b). We had postulated that such binding occurred through specific recognition in the major groove of N7 and O6 groups on neighboring guanine residues. In the B-form, the major groove is too wide to allow such recognition. On the other hand, docking experiments, using coordinates for d(CCCCGGGG) obtained from the Brookhaven data base, demonstrate that such recognition should be favorable in the A-form (Xu et al., 1993b). Precedent for this type of recognition can be found in the work of Hingerty et al. (1982) whose crystal structure of tRNA showed a $Co(NH_3)_6^{3+}$ cation bound in the major groove of a duplex region, bridging neighboring guanine residues. Recently, exactly this type of recognition was observed by $Co(NH_3)_6^{3+}$ in crystal structures of A-form decameric oligonucleotides (Gao et al., 1995).

The potential of chiral metal complexes to recognize different DNA conformations has prompted considerable research activity. The interactions of the stereoisomers of [Ru(*o*-phen)]$^{2+}$ have been particularly well-studied (Barton et al., 1984; Rehmann & Barton, 1990; Satyanarayana et al., 1992, 1993). The two stereoisomers of [Ru(*o*-phen)]$^{2+}$ show only modest differences in binding free energy and appear to associate primarily through a surface binding mode. Nonetheless, different proton chemical shifts are observed for Δ-[Ru(*o*-phen)]$^{2+}$ compared to Λ-[Ru(*o*-phen)]$^{2+}$ when these two enantiomers are bound to the oligomers d(GTG-CAC)$_2$ and d(CGCGCG)$_2$ (Rehmann & Barton, 1990). Hence, chiral discrimination by DNA can occur even in the absence of large differences in binding free energy between two stereoisomers. Such behavior is consistent with our view of the interplay between electrostatic association and specific recognition of structural features on the DNA.

In order to refine our understanding of electrostatic and topological influences on DNA recognition, we have studied the interaction between DNA and the Λ and Δ isomers of $Co(en)_3^{3+}$. In this case, chiral recognition is expected to occur primarily through hydrogen bonding, in distinct contrast to the recognition observed for [Ru(*o*-phen)]$^{2+}$. To the extent that cations such as $Co(en)_3^{3+}$ can serve as simple model compounds for basic amino acids, this work may also provide insight into the recognition of DNA by basic proteins such as protamines and histones. Moreover, it is clear that hydrogen bonding provides a potentially very useful tool in designing metal ion complexes as sequence-specific DNA-binding ligands.

In this paper we examine how the binding environments of the two $Co(en)_3^{3+}$ enantiomers depend on base sequence. In obtaining this information, we have applied ^{59}Co NMR measurements. We complement this information with CD data, which reflect how DNA conformations are modulated by interaction with the two isomers of $Co(en)_3^{3+}$. We have also performed ^{1}H NMR measurements that will be the subject of another communication.

MATERIALS AND METHODS

The synthesis and purification of DNA were performed as described in previous papers (Xu et al., 1993a,b). Racemic tris(ethylenediamine)cobalt(III) chloride, sodium chloride, ethylenediamine, and (+)-tartaric acid were purchased from Aldrich. (−)-Tartaric acid was purchased from Lancaster Synthesis. The resolution of tris(ethylenediamine)cobalt(III) chloride was accomplished as described by Rochow (1960): 7 g of tris(ethylenediamine)cobalt(III) chloride and 5.3 g of (−)-tartaric acid (1:3 molar ratio) were weighed and dissolved in 25 mL distilled water. The pH of the solution was adjusted with ethylenediamine to 7.0. The pH-

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balanced solution was heated on a steam bath for 1 h. The solution volume was then reduced by evaporation to 12 mL. The solution was refrigerated overnight. The crystals that formed were filtered and washed twice with 40% ethanol solution. The weight of this enantiomer complex was 3.9 g, and its specific rotation, $[\alpha]_D$ measured on a polarimeter, was −97°. The enantiomer complex and 1 g of sodium chloride (1:2 molar ratio) were dissolved in 20 mL of hot water. The solution was stirred and heated on a steam bath for 1 h, which reduced its volume to 10 mL. Δ-Tris-(ethylenediamine)cobalt(III) chloride was recrystallized twice at room temperature, and the specific rotation of this resolved enantiomer was measured to be −161 ± 5° compared to a literature value of −155° (Werner, 1912). Λ-Tris(ethylenediamine)cobalt(III) chloride was resolved in a similar manner using (+)-tartaric acid. The specific rotation was found to be 161 ± 5° (literature value +155°). The extinction coefficient of tris(ethylenediamine)cobalt(III) chloride used for concentration determination is $\epsilon_{466} = 88.0\ M^{-1}cm^{-1}$. The separated enantiomers used for the ^{59}Co NMR experiments were dissolved in and lyophilized against D_2O three times before final preparation of stock solutions in 99.96% D_2O.

Circular dichroism measurements were performed on a Jasco 600 instrument. The sample temperature was ambient. CD data were transformed into molar ellipticity $[\theta]$ in units of deg cm²/dmol 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.

^{59}Co NMR experiments were performed on the GE-Omega 500 MHz instrument. Chemical shifts for $Co(en)_3Cl_3$ titrations were referenced to a 0.1 M $Co(en)_3Cl_3$ solution at 21.4 °C. The ^{59}Co observation frequency was 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

$^{59}Co(en)_3^{3+}$ NMR in Simple Salt Solution Shows a Dominance of the Quadrupolar Relaxation Mechanism. The relaxation behavior of $^{59}Co(en)_3^{3+}$ in simple salt solution differs qualitatively from that of $^{59}Co(NH_3)_6^{3+}$(Craighead et al., 1975; Rose & Bryant, 1979). For the latter, the transverse relaxation behavior is dominated by scalar coupling to the ^{14}N nuclei, with a minor contribution from the quadrupolar mechanism. The effective ^{59}Co correlation time is thus the ^{14}N T_1, and the ^{59}Co line width increases with increasing temperature. In contrast, as is illustrated in Figure 1, the quadrupolar mechanism dominates for $^{59}Co(en)_3^{3+}$ below 30 °C, resulting in an initial decrease in the line width with increasing temperature. For $Co(en)_3^{3+}$, at room temperature, the contributions from the two relaxation mechanisms are nearly identical. This difference in relaxation behavior reflects in large part the intrinsically longer ^{14}N T_1 for $Co(NH_3)_6^{3+}$ compared to $^{59}Co(en)_3^{3+}$ and also the larger quadrupolar contribution to the ^{59}Co transverse relaxation rate for the latter. The decreased ^{14}N T_1 of $Co(en)_3^{3+}$ compared to $Co(NH_3)_6^{3+}$ in turn reflects a decrease in symmetry around the ^{14}N nucleus. As a consequence, at 21.4 °C, the scalar coupling contribution to the ^{59}Co transverse relaxation rate of $Co(en)_3^{3+}$ is only 145 s^{-1}, whereas the corresponding contribution to the $Co(NH_3)_6^{3+}$ relaxation rate is 358 s^{-1}. In contrast, the quadrupolar contribution to the $^{59}Co(en)_3^{3+}$ relaxation rate is 140 s^{-1} under these conditions, whereas the corresponding contribution to the $^{59}Co(NH_3)_6^{3+}$ relaxation rate is only 24 s^{-1}. This difference in the quadrupolar contribution reflects a less symmetrical distribution of the six nitrogen ligands around the central cobalt ion for $Co(en)_3^{3+}$ compared to $Co(NH_3)_6^{3+}$, and to a lesser extent the larger size and thus slower tumbling motions of $Co(en)_3^{3+}$. In simple salt solution, at room temperature, the effective rotational correlation time may be estimated as 10 ps for $^{59}Co(NH_3)_6^{3+}$ and as 30 ps for $^{59}Co(en)_3^{3+}$ (Craighead et al., 1975; Hartmann & Sillescu, 1964). Using these estimates, the quadrupolar coupling constants (see below) are calculated to be 3.4 MHz for $^{59}Co(en)_3^{3+}$ and 2.4 MHz for $^{59}Co(NH_3)_6^{3+}$.

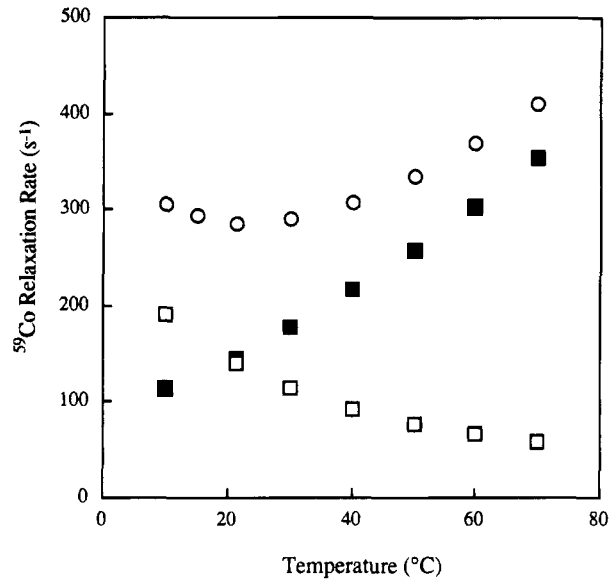

FIGURE 1: Temperature dependence of $^{59}Co(en)_3^{3+}$ R_2 (○) and R_1 (□) relaxation rates for a 0.1 M solution of $Co(en)_3Cl_3$. Under extreme narrowing conditions, R_1 equals the quadrupolar contribution to R_2. Also plotted is the scalar coupling contribution, R_{2sc} (■), which is the difference between these R_2 and R_1.

$^{59}Co(en)_3^{3+}$ NMR Mirrors the DNA Sequence Dependence Observed for $^{59}Co(NH_3)_6^{3+}$. On the basis of the dominant relaxation mechanism of $^{59}Co(NH_3)_6^{3+}$ and on CD changes, we have categorized DNA oligomers into three groups (Xu et al., 1993a). For class 1 oligonucleotides, the quadrupolar mechanism dominates, and the effective rotational correlation time of $^{59}Co(NH_3)_6^{3+}$ is close to that of a DNA octamer. $^{59}Co(NH_3)_6^{3+}$ resonances are upfield shifted by 30−40 ppm. All of the class 1 oligonucleotides show significant changes in CD spectra in the presence of $^{59}Co(NH_3)_6^{3+}$. Class 1 oligonucleotides include d(GGCCGGCC), d(CCCCGGGG) and d(*CG*CG*CG*CG). For the latter oligonucleotide, *C stands for 5-methylated cytosine, and the oligonucleotide undergoes a B-Z transition upon titration with $Co(NH_3)_6^{3+}$. For class 2 oligonucleotides, the scalar coupling mechanism dominates, indicating that the rotational mobility of $^{59}Co(NH_3)_6^{3+}$ is largely independent of DNA tumbling. Resonances are shifted upfield by about 19 ppm. $Co(NH_3)_6^{3+}$ does not affect significantly the CD spectra of any of the oligonucleotides in this group. Among this group are d(GGAATTCC) and d(CATATATG). Class 3 oligonucleotides give $^{59}Co(NH_3)_6^{3+}$ effective rotational correlation times that are intermediate between the above two cases, whereas $^{59}Co(NH_3)_6^{3+}$ chemical shifts are close to those observed for

class 2 oligonucleotides. For class 3 oligonucleotides, no significant CD changes are observed in the presence of Co-$(NH_3)_6^{3+}$. Among this group are d(GCGCGCGC) and d(CTCTAGAG).

In contrast to the situation for $^{59}Co(NH_3)_6^{3+}$, for all of the DNA octamer solutions that we have studied, the relaxation behavior of $^{59}Co(en)_3^{3+}$ shows the characteristic quadrupolar temperature dependence. This difference in relaxation behavior parallels the difference observed in simple salt solutions and appears to reflect not so much an intrinsic difference in rotational dynamics as the fact, discussed above, that the transition between scalar coupling and quadrupolar mechanisms occurs at shorter τ_c's for $^{59}Co(en)_3^{3+}$ than for $^{59}Co(NH_3)_6^{3+}$.

The small size of the oligonucleotides studied places an upper limit of a few nanoseconds on the effective rotational correlation time of $^{59}Co(en)_3^{3+}$. Under these conditions, "near-extreme narrowing" behavior applies, and the effective correlation time of the spin $^7/_2$ ^{59}Co nucleus can be estimated from the following two equations (Halle & Wennerström, 1981b):

$$R_1 = \frac{2\pi^2}{49}\chi^2[0.2J(\omega) + 0.8J(2\omega)] \quad (1)$$

$$R_2 = \frac{2\pi^2}{49}\chi^2[0.3J(0) + 0.5J(\omega) + 0.2J(2\omega)] \quad (2)$$

Here χ is the quadrupole coupling constant, a quantity that reflects the magnitude of the electric field gradient at the nucleus and thus reflects the symmetry of inner sphere coordination surrounding the cobalt nucleus. $J(\omega)$ is a reduced spectral density function that characterizes the frequency distribution of the motions governing the quadrupolar interaction. Isotropic rotational motion of the quadrupolar nucleus gives a Lorentzian spectral density function

$$J(\omega) = \frac{\tau_c}{1 + \omega^2\tau_c^2} \quad (3)$$

where τ_c is the correlation time describing the time scale of the isotropic motions that randomize the electric field gradient. For $Co(en)_3^{3+}$ trapped near the DNA surface, it is not obvious that a single correlation time will suffice to describe the decay of quadrupolar correlation. For example, rapid motions may occur on a time scale that is well separated from those reflecting the DNA tumbling dynamics, and may partially randomize the quadrupolar interaction. Complete randomization would result from the tumbling of the DNA oligomer. Under these circumstances, if correlation times are determined from transverse and longitudinal relaxation rates, then the effective correlation times will still reflect slow-motion processes, and the effective coupling constant will be reduced by an amount reflecting the contribution of the rapid motions (Halle & Wennerström, 1981a; Xu et al., 1993b). Another situation resulting in similar consequences would be rapid exchange among rotationally mobile and rotationally immobilized environments on the DNA (Xu et al., 1993b).

The DNAs that we have studied in $Co(en)_3^{3+}$ solution are the class 1 oligonucleotides d(GGCCGGCC), d(CCCCGGGG), and d(*CG*CG*CG*CG), the class 2 oligonucleotides d(GGAATTCC) and d(CATATATG), and the class 3 oligonucleotides d(GCGCGCGC) and d(CTCTAGAG). d(*CG*CG*CG*CG) forms left-handed Z-DNA in the presence of $Co(en)_3^{3+}$. All the other DNAs remain right-handed as monitored by circular dichroism. For solutions of resolved isomers with one $Co(en)_3^{3+}$ per duplex, the rotational correlation times and quadrupole coupling constants of $Co(en)_3^{3+}$ were calculated from ^{59}Co transverse and longitudinal relaxation measurements. The results of such calculations are shown in Tables 1 and 2 for samples at 21.4 °C.

Table 1: ^{59}Co NMR Parameters of Δ-$Co(en)_3^{3+}$

class[a]	oligonucleotide	R_2 (s^{-1})	R_1 (s^{-1})	σ (ppm)	χ_{eff} (MHz)	τ_{eff} (ns)
1	CCCCGGGG	6510	1396	−29.0	3.86	2.63
	GGCCGGCC	5563	1807	−65.0	3.73	1.84
	*CG*CG*CG*CG	2140	1063	−23.3	2.7	1.14
2	GCGCGCGC	2195	837.7	−31.1	2.54	1.56
	CTCTAGAG	1167	658	−16.9	2.09	0.96
3	GGAATTCC	785	513	−16.4	1.84	0.76
	CATATATG	763	541	−16.6	1.91	0.65

[a] Classification is based on the relaxation behavior of $^{59}Co(NH_3)_6^{3+}$ (Xu et al., 1993).

Table 2: ^{59}Co NMR parameters of Λ- $Co(en)_3^{3+}$

class[a]	oligonucleotide	R_2 (s^{-1})	R_1 (s^{-1})	σ (ppm)	χ_{eff} (MHz)	τ_{eff} (ns)
1	CCCCGGGG	3454	991	−27.65	2.99	2.07
	GGCCGGCC	2759	1219	−39.63	2.96	1.32
	*CG*CG*CG*CG	3196	1411	−31.8	3.18	1.33
2	GCGCGCGC	1141	608	−25.2	2.02	1.04
	CTCTAGAG	896	588	−16.9	1.97	0.75
3	GGAATTCC	836	536	−16.9	1.88	0.78
	CATATATG	764	558	−16.4	1.95	0.61

[a] Classification is based on the relaxation behavior of $^{59}Co(NH_3)_6^{3+}$ (Xu et al., 1993).

As is illustrated in Figure 2, for d(GGCCGGCC) and d(*CG*CG*CG*CG), all of the class 1 oligonucleotides show two separate $^{59}Co(en)_3^{3+}$ peaks in the ^{59}Co NMR spectrum of racemic $Co(en)_3^{3+}$ in low-salt oligonucleotide solution. One of these peaks is always broader and shifted further upfield. The other is narrower and shifted less upfield. As illustrated for d(GGCCGGCC) in Figure 3 and as we have reported for d(CCCCGGGG), the two peaks approach each other as the temperature is raised toward the DNA melting point (Xu et al., 1993b). Based on ^{59}Co NMR chemical shifts and relaxation rates, these $^{59}Co(en)_3^{3+}$ peaks were assigned using resolved Δ- and Λ-$Co(en)_3^{3+}$. For d(GGCCGGCC) and d(CCCCGGGG), the broader upfield peak corresponds to the Δ-$Co(en)_3^{3+}$ isomer and the narrower downfield one corresponds to the Λ-$Co(en)_3^{3+}$ isomer.

The ^{59}Co NMR spectrum of racemic $Co(en)_3^{3+}$ in d(*CG*CG*CG*CG) solution is qualitatively similar to that observed for the other class 1 oligomers. A broad upfield peak and a narrower downfield peak are observed. However, further experiments with resolved $Co(en)_3^{3+}$ enantiomers confirm that in solutions of this left-handed DNA the broad upfield peak corresponds to Λ-$Co(en)_3^{3+}$ and the narrow downfield peak corresponds to Δ-$Co(en)_3^{3+}$.

As shown in Figure 4 for d(GGCCGGCC), the longitudinal and transverse relaxation rates of Δ- and Λ-$Co(en)_3^{3+}$ show the characteristic quadrupolar temperature dependences. These data may be analyzed using eqs 1−3 in order to obtain effective correlation times and quadrupole coupling constants.

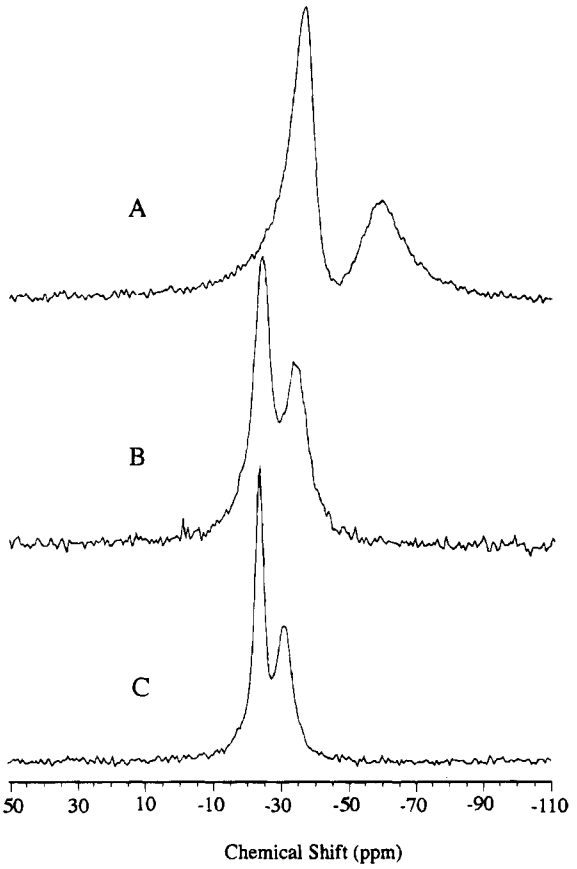

FIGURE 2: ^{59}Co NMR spectra of racemic ^{59}Co(en)$_3^{3+}$ in oligonucleotide solution. A, 1.47 mM strand concentration of d(GGCCGGCC), 0.75 mM Co(en)$_3$Cl$_3$. B, 2.17 mM strand concentration of d(*CG*CG*CG*CG), 0.97 mM Co(en)$_3$Cl$_3$. C, 1.39 mM strand concentration of d(GCGCGCGC), 0.68 mM Co(en)$_3$Cl$_3$. For all spectra, the temperature is 21.4 °C.

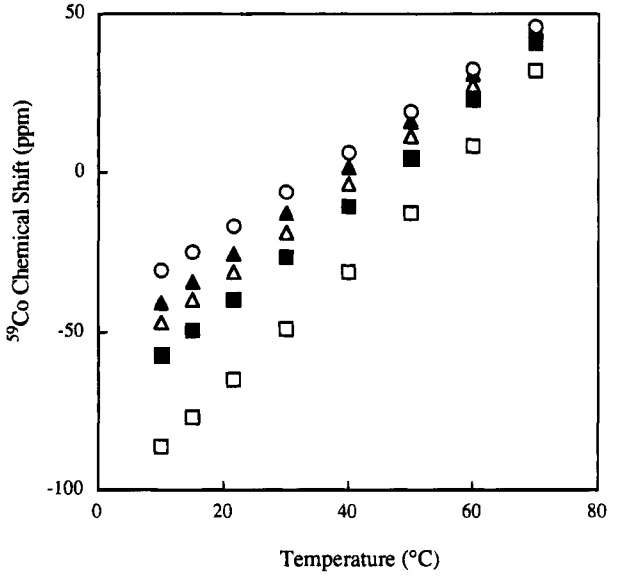

FIGURE 3: ^{59}Co NMR chemical shifts of Co(en)$_3^{3+}$ enantiomers in oligonucleotide solution. For the 2.68 mM d(GGCCGGCC) strand: (□) 1.35 mM Δ-Co(en)$_3^{3+}$, (■) 1.32 mM Λ-Co(en)$_3^{3+}$. For the 2.1 mM d(GCGCGCGC) strand: (Δ) 1.1 mM Δ-Co(en)$_3^{3+}$, (▲) 1.0 mM Λ-Co(en)$_3^{3+}$. For the 1.88 mM d(CATATATG) strand: (○) 0.949 mM Δ-Co(en)$_3^{3+}$. Indistinguishable chemical shifts were obtained for 0.949 mM Λ-Co(en)$_3^{3+}$.

It is worth noting that, according to eq 1, a maximum in R_1 should occur for a correlation time of 1.64 ns. The modest increase in the longitudinal relaxation rate with temperature

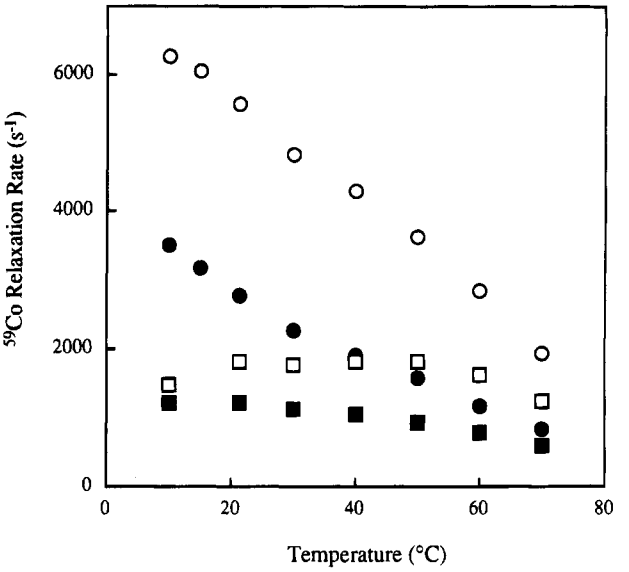

FIGURE 4: ^{59}Co(en)$_3^{3+}$ NMR relaxation rates in d(GGCCGGCC) solution. The DNA strand concentration is 2.68 mM. R_1 (□) and R_2 (○) for a solution containing 1.35 mM Δ-Co(en)$_3^{3+}$. R_1 (■) and R_2 (●) for a solution containing 1.32 mM Λ-Co(en)$_3^{3+}$.

that occurs at low temperatures reflects this expectation.

As is summarized in Tables 1 and 2, at 21.4 °C and at a ratio of one Co(en)$_3^{3+}$ per DNA duplex, the effective room temperature rotational correlation times of Δ- and Λ-Co(en)$_3^{3+}$ in solution with the class 1 oligonucleotides d(GGCCGGCC), d(CCCCGGGG) and d(*CG*CG*CG*CG) vary between 1.1 and 2.0 ns. For comparison, as calculated from the Stokes–Einstein equation, the rotational correlation time of a DNA octamer is around 2.2 ns at 21.4 °C (Xu et al., 1993b). Quadrupole coupling constants for the class 1 oligonucleotides are relatively large, close to or exceeding the predicted value of 3.4 MHz. For each of the right-handed DNAs, the Δ isomer shows larger chemical shifts, effective coupling constants, and effective correlation times than does the Λ isomer. For the left-handed DNA, the reverse trend is evident. Hence, our observations suggest a general rule that DNA inhibits more strongly the mobility of Co(en)$_3^{3+}$ of the same helical sense.

In contrast to above DNA oligomers, the class 2 oligomers d(CATATATG) and d(GGAATTCC) show no discrimination between the Co(en)$_3^{3+}$ enantiomers. For a racemic mixture of Co(en)$_3^{3+}$, there is a single peak in the ^{59}Co spectrum. Temperature-dependent ^{59}Co NMR experiments of separated isomers in solutions of these two oligomeric DNAs, show that both Δ- and Λ-Co(en)$_3^{3+}$ have the same chemical shifts and relaxation rates (Figures 3 and 5). Effective rotational correlation times are significantly less than 1 ns, and effective quadrupole coupling constants are less than 2 MHz (Tables 1 and 2).

Somewhat unexpectedly, racemic Co(en)$_3^{3+}$ in solution with the class 3 oligomer d(GCGCGCGC) solution also shows two ^{59}Co NMR peaks (Figures 2 and 6). Experiments performed with the separated enantiomers identify the broader, upfield shifted peak as Δ-Co(en)$_3^{3+}$ and the downfield shifted peak as Λ-Co(en)$_3^{3+}$. The effective rotational correlation times for these two enantiomers were determined to be 1.57 and 1.04 ns for the Δ and Λ isomers, respectively.

Although the chemical shifts of Co(en)$_3^{3+}$ enantiomers in d(CTCTAGAG) solution are the same, the relaxation rates differ significantly (Figure 7 and Tables 1 and 2). Both

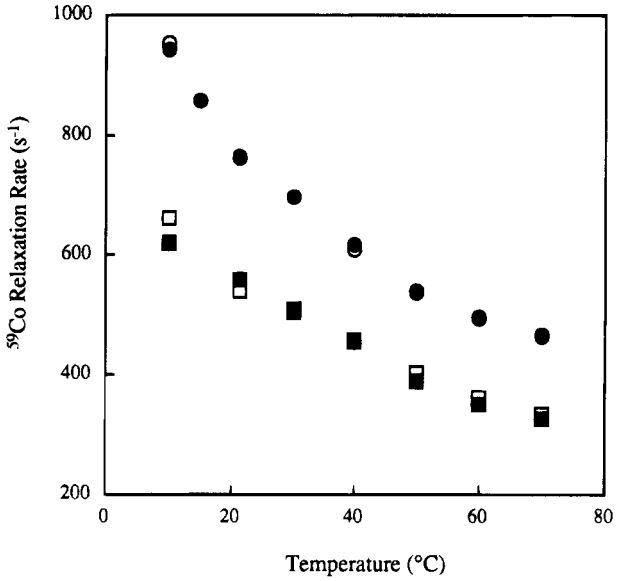

FIGURE 5: $^{59}Co(en)_3^{3+}$ NMR relaxation rates in d(CATATATG) solution. The DNA strand concentration is 1.88 mM. R_1 (□) and R_2 (○) for a solution containing 0.949 mM Δ-$Co(en)_3^{3+}$. R_1 (■) and R_2 (●) for a solution containing 0.949 mM Λ-$Co(en)_3^{3+}$.

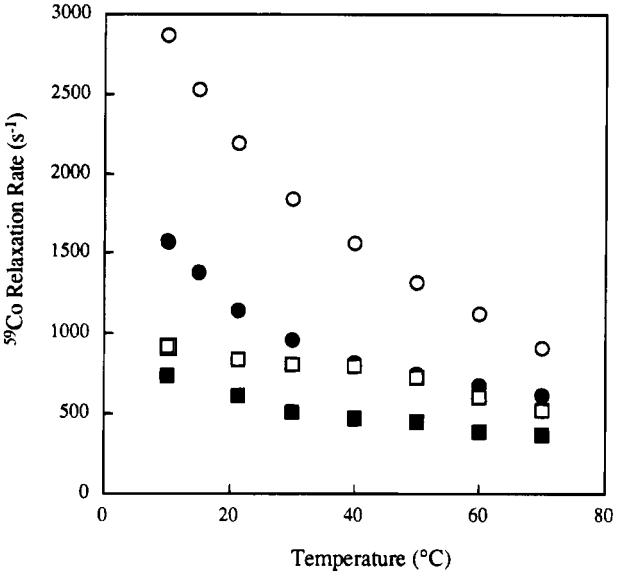

FIGURE 6: $^{59}Co(en)_3^{3+}$ NMR relaxation rates in d(GCGCGCGC) solution. The DNA strand concentration is 2.1 mM. R_1 (□) and R_2 (○) for a solution containing 1.1 mM Δ-$Co(en)_3^{3+}$. R_1 (■) and R_2 (●) for a solution containing1.0 mM Λ-$Co(en)_3^{3+}$.

longitudinal and transverse relaxation rates of Δ-$Co(en)_3^{3+}$ are larger than those of Λ-$Co(en)_3^{3+}$. The effective rotational correlation times are 0.96 and 0.75 ns for the Δ and Λ isomers, respectively.

The Δ and Λ Isomers of $Co(en)_3^{3+}$ Both Perturb the Structure of Duplex Oligonucleotides, but in Nonidentical Ways. d(CCCCGGGG) undergoes a B-A transition in the presence of $Co(NH_3)_6^{3+}$. As we have discussed elsewhere, Δ-$Co(en)_3^{3+}$ and Λ-$Co(en)_3^{3+}$ change the CD of this oligomer in dramatic but nonidentical ways (Xu et al., 1993b). As illustrated in Figure 8, the CD of this oligomer changes in the presence of both stereoisomers, but does so much more dramatically in the presence of Δ-$Co(en)_3^{3+}$. The intense peak observed at 214 nm suggests that Δ-$Co(en)_3^{3+}$ induces significant base tilting. In addition, the peak intensity is greater at 260 nm than at 290 nm. Moreover, the CD spectra of d(GGCCGGCC) in the presence of Δ-$Co(en)_3^{3+}$ and in

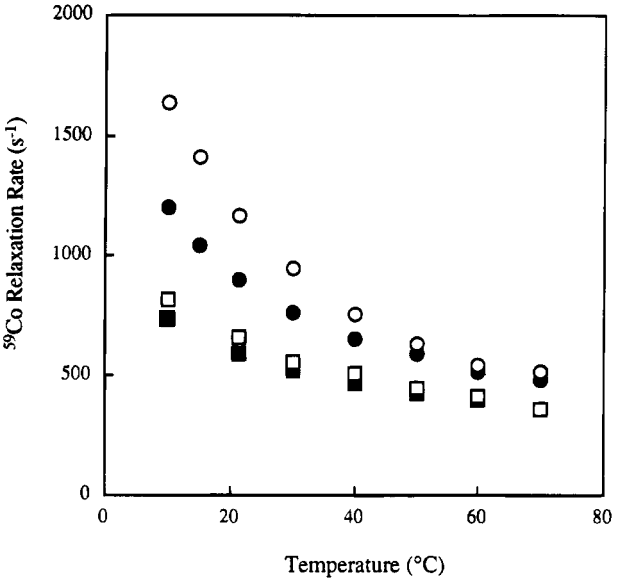

FIGURE 7: $^{59}Co(en)_3^{3+}$ NMR relaxation rates in d(CTCTAGAG) solution. The DNA strand concentration is 1.93 mM. R_1 (□) and R_2 (○) for a solution containing 0.97 mM Δ-$Co(en)_3^{3+}$. R_1 (■) and R_2 (●) for a solution containing 1.02 mM Λ-$Co(en)_3^{3+}$.

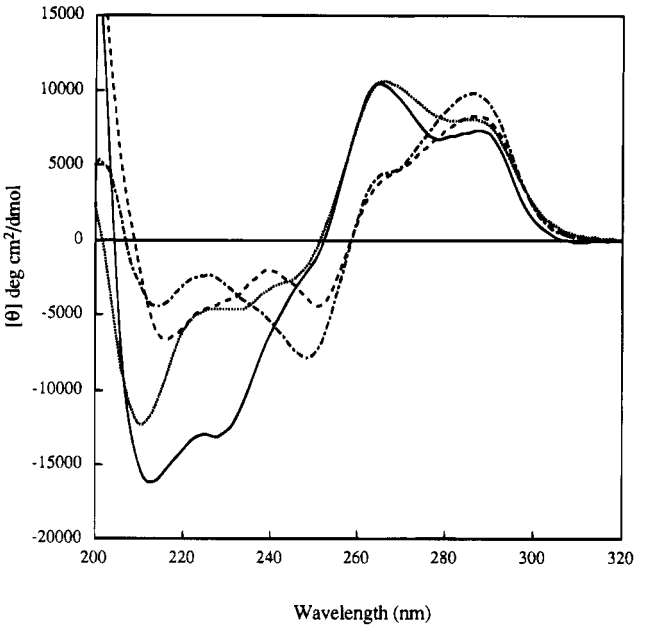

FIGURE 8: CD spectra of 7.8 μM d(GGCCGGCC) (a) in the presence of 39.1 μM Δ-$Co(en)_3^{3+}$ (solid curve) and (b) in the presence of 37.0 μM Λ-$Co(en)_3^{3+}$ (dashed curve), (c) 8.75 μM d(GGCCGGCC) in the presence of 80% TFE and 3 mM NaCl (dotted curve), and (d) 8.6 μM d(GGCCGGCC) in the presence of 0.1 M NaCl (dash−dot curve).

80% TFE solution are notably similar. Since TFE is a solvent known to induce the B-A transition, this indicates that Δ-$Co(en)_3^{3+}$ also induces a B-A transition of d(GGCCGGCC). On the other hand, the CD spectrum of this oligomer in the presence of Λ-$Co(en)_3^{3+}$ is more conservative. Moreover, as is observed for d(GGCCGGCC) in the presence of 0.1 M NaCl, the peak intensity of this oligomer in the presence of Λ-$Co(en)_3^{3+}$ is greater at 290 nm than at 260 nm. Overall, the CD spectrum of d(GGCCGGCC) in the presence of Λ-$Co(en)_3^{3+}$ is similar to that observed in $Co(NH_3)_6^{3+}$ solution (Xu et al., 1993a).

Specific recognition plays a role in the $Co(NH_3)_6^{3+}$-induced B-Z transition of d(*CG*CG*CG*CG) (Gessner et al., 1985). As shown by the CD spectra in Figure 9, the

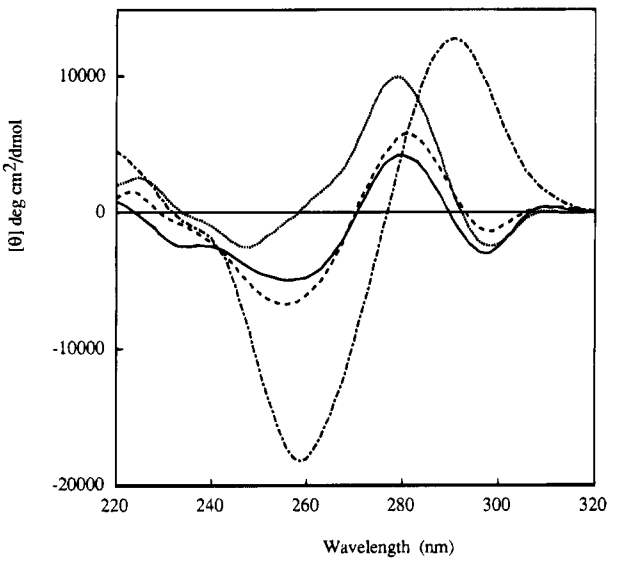

FIGURE 9: CD spectra of 5.8 μM d(*CG*CG*CG*CG) (a) in the presence of 13 μM Δ-Co(en)$_3^{3+}$ (solid curve), (b) in the presence of 14 μM Λ-Co(en)$_3^{3+}$ (dashed curve), (c) in the presence of 12 μM Co(NH$_3$)$_6^{3+}$, and (d) in the presence of 0.1 M NaCl (dash−dot curve).

two enantiomers of Co(en)$_3^{3+}$ also induce the peak inversion that is characteristic of a transition from B to Z forms. There appear to be modest differences in the structural effects of the two enantiomers. At 298 nm, the negative intensity is stronger for the spectrum obtained in the presence of Λ-Co(en)$_3^{3+}$ compared to that obtained in the presence of Δ-Co(en)$_3^{3+}$. Also, the positive intensity at 280 nm and the negative intensity at 258 nm are stronger for d(*CG*CG*CG*CG) in the presence of Δ-Co(en)$_3^{3+}$ than in the presence of Λ-Co(en)$_3^{3+}$. Nonetheless, as is illustrated in Figure 9, the spectra obtained in the presence of either enantiomer differ significantly from those obtained at high Co(NH$_3$)$_6^{3+}$ concentrations (or at high NaCl concentrations). Whereas the NaCl-, Co(NH$_3$)$_6^{3+}$- and Co(en)$_3^{3+}$-induced Z-DNA structures each show a negative peak at around 300 nm and a positive peak at around 280, the Λ- and Δ-Co(en)$_3^{3+}$ induced structures show an additional negative peak at 260 nm. As shown in Figure 9, similar negative peak is seen for the B-form spectrum of d(*CG*CG*CG*CG) that is observed in the presence of 0.1 M NaCl. However, the observed spectrum does not appear to be a simple linear combination of B- and Z- form spectra. Moreover, additional titration of d(*CG*CG*CG*CG) with Co(en)$_3^{3+}$resulted in no further noticeable changes in the CD spectrum. For none of the class 2 or class 3 oligonucleotides are significant changes observed in CD spectra upon titration with (either stereoisomer of) Co(en)$_3^{3+}$.

DISCUSSION

Cation NMR studies of $^{43}Ca^{2+}$, $^{25}Mg^{2+}$, and ^{59}Co(NH$_3$)$_6^{3+}$ in polymeric DNA solutions demonstrate the existence of significant binding heterogeneity for these cations (Braunlin et al., 1987a,b, 1889, 1991, 1992; Braunlin & Xu, 1992; Xu et al., 1993a,b). The picture that arises is of an equilibrium between a small class (or classes) of specifically bound cation and a larger class of loosely associated, nonspecifically bound cation. The two classes may be distinguished on the basis of characteristic relaxation behaviors and chemical shifts. For $^{43}Ca^{2+}$ and ^{59}Co(NH$_3$)$_6^{3+}$, an increase in the fraction of specifically bound cation correlates with an increase in the GC content of the DNA (Braunlin et al., 1992; Braunlin & Xu, 1992). The binding of ^{59}Co(NH$_3$)$_6^{3+}$ to bacterial DNAs of variable GC content was examined in some detail. Simultaneous fitting of the relaxation and chemical shift results were compared with published dinucleotide frequencies. A striking correlation was found with the relative fraction of GG dinucleotide sequences (compared to other dinucleotide sequences) and the relative fraction of tight binding sites (compared to delocalized binding sites). A structural rationale for the involvement of GG sequences was advanced on the basis of a favorable molecular electrostatic potential in the major groove (Braunlin & Xu, 1992) and on a crystal structure showing ^{59}Co(NH$_3$)$_6^{3+}$ bound to a duplex (A-form) region of t-RNA (Hingerty et al., 1982).

Subsequent oligonucleotide studies supported these ideas. Thirteen octanucleotides were examined and could be divided into three classes on the basis of their effects on ^{59}Co(NH$_3$)$_6^{3+}$ NMR chemical shifts and relaxation rates (Xu et al., 1993a). Class 1 octanucleotides showed significantly enhanced ^{59}Co upfield shifts and gave temperature dependences characteristic of a dominant quadrupolar relaxation mechanism. Effective quadrupole coupling constants were obtained that were comparable to those estimated in simple salt solution, and effective correlation times reflected the overall tumbling motions of the octamer duplexes. With the exception of *CG*CG*CG*CG, which undergoes a ^{59}Co(NH$_3$)$_6^{3+}$-induced B-Z transition, all of the class 1 oligonucleotides possessed neighboring, same-strand guanine residues. Moreover, all class 1 oligonucleotides showed large CD changes upon titration with ^{59}Co(NH$_3$)$_6^{3+}$. The nature of the CD changes suggested Co(NH$_3$)$_6^{3+}$-induced structural transitions toward A-DNA characteristics (Xu et al., 1993a,b). Such transitions would allow bound Co(NH$_3$)$_6^{3+}$ cations to bind in the major groove, making simultaneous contact with N7 and O6 residues on neighboring guanines.

At the other extreme, class 2 oligonucleotides showed the characteristic scalar coupling temperature dependence, as expected for significantly enhanced rotational mobility of bound ^{59}Co(NH$_3$)$_6^{3+}$ cations. Such class 2 oligonucleotides tended to be AT rich, and showed no significant CD changes upon titration with Co(NH$_3$)$_6^{3+}$.

Intermediate ^{59}Co(NH$_3$)$_6^{3+}$ NMR behavior was found for class 3 oligonucleotides, for which some inhibition of rotational mobility could be discerned. Class 3 oligonucleotides showed no significant CD changes upon titration with Co(NH$_3$)$_6^{3+}$. Among the class 3 oligonucleotides was the 100% GC oligomer GCGCGCGC.

Compared to ^{59}Co(NH$_3$)$_6^{3+}$, the contribution of the scalar coupling mechanism to the ^{59}Co relaxation behavior of ^{59}Co(en)$_3^{3+}$ is significantly reduced in simple salt solution, and is effectively overwhelmed in oligonucleotide solution by the quadrupolar mechanism. The origin of this fundamental difference in relaxation behavior can be traced to a decrease in the ^{14}N T_1 and an increase in the ^{59}Co quadrupole constant for ^{59}Co(en)$_3^{3+}$ compared to ^{59}Co(NH$_3$)$_6^{3+}$. A consequence of this fundamental difference in relaxation behavior is that the transition from scalar coupling to quadrupolar mechanisms no longer provides a useful criterion for a transition from site binding to a more delocalized binding mode. Instead, for ^{59}Co(en)$_3^{3+}$ binding to oligonucleotides, the magnitudes of the effective quadrupole coupling constant and correlation time provide an analogous criterion. On the basis of this criterion, the results summarized in Tables 1 and 2 show that, for both stereoisomers of Co(en)$_3^{3+}$, the same

ordering of DNA oligonucleotides is found as was previously found for $^{59}Co(NH_3)_6^{3+}$.

Class 1 oligonucleotides show effective ^{59}Co correlation times that reflect the overall tumbling of a DNA octamer. These oligonucleotides also show effective quadrupole coupling constants that are comparable in magnitude to those estimated for the free $^{59}Co(en)_3^{3+}$ cation. Large $Co(en)_3^{3+}$-induced structural perturbations are evident from the CD spectra, and these perturbations are in the direction of more A-like characteristics.

Class 2 oligonucleotides show effective ^{59}Co correlation times that are significantly reduced compared to the rotational correlation time for oligonucleotide tumbling. Effective quadrupole coupling constants are also reduced significantly in magnitude compared to those estimated for the free $^{59}Co(en)_3^{3+}$ cation. No significant structural perturbations can be deduced from the CD spectra.

Class 3 oligonucleotides show ^{59}Co correlation times and effective quadrupole coupling constants that are intermediate between those of classes 1 and 2. No significant structural perturbations can be deduced from the CD spectra.

In terms of recognition of the two stereoisomers of $Co(en)_3^{3+}$, the three oligonucleotide classes show notable differences. For the right-handed class 1 oligomers, d(CCCCGGGG) and d(GGCCGGCC), larger τ_{eff} and χ_{eff} values are observed for the Δ isomer compared to the Λ isomer, consistent with reduced rotational mobility of the former. Reduced rotational mobility in turn suggests more specific association of the Δ isomer. This notion is further supported by the observed chemical shifts, which are more upfield shifted for the Δ isomer compared to the Λ isomer. Interestingly, for the left-handed oligomer d(*CG*CG*CG*CG) it is the Λ isomer that is associated with larger upfield shifts and larger τ_{eff} and χ_{eff} values.

For class 1 oligomers, structural differences monitored by CD correlate well with differences in the rotational dynamics of the bound cobalt complex: the more strongly the rotation of the isomer is inhibited, the more severely the DNA conformation is perturbed. These differential effects of the two enantiomers likewise correlate well with the reported differences between the binding behaviors of the two enantiomers. On the basis of proton chemical shifts of the DNA, Watt et al. (1994) report that the intrinsic affinity for d(CCAATCCGGATTG) is 10 times greater for the Δ isomer than for the Λ isomer.

For class 2 oligomers, no evidence for differential recognition of the two stereoisomers of $Co(en)_3^{3+}$ can be observed. The $^{59}Co(en)_3^{3+}$ chemical shifts and relaxation rates in solutions of such oligomers are identical for the two isomers.

Class 3 oligomers represent an intermediate case. Racemic $Co(en)_3^{3+}$ in d(GCGCGCGC) solution gives clearly resolved ^{59}Co peaks, and the relaxation behaviors of the two isomers are distinct. For d(CTCTAGAG), although identical ^{59}Co chemical shifts are observed, the two isomers can nonetheless be distinguished on the basis of their ^{59}Co NMR relaxation behavior.

Attempts to localize bound polyamines on oligonucleotides in solution by NOE measurements have failed as a consequence of the significant mobility of these cations on the DNA (Wemmer et al., 1985). In contrast to this situation, our ^{59}Co NMR data have shown that $Co(NH_3)_6^{3+}$ and the isomers of $Co(en)_3^{3+}$ are significantly inhibited in their rotational mobility when bound specifically to G-rich DNAs.

Given the similar DNA sequence-dependent behavior observed for the two stereoisomers of $Co(en)_3^{3+}$ and $Co(NH_3)_6^{3+}$, it is quite likely that these cations bind in similar locations on the DNA helix. We have obtained proton NMR NOESY data that demonstrate recognition of Δ-$Co(en)_3^{3+}$ in the major groove of d(CCCCGGGG), near the center of the duplex (work in progress). These results thus support the hypothesis that cations such as $Co(en)_3^{3+}$ and $Co(NH_3)_6^{3+}$ bind in the major groove of A-DNA, making simultaneous contact with electronegative groups on neighboring guanines. The fact that we are unable to obtain measureable NOEs from Λ-$Co(en)_3^{3+}$ is consistent with our ^{59}Co NMR and CD results, which amply demonstrate that the binding modes of these two stereoisomers of $Co(en)_3^{3+}$ are not identical.

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