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Minor groove intercalation of Δ-[Ru(Me$_2$phen)$_2$dppz]$^{2+}$ to the hexanucleotide d(GTCGAC)$_2$ †

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Received 28th June 2001, Accepted 14th January 2002
First published as an Advance Article on the web 4th February 2002

The metal complex Δ-[Ru(Me$_2$phen)$_2$dppz]$^{2+}$ (Me$_2$phen = 2,9-dimethyl-1,10-phenanthroline; dppz = dipyrido-[3,2-*a*:2′,3′-*c*]phenazine) has been synthesised and its binding to the hexanucleotide d(GTCGAC)$_2$ studied by ^{1}H NMR spectroscopy. The crystal structure of *rac*-[Ru(Me$_2$phen)$_2$dppz](PF$_6$)$_2$·2H$_2$O has been determined. Addition of Δ-[Ru(Me$_2$phen)$_2$dppz]$^{2+}$ to d(GTCGAC)$_2$ induced significant broadening of the Me$_2$phen and dppz resonances and large upfield shifts of the dppz resonances, consistent with the metal complex binding through intercalation of the dppz ligand. Also indicative of intercalation are the observed upfield shifts of the T$_2$ and G$_4$ imino protons and the 17 °C increase in the melting temperature of the hexanucleotide upon addition of the metal complex. NOE cross-peaks from the Me$_2$phen protons were only observed to hexanucleotide minor groove H1′ and H4′/H5′/H5″ protons in NOESY spectra of the hexanucleotide with added metal complex. In addition, NOEs were also observed between the H13/14 protons of the dppz ligand and the hexanucleotide major groove T$_2$methyl and sugar H2′/H2″ protons. From the combined NMR data it is concluded that the dppz-based ruthenium(II) complex Δ-[Ru(Me$_2$phen)$_2$dppz]$^{2+}$ intercalates from the minor groove of the hexanucleotide mini-duplex d(GTCGAC)$_2$.

Introduction

The use of inert transition metal complexes for DNA recognition and DNA mediated electron transfer has attracted considerable interest over the past decade, and has been the subject of a variety of recent review articles.[1–3] Of the DNA binding complexes that have been studied, those based upon the dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz) ligand have probably attracted the most interest. Complexes such as [Ru(phen)$_2$dppz]$^{2+}$ (phen = 1,10-phenanthroline) bind DNA strongly through intercalation of the dppz ligand.[4–6] Furthermore, the luminescent properties of the dppz-based complexes have led to their application as DNA 'light switches' and probes for long range DNA mediated electron transfer studies.[7–13] Despite the considerable interest and the number of studies that have used dppz-based complexes, the mode of DNA binding remains unresolved and the centre of controversy.

From both photophysical studies and NMR data, Barton and co-workers have proposed that [Ru(phen)$_2$dppz]$^{2+}$ intercalates from the DNA major groove.[14–16] Alternatively, Nordén and co-workers, on the basis of the similarity of the binding geometry to that of the proven minor groove intercalating agent actinomycin D and photophysical studies using T4-DNA, have proposed that [Ru(phen)$_2$dppz]$^{2+}$ intercalates from the minor groove.[17,18] Furthermore, Lincoln and Nordén, demonstrated by linear dichroism that [Ru(phen)$_3$]$^{2+}$ (which has been shown to bind from the minor groove)[19] was capable of semi- or quasi-intercalation, and that the binding geometry of [Ru(phen)$_3$]$^{2+}$ was similar to that of [Ru(phen)$_2$dppz]$^{2+}$.[20]

In the absence of a crystal structure, NMR spectroscopy is the best technique available to characterize the DNA binding of complexes such as [Ru(phen)$_2$dppz]$^{2+}$. However, NMR studies of oligonucleotide binding by dppz complexes have been difficult, due to: the lack of DNA binding sequence specificity; broad resonances (due to intermediate exchange kinetics); and the abundance of overlapping resonances in the aromatic region. Dupureur and Barton have used selective deuteration of the phen and dppz ligands of Δ- and Λ-[Ru(phen)$_2$dppz]$^{2+}$ to simplify the spectra of the metal complex bound to a hexanucleotide.[14,15] This approach resulted in the assignment of several NOE cross-peaks from the Δ-enantiomer to the hexanucleotide major groove protons, thereby suggesting that the metal complex intercalates from the major groove.[15] No intermolecular NOEs between the hexanucleotide and the Λ-[Ru(phen)$_2$dppz]$^{2+}$ were detected.[15]

In an attempt to observe a greater number of metal complex–hexanucleotide intermolecular NOEs, so as to further characterize the DNA binding, we have used the 2,9-dimethyl-1,10-phenanthroline analogue of Δ-[Ru(phen)$_2$dppz]$^{2+}$, *i.e.* Δ-[Ru(Me$_2$phen)$_2$dppz]$^{2+}$, as shown in Fig. 1. The incorporation of the methyl groups onto the phenanthroline ligands has three advantages. (1) The methyl groups provide strong signals (compared to the aromatic protons) from which intermolecular NOEs can be observed. (2) The aromatic region is simplified, due to the reduction of the number of aromatic resonances and the degree of *J*-coupling that is observed. (3) The assignment of the resonances from the bound metal complex is facilitated.

† Electronic supplementary information (ESI) available: a figure showing the aromatic region of the hexanucleotide with added Δ-[Ru(Me$_2$phen)$_2$dppz]$^{2+}$ at a metal complex:duplex ratio of 0.9 (A) and 1.8 (B). For the crystal structure of [Ru(Me$_2$phen)$_2$dppz]$^{2+}$ the positional atomic coordinates, bond lengths, and bond angles and details of least-squares planes calculations are presented in Tables S1 to S4. See http://www.rsc.org/suppdata/dt/b1/b105689c/

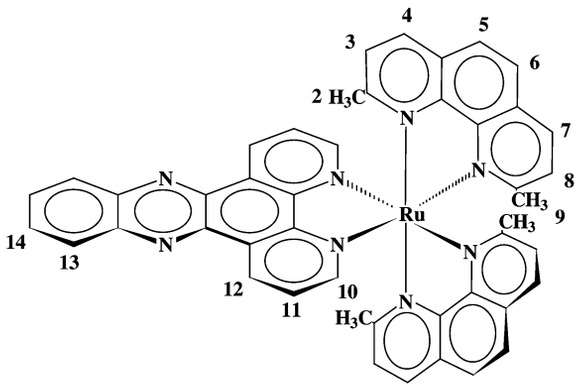

Fig. 1 Structure and atom numbering of Δ-[Ru(Me$_2$phen)$_2$dppz]$^{2+}$.

The phenanthroline 2-methyl group provides a link to the dppz ligand due to its closeness to the H10 proton, compared with the corresponding distance from the 9-methyl group. This allows the assignment of the Me$_2$phen and the dppz resonances. In addition, while the methyl groups will affect the intercalation process to some degree, simple molecular modelling suggests that the [Ru(Me$_2$phen)$_2$dppz]$^{2+}$ complex can still fully intercalate. In this paper we report a ^{1}H NMR study of the binding of Δ-[Ru(Me$_2$phen)$_2$dppz]$^{2+}$ to the hexanucleotide d(GTCGAC)$_2$. As ruthenium(II) dppz complexes show no sequence selectivity in their DNA binding,[15] it was not possible to study the binding of Δ-[Ru(Me$_2$phen)$_2$dppz]$^{2+}$ bound to an oligonucleotide at a single site. Consequently, to aid comparison with earlier NMR studies of the binding of [Ru(phen)$_2$dppz]$^{2+}$ and closely related complexes to d(GTCGAC)$_2$,[14,15,21] we have used the same hexanucleotide to study the DNA binding of Δ-[Ru(Me$_2$phen)$_2$dppz]$^{2+}$.

Experimental

Materials

The hexanucleotide d(GTCGAC)$_2$ was obtained from GeneWorks, South Australia. Ruthenium(III) chloride hydrate, 1,10-phenanthroline, 2,9-dimethyl-1,10-phenanthroline hydrate, potassium hexafluorophosphate, aluminium oxide, activated, neutral, Brockmann I, Amberlite IRA-400(Cl) ion exchange resin, D$_2$O (99.96%) and dibenzoyl-L-tartaric acid were obtained from Aldrich Chemical Company.

Ligand synthesis

The dppz ligand was prepared by the method of Dupureur and Barton.[15] A mixture of 1,10-phenanthroline-5,6-dione[22] (1.0 g, 4.8 mmol) and 1,2-phenylenediamine (0.6 g, 5.7 mmol) in ethanol (400 mL) was stirred for 2 h at 50 °C and then at room temperature overnight. The resulting solution was reduced in volume by rotary evaporation at 50 °C to yield a cream-coloured product. The crude product was left to stand for 8 h, methanol–water (10:90) was then added, and the product was filtered and recrystallized from methanol to give a cream solid. Yield: 0.96 g, 80%.

Metal complex synthesis

[Ru(Me$_2$phen)$_2$Cl$_2$] was synthesised as previously described.[21] [Ru(Me$_2$phen)$_2$dppz]$^{2+}$ was prepared by refluxing 1.0 g of [Ru(Me$_2$phen)$_2$Cl$_2$] and 0.52 g (1.2 equiv) of dppz in 500 mL of 75% ethanol for 5 h. The volume was then reduced (50 mL), the solution cooled, and excess KPF_6 added. The resultant orange precipitate was filtered and washed with water (100 mL) and then diethyl ether (50 mL). The solid was dissolved in acetonitrile (20 mL) and applied to the head of a column (5 × 30 cm) of activated aluminum oxide (neutral Brockmann 1). The orange band was eluted with acetonitrile, and to this fraction was added water (20 mL) to yield fine orange needles (0.99 g, 76%). ^{1}H NMR (d$_6$-acetone): δ 9.60 (d, J = 8.10 Hz, 1H), 8.94 (d, J = 8.42 Hz, 1H), 8.50 (d, J = 8.10 Hz, 1H), 8.47 (d, J = 8.42 Hz, 1H), 8.43 (dd, J = 6.6, 3.48 Hz, 2H), 8.31 (d, J = 8.80 Hz, 1H), 8.15 (dd, J = 6.6, 3.48 Hz, 2H), 8.04 (d, J = 8.42 Hz, 1H), 7.88 (d, J = 5.60 Hz, 1H), 7.72 (dd, J = 8.15, 5.4 Hz, 1H), 7.44 (d, J = 8.24 Hz, 1H), 2.18 (s, 3H), 2.11 (s, 3H). Anal. calc. for [Ru(Me$_2$phen)$_2$dppz](PF$_6$)$_2$·H$_2$O: C, 49.88; H, 3.28; N, 10.11%. Found: C, 50.26; H, 3.15; N, 9.81%.

Crystallography

Structure determination. *Rac*-[Ru(Me$_2$phen)$_2$dppz](PF$_6$)$_2$·2H$_2$O: $C_{46}H_{38}F_{12}N_8O_2P_2Ru$, M = 1125.86, monoclinic, space group $P2_1/n$ (no. 14), a = 14.5556(8), b = 16.8090(9), c = 19.300(1) Å, β = 100.736(1)°, V = 4639.4(4) Å, D_c = 1.612 g cm^{-3}, Z = 4, crystal size 0.35 × 0.11 × 0.09 mm, orange needle, λ(MoKα) = 0.71073 Å, μ(MoKα) = 5.05 cm^{-1}, $2\theta_{max}$ = 56.6°, T = 294 K, N_{ind} = 11 393 (R_{merge} = 0.022), N_{obs} = 7861 [$I > 2.5\ \sigma(I)$], N_{var} = 658, residuals $R(F)$ = 0.050, $R_w(F)$ = 0.049, GoF(all) = 3.96, $\Delta\rho_{min,max}$ = −0.46, 0.68 e Å^{-3}.

Data collection, structure solution and refinement. Data were collected on a Bruker SMART 1000 CCD diffractometer. The data integration and reduction were undertaken with SAINT and XPREP,[23] and absorption corrections were applied using SADABS.[23] The data reduction included the application of Lorentz and polarisation corrections. The structure was solved by direct methods using SHELXS-86[24] and refined using full-matrix least-squares methods with teXsan.[25] Hydrogen atoms were included at calculated sites with isotropic thermal parameters based on that of the riding atom. Non-hydrogen atoms were refined anisotropically except for the partially occupied solvent sites (assigned as oxygen atoms with occupancies fixed on the basis of observed peak intensities) and the minor sites of a rotationally disordered PF_6^- anion. Neutral atom scattering factors were taken from International Tables.[26] Anomalous dispersion effects were included in F_c;[27] the values for $\Delta f'$ and $\Delta f''$ were those of Creagh and McAuley.[28] The values for the mass attenuation coefficients are those of Creagh and Hubbell.[29] All other calculations were performed using the teXsan[25] crystallographic software package of Molecular Structure Corporation.

An ORTEP[30] plot is shown in Fig. 2. Positional atomic coordinates, bond lengths, and bond angles and details of least-squares planes calculations are presented in Tables S1–S4 of the ESI.† Observed and calculated structure factors, thermal parameters for non-hydrogen atoms, positional and thermal parameters of the hydrogen atoms, torsion angles, and close inter- and intra-molecular contacts are available upon request from the author.

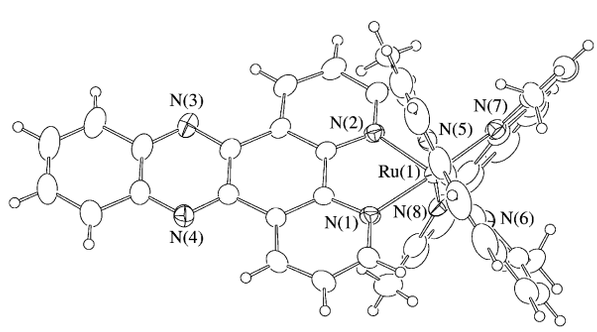

Fig. 2 ORTEP plot (30% thermal ellipsoids) with partial atom numbering scheme.

CCDC reference number 168297.

See http://www.rsc.org/suppdata/dt/b1/b105689c/ for crystallographic data in CIF or other electronic format.

Enantiomer resolution

The enantiomers of $[Ru(Me_2phen)_2dppz]^{2+}$ were resolved on a Sephadex SP-C 25 column (40 × 2.5 cm) using 0.1 M disodium dibenzoyl-L-tartrate, 0.2 M sodium chloride, and 20% acetone as the eluent. The enantiomeric purity of the chloride salt was assayed by CD spectroscopy, with the Δ-enantiomer displaying negative circular dichroism at 310 and 464 nm.

Sample preparation for NMR analysis

The hexanucleotide was dissolved in 0.65 mL of phosphate buffer (10 mM, pH 7) containing 20 mM NaCl, 0.1 mM EDTA and a trace of DSS 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 hexanucleotide concentration was determined from the A_{260} absorbance using an extinction coefficient of 6600 M^{-1} cm^{-1} per nucleotide.[31]

Instrumental methods

400 MHz ^{1}H NMR spectra were recorded on a Varian Unity*plus*-400 spectrometer. NOESY spectra were recorded by the method of States *et al.*,[32] using 2048 data points in t_2 for 256 t_1 values with a pulse repetition delay of 1.7 s. DQFCOSY experiments were accumulated using 2048 data points in t_2 for 256–310 t_1 values with a pulse repetition delay of 1.7 s. Spectra recorded in 90% H_2O–10% D_2O were collected using the WATERGATE solvent suppression technique of Piotto *et al.*[33] Circular dichroism spectra (CD) were recorded at ambient temperature on a Jasco 500C spectropolarimeter.

Molecular modelling

The coordinates for the metal complex were taken from the crystal structure. The hexanucleotide model was constructed using HyperChem molecular modelling software[34] and the metal complex was manually docked. Energy minimisation by Polak–Ribiere conjugate-gradient refinement was carried out with the metal complex treated as a rigid group, a sound approximation given the rigidity of each of the ligands and the strained nature of the complex. Models were investigated with the metal complex intercalated between each of the unique base-pair combinations and a variety of orientations of the complex were used as starting points. Minimisation was continued until the RMS energy gradient was less than 0.2 kJ $Å^{-1}$ mol^{-1}.

Results

Rac-$[Ru(Me_2phen)_2dppz](PF_6)_2\cdot 2H_2O$ crystal structure

The crystal structure analysis confirms that simultaneous coordination of two Me_2phen ligands and the dppz ligand is possible but there is evidence of significant steric strain arising from the bulk introduced by the methyl substituents. For instance, the Ru–N(Me_2phen) [2.092(3)–2.125(3) Å] and Ru–N(dppz) [2.082(3)–2.084(3) Å] bond lengths are significantly longer than the Ru–N(phen) [2.065(6)–2.073(6) Å] and Ru–N(dpq) [2.043(5)–2.063(5) Å] bonds lengths observed in the closely related complex $[Ru(phen)_2dpq]^{2+}$.[35] In addition, the Me_2phen ligands are significantly bowed as a result of interactions between the methyl groups and the nitrogen donor atoms of adjacent ligands. Deviations from the least-squares planes are in the range of ±0.18 Å for both of these ligands. The methyl groups adjacent to the dppz ligand do not protrude toward the dppz as far as the nitrogen donors but will impact somewhat on the extent to which the complex can intercalate.

Assignment of the ^{1}H NMR resonances

The ^{1}H NMR spectrum of the free $d(GTCGAC)_2$ was assigned by standard techniques.[36–38] Analysis of short mixing time NOESY spectra and the observation of imino protons from the non-terminal base pairs indicated that the hexanucleotide adopted a B-type mini double helix in solution.

The resonances from both the metal complex and hexanucleotide become significantly broadened upon addition of the metal complex to the hexanucleotide at 25 °C, indicating intermediate exchange binding kinetics. At lower temperatures (<10 °C) slow exchange binding kinetics are observed for some resonances, in agreement with the $[Ru(phen)_2dppz]^{2+}$–hexanucleotide binding results of Dupureur and Barton.[14,15] However, at temperatures below 10 °C the resonances are extremely broad. At 45 °C the spectrum of $d(GTCGAC)_2$ with added Δ-$[Ru(Me_2phen)_2dppz]^{2+}$ is sufficiently resolved so that the resonances from both the hexanucleotide and the metal complex can be assigned (see Fig. 3).

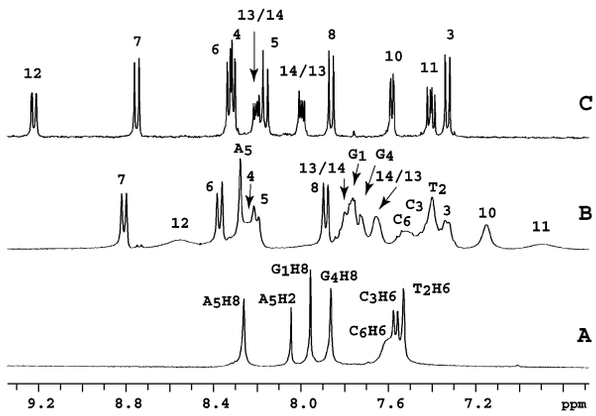

Fig. 3 ^{1}H NMR spectra of the aromatic region of the free hexanucleotide (A), the hexanucleotide (1.4 mM) with added Δ-$[Ru(Me_2phen)_2dppz]^{2+}$ at a metal complex:duplex ratio of 0.9 (B) and the free metal complex (C). All spectra were obtained in the same aqueous buffer [10 mM phosphate (pH 7) containing 20 mM NaCl and 0.1 mM EDTA] at 45 °C.

The resonances from the bound metal complex were assigned by a combination of two-dimensional NMR experiments. The relative closeness of the H10 proton to the 2-methyl compared with the 9-methyl group allows the assignment of these protons in a NOESY spectrum. Once the methyl groups have been specifically assigned the remaining Me_2phen protons are easily assigned through their various connectivities in NOESY and DQFCOSY spectra. Similarly, given the assignment of the H10 resonance the H11 and H12 resonances can then be assigned through their respective coupling in a DQFCOSY spectrum. The dppz H13/H14 resonances can then be non-specifically assigned through their coupling in a DQFCOSY experiment. Although some of the hexanucleotide resonances still exhibited considerable exchange broadening at 45 °C, nearly all the resonances from the bound $d(GTCGAC)_2$ could be assigned through their connectivities in NOESY and DQFCOSY spectra.[36–38]

Δ-$[Ru(Me_2phen)_2dppz]^{2+}$–$d(GTCGAC)_2$ interactions – one-dimensional NMR

The metal complex was initially titrated into the hexanucleotide solution to a metal complex:hexanucleotide duplex ratio of 0.9, at 25 °C. Owing to the overlap and broadness of the resonances, it was not possible to determine the chemical shift of most of the resonances from the metal complex and hexanucleotide at each point in the titration. However, upon titration of the metal complex up to a metal complex:hexanucleotide ratio of 1.8, at 35 and 45 °C (see ESI †), it was observed that the chemical shifts of the resonances from the metal complex remained constant (±0.01 ppm). This suggests that the metal complex predominantly exists in the hexanucleotide-bound form throughout the

Table 1 Change in chemical shift (ppm) for the $d(GTCGAC)_2$ resonances upon addition of Δ-$[Ru(Me_2phen)_2dppz]^{2+}$ at a metal complex:duplex ratio of 0.9, in 10 mM phosphate (pH 7) containing 20 mM NaCl at 25 and 45 °C. Numbers in parentheses are tentative assignments. ND = not determined

	Hexanucleotide proton							
	H8/H6		H5/H2		Me		H1′	
	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C
G_1	−0.22	−0.19					−0.15	−0.21
T_2	−0.10	−0.11			0.08	−0.05	−0.48	−0.43
C_3	−0.09	−0.15	0.00	−0.14			ND	−0.55
G_4	−0.18	−0.12					−0.08	−0.19
A_5	0.09	0.02	ND	(−0.26)			−0.34	−0.39
C_6	−0.11	0.00	(0.19)	−0.02			0.00	−0.04

Table 2 ^1H NMR chemical shifts of the free Δ-$[Ru(Me_2phen)_2dppz]^{2+}$ and the hexanucleotide-bound Δ-$[Ru(Me_2phen)_2dppz]^{2+}$, in 10 mM phosphate buffer (pH 7) containing 20 mM NaCl at 45 °C. The assignment of the H14 and H13 resonances could be inverted, *i.e.* H13 = 7.99 and 7.64 ppm

Ligand proton	Free Δ-$[Ru]^{2+}$ complex/ppm	$d(GTCGAC)_2$-bound Δ-$[Ru]^{2+}$ complex/ppm	Change in shift upon binding/ppm
dppz			
H14	7.99	7.64	−0.35
H13	8.20	7.78	−0.42
H12	9.22	8.55	−0.67
H11	7.40	6.89	−0.51
H10	7.58	7.15	−0.43
Me_2phen			
9-Methyl	2.04	2.01	−0.03
H8	7.86	7.88	0.02
H7	8.75	8.81	0.06
H6	8.33	8.37	0.04
H5	8.16	8.20	0.04
H4	8.31	8.25	−0.06
H3	7.33	7.33	0.00
2-Methyl	1.91	1.85	−0.06

titration, consistent with the established high DNA binding affinity of dppz-based ruthenium(II) complexes.[14,15]

NMR spectra of the metal complex-bound hexanucleotide, at a metal complex:hexanucleotide duplex ratio of 0.9, were recorded over a range of temperatures. At all temperatures, addition of the metal complex induced significant changes in the chemical shift of the resonances from the hexanucleotide (see Table 1). All the aromatic H8 and H6 resonances exhibited significant upfield shifts (except for A_5 and T_6 at 45 °C). However, the metal complex binding induced greater shifts for most of the sugar H1′ protons. Hexanucleotide binding induced large upfield shifts for the dppz protons (see Table 2), consistent with the metal complex binding by intercalation. Alternatively, only relatively small shifts (≤0.06 ppm) were observed for the Me_2phen protons. This is consistent with selective intercalation by the dppz ligand. Addition of the metal complex to the hexanucleotide also induced extensive broadening and upfield shifts of the T_2 and G_4 imino resonances. At 25 °C where both imino protons could be assigned, upfield shifts of 0.59 and 0.35 ppm were observed for the T_2 and G_4 imino resonances respectively. The large upfield shifts and extensive broadening observed for the imino resonances are again consistent with the metal complex binding by intercalation.[15,39] The broadening of the imino, and amino, resonances precluded the observation of NOE cross-peaks from these protons in NOESY experiments.

DNA binding by intercalation is also generally characterised by an increase in the melting temperature of the DNA.[15] For the hexanucleotide, changes to the melting temperature can be determined from the transition midpoint of the temperature dependence curve of the resonances from the hexanucleotide. The chemical shift changes reflect the conversion from a duplex state to the totally base-destacked single state for the

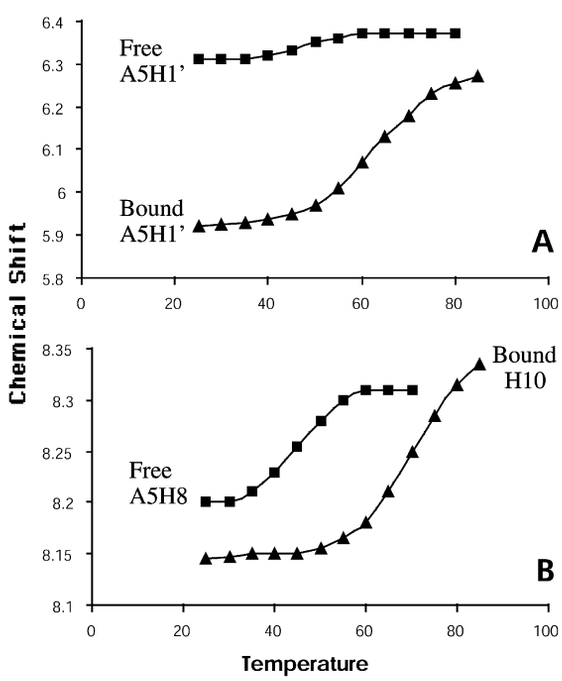

Fig. 4 Melting curves (data points connected by a smoothed line) of the free $d(GTCGAC)_2$ duplex (1.4 mM) (■) and the Δ-$[Ru(Me_2phen)_2dppz]^{2+}$-bound hexanucleotide duplex (▲). (A) The chemical shift of the A_5H1' resonance of the free hexanucleotide and the metal complex-bound hexanucleotide as a function of temperature. (B) The A_5H8 resonance of the free hexanucleotide and the H10 resonance (δ + 1.0 ppm) of the metal complex bound to the hexanucleotide as a function of temperature.

hexanucleotide at millimolar concentrations. Fig. 4 shows the chemical shift of several resonances from the free and metal complex-bound hexanucleotide as a function of temperature. The transition midpoint of the temperature dependence curve of the free hexanucleotide was determined to be 45 °C, in agreement with the results reported by Dupureur and Barton for the same hexanucleotide.[15] Addition of the metal complex increased the midpoint of the transition of the hexanucleotide by 17 °C, consistent with intercalation and the results of Dupureur and Barton for Δ-$[Ru(phen)_2dppz]^{2+}$.[15]

Δ-$[Ru(Me_2phen)_2dppz]^{2+}$–$d(GTCGAC)_2$ interactions – two-dimensional NMR

NOESY spectra of the hexanucleotide with added metal complex were recorded at temperatures in the range 3–60 °C, using mixing times ranging from 100 to 350 ms, to obtain a more detailed picture of the metal complex binding. As in previous NMR studies of the oligonucleotide binding by dppz complexes, it was not possible to observe all expected intraduplex NOE cross-peaks expected for a B-type DNA helix at any one temperature. This is presumably due to selective exchange broadening of some of the resonances from the hexanucleotide. However, a considerable number of NOE cross-peaks between the metal complex and the hexanucleotide could be assigned, particularly at 45 °C (see Fig. 5 and Table 3). At 45 °C the

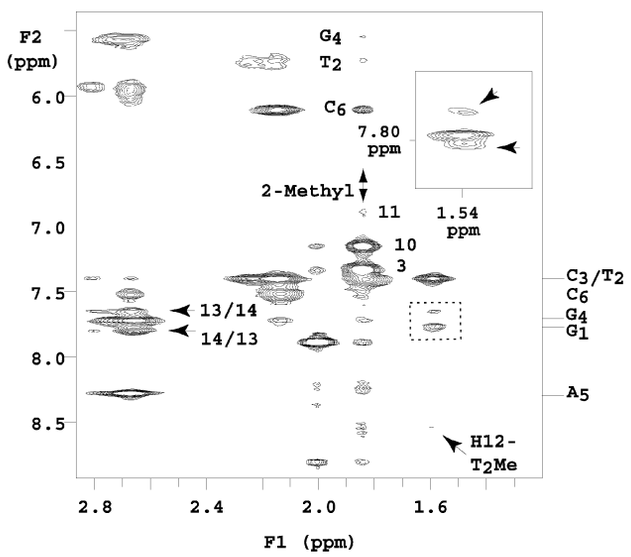

Fig. 5 Expansion of the NOESY spectrum (350 ms mixing time) of Δ-$[Ru(Me_2phen)_2dppz]^{2+}$ and $d(GTCGAC)_2$, at a metal complex:duplex ratio of 0.9 at 45 °C. The expansion shows the NOE connectivities from the hexanucleotide base H8/H6 (7.4 to 8.3 ppm) and sugar H1′ (5.5 to 6.2 ppm) protons and metal complex aromatic protons (6.8 to 8.8 ppm) to the hexanucleotide sugar H2′/H2″ and T_2Me protons and the metal complex methyl protons (1.3 to 2.8 ppm). NOEs between the metal complex H13/H14 protons and the hexanucleotide G_4H2′ and G_1H2′/H2″ protons are indicated. The NOEs between the metal complex H13/H14 protons and the hexanucleotide T_2Me protons are shown (in the dashed box). The insert is an expansion of the NOEs inside the dashed box but from a NOESY spectrum at 35 °C, where the H13/H14 to T_2Me protons NOEs (indicated by arrows) are more clearly observed.

hexanucleotide duplex will have started to melt; however, from the temperature dependence curves shown in Fig. 4 it is concluded that the duplex will still be largely intact. Most of the intermolecular NOEs observed at 45 °C were also detected in NOESY spectra recorded at 35 °C (see Fig. 6), although with lower resolution due to increased peak broadening. Intermolecular NOEs are observed from the Me_2phen ligand protons to the hexanucleotide H1′ and H4′/H5′/H5″ protons. In particular, to the H1′ protons of T_2, G_4, A_5 and C_6 and to the H4′/H5′/H5″ protons of C_3 and G_4. The sugar H1′ and H4′/H5′/H5″ protons are located in (or are most accessible from) the

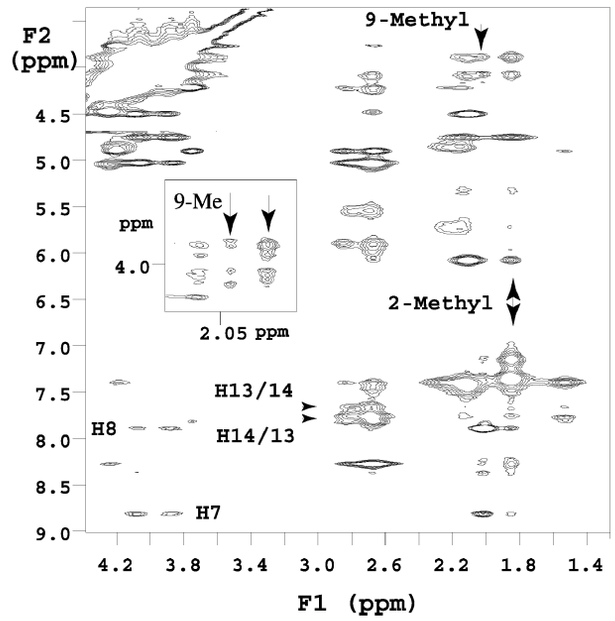

Fig. 6 Expansion of the NOESY spectrum (350 ms mixing time) of Δ-$[Ru(Me_2phen)_2dppz]^{2+}$ and $d(GTCGAC)_2$, at a metal complex : duplex ratio of 0.9 at 35 °C. NOEs from the Me_2phen H8, H7, 2-methyl and 9-methyl protons to hexanucleotide minor groove protons and NOEs from the dppz ligand H13/14 protons to hexanucleotide major groove protons are indicated. The insert shows the NOEs from the 2-methyl (and C_3H2′) and 9-methyl groups to the H4′/H5′/H5″ protons of the hexanucleotide (indicated by arrows) but at 45 °C where the cross-peaks are more clearly resolved.

hexanucleotide minor groove. No NOEs were observed from the Me_2phen protons to hexanucleotide major groove protons (H8/H6, TMe, H2′/H2″ and H3′). By contrast, intermolecular NOEs are only observed from the dppz H13/H14 protons to the hexanucleotide major groove protons, in particular the G_4H2′, G_1H2′/H2″ and T_2Me protons. The NOE data indicate that Δ-$[Ru(Me_2phen)_2dppz]^{2+}$ intercalates from the minor groove. In this binding mode the non-intercalating Me_2phen ligands would be located in the minor groove with the dppz ligand intercalated between the stacked bases and, due to the length of the dppz ligand, the H13/14 protons project out into the major groove.

The strongest NOE cross-peaks observed from the metal complex are to the hexanucleotide G_4 protons, indicating that the metal complex intercalates predominantly on either side of the G_4 base. However, the intermolecular NOEs observed between the H14 and the G_1H2′/H2″ protons indicate that the metal complex also intercalates between the A_5C_6 residues, indicating that Δ-$[Ru(Me_2phen)_2dppz]^{2+}$ demonstrates little sequence selectivity. As the metal complex exchanges relatively rapidly between the various binding sites, the observed intermolecular NOEs only represent a time-averaged indication of the binding of the metal complex with the hexanucleotide. Consequently, the NOE results only give an indication of the dominant binding modes and minor binding modes cannot be discounted.

Binding models

The metal complex did not bind exclusively at one site, and even if it had, rotation about the C_2 axis of the complex would result in averaging of contacts at all binding sites except the central C_3G_4. Consequently, only broad exchange-averaged resonances were observed from the metal complex and hexanucleotide, and therefore, the NMR data did not allow for the determination of a quantitative structure. However, a series of simple binding models was constructed to examine the proposed minor groove intercalation of the Δ-$[Ru(Me_2phen)_2dppz]^{2+}$ complex. For intercalation between the bases, the proximity of

Table 3 NOE cross-peaks observed between the Δ-[Ru(Me_2phen)$_2$dppz]$^{2+}$ and d(GTCGAC)$_2$, at a metal complex:duplex ratio of 0.9, in 10 mM phosphate (pH 7) containing 20 mM NaCl at 35 and/or 45 °C. The relative intensities of the NOEs are indicated (S = strong; M = medium; W = weak; and VW = very weak)

Metal complex proton	Hexanucleotide protons
H14	G_4H2′(S), G_1H2′(W), G_1H2″(W), T_2Me(M)
H13	G_4H2′(S), G_1H2′(W), G_1H2″(W), T_2Me(M)
H12	T_2Me(VW)
H10	C_3H4′(W)
9-Methyl	C_3H4′(M), G_4H4′/H5′/H5″(M)
H8	C_3H4′(M), G_4H4′/H5′/H5″(M)
H7	C_3H4′(M), G_4H4′/H5′/H5″(M)
H3	C_6H1′(M), G_4H1′(W)
2-Methyl	T_2H1′(W), G_4H1′(VW), A_5H1′(VW), C_6H1′(M), G_4H4′/H5′/H5″(M)

the metal complex 2-methyl protons to the guanine edges of the base pairs was the limiting factor to the extent that the dppz ring could be inserted into the base stack. Despite the potential for steric clashes, it was found that the metal complex could intercalate into B-type DNA such that the dppz ring just projected into the major groove. The ability of the metal complex to fully intercalate is aided by the significantly bowed Me_2phen ligands, as shown in the crystal structure. Minimised energies for binding at all three unique base-pair combinations differed over a range of only 10 kJ mol^{-1}, consistent with the lack of selectivity. It was also possible to produce minimised models with different orientations of the metal complex with respect to the DNA, again indicating a lack of selectivity. Close interproton contacts were observed in these models that were consistent with all observed intermolecular NOE cross-peaks. Models of G_4A_5 (see Fig. 7) and A_5C_6 intercalation from the minor groove were sufficient to provide explanations for all observed NOE contacts, but we would not rule out C_3G_4 intercalation. More importantly, all contacts were found to be consistent with intercalation from the minor groove, with only one observed NOE (H12 to T_2Me) consistent with intercalation from either groove.

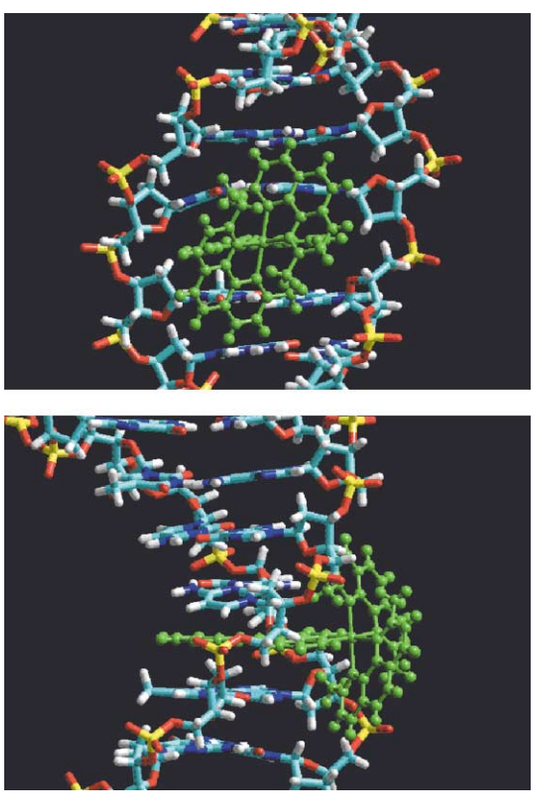

Fig. 7 Two views of a molecular model of Δ-[Ru(Me_2phen)$_2$dppz]$^{2+}$ bound to the hexanucleotide d(GTCGAC)$_2$, generated using HyperChem 5.0.[34] The model of the hexamer duplex was generated in HyperChem and the metal complex intercalated between the G_4 and A_5 residues. Energy minimisation of the hexamer was then carried out to convergence.

Discussion

Despite the considerable interest in the DNA binding of ruthenium(II) complexes based on the dppz ligand the mode of binding remains unresolved. An important aspect of the DNA binding mode, and a point of considerable controversy, is the determination of the groove from which the dppz complex intercalates. We have used the Δ-[Ru(Me_2phen)$_2$dppz]$^{2+}$ complex to study this aspect of DNA binding by dppz-based ruthenium(II) complexes.

While there is significant strain, due to the methyl groups, it has been demonstrated that the complex [Ru(Me_2phen)$_2$dppz]$^{2+}$ can be made and resolved. No experiments were specifically conducted to determine how stable this complex is. However, no noticeable degradation of the NMR resonances from the metal complex was observed in samples that were repeatedly used, for both the free and hexanucleotide-bound metal complex. From the crystal structure it was observed that the Me_2phen ligands are significantly bowed. This results in the methyl groups adjacent to the dppz ligand not protruding towards the dppz as far as may be expected. Hence, while the methyl groups will impact somewhat on the extent to which the metal complex can intercalate, they do not inhibit the complex from intercalating such that the dppz ligand projects into the opposite groove.

Addition of the metal complex to the hexanucleotide induced considerable upfield shifts for the metal complex dppz protons and the hexanucleotide imino protons. These resonances also displayed significant broadening, indicating intermediate exchange binding kinetics. The addition of the metal complex caused a 17 °C increase in the midpoint of the temperature dependence curve of the resonances from the hexanucleotide. These observations are in agreement with those reported by Dupureur and Barton in their hexanucleotide binding study of Δ-[Ru(phen)$_2$dppz]$^{2+}$,[15] and strongly suggest that Δ-[Ru(Me_2-phen)$_2$dppz]$^{2+}$ binds the hexanucleotide by intercalation. The observed pattern of intermolecular NOEs confirms that the metal complex binds by intercalation, as all the observed NOEs from the Me_2phen ligands are to the minor groove while those from the terminal protons on the dppz ligand are to the major groove. Furthermore, from the observed pattern of intermolecular NOEs it is concluded that Δ-[Ru(Me_2phen)$_2$dppz]$^{2+}$ intercalates from the minor groove. As the Me_2phen ligands are significantly bowed and the molecular modelling demonstrates that the Δ-[Ru(Me_2phen)$_2$(dppz)]$^{2+}$ complex can fully intercalate, we believe that the proposed minor groove binding is not a consequence of the methyl groups. The minor groove binding mode conclusion is consistent with the results of our recent hexanucleotide binding study of Δ- and Δ-[Ru(Me_2phen)$_2$-dpq]$^{2+}$,[21] thereby suggesting that ruthenium(II) polypyridyl complexes favor minor groove binding as originally proposed by Lincoln *et al.*[17]

Using the same hexanucleotide used in this study and the

Δ-[Ru(phen)$_2$(dppz)]$^{2+}$ complex, Dupureur and Barton proposed major groove binding.[15] This conclusion was based on the observation of an NOE from the H12 of the metal complex to the A_5H8 of the hexanucleotide. In our minor groove binding model, the metal complex H12 proton is positioned near the centre of the stacked bases. Consequently, it is possible that an NOE could be observed from the H12 protons to a major groove proton. Indeed, we observed a weak NOE from the H12 protons to the T_2Me protons.

Lincoln *et al.* have suggested that dppz-based complexes bind DNA in a cooperative manner.[40] This indicates that there could be two metal complexes bound to 50% of the hexanucleotide in the experiments where the ratio of the metal complex:hexanucleotide duplex is 0.9. However, the distribution of the bound metal complex should not affect the proposed binding model. The minor groove binding mode shown in Fig. 7 is only a qualitative model, based on observed NOEs from two or more binding sites.

While it may not appear that intercalation would be favoured in the minor groove on steric grounds, many DNA binding studies of relatively bulky molecules have demonstrated the flexibility of DNA. For example, Önfelt *et al.* reported that the bis-intercalating complex [μ-c4(cpdppz)$_2$-(phen)$_4$Ru$_2$]$^{4+}$ intercalates such that both Ru(phen)$_2$ moieties are in the same groove.[41] For this type of binding to occur the ruthenium bis-intercalator must thread through the DNA strands, thereby demonstrating the 'large amplitude of conformational change' that can occur in DNA.[41]

It has been clearly demonstrated that metallointercalators based on the 9,10-phenanthrenequinone diimine (phi) ligand intercalate from the major groove.[42–45] In particular, as the complex Δ-[Rh(phen)$_2$phi]$^{3+}$ intercalates from the major groove,[42] the results of this study could indicate that the structure of the intercalating ligand plays an important role in determining the DNA binding site. For the phi-based intercalators, the long axis of the aromatic rings run parallel to the long axis of the base-pairs, whereas for the dppz complexes the long axis of the aromatic rings run perpendicular to the long axis of the base-pairs. This difference in shape could lead to the observed difference in the binding modes. However, this proposal is not consistent with the groove binding preference of organic drugs that bind DNA by intercalation. For example, both actinomycin (long axis of the aromatic rings parallel to the long axis of the base-pairs) and the anthracycline drugs (aromatic rings perpendicular to the base-pairs) intercalate from the minor groove,[46] as do most positively charged organic drugs.[46] Hence, the difference in the preferred groove for binding by the metallointercalators may be related to the shape of the intercalating ligand, but probably only as a consequence of the steric bulk provided by the remainder of the octahedral complex.

Haq *et al.* have demonstrated that the intercalative binding of [Ru(phen)$_2$dppz]$^{2+}$ with DNA is entirely entropically driven, with hydrophobic interactions, changes in hydration and the release of counter ions being the dominant driving forces.[47] Hence, while the steric interactions of the non-intercalating part of the complex with the DNA grooves may affect the binding preference, the sequestering of the phenanthroline ligands from the water is also probably important. Rhodium(III) complexes containing the phi ligand and either aromatic or aliphatic ancillary ligands have been shown to intercalate from the major groove.[42–45] However, it has not been established whether dppz-based complexes with non-aromatic ancillary ligands will intercalate from the DNA minor groove in a similar fashion to [Ru(Me$_2$phen)$_2$dppz]$^{2+}$.

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