# Binding of Co(III) to a DNA Oligomer via Reaction of $[Co(NH_3)_5(OH_2)]^{3+}$ with $(5medC\text{-}dG)_4$[†]

David M. Calderone, Edgardo J. Mantilla, Michael Hicks, Daniel H. Huchital, W. Rorer Murphy, Jr., and Richard D. Sheardy*

*Department of Chemistry, Seton Hall University, 400 South Orange Avenue, South Orange, New Jersey 07079-2694*

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ABSTRACT: The interaction specificities of cobalt(III) ammines with the self-complementary eight-base pair DNA oligomer $(5medC\text{-}dG)_4$ have been investigated. Standard protocol for preparing DNA samples calls for heat annealing the DNA oligomer in phosphate buffer in the absence or presence of cobalt(III) ammine complex for 2 min at 80 °C, followed by slow cooling to 25 °C. An alternative method for DNA preparation is incubation of the oligomer in the presence of the cobalt(III) complex at 37 °C followed by exhaustive dialysis. The conformational properties of the thus-treated DNA oligomer were determined by inspection of the UV and CD spectra at 25 and 95 °C and thermal denaturation studies. With heat annealing in the absence of any cobalt(III) complex, $(5medC\text{-}dG)_4$ assumes a double-stranded, right-handed B conformation at 25 °C. Upon heat annealing in the presence of 200 $\mu$M $[Co(NH_3)_6]^{3+}$, $(5medC\text{-}dG)_4$ assumes a double-stranded, left-handed Z conformation at 25 °C. In contrast, the CD and UV spectra of $(5medC\text{-}dG)_4$ heat annealed in the presence of 200 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$ are consistent with a distorted B-like conformation at 25 °C. Incubation of the oligomer in the presence of $[Co(NH_3)_5(OH_2)]^{3+}$ results in modification of the conformational properties of the oligomer at both 25 and 95 °C relative to the untreated oligomer. The extent of modification depends upon the incubation concentration of $[Co(NH_3)_5(OH_2)]^{3+}$ and the reaction time. Atomic absorption (AA) analyses of these treated DNA samples indicate a high degree of cobalt association to the oligomer. These studies suggest that $[Co(NH_3)_5(OH_2)]^{3+}$ reacts with the oligomer, resulting in tight binding of the cobalt(III) metal center to the DNA lattice. The combined results are interpreted in terms of coordination of the cobalt(III) to N7 of guanine with subsequent loss of the aquo group from $[Co(NH_3)_5(OH_2)]^{3+}$.

A number of small metal complexes have been shown to interact with DNA via coordination of the nitrogenous bases to the metal with loss of a labile ligand from the metal. For example, $[Ru(NH_3)_5(OH_2)]^{2+}$ has been shown to bind to calf thymus DNA primarily at N7 of guanine bases (Clark et al., 1986). Cisplatin, *cis*-$Pt(NH_3)_2Cl_2$, cross-links at GpG sites in an intrastrand manner and at GpC sites in an interstrand manner, producing GN7-Pt-GN7 linkages (Brabek et al., 1992; Bruhn et al., 1990; Hopkins et al., 1992, and references cited therein). It has also been suggested that *cis*-$[Ru(NH_3)_2Cl_2]^{2+}$ behaves in a manner similar to that of cisplatin (Clark, 1980).

Reversible interactions of small metal complexes with nucleic acids have also been noted. It is well-known that cobalt(III) hexammine, $[Co(NH_3)_6]^{3+}$, induces the B to Z transition in both DNA polymers and oligomers possessing dC-dG and 5medC-dG (where 5medC is 5-methylcytidine) dinucleotide repeats (Behe & Felsenfeld, 1981; Winkle & Sheardy, 1990; Winkle et al., 1991; Lu et al., 1992). The stabilization of Z-DNA by $[Co(NH_3)_6]^{3+}$ has been attributed to specific hydrogen bonds between three of the ammine groups with guanine and phosphate acceptor sites on the DNA helix (Gessner et al., 1985). This reagent has also been shown to induce an unusual non-Z-like structure in a DNA oligomer containing a $(dC\text{-}dG)_4$ segment (Winkle et al., 1992) and to induce the B to A transition in oligomers of sequence d(CCCCGGGG) (Xu et al., 1993). $[Co(NH_3)_6]^{3+}$ also binds to polymeric native DNA with high affinity and with a preference to GC rich DNAs (Braunlin et al., 1987; Braunlin & Xu, 1992).

Divalent and trivalent metal complexes also bind to tRNAs with high affinities. Co(II), $Mg^{2+}$, and Mn(II) display cooperativity in binding and have both weak and strong binding sites. The cooperativity and association constant depend upon the nature of the cation (Danchin, 1972). The binding of multivalent transition metal complexes to yeast tRNA also stabilizes the native conformer of the RNA molecule. The relative effectiveness of the stabilization depends upon the cation, its charge, and the coordinated ligands (Karpel et al., 1975). A crystallographic study of the binding of Co(II) to tRNA reveals coordination of N7 of G15 to the cobalt with water occupying the other coordination sites of the octahedron (Jack et al., 1977).

Studies have also investigated the interactions of such metal complexes with nucleosides and nucleotides. The locale of metal complexation to the base depends upon the coordination number as well as the size of the metal. Evidence for the binding of simple Co(III) complexes to nucleoside bases indicates outer sphere coordination in both crystal structures and solution (Marzilli, 1981, and references cited therein). Additional evidence for such interactions of purine nucleotides with $[Co(NH_3)_6]^{3+}$, $[Co(NH_3)_5Cl]^{2+}$, and $[Co(NH_3)_4Cl_2]^{+}$ has been gleaned from FT-IR and $^1$H-NMR studies (Tajmir-Riahi, 1991).

Our research has also been directed toward delineating the interaction specificities of small metal complexes with

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* To whom correspondence should be sent.

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nucleic acids (Carlson et al., 1993). One particular area of interest is investigating how replacement of one of the ammine ligands of $[Co(NH_3)_6]^{3+}$ with a more labile ligand (e.g., $H_2O$) would alter its interaction with nucleic acids. For these studies, we investigated the interactions of $[Co(NH_3)_5(OH_2)]^{3+}$ with the synthetic DNA oligomer **Z8**, $(5medC\text{-}dG)_4$.

## MATERIALS AND METHODS

*General.* All chemicals were reagent grade and used directly unless otherwise noted. The DNA oligomer **Z8**, $(5medC\text{-}dG)_4$, was synthesized on an Applied Biosystem 380B DNA Synthesizer (Foster City, CA) via the phosphoramadite method (Caruthers, 1988) and purified by reverse phase HPLC as previously reported (Sheardy, 1988). After the second purification, the oligomer was exhaustively dialyzed vs water and then lyophilized to dryness. DNA purity was confirmed by analytical HPLC and gel electrophoresis. $[Co(NH_3)_6]^{3+}$ was obtained from Kodak (Rochester, NY) and used without further purification. $[Co(NH_3)_5(OH_2)]^{3+}$ was prepared according to Basolo and Murman (1953). Standard buffer for CD and UV determinations was 50.0 mM phosphate buffer (pH 7.0), 0.05 mM EDTA with NaCl, and/or cobalt(III) complex added to various concentrations.

*Preparation of Cobalt-Modified DNA.* Lyophilized DNA samples were dissolved in phosphate buffer with 50 mM NaCl to give a final DNA concentration of ca. 40−75 $\mu$M in base pairs. $[Co(NH_3)_6]^{3+}$ or $[Co(NH_3)_5(OH_2)]^{3+}$ was then added to give final concentrations of the cobalt complex from 100 to 400 $\mu$M. These conditions were chosen to ensure duplex stability of the oligomer, to have a [cobalt complex]/[DNA] for sufficient reaction, and to preclude possible precipitation at higher concentrations of DNA and/or cobalt complex. For untreated **Z8** in buffer alone or **Z8** in buffer with $[Co(NH_3)_6]^{3+}$, samples were prepared by heating at 80 °C for 2 min followed by slow cooling to room temperature. Samples of **Z8** in the presence of $[Co(NH_3)_5(OH_2)]^{3+}$ were prepared in one of two fashions: (1) heat annealing by heating at 80 °C for 2 min followed by slow cooling to room temperature or (2) incubation by heating at 37 °C (without prior heat annealing) for various times. A method found to effectively remove all nontightly bound cobalt complex was exhaustive dialysis vs 200 mM NaCl in water followed by exhaustive dialysis vs pure water (D. M. Calderone and R. D. Sheardy, unpublished results). After dialysis, the aqueous samples were then lyophilized to dryness. Prior to any subsequent characterization, the DNA samples were reconstituted in standard buffer at various NaCl concentrations, heated at 80 °C for 2 min, and slowly cooled to room temperature.

*Circular Dichroism Studies.* The CD spectra of untreated **Z8** and treated **Z8** samples prepared in the presence of cobalt(III) complex by any method were determined at 25 or 95 °C with an AVIV 62A DS circular dichroism spectropolarimeter.

*Thermal Denaturation Studies.* The UV spectra of untreated **Z8** and treated **Z8** samples prepared in the presence of cobalt(III) complex by any method were determined with a Gilford Response II UV/VIS spectrophotometer. For thermal denaturation studies, all samples treated with $[Co(NH_3)_5(OH_2)]^{3+}$ were dialyzed as described above prior to use. Thermal denaturation studies were carried out as previously described by monitoring the transition at 280 nm (Sheardy et al., 1994). The data were transferred to a personal computer for determination of the transition temperature, $T_m$. The $T_m$ values reported here were determined as the inflection point of the $A_{280}$ vs $T$ plots via examination of the first derivatives.

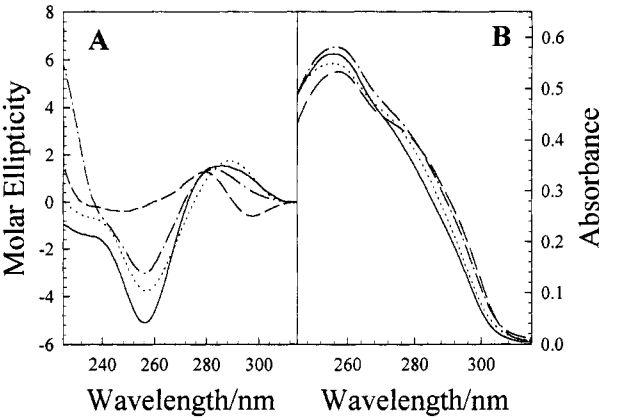


FIGURE 1: CD spectra (A) and UV spectra (B) at 25 °C of **Z8** in standard buffer with 50 mM NaCl (solid line), **Z8** heat annealed in standard buffer, 50 mM NaCl, and 200 $\mu$M $[Co(NH_3)_6]^{3+}$ without subsequent dialysis (dash), **Z8** heat annealed in standard buffer, 50 mM NaCl, and 200 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$ without subsequent dialysis (dash-dot), and **Z8** incubated for 48 h at 37 °C in standard buffer, 50 mM NaCl, and 200 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$, followed by exhaustive dialysis, lyophilization, and reconstitution in standard buffer wtih 50 mM NaCl (dot). The concentration of DNA for these samples was ca. $4.2 \times 10^{-5}$ M in base pairs.

*Graphite Furnace Atomic Absorption (AA) Studies.* In order to determine any tightly bound cobalt(III) for **Z8** incubated with 200 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$ followed by exhaustive dialysis, AA spectra were recorded with a Polarized Zeeman Spectrometer Z-8270 from Hitachi using a platform graphite tube with Argon purge. The temperature program involved drying from 80 to 140 °C, ashing at 200 °C for a 20 s hold, ramping to 800 °C for a 30 s hold, and then atomizing at 2700 °C and reading the absorbance at 240.7 nm. Readings were performed in triplicate using a standard SSC-300 Hitachi Autosampler and compared to an external standard curve at 20, 50, and 100 ppb.

## RESULTS

*How Does $[Co(NH_3)_5(OH_2)]^{3+}$ Affect the Conformational State of* **Z8***?* **Z8** is an alternating purine−pyrimidine 8-mer which has been shown to undergo the NaCl-induced B−Z transition (Sheardy, 1991; Sheardy et al., 1993). Figure 1A shows the CD spectra of untreated **Z8**, as well as **Z8** under a variety of conditions. In the absence of any cobalt complex, the CD spectrum of **Z8** is typical for the right-handed B conformation with a peak at 280 nm and a trough at 255 nm. Heat annealing **Z8** in the presence of increasing concentrations of $[Co(NH_3)_6]^{3+}$ induces CD spectral changes up to 150 $\mu$M in cobalt complex with no further spectral changes at higher concentrations. The CD spectrum of this oligomer in the presence of 200 $\mu$M $[Co(NH_3)_6]^{3+}$ is characteristic of the left-handed Z conformation with a trough at 295 nm and a peak at 275 nm (Sheardy, 1991). Heat annealing **Z8** in the presence of increasing concentrations of $[Co(NH_3)_5(OH_2)]^{3+}$ also induces CD spectral changes up to 150 $\mu$M in cobalt complex with no additional spectral changes at higher concentrations. However, the CD spectrum in the presence of 200 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$ is only

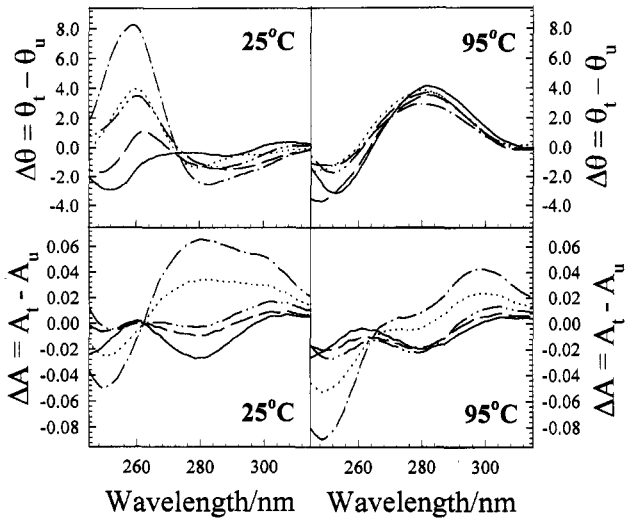


FIGURE 2: Effect of increasing the concentration of $[Co(NH_3)_5(OH_2)]^{3+}$ in the incubation of **Z8** at 37 °C for 48 h, followed by exhaustive dialysis, on the molar ellipticity and UV absorption of the DNA oligomer. Spectra for untreated and treated **Z8** (ca. 4.2 × $10^{-5}$ M in base pairs) were determined in standard buffer with 200 mM NaCl at 25 and 95 °C. The upper panels show the CD difference spectra plotted as $\Delta\theta = \theta_t - \theta_u$, where $\theta_t$ and $\theta_u$ are the molar ellipticities of the treated and untreated oligomer, respectively. The lower panels show the UV absorption difference spectra plotted as $\Delta A = A_t - A_u$, where $A_t$ and $A_u$ are the absorbances of the treated and untreated oligomer, respectively. The concentration of $[Co(NH_3)_5(OH_2)]^{3+}$ in the incubation was 100 $\mu$M (solid line), 200 $\mu$M (dash), 250 $\mu$M (dot), 300 $\mu$M (dash-dot), or 400 $\mu$M (dash-dot-dot).

slightly different from that of untreated **Z8** with a peak at 278 nm and a shallow trough at 256 nm (Figure 1A). This initial result suggests that $[Co(NH_3)_5(OH_2)]^{3+}$ modifies the DNA oligomer in such a fashion as to prevent the B−Z conformational transition. **Z8** was then incubated at 37 °C for 48 h in the presence of 200 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$, followed by exhaustive dialysis. The resultant CD spectrum of this sample is also consistent with that of an altered B-like structure with a peak at 279 nm and a trough at 255 nm.

The UV spectra of the same samples above are shown in Figure 1B. The appearance of the shoulder at 280 nm and the hypochromism at 260 nm for the oligomer in the presence of 200 $\mu$M $[Co(NH_3)_6]^{3+}$ is characteristic of Z-DNA (Behe & Felsenfeld, 1981). However, the spectra of the oligomer treated with $[Co(NH_3)_5(OH_2)]^{3+}$ by either the heat annealing or incubation followed by dialysis methods differ from those of both the untreated sample and the sample treated with $[Co(NH_3)_6]^{3+}$. These data also suggest a distorted B-like structure for the oligomer treated with $[Co(NH_3)_5(OH_2)]^{3+}$.

*How Does the Incubation Concentration of $[Co(NH_3)_5(OH_2)]^{3+}$ Affect the Degree of Modification of* **Z8***?* The **Z8** oligomer was incubated with increasing concentrations of $[Co(NH_3)_5(OH_2)]^{3+}$ for 48 h at 37 °C, followed by exhaustive dialysis, in order to monitor the extent of DNA modification. The CD and UV spectra for any DNA oligomer depend upon a number of factors, such as sequence, conformational state, extent of base stacking, and the position of the duplex−random coil equilibrium. Environmental conditions (e.g., ionic strength of solution, temperature, etc.) will certainly influence the last three factors as well. In addition, the binding of cobalt to the DNA backbone would also alter the conformational properties of the oligomer. In order to emphasize the difference between cobalt-modified **Z8** and untreated **Z8**, we chose to compare their respective CD and

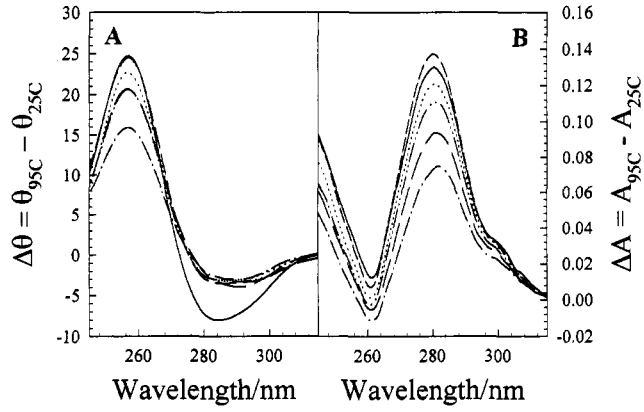


FIGURE 3: Effect of increasing the concentration of $[Co(NH_3)_5(OH_2)]^{3+}$ in the incubation of **Z8** with $[Co(NH_3)_5(OH_2)]^{3+}$ at 37 °C, followed by exhaustive dialysis, on the CD and UV spectral characteristics. Spectra for untreated and treated **Z8** (ca. 4.2 × $10^{-5}$ M in base pairs) were determined in standard buffer with 200 mM NaCl at 25 and 95 °C. Panel A shows the CD temperature difference spectra, and panel B shows the UV temperature difference spectra for the untreated and treated **Z8**. Here, $\Delta\theta = \theta_{95C} - \theta_{25C}$ and $\Delta A = A_{95C} - A_{25C}$, where the subscripts designate the temperature at which the spectra were recorded. The concentration of $[Co(NH_3)_5(OH_2)]^{3+}$ in the incubation was 0 $\mu$M (solid line), 100 $\mu$M (short dash), 200 $\mu$M (dot), 250 $\mu$M (long dash), 300 $\mu$M (dash-dot), or 400 $\mu$M (dash-dot-dot).

UV spectra through the use of difference spectra.

The CD difference spectra (plotted as the CD spectrum of a particular treated sample minus the CD spectrum of the untreated **Z8**) shown in Figure 2 indicate that treatment of **Z8** with 100 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$ only slightly alters the conformation of the DNA oligomer at 25 °C. However, treatment of **Z8** at higher concentrations of $[Co(NH_3)_5(OH_2)]^{3+}$ increases the molar ellipticity at 255 nm relative to untreated **Z8**, with the greatest degree of alteration at 300 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$. In contrast to the trend, incubation at 400 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$ shows a lesser degree of alteration than incubation at 300 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$. The CD difference spectra at 95 °C are quite similar to each other and also point to conformational deviations from untreated **Z8** at this temperature.

The UV difference spectra (plotted as the UV spectrum of a particular sample minus that of untreated **Z8**) in Figure 2 show the same trends as the CD difference spectra. At 25 °C, the greatest conformational deviation from untreated **Z8** is observed for **Z8** incubated with 300 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$. These spectra are characterized by dramatic increases in the absorptions at 280 nm and slight decreases in the absorptions at 255 nm. Dramatic differences in the UV spectra are also observed at 95 °C.

Another revealing way to depict the conformational changes imparted to **Z8** by treatment with $[Co(NH_3)_5(OH_2)]^{3+}$ is to examine the CD and UV difference spectra plotted as the spectrum obtained at 95 °C minus the spectrum obtained at 25 °C for a particular sample (Figure 3). The CD difference spectra (Figure 3A) indicate a decrease in the difference of the ellipticities at 255 nm and an increase in the difference in ellipticities at around 288 nm upon treatment with the cobalt complex. The magnitude of the difference in ellipticities at 255 nm depends upon the concentration of $[Co(NH_3)_5(OH_2)]^{3+}$ in the incubation, with the greatest deviation occurring with treatment of **Z8** with 300 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$. Similar trends are observed in the UV difference spectra (Figure 3B). For example, the sample

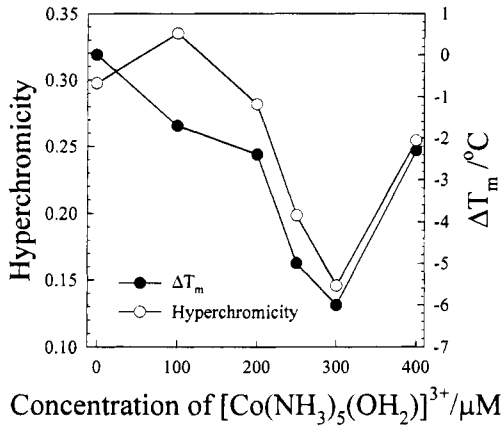


FIGURE 4: Effect of increasing the concentration of $[Co(NH_3)_5(OH_2)]^{3+}$ in the incubation of **Z8** with $[Co(NH_3)_5(OH_2)]^{3+}$ at 37 °C, followed by exhaustive dialysis, on the thermal denaturation. Thermal denaturation studies were carried out with untreated and treated **Z8** samples in standard buffer wtih 200 mM NaCl and the data transferred to a personal computer for analysis. Hyperchromicity is calculated as $(A_{280,95C} - A_{280,25C})/A_{280,25C}$, where $A_{280,95C}$ and $A_{280,25C}$ are the absorbances of the sample at 280 nm at 95 and 25 °C, respectively. The change in thermal denaturation temperature, $\Delta T_m$, is $T_{m,t} - T_{m,u}$, where $T_{m,t}$ and $T_{m,u}$ are the thermal denaturation temperatures of treated and untreated samples, respectively. The $T_m$ values were determined as the inflection point of absorbance vs temperature plots via first derivatives. The DNA concentration for all samples for these data was ca. $4.2 \times 10^{-4}$ M in base pairs.

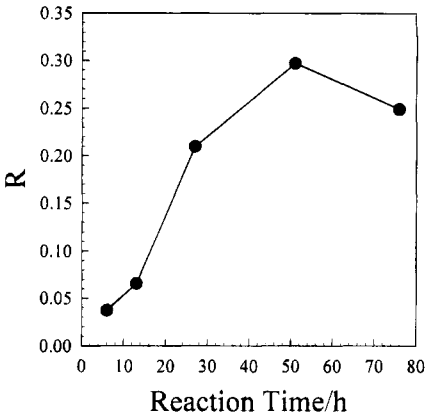


FIGURE 5: Effect of incubation time for cobalt binding to **Z8** incubated in standard buffer, 50 mM NaCl, and 200 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$ at 37 °C, followed by exhaustive dialysis. The binding ratio, $R$, is determined as number of atoms of cobalt bound per base pair of DNA. The concentration of cobalt was determined by atomic absorption as detailed in Materials and Methods.

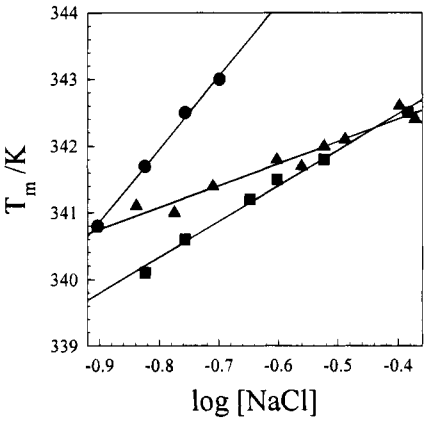


FIGURE 6: Plots of $T_m$ vs log[NaCl] for various **Z8** samples: untreated **Z8** (circles), **Z8** heat annealed in the presence of 200 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$, followed by exhaustive dialysis (squares), and **Z8** incubated with 200 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$ for 48 h at 37 °C, followed by exhaustive dialysis (triangles). All DNA samples were prepared in 5 mM phosphate buffer (pH 7.0) with NaCl added to give [NaCl] ranging from 125 to 400 mM ([DNA] = $4.2 \times 10^{-4}$ M in base pairs). Thermal denaturation studies and $T_m$ determinations were carried out as described in Materials and Methods.

treated at 300 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$ displays a nearly 50% decrease in the UV absorption difference relative to untreated **Z8**.

Figure 4 shows that the hyperchromicity in the thermal denaturation of treated **Z8** dramatically decreases with an increase in incubation concentration of $[Co(NH_3)_5(OH_2)]^{3+}$ from 100 to 300 $\mu$M (i.e., from nearly 32% for untreated **Z8** to only 13% for **Z8** treated with 300 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$). Treatment of **Z8** with 400 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$ leads to a lesser decrease in hyperchromicity. This trend is completely consistent with the CD and UV spectral changes shown in Figures 2 and 3. It is interesting to note that $\Delta T_m$ data ($\Delta T_m = T_{m,t} - T_{m,u}$, where $T_{m,t}$ and $T_{m,u}$ are the $T_m$ values for the treated and untreated **Z8** samples, respectively) generally parallel the hyperchromicity data. Increasing the incubation concentration of $[Co(NH_3)_5(OH_2)]^{3+}$ from 100 to 300 $\mu$M results in a decrease of the $T_m$ of the oligomer relative to untreated **Z8**, consistent with increasing modification of the oligomer. There is apparently a lesser degree of modification when **Z8** is incubated with 400 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$. As discussed below, this decrease in $T_m$ does not necessarily indicate a decrease in helix stability for the treated samples.

*Does the Incubation of* ***Z8*** *with* $[Co(NH_3)_5(OH_2)]^{3+}$ *Result in Binding of Cobalt(III) to the Oligomer?* Incubation of **Z8** in the presence of 200 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$ at 37 °C for times ranging from 8 to 72 h, followed by exhaustive dialysis, was carried out in order to assess any uptake of cobalt by the oligomer. In order to test for the presence of tightly bound cobalt, two different assays were carried out. A direct approach for detection of bound cobalt is AA (atomic absorption) analysis of the treated **Z8** samples. The results (Figure 5) indicate that, in general, the amount of tightly bound cobalt increases with increasing time of incubation and then levels off at reaction times greater than ca. 48 h. At the long reaction times, the binding ratio $R$ ($R$ = atoms of bound cobalt to DNA base pairs) is on the order of 0.25 or 1 cobalt bound for every 3.5−4 base pairs.

An indirect method for establishing binding of cobalt to the DNA oligomers is determination of the $T_m$ of a specifically treated sample as a function of [NaCl]. Here, untreated **Z8** and **Z8** heat annealed in the presence of 200 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$, followed by exhaustive dialysis, or incubated for 48 h with 200 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$, followed by exhaustive dialysis, were prepared in standard buffer at NaCl concentrations ranging from 125 to 400 mM and subjected to thermal denaturation. Plots of $T_m$ vs log[NaCl] for the three samples are shown in Figure 6. As can be seen, both treated DNA oligomers have lower $T_m$ values than the untreated oligomer at NaCl concentrations greater than 125 mM. The slopes of the least squares regression lines ($r^2 > 0.955$ for all regressions) for the treated samples are quite different from that of the untreated sample as well as quite different from each other. Hence, the treated samples have a dramatic decrease in the salt dependence on their melting temperatures relative to the untreated sample.

## DISCUSSION

The data presented here indicate that $[Co(NH_3)_5(OH_2)]^{3+}$ does not induce the B−Z transition in the alternating purine−pyrimidine oligomer **Z8** but apparently does modify the oligomer. The degree of modification depends upon the method of treatment, the concentration of the cobalt complex, and the length of incubation. The CD and UV spectral data presented in Figure 1 suggest that heat annealing **Z8** in the presence of $[Co(NH_3)_5(OH_2)]^{3+}$ modifies the oligomer to an altered B-like conformation. Since the heat annealing was carried out at 80 °C for 2 min, it is likely that most of the oligomer was single-stranded during that treatment. In order to investigate the interaction of $[Co(NH_3)_5(OH_2)]^{3+}$ with duplex DNA, the oligomer was incubated in the presence of the cobalt complex at 37 °C [the $T_m$ of **Z8** under the conditions used is about 70 °C (R. D. Sheardy and S. Marotta, unpublished results)]. As shown by the CD and UV difference spectra shown in Figures 2−4, such incubation of **Z8** in the presence of increasing concentrations of $[Co(NH_3)_5(OH_2)]^{3+}$ also leads to the modified B-like conformation even after exhaustive dialysis.

The data presented clearly demonstrate that the treated oligomer is conformationally distinct from the untreated oligomer in both the native (i.e., at 25 °C) and denatured (i.e., at 95 °C) states. The melting profiles indicate complete thermally induced transitions for both treated and untreated oligomers, as shown by establishment on an upper base line at temperatures greater than 88 °C (data not shown). However, the CD and UV data at 95 °C indicate that the treated samples are quite different from the untreated sample. Surprisingly, treatment of the oligomer at 400 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$ apparently results in a less modified oligomer than treatment at 300 $\mu$M cobalt complex as shown by the smaller changes in molar ellipticity, hyperchromicity upon denaturation, and $\Delta T_m$ values.

Incubation of $[Co(NH_3)_5(OH_2)]^{3+}$ in a variety of buffers or in water alone at 37 °C but in the absence of DNA slowly leads to degradation of the cobalt complex as shown by both UV−vis spectral changes for the complex and formation of a precipitate in the reaction eppendorf tube (D. M. Calderone and R. D. Sheardy, unpublished results). It is likely that the decomposition products are insoluble dimers and multimers of the cobalt complex (Cotton & Wilkinson, 1988). At low concentrations of $[Co(NH_3)_5(OH_2)]^{3+}$, the presence of the DNA oligomer actually inhibits this degradation since the formation of the precipitate is not observed under these conditions. However, at higher concentrations of $[Co(NH_3)_5(OH_2)]^{3+}$, there apparently is sufficient competition from self-reaction to reaction with the DNA oligomer. Thus, the smaller extent of cobalt modification of **Z8** at 400 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$ is apparently due its concentration dependent self-destruction resulting in a less effective concentration of cobalt complex for reaction with the DNA. It should be noted that the equilibrium constant for the reaction

$$[Co(NH_3)_5(OH_2)]^{3+} + Cl^- \rightleftharpoons [Co(NH_3)_5Cl]^{2+} + H_2O$$

is near unity at 25 °C (Bowser, 1993, and references cited therein). However, the large excess of $H_2O$ to $Cl^-$ (i.e., 55 M vs 50 mM, respectively) drives the equilibrium to the side of the aquated form. Hence, the predominant species responsible for the modification of the DNA oligomer is $[Co(NH_3)_5(OH_2)]^{3+}$.

Table 1: Influence of NACl on the Thermal Denaturations of Untreated and Treated **Z8**[a]

| sample | $T_m$ (°C) | $\Delta H°$ (kcal/mol) | $\delta T_m/\delta$ log[NaCl] | $\Delta n$ |
|---|---|---|---|---|
| untreated **Z8** | 69.6 ± 0.4 | 45.2 ± 2.3 | 10.93 | 0.92 |
| treated **Z8**, heat annealed | 67.7 ± 0.4 | 48.0 ± 2.4 | 5.36 | 0.48 |
| treated **Z8**, incubated | 68.5 ± 0.4 | 45.0 ± 2.2 | 3.30 | 0.28 |

[a] Values determined for **Z8** in 200 mM NaCl.

The data presented in Figure 5 indicate a slow uptake of cobalt by **Z8** at reaction times less than 30 h, with a leveling off in the uptake at longer reaction times. This leveling off may be due to the degradation of the cobalt complex after the longer reaction times or due to completed reaction of the cobalt complex with the DNA oligomer. Since the degradation of the cobalt complex is apparently inhibited in the presence of DNA (as noted above), it is possible that the oligomer is saturated with cobalt at 1 cobalt for every 3.5−4 base pairs after ca. 48 h of reaction. In such a circumstance, the binding of 1 cobalt would result in a "site exclusion" effect such as noted for simple intercalators such as daunomycin with a site size of 2.8−3.6 base pairs (Chaires et al., 1987). Finally, the observation that all melting profiles for the data presented in Figures 4 are reversible also suggests that the cobalt is tightly bound.

Due to the lability of the aquo group, it is likely that coordination of the cobalt to sites on the DNA is occurring, with concomitant loss of water from the cobalt. The question naturally arises as to the site of cobalt binding. Binding to phosphate is ruled out due to the lability of the phosphate−cobalt bond (Cotton & Wikinson, 1988). The most likely site is N7 of guanine bases in **Z8**, as observed in the reaction of calf thymus DNA with $[Ru(NH_3)_5(OH_2)]^{2+}$ (Clark et al., 1986) and the binding of Co(II) to tRNA (Jack et al., 1977). In order to address the question of base or sequence specificity in the binding of the cobalt to DNA, we have synthesized a series of DNA oligomers with single G, GG, GC, or CG sites. Preliminary results, to be presented elsewhere, indicate that $[Co(NH_3)_5(OH_2)]^{3+}$ displays a sequence specificity with enhanced binding to those oligomers with the single GG and GC sites (R. D. Sheardy, M. Hicks, D. Calderone, C. Villella, G. Wharton, III, D. H. Huchital, and W. R. Murphy, Jr., manuscript in preparation, 1995).

The lower $T_m$ values for the modified oligomers arising from the incubation of **Z8** with increasing concentrations of $[Co(NH_3)_5(OH_2)]^{3+}$ suggest that they are less stable than the unmodified oligomer. In order to assess the thermodynamic stabilities of the untreated and treated **Z8** samples, curve analysis of the melting profiles was carried out according to Marky and Breslauer (1987). Assuming a two-state transition, the enthalpy change for the melting of a slef-complementary DNA oligomer can be determined:

$$\Delta H° = -6RT_m^2(\delta\alpha/\delta T)_{T=T_m} \quad (1)$$

where $\Delta H°$ is the standard enthalpy change, $T_m$ is the inflection point of the $\alpha$ vs $T$ melting profile, and $\alpha$ is the fraction of single strands. Enthalpic comparisons of untreated **Z8** and **Z8** heat annealed or incubated in the presence of 200 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$ followed by exhaustive dialysis are revealed in Table 1. At 200 mM NaCl, the different samples have unique $T_m$ values, but the enthalpies

of thermal denaturations are quite similar. Hence, the $T_m$ values alone cannot be used to assess thermodynamic stability.

The coordination of cobalt(III) metal to the DNA substrate would dramatically alter the charge density of the oligomer. According to the terms of the polyelectrolyte theories (Manning, 1978; Record et al., 1978, 1981), this alteration should thus change the number of sodium counterions bound to the lattice. At low concentrations of NaCl (less than 400 mM), there is a linear relationship between $T_m$ and log[NaCl]; the slope of the resultant line reflects the linkage between $Na^+$ binding and the duplex to single strand transition. Record et al. (1978) have shown that

$$\delta T_m/\delta \log[\mathrm{NaCl}] = (2.303RT_m^2/\Delta H^\circ)\Delta n \qquad (2)$$

where $\Delta n$ is the differential ion binding term. The $\Delta n$ term represents the release of counterions upon denaturation (i.e., double-stranded DNA has a higher charge density than single-stranded DNA). Using the experimentally determined slopes for untreated **Z8** and **Z8** heat annealed or incubated in the presence of 200 $\mu$M $[Co(NH_3)_5(OH_2)]^{3+}$, the values of $T_m$, and the values of $\Delta H^\circ$ at 200 mM NaCl in Table 1, $\Delta n$ values were determined and tabulated in the last column of Table 1. As can be seen, there is a dramatic decrease in the differential ion binding term ($\Delta n$) for both treated DNA oligomers. For untreated **Z8**, $\Delta n = 0.92$, indicating that 0.92 Na ions are released per 8 base pairs of duplex upon melting. This value corresponds to 0.066 Na ions released per phosphate. In contrast, only 0.48 and 0.28 $Na^+$ per duplex (i.e., 0.034 Na ions per phosphate and 0.020 Na ions per phosphate) are released, respectively, for **Z8** heat annealed or incubated in the presence of $[Co(NH_3)_5(OH_2)]^{3+}$. These data also point to tightly bound cobalt.

Tajmir-Riahi et al. (1993) observed direct metal−base binding via IR spectroscopy when calf thymus DNA was treated with cobalt pentammine at high cobalt/base pair ratios. The results presented here strongly suggest that $[Co(NH_3)_5(OH_2)]^{3+}$ binds to DNA in a manner consistent with coordination to the cobalt metal center with concomitant loss of the labile aquo group. Since such coordination would most likely involve N7 of guanine, the cobalt center would reside in the major groove where hydrogen bonding from the ammines to the DNA would be favorable (Seeman et al., 1976). Although $[Co(NH_3)_5(OH_2)]^{3+}$ is octahedral, the ammines occupying the positions above and below the plane occupied by the leaving group should not interfere with the interaction due to their small van der Waals radii. In fact, the binding may even be enhanced by their presence due to possible hydrogen-bonding interactions between these ammines and acceptor sites on the nucleic acid backbone as described for the binding of $[Co(NH_3)_6]^{3+}$ to $(dC\text{-}dG)_3$ (Gessner et al., 1985).

The interaction of $[Co(NH_3)_5(OH_2)]^{3+}$ with **Z8** modifies the conformational properties of the DNA oligomer at both 25 and 95 °C. At high concentrations of $[Co(NH_3)_5(OH_2)]^{3+}$ and long reaction times, the modification is consistent with coordination of the cobalt to GN7 via loss of the aquo group. The results are also consistent with interstrand cross-linking involving the GC sites such as observed with *cis*-platin (Hopkins et al., 1992), and it is tempting to suggest that. However, more experimental evidence is mandated to confirm any cross-linking. These data do indicate that simple cobalt(III) complexes possessing labile groups may have surprisingly interesting DNA binding properties. In order to delineate the nature of the adducts formed, we are currently investigating the interaction specificities of $[Co(NH_3)_5(OH_2)]^{3+}$ and other analogues to a variety of synthetic DNA oligomers.

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