NMR Study of the Sequence-Specific Binding of the Δ-Tris(ethylenediamine)cobalt(III) Cation with d(TCGGGATCCCGA)$_2$

John V. Fry and J. Grant Collins*

School of Chemistry, University College,
University of New South Wales,
Australian Defence Force Academy,
Canberra 2600, Australia

Received August 2, 1996

Introduction

There has been considerable interest in the interactions between coordinatively saturated transition metal ion complexes and DNA, as the study of these interactions can provide information on DNA structure and recognition.[1,2] To date, most work has centered on metal complexes of intercalating ligands.[3−10] However, recent studies have shown that simple metal complexes of nonintercalating ligands, such as NH_3 and ethylenediamine, can also be used to probe the basis of DNA-sequence-specific binding and conformational transitions.[11−17] Braunlin and coworkers showed that $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$ can specifically bind DNA molecules that contain runs of two or more guanine residues and induce a conformational transition toward A-type DNA,[11−13] while, more recently, Robinson and Wang used NOE measurements to show that the B to A conformational change is most likely due to the metal complex binding to the guanine bases in the major groove.[17] However, in these studies and ^{1}H NMR studies from our laboratory on the binding of $Co(en)_3^{3+}$ and related complexes to oligonucleotides,[14−16] it has not been possible to unambiguously show that the metal complexes selectively bind GG sequences. As electrostatic interactions between the metal ion and the polyanion oligonucleotide appear to dominate binding,[15] although specific recognition may occur along with these electrostatic interactions, we sought to examine the binding of Δ-$Co(en)_3^{3+}$ to an oligonucleotide containing a GGG sequence. A GGG sequence has been shown to have the greatest negative electrostatic potential of all DNA base triplets.[18] In this study, we report the binding of Δ-$Co(en)_3^{3+}$ to a dodecanucleotide containing the GGG sequence d($T_1C_2G_3G_4G_5A_6T_7C_8C_9C_{10}G_{11}A_{12}$)$_2$. Specific NOE cross peaks between the G_3 and G_4 H8 protons and the metal complex methylene protons are observed, which unambiguously shows that Δ-$Co(en)_3^{3+}$ selectively binds GG sequences in the major groove. Interestingly, no oligonucleotide conformational transition toward A-type DNA was observed.

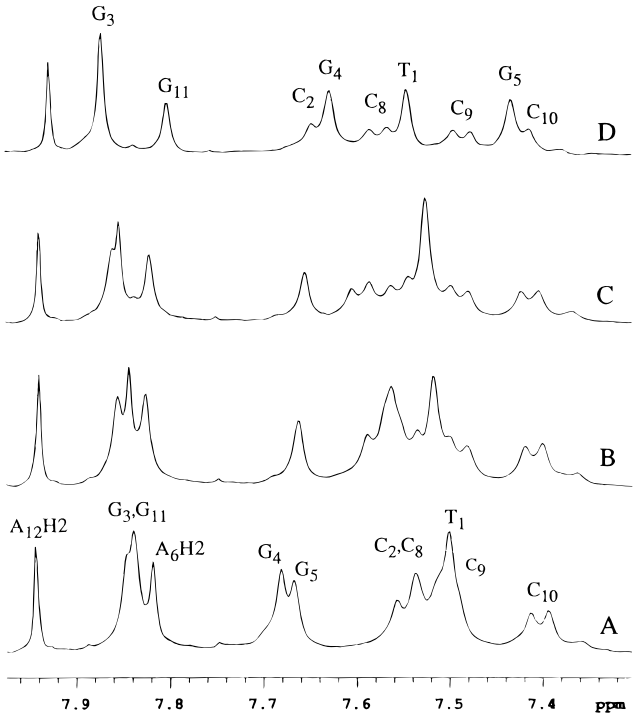

Figure 1. Parts of the aromatic base proton regions of the ^{1}H NMR spectra of d(TCGGGATCCCGA)$_2$ (1.1 mM) at Δ-$Co(en)_3^{3+}$ to dodecanucleotide ratios of (A) 0, (B) 0.3, (C) 0.6, and (D) 1.5 in 10 mM phosphate buffer (pH 7) containing 20 mM NaCl at 25 °C.

Experimental Section

Materials. The dodecanucleotide d(TCGGGATCCCGA)$_2$ was obtained from Bresatec Ltd. The resolved Δ-enantiomer of $Co(en)_3Cl_3$ was a gift from Dr. Rodney Geue and Prof. Alan Sargeson, Research School of Chemistry, Australian National University. D_2O (99.96% D) was obtained from Aldrich Chemical Co., while all other reagents used were of analytical grade.

Sample Preparation. The dodecanucleotide (0.7 μmol) was dissolved in 0.65 mL of phosphate buffer (10 mM, pH 7) containing 20 mM NaCl, 0.5 mM EDTA, and a trace of DSS as an internal chemical shift reference. The dodecanucleotide concentration was determined from the A_{260} absorbance using an extinction coefficient of 6600 M^{-1} cm^{-1} per nucleotide. Aliquots of stock solutions of Δ-$Co(en)_3^{3+}$ were titrated directly into the NMR tube.

Instrumental Methods. ^{1}H NMR spectra were recorded at 25 °C on a Varian Unity*plus* 400 spectrometer operating at 400 MHz. One-dimensional spectra recorded in 90% H_2O/10% D_2O were collected using the WATERGATE solvent suppression technique of Piotto et al.[19] Two-dimensional phase-sensitive NOESY (100 and 250 ms mixing times) and DQFCOSY spectra were accumulated using 2048 data points in t_2 for 256−310 t_1 values with a pulse repetition delay of 1.7 s.

Results and Discussion

Parts of the aromatic regions of the NMR spectra of d(TCGGGATCCCGA)$_2$ at various ratios of added Δ-$Co(en)_3^{3+}$ are shown in Figure 1. The G_5H8 resonance displays a large

(1) Pyle, A. M.; Barton, J. K. In *Progress in Inorganic Chemistry*; Lippard, S. J., Ed.; Wiley Interscience: New York, 1990; Vol. 38, p 413.
(2) Sigman, D. S.; Mazumder, A.; Perrin, D. M. *Chem. Rev.* **1993**, *93*, 2295.
(3) Nordén, B.; Tjerneld, F. *FEBS Lett.* **1976**, *67*, 368.
(4) Barton, J. K.; Danishefsky, A. T.; Goldberg, J. M. *J. Am. Chem. Soc.* **1984**, *106*, 2172.
(5) Pyle, A. M.; Rehmann, J. P.; Meshoyer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1989**, *111*, 3051.
(6) Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. *Biochemistry* **1993**, *32*, 2573.
(7) Hudson, B. P.; Dupureur, C. M.; Barton, J. K. *J. Am. Chem. Soc.* **1995**, *117*, 9379.
(8) Terbrueggen, R. H.; Barton, J. K. *Biochemistry* **1995**, *34*, 8227.
(9) Eriksson, M.; Leijon, M.; Hiort, C.; Nordén, B.; Graslund, A. *Biochemistry* **1994**, *33*, 5031.
(10) Lincoln, P.; Broo, A.; Nordén, B. *J. Am. Chem. Soc.* **1996**, *118*, 2644.
(11) Xu, Q.; Shoemaker, R. K.; Braunlin, W. H. *Biophys. J.* **1993**, *65*, 1039.
(12) Xu, Q.; Jampani, S. R. B.; Braunlin, W. H. *Biochemistry* **1993**, *32*, 11754.
(13) Xu, Q.; Jampani, S. R. B.; Deng, H.; Braunlin, W. H. *Biochemistry* **1995**, *34*, 14059.
(14) Watt, T. A.; Collins, J. G.; Arnold, A. P. *Inorg. Chem.* **1994**, *33*, 609.
(15) Svensson, B.; Woodward, C. E.; Arnold, A. P.; Collins, J. G.; Lafitani, J. *J. Phys. Chem.* **1995**, *99*, 10412.
(16) Watt, T. A.; Tong, C.; Arnold, A. P.; Collins, J. G. *Biochem. Mol. Biol., Int.* **1996**, *38*, 383.
(17) Robinson, H.; Wang, A. H.-J. *Nucleic Acids Res.* **1996**, *24*, 676.
(18) Pullman, A.; Pullman, B. *Q. Rev. Biophys.* **1981**, *14*, 289.
(19) Piotto, M.; Saudek, V.; Sklenar, V. *J. Biomol. NMR* **1992**, *2*, 661.

S0020-1669(96)00937-8 CCC: $14.00

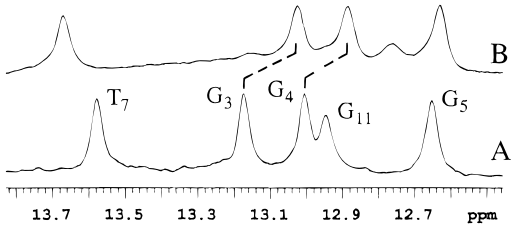

Figure 2. ^{1}H NMR spectra of the imino protons of (A) d(TCGGGATCCCGA)$_2$ (1.1 mM) and (B) d(TCGGGATCCCGA)$_2$ with added Δ-Co(en)$_3^{3+}$, at a metal complex to dodecanucleotide ratio of 4, in 90% H_2O/10% D_2O 10 mM phosphate buffer (pH 7) containing 20 mM NaCl at 17 °C.

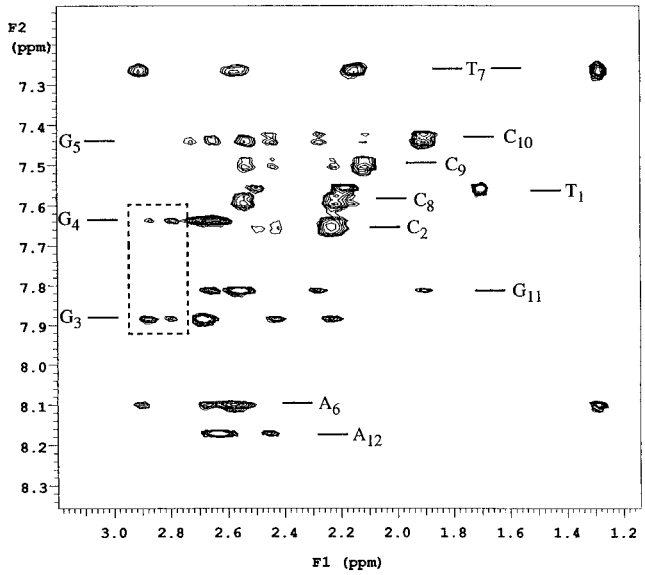

Figure 3. Expansion of the NOESY spectrum (100 ms mixing time) of d(TCGGGATCCCGA)$_2$, with added Δ-Co(en)$_3^{3+}$, at a metal complex to dodecanucleotide ratio of 1.5, in 10 mM phosphate buffer (pH 7) containing 20 mM NaCl at 25 °C. The expansion shows the aromatic base (7.3−8.3 ppm) to sugar H2′/H2″, T-methyl, and Δ-Co(en)$_3^{3+}$ methylene proton region (1.2−3.0 ppm). The cross peaks inside the dashed box show the NOEs between the H8 of G_3 and G_4 and the Δ-Co(en)$_3^{3+}$ methylene protons (2.79 and 2.88 ppm).

upfield shift (0.24 ppm) upon addition of the metal complex, while the G_3H8 and G_4H8 resonances show only relatively small chemical shift movements (<0.05 ppm). The base-paired cytosines (C_8, C_9, and C_{10}) also only show relatively small chemical shift movements. The imino resonances in the NMR spectrum of the dodecanucleotide collected in 90% H_2O/10% D_2O were examined to determine the extent of the base pairing before and after the addition of Δ-Co(en)$_3^{3+}$. Figure 2 shows the imino resonances of the free and metal complex bound dodecanucleotide at 17 °C. In each spectrum, five resonances are observed, indicating that only the terminal residue does not form a stable base pair at 17 °C. However, in the spectrum of the metal complex bound dodecanucleotide, the less intense, relatively broader imino resonance from the C_2·G_{11} base pair indicates that the penultimate nucleotide residue exhibits significant fraying.

Figure 3 shows an expansion of a 100 ms mixing time NOESY spectrum of d(TCGGGATCCCGA)$_2$ with added Δ-Co(en)$_3^{3+}$ at a metal complex to dodecanucleotide ratio of 1.5. In this spectrum NOEs are observed between the base H8/H6 protons and the H2′/H2″ protons from their own and 5′-deoxyribose rings, consistent with a right-handed oligonucleotide duplex.[20−22] In addition, NOE cross peaks of medium intensity between the methylene protons of the metal complex and the H8 of both G_3 and G_4 are also observed. No NOE cross peak

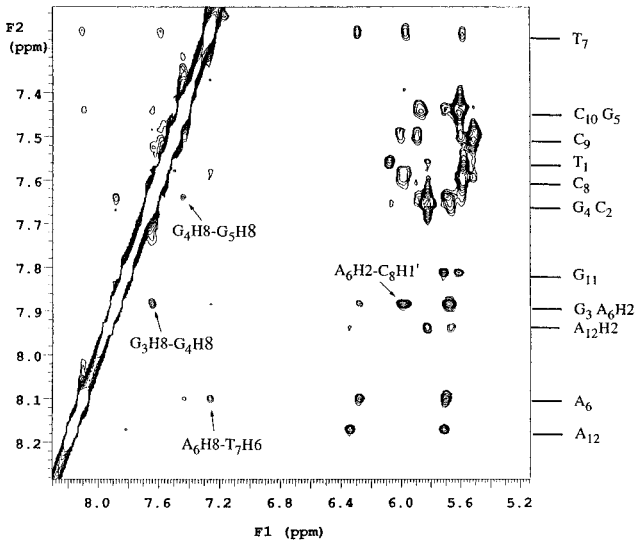

Figure 4. Expansion of the NOESY spectrum (100 ms mixing time) of d(TCGGGATCCCGA)$_2$ (1.1 mM), with added Δ-Co(en)$_3^{3+}$, at a metal complex to dodecanucleotide ratio of 1.5, in 10 mM phosphate buffer (pH 7) containing 20 mM NaCl at 25 °C. The expansion shows the aromatic base (7.3−8.2 ppm) to aromatic base and sugar H1′ region (5.2−8.2 ppm).

from the Δ-Co(en)$_3^{3+}$ to any other H8/H6 proton was observed, even at a metal complex to duplex ratio of 4. Because the H8 protons of guanine residues are located in the dodecanucleotide major groove, these results indicate that the metal complex selectively binds in the major groove at the G_3G_4 sequence. In support of this, an NOE cross peak was also observed between the methylene protons of the metal complex and the coincident G_3 and G_4 H3′ resonances. This study provides the first unambiguous evidence that complexes such as Δ-Co(en)$_3^{3+}$ selectively target GG sequences in the major groove of the oligonucleotide.

In contrast to previous studies,[11−13,17] analysis of the relative NOE cross peak intensities demonstrated that the metal complex bound dodecanucleotide adopts a B-type conformation in solution. First, the NOE cross peak intensity from each base H8/H6 to its own sugar protons decreases in the order H2′ > H1′ > H3′, and second, the NOE from each H8/H6 to its own H2′ was larger than that to the H2′ proton on the 5′-sugar.[20−22] Additionally, in the DQFCOSY spectrum of the dodecanucleotide with added Δ-Co(en)$_3^{3+}$, strong H1′−H2′ cross peaks were observed for all clearly resolvable sugar residues, consistent with the 2′-endo sugar pucker of B-type DNA.[23] These results indicate that metal complexes such as Δ-Co(en)$_3^{3+}$ can selectively bind GG sequences in B-type DNA. However, a number of NOE cross peaks in the NOESY spectra suggest that Δ-Co(en)$_3^{3+}$ binding has induced some sequence-dependent structural changes that are superimposed on the standard B-type DNA. For example, a particularly strong NOE between the A_6 H2 and C_8H1′ (Figure 4) suggests significant propeller twisting of the C_8·G_5 and T_7·A_6 base pairs.

It is not possible to generate a detailed description of the Δ-Co(en)$_3^{3+}$−d(TCGGGATCCCG)$_2$ binding from the NOE data presented here because specific individual protons on the metal complex cannot be assigned. It has been postulated that

(20) Scheek, R. M.; Boelens, R.; Russo, N.; van Boom, J. H.; Kaptein, R. *Biochemistry* **1984**, *23*, 1371.
(21) Feigon, J.; Leupin, W.; Denny, W. A.; Kearns, D. R. *Biochemistry* **1983,** *22*, 5943.
(22) Patel, D. J.; Shapiro, L.; Hare, D. *J. Biol. Chem.* **1986,** *261*, 1223.
(23) Zhou, N.; Manogaran, S.; Zon, G.; James, T. L. *Biochemistry* **1988**, *27*, 6013.

$Co(NH_3)_6^{3+}$ binds GG sequences in A-type DNA through specific hydrogen bonds from the metal complex to the N7 and O6 groups of adjacent guanines.[11,12,17] The NOE results presented here are consistent with this model. In canonical B-type DNA, the major groove is too wide to allow simultaneous contact of the metal complex with the N7 and O6 groups of adjacent guanines.[13] However, a relatively strong NOE between the G_3H8 and G_4H8 was observed (see Figure 4). This NOE interaction, which is considerably stronger than the equivalent cross peak in the free dodecanucleotide, indicates a reduced G_3H8−G_4H8 distance. This drawing together of the G_3 and G_4 bases would then allow the formation of the hydrogen bonds from the metal complex to the adjacent guanines, consistent with the proposed model.

The observed intermolecular NOE cross peaks indicate that the cobalt(III) complex binds the G_3G_4 sequence in d(TCGGGATCCCGA)$_2$ with significantly greater selectivity than was previously found for the G_7G_8 sequence in d(CAATCCGGATTG)$_2$.[16] What is the basis for this increased binding selectivity? It has been shown that the GGG sequence has the greatest negative electrostatic potential of any three-base DNA sequence,[18] with the central G being the most negative followed by the 5′-G and last the 3′-G.[24] The Δ-$Co(en)_3^{3+}$ complex was shown to bind selectively at the G_3G_4 bases, in agreement with the negative electrostatic potential ordering. This may suggest that the increased binding selectivity is simply due to the increased electrostatic interactions. This proposal is consistent with recent studies which have shown that simple metal cations, for example Mn^{2+} and Zn^{2+}, selectively bind oligonucleotides at the sites of greatest negative electrostatic potential, such as GG and GGG sequences.[24−26] Alternatively, the increased selectivity could be due to a dodecanucleotide conformational change that increases the binding affinity at the G_3G_4 binding site. The NMR spectra of d(TCGGGATCCCGA)$_2$ with added metal complex indicates that although the dodecanucleotide maintains the basic B-type conformation, the Δ-$Co(en)_3^{3+}$ binding does induce some conformational changes in the central part of the dodecanucleotide. For example, the guanine residue that exhibited the largest H8 chemical shift movement on addition of Δ-$Co(en)_3^{3+}$ was the G_5 not the G_3G_4 metal complex binding site. Furthermore, the NOESY data suggest that the C_8·G_5 and T_7·A_6 base pairs are significantly propeller-twisted while the G_3 and G_4 bases are drawn closer together upon the binding of the metal complex.

IC960937U

(24) Jia, X.; Zon, G.; Marzilli, L. G. *Inorg. Chem.* **1991**, *30*, 228.
(25) Froystein, N. A.; Sletten, E. *Acta Chem. Scand.* **1991**, *45*, 219.
(26) Froystein, N. A.; Davis, J. T.; Reid, B. R.; Sletten, E. *Acta Chem. Scand.* **1993**, *47*, 649.