Resonance Raman Probing of the Interaction between Dipyridophenazine Complexes of Ru(II) and DNA

Colin G. Coates,[†] Luc Jacquet,[‡] John J. McGarvey,*,[†] Steven E. J. Bell,[†] Ala H. R. Al-Obaidi,[†] and John M. Kelly*,[‡]

Contribution from the School of Chemistry, Queen's University of Belfast, Belfast BT9 5AG, Northern Ireland, and the Chemistry Department, Trinity College, University of Dublin, Dublin 2, Ireland

Received January 7, 1997[⊗]

Abstract: Resonance Raman (RR) spectroscopy has been used to probe the interaction between dipyridophenazine (dppz) complexes of ruthenium(II), $[Ru(L)_2(dppz)]^{2+}$ (L = 1,10-phenanthroline (**1**) and 2,2-bipyridyl (**2**)), and calf-thymus DNA. Ground electronic state RR spectra at selected probe wavelengths reveal enhancement patterns which reflect perturbation of the dppz-centered electronic transitions in the UV−vis spectra in the presence of DNA. Comparison of the RR spectra recorded of the short-lived MLCT excited states of both complexes in aqueous solution with those of the longer-lived states of the complexes in the DNA environment reveals changes to excited state modes, suggesting perturbation of electronic transitions of the dppz ligand in the excited state as a result of intercalation. The most prominent feature, at 1526 cm^{-1}, appears in the spectra of both **1** and **2** and is a convenient marker band for intercalation. For **1**, the excited state studies have been extended to the Δ and Λ enantiomers. The marker band appears at the same frequency for both but with different relative intensities. This is interpreted as reflecting the distinctive response of the enantiomers to the chiral environment of the DNA binding sites. The results, together with some analogous data for other potentially intercalating complexes, are considered in relation to the more general application of time-resolved RR spectroscopy for investigation of intercalative interactions of photoexcited metal complexes with DNA.

Introduction

In recent years, considerable attention has been devoted to the design of small, non-radioactive molecules capable of selectively binding to nucleic acids.[1−4] To characterize the nature of the interactions involved and to explore the possible photophysical implications, a range of techniques have been brought to bear, including time-resolved luminescence methods,[3] linear dichroism,[4a] and calorimetry.[4b]

Resonance Raman (RR) and time-resolved resonance Raman (TR[3]) methods are particularly effective for probing the vibrational structure of the excited states of transition metal and organometallic complexes.[5] An attractive feature of these techniques is versatility, enabling studies in both homogeneous and heterogeneous media, reflected in the latter instance in their ability to provide valuable insight on the effect of microheterogeneous environments such as colloids or micelles.[6] Previous RR investigations of the interaction of Ru(II) complexes with DNA have been limited to cases of relatively low binding affinities,[6,7] most probably nonintercalative. Some evidence of porphyrin intercalation into DNA has however been detected successfully by the RR technique.[8]

Due to their high affinity toward DNA, mixed ligand complexes of the type $[Ru(L)_2dppz]^{2+}$ (L = 1,10-phenanthroline (phen) (**1**), 2,2′-bipyridine (bpy) (**2**); dppz = dipyrido[3,2-*a: 2′,3′-c*]phenazine) have been investigated in some detail.[3,4] Barton and co-workers have proposed an intercalative binding mode of the dppz ligand with DNA, resulting in a significant enhancement of the emission from the lowest MLCT excited state[3b] and prompting the proposal that the complex can act as a molecular "light switch" reporter of DNA intercalation. Recent studies on the interaction with DNA of the enantiomers of **1** by single photon counting, circular dichroism, and calorimetric methods[4a,b] suggest the possibility of detecting differences in the response of the enantiomers to the DNA environment.

We report here the use of RR spectral techniques as a probe of the interaction of **1** and **2** with DNA. In particular, we have employed transient resonance Raman (TR[2]) spectroscopy as a further handle on the photophysics in DNA of the racemic forms of both **1** and **2** and of the Δ and Λ enantiomers of the former. The aim of the latter study was to explore whether TR[2] techniques might be effective in discerning possible distinctive

[†] Queen's University.
[‡] Trinity College.
[⊗] Abstract published in *Advance ACS Abstracts,* July 1, 1997.
(1) (a) Jacquet, L.; Kelly, J. M.; Kirsch-De Mesmaeker, A. *J. Chem. Soc., Chem.* Commun. **1995**, 913 and references therein. (b) Coates, C. G.; Jacquet, L.; McGarvey, J. J.; Bell, S. E. J.; Kelly, J. M.; Al-Obaidi, A. H. R. *J. Chem. Soc., Chem. Commun.* **1996**, 35.
(2) Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. *Biochemistry* **1992**, *31*, 9319.
(3) (a) Friedman, A. E.; Chambron, J.-C.; Sauvage, J.-P.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 4960. (b) Friedman, A. E.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *Nucleic Acids Res.* **1991**, *19*, 2595. (c) Jenkins, Y.; Friedman, A. E.; Turro, N. J.; Barton, J. K. *Biochemistry* **1992**, *31*, 10809. (d) Hartshorn, R. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 5919. (e) Arkin, M. R.; Stemp, R. E.; Holmin, R. E.; Barton, J. K.; Hormann, A.; Olson, E. J. C.; Barbara, P. F. *Science* **1996**, *273*, 475.
(4) (a) Hiort, C. H.; Lincoln, P.; Norden, B. *J. Am. Chem. Soc.*, **1993**, *115*, 3448. (b) Hag, I.; Lincoln, P.; Suh, D.; Norden, B.; Chowdhry, B. Z.; Chaires, J. B. *J. Am. Chem. Soc.* **1995**, *117*, 4788. (c) Turro, C.; Bossman, S. H.; Jenkins, Y.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc.* **1995**, *117*, 9026.
(5) (a) Al-Obaidi, A. H. R.; Gordon, K. C.; McGarvey, J. J.; Bell, S. E. J.; Grimshaw, J. *J. Phys. Chem.* **1993**, *97*, 10942 and references therein. (b) Strouse, G. F.; Schoonover, J. R.; Duesing, R.; Boyde, S.; Jones, W. E., Jr.; Meyer, T. J. *Inorg. Chem.* **1995**, *34*, 473 and references therein.

(6) Turro, C.; Bossman, S. H.; Leroi, G. E.; Barton, J. K.; Turro. N. J. *Inorg. Chem.* **1994**, *33*, 1344.
(7) Tysoe, S. A.; Morgan, R. J.; Baker, D.; Strekas, T. C. *J. Phys. Chem.* **1993**, *97*, 1707.
(8) Scheider, J. H.; Odo, J.; Nakamoto, K. *Nucleic Acids Res.* **1988**, *16*, 10232.

interactions of the MLCT excited states of the enantiomers with the chiral, microheterogenous environment of the DNA.

Experimental Section

Chemicals. Calf thymus DNA and $RuCl_3 \cdot xH_2O$ were purchased from Aldrich Sigma, phenanthroline and bipyridine[9] from Merck. Λ and Δ Ru(phen)$_2$phendione(PF$_6$)$_2$ were a gift from P. Lincoln and B. Norden. Dppz was prepared as described by Norden et al.[4a] Literature methods[3] were used for preparation of racemic [Ru(phen)$_2$dppz]Cl$_2$ and [Ru(bpy)$_2$dppz]Cl$_2$. DNA was dialyzed three times in pH 7 phosphate buffer (10^{-2} M). UV−vis spectrophotometry was employed to check DNA purity (A_{260}:A_{280} > 1.9) and concentration (ϵ = 6600 dm^3 mol^{-1} cm^{-1} at 260 nm). All measurements were obtained from solutions which were ∼10^{-4} M in metal complex. An aerated phosphate buffer (10 mM phosphate, 50 mM NaCl, pH 7) was used to provide an aqueous environment. The ratio of [DNA phosphate]:[Ru] employed throughout was 20:1, to ensure strong interaction of the complex with binding sites.

[Ru(dppz)$_3$]Cl$_2$. $RuCl_3 \cdot xH_2O$ (260 mg:1.0 mmol) was refluxed for 24 h with excess dppz in ethanol (1.0 g:3.5 mmol). The evolution of the reaction was followed by UV−vis spectrometry. After reaction completion, the mixture was purified on Sephadex SP-C25 (eluent water/ethanol 20:80, HCl 0.5 M) and precipitated as the PF$_6$ salt. NMR in DMSO$_{d_6}$: 9.68 (d), 8.56 (dd), 8.41 (d), 8.22 (dd), 7.99 (dd). Overall yield 12%. The chloride salt was obtained by ion exchange chromatography: Sephadex A25 (1 cm × 10 cm).

Δ and Λ [Ru(phen)$_2$dppz](PF$_6$)$_2$. A 20 mg:0.021 mmol mixture of Λ or Δ [Ru(phen)$_2$phendione](PF$_6$)$_2$ was refluxed with an excess of 1,2-diaminobenzene (10 mg:0.092 mmol) in a 5/1 EtOH:CH$_3$CN mixture in the presence of *p*-toluenesulfonic acid for 24 h. The complexes were purified on Sephadex SP-C25 using a water:EtOH 5/1, NaCl (0.3 M) mixture as eluent and precipitated as PF$_6$ salts: overall yields 85%.

Instrumentation. UV−vis absorption spectra were recorded with a diode array spectrophotometer (Hewlett Packard Model 8452A).

Ground state Raman spectra were recorded with a CCD detector (Princeton Instruments Model LN/UV 1152) coupled to a Jobin-Yvon HR640 spectrometer. The excitation source was an Ar$^+$ laser (Spectra Physics Model 2025) with outputs at 351 and 363.8 nm and in the visible range 450−528 nm.

Laser flash photolysis and time resolved excited-state absorption (ESA) measurements employed rapid response spectrophotometric detection coupled to a digitiser (Tektronix 7912AD) for single channel transient kinetic studies and a gated dual diode array multichannel detector (Princeton Instruments Model DIDA 700G) to record ESA spectra.[5a,10] For both flash photolysis and transient Raman studies (see below), excitation wavelengths covering the range 355−600 nm were provided by Q-switched Nd:YAG lasers (Spectra Physics Models DCR2 and GCR3) operating at 355 or 532 nm, coupled to a dye laser or a stimulated Raman wavelength-shifting cell filled with H$_2$ or CH$_4$.

Excited state resonance Raman spectra were generated by the well-known single-color pump and probe method[10,11] in which the leading edge of the laser pulse incident on the sample pumps the molecules into the excited state and the trailing edge probes the Raman scattering.

Samples were contained in spinning cells in order to minimize the possibility of thermal degradation and/or photodegradation, especially where relatively long spectral accumulation times (10−15 min) at a pulse repetition rate of 10 Hz were required to obtain transient RR signals of good signal quality. The transient spectra were recorded with a multichannel detector (EG&G OMA III with Model 1420B intensified detector) coupled to a triple spectrometer of simple, in-house design, described earlier.[10] Incident pulse energies were typically *ca.* 3 mJ for the excited state RR experiments and somewhat higher for flash photolysis and ESA measurements, in the region of 10 mJ. The concentration of **1** and **2** used for resonance Raman (ground state and
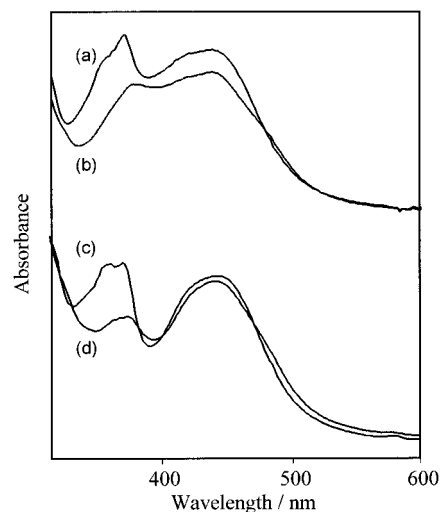
(9) Ru(bpy)$_3$$^{2+}$ and Ru(phen)$_3$$^{2+}$ were available in high purity from previous work.

(10) Gordon, K. C.; McGarvey, J. J. *Inorg. Chem.* **1991**, *30*, 2986.

(11) Dallinger, R. F.; Woodruff, W. H. *J. Am. Chem. Soc.* **1979**, *101*, 4391.

Figure 1. UV−vis spectra of [Ru(phen)$_2$(dppz)]$^{2+}$ (a and b) and [Ru(bpy)$_2$(dppz)]$^{2+}$ (c and d): (a and c) in buffer; (b and d) [DNA-phosphate]:[Ru] ratio of 20:1.

transient) and flash photolysis studies was typically 10^{-4} mol dm^{-3}, throughout the investigation.

Results

While the primary focus of this study was the behavior of the lowest MLCT excited states of **1** and **2** in the DNA environment, a parallel study of the ground electronic states, especially of the resonance Raman spectroscopy using CW excitation, provides an important baseline for comparison.

Ground State. Figure 1 shows the changes which occur in the UV−vis spectra for both **1** and **2** in the presence of DNA. The spectral changes are consistent with published data.[4a] The most striking effect is observed in the absorption band near 374 nm, assignable to an intraligand (IL) transition of the dppz entity, involving a marked decrease in intensity coupled with a red shift in λ_{max} to 382 nm. The broad, poorly-resolved absorption in the visible region, arising from MLCT transitions to both dppz and phen/bpy ligands, also undergoes a slight intensity decrease and further band broadening in the presence of DNA, with an isosbestic point at 480 nm.

Ground state resonance Raman spectra of **1** and **2** were recorded at excitation wavelengths of 457.9 and 488 nm, both in buffer solution and in the presence of DNA. Figure 2 shows the spectra at both excitation wavelengths for **1** in the two environments. To assist with the assignment of vibrational modes, a ground state resonance Raman spectrum was also recorded of the homoleptic complex [Ru(dppz)$_3$]$^{2+}$ at an excitation wavelength of 457.9 nm, enabling the identification of dppz-associated vibrations at 1600, 1575, 1495, 1472, 1448, 1407, 1359, 1311, 1264, and 1188 cm^{-1} (Figure 3). Two significant points emerge from comparison with the RR spectra of **1**. Firstly, in the absence of DNA, it is apparent (Figure 2) on switching from 457 to 488 nm excitation that the relative intensities of dppz features increase with respect to phen features, suggesting that the degree of resonance enhancement of dppz vibrational modes becomes greater at longer wavelengths. Secondly, it is clear that a lowering in intensity of dppz features with respect to phen features occurs upon DNA addition, but the extent of this effect evidently decreases toward longer probe wavelengths. Indeed, in the spectra recorded with 488 nm excitation the difference in degree of enhancement between the dppz and phen modes is negligible. An entirely analogous situation holds for the [Ru(bpy)$_2$dppz]$^{2+}$ complex.

To explore the region of dppz-centered intraligand absorption, ground state resonance Raman spectra of the complexes were
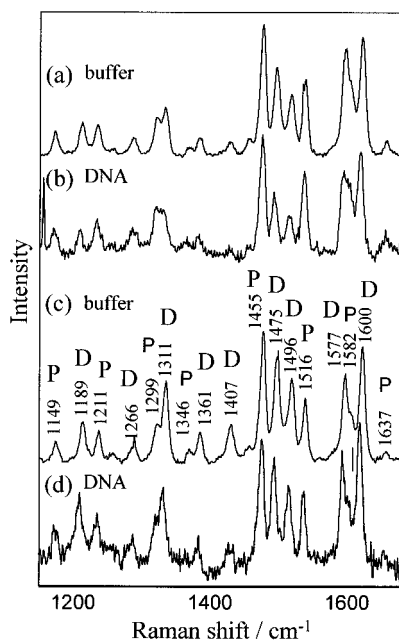
Figure 2. Ground state RR spectra of [Ru(phen)$_2$(dppz)]$^{2+}$ recorded at λ_{ex}: 457.9 nm (a and b); 488 nm (c and d); (a and c) in buffer; (b and d) [DNA-phosphate]:[Ru] ratio of 20:1. P and D denote phen and dppz modes.
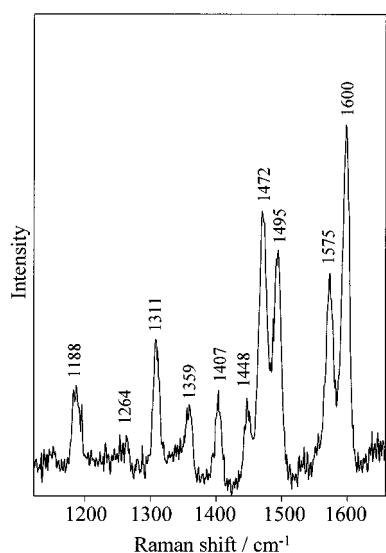

Figure 3. Ground state RR spectra of Ru(dppz)$_3^{2+}$ in ethanol (solvent bands subtracted) recorded at λ_{ex}: 457.9 nm.

recorded with 363.8 nm excitation, both in buffer solution and DNA bound, as before. Spectra recorded at this wavelength for both **1** and **2** in buffer alone are very similar, consisting largely of modes assignable to dppz (Figure 4). Closer inspection, however, shows minor differences, with weak features attributable to bpy or phen in the respective spectra. In the presence of DNA a marked decrease in resonance enhancement of dppz features is evident across the entire spectrum for either complex. Although weak, the remaining features can be observed to be in the same positions as the bpy/phen modes identified in the spectra recorded in buffer alone.

Excited State. In aqueous buffer solution, in the absence of DNA, we have been unable to observe excited state absorption (ESA) spectra on a nanosecond time scale for either **1** or **2**. This observation is consistent with the reported[3] absence of luminescence from either complex in aqueous solution on this time scale. Very recent work[3e] by Barton, Barbara, *et al.* reports a lifetime of 250 ps for Δ-Ru(phen)$_2$dppz^{2+}. However, ESA

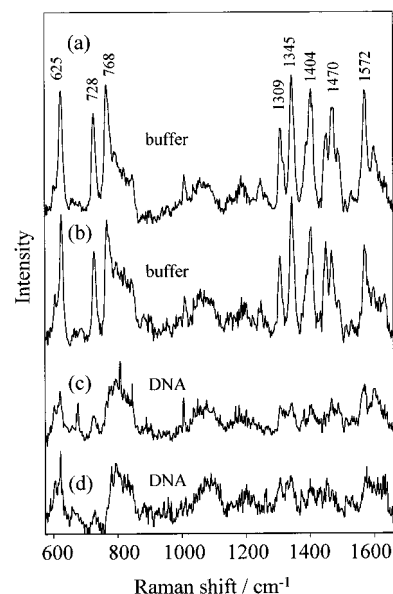**Figure 4.** Ground state RR spectra of [Ru(bpy)$_2$(dppz)]$^{2+}$ (a and c) and [Ru(phen)$_2$(dppz)]$^{2+}$ (b and d) at λ_{ex} = 363.8 nm: (a and b) in buffer; (c and d) [DNA-phosphate]:[Ru] ratio of 20:1.

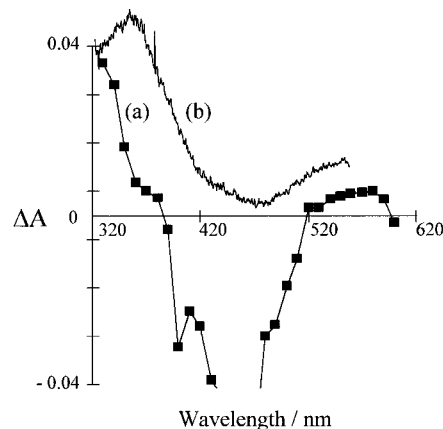**Figure 5.** Transient absorption spectra of [Ru(phen)$_2$(dppz)]$^{2+}$ recorded in the presence of DNA, λ_{ex} = 355 nm: (a) point-by-point absorbance difference (ΔA) spectrum uncorrected for ground state depletion; (b) excited state absorption spectrum, recorded with use of a gated multichannel diode array, 30 ns after laser excitation (spectrum corrected for ground state absorption).

spectra in the presence of DNA are readily observed on the nanosecond time scale. The point-by-point absorbance difference (ΔA) spectrum for **1** is shown in Figure 5a, exhibiting features both to the blue and to the red of the ground state depletion. Also shown (Figure 5b) is an ESA spectrum of **1** recorded 30 ns after laser excitation and corrected for ground state depletion. Spectra of **2** recorded under identical conditions are very similar. Decay of the ΔA signal in the presence of DNA was clearly nonexponential, consistent with the independent findings from earlier luminescence decay measurements.[3c,e]

Transient resonance Raman experiments were performed with use of the single-color laser pulse technique at excitation wavelengths of 480 and 355 nm on complexes **1** and **2** in the presence and absence of DNA. Figure 6 shows spectra recorded for **1** with pulsed 480 nm excitation. For the complex in buffer solution (trace a), the pulse energy is sufficiently low and the excited state lifetime sufficiently short that no appreciable population of the excited state occurs and the spectrum mainly displays ligand vibrations, enhanced in resonance with electronic transitions from the ground state. Following the addition of DNA, however, a spectrum recorded at the same pulse energy
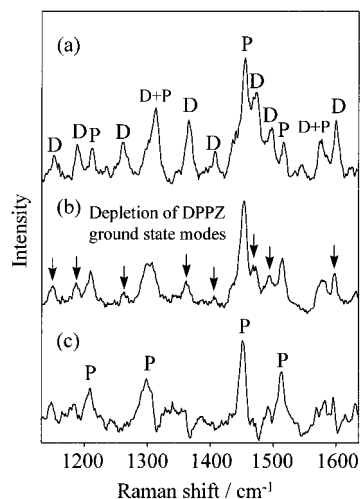
Figure 6. Excited state resonance Raman spectra of [Ru(phen)₂(dppz)]²⁺ recorded with use of the single-color laser pulse technique (laser pulse duration *ca.* 8 ns; see Experimental Section for details) at $\lambda_{ex} = 480$ nm; pulse energy 3 mJ: (a) in buffer; (b) [DNA-phosphate]:[Ru] ratio of 20:1; (c) a subtraction spectrum, (b − a), scaled for complete removal of residual dppz ground state features.
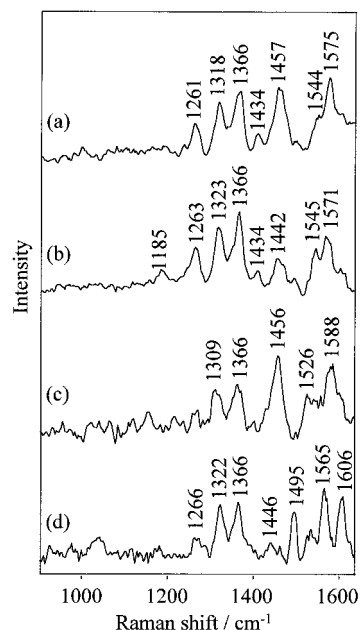

Figure 7. Excited state resonance Raman spectra of [Ru(phen)₂(dppz)]²⁺ (a and c) and [Ru(bpy)₂(dppz)]²⁺ (b and d) recorded with the single-color laser pulse method (laser pulse duration *ca.* 8 ns) at $\lambda_{ex} = 355$ nm (pulse energy was such that essentially 100% conversion was achieved): (a and b) in buffer; (c and d) [DNA phosphate]:[Ru] ratio of 20:1.

as for trace a showed a marked diminution of ground state dppz features, consistent with depletion of the ground state by the laser flash under these conditions (trace b). Subtraction of residual dppz ground state modes (b − a), reveals modes attributable to neutral phen. A similar situation obtains for the spectra of **2** recorded under equivalent conditions. In this case (spectra not shown), only modes attributable to neutral bpy ligand remain, following subtraction of dppz features.

Spectra recorded in the presence and absence of DNA for both **1** and **2** at higher pulse energies and at an excitation wavelength of 355 nm are shown in Figure 7. The conditions in this case in either environment are such that a large conversion to the MLCT excited state of the complex under study is brought about. Consistent with this, the RR spectra recorded for **1** and **2** in buffer (Figure 7, traces a and b) clearly reveal several modes
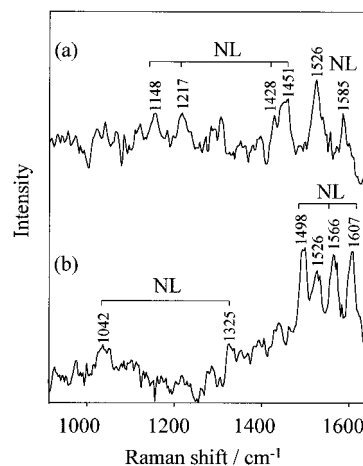

Figure 8. Scaled subtractions of spectra shown in Figure 7. [Spectra recorded in the presence of DNA] − [spectra recorded in aqueous buffer solution] of (a) [Ru(phen)₂(dppz)]²⁺ and (b) [Ru(bpy)₂(dppz)]²⁺. Each subtraction is scaled for complete removal of the 1366 cm⁻¹ dppz•⁻ feature. NL denotes neutral ligand modes of the corresponding ancillary ligands.

which can be attributed to the coordinated dppz•⁻ radical anion, on the basis of their common observation in the spectra of **1** and **2** and the fact that they increase with increasing laser power. The addition of DNA resulted in significant changes to the excited state spectra for both **1** and **2**, as shown by traces c and d in Figure 7. Initial inspection indicates that the intensities of several dppz•⁻ features decrease with respect to the corresponding neutral ligand modes of the ancillary ligands. Figure 8 shows the result of subtracting spectra recorded in buffer for each complex from those recorded in the presence of DNA. [The subtractions were scaled to the 1366-cm⁻¹ band (Figure 7) in order to cancel the dppz•⁻ features.] For each subtracted spectrum, it is evident that the remaining features are attributable mainly to neutral ligand vibrations (L = phen or bpy), possibly enhanced through resonance with LMCT transitions in the respective [Ruᴵᴵᴵ(L)₂dppz•⁻]²⁺ excited states populated within the laser pulse. These neutral ligand modes are more evident in the spectra of **1** than of **2** simply as a consequence of the greater RR scattering cross section of the bpy compared to the phen ligand.[12,13] More significantly, however, the subtracted spectra for both complexes show a prominent additional feature at 1526 cm⁻¹, which is not due to neutral ligand in the [Ruᴵᴵᴵ(L)₂dppz•⁻]²⁺ excited state, nor to ground state dppz.

This band appeared consistently in spectra recorded in many independently prepared samples of **1** and **2** in aqueous buffer *but only in the presence of* DNA. Moreover, the band could be observed at the commencement of a period of spectral accumulation and, apart from the expected improvement in signal to noise with accumulation time, there was no increase in relative signal height, effectively ruling out the possibility that the feature might have been the result of sample photodecomposition. A feature at 1526 cm⁻¹ was also observed in the RR spectra of **1** and **2** in acetonitrile, recorded with use of pulsed 355 nm excitation, under conditions of power and beam focus similar to those employed for the experiments in DNA shown in Figure 7. In addition to the 1526-cm⁻¹ band, several other features attributable to dppz•⁻ were evident in the spectra recorded in MeCN solution, as well as a number of bands due to neutral ligand modes (phen or bpy in **1** and **2**, respectively).[14]

(12) Kumar, C. V.; Barton, J. K.; Turro. N. J.; Gould, I. R. *Inorg. Chem.* **1987**, *26*, 1455.
(13) Chang, Y. J.; Xu, X.; Yabe, T.; Yu, S.; Anderson, D. R.; Orman, L. K.; Hopkins, J. B. *J. Phys. Chem.* **1990**, *94*, 729.
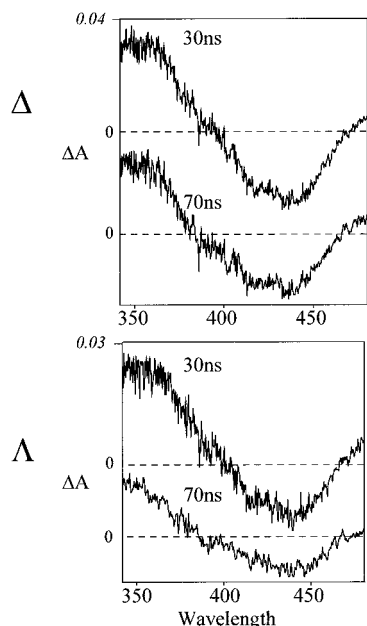
Figure 9. ESA spectra recorded of enantiomers of [Ru(phen)₂(dppz)]²⁺ in the presence of DNA, λ_{ex} = 355 nm, at 30 and 70 ns after laser excitation, uncorrected for ground state depletion.

For comparative purposes, transient RR spectra were also recorded for the complex [Ru(bpy)₃]²⁺ at the same two excitation wavelengths, 480 and 355 nm. In contrast to the situation with **1** and **2**, the spectra observed for Ru(bpy)₃²⁺ were independent of the environment, i.e. buffer solution or DNA.

Studies with Enantiomers. Transient resonance Raman and ESA spectra were also recorded for optically pure Δ and Λ enantiomers of **1**. At a given pulse energy, the ESA spectra recorded for the two enantiomers in the presence of DNA over the spectral range 300−400 nm were indistinguishable, closely resembling the spectrum of the racemate of **1** shown in Figure 5. Figure 9 shows ESA spectra for each of the DNA-bound enantiomers of **1** recorded at delays of 30 and 70 ns after laser excitation. The more rapid decay of the Λ enantiomer is apparent and in line with the lower luminescence quantum yield reported for this enantiomer when intercalated.[4a]

Transient resonance Raman spectra recorded for each enantiomer by the single-color technique with 355 nm excitation are shown in Figure 10. As in the experiments with the racemates of **1** and **2**, the pulse energy used was sufficient to ensure a high degree of conversion to the MLCT excited state in both the presence and absence of DNA. Following the approach used above, the transient RR spectra recorded for the individual enantiomers in buffer solution were subtracted from the corresponding spectra of the DNA-bound species. The results are displayed in traces a and b of Figure 10. It is apparent that the intensity of the 1526-cm⁻¹ band is greater for the Δ enantiomer than for the Λ form. For comparison, trace c shows the average of the spectra of the individual enantiomers and trace d the corresponding spectrum of the racemate of **1**. The excited state RR spectra of the two enantiomers have also been recorded in acetonitrile and are shown in Figure 11. In this case the 1526-cm⁻¹ band again appears, but the relative intensity is identical for the two enantiomers.

---

(14) Attempts to record RR spectra of the dppz•⁻ radical anion, electrochemically generated from the dppz ligand in a thin layer electrode cell, proved impossible due to strong sample luminescence: Waterland, M. R.; Gordon, K. C.; McGarvey, J. J.; Jayaweera, P. M. Paper in preparation.
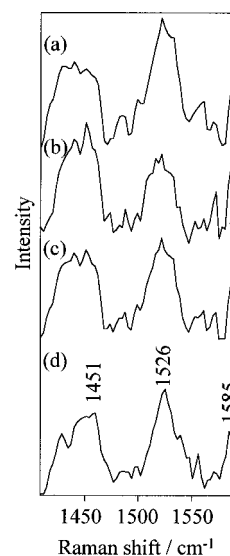

Figure 10. Scaled subtractions of the transient resonance Raman spectra of the Δ and Λ enantiomers of [Ru(phen)₂(dppz)]²⁺ recorded with use of the single-color laser pulse technique at λ_{ex} = 355 nm: spectra recorded in the presence of DNA ([DNA phosphate]:[Ru] ratio of 20:1) − spectra recorded in aqueous buffer solution. The region surrounding the 1526-cm⁻¹ feature only is displayed: (a) Δ; (b) Λ; (c) average of Δ and Λ spectra (i.e. {spectra a + b}/2]; (d) the racemic mixture.
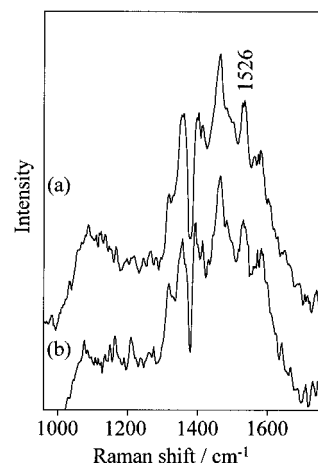

Figure 11. Excited state resonance Raman spectra of enantiomers of [Ru(phen)₂(dppz)]²⁺ in acetonitrile, recorded within the pulse duration (<10 ns) at λ_{ex} = 355 nm; pulse energy 3 mJ: (a) Λ form; (b) Δ form.

Discussion

Consideration of both the ground and excited state resonance Raman (RR) spectra illustrates the value of RR spectroscopy as a probe of intercalation, with the ability to highlight changes in the vibrational mode patterns of the ligands involved. Discussion of appropriate spectral features of the ground states is a necessary prelude to examination of the excited state data, which are the primary focus of the overall investigation.

The most marked changes which occur in the ground state (CW laser-excited) RR spectra of complexes **1** and **2** (Figures 2 and 4) are associated with the intercalating dppz ligand. Consideration of the data in Figure 2 enables at least a partial resolution of the contributions to the broad MLCT absorption in the visible region near 450 nm (Figure 1). In particular, the increase in relative intensities of dppz features with respect to phen features on moving from 457 to 488 nm excitation of **1** suggests that the dppz component of the MLCT absorption lies to the red of the phen component (or in the corresponding case of **2**, the bpy component), consistent with the respective degrees
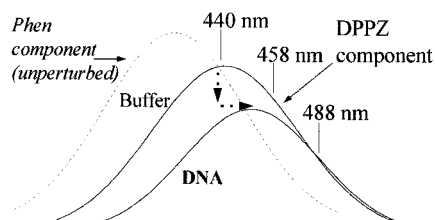
of π-acceptor character of the ligands. Upon addition of DNA, a lowering in intensity of dppz features with respect to phen modes, which is evident in the spectra probed at 457 nm (Figure 2a,b) but hardly discernible in the corresponding spectra recorded with 488 nm excitation (Figure 2c,d), suggests that upon intercalation the dppz component of the MLCT absorption band undergoes not only a lowering in intensity but also a red shift.[15] The corresponding spectra for **2** point to a similar conclusion. This link between MLCT band shift and the intercalation of a ligand L appears to be more widely applicable, as studies[16] on the complex [Ru(tap)$_2$dppz]$^{2+}$ (tap = 1,4,5,8-tetraazaphenanthrene) seem to demonstrate. In this case, charge-transfer transitions to tap and dppz ligands are more clearly separated than in **1** or **2**, with λ_{max} located at 454 and 412 nm, respectively, in aqueous buffer. Addition of DNA results in a well-defined decrease in intensity and accompanying red shift to 420 nm of the 412-nm band, but the 454-nm band remains largely unperturbed.

The higher energy band near 360 nm in the spectra of **1** and **2** is primarily attributable to dppz-centered intraligand absorption, as the RR spectra recorded with CW excitation at 363.8 nm (Figure 4) indicate. The observed vibrational features are largely assignable to dppz modes,[17] and the decreases in band intensities upon addition of DNA are readily correlated with the significant lowering in intensity of the intraligand band (Figure 1) upon intercalative binding to DNA.

In aqueous buffer solution, in the absence of DNA, decay of the lowest MLCT excited states of these complexes is extremely efficient. The well-established enhancement of the MLCT-centered emission, which occurs[3b] upon addition of DNA, is indicative of a longer excited state lifetime under these conditions, and the readily observed ESA spectra of the lowest MLCT excited states of **1** or **2** in the presence of DNA (Figure 5) are consistent with this. The absorption features appearing to the blue and to the red of the ground state depletion are attributable to $\pi^*-\pi^*$ transitions of the coordinated dppz$^{\bullet-}$ ligand on which the electron is localized in the MLCT excited state, as confirmed by the transient resonance Raman spectra referred to earlier (Figure 7) and considered further below.

The marked diminution of ground state dppz features in the 480-nm pulsed laser-excited RR spectrum of **1** (Figure 6) upon addition of DNA is the result of depletion of the ground state by the laser flash. That this is most likely the case and that the depletion of dppz modes is not merely the result of a decrease in the degree of resonance enhancement at the probe wavelength, caused by shifts in the ground state UV–vis absorption spectrum upon addition of DNA (Figure 1), is suggested by the fact that 480 nm is an isosbestic point for the spectra shown in Figure 1. The modes due to neutral phen which emerge when residual dppz modes are subtracted (trace c, Figure 6) cannot be ground

state phen modes arising from resonance with the ground electronic state MLCT transition since the marked reduction in intensity of dppz modes indicates significant depletion of the ground state by the laser pulse. (Spectra recorded at a similar wavelength, 488 nm, but using *CW excitation* show no change in band intensities upon addition of DNA.) Furthermore, the intensity distribution for the phen modes in Figure 6 is not that expected for resonance with the electronic ground state transition. The most reasonable conclusion is that these phen modes may arise as a result of resonance with a LMCT [phen → Ru^{III}] transition of the excited state species, expected to be populated by excitation at 480 nm if the excited state lifetime is increased upon intercalation to DNA. Again, this is consistent with the earlier reports[3b,e] of enhanced and longer-lived luminescence from the lowest MLCT excited state of the DNA-intercalated complex.

The appearance of several features attributable to the radical-like dppz$^{\bullet-}$ ligand in the 355 nm excited RR spectra of **1** and **2** recorded in buffer (Figure 7) points to electron localization on the dppz moiety in the lowest MLCT excited states of both complexes. The new feature common to the excited state RR spectra of both, which appears at 1526 cm^{-1}, *but only upon the addition of* DNA (Figure 8), is also assigned as a mode of the dppz$^{\bullet-}$ radical anion. Evidently, it arises through resonance with a $\pi^*-\pi^*$ transition of dppz$^{\bullet-}$, which is distinct from that observed in the absence of DNA and is apparently linked to the intercalative interaction of the dppz ligand with DNA binding sites. The fact that the appearance of the band is independent of the ancillary ligands is consistent with this proposal, since they do not intercalate with the binding sites. Further measurements in progress[16] illustrate the more general use of the transient RR technique to probe the excited states of other intercalating or partially intercalating complexes. One example[18] is the complex [Ru(bpy)$_2$(HAT)]$^{2+}$ (HAT = 1,4,5,8,9,12-hexaazatriphenylene). In this case[16] the lifetime of the lowest MLCT excited state is measurable in both aqueous buffer and DNA. While the ESA spectra in the two environments are indistinguishable, preliminary results[16] from transient RR measurements show enhancement patterns of excited state modes of HAT$^{\bullet-}$ in the DNA-bound complex, which are different from the pattern in aqueous solution, thus providing more concrete evidence of intercalative interaction of the HAT ligand in this instance. The contrasting observation in relation to the RR spectra of the lowest MLCT excited state of Ru(bpy)$_3$$^{2+}$ is interesting. In this case, the spectra recorded in buffer solution or in the presence of DNA are identical, as should be expected since this complex is known to bind to DNA without intercalation.

Since the feature at 1526 cm^{-1} attributed to a mode of dppz$^{\bullet-}$ appears in the excited state resonance Raman spectra of **1** and **2** recorded in both the DNA and acetonitrile environments but not in the aqueous buffer solutions, it is reasonable to regard the band as characteristic of a nonaqueous environment. While it is not possible to make a precise assignment to a particular vibrational mode of dppz$^{\bullet-}$ with the data currently available, its appearance in the excited state both in CH$_3$CN and when the complex is bound to DNA, but not in aqueous solution, suggests that the vibration is particularly sensitive to the complexation/protonation of the dppz$^{\bullet-}$ ligand by water, which has been proposed[3] as the reason for the short lifetime of the excited state in aqueous environments.

It is significant that although the transient RR spectra of the individual, DNA-bound Δ and Λ enantiomers of **1** (Figure 10)

---

(15) The enhancement patterns observed in the ground state RR spectra at each probe wavelength in the presence and absence of DNA suggest a lowering of intensity coupled with a red shift of the dppz component of the MLCT absorption upon intercalation, represented schematically here:

(16) Coates, C. Ph.D. Thesis, QUB, 1996; to be published.
(17) There are minor differences between the spectra of **1** and **2**. Closer inspection reveals weak features attributable to phen or bpy, respectively, suggesting a small degree of overlap in this spectral region between the dppz intraligand absorption and the MLCT transitions to bpy/phen ligands.

(18) De Buyl, F.; Kirsch-De Mesmaeker, A.; Tossi, A.; Kelly, J. M. *J. Photochem. Photobiol. A, Chem.* **1991**, *60*, 27.

both reveal the same 1526-cm^{-1} mode as recorded for the racemate (Figure 8), the relative intensity of the band is greater in the case of the Δ enantiomer. In contrast, in CH$_3$CN, the feature appears *with the same relative intensity* in the transient RR spectra of the individual enantiomers of **1**.

To account for the intensity differences in the 1526-cm^{-1} mode in the transient RR spectra of the two enantiomers in the presence of DNA, one might look first for differences between the ESA spectra of the two forms. Although no immediately obvious differences appear in the respective ESA spectra (Figure 9), the explanation for the differences in the resonance Raman data may still lie in this direction, due to the ability of the RR technique to probe subtle differences in the relative intensities of transitions at a given probe wavelength. It is possible to envisage two closely overlapped transitions within the 300−400 nm absorption envelope of the dppz$^{•−}$ entity, with one yielding an enhancement pattern which is more evident in the RR spectrum seen in water while the other gives rise to a similar enhancement pattern but with the additional 1526-cm^{-1} mode as observed in the nonaqueous medium (CH$_3$CN or DNA). One possible view is that in going from an aqueous to a "nonaqueous" environment small shifts in opposite directions of the overlapped transitions at the probe wavelength may occur, resulting in slight differences to the relative intensities of the transitions at this wavelength, but having a negligible effect on the global change in the ESA spectrum, which is a summation of contributing transitions. Furthermore, it is clearly a perturbation of a dppz$^{•−}$-localized transition that is responsible for the apparent depletion of dppz$^{•−}$ modes relative to neutral ligand modes, which remain unperturbed upon intercalation.

In a recent study using linear dichroism to probe the enantioselectivity of the DNA binding of **1** Norden et al[19] have proposed that small but distinct differences may arise between the two intercalated enantiomers in regard to the inclination of the intercalating dppz ligand (itself perpendicular to the DNA helix axis) with respect to a horizontal plane. The angle of inclination is positive for both, but somewhat larger for the Λ form than for the Δ form. The significance of the excited state RR spectra reported here for the two enantiomers is that we now have a spectroscopic handle that allows a clear distinction between the two, with the intensity variations apparently reflecting the distinctive response of the Δ and Λ forms to the chiral environment associated with the DNA binding sites.

Characterization of the binding of the electronic ground states of enantiomeric metal complexes to DNA is at a well-developed stage, particularly as a result of the application of NMR spectroscopy.[20] The situation with regard to the binding of metal complex excited states is less well-developed. While time-resolved electronic spectroscopy (absorption and luminescence) has been used for detailed kinetic analysis,[3e] spectral overlap may sometimes be a compromising feature.[3e] Transient resonance Raman spectroscopy, with the ability to selectively probe ligand vibrational modes, even where spectral overlap occurs, clearly has potential in this respect. The results of the investigation reported here have provided a preliminary demonstration of how the technique can be used to distinguish the excited state intercalative interactions with DNA of metal complex enantiomers. The ability to probe the excited state interactions of chiral metal complexes is relevant to a number of aspects of the behavior of photoactivated, DNA-bound complexes, including photocleavage and electron transfer processes. In the latter case, for instance, the efficiency of electron transfer quenching of the excited states of metal complexes bound to DNA has been shown to be dependent on metal complex chirality.[3e,21] Our results suggest that the use of transient resonance Raman spectroscopy to probe the response of chiral molecules to chiral environments is promising and worthy of further development.

(19) Lincoln, P.; Broo, A.; Norden, B. *J. Am. Chem. Soc.* **1996**, *118*, 2644.
(20) Dupureur, C. M.; Barton, J. K. *Inorg. Chem.* **1997**, *36*, 33.

(21) Lincoln, P.; Tuite, E.; Norden, B. *J. Am. Chem. Soc.* **1997**, *119*, 1454.