^{1}H NMR Study of the Enantioselective Binding of the Tris(ethylenediamine)cobalt(III) Cation with the Dodecanucleotide d(CAATCCGGATTG)$_2$

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Coordinatively saturated transition metal ion complexes, which can only bind to DNA through weak noncovalent interactions, have been shown to be excellent probes for differentiating DNA conformations and sequence-specific microheterogeneity in the duplex.[1] An area of particular interest is the enantiomeric selectivity that some of these chiral metal ion complexes show in their binding to DNA.[2-5] The specific structural information gained from these studies is useful for the design of sequence- or structure-specific binding drugs.

Chiral, octahedral transition metal ion complexes of intercalating ligands such as 1,10-phenanthroline can bind to DNA in an enantiomerically selective fashion.[2-5] However, the question of enantioselective DNA-binding by complexes of *non*intercalating ligands is not yet resolved. While small, chiral metal complexes that can form multiple hydrogen bonds with the DNA have the potential to be enantioselective in their binding, $Co(en)_3^{3+}$ has been reported to show no enantiomeric selectivity in its binding to calf thymus DNA.[6] On the other hand, the Λ-enantiomer has been shown to bind preferentially to the mononucleotide guanosine 5′-monophosphate.[7] The wide range of microstructures in heterogeneous calf thymus DNA may have masked any sequence- or structure-specific binding of one enantiomer of $Co(en)_3^{3+}$ over the other and produced an overall binding average. We have therefore examined, using ^{1}H NMR spectroscopy, the binding of both enantiomers of $Co(en)_3^{3+}$ to the dodecanucleotide d(CAATCCGGATTG)$_2$, a small segment of B-type DNA that contains several guanosine residues in the central region of the duplex.

Experimental Section

Materials. The dodecanucleotide d(CAATCCGGATTG)$_2$ and Sephadex G-15 gel-filtration resin were obtained from Pharmacia P-L Biochemicals. The cation-exchange resin AG 50W-X8 was purchased from BioRad and the D_2O (99.96 %D) was obtained from Aldrich Chemical Co. The resolved Δ- and Λ- enantiomers of $Co(en)_3Cl_3$ were gifts from Dr. Rodney Geue and Prof. Alan Sargeson, Research School of Chemistry, Australian National University. All other reagents used were of analytical grade.

Sample Preparation. The dodecanucleotide (1–2 μmol) was dissolved in 0.7 mL of phosphate buffer (10 mM, pH 7) containing 100 mM NaCl, 1 mM EDTA, and DSS as an internal chemical shift reference. Aliquots of stock solutions of the respective enantiomers of $Co(en)_3^{3+}$ were titrated directly into the NMR tube. The metal complexes were removed from the dodecanucleotide by repeated batch treatment of the bound $Co(en)_3^{3+}$-dodecanucleotide mixture with cation exchange resin at 40 °C. Low molecular weight compounds including NaCl were removed from the nucleotide by gel-filtration using Sephadex G-15 (1 cm × 28 cm column).

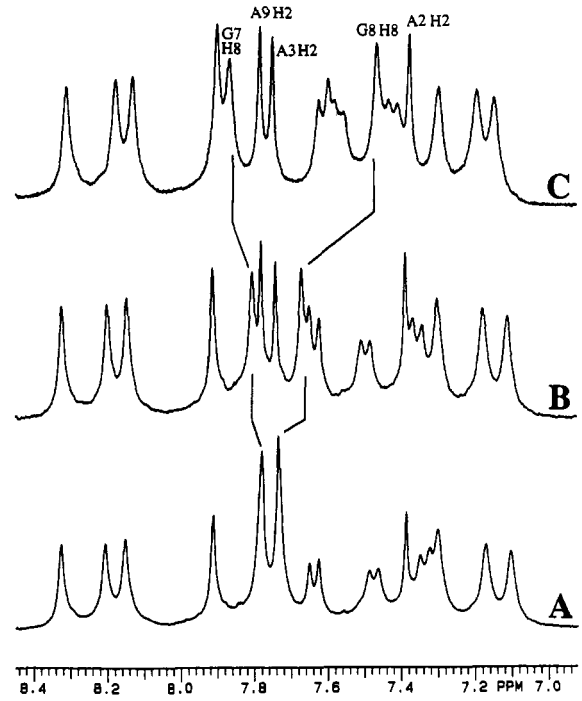

Figure 1. ^{1}H NMR spectrum of the aromatic base protons of d(CAATCCGGATTG)$_2$ at Δ-$Co(en)_3^{3+}$ to nucleotide ratios of (A) 0, (B) 1.0, and (C) 7.0.

Instrumental Methods. ^{1}H NMR spectra were recorded at 25 °C on a Varian XL-300 spectrometer operating at 300 MHz. Two-dimensional phase-sensitive NOESY spectra were recorded by the method of States et al.,[8] with a pulse repetition delay of 1.9 s and a mixing time of 250 ms. The dodecanucleotide concentration was determined from the manufacturer's specific absorbance A_{260} = 18.1 absorbance units per 1 cm pathlength per milligram of nucleotide per milliliter.

Determination of Binding Constants. We have estimated the relative binding affinities of the $Co(en)_3^{3+}$ enantiomers by fitting the NMR data to a simple M + DNA = M–DNA binding model. The model assumes that the chemical shift changes are caused exclusively by the binding of one metal complex in the immediate vicinity of the proton being monitored. A more elaborate analysis of the binding parameters would be possible if a detailed picture of the binding stoichiometry and binding site size (base-pair exclusion zone) were known. Binding constants were determined from the ^{1}H NMR spectra by following the exchange-averaged chemical shifts of the aromatic base protons of the dodecanucleotide as a function of added $Co(en)_3^{3+}$. This data was fitted to the binding isotherm by nonlinear least-squares methods, using our implementation of the program KINET.[9]

Results and Discussion

The assignment of the nonexchangeable proton resonances and solution conformation of the dodecanucleotide have been previously reported.[10] Figure 1 shows the aromatic base proton region of the ^{1}H NMR spectrum of d(CAATCCGGATTG)$_2$ at various ratios, R, of Δ-$Co(en)_3^{3+}$ to the dodecamer. Only one set of resonances was observed at all ratios (R = 0–11) indicating fast exchange, on the NMR time scale, between the free and bound forms. Addition of Λ-$Co(en)_3^{3+}$ to the dodecanucleotide produces a different pattern of chemical shift movements, shown in Figure 2, indicating different enantiomeric binding to the dodecanucleotide. This dissimilar chemical shift behavior shown by the two enantiomers could result from a difference in binding mode and/or a difference in binding constant. The chemical shift movements of the central 5′-CCGG-3′ section were sufficiently

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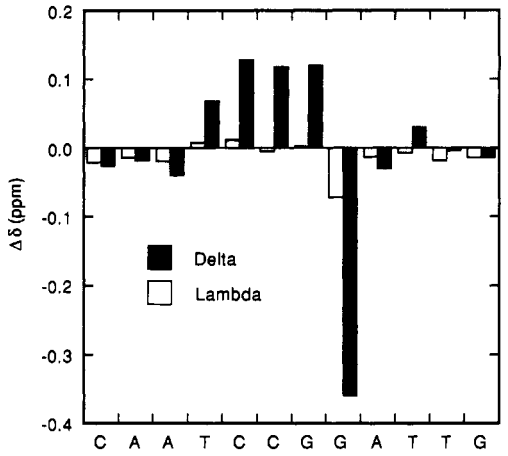

Figure 2. Chemical shift movements (at metal complex: nucleotide ratio, $R = 11$) of the base H8 and H6 protons of d(CAATCCGGATTG)$_2$, caused by addition of Δ- and Λ-Co(en)$_3^{3+}$. The individual bases (C1–G12) are listed on the horizontal axis.

large to permit their fitting by nonlinear least-squares methods. Estimates of the binding constants for the interaction of the dodecamer with the enantiomers of Co(en)$_3^{3+}$ are 1000 ± 500 M^{-1} (Δ) and 100 ± 50 M^{-1} (Λ).

Competitive binding experiments confirmed the stronger binding of the Δ-enantiomer. The Δ-isomer produces a much larger upfield chemical shift movement of the G8–H8 proton resonance than does the Λ-isomer. The Δ-form is not displaced significantly from the nucleotide by addition of a large excess of Λ-isomer. For example, the 0.169 ppm upfield chemical shift change of the G8–H8 resonance, observed in the presence of the Δ-isomer at $R = 3$, was only minimally reduced (0.008 ppm) by the addition of a 5-fold excess of the Λ-form. On the other hand, addition of an equimolar quantity of the Δ-form to a solution containing the nucleotide and the Λ-form results in the same chemical shift that is observed in the presence of the Δ-isomer alone.

Addition of the Δ-enantiomer to the dodecanucleotide results in larger chemical shift changes for resonances due to the H8 and H6 protons of the central 5′-CCGG-3′ region than H8/H6 protons of bases near the ends of the duplex. These protons are found in the major groove of the duplex.[11] Furthermore, while the A–H8 protons exhibit a relatively small chemical shift movement on addition of the Δ-enantiomer, the A–H2 protons that are located in the minor groove show an even smaller chemical shift change. The A–H2 protons are normally very sensitive to metal ion complex or drug binding in the minor groove.[12,13] We conclude that Δ-Co(en)$_3^{3+}$ binds in the major groove, near the center of the nucleotide.

NOESY experiments were performed to obtain the chemical shift movements of the resonances due to the dodecanucleotide sugar protons. In a similar fashion to the base H8/H6 resonances, the sugar H1′, H2′, and H2″ resonances that exhibited the largest movements belong to the central 5′-CCGG-3′ segment.

In a right-handed nucleotide helix, the aromatic H8 and H6 protons exhibit an NOE to their own H1′, H2′, and H2″ sugar

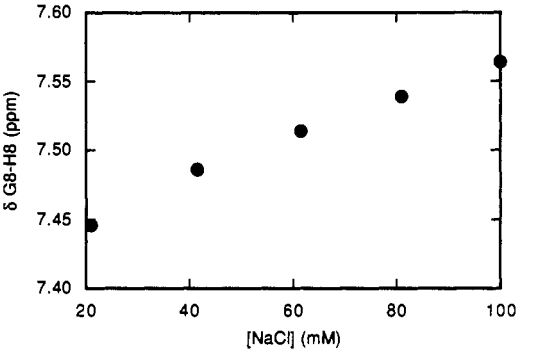

Figure 3. Chemical shift of the nucleotide G8–H8 proton, in the presence of Δ-Co(en)$_3^{3+}$ ($R = 3$), as a function of added NaCl.

protons as well as to the H1′, H2′, and H2″ protons of the flanking 5′-sugar, but not to the corresponding protons of the adjacent 3′-sugar.[14,15] In a standard B-type duplex, the distance between the base H8/H6 proton to its own H2′ is approximately 2 Å and is approximately 4 Å to the H2′ proton on the flanking 5′ sugar.[14,15] For an A-type helix the opposite is found, with the shorter distance being to the H2′ proton of the 5′ sugar.[14,16] Analysis of the NOESY connectivities between the base H8/H6 and sugar H-2′/H-2″ shows that the Co(en)$_3^{3+}$–d(CAATCCGGATTG)$_2$ complex has maintained the basic B-type conformation, with possible minor conformational changes in the central segment.

In the presence of the Δ-Co(en)$_3^{3+}$, the chemical shifts of the protons in the central region of the nucleotide vary significantly with the concentration of added NaCl, over the range 20–100 mM, as shown in Figure 3. In the absence of the metal complex, the chemical shifts of the base protons of the nucleotide are essentially independent of NaCl concentration over this range. The chemical shift changes in the presence of metal complex were in the direction of the free nucleotide, as the concentration of added NaCl was increased, indicating that the binding constant decreases with increasing NaCl concentration. This inverse dependence on the ionic strength suggests that electrostatic interactions contribute significantly toward the stability of the Co(en)$_3^{3+}$-d(CAATCCGGATTG)$_2$ complex.

While electrostatic interactions may be predominantly responsible for the stabilization of the complex, these nonspecific forces are unlikely to be responsible for the observed enantiomeric selectivity. Two important factors that may be responsible for selectivity are the ability to form multiple hydrogen bonds from coordinated amines to the nucleotide and the shape selectivity caused by van der Waals interactions with the walls of the major groove. Complementary molecular modeling studies, currently underway, may resolve these issues.

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