A ^{1}H NMR Study of the Binding of Δ-[Ru(phen)$_2$DPQ]$^{2+}$ to the Hexanucleotide d(GTCGAC)$_2$. Evidence for Intercalation from the Minor Groove

Ivan Greguric,[†] Janice R. Aldrich-Wright,[*,†] and
J. Grant Collins[*,‡]

Department of Chemistry
University of Western Sydney, Macarthur
P.O. Box 555, Campbelltown, New South Wales, Australia
School of Chemistry, University College
University of New South Wales
Australian Defence Force Academy
Canberra, 2600, Australia

There has been considerable interest in the DNA binding properties of inert transition metal complexes that are capable of binding to DNA by intercalation.[1–3] These complexes can be used to help establish the principles of nucleic acid recognition,[4–8] probe the tertiary structure of nucleic acids,[9,10] and examine electron transfer mediated by DNA.[11,12] The determination of the DNA binding geometry is a very important aspect in the study of the interactions of metallointercalators with nucleic acids. While it has been unambiguously demonstrated that metallointercalators based on the 9,10-phenanthrene-quinone diimine (phi) ligand intercalate from the major groove,[7,13,14] the DNA binding location of Ru(II) polypyridyls is not yet firmly established. On the basis of NMR evidence, Dupureur and Barton have shown that Δ-[Ru(phen)$_2$DPPZ]$^{2+}$ (DPPZ = dipyridophenazine) intercalates from the major groove.[15] However, on the basis of a similarity of the DNA binding geometry of Δ-[Ru(phen)$_2$DPPZ]$^{2+}$ with actinomycin D, Lincoln et al. have proposed that the metal complex may intercalate from the minor groove.[16] In this paper, we report a NMR study of the binding of Δ-[Ru(phen)$_2$DPQ]$^{2+}$ (DPQ = dipyrido[2,2-*d*:2′,3′-*f*]quinoxaline), an analogue of Δ-[Ru-(phen)$_2$DPPZ]$^{2+}$, to the hexanucleotide d(GTCGAC)$_2$.[17] Evidence is presented which demonstrates that this particular metal complex intercalates from the DNA minor groove.

Figure 1 shows the ^{1}H NMR spectrum of d(GTCGAC)$_2$ as a function of added Δ-[Ru(phen)$_2$DPQ]$^{2+}$, as well as the spectrum of the free metal complex. The assignment of the resonances of the bound metal complex were established from 2D NOESY
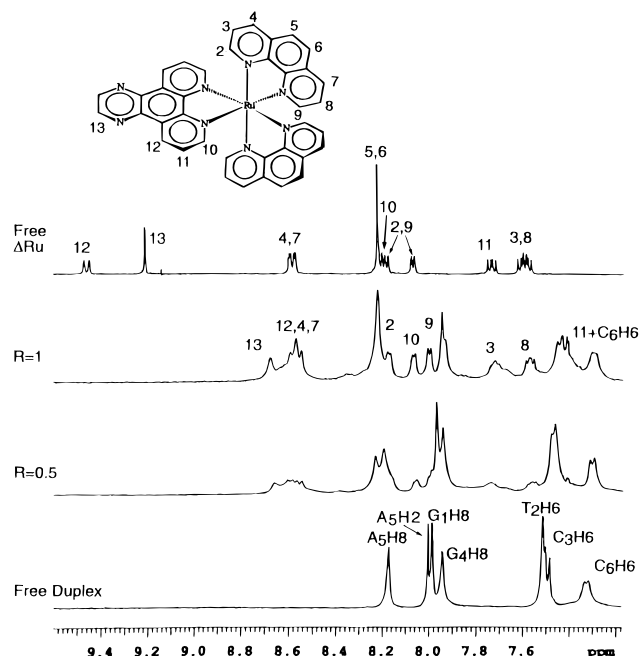

Figure 1. ^{1}H NMR spectra of the aromatic region of Δ-[Ru(phen)$_2$-DPQ]$^{2+}$–d(GTCGAC)$_2$ at metal complex to duplex ratios (*R*) of 0 (1 mM in duplex), 0.5, and 1, in 10 mM phosphate (pH 7) containing 20 mM NaCl . The NMR spectrum of the free metal complex (1 mM in identical buffer) and its structure are also given. All NMR spectra were acquired on a Varian Unity*plus* 400 spectrometer.

and COSY experiments (examples of the 2D spectra are shown in the Supporting Information, as is a table of the chemical shifts of the free and hexanucleotide bound metal complex). The large upfield chemical shift movements observed for the DPQ H11 (0.44 ppm), H12 (0.87 ppm), and H13 (0.53 ppm) resonances upon addition of the metal complex to the hexanucleotide are consistent with the metal complex binding the hexanucleotide by intercalation.[7,13–15] The broadening of the metal complex and hexanucleotide resonances, due to intermediate exchange binding kinetics (on the NMR time scale), is also consistent with intercalation. The intermediate exchange kinetics could not be changed to slow exchange by altering the temperature, with spectra recorded at 10 °C and below exhibiting significantly broader resonances than those observed in the 25 °C spectrum shown in Figure 1. The large upfield shifts observed for the DPQ resonances, coupled with the very small shifts of the phenanthroline resonances in the spectra of the hexanucleotide with added metal complex, indicate that the DPQ ligand selectively intercalates. The chemical shift assignments of the free and metal complex bound hexanucleotide are given in Table 1. It is observed that the resonances from the d(GTCGAC)$_2$ minor groove H1′ protons of the central TCGA region exhibit larger chemical shift movements than the corresponding major groove protons (H8/H6, H2′, and H3′) upon addition of the metal complex. Surprisingly, the A$_5$H2 resonance exhibited only a small (0.06 ppm) upfield shift.

Figure 2 shows the base H8/H6 to sugar H1′ and H3′ (in part) region of a NOESY spectrum of the hexanucleotide with added Δ-[Ru(phen)$_2$DPQ]$^{2+}$. In a right-handed B-type nucle-

[†] University of Western Sydney, Macarthur.
[‡] University College, University of New South Wales.
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(17) [Ru(phen)$_2$DPQ]$^{2+}$ was prepared by the addition of the ligand dipyrido[3,2-*d*:2′,3′-*f*]quinoxaline (DPQ) to Ru(phen)$_2$Cl$_2$. The synthesis of the DPQ ligand and the [Ru(phen)$_2$DPQ]$^{2+}$ complex will be reported elsewhere. The Δ-enantiomer was resolved on CM-25 Sephadex using potassium antimonyl tartrate as the chiral eluent. The Δ-[Ru(phen)$_2$DPQ]$^{2+}$ complex was judged to be >95% enantiomerically pure by CD spectroscopy and by analysis of its ^{1}H NMR spectrum with added potassium antimonyl tartrate compared to spectra of various enantiomeric mixtures with added potassium antimonyl tartrate. The hexanucleotide d(GTCGAC)$_2$ was purchased from Bresatec Ltd., Australia.

Table 1. ¹H NMR Chemical Shifts (in ppm) of d(GTCGAC)$_2$ and d(GTCGAC)$_2$ with Added Δ-[Ru(phen)$_2$DPQ]$^{2+}$ at a Metal Complex to Duplex Ratio of 1[a]

	oligonucleotide proton				
	H8/H6	H1′	H2′	H2″	H3′
G$_1$	8.01 (7.96)	6.07 (5.93)	2.73 (2.61)	2.81 (2.72)	4.85 (4.83)
T$_2$	7.52 (7.42)	6.21 (6.06)	2.25 (2.20)	2.59 (2.45)	4.93 (4.90)
C$_3$	7.51 (7.46)	5.72 (5.52)	2.05 (1.91)	2.42 (2.28)	4.87 (4.80)
G$_4$	7.96 (7.94)	5.64 (5.53)	2.74 (2.72)	2.79 (2.72)	5.03 (5.03)
A$_5$	8.19 (8.23)	6.29 (6.04)	2.65 (2.66)	2.89 (2.83)	5.02 (5.01)
C$_6$	7.35 (7.31)	6.07 (6.05)	2.09 (2.09)	2.09 (2.09)	4.48 (4.47)

[a] Numbers in parentheses are added Δ-[Ru(phen)$_2$DPQ]$^{2+}$. Solution: 10 mM phosphate (pH 7) containing 20 mM NaCl at 25 °C.

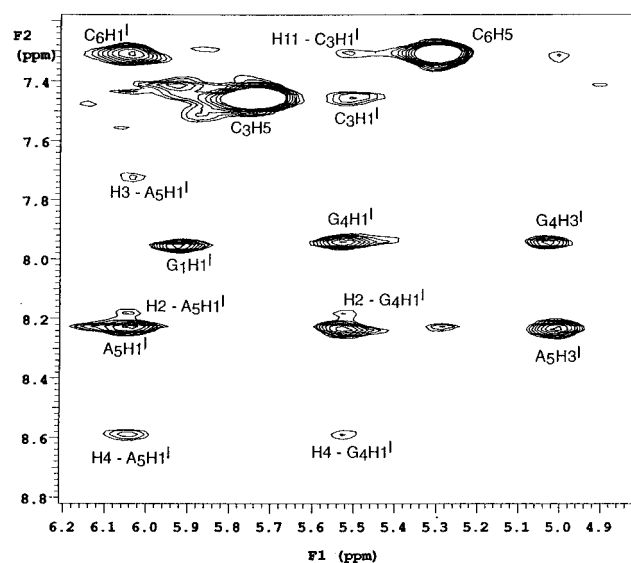**Figure 2.** Expansion of the NOESY spectrum (350 ms mixing time) of Δ-[Ru(phen)$_2$DPQ]$^{2+}$ and d(GTCGAC)$_2$ at a metal complex to duplex ratio of 1, showing the hexanucleotide base and metal complex protons (7.3 to 8.8 ppm) to hexanucleotide sugar H1′ and H3′ (in part) region. NOEs between metal complex H2, H3, H4, and H11 protons and hexanucleotide sugar H1′ protons are shown. The NOE crosspeak between the metal complex H4 and hexanucleotide A$_5$H1′ was also observed in 150 and 250 ms mixing time NOESY spectra. The relatively intense crosspeak at 7.42 ppm (F2) and 5.93 ppm (F1) is the NOE between the T$_2$H6 and G$_1$H1′. No NOEs from the metal complex to the major groove G$_4$H3′ and A$_5$H3′ protons are observed, consistent with minor groove binding.

otide helix, the aromatic H8 and H6 protons exhibit an NOE to their own H1′ sugar proton as well as to the H1′ proton of the flanking 5′-sugar (the sugar of the nucleotide residue in the 5′-direction).[18−20] In addition to these intraduplex sequential NOE crosspeaks, intermolecular NOE crosspeaks between the Δ-[Ru-(phen)$_2$DPQ]$^{2+}$ and the hexanucleotide are also observed. NOE crosspeaks from the metal complex H2, H3, and H4 protons to the minor groove G$_4$H1′ and A$_5$H1′ protons are observed in

Figure 2, along with an NOE between the H11 and C$_3$H1′ resonance. Additional NOE crosspeaks between the metal complex and the hexanucleotide H4′ protons (located in the minor groove), H5′/5″ protons (most accessible from the minor groove) and T-methyl protons (located in the major groove) were also observed. Specifically, intermolecular NOEs were detected between the following: H4 and A$_5$H4′ and A$_5$H5′/H5″ protons; H10 and C$_3$H4′ and C$_3$H5′/H5″ protons; H13 and T$_2$-methyl protons (Supporting Information).

Taken in conjunction, the observed 2D NOE crosspeaks and the 1D chemical shift movements observed upon addition of Δ-[Ru(phen)$_2$DPQ]$^{2+}$ to the hexanucleotide provide a good description of the metal complex binding. The nonintercalating phenanthroline ligands are located in the minor groove with the DPQ ligand intercalated between the T$_2$·A$_5$ and C$_3$·G$_4$ base pairs.[21] The H13 proton on the DPQ ligand extends into the major groove, thereby giving an NOE to the T$_2$-methyl protons. The observation that the DPQ H12 exhibits a larger upfield shift than the H13 in the spectra of the hexanucleotide with added metal complex indicates that the H13 proton is not located in the maximum shielding region of the base pairs. This is consistent with the proposal that the H13 proton extends into the major groove.

Resonances from a minor form of the bound metal complex were also observed. This minor hexanucleotide binding form (approximately 15% of the metal complex) exhibits intermediate exchange in its hexanucleotide binding, but is in slow exchange with the major hexanucleotide binding form. This is evidenced by the observation of two distinct sets of Δ-[Ru(phen)$_2$DPQ]$^{2+}$ crosspeaks in COSY experiments, even at 45 °C (see Supporting Information). At metal complex to hexanucleotide ratios greater than 1:1, no change in the relative intensities of the resonances from the metal complex was observed. It was not possible to establish the binding position of this minor form.

In conclusion, the results presented here demonstrate that the octahedral metallointercalator Δ-[Ru(phen)$_2$DPQ]$^{2+}$ intercalates from the minor groove. This study, to our knowledge, provides the first strong evidence that Ru(II) polypyridyl metallointercalators can intercalate from the DNA minor groove. As complexes such as Δ-[Rh(phen)$_2$phi]$^{3+}$ bind in the major groove,[13] the results of this study suggest that the structure of the intercalating ligand of the metallointercalator plays an important role in determining the DNA binding site.

Supporting Information Available: Expansions of NOESY spectra of Δ-[Ru(phen)$_2$DPQ]$^{2+}$ and d(GTCGAC)$_2$, at a metal complex to duplex ratio of 1, showing the: aromatic−aromatic; aromatic−sugar H4′/H5′/H5″; and aromatic−sugar H2′/H2″ and T-methyl regions; an expansion of a COSY spectrum of Δ-[Ru(phen)$_2$DPQ]$^{2+}$ and d(GTC-GAC)$_2$, at a metal complex to duplex ratio of 1 at 45 °C, showing the aromatic−aromatic region; and a table of the chemical shift assignments of the free and hexanucleotide bound metal complex (5 pages). See any current masthead page for ordering and Internet access instructions.

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(21) As the metal complex intercalates between either T$_2$ and C$_3$ or the symmetrically related G$_4$ and A$_5$, only a partial loss of intensity of the sequential H8/H6−H1′/H2′/H2″ NOEs would be expected in the NOESY spectra. Because the hexanucleotide resonances exhibit different degrees of broadening on addition of Δ-[Ru(phen)$_2$DPQ]$^{2+}$, it was not possible to detect intercalation by the observation of a partial loss of intensity of particular sequential NOEs.