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Interaction of polypyridyl ruthenium(II) complexes containing non-planar ligands with DNA

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2-(2-Chlorophenyl)imidazo[4,5-*f*]1,10-phenanthroline (CIP) or 2-(2-nitrophenyl)imidazo[4,5-*f*]1,10-phenanthroline (NIP) and their complexes $[Ru(bpy)_2(CIP)]^{2+}$ and $[Ru(bpy)_2(NIP)]^{2+}$ (bpy = 2,2′-bipyridine) have been synthesized and characterized. The binding of the two complexes to calf thymus DNA has been investigated with spectrophotometric methods and viscosity measurements. The experimental results indicate that the two complexes bind to DNA through a partial intercalative mode that is different from the bonding mode for their parent compound, $[Ru(bpy)_2(PIP)]^{2+}$ (PIP = 2-phenylimidazo[4,5-*f*]1,10-phenanthroline). The crystal structure of $[Ru(bpy)_2(CIP)][ClO_4]_2\cdot 2H_2O$ was determined by X-ray diffraction analysis; the imidazo[4,5-*f*]1,10-phenanthroline moiety is not coplanar with the 2-chlorophenyl ring, having a dihedral angle of 44.5° in the CIP.

There has been substantial interest in understanding the DNA binding properties of polypyridyl ruthenium(II) complexes in the hope of developing novel probes of nucleic acid structure and sites.[1–6] The strong visible absorbance and the luminescent characteristics of the ruthenium(II) complexes and their perturbations on binding to DNA provide a convenient handle for monitoring the DNA binding process. Since pioneering studies by Barton and co-workers[1a–c] showed that optically active isomers of $[Ru(phen)_3]^{2+}$ (phen = 1,10-phenanthroline) bind to DNA with distinctive characteristics, the binding of the complex to DNA has been actively studied and many new structural analogues based on the prototype $[Ru(phen)_3]^{2+}$ have been also synthesized and investigated.

However, most of these reported complexes contain only planar aromatic ligands and investigations of polypyridyl ruthenium(II) complexes with non-planar ligands as DNA-binding reagents have been relatively few. In fact, some of these complexes also exhibit interesting properties upon binding to DNA. For example, although the DIP (DIP = 4,7-diphenyl-1,10-phenanthroline) ligand in $[Ru(DIP)_3]^{2+}$ is not flat, with phenyl groups twisted out of the phenanthroline plane,[7] experimental data are consistent with intercalation binding by this ligand[1f] and show it can distinguish between left- and right-handed DNA helices.[1a,b] Morgan *et al.*[8] have also addressed two ruthenium(II) complexes with out-of-plane ligands, $[Ru(bpy)_2(qpy)]^{2+}$ (qpy = quaterpyridyl) and $[Ru(bpy)_2(dpp)]^{2+}$ (dpp = 2,3-di-2-pyridylpyrazine). The former can intercalate DNA, whereas the latter cannot.

In this paper we report the DNA binding behaviour of two bpy ruthenium(II) complexes with a non-flat ligand, 2-(2-chlorophenyl)imidazo[4,5-*f*]1,10-phenanthroline (CIP) or 2-(2-nitrophenyl)imidazo[4,5-*f*]1,10-phenanthroline (NIP). In each complex two bpy are used as co-complexation ligands with CIP or NIP, because bpy has been previously demonstrated to be at best only minimally efficient at inducing intercalative binding with DNA,[1b,f] allowing us to focus on the influence of the conformation of CIP or NIP on the interaction.

Experimental

Syntheses

The complex *cis*-$[Ru(bpy)_2Cl_2]\cdot 2H_2O$, 1,10-phenanthroline-5,6-dione and $[Ru(bpy)_2(PIP)][ClO_4]_2\cdot 3H_2O$ (PIP = 2-phenylimidazo[4,5-*f*]1,10-phenanthroline) were prepared according to the literature procedures.[9–11] Other materials were commercially available.

CIP. The compound was synthesized according to the method for the preparation of imidazole rings established by Steck and Day.[12] A mixture of 2-chlorobenzaldehyde (3.5 mmol, 0.4 cm^3 of 98% solution), 1,10-phenanthroline-5,6-dione (2.5 mmol, 0.525 g), ammonium acetate (50 mmol, 3.88 g) and glacial acetic acid (7 cm^3) was refluxed for about 2 h, then cooled to room temperature and diluted with water (*ca.* 25 cm^3). Dropwise addition of concentrated aqueous ammonia gave yellow precipitates, which were collected and washed with water. The crude products were purified by silica gel filtration (60–100 mesh, ethanol). The principal yellow band was collected. Crystalline solids were obtained by slow evaporation of the solution, then were dried at 50 °C *in vacuo*. Yield 0.678 g, 82% (Found: C, 68.85; H, 3.3; N, 17.0. Calc. for $C_{19}H_{11}ClN_4$: C, 69.1; H, 3.4; N, 17.0%). $\tilde{\nu}_{max}/cm^{-1}$ 3423 (N–H) 3057 (C–H) and 1609 (C=N). ^1H NMR [$(CD_3)_2SO$]: δ 13.83 (s, 1 H), 9.05 (d, 1 H), 9.01 (d, 1 H), 8.95 (d, 1 H), 8.86 (d, 1 H), 7.94 (d, 1 H), 7.89–7.80 (m, 2 H), 7.70 (d, 1 H) and 7.60–7.53 (m, 2 H).

NIP·0.5H_2O. This compound was obtained with a procedure analogous to that for CIP, using 2-nitrobenzaldehyde in place of 2-chlorobenzaldehyde. Yield 0.656 g, 75% (Found: C, 64.85; H, 3.4; N, 19.8. Calc. for $C_{19}H_{12}N_5O_{2.5}$: C, 65.2; H, 3.45; N, 20.0%). $\tilde{\nu}_{max}/cm^{-1}$ 3402 (N–H), 3057 (C–H), 1609 (C=N) and 1532 (NO_2). ^1H NMR [$(CD_3)_2SO$]: δ 14.11 (s, 1 H), 9.05 (d, 1 H), 9.00 (d, 1 H), 8.86 (d, 1 H), 8.71 (d, 1 H), 8.08 (d, 1 H), 7.96 (d, 1 H), 7.90–7.84 (m, 2 H) and 7.80–7.75 (m, 2 H).

$[Ru(bpy)_2(CIP)][ClO_4]_2\cdot 2H_2O$. A mixture of *cis*-$[Ru(bpy)_2Cl_2]\cdot 2H_2O$ (0.5 mmol, 0.261 g), CIP (0.5 mmol, 0.165 g), methanol (20 cm^3) and water (10 cm^3) was refluxed under argon for 2 h to give a clear red solution. After most of the methanol solvent was removed under reduced pressure, a red precipitate was obtained by dropwise addition of a saturated aqueous $NaClO_4$ solution. The product was purified by column chromatography on alumina using acetonitrile–toluene (2:1 v/v) as eluent and then dried *in vacuo*. Yield 0.313 g, 64% (Found: C,

48.0; H, 3.0; N, 11.7. Calc. for $C_{39}H_{31}Cl_3N_{10}O_{10}Ru$: C, 47.6; H, 3.2; N, 11.45%). $\tilde{\nu}_{max}$/cm^{-1} 3428 (N–H), 3074 (C–H), 1606 (C=N) and 1092 (ClO_4^-). λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) (water) 457 (14100), 283 (80000), 255 (36900) and 211 (41300). ^{1}H NMR [$(CD_3)_2SO$]: δ 14.55 (s, 1 H), 9.08 (dd, 2 H), 8.89 (d, 2 H), 8.85 (d, 2 H), 8.23 (t, 2 H), 8.12–8.09 (m, 4 H), 7.92–7.91 (m, 3 H), 7.88 (d, 2 H), 7.77 (d, 2 H), 7.64–7.60 (m, 6 H) and 7.35 (s, 1 H).

[Ru(bpy)$_2$(NIP)][ClO$_4$]$_2$·H$_2$O. This complex (deep red) was synthesized in an identical manner to that described for [Ru(bpy)$_2$(CIP)][ClO$_4$]$_2$·2H$_2$O, with 0.5 mmol, 0.175 g NIP·0.5H$_2$O in place of CIP. Yield 0.28 g, 59% (Found: C, 48.15; H, 2.8; N, 13.0. Calc. for $C_{39}H_{31}Cl_3N_{10}O_{10}Ru$: C, 48.0; H, 3.0; N, 13.0%). $\tilde{\nu}_{max}$/cm^{-1} 3430 (N–H), 3070 (C–H), 1601 (C=N), 1534 (NO_2) and 1091 (ClO_4^-). λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) (water) 457 (17400), 283 (88900), 255 (53900) and 205 (51500). ^{1}H NMR [$(CD_3)_2SO$]: δ 8.89–8.83 (m, 4 H), 8.28 (d, 1 H), 8.2 (t, 2 H), 8.08 (t, 2 H), 7.88 (d, 2 H), 7.82 (d, 2 H), 7.76–7.70 (m, 6 H), 7.59–7.57 (m, 4 H), 7.54 (t, 1 H) and 7.37 (t, 2 H).

CAUTION: Perchlorate salts of metal complexes with organic ligands are potentially explosive, and only small amounts of the material should be prepared and handled with great care.

Measurements

The analyses (C, H and N) were performed using a Perkin-Elmer 240Q elemental analyser. Infrared spectra were obtained with a Nicolet 170SX-FTIR spectrophotometer and KBr discs, UV/VIS spectra on a Shimadzu MPS-2000 spectrophotometer and ^{1}H NMR spectra on a Bruker ARX-300 spectrometer with $(CD_3)_2SO$ as solvent and Me_4Si as an internal standard. Steady-state emission experiments were performed with a Shimadzu RF-5000 fluorescence spectrometer. Time-resolved emission measurements were conducted with the same detection system and procedure as described previously.[11]

All the experiments involving the interaction of the complexes with DNA were carried out in aerated buffer (5 mmol dm^{-3} Tris–HCl, pH 6.8, 50 mmol dm^{-3} NaCl). Solutions of calf thymus DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm of *ca.* 1.9 : 1, indicating that the DNA was sufficiently free of protein.[13] The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 dm^3 mol^{-1} cm^{-1}) at 260 nm.[14]

Viscosity experiments used a Ubbelodhe viscometer, immersed in a thermostatted water-bath maintained at 28 (±0.1) °C. The DNA samples, approximately 200 base pairs in average length, were prepared by sonication in order to minimize complexities arising from DNA flexibility.[15] Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* the concentration of ruthenium(II) complex, where η is the viscosity of DNA in the presence of complex and η_0 that of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t) corrected for that of buffer alone (t_0), $\eta = t - t_0$.[3b]

Crystallography

The red prismatic crystals of [Ru(bpy)$_2$(CIP)][ClO$_4$]$_2$·2H$_2$O were grown from the diffusion of diethyl ether vapour into a concentrated acetonitrile solution of the complex. A single crystal of dimensions 0.60 × 0.44 × 0.34 mm was used for data collection.

Crystal data and data collection parameters. $C_{39}H_{27}Cl_3N_8O_8Ru$·2H$_2$O, M = 979.14, monoclinic, space group $P2_1/n$, a = 14.283(2), b = 16.671(2), c = 17.596(2) Å, β = 91.02(1)°, U = 4189.2(9) Å^3, Z = 4, D_c = 1.552 g cm^{-3}, μ(Mo-Kα) = 0.632 mm^{-1}, F(000) = 1984, T = 295 K.

7159 Reflections were measured (6582 unique, R_{int} = 0.0204) on a Siemens P4 diffractometer in the range 1.68 < θ < 25.98°, $0 \leqslant h \leqslant 16$, $0 \leqslant k \leqslant 20$, $-20 \leqslant l \leqslant 20$, operating in ω scan mode and using graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å). A semiempirical absorption correction *via* ψ scans was applied.

Table 1 Selected bond lengths (Å) and angles (°) for [Ru(bpy)$_2$(CIP)][ClO$_4$]$_2$·2H$_2$O

Ru–N(3)	2.064(2)	Ru–N(6)	2.052(3)
Ru–N(4)	2.075(2)	Ru–N(7)	2.061(3)
Ru–N(5)	2.063(3)	Ru–N(8)	2.063(3)
N(6)–Ru–N(7)	86.73(10)	N(3)–Ru–N(4)	79.63(9)
N(6)–Ru–N(5)	78.83(10)	C(12)–N(3)–Ru	128.2(2)
N(7)–Ru–N(5)	95.21(10)	C(13)–N(3)–Ru	114.08(18)
N(6)–Ru–N(8)	97.80(10)	C(15)–N(4)–Ru	128.1(2)
N(7)–Ru–N(8)	79.09(10)	C(14)–N(4)–Ru	114.25(19)
N(5)–Ru–N(8)	173.60(10)	C(24)–N(5)–Ru	115.1(2)
N(6)–Ru–N(3)	95.26(10)	C(20)–N(5)–Ru	126.4(2)
N(7)–Ru–N(3)	177.23(10)	C(25)–N(6)–Ru	116.0(2)
N(5)–Ru–N(3)	87.09(10)	C(29)–N(6)–Ru	125.4(2)
N(8)–Ru–N(3)	98.70(10)	C(30)–N(7)–Ru	124.9(2)
N(6)–Ru–N(4)	173.97(10)	C(34)–N(7)–Ru	115.0(2)
N(7)–Ru–N(4)	98.49(10)	C(39)–N(8)–Ru	126.4(2)
N(5)–Ru–N(4)	97.61(10)	C(35)–N(8)–Ru	115.2(2)
N(8)–Ru–N(4)	86.20(10)		

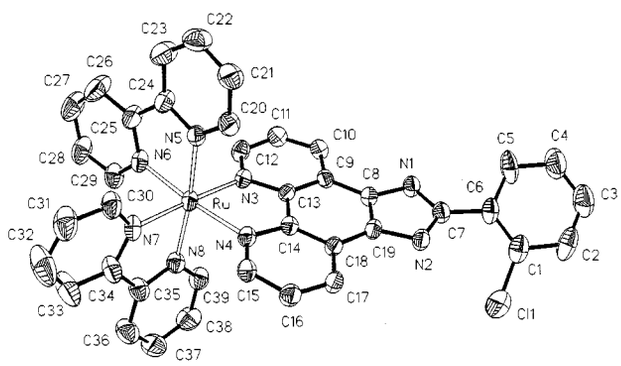

Fig. 1 An ORTEP drawing of [Ru(bpy)$_2$(CIP)]$^{2+}$ and the atom numbering.

Structure solution and refinement. The structure was solved by the direct method and refined anisotropically on F^2 by full-matrix least-squares techniques using the SHELXTL 97 program.[16] All hydrogen atoms were generated geometrically (C–H 0.96 Å). The final refinement gave R = 0.0506, R' = 0.1286. The final difference map had peaks between −0.536 and 1.179 e Å^{-3}.

CCDC reference number 186/1195.

See http://www.rsc.org/suppdata/dt/1999/19/ for crystallographic files in .cif format.

Results and discussion

Crystal structure

The molecular structure of [Ru(bpy)$_2$(CIP)][ClO$_4$]$_2$·2H$_2$O has been confirmed by single crystal X-ray diffraction analysis. It consists of a [Ru(bpy)$_2$(CIP)]$^{2+}$ cation, two disordered ClO_4^- and two water molecules, one of which occupies two positions (O10W and O11W) with an occupancy of 0.5, respectively; the other has full occupancy and forms a hydrogen bond with the imidazole hydrogen, H(2N), O9W···H(2N) 2.84 Å. An ORTEP[17] drawing of the cation with atomic numbering scheme is depicted in Fig. 1. Selected bond lengths and angles are summarized in Table 1.

As shown in Fig. 1, the central ruthenium atom is chelated by two bpy ligands oriented in a *cis* geometry and a CIP ligand. The co-ordination geometry about the ruthenium atom is that of a distorted octahedron, with a bite angle of 79.2° averaged over the three bidentate ligands. This distortion from an ideal

octahedral geometry is due to the customary narrow bite angles of the bipyridine moieties, as seen in other ruthenium–bipyridine complexes.[18] The torsion angles between the pyridine pairs of two bpy ligands of the complex are non-equivalent, one being 2 and the other 8°; however, they are all located in the range expected for this type of compound such as $[Ru(bpy)_2(gly)]^+$ (gly = glycinate) (1.4 and 7.4°),[19] $[Ru(bpy)_2(ip)]^{2+}$ [ip = imidazo[4,5-*f*]1,10-phenanthroline] (5.7 and 8.6°),[12] $[Ru(bpy)_2(phen)]^{2+}$ (6.4 and 10.3°) and $[Ru(bpy)_2(mphen)]^{2+}$ (mphen = 5-methyl-1,10-phenanthroline) (1.9 and 12.3°).[20] In the CIP ligand the ip moiety is planar with an average deviation of 0.0385 Å from the least-squares plane, but the 2-chlorophenyl group is remarkably twisted with respect to the ip plane forming a dihedral angle of 44.5° to minimize possible steric interaction between the substituent Cl and the imidazole ring. Although the crystal structure of $[Ru(bpy)_2(NIP)]^{2+}$ is not known, it is guessed that the NIP also might possess a non-planar conformation because the NO_2 group in NIP has a larger steric volume than the substituent Cl. The arrangement of CIP or NIP is different from the geometry of their parent compound, PIP, all atoms of which basically lie on a plane.[21]

The mean Ru–N bond length (2.063 Å) is comparable with those published for $[Ru(bpy)_3]^{2+}$ (2.056 Å),[22] $[Ru(bpy)_2(phen)]^{2+}$ (2.069 Å)[20] and $[Ru(phen)_3]^{2+}$ (2.063 Å),[23] although there are larger differences in size and shape for bpy, phen and CIP. There are two ways of explaining why these Ru–N bond lengths are similar to each other. One may be that the changes of σ bonding are almost balanced by those of π bonding in Ru–N with the changes of these ligand structures such that the interatomic Ru–N are basically constant.[22] Another possible interpretation is that these bonds are not particularly sensitive to the total electronic density, as seen in the structures of $[Ru(bpy)_3]$, $[Ru(bpy)_3]^{2+}$ and $[Ru(bpy)_3]^{3+}$.[24]

An interesting feature of the crystal structure is the packing of the complex cation with respect to its chirality. A pair of $[Ru(bpy)_2(CIP)]^{2+}$ enantiomers (Δ and Λ) are arranged asymmetrically together by the mutual penetration of the two CIP ligands. The closest distance between the two imidazo[4,5-*f*]-1,10-phenanthroline planes is 3.7 Å. Such a close packing arrangement implies that there are some 'π–π' stacking interactions between the aromatic systems of the two intruding CIP ligands.

Absorption spectroscopic studies

The electronic absorption spectra of the two complexes mainly consist of four well resolved bands, similar in shape to those of $[Ru(bpy)_3]^{2+}$.[25] The lowest energy absorption bands at 457 nm and the middle intensity peaks at 255 nm are assigned to metal-to-ligand charge transfer (MLCT) transitions;[25] the two bands with maxima of 283 and 211 nm for $[Ru(bpy)_2(CIP)]^{2+}$ and 283 and 205 nm for $[Ru(bpy)_2(NIP)]$ are attributed to intraligand π–π* transitions by comparison with the spectrum of $[Ru(bpy)_3]^{2+}$.[25]

The interaction of the two complexes with DNA was investigated using absorption spectra. The electronic spectral trace of the two complexes titrated with DNA is given in Fig. 2, using $[Ru(bpy)_2(CIP)]^{2+}$ as an example. In both cases, although no red shift was found, notable hypochromicities were observed. The MLCT transitions at 457 nm show a decrease in intensity with a maximum value of 12% as the amount of DNA was increased. The spectroscopic changes suggest that there are some interactions between the complexes and DNA. However, it is noteworthy that the hypochromicity of the MLCT peaks is much smaller than that of their parent complex, $[Ru(bpy)_2(PIP)]^{2+}$ (21.9%), in which PIP can insert deeply into DNA base pairs.[11] Thus it is believed that the binding mode of the two complexes to DNA is likely different from that of $[Ru(bpy)_2(PIP)]^{2+}$.

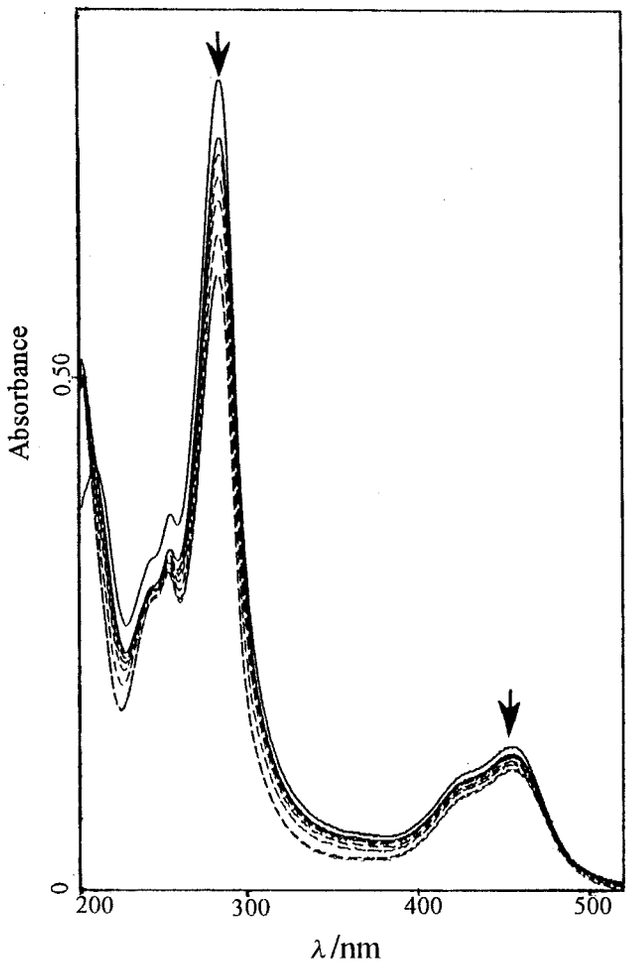

Fig. 2 Electronic spectral traces of $[Ru(bpy)_2(CIP)]^{2+}$ in Tris–HCl buffer upon addition of calf thymus DNA. [Ru] = 10 μmol dm^{-3}, [DNA] = (0–5) × 10^{-4} mol dm^{-3}.

Luminescence studies

In water or CH_3CN, $[Ru(bpy)_2(CIP)]^{2+}$ can emit luminescence with similar emission maxima, 611 and 613 nm, but different lifetimes, 624 and 1579 ns, respectively. Its quantum yields relative to $[Ru(bpy)_3]^{2+}$ are 0.78 in water and 0.96 in CH_3CN, comparable to those of $[Ru(bpy)_2(PIP)]^{2+}$ (0.81 and 0.94). For $[Ru(bpy)_2(NIP)]^{2+}$ no emission is observed in the two media. This could be explained in the terms of the photoexcited electron being captured by the strong electron-accepting group, NO_2, in the NIP ligand and being unable to give emission to return to the ground state. A similar case was observed for ruthenium(II) complexes containing 5-nitro-1,10-phenanthroline.[26]

The results of the emission titrations for $[Ru(bpy)_2(CIP)]^{2+}$ with DNA are illustrated with the titration curves in Fig. 3. Upon addition of DNA the emission intensity grows steadily to around 1.9, and the lifetime increases from 624 to 1310 ns. The magnitudes of emission enhancement are much larger than that for $[Ru(bpy)_3]^{2+}$,[1*f*] but obviously smaller than that of the structurally related DNA intercalator $[Ru(bpy)_2(PIP)]^{2+}$.[11] Although the emission enhancement and lifetime increase could not be regarded as a criterion for binding mode, they are related to the extent to which the complex gets into a hydrophobic environment inside the DNA and avoids the quenching effect of solvent water molecules. Therefore we infer that $[Ru(bpy)_2(CIP)]^{2+}$ inserts less deeply into the hydrophobic environment of DNA than does $[Ru(bpy)_2(PIP)]^{2+}$.

Steady-state emission quenching experiments using $[Fe(CN)_6]^{4-}$ as quencher support the above proposal. As shown in Fig. 4, in the absence of DNA, $[Ru(bpy)_2(CIP)]^{2+}$ was effi-

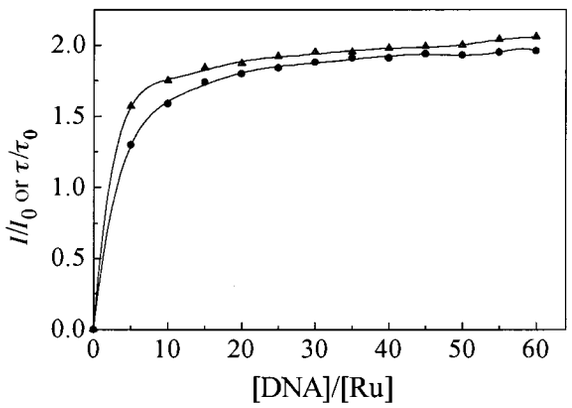

Fig. 3 Plots of relative emission intensity (●) and excited state lifetime (▲) *versus* DNA : Ru ratio for $[Ru(bpy)_2(CIP)]^{2+}$ ([Ru] = 2 μmol dm^{-3}).

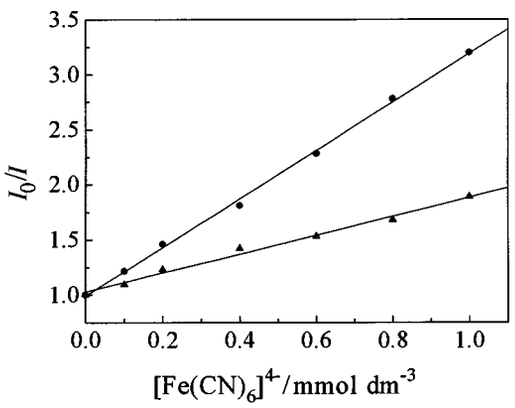

Fig. 4 Emission quenching curves of $[Ru(bpy)_2(CIP)]^{2+}$ with increasing concentration of quencher $[Fe(CN)_6]^{4-}$ in the absence (●) and presence (▲) of DNA. [Ru] = 2 μmol dm^{-3}, DNA : Ru = 40 : 1.

ciently quenched by the quencher, resulting in a linear Stern–Volmer plot (slope 2.2, correlation coefficient 0.999). In the presence of DNA the slope of the plot is remarkably decreased (slope 0.86, correlation coefficient 0.995), but not nearly equal to zero just like that of $[Ru(bpy)_2(PIP)]^{2+}$.[11] The ion $[Fe(CN)_6]^{4-}$ has been shown to be able to distinguish differently bound ruthenium(II) species.[27] Positively charged 'free' complex ions should be readily quenched by $[Fe(CN)_6]^{4-}$ when the complex bound to DNA can be protected from the quencher because highly negatively charged $[Fe(CN)_6]^{4-}$ would be repelled by the negative DNA phosphate backbone, hindering quenching of the emission of the bound complex. Therefore a larger slope for the Stern–Volmer curve parallels poorer protection. So $[Ru(bpy)_2(CIP)]^{2+}$ binds less tightly than does $[Ru(bpy)_2(PIP)]^{2+}$. This is just the expected result on the basis that PIP possesses a flatter conformation than CIP, which would lead PIP to intercalate more easily into DNA than does CIP.

In previous studies on DNA-complex binding biexponential emission decay curves were observed. They were usually ascribed to two different binding forms.[1b,c,j,k,8] For $[Ru(bpy)_2(CIP)]^{2+}$ the emission decay curves fit well with monoexponential functions. So we speculate that it interacts with DNA through a binding mode[11] and that the bound component exchanges rapidly with coexisting free component.[1c]

For $[Ru(bpy)_2(NIP)]^{2+}$ no luminescence was detected, either alone in aqueous solution or in the presence of DNA. The quenching is caused by the strong electron-accepting group, NO_2, in the complex structure itself. So it is not sensitive to the environment. The observed non-emissive behaviour of $[Ru(bpy)_2(NIP)]^{2+}$ does not imply it cannot interact with DNA.

Viscosity measurements

Optical photophysical probes generally provide necessary, but not sufficient, clues to support a binding model. Hydrodynamic measurements that are sensitive to length change (*i.e.* viscosity and sedimentation) are regarded as the least ambiguous and most critical tests of a binding model in solution in the absence of crystallographic structural data.[2a,b] A classical intercalation model demands that the DNA helix lengthens as base pairs are separated to accommodate the bound ligand, leading to the increase of DNA viscosity. In contrast, a partial, non-classical intercalation of ligand could bend (or kink) the DNA helix, reduce its effective length and, concomitantly, its viscosity.[2a,b]

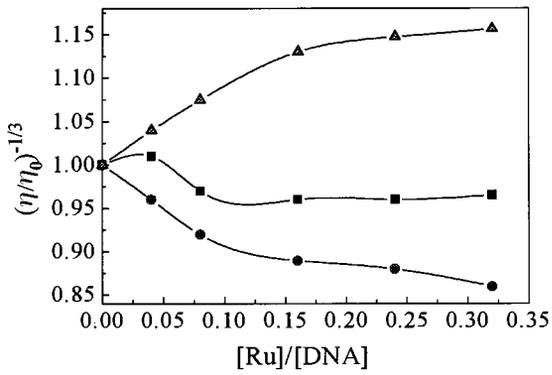

Fig. 5 Effect of increasing amounts of $[Ru(bpy)_2(PIP)]^{2+}$ (▲), $[Ru(bpy)_2(CIP)]^{2+}$ (■) and $[Ru(bpy)_2(NIP)]^{2+}$ (●) on the relative viscosity of calf thymus DNA.

The effect of *rac*-$[Ru(bpy)_2(PIP)]^{2+}$, $[Ru(bpy)_2(CIP)]^{2+}$ and $[Ru(bpy)_2(NIP)]^{2+}$ on the viscosity of rod-like DNA is shown in Fig. 5. The complex $[Ru(bpy)_2(PIP)]^{2+}$ can increase the viscosity of DNA, consistent with the classical intercalation mode; the last two complexes decreased DNA viscosity as the molar ratio of ruthenium(II) complex to DNA was increased, similar to experiments on interactions of DNA with Δ-$[Ru(phen)_3]^{2+}$.[2a] The experimental results suggest the two complexes could bind to DNA not by the classical intercalation binding mode but by the partial, non-classical intercalation model. This may be related to the molecular structures of the complexes. Since the steric constraint to coplanarity of the phenyl containing the larger substituent group and ip moiety in the CIP or NIP is obviously more severe than that for DIP in $[Ru(DIP)_3]^{2+}$ or qpy in $[Ru(bpy)_2(qpy)]^{2+}$, in which the torsion between the aromatic rings is involved in the interaction of the smaller hydrogen atoms,[8] the CIP or NIP ligand could not completely intercalate {like $[Ru(DIP)_3]^{2+}$ or $[Ru(bpy)_2(qpy)]^{2+}$ does}, at most they could penetrate their substituted phenyl moieties into the DNA base pairs, leaving the other part of the ligand in the groove. The partial intercalation may act as a "wedge" to pry apart one side of a base-pair stack, as observed for the Δ-$[Ru(phen)_3]^{2+}$,[2a,b] but not fully separate the stack as required by the classical intercalation mode. This would cause a static bend or kink in the helix and decrease the viscosity of DNA.

In conclusion, based on the variations of photophysical properties and viscosity measurement on binding to DNA, as well as the crystal structure, it is concluded that the torsion between the aromatic rings of CIP or NIP, due to the introduction of the bulky substituent, results in $[Ru(bpy)_2(CIP)]^{2+}$ or $[Ru(bpy)_2(NIP)]^{2+}$ only partially intercalating into DNA, differing from the binding behaviour of their parent complex, $[Ru(bpy)_2(PIP)]^{2+}$.[11]

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