NMR Evidence for Sequence-Specific DNA Minor Groove Binding by Bis(ethylenediamine)platinum(II)

Craig A. Franklin, 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 April 30, 1996[⊗]

^{1}H NMR spectroscopy was used to study the binding of $Pt(en)_2^{2+}$ to the dodecanucleotides $d(CAATCCGGATTG)_2$ and $d(TCGGGATCCCGA)_2$. Addition of $Pt(en)_2^{2+}$ to $d(CAATCCGGATTG)_2$ induced a significant and selective change in the chemical shift of the A_3H2 resonance but only small shifts for the major groove base resonances. Similarly, addition of $Pt(en)_2^{2+}$ to $d(TCGGGATCCCGA)_2$ induced a selective change in the chemical shift of the A_6H2 resonance. In the NOESY spectrum of $d(CAATCCGGATTG)_2$ with added $Pt(en)_2^{2+}$ intermolecular NOE cross-peaks between the methylene protons of the metal complex and the H2 of A_3 and A_9, as well as the H1′ of T_4, T_{10}, and T_{11} were observed. Importantly, no NOE cross-peaks from the metal complex to either major groove protons or guanine and cytosine protons were observed. In the NOESY spectrum of $d(TCGGGATCCCGA)_2$ with added $Pt(en)_2^{2+}$ intermolecular NOE cross-peaks from the metal complex were only observed to the H2 and H1′ protons of A_6 and the T_7H1′ proton. The results demonstrate that the square planar metal complex $Pt(en)_2^{2+}$ selectively binds AT sequences in the DNA minor groove.

Introduction

There has been considerable interest in the noncovalent binding ability of transition metal complexes with DNA, as the study of these interactions can increase our understanding of the principles of DNA recognition.[1−10] The rigid geometry of the transition metal complexes provides a defined scaffold for the addition of organic ligands that can control site selective binding.[1,2,8,10] While most investigations have focused on intercalating metal complexes, recent studies have shown that coordinatively saturated octahedral metal complexes of nonintercalating ligands also have the ability to selectively recognize specific DNA sequences and conformations.[4,5] Simple amine complexes such as $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$ bind in the major groove of DNA in both a sequence and DNA structure selective manner.[4,5,9] Furthermore, these nonintercalating complexes are capable of inducing DNA conformational transitions, from B-type DNA to either Z- or A-type DNA, depending upon the DNA base sequence.[5,11]

One aspect that has emerged from the study of intercalating and nonintercalating metal complex−DNA binding is the importance of shape selection, that is, matching the shape and symmetry of the metal complex with that of the DNA binding site.[1] As octahedral metal complexes have been predominantly utilized in DNA binding studies, we sought to examine the effect of changing the shape of the coordination geometry of the metal complex. In this paper we report an examination of the DNA binding of the square planar metal complex $Pt(en)_2^{2+}$. It has been proposed that $Co(NH_3)_6^{3+}$ and Δ-$Co(en)_3^{3+}$ can selectively interact with DNA by forming hydrogen bonds with the N7 and O6 groups of two adjacent guanines in the major groove.[4,5,12] The square planar complex $Pt(en)_2^{2+}$ is also capable of interacting with DNA in this manner. However, a recent report proposed that square planar peptide complexes of Ni(II) selectively associate with A/T rich regions in the minor groove.[13]

Here we report a ^{1}H NMR study of the binding of $Pt(en)_2^{2+}$ to the self-complementary dodecanucleotides d(CAATCCGGATTG) and d(TCGGGATCCCGA). Both dodecanucleotides were designed to contain both GG and AT sequences, with d(CAATCCGGATTG) containing a central GG sequence and two potential A/T rich binding regions, while d(TCGGGATCCCGA) contains only a single AT sequence flanked by G/C rich regions. The results of this study show that the $Pt(en)_2^{2+}$ complex does not bind GG sequences or in the major groove, but does selectively bind an AT sequence in the DNA minor groove.

Experimental Section

Materials. The dodecanucleotides $d(CAATCCGGATTG)_2$ and $d(TCGGGATCCCGA)_2$ were obtained from Bresatec Ltd. South Australia. $[Pt(en)_2]Cl_2$ and D_2O (99.96%) were obtained from Aldrich Chemical Company, while CM-Sephadex was obtained from Pharmacia.

Sample Preparation. Dodecanucleotides were converted into the Na^+ form using a small CM-sephadex column. The dodecanucleotide was dissolved in 0.7 mL of phosphate buffer (10 mM, pH 7) containing 20 mM NaCl and 0.1 mM EDTA, and a trace of DSS was added as an internal chemical shift reference. For experiments carried out in D_2O the sample was repeatedly freeze-dried from D_2O and finally made up in 99.96% D_2O. The dodecanucleotide concentration was determined

[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1996.

(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) Krotz, A. H.; Kuo, L. Y.; Shields, T. P.; Barton, J. K. *J. Am. Chem. Soc.* **1993**, *115*, 3877.
(3) David, S. S.; Barton, J. K. *J. Am. Chem. Soc.* **1993**, *115*, 2984.
(4) Xu, Q.; Shoemaker, R. K.; Braunlin, W. H. *Biophys. J.* **1993**, *65*, 1039.
(5) Xu, Q.; Jampani, S. R. B.; Braunlin, W. H. *Biochemistry* **1993**, 32, 11754.
(6) Collins, J. G.; Shields, T. P.; Barton, J. K. *J. Am. Chem. Soc.* **1994**, *116*, 9840.
(7) Eriksson, M.; Leijon, M.; Hiort, C.; Norden, B.; Graslund, A. *Biochemistry* **1994**, *33*, 5031.
(8) Hudson, B. P.; Dupureur, C. M.; Barton, J. K. *J. Am. Chem. Soc.* **1995**, *117*, 9379.
(9) Xu, Q.; Jampani, S. R. B.; Deng, H.; Braunlin, W. H. *Biochemistry*, **1995**, 34, 14059.
(10) Terbrueggen, R. H.; Barton, J. K. *Biochemistry* **1995**, *34*, 8227.
(11) Gessner, R. V.; Quigley, G. J.; Wang, A. H.-J.; van der Marel, G. A.; van Boom, J. H.; Rich, A. *Biochemistry* **1985**, *24*, 237.
(12) Hingerty, B. E.; Brown, R. S.; Klug, A. *Biochem. Biophys. Acta* **1982**, *697*, 78.
(13) Liang, Q.; Denney Eason, P.; Long, E. C. *J. Am. Chem. Soc.* **1995**, *117*, 9625.

S0020-1669(96)00477-6 CCC: $12.00

from the A_{260} absorbance using an extinction coefficient of 6600 M^{-1} cm^{-1} per nucleotide.[14] Aliquots of stock solutions of $Pt(en)_2^{2+}$ were titrated directly into the NMR tube.

Instrumental Methods. ^1H NMR spectra were recorded 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*.[15] Two dimensional phase sensitive NOESY spectra were recorded by the method of States *et al*.,[16] using 2048 data points in t_2 for 256−320 t_1 values with a pulse repetition delay of 1.7 s for various mixing times. NOESY spectra recorded in 90% H_2O/10% D_2O were obtained using the standard NOESY pulse sequence with the WATERGATE sequence incorporated as a read pulse. DQFCOSY experiments were accumulated using 2048 data points in t_2 for 256 t_1 values with a pulse repetition delay of 1.7 s. NOESY spectra were apodized with either a shifted sinebell or Gaussian function. No significant changes in the intensity of the intermolecular NOEs (compared to the intra-dodecanucleotide NOEs) were observed with the different processing functions.

Determination of the $Pt(en)_2^{2+}$−d(TCGGGATCCCGA)$_2$ Binding Constant. The binding affinity of $Pt(en)_2^{2+}$ with the dodecanucleotide d(TCGGGATCCCGA)$_2$ was determined by fitting the NMR data to a simple M + DNA = M−DNA binding model as previously described.[17] 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 model would be required to determine the binding constant of $Pt(en)_2^{2+}$ with d(CAATCCGGATTG)$_2$, due to the 2:1 binding stoichiometry and the possibility of several binding positions within each A/T region.

Results

Assignment of the Proton Resonances of d(CAATCCGGATTG)$_2$ and d(TCGGGATCCCGA)$_2$. The NMR resonances of the free dodecanucleotides were assigned from a combination of NOESY and DQFCOSY spectra, according to established methods.[18−20] In the NOESY spectrum of a right-handed duplex each base H8 and H6 proton should exhibit an NOE cross-peak to its own sugar H1′, H2′ and H2″ protons as well as to the H1′, H2′ and H2″ protons on the flanking 5′ nucleotide residue.[19,20] In the NOESY spectrum of both d(CAATCCGGATTG)$_2$ and d(TCGGGATCCCGA)$_2$ an NOE is observed from each base H8 and H6 resonances to its own sugar H1′/H2′/H2″ protons as well as to H1′/H2′/H2″ protons of the sugar of the nucleotide residue in the 5′ direction. In a B-type duplex the distance between the base H8/H6 proton to its own H2′ is approximately 2 Å and approximately 4 Å to the H2′ proton on the flanking 5′ sugar.[20] As the NOE cross-peak from each base H8/H6 proton to its own H2′ proton is larger than the cross-peak to the corresponding H2′ proton on the 5′ sugar, it is concluded that both dodecanucleotides adopt a B-type conformation in solution.

The imino resonances in the NMR spectra of the dodecanucleotides collected in 90% H_2O/10% D_2O were examined to determine the extent of the base-pairing. Figure 1 shows the imino resonances of both dodecanucleotides at 17 °C. In each spectrum only five resonances are observed, indicating that the terminal residue does not form a stable base-pair at 17 °C. Furthermore, the penultimate nucleotide residues also exhibit significant fraying, as shown by the less intense, relatively broader imino resonances from the $A_2{\cdot}T_{11}$ and $C_2{\cdot}G_{11}$ base-pairs in d(CAATCCGGATTG)$_2$ and d(TCGGGATCCCGA)$_2$ respectively.

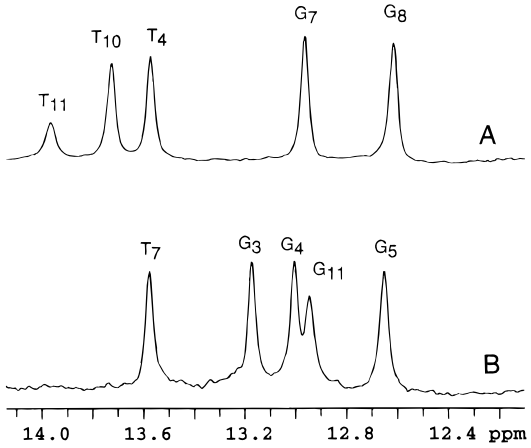

Figure 1. ^1H NMR spectrum of the imino protons of (A) d(CAATCCGGATTG)$_2$ (1.3 mM) and (B) d(TCGGGATCCCGA)$_2$ (1.1 mM) in 90% H_2O/10% D_2O at 17 °C.

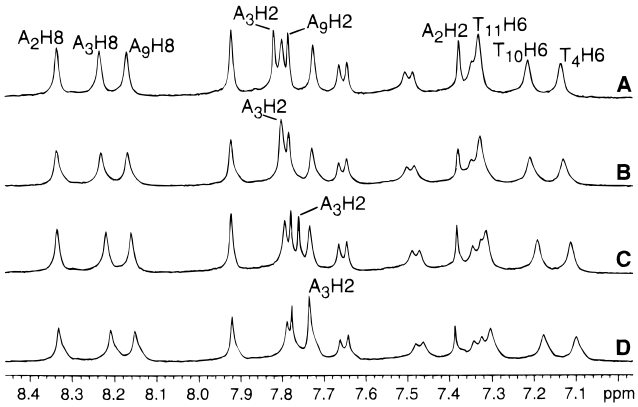

Figure 2. ^1H NMR spectrum of the aromatic base protons of d(CAATCCGGATTG)$_2$ (1.2 mM) at $Pt(en)_2^{2+}$ to dodecanucleotide ratios of (A) 2.0, (B) 1.5, (C) 0.5, and (D) 0.

Titration of d(CAATCCGGATTG)$_2$ and d(TCGGGATCCCGA)$_2$ with $Pt(en)_2^{2+}$. The NMR spectra of d(CAATCCGGATTG)$_2$ at various ratios of added $Pt(en)_2^{2+}$ are shown in Figure 2. Only one set of dodecanucleotide resonances were observed upon addition of the metal complex at all molar ratios of metal complex to dodecanucleotide. This indicates fast-exchange (on the NMR time scale) between the free dodecanucleotide and the metal complex bound form. The addition of $Pt(en)_2^{2+}$ induces relatively small changes in the chemical shifts of the major groove purine H8 and pyrimidine H6 resonances. The A_3H8, T_4H6, T_{10}H6, and T_{11}H6 show slightly larger shifts than the other major groove protons (see Table 1). The minor groove A_2H2 and A_9H2 resonances also show only small chemical shift changes upon addition of the metal complex, however, the A_3H2 resonance shifts 0.21 ppm downfield. This large and selective change in chemical shift for the A_3H2 resonance is consistent with the $Pt(en)_2^{2+}$ complex binding in the minor groove near the A_3 residue. However, observed chemical shift differences alone are insufficient evidence to establish the binding site of the metal complex.

The NMR spectra of d(TCGGGATCCCGA)$_2$ at various ratios of added $Pt(en)_2^{2+}$ are shown in Figure 3. Again, the addition of $Pt(en)_2^{2+}$ to d(TCGGGATCCCGA)$_2$ induces only small changes in the chemical shifts of the resonances from major groove protons (see Table 2), with the A_6H8 and T_7H6 displaying the largest shifts (0.04 and 0.06 ppm respectively).

(14) Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning*: Cold Spring Harbor Laboratory: 1982.
(15) Piotto, M.; Saudek, V.; Sklenar, V. *J. Biomol. NMR* **1992**, *2*, 661.
(16) States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286.
(17) Watt, T. A.; Collins, J. G.; Arnold, A. P. *Inorg. Chem.* **1994**, *33*, 609.
(18) Scheek, R. M.; Boelens, R.; Russo, N.; van Boom, J. H.; Kaptein, R. *Biochemistry* **1984**, *23*, 1371.
(19) Feigon, J.; Leupin, W.; Denny, W. A.; Kearns, D. R. *Biochemistry* **1983,** *22*, 5943.
(20) Patel, D. J.; Shapiro, L.; Hare, D. *J. Biol. Chem.* **1986,** *261*, 1223.

Table 1. ^{1}H NMR Chemical Shifts (in ppm) of d(CAATCCGGATTG)$_2$ and the Chemical Shift Differences Induced by the Addition of Pt(en)$_2^{2+}$ (Numbers in Parentheses), at a Metal Complex to Dodecanucleotide Ratio of 20[a]

	oligonucleotide proton				
	H8/H6	AH2	H1′	H2′	H2″
C_1	7.65 (−0.02)		5.62 (0.00)	1.76 (0.05)	2.30 (0.03)
A_2	8.33 (0.00)	7.39 (−0.01)	5.93 (**−0.07**)	2.80 (0.01)	2.93 (0.01)
A_3	8.21 (**0.05**)	7.74 (**0.21**)	6.23 (−0.02)	2.60 (**0.05**)	2.93 (0.00)
T_4	7.10 (**0.08**)		5.88 (**−0.05**)	2.01 (**0.12**)	2.45 (−0.03)
C_5	7.47 (0.04)		5.94 (0.03)	2.06 (**0.08**)	2.44 (−0.01)
C_6	7.33 (0.01)		5.51 (0.00)	1.86 (−0.03)	2.25 (0.02)
G_7	7.79 (0.03)		5.51 (0.02)	2.61 (0.04)	2.68 (**0.05**)
G_8	7.74 (0.04)		5.67 (0.02)	2.60 (0.01)	2.73 (**0.05**)
A_9	8.15 (0.03)	7.78 (0.01)	6.22 (−0.01)	2.60 (**0.05**)	2.90 (0.03)
T_{10}	7.18 (**0.06**)		5.96 (0.02)	1.94 (**0.10**)	2.48 (−0.03)
T_{11}	7.30 (**0.07**)		5.93 (**−0.09**)	1.96 (**0.10**)	2.37 (**0.06**)
G_{12}	7.92 (0.00)		6.15 (0.03)	2.59 (0.01)	2.36 (0.03)

[a] Significant chemical shift differences (0.05 ppm or more) are shown in bold face. Positive numbers indicate a downfield shift.

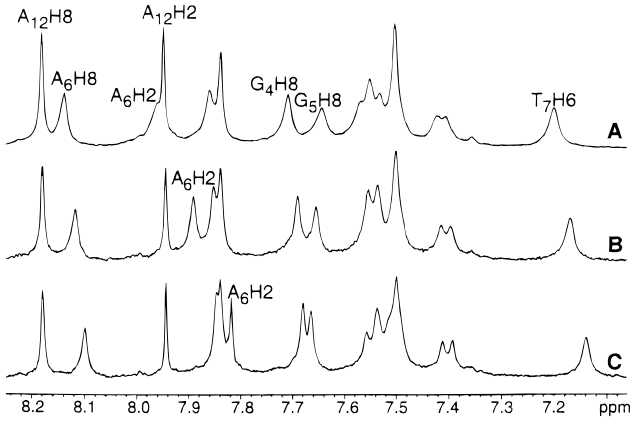

Figure 3. ^{1}H NMR spectrum of the aromatic base protons of d(TCGGGATCCCGA)$_2$ (0.9 mM) at Pt(en)$_2^{2+}$ to dodecanucleotide ratios of (A) 4 (B) 1, and (C) 0.

The minor groove A_6H2 resonance exhibits a relatively large downfield shift (0.14 ppm), again possibly suggesting that the metal complex binds at the AT sequence. No chemical shift changes were observed for the H8 or H2 of A_{12} upon addition of the Pt(en)$_2^{2+}$. From the analysis of the chemical shift changes of the A_6H2 resonance as a function of added metal complex (from R = 0 to 10), the Pt(en)$_2^{2+}$−d(TCGGGATCCCGA)$_2$ binding constant was estimated to be 2500 ± 300 M^{-1}.

In titration experiments carried out in 90% H_2O/10%D_2O at 10 °C, no significant change (less than 0.03 ppm) in the chemical shifts, or the relative intensities, of the imino resonances was observed for either dodecanucleotide upon addition of Pt(en)$_2^{2+}$.

NOESY Spectra of the Pt(en)$_2^{2+}$-Bound Dodecanucleotides. NOESY spectra of d(CAATCCGGATTG)$_2$ were recorded with added Pt(en)$_2^{2+}$ at metal complex to dodecanucleotide ratios (*R*) of 2, 4, and 20. At $R = 2$, intermolecular NOE cross-peaks are observed from the A_3H2 and A_9H2 to the metal complex methylene protons (Figure 4). Figure 5 shows the expansion of the dodecanucleotide aromatic resonances to the sugar H2′/H2″ region at R = 4. In this spectrum the intermolecular NOE cross-peak from the A_3H2 to the metal complex methylene protons is more easily seen. A weaker cross-peak between the A_2H2 and the metal methylene protons is also observed. Due to the overlap of the A_3 and A_9 H2′ resonances with the Pt(en)$_2^{2+}$ resonance, it was not possible to unambiguously assign NOE cross-peaks from the A_3H1′ and A_9H1′ to the metal complex. However, NOE cross-peaks from the metal methylene protons to the T_4H1′, T_{10}H1′, and T_{11}H1′ were observed at $R = 2$, 4, and 20. As the sugar H1′ and adenine H2 protons lie in the dodecanucleotide minor groove, the results strongly suggest that the Pt(en)$_2^{2+}$ complex binds in the minor groove at the A_3T_4 region (and equivalent A_9T_{10} sequence). Importantly, no intermolecular NOE cross-peaks between the metal complex and d(CAATCCGGATTG)$_2$ major groove protons were observed.

The proposal that Pt(en)$_2^{2+}$ binds in the minor groove at AT base sequences was confirmed by the analysis of the NOESY spectra of d(TCGGGATCCCGA)$_2$ with added Pt(en)$_2^{2+}$. Figure 6 shows an expansion of the NOESY spectrum of d(TCGGGATCCCGA)$_2$ at a metal complex to dodecanucleotide ratio of 4. An intermolecular NOE cross-peak between the A_6H2 and the Pt(en)$_2^{2+}$ methylene protons is clearly observed. Furthermore, NOE cross-peaks from the metal complex methylene protons to the A_6H1′ and T_7H1′ were also observed (data not shown). No NOE cross-peaks from the Pt(en)$_2^{2+}$ methylene protons to guanine or cytosine protons were observed, even at a metal complex to dodecanucleotide ratio of 4. These results again indicate that Pt(en)$_2^{2+}$ binds selectively at an AT sequence, and in the minor groove.

Due to the fast rate of exchange between the Pt(en)$_2^{2+}$ amine and water protons in the pH 7 buffer, it was not possible to obtain NOE data from the Pt(en)$_2^{2+}$ amine protons in NOESY spectra collected in 90% H_2O/10% D_2O.

Solution Conformation of the Pt(en)2^{2+}-Bound Dodecanucleotide. In the NOESY spectra of d(CAATCCGGATTG)$_2$ and d(TCGGGATCCCGA)$_2$ with added Pt(en)$_2^{2+}$ (Figures 5 and 6) a NOE cross-peak is observed from each base H8/H6 resonance to its own H2′/H2″ protons as well as to the H2′/H2″ protons on the 5′-flanking residue. It is also observed in Figures 5 and 6 that the NOE cross-peak from each base H8/H6 to its own H2′ proton is significantly stronger than the cross-peak to the H2′ proton on the flanking 5′ residue. These observations indicate that the dodecanucleotides maintain the basic B-type conformation in solution upon Pt(en)$_2^{2+}$ binding. In the NOESY spectra of d(CAATCCGGATTG)$_2$ with added Pt(en)$_2^{2+}$ NOE connectivities are observed from the A_3H2 to the A_2H2, T_4H1′, and T_{11}H1′, as well as from the A_9H2 to the T_{10}H1′ and C_5H1′. These intra- and interstrand NOE cross-peaks are consistent with propeller twisting in the A_2A_3T4 region (and the symmetrically related $A_9T_{10}T_{11}$ region).[21,22] Propeller twisting can result in the walls of the minor groove being drawn in, thereby resulting in a narrower minor groove.[21,22] Equivalent cross-peaks were, however, observed in the NOESY spectrum of the free dodecanucleotide, indicating that the propeller twisting is not induced or significantly altered by the Pt(en)$_2^{2+}$ binding.

(21) Embrey, K. J.; Searle, M. S.; Craik, D. J. *Biochem. Int.* **1991**, *24*, 567.
(22) Singh, M. P.; Plouvier. B.; Hill, G. C.; Gueck, J.; Pon, R. T.; Lown, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 7006.

Table 2. ^{1}H NMR Chemical Shifts (in ppm) of d(TCGGGATCCCGA)$_2$ and the Chemical Shift Differences Induced by the Addition of Pt(en)$_2^{2+}$ (Numbers in Parentheses), at a Metal Complex to Dodecanucleotide Ratio of 4[a]

	oligonucleotide proton				
	H8/H6	AH2	H1′	H2′	H2″
T_1	7.50 (0.01)		6.07 (0.01)	2.12 (−0.02)	2.47 (−0.01)
C_2	7.55 (0.00)		5.58 (0.00)	2.11 (−0.03)	2.37 (0.01)
G_3	7.84 (0.02)		5.47 (0.01)	2.62 (0.03)	2.67 (0.00)
G_4	7.68 (−0.03)		5.71 (0.01)	2.58 (0.01)	2.74 (0.00)
G_5	7.66 (−0.01)		5.64 (0.00)	2.59 (−0.01)	2.73 (−0.01)
A_6	8.10 (0.04)	7.82 (**0.14**)	6.23 (−0.02)	2.56 (0.03)	2.92 (−0.01)
T_7	7.14 (**0.06**)		5.93 (−**0.06**)	2.04 (**0.09**)	2.49 (−**0.05**)
C_8	7.54 (0.03)		5.98 (−0.01)	2.18 (0.04)	2.45 (0.00)
C_9	7.50 (0.02)		5.90 (−0.01))	2.08 (0.01)	2.40 (0.01)
C_{10}	7.40 (0.02)		5.48 (0.01)	1.81 (0.03)	2.19 (0.02)
G_{11}	7.84 (0.01)		5.69 (−0.01)	2.55 (0.04)	2.64 (0.02)
A_{12}	8.18 (0.00)	7.94 (0.00)	6.34 (0.00)	2.63 (0.00)	2.46 (−0.02)

[a] Significant chemical shift differences (0.05 ppm or more) are shown in bold face. Positive numbers indicate a downfield shift.

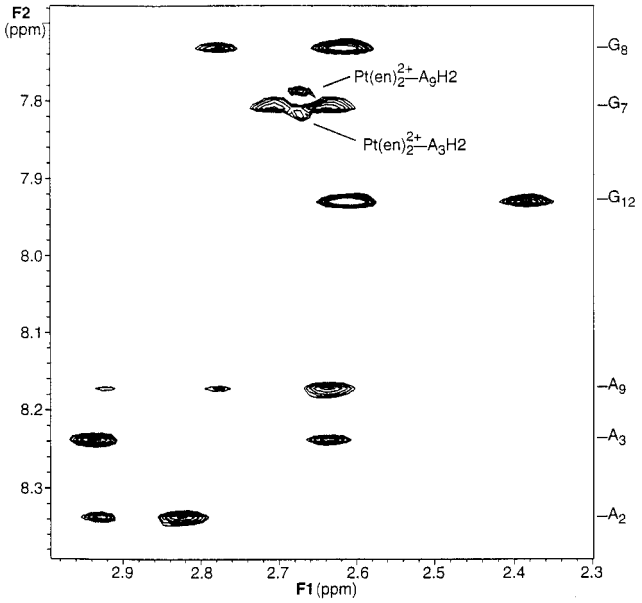

Figure 4. Expansion of the NOESY spectrum (250 ms mixing time) of Pt(en)$_2^{2+}$ and d(CAATCCGGATTG)$_2$ (1.2 mM), at a metal complex to dodecanucleotide ratio of 2, showing part of the aromatic base (7.7−8.4 ppm) to Pt(en)$_2^{2+}$ methylene protons (2.66 ppm) and sugar H2′/H2″ protons (2.3−3.0 ppm) region.

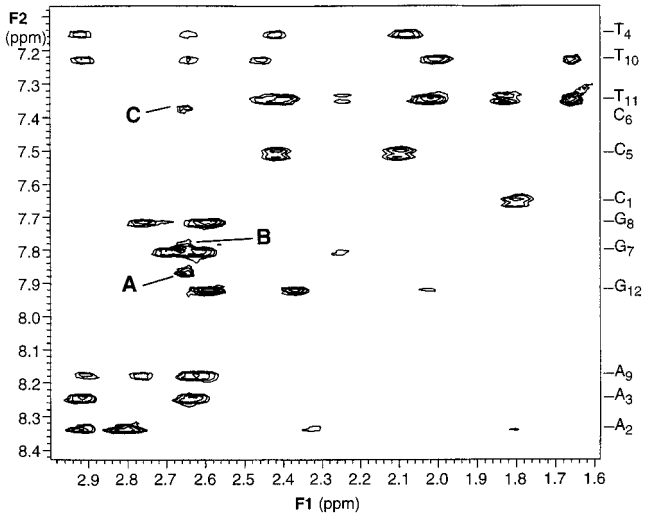

Figure 5. Expansion of the NOESY spectrum (250 ms mixing time) of Pt(en)$_2^{2+}$ and d(CAATCCGGATTG)$_2$ (1.2 mM), at a metal complex to dodecanucleotide ratio of 4, showing the full aromatic base (7.1−8.4 ppm) to sugar H2′/H2″ (1.7−3.0 ppm) and Pt(en)$_2^{2+}$ methylene protons (2.65 ppm) region. Labeling of cross-peaks: A, A$_3$H2−Pt(en)$_2^{2+}$; B, A$_9$H2−Pt(en)$_2^{2+}$; C, A$_2$H2−Pt(en)$_2^{2+}$.

The NOESY (and DQFCOSY) experiments suggest that the Pt(en)$_2^{2+}$ binding does not significantly alter the conformation of d(CAATCCGGATTG)$_2$. However, as the intermolecular NOE cross-peaks from the A$_3$H2 and A$_9$H2 to the metal complex are of similar intensity, the selective and relatively large downfield shift observed for the A$_3$H2 resonance suggests that minor conformational changes are induced upon Pt(en)$_2^{2+}$ binding.

Discussion

The addition of Pt(en)$_2^{2+}$ to both dodecanucleotides induced a relatively large and selective change in the chemical shift of an adenine H2 resonance, the A$_3$H2 in d(CAATCCGGATTG)$_2$ and the A$_6$H2 in d(TCGGGATCCCGA)$_2$. Alternatively, only relatively small chemical shift differences were observed for all major groove proton resonances. In the two dimensional experiments, NOE connectivities between the methylene protons of the metal complex and various dodecanucleotide minor groove protons were observed (see Figure 7). These results indicate that the Pt(en)$_2^{2+}$ binds in the DNA minor groove. As the metal complex exhibited fast exchange (on the NMR time scale) in its binding to both dodecanucleotides, the observation

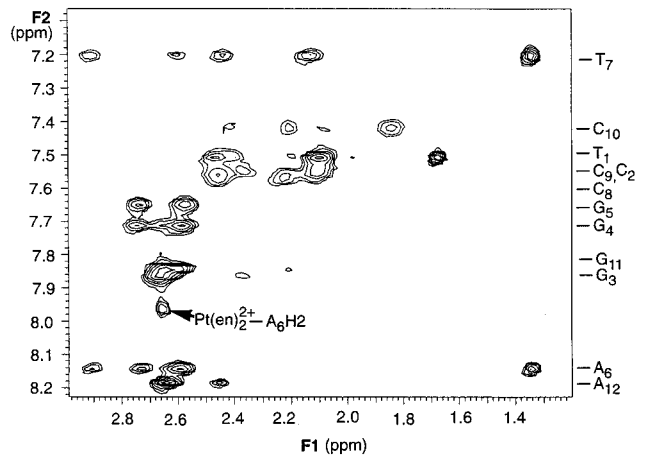

Figure 6. Expansion of the NOESY spectrum (250 ms mixing time) of Pt(en)$_2^{2+}$ and d(TCGGGATCCCGA)$_2$ (0.9 mM), at a metal complex to dodecanucleotide ratio of 4, showing the aromatic base (7.1−8.2 ppm) to Pt(en)$_2^{2+}$ methylene protons (2.65 ppm) and sugar H2′/H2″ and TMe protons (1.3−2.9 ppm) region.

of intermolecular NOE cross-peaks from the metal complex to various minor groove AH2, AH1′, and TH1′ resonances is strong evidence that the Pt(en)$_2^{2+}$ selectively binds at AT sequences. With the self-complementary dodecanucleotide d(CAATCCG-

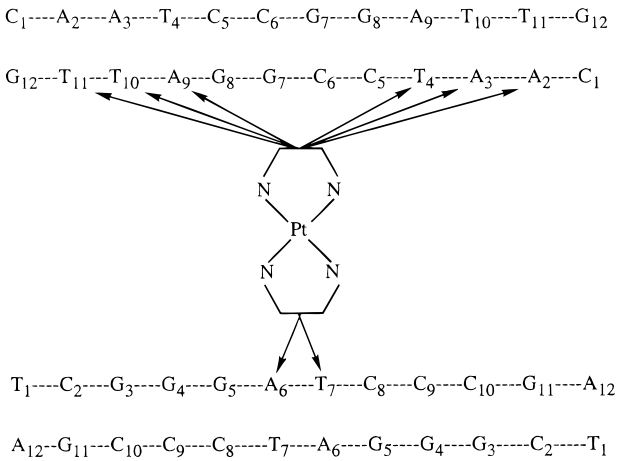

Figure 7. Schematic showing the summary of the observed intermolecular NOEs between $Pt(en)_2^{2+}$ and dodecanucleotides d(CAATCCGGATTG)$_2$ and d(TCGGGATCCCGA)$_2$. The intermolecular NOEs from the equivalent $Pt(en)_2^{2+}$ methylene protons are shown to only one of the two symmetric strands for each nucleotide duplex. Arrows to adenine (A) residues indicate NOEs to the AH2 and sugar H1′ protons, while arrows to thymine (T) residues indicate an NOE to the sugar H1′ protons.

GATTG)$_2$ the metal complex bound at the A_3T_4 and A_9T_{10} sequences, which are located in A/T tracts of the dodecanucleotide. The requirement for only a single AT sequence was demonstrated in binding experiments with the dodecanucleotide d(TCGGGATCCCGA)$_2$. The $Pt(en)_2^{2+}$ selectively bound in the minor groove at the isolated AT sequence. The NOE data presented in this study does not allow a detailed model of $Pt(en)_2^{2+}$−DNA binding to be generated. However, the simple molecular model of d(CAATCCGGATTG)$_2$ with the $Pt(en)_2^{2+}$ complex bound in an edge-on fashion (Figure 8) does show the complementarity of the metal complex with the minor groove. The model also shows the expected (from the NOE data) two base-pair binding site.

While there are examples of organic drugs that contain a coordinated metal and bind in the DNA minor groove,[23−25] it has been recently proposed that simple nonintercalating hexaamine octahedral complexes, eg. $Co(en)_3^{3+}$, target the DNA major groove.[4,5] The results of this study indicate that the square planar $Pt(en)_2^{2+}$ complex noncovalently binds in the DNA minor groove. The reason for the AT minor groove binding may be similar in origin to the considerable number of cationic organic groove binding drugs which also show a preference for AT sequences in the DNA minor groove.[26−29] In the narrower minor groove found at AT rich regions the drug binding is

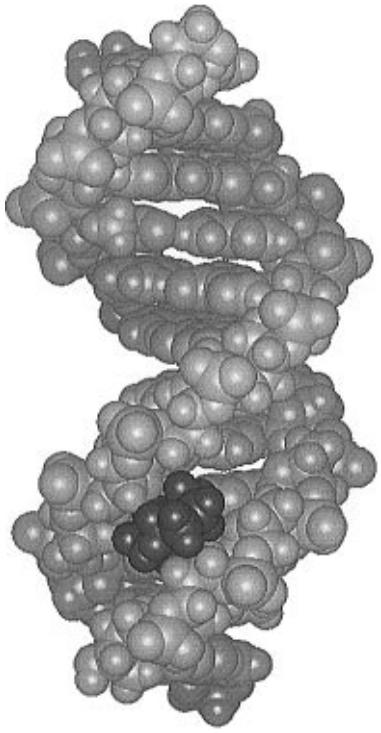

Figure 8. Simple computer generated CPK model showing $Pt(en)_2^{2+}$ (black) binding in the minor groove of d(CAATCCGGATTG)$_2$ (gray) at a 5′-AT region. The model shows the good fit of the metal complex with the minor groove when the $Pt(en)_2^{2+}$ binds edge-on. The model also shows that the metal complex covers two base-pairs, consistent with the NOE data.

stabilized by close van der Waals interactions with the groove, as well as by hydrogen bonds to N3 atoms of adenines or O2 atoms of thymines.[26] Additionally, more favorable electrostatic interactions are obtained in the minor groove at AT sequences which have a greater negative electrostatic potential than GC sequences.[26]

Liang *et al*. proposed that square planar peptide complexes of Ni(II) noncovalently bind at A/T rich regions in the minor groove.[13] The association constant determined for the binding of $Pt(en)_2^{2+}$ with d(TCGGGATCCCGA)$_2$ is considerably lower than the 10^5 M^{-1} DNA binding affinity reported for the Ni(II) complexes.[13] While this indicates that the DNA binding of the Ni(II) peptide and $Pt(en)_2^{2+}$ complexes are mediated by different intermolecular interactions, it does suggest that square planar complexes will, in general, bind in the DNA minor groove. Hence, it may be possible to gain a better understanding of the relative contributions of the various intermolecular forces involved in minor groove recognition by studying the DNA binding of a range of metal complexes related to $Pt(en)_2^{2+}$.

IC9604775

(23) Manderville, R. A.; Ellena, J. F.; Hecht, S. M. *J. Am. Chem. Soc.* **1995**, *117*, 7891.
(24) Gao, X.; Patel, D. J. *Biochemistry* **1989**, *28*, 751.
(25) Keniry, M. A.; Banville, D. L.; Simmonds, P. M.; Shafer, R. *J. Mol. Biol.* **1993**, *231*, 753.
(26) Neidle, S. DNA *Structure and Recognition*; IRL Press: Oxford, England, 1994.
(27) Fede, A.; Labhardt, A.; Bannwarth, W.; Leupin, W. *Biochemistry* **1991**, *30*, 11377.
(28) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *J. Mol. Biol.* **1985**, *183*, 553.
(29) Lane, A. N.; Jenkins, T. C.; Brown, T.; Neidle, S. *Biochemistry* **1991**, *30*, 1372.