Ruthenium(II) complex of 2-(9-anthryl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline: synthesis, spectrophotometric pH titrations and DNA interaction

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A Ru(II) complex of $[(bpy)_2Ru(aip)](ClO_4)_2 \cdot H_2O$ (bpy = 2,2′-bipyridine, aip = 2-(9-anthryl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline) was newly synthesized. The ground- and excited-state acid–base properties of the complex were studied by UV–visible and emission spectrophotometric pH titrations. The excited-state ionization constants of $pK_{a1}^* = 2.57$, $pK_{a2}^* = 8.47$ are comparable to and 1.3 orders of magnitude greater than the ground-state ones, respectively. The interaction of the complex with calf thymus DNA was investigated by UV–visible and emission spectroscopy, steady-state emission quenching by $[Fe(CN)_6]^{4-}$, competitive binding with ethidium bromide, reverse salt titrations, DNA melting experiments, as well as viscosity measurements. The results indicated that the complex bound to the DNA by interaction of the anthryl moiety of the complex with an intrinsic binding constant of the order of 10^4 M^{-1} in buffered 50 mM NaCl at room temperature, and non-electrostatic binding free energy made a predominant contribution to the binding free energy.

1. Introduction

Ruthenium(II) polypyridyl complexes have been substantially investigated in view of their extensive applications in the field of photochemistry, photophysics and biochemistry in the past decade.[1–4] Owing to their excellent chemical stability, facile electron transfer, strong luminescent emission and relatively long-lived excited states, ruthenium(II) complexes binding to DNA are of considerable interest as DNA structural probes, DNA footprinting and sequence specific cleaving agents, and DNA metallointercalators in probing DNA-mediated electron transfer.[5–9] Ru(II) complexes with protonatable/deprotonatable sites are of great importance for understanding the effects of pH on biomolecules,[10,11] *e.g.* interesting pH-dependent DNA damage and selectivity towards cancer cells have recently been demonstrated.[10] Ru(II) complexes containing an imidazole group have been well studied with respect to the reversible acid–base interconversion which may induce a large energy perturbation,[12,13] and affect the DNA binding properties. We have recently studied the DNA and RNA binding properties of a dinuclear Ru(II) complex,[14] and the mononuclear structurally analogous Ru(II) complexes with deprotonatable/protonatable imidazole[12,15,16] and ferrocenyl[17] groups.

The anthryl derivatives have been investigated in the areas of molecular and ionic recognition or sensing, and the highly selectively sequence recognition of DNA bases[18–20] due mostly to the modulation of the emission properties of the anthryl-containing chromophores. However, the emission of the anthryl chromophore was produced by excitation using ultraviolet light which is harmful to biological tissues. In this paper we report on the effects of pH on the acid–base and DNA binding properties of a new Ru(II) complex containing both anthryl and imidazole chromophores, demonstrating that the optical properties of the Ru(II) complex, particularly the emission of the Ru(II) complex under the excitation of visible light rather than ultraviolet light, can serve as a reporter on the changes in pH and the intercalation binding of the anthryl moiety on the complex into the DNA duplex.[21] The results will provide important fundamental data for developing highly sensitive DNA sequence probes by using visible light excitation in the future.

2. Experimental

2.1 Physical measurements

Infrared spectra were recorded on a Nicolet Avtar 360 FT-IR spectrophotometer as KBr disks. ^1H NMR spectra were collected on a Bruker DRX-500 NMR spectrometer with DMSO-d_6 as solvent. All chemical shifts are given relative to TMS. Elemental analyses were obtained on a Vario EL elemental analyzer. UV–visible absorption spectra were recorded on a GBC Cintra 10e UV–visible spectrophotometer. Emission spectra were obtained on a Shimadzu RF-5301PC spectrofluorophotometer at room temperature. UV–visible and emission spectrophotometric pH titrations were carried out in aqueous solution containing a Britton–Robinson buffer and 0.2 M NaCl to keep a constant ionic strength. Other experiments involving the interaction of the complex with DNA were conducted in doubly distilled water buffered at

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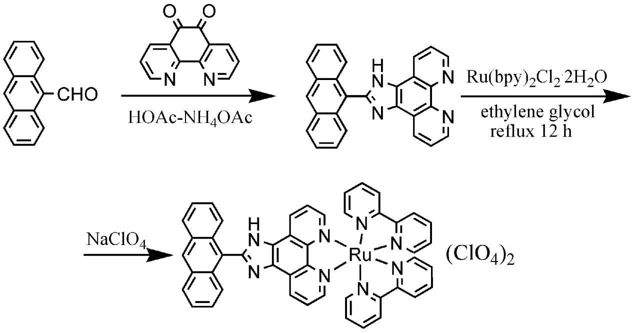

Scheme 1 The synthetic route to [(bpy)$_2$Ru(aip)](ClO$_4$)$_2$.

pH = 7.10 with 5 mM Tris–HCl, at 50 mM NaCl. The solutions of calf thymus DNA (ct-DNA) gave ratios of UV absorbance at 260 nm to that at 280 nm of about 1.8–1.9 : 1, indicating that the DNA was sufficiently free of protein.[22] The concentration per nucleotide of DNA was determined spectrophotometrically by assuming $\varepsilon_{260\,\mathrm{nm}} = 6600\ \mathrm{M\ cm^{-1}}$.[23] Viscosity measurements were made by using an Ubbelohde viscometer, immersed in a thermostated water-bath maintained at 32.16 ± 0.05 °C. DNA samples, approximately 200 base pairs in average length, were prepared by sonication in order to minimize contributions to the viscosity arising from DNA flexibility. Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* the ratio of the concentration of ruthenium(II) complex to that of DNA, where η and η_0 are the viscosities of DNA solutions in the presence and absence of the complex, respectively. Viscosity values were calculated from the observed flow times of DNA containing solutions (t) corrected for that of buffer alone (t_0), $\eta = t - t_0$. DNA melting studies were carried out in 1.5 mM Na_2HPO_4, 0.5 mM NaH_2PO_4 and 0.25 mM Na_2EDTA by continuous heating from 50 to 92 °C at a rate of 1 °C min^{-1}. Data were present as $(A - A_0)/(A_f - A_0)$ *vs.* temperature, where A_f, A_0, A are the final, initial and observed absorbance at 260 nm, respectively.

2.2 Preparation of [(bpy)$_2$Ru(aip)](ClO$_4$)$_2$ · H$_2$O

The synthetic route to [(bpy)$_2$Ru(aip)](ClO$_4$)$_2$ · H$_2$O is described in Scheme 1. A mixture of [Ru(bpy)$_2$Cl$_2$] · 2H$_2$O[24] (0.13 g, 0.25 mmol) and aip[24] (0.099 g, 0.25 mmol) in ethylene glycol (7.0 cm^3) was heated under a nitrogen atmosphere for 12 h at 126 °C to give a clear red solution. The red-orange precipitate appeared after addition of 4-fold excess of saturated aqueous $NaClO_4$ solution, was filtered and washed with diethyl ether. ***Caution****! All the perchlorate salts are potentially explosive and therefore should be handled in small quantity with care*. The crude product was then recrystallized from CH_3CN–Et_2O. Yield: 0.24 g (91%). IR: ν_{max} (KBr, cm^{-1}): 3429 (vs, $\nu_{O\text{-}H}$), 1602 (s, $\nu_{C{=}N}$), 1091 (vs, ν ClO_4^-). Anal. Calcd for $C_{47}H_{32}N_8Cl_2O_8Ru \cdot H_2O$ (%): C, 54.98; H, 3.34; N, 10.91. Found: C, 54.83; H, 3.74; N, 11.14%. ^{1}H NMR (500 MHz, DMSO-d$_6$): δ/ppm 14.82 (s, 1H), 9.19 (s, 1H), 8.89 (dd, 5H), 8.30 (d, 2H), 8.25 (t, 2H), 8.16 (t, 2H), 8.11 (d, 2H), 7.96 (dd, 2H), 7.90 (d, 2H), 7.85 (d, 2H), 7.72 (d, 2H), 7.64 (t, 2H), 7.59 (t, 4H), 7.41 (t, 2H). UV–vis in H$_2$O: λ/nm ($\varepsilon \times 10^4$ M^{-1} cm^{-1}): 253 (14.7), 282 (8.5), 367 (1.6), 386 (1.5), 460 (1.8).

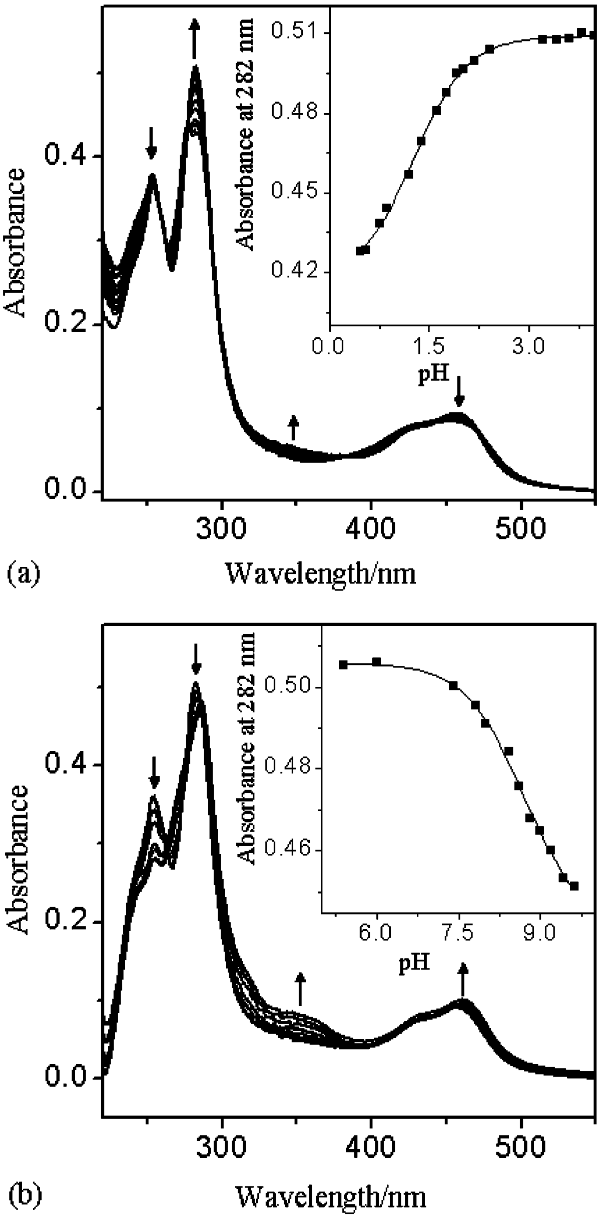

Fig. 1 The changes of the UV–visible absorption spectra of the Ru(II) complex upon raising pH from 0.0500 to 4.00 (a) and from 5.50 to 10.0 (b).

3. Results and discussion

3.1 The pH effects on UV–visible and emission spectra

UV–visible and emission spectrophotometric pH titrations were carried out over pH range 0.05–10.00. The UV–visible spectra of the aqueous solution of the complex as a function of pH are shown in Fig. 1, and show that the complex underwent two deprotonation processes. Upon increasing the pH from 0.0500 to 4.00, the absorbance for the bands of intraligand $\pi \rightarrow \pi^*$ transition at 253 nm and the metal-to-ligand charge transfer (MLCT) at 460 nm slightly decreased, while that for the other intraligand $\pi \rightarrow \pi^*$ transition band at 283 nm increased. The spectral changes observed above are assigned

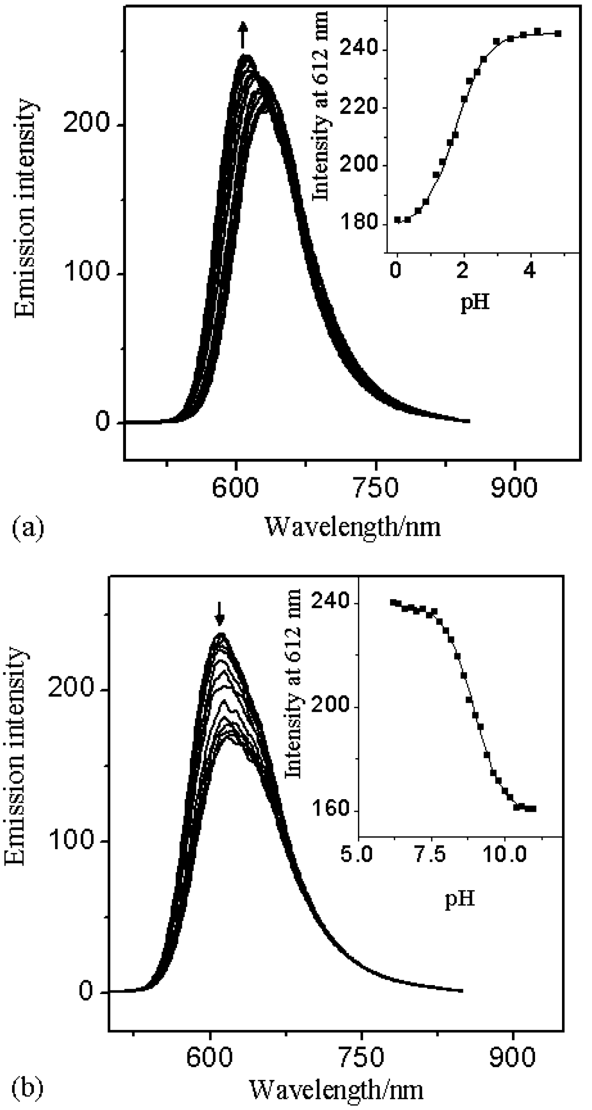

Fig. 2 The pH effects on the emission spectra of the Ru(II) complex upon raising pH from 0.0500 to 4.00 (a) and from 6.00 to 10.0 (b).

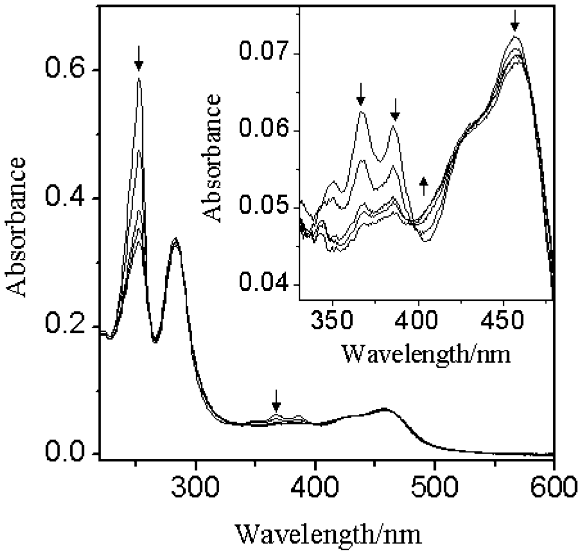

Fig. 3 The UV–visible absoption spectra of the Ru(II) complex (3.97 μM) in the absence and the presence of increasing amounts of DNA (0.00–68.0 μM) in buffered 50 mM NaCl.

to the dissociation of the proton on the protonated imidazole ring. As the pH increased from 5.50 to 10.0, the second deprotonation for the neutral imidazole ring occurred and was characterized by a decrease in the intensities of the bands at 253 and 282 nm, but an increase in the intensities of the bands at ~350 nm and 460 nm. Two ground-state ionization constants of $\mathrm{p}K_{a1} = 1.27 \pm 0.03$, $\mathrm{p}K_{a2} = 8.69 \pm 0.08$ were obtained by nonlinear sigmoidal fit of the data in the insets of Fig. 1(a) and Fig. 1(b).

The pH effects on the emission spectra are shown in Fig. 2. Over the pH range studied, the complex exhibited emission maxima at ~612 nm which are characteristic of ^{3}MLCT luminescene (dπ(Ru) → π*(ligand)). Upon increasing the pH from 0.0500 to 4.00, the emission bands were blue-shifted from 633 nm to 609 nm with increasing intensities. Upon further raising the pH from 6.00 to 10.5, the emission intensities decreased as the maxima red-shifted from 613 nm to 617 nm. Obviously, the changes in the emission spectra observed above are attributed to two distinctly different excited state deprotonation processes as described above for the ground-state deprotonation. The excited-state ionization constants, $\mathrm{p}K_a^*$, could be roughly evaluated by eqn (1):[14]

$$\mathrm{p}K_a^* = \mathrm{p}K_a + (0.625/T)(\nu_B - \nu_{HB}) \qquad (1)$$

where ν_B and ν_{HB} are approximately equal to the emission maxima for the basic and acidic species, respectively. Excited-state ionization constants of $\mathrm{p}K_{a1}^* = 2.57$, $\mathrm{p}K_{a2}^* = 8.47$ were derived. The value of $\mathrm{p}K_{a2}^*$ is comparable to the corresponding ground-state one $\mathrm{p}K_{a2}$, while the value of $\mathrm{p}K_{a1}^*$ is 1.3 orders of magnitude greater than $\mathrm{p}K_{a1}$, indicating that the protonated complex of $[(bpy)_2Ru(Haip)]^{3+}$ is more basic in the excited state than in the ground state, and that the excited electron on $[(bpy)_2Ru(Haip)]^{3+}$ is mainly delocalized on aip rather than on bpy. However, the excited electron for $[(bpy)_2Ru(aip)]^{2+}$ was delocalized on bpy. This is an interesting example where the protonation/deprotonation process switched the MLCT emissive routes on the Ru(II) complex.

3.2 DNA binding properties

3.2.1 UV–vis absorption spectra. The UV–visible absorption spectra of the complex in the absence and the presence of ct-DNA are illustrated in Fig. 3. Upon addition of ct-DNA, the visible MLCT band at 460 nm and π–π* band at 282 nm were little disturbed due to the fact that these two bands are of mainly bpy-localized in nature.[14] However the π–π* bands at 253 nm and 367 nm showed hypochromicities of 43% and 23%, respectively. The evident hypochromicities observed for these two bands may reveal that these two bands are mostly aip-related in nature, and the band at 367 is clearly associated with the anthryl group on comparison with the UV spectra of the analogue Ru(II) complexes[12,14,15,17] without the anthryl group. Since the most probable twisting angle between the molecular planes of the anthracene and imidazole moieties is about 15°,[25] it is possible that the anthryl group intercalates

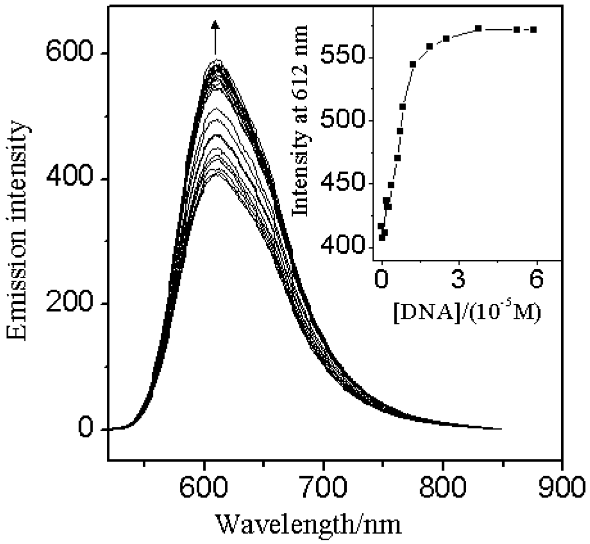

Fig. 4 The changes in the emission spectra (λ_{ex} = 463 nm) of the Ru(II) complex (3.97 μM) upon addition of DNA (0.00–60.0 μM).

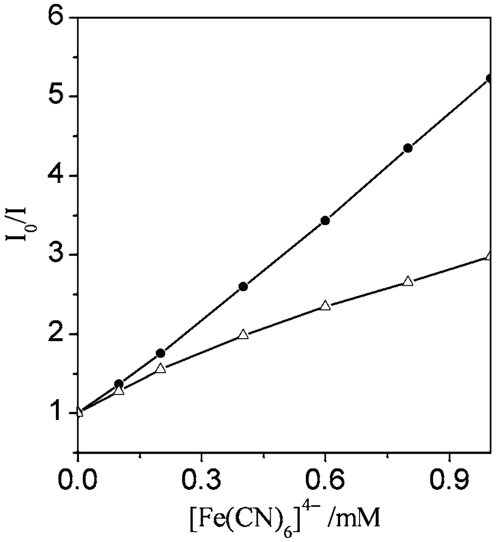

Fig. 5 Emission quenching of the Ru(II) complex (3.97 μM) with increasing concentration of [Fe(CN)$_6$]$^{4-}$ in the absence (●) and presence (△) of DNA: [Ru] = 3.97 μM, [DNA] : [Ru] = 8.50 : 1.

into the DNA helix as evidenced by (9-anthrylmethyl)ammonium chloride.[20] To further illustrate the binding strength of the complex to DNA, the intrinsic binding constant with ct-DNA was determined from the decay of the absorbance monitored at 253 nm by employing eqn (2):[26]

$$\frac{[\mathrm{DNA}]}{(\varepsilon_a - \varepsilon_f)} = \frac{[\mathrm{DNA}]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)} \qquad (2)$$

where [DNA] is the concentration of DNA in base pairs, ε_a is the apparent absorption coefficient calculated as A_{obsd}/[Ru], and ε_f and ε_b represent the extinction coefficients for the free and bound forms of the ruthenium complex. The intrinsic DNA binding constant K_b was derived to be (4.9 ± 0.3) × 10^4 M^{-1} in buffered 50 mM NaCl which is close to those for some known DNA intercalators, such as K_b = 2.1 × 10^4 M^{-1} for [Ru(bpy)$_2$(ddt)]$^{2+}$ (ddt = 3-(pyrazin-yl)-5,6-diphenyl-*as*-trazine),[27] 6.3 × 10^4 M^{-1} for [Ru(bpy)$_2$(dpt)]$^{2+}$ (dpt = 3-(pyrazin-yl)-*as*-triazino[5,6-*f*]phenanthrene),[27] 1.7 × 10^5 M^{-1} for [Ru(bpy)$_2$(atatp)]$^{2+}$ (atatp = acenaphtheno[1,2-*b*]-1,4,8,9-tetraazatriphenylene),[28] and some anthryl-containing compounds (K_b ≈ 10^4 M^{-1}),[20,29] but much lower in comparison with those (K_b > 10^6 M^{-1}) for dppz–based intercalators.[30,31]

3.2.2 Luminescence spectroscopic studies. As shown in Fig. 4, the emission intensities were enhanced by ~50% upon addition of DNA. Scatchard analysis[28] of the luminescence data gave a binding constant of (7.8 ± 0.8) × 10^4 M^{-1} and a site size of *s* = 2.6 ± 0.2 base pairs which are reasonable on comparison with those (K_b = 7.8 × 10^4 M^{-1}, *s* = 4.8) for AMAC (9-anthrylmethyl ammonium chloride).[20] The results of steady-state emission quenching experiments using [Fe(CN)$_6$]$^{4-}$ as the quencher are illustrated in Fig. 5. In the absence of DNA, the emission of the Ru(II) complex was efficiently quenched by [Fe(CN)$_6$]$^{4-}$. However, on addition of DNA, the quenching efficiency of the Ru(II) complex bound to DNA by [Fe(CN)$_6$]$^{4-}$ was decreased relative to that of the free complex, which may be explained by the fact that the Ru(II) complex was partially protected from the quencher to some extent by the array of negative charges along the DNA phosphate backbone hindering quenching of the emission of the bound complex. However, the protection of the Ru(II) complex from emission quenching by [Fe(CN)$_6$]$^{4-}$ is not very effective. The phenomenon is similar to those observed for [Ru(phen)$_3$]$^{2+}$ [2,8] and [Ru(bpy)$_2$IP]$^{2+}$.[32] Furthermore, competitive binding experiments with the proven DNA intercalator ethidium bromide (EB) may give further information about the DNA binding properties of the Ru complex, since a DNA intercalator could replace EB from the DNA bound EB. The addition of complex to DNA pretreated with EB caused an appreciable reduction in emission intensity of DNA-bound EB by 53% relative to that observed in the absence of this Ru complex (Fig. 6). The quenching plot of I_0/I *vs.* [Ru]/[DNA] is in good agreement with the linear Stern–Volmer equation (inset in Fig. 6), and the quenching constant was derived to be 1.64. We can also determine from the data in Fig. 6 that 50% of EB molecules were replaced from DNA bound EB at a concentration ratio of [Ru]/[EB] ≈ 3. By taking a DNA binding constant of 2.6 × 10^5 M^{-1} for EB,[33] an apparent DNA binding constant of 8.6 × 10^4 M^{-1} was derived (K_b(EB)/3)[34] for the Ru(II) complex in this study. This value is in good agreement with that derived by direct luminescence spectroscopic measurements.

3.2.3 Viscosity study. The viscosity of a DNA solution is sensitive to the addition of organic drugs and metal complexes bound by intercalation. To further explore the interaction properties between the Ru(II) complex and the DNA, the specific relative viscosity of DNA was examined by varying the concentrations of the added complex and the results are presented in Fig. 7. A classical intercalation model results in lengthening the DNA helix, leading to an increase in the DNA

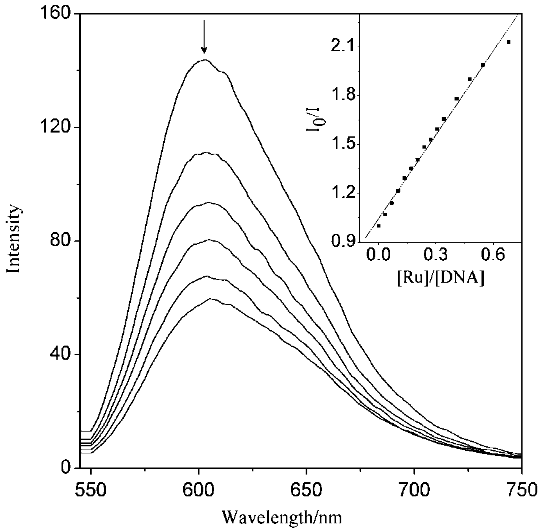

Fig. 6 The changes in emission spectra of EB–DNA ([DNA] : [EB] = 5 : 1) upon addition of the Ru(II) complex. Inset: fluorescence quenching curve of DNA-bound EB.

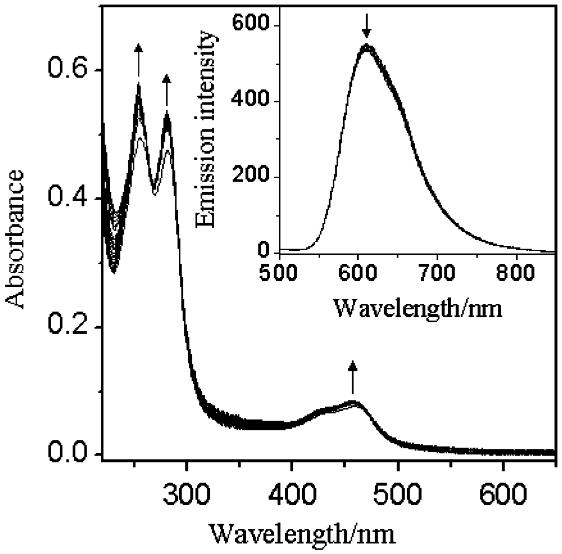

Fig. 8 The changes of absorption and (inset) emission spectra of the Ru(II) complex upon raising the concentration of NaCl from 0.000 to 1.50 M at [Ru] = 4.20 μM and [DNA] = 32.7 μM.

viscosity. In contrast, a partial or nonclassical intercalation of the ligand could bend (or kink) the DNA helix, reducing the effective length, and its viscosity concomitantly.[9–18] The relative viscosity of ct-DNA bound with the Ru complex increased with increasing complex concentration similarly to the behavior of some known DNA intercalators,[20,35] indicative of a classical intercalation of the complex to the DNA.

3.2.4 Salt effects. The reverse salt titration is an efficient method to distinguish binding modes between the bound molecules and DNA. The groove-bound molecules can be easily released from the helix by increasing the ionic strength, while this is difficult for the intercalate-bound molecules.[36,37] The changes in UV–visible and emission spectra of the Ru(II) complex in the presence of the DNA upon increasing the concentration of NaCl added are shown in Fig. 8. The spectra were only partially recovered on different contents even on increasing NaCl concentration up to 1.5 M in comparison with the spectra shown in Fig. 3 and Fig. 4. This is consistent with an intercalative model. The insensitivity of the release of the complex to the NaCl concentration also supports intercalative binding by the anthryl moiety. This can be understood by the fact that the intercalative anthryl moiety is far away from the positively charged Ru(II) center and the intercalation of the anthryl moiety resulted in less release of sodium ions.

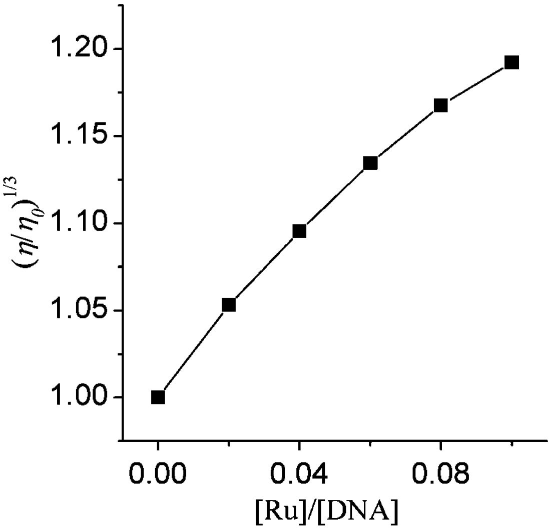

Fig. 7 The changes in the relative viscosities of DNA with increasing concentrations of the Ru(II) complex in buffered 50 mM NaCl.

The dependence of intrinsic DNA binding constant of the complex on [Na$^+$] was also investigated. The binding constants decreased with increasing salt concentration. A plot of logK_b *vs.* [Na$^+$] was linear below 0.1 M NaCl (Fig. 9). According to the polyelectrolyte theory developed by Record *et al.*,[37] the observed binding constant K_b is a function of the charge on the cation (Z), the fraction of counterion associated with each DNA phosphate (Ψ) which is generally taken to be 0.88 for double-stranded B-form DNA. A slope in a plot of logK_b *vs.* log[Na$^+$] is equal to SK in eqn (3):

$$SK = \delta \log K/\delta \log K[\mathrm{Na}^+] = -Z\Psi \quad (3)$$

$$\Delta G_{\mathrm{obs}} = -RT \ln K_{\mathrm{obs}} \quad (4)$$

$$\Delta G_{\mathrm{pe}} = SKRT \ln [\mathrm{Na}^+] \quad (5)$$

$$\Delta G_{\mathrm{t}} = \Delta G_{\mathrm{obs}} - \Delta G_{\mathrm{pe}} \quad (6)$$

The binding free energy can be calculated from eqn (4). Electrostatic (ΔG_{pe}) and nonelectrostatic (ΔG_{t}) portions of the free energy can be calculated from eqn (5) and (6), respectively. An SK value of −0.85 for the interaction of the complex (Fig. 9) yielded a charge Z of 0.97 which is less than two positive charges carried by the Ru(II) complex. A nonelectrostatic free energy ΔG_{t} was derived to be −20.4 kJ mol^{-1},

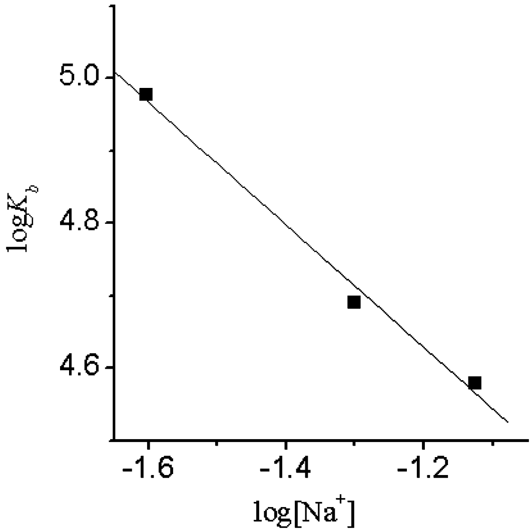

Fig. 9 The dependence of the DNA binding constant of the Ru(II) complex on the salt concentrations.

and electrostatic free energy ΔG_{pe} of -6.3 kJ mol^{-1} in 50 mM NaCl. It is apparent that the electrostatic contribution to the free energy is much less than the nonelectrostatic portion, strongly supporting the theory that that the anthryl moiety is intercalated between the base pairs of the DNA helix, otherwise, if a closer moiety than the anthryl on the complex intercalated in the DNA, it would result in a much more increased contribution of the electrostatic part, as we recently observed for a dinuclear Ru(II) complex of $[(bpy)_2Ru(ebipcH_2)Ru(bpy)_2](ClO_4)_4$, where ebipcH$_2$ = *N*-ethyl-4,7-bis([1,10]-phenanthroline-[5,6-*f*]imidazol-2-yl)carbazole.[14]

3.2.5 DNA melting studies. A further evidence for the intercalation of the Ru(II) complex into the helix was obtained from the DNA melting studies. Intercalation of small molecules into the helix is known to increase the helix melting tempetature (T_m), the temperature at which the double helix denatures into single-stranded DNA.[38] The extinction coefficient of DNA bases at 260 nm in the double-helical form is much less than in the single-stranded form, hence, melting of the helix leads to an increase in the absorption at this wavelength.[20] The DNA melting temperatures in the absence and in the presence of different concentrations of the Ru(II) complex are present in Fig. 10. The melting temperature (T_m) of the free duplex is 66.8 °C. In the presence of $[(bpy)_2Ru(aip)](ClO_4)_2$, it increased to 84.8 °C ($\Delta T_m = 18.0$ °C) with the [DNA]/[Ru] = 10 : 1. The observation that the complex increased the T_m is consistent with that expected for intercalators and indicates stabilization by the metal complex of the double helix. Classical intercalators generally give ΔT_m valued of >5 °C at the concentration ratio of [DNA]/[Ru] = 10 : 1.[39,40] The large increase in the DNA melting temperatures upon binding of the Ru(II) complex in this study to the DNA, strongly supports for intercalation of the complex into the helix.

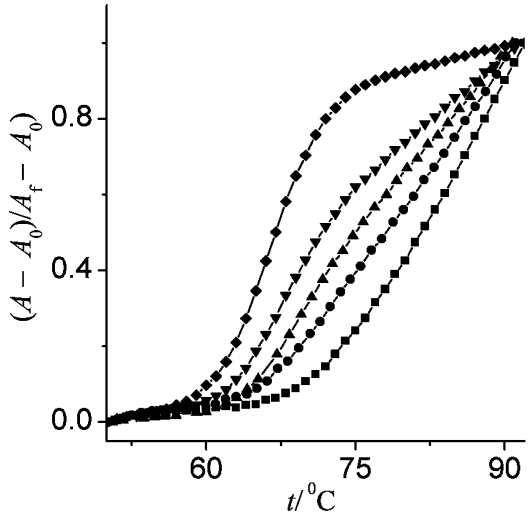

Fig. 10 The melting curves of ct-DNA (60.0 μM) at 260 nm in the absence and the presence of the Ru(II) complex with different ratios of [DNA] to [Ru]. DNA alone (◆); 40 : 1(▼); 25 : 1(▲); 15 : 1(●); 10 : 1(■).

4. Conclusions

A newly synthesized Ru(II) complex was demonstrated to be a sensitive spectroscopic pH sensor and switch for the MLCT emissive route, and a DNA intercalator. The anthryl group was shown to be the intercalative moiety on the complex by the following points: the anthryl group-based UV bands caused evident hypochromism upon binding the complex to the DNA; the DNA binding constant is of the same order of magnitude as those for proven anthryl-related DNA intercalators; the electrostatic binding free energy is much smaller than the non-electrostatic one. DNA melting studies showed ΔT_m values of 18 °C at [DNA] : [Ru] = 10 : 1. Further applications of the complex for selective sequence recognition of nucleic acid are in progress.

Acknowledgements

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References

1 A. F. Nogueira, S. H. Toma, M. Vidotti, A. L. B. Formiga, S. I. C. de Torresi and H. E. Toma, *New J. Chem.*, 2005, **29**, 320.
2 H. Xu, K. C. Zheng, H. Deng, L. J. Lin, Q. L. Zhang and L. N. Ji, *New J. Chem.*, 2003, **27**, 1255.
3 E. Ruba, J. R. Hart and J. K. Barton, *Inorg. Chem.*, 2004, **43**, 4570.
4 C. Metcalfe and J. A. Thomas, *Chem. Soc. Rev.*, 2003, **32**, 215.
5 C. Moucheron, A. K. D. Mesmaeker and S. Choua, *Inorg. Chem.*, 1997, **36**, 584.
6 Y. Liu, A. Chouai, N. N. Degtyareva, D. A. Lutterman, K. R. Dunbar and C. Turro, *J. Am. Chem. Soc.*, 2005, **127**, 10796.
7 L. N. Ji, X. H. Zhou and J. G. Liu, *Coord. Chem. Rev.*, 2001, **216**, 513.
8 J. Olofsson, L. M. Wilhelmsson and P. Lincoln, *J. Am. Chem. Soc.*, 2004, **126**, 15458.
9 K. E. Duncan, D. T. Odom and J. K. Barton, *Chem. Rev.*, 1999, **99**, 2797.

10 C. S. Allardyce, P. J. Dyson, D. J. Ellis and S. L. Heath, *Chem. Commun.*, 2001, 1396.
11 D. Bashford and M. Karplus, *Biochemistry*, 1990, **29**, 10219.
12 G. Y. Bai, K. Z. Wang, Z. M. Duan and L. H. Gao, *J. Inorg. Biochem.*, 2004, **98**, 1017.
13 H. Chao, R. H. Li, B. H. Ye, H. Li, X. L. Feng, J. W. Cai, J. Y. Zhou and L. N. Ji, *J. Chem. Soc., Dalton Trans.*, 1999, 3711.
14 F. R. Liu, K. Z. Wang, G. Y. Bai, Y. Z. Zhang and L. H. Gao, *Inorg. Chem.*, 2004, **43**, 1799.
15 G. Y. Bai, B. Dong, Y. Y. Lü, K. Z. Wang, L. P. Jin and L. H. Gao, *J. Inorg. Biochem.*, 2003, **97**, 207.
16 S. D. Jiang, K. Z. Wang, F. R. Liu and Y. A. Zhang, *Acta. Chim. Sinica*, 2005, **63**, 783.
17 M. J. Han, K. Z. Wang, Y. Y. Lü and L. H. Gao, *Chem. J. Chin. Univ.*, 2004, **25**, 2221.
18 C. V. Kumar and E. H. J. Asuncion, *J. Chem. Soc., Chem. Commun.*, 1992, 470.
19 C. V. Kumar, E. H. A. Punzalan and W. B. Tan, *Tetrahedron*, 2000, **56**, 7027.
20 C. V. Kumar and E. H. Asuncion, *J. Am. Chem. Soc.*, 1993, **115**, 8547.
21 After completion of this work, a publication of a analogue complex appeared: M. Mariappan and B. G. Maiya, *Eur. J. Inorg. Chem.*, 2005, 2164.
22 J. G. Liu, Q. L. Zhang, X. F. Shi and L. N. Ji, *Inorg. Chem.*, 2001, **40**, 5045.
23 M. E. Reichmann, S. A. Rice, C. A. Thmas and P. Doty, *J. Am. Chem. Soc.*, 1954, **76**, 3047.
24 Z. Q. Ji, S. P. D. Huang and A. R. Gaudalupe, *Inorg. Chim. Acta*, 2000, **305**, 127.
25 A. J. Mondal, G. Ramakrishna, A. K. Singh, H. N. Ghosh, M. Mariappan, B. G. Maiya, T. Mukherjee and D. K. Palit, *J. Phys. Chem. A*, 2004, **108**, 7843.
26 A. Wolf, G. H. Shimer Jr. and T. Meehan, *Biochemistry*, 1987, **26**, 6392.
27 H. Deng, J. W. Cai, H. Xu, H. Zhang and L. N. Ji, *Dalton Trans.*, 2003, 325.
28 Q. X. Zhen, Q. L. Zhang, J. G. Liu, B. H. Ye, L. N. Ji and L. Wang, *J. Inorg. Biochem.*, 2000, **78**, 293.
29 R. Ostaszewski, E. Wilczynska and M. Wolszczak, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 2995.
30 D. L. Carlson, D. H. Huchital, E. J. Mantilla, R. D. Sheardy and W. R. Murphy, *J. Am. Chem. Soc.*, 1993, **115**, 6424.
31 P. Lincoln, A. Broo and B. Norden, *J. Am. Chem. Soc.*, 1996, **118**, 2644.
32 J. Z. Wu, B. H. Ye, L. Wang, L. N. Ji, J. Y. Zhou, R. H. Li and Z. Y. Zhou, *J. Chem. Soc., Dalton Trans.*, 1997, 1395.
33 T. Biver, F. Secco and M. R. Tinè, *J. Inorg. Biochem.*, 2004, **98**, 33.
34 Y. Y. Lü, L. H. Gao, M. J. Han and K. Z. Wang, *Eur. J. Inorg. Chem.,* DOI: 10.1002/ejic.200500748.
35 S. Satyanarayana, J. C. Dabrowiak and J. B. Chaires, *Biochemistry*, 1992, **31**, 9319.
36 L. S. Ling, Z. K. He and Y. E. Zeng, *Spectrochim. Acta, Part A*, 1999, **55**, 1297.
37 M. T. Record, C. F. Anderson and T. M. Lohman, *Rev. Biophys.*, 1978, **11**, 103.
38 D. J. Patel, *Acc. Chem. Res.*, 1979, **12**, 118.
39 S. J. Heater, M. W. Carrano, D. Rains, R. B. Walter, D. Ji, Q. Yan, R. S. Czernuszewic and C. Carrano, *Inorg. Chem.*, 2000, **39**, 3881.
40 C. M. Dupureur and J. K. Barton, *Inorg. Chem.*, 1997, **36**, 33.