Interaction of $[Pt(en)(phen)]^{2+}$ and $[Pt(en)(phi)]^{2+}$ with the Hexanucleotide $d(GTCGAC)_2$: Evidence for Minor Groove Binding

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Introduction

There is considerable interest in the interactions of inert transition metal complexes with nucleic acids. Metal complexes have been used in studying the principles of nucleic acid recognition,[1−5] in probing the tertiary structures of nucleic acids,[6−8] as artificial nucleases,[9,10] as luminescent probes for DNA,[11−13] and in examining electron transfer mediated by DNA.[14−20] If new metallointercalators are to be designed and used for their many potential DNA-related applications, it is important to establish those factors that govern the groove access of the intercalator. Octahedral rhodium(III) complexes based upon the phenanthrenequinone diimine (phi) ligand have been unambiguously shown to intercalate from the DNA major groove.[5] However, the groove-binding preference of octahedral polypyridyl-based metallointercalators is still the subject of controversy.[21−26]

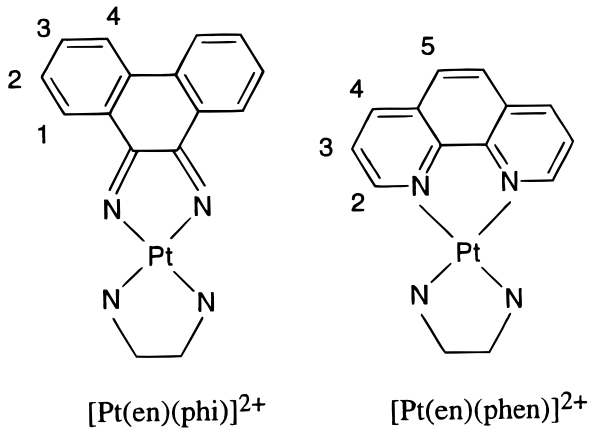

Figure 1. Structure and numbering of $[Pt(en(phen)]^{2+}$ and $[Pt(en-(phi)]^{2+}$ complexes.

Square planar metal complexes containing various aromatic moieties have also been shown to bind DNA by intercalation.[27−29] However, the groove access of this type of metallointercalator has yet to be firmly established. The polypyridyl complex $[Pt(tpy)(HET)]^+$ (where tpy = 2,2′:6′,2″-terpyridyl and HET = 2-hydroxyethanethiolate) has been shown to intercalate between the bases of a dinucleotide from the major groove.[29] However, it is unclear if the observed major groove binding with a dinucleotide is truly representative. In this study, our aim was to examine the DNA binding of two square planar metallointercalators with a hexanucleotide rather than a dinucleotide. While a hexanucleotide is still only a small segment of DNA, NMR and X-ray studies have shown that hexanucleotides do form mini double helices.[22,26,30,31] In this note, we report a ^1H NMR study of the binding of $[Pt(en)(phen)]^{2+}$ (en = ethylenediamine) and $[Pt(en)(phi)]^{2+}$ (shown in Figure 1) to the hexanucleotide $d(GTCGAC)_2$.

Experimental Section

Materials. The hexanucleotide $d(GTCGAC)_2$ was obtained from Geneworks Ltd. Potassium tetrachloroplatinate, 1,10-phenanthroline, 9,10-diaminophenanthrene, and D_2O (99.96%) were obtained from Aldrich Chemical Co.

$[Pt(en)(phen)]Cl_2$. A suspension of $[Pt(phen)Cl_2]$ (0.07 g, 0.16 mmol), made by an adaptation of the method of Palocsay et al.,[32] in water (100 mL) was brought to a gentle reflux for 1 h. A solution of 1,2-diaminoethane (0.01 mL, 0.16 mmol) in water (100 mL) was added dropwise, and the mixture was kept at a gentle reflux for 10 h. Another equivalent of 1,2-diaminoethane was added, and the solution was refluxed again for 10 h. The pale yellow solution was filtered and

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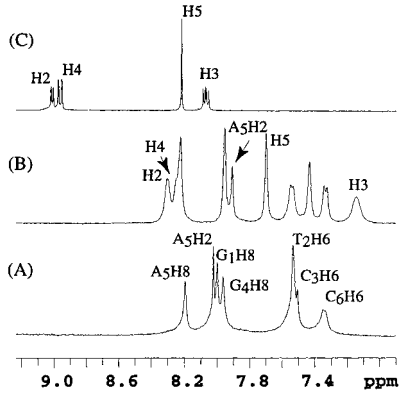

Figure 2. ^{1}H NMR spectra of the free d(GTCGAC)$_2$ (1.4 mM) (A), of the d(GTCGAC)$_2$ with added [Pt(en)$_2$(phen)]$^{2+}$ at $R = 0.9$ (B), and of the free metal complex (C) in 10 mM phosphate buffer (pH 7) containing 20 mM NaCl at 25 °C.

reduced to dryness by rotary evaporation. The crude product was recrystallized from water to afford a yellow powder. Yield: 0.02 g, 25%; ϵ_{283} 17 830 M^{-1} cm^{-1}. ^{1}H NMR (D$_2$O): δ 3.00 (s, 4H), 8.05 (dd, 2H), 8.09 (s, 2H), 8.89 (d, 2H), 8.96 (d, 2H). MS (ESMS, CH$_3$CN, MW = 506.3) *m*/*z*: 435.2 (M − 2Cl$^-$)$^+$.

[Pt(en)(phi)]Cl$_2$. [Pt(en)Cl$_2$] (0.60 g, 1.83 mmol), made by an adaptation of the method of Fanizzi et al.,[33] was added to 9,10-diaminophenanthrene (1.00 g, 4.80 mmol) and water (31 mL), and the mixture was refluxed vigorously (4 h) in the dark. The mixture was filtered to remove excess 9,10-diaminophenanthrene, and excess concentrated sodium perchlorate was added to the filtrate, which was refrigerated overnight. The tan product was filtered and recrystallized from water. The dark yellow product was converted to the chloride salt using IRA 400 (Cl) Amberlite. The resulting orange solution was reduced to dryness and then washed with methanol and ether. The crude product was dissolved in water, filtered, and then freeze-dried. The resulting brown powder was stored in a desiccator. Yield: 0.22 g, 22%; ϵ_{260} 3931 M^{-1} cm^{-1}. ^{1}H NMR (D$_2$O): δ 2.82 (s, 4H), 7.53 (t, 2H), 7.79 (t, 2H), 8.28 (d, 2H), 8.30 (d, 2H). MS (ESMS, CH$_3$CN, MW = 532.3) *m*/*z*: 461.0 (M − 2Cl$^-$)$^+$.

NMR Spectroscopy. ^{1}H NMR spectra were recorded on a Varian Unity*plus*-400 spectrometer operating at 400 MHz for the ^{1}H nuclei. Two-dimensional DQFCOSY spectra were recorded with 2048 points over a spectral width of 4200 Hz in the t2 dimension for 256 t1 values, with a recycle delay of 1.7 s. Two-dimensional phase-sensitive NOESY spectra were recorded by the method of States et al.,[34] with a pulse repetition delay of 1.7 s for the mixing times of 100, 250, and 350 ms. Spectra recorded in 90:10 H$_2$O/D$_2$O were recorded using the WATERGATE solvent suppression technique.[35]

Results and Discussion

One-Dimensional NMR Experiments. The ^{1}H NMR resonances from the hexanucleotide d(GTCGAC)$_2$ have been previously assigned, and analyses of DQFCOSY and short mixing time NOESY spectra have indicated that the hexanucleotide adopts a mini B-type DNA helix in aqueous solution even at low ionic strength.[22,26]

Figure 2 shows the NMR spectrum of the free d(GTCGAC)$_2$ and the hexanucleotide with added [Pt(en)(phen)]$^{2+}$ at a metal complex-to-hexanucleotide duplex ratio (*R*) of 0.9. The phenanthroline resonances from the metal complex exhibit significant upfield shifts: H2, −0.66 ppm; H3, −0.90 ppm; H4, −0.64 ppm; and H5, −0.51 ppm. The upfield shifts and significant broadening, resulting from intermediate to fast exchange (on the NMR time scale) of the phenanthroline resonances, are consistent with the metal complex binding the hexanucleotide by intercalation. The addition of [Pt(en)(phen)]$^{2+}$ induces relatively small changes in the chemical shift of the hexanucleotide H8 and H6 resonances (see page S1 of Supporting Information). The A$_5$H2, however, shifts 0.12 ppm upfield (see Figure 2) upon the addition of [Pt(en)(phen)]$^{2+}$. The sugar H1′ protons of G$_1$, T$_2$, C$_3$, and G$_4$ also exhibit upfield shifts of >0.05 ppm. The relatively larger shifts for the A$_5$H2 and the sugar H1′ protons, located in the minor groove, tentatively indicate that [Pt(en)(phen)]$^{2+}$ intercalates from the minor groove.

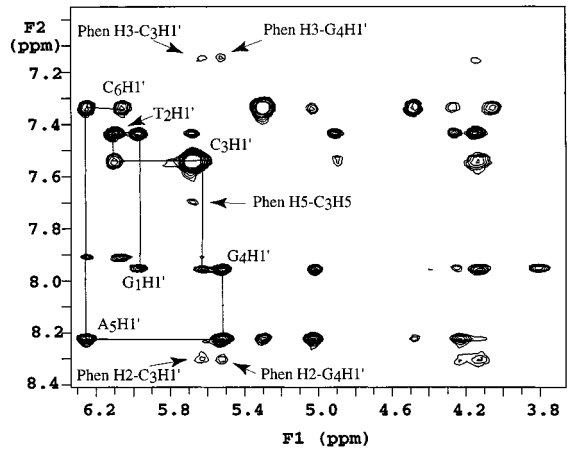

Figure 3. Expansion of a NOESY spectrum (350 ms mixing time) of d(GTCGAC)$_2$ (1.4 mM) with added [Pt(en)(phen)]$^{2+}$ at $R = 0.9$ in a 10 mM phosphate buffer (pH 7) containing 20 mM NaCl at 25 °C. The expansion shows the NOE's between the metal complex and hexanucleotide aromatic protons (7.1−8.4 ppm) and the sugar H1′, H3′, H4′, and H5′/H5″ protons (3.8−6.3 ppm). The intermolecular NOE cross-peaks between [Pt(en)(phen)]$^{2+}$ and the hexanucleotide are indicated. The sequential NOE connectivities are also shown.

The addition of [Pt(en)(phi)]$^{2+}$ to the hexanucleotide also induced significant broadening and upfield shifts of the phi resonances at $R = 0.9$ (see page S2 of Supporting Information): H1, −0.90 ppm; H2, −0.94 ppm; H3, −0.54 ppm; and H4, −0.57 ppm. At low temperatures (<15 °C), the phi H2 resonance splits into two broad peaks due to slow exchange binding kinetics. These observations are consistent with the [Pt(en)(phi)]$^{2+}$ binding the hexanucleotide by intercalation. The addition of [Pt(en)(phi)]$^{2+}$ induced only relatively small changes in the chemical shift for the hexanucleotide resonances, with only the A$_5$H2, G$_1$H1′/H2′, and C$_3$H2″ exhibiting shifts of ≥0.05 ppm.

The ^{1}H NMR spectra of d(GTCGAC)$_2$ with added [Pt(en)(phen)]$^{2+}$ or [Pt(en)(phi)]$^{2+}$ were recorded in 90:10 H$_2$O/D$_2$O at 15 °C. In both cases, two broad imino proton resonances were observed (from T$_2$ and G$_4$), indicating that the hexanucleotide duplex was still intact, with only the terminal residue not forming a stable base pair upon the addition of either metal complex.

Two-Dimensional NOESY Experiments. [Pt(en)(phen)]$^{2+}$. In addition to the expected intraduplex NOE cross-peaks for a B-type DNA conformation, NOE's are also observed between the metal complex and the hexanucleotide in NOESY spectra of d(GTCGAC)$_2$ with added [Pt(en)(phen)]$^{2+}$ (see Figure 3 and Table 1). NOE's from the phen H5 are only observed to the major groove protons, in particular, the C$_3$H5 and T$_2$Me protons. Alternatively, intermolecular NOE's from the phen H2 and H3 protons are observed at both major groove (C$_3$H2′/H2″ and G$_4$-H2′/H2″) and minor groove (C$_3$H1′ and G$_4$H1′) hexanucleotide protons. Additional NOE contacts from the phen H2 to sugar

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Table 1. NOE Cross-Peaks Observed between the Platinum(II) Complexes $[Pt(en)(phen)]^{2+}$ and$[Pt(en)(phi)]^{2+}$ and the Hexanucleotide $d(GTCGAC)_2$[a]

	hexanucleotide proton
$[Pt(en)(phen)]^{2+}$	
H2	C_3H1', G_4H1', G_4H2'/H_2'', $C_3/G_4H4'/H5'/H5''$ (A_5H1', T_2H1', C_3H2')
H3	C_3H1', G_4H1', G_4H2'/H_2'', $C_3H2'/H2''$
H4	(C_3H5, T_2Me, C_3H1', G_4H1', $G_4H2'/H2''$, C_3H2')
H5	C_3H5, T_2Me (C_3H_2')
en	(A_5H2)
$[Pt(en)(phi)]^{2+}$	
H1	(G_4H1', A_5H1', T_2H1')
H2	(G_4H1', $G_4H2'/H2''$, $T_2H2'/H2''$)
H3	C_3H5, T_2Me (A_5H1', G_4H8, $G_4H2'/H2''$, T_2H2'/H_2'')
H4	T_2Me

[a] NOE's only observed (or unambiguously assigned) in spectra at a metal complex-to-duplex-ratio of 2 are shown in parentheses.

H4′/H5′/H5″ protons, located in the minor groove, are also observed. In the NOESY spectra of the hexanucleotide with added $[Pt(en)(phen)]^{2+}$ at $R = 0.9$ (Figure 3), the phen H4 resonance is coincidental with the A_5H8 resonance; however, at $R = 2$, the phen H4 is clearly resolved (see page S3 of Supporting Information). At $R = 2$, NOE's are observed from the phen H4 to the hexanucleotide major groove (e.g., C_3H5 and T_2Me) and minor groove (e.g., G_4H1') protons.

The observed intermolecular NOE contacts suggest that the $[Pt(en)(phen)]^{2+}$ intercalates from the minor groove with the leading edge of the phen (H5) projecting out into the major groove. Binding in this fashion would position the phen H2, H3, and H4 protons between the base pairs at the intercalation site within close proximity to both major groove and minor groove hexanucleotide protons. Also consistent with this binding model is the tentative assignment of an NOE from the en protons to the minor groove A_5H2 proton in the NOESY spectra of the hexanucleotide with added $[Pt(en)(phen)]^{2+}$ at $R = 2$ (see page S3 of Supporting Information).

In the NOESY spectra of the hexanucleotide with added $[Pt(en)(phen)]^{2+}$ at $R = 0.9$, the bulk of the intermolecular NOE's are to the C_3 and G_4 protons. This suggests that $[Pt(en)(phen)]^{2+}$ binds predominantly between the C_3 and G_4 bases. However, the observed intermolecular NOE's to the T_2Me indicate that there is also binding between the G_4 and A_5 bases.

$[Pt(en)(phi)]^{2+}$. In the NOESY spectra of the hexanucleotide with added $[Pt(en)(phi)]^{2+}$ at $R = 0.9$, only a few intermolecular NOE cross-peaks were observed−phi H3 to the C_3H5 and T_2Me protons and phi H4 to the T_2Me protons. However, at $R = 2$, a greater number of NOE's between the metal complex and hexanucleotide protons were observed. In the NOESY spectrum at $R = 2$ at 25 °C, an NOE between the metal complex H4 and the T_2Me is clearly observed (see page S4 of Supporting Information); however, the H1 and H3 resonances are coincidental. At 40 °C, the phi H1 and H3 are just resolved (see Figure 4). In this spectrum, relatively strong NOE's between the phi H1 and G_4H1' protons, between the phi H2 and $G_4H2'/H2''$ proton, and between the phi H3 and T_2Me proton are observed. In addition, a range of other weaker NOE's are also observed,

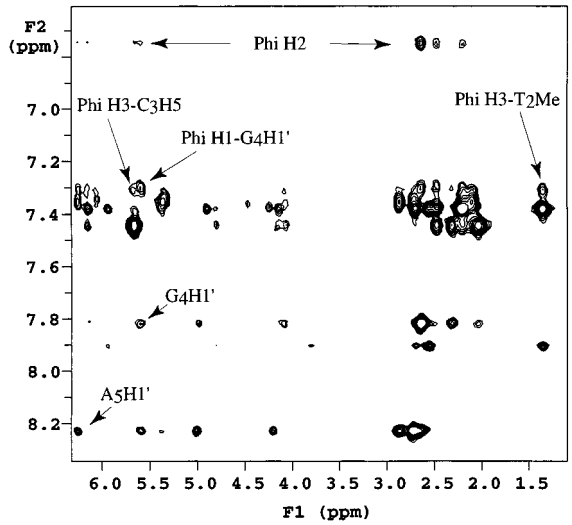

Figure 4. Expansion of a NOESY spectrum (350 ms mixing time) of $d(GTCGAC)_2$ (1.3 mM) with added $[Pt(en)(phi)]^{2+}$ at $R = 2$ in a 10 mM phosphate buffer (pH 7) containing 20 mM NaCl at 40 °C. The expansion shows the NOE connectivities between the metal complex and hexanucleotide aromatic protons (6.7−8.3 ppm) and the hexanucleotide sugar protons (1.5−6.3 ppm). Some of the intermolecular NOE cross-peaks between $[Pt(en)(phi)]^{2+}$ and the hexanucleotide are indicated.

including NOE's from the phi H3 to the C_3H5 and G_4H8 (data not shown) protons and from the phi H2 to the G_4H1' proton. No clear intermolecular NOE cross-peaks between the phi H4 and the hexanucleotide are observed at 40 °C.

The observed intermolecular NOE's (see Table 1) suggest that the metal complex intercalates from the hexanucleotide minor groove. Relatively strong NOE cross-peaks are observed from the phi H3 and H4 protons (on the leading edge) to the hexanucleotide major groove C_3H5 and T_2Me protons, while NOE's from the phi H1 and H2 to the G_4H1' proton (minor groove) are observed. All the observed intermolecular NOE's are consistent with a binding model where the metal complex intercalates from the minor groove at the GA/TC site with the leading edge of the phi rings extending into the major groove (see page S5 of Supporting Information).

These results suggest that both $[Pt(en)(phen)]^{2+}$ and $[Pt(en)(phi)]^{2+}$ bind DNA by intercalation and, more importantly, from the minor groove. As $[Pt(tpy)(HET)]^{+}$ intercalated from the major groove side of a dinucleotide and $[Pt(en)(phen)]^{2+}$ and $[Pt(en)(phi)]^{2+}$ were found to intercalate from the minor groove of a hexanucleotide, it could be speculated that the formation of structured grooves is a factor in the determination of groove access.

Supporting Information Available: The ^1H NMR spectrum of free $d(GTCGAC)_2$ and with added $[Pt(en)_2(phi)]^{2+}$, the NOESY spectra of $d(GTCGAC)_2$ with added $[Pt(en)(phen)]^{2+}$ at $R = 2$ and added $[Pt(en)(phi)]^{2+}$ at $R = 2$ at 25 °C, and a model of $[Pt(en)(phi)]^{2+}$ intercalated between the G_4 and A_5 bases of $d(GTCGAC)_2$. This material is available free of charge via the Internet at http://pubs.acs.org.

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