Resonance-Raman probing of the interaction between dipyridophenazine complexes of ruthenium(II) and DNA

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The resonance-Raman spectroscopic technique is an effective probe of the interaction between dipyridophenazine (dppz) complexes of ruthenium(II) and calf-thymus DNA, providing evidence that DNA addition results in changes to electronic transitions of the intercalating dppz ligand in both ground and excited states.

Design of small, non-radioactive molecules, capable of selectively binding to nucleic acids is an area on which considerable attention has been focused.[1–7] Selective spectroscopic methods are essential to successfully characterise the nature and photophysical implications of the interaction which exists.

Mixed-ligand complexes of the type $[RuL_2(dppz)]^{2+}$ {L = 2,2′-bipyridine (bpy), 1,10-phenanthroline (phen) ; dppz = dipyrido[3,2 : *a*-2′,3′ : *c*]phenazine} have been investigated extensively as luminescent probes of nucleic acids.[1–4] Through observation of luminescent parameters, Barton,[1,2] Norden[3] and their co-workers have proposed an intercalative binding mode of the dppz ligand with DNA, the extended, planar aromatic surface allowing for extensive hydrophobic stacking between base pairs. The interaction results in a decrease in efficiency of non-radiative relaxation from the MLCT excited state (in which electron localisation is on the dppz ligand) to the ground state, and the very weak emission in aqueous solution is greatly enhanced upon binding.[1,2]

We now report use of resonance-Raman spectroscopy (RRS) coupled with flash photolysis techniques as a significant probe[5] of the interaction between the complexes $[RuL_2(dppz)]^{2+}$ and calf-thymus DNA.[5,6] While changes occur in the ground state RR spectra we wish here to emphasise significant changes in the transient RR spectra of the lowest lying excited state since it is the photophysical properties of this state which have attracted so much interest.

The influence of DNA on the UV–VIS absorption and ground-state RR spectra† is illustrated for $[Ru(phen)_2(dppz)]^{2+}$ in Fig. 1. The intensity changes and shifts in the UV–VIS band maxima are consistent with published data.[3a] Very similar effects are apparent in the absorption spectra of $[Ru(bpy)_2(dppz)]^{2+}$. Changes in the enhancement patterns of the ground-state resonance-Raman spectra of both complexes in the presence of DNA are consistent with changes to the UV–VIS absorption spectrum (Fig. 1). For example, slight intensity changes in the RR spectra recorded at 457.9 nm can be shown to be the result of a lowering in intensity of the dppz component of the MLCT absorption since subtraction of the spectrum recorded in DNA solution from that of the complex in buffer produced a spectrum consisting only of modes attributable to dppz vibrations (see Fig. 1). At 363.8 nm, rather larger changes occur in the RR spectra, consistent with the larger changes in the UV–VIS in this region. A full analysis of such experiments will be presented elsewhere.

In aqueous buffer solution in the absence of DNA, decay of the lowest excited states of these complexes is extremely efficient and no luminescence has been detected on a nanosecond timescale.[1] Likewise, we have been unable to observe excited-state absorption (ESA) spectra on this timescale. In the DNA-intercalated form, however, significant emission enhancement has been reported.[1,2] The ESA spectra in the presence of DNA are also readily observed, as exemplified by the absorbance difference spectrum (ΔA) of the excited state of $[Ru(phen)_2(dppz)]^{2+}$ in Fig. 1. {The corresponding spectrum for $[Ru(bpy)_2(dppz)]^{2+}$–DNA is essentially identical.} The features observed to higher wavenumber of the ground-state bands are therefore attributed to π^*–π^* transitions of the coordinated

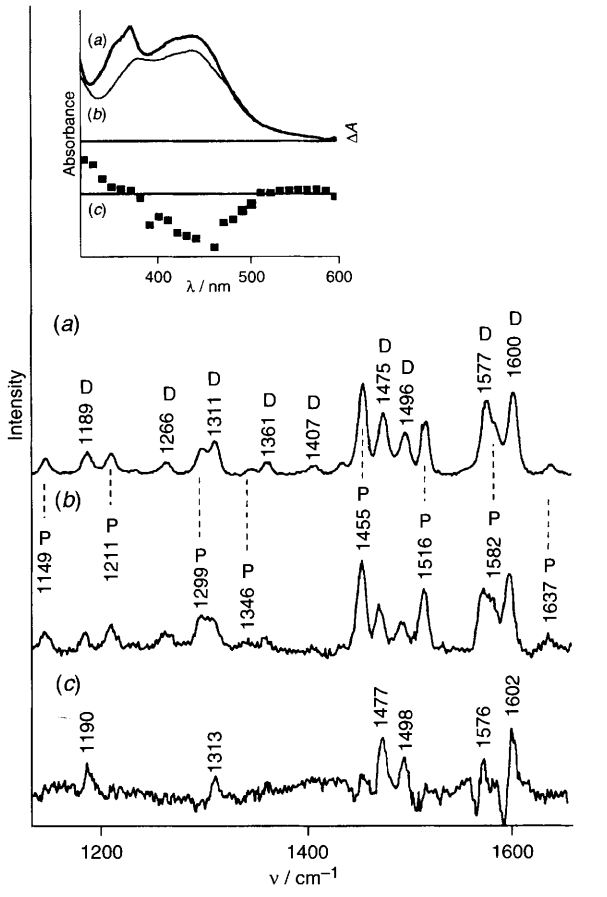

Fig. 1 Ground state RR spectra of $[Ru(phen)_2(dppz)]^{2+}$ recorded at λ_{ex} = 457.9 nm (*a*) in buffer; (*b*) [DNA phosphate] : [Ru] ratio of 20 : 1; (*c*) is a subtraction spectrum (*a*) — (*b*) scaled for complete removal of phen features. The parentage of the most prominent peaks is denoted by P = phen or D = dppz. For assignment purposes, a ground-state resonance-Raman spectrum was recorded of the homoleptic complex $[Ru(dppz)_3]^{2+}$ using 457.9 nm excitation, which gave dppz vibrations at 1600, 1574, 1497, 1474, 1450, 1406, 1360, 1310, 1264 and 1187 cm^{-1}. {Inset: UV–VIS spectra of $[Ru(phen)_2(dppz)]^{2+}$: (*a*) in buffer; (*b*) [DNA phosphate] : [Ru] ratio of 20 : 1; (*c*) is a transient absorbance difference spectrum recorded for aerated solution (*b*), λ_{ex} 416 nm (10 mJ pulse energy).}

radical-like dppz$^{\cdot-}$ ligand which carries the electron density in the MLCT excited state.

The addition of DNA resulted in significant changes to the excited-state RR spectra of both complexes, as illustrated in Fig. 2 which displays pure excited-state spectra acquired in buffer solution and in presence of DNA, at an excitation wavelength of 354.7 nm. Fig. 3 shows the result of subtracting spectra recorded in buffer from those recorded in the presence of DNA. The subtractions were scaled on the band at 1366 cm^{-1} (Fig. 2) in order to remove the dppz$^{\cdot-}$ contributions. It is evident in the case of the subtracted spectrum for [Ru(bpy)$_2$(dppz)]$^{2+}$ that the remaining features are mainly due to neutral ligand bpy (readily identifiable from the relative intensities and band positions[8]) in the [Ru^{III}(bpy)$_2$(dppz$^{\cdot-}$)]$^{2+}$ excited state. An equivalent situation seems to arise for the [Ru(phen)$_2$(dppz)]$^{2+}$ complex, though it is less clear cut in this instance, since phen is a much weaker Raman scatterer than bpy in such an excited state.[9] It is this

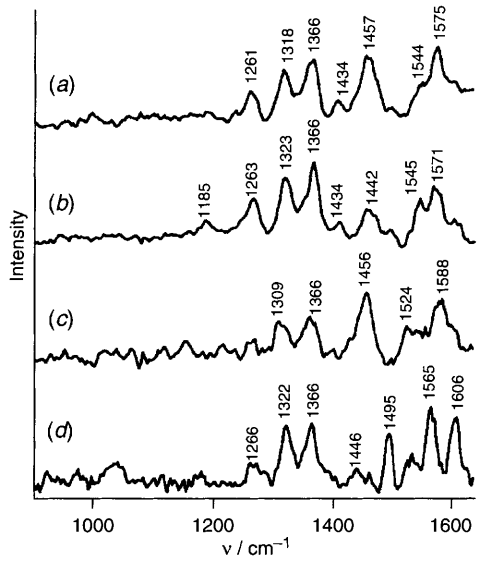

Fig. 2 Pure excited-state resonance-Raman spectra of [Ru(phen)$_2$(dppz)]$^{2+}$ [(*a*) and (*c*)] and [Ru(bpy)$_2$(dppz)]$^{2+}$ [(*b*) and (*d*)] recorded within the laser pulse duration (< 10 ns) at λ_{ex} = 355 nm (pulse energy was such that full conversion was obtained): (*a*) and (*b*) in buffer; (*c*) and (*d*) [DNA phosphate] : [Ru] ratio of 20 : 1

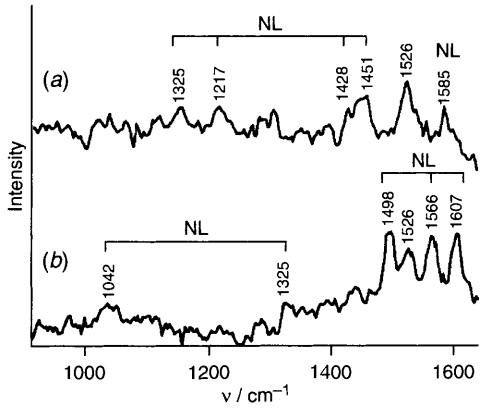

Fig. 3 Scaled subtractions of spectra shown in Fig. 2. Spectra recorded in presence of DNA − spectra recorded in aqueous buffer solution of (*a*) [Ru(phen)$_2$(dppz)]$^{2+}$ and (*b*) [Ru(bpy)$_2$(dppz)]$^{2+}$. Each subtraction is scaled for complete removal of the 1366 cm^{-1} dppz$^{\cdot-}$ feature. NL denotes neutral ligand modes of the corresponding ancillary ligands.

which gives rise to the large differences in the excited state RR spectra of the two complexes with DNA.

The subtracted spectra (Fig. 3) for both complexes show a prominent new feature at 1526 cm^{-1} which is not due to neutral ligand in the [Ru^{III}L$_2$(dppz$^{\cdot-}$)]$^{2+}$ excited state. It seems obvious to assign it as a mode of dppz$^{\cdot-}$ but one which is not present in the excited-state RR spectra recorded in buffer solution in the absence of DNA (other new features may exist but are too weak to be confidently distinguished from the spectral noise). This mode apparently arises through resonance with a π^*–π^* transition of dppz$^{\cdot-}$ which is distinct from that observed in the absence of DNA and attributable to the intercalative interaction of the dppz ligand with the binding sites. The identity of the ancillary ligand appears to have no influence on this effect which is what would be expected if it is the dppz ligand which is intercalated. Although we do not have sufficient information to assign the feature at this stage the finding is an important one, providing a marker band which signals the intercalative interaction of the dppz ligand with DNA. The present findings are a demonstration of the utility of resonance-Raman spectroscopy as an effective probe[5] of the interactions between metal complexes and DNA.‡

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Footnotes

† Preparation of [RuL$_2$(dppz)]Cl$_2$ and purification of DNA (ε = 6600 dm^3 mol^{-1} cm^{-1} at 260 nm) were performed by literature methods.[1,7] Concentrations and purity checks were determined spectrophotometrically. All measurements were obtained from solutions which were ≈ 10^{-4} mol dm^{-3} in metal complex, in an aerated phosphate buffer (10 mmol dm^{-3} phosphate, 50 mmol dm^{-3} NaCl, pH 7). The ratio of [DNA phosphate] : [Ru] employed throughout was 20 : 1, to ensure maximum interaction of the complex with binding sites. A CW Ar$^+$ laser was used for ground-state resonance-Raman studies. A Nd : YAG pulsed laser was employed as a pump source for flash photolysis and transient resonance Raman experiments.[10]

‡ For comparative purposes, RR spectra were recorded of the complex [Ru(bpy)$_3$]$^{2+}$ at each of these wavelengths. This complex is known to bind to the helix without intercalation. As expected, there was no difference between spectra recorded of the free complex in buffer solution and in presence of DNA.

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