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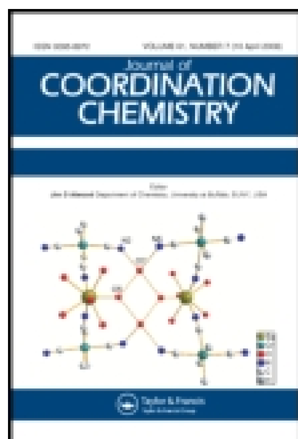

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Spectroscopy studies on DNA binding of two ruthenium complexes $[Ru(MeIm)_4(L)]^{2+}$ (L = iip and tip)

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Spectroscopy studies on DNA binding of two ruthenium complexes $[Ru(MeIm)_4(L)]^{2+}$ (L = iip and tip)

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Two ruthenium(II) complexes $[Ru(MeIm)_4(L)]^{2+}$ (L = 2-(imidazo-4-group)-1H-imidazo-[4,5-f][1,10]phenanthroline, 2-(thiophene-2-group)-1H-imidazo[4,5-f][1,10]phenanthroline, MeIm = 1-methylimidazole) have been synthesized according to literature and structurally characterized. The interaction of the complexes with calf thymus DNA has been explored using electronic absorption titration, competitive binding experiment, circular dichroism, thermal denaturation, and viscosity measurements. The results show that both complexes could bind DNA in a intercalation mode and the DNA-binding affinity of $[Ru(MeIm)_4(tip)]^{2+}$ ($K_b = (7.2 \pm 0.3) \times 10^5\,(\text{mol L}^{-1})^{-1}$) is greater than that of $[Ru(MeIm)_4(iip)]^{2+}$ ($K_b = (6.1 \pm 0.2) \times 10^5\,(\text{mol L}^{-1})^{-1}$).

Keywords: Ru(II) complexes; DNA binding; Spectral property

1. Introduction

Transition metal complexes with efficient DNA binding and cleavage properties under physiological conditions have found wide applications in nucleic acid chemistry [1–3]. Studies on small molecule binding to DNA are very important in the development of new therapeutic reagents and DNA molecular probes [4–6]. In general, ruthenium(II) complexes bind to DNA in a non-covalent interaction such as electrostatic binding, groove binding [7], intercalative binding or partial intercalative binding [8, 9]. Many important applications of these complexes require that the complexes bind to DNA in an intercalative mode and it is already known that intercalative ligands govern the DNA-binding modes and affinities. Therefore, a great deal of work has been done on modifying the intercalative ligand [10–13]. However, most of the reported Ru(II) complexes are less soluble in water due to their big polycyclic heteroaromatic hydrophobic ligands. Recently, we reported the DNA-binding of $[Ru(MeIm)_4(dppz)]^{2+}$ (MeIm = 1-methylimidazole, dppz = dipyrido[3,2-a:20,30-c]phenazine) [14] and that the aqueous solubility of $[Ru(MeIm)_4(dppz)]^{2+}$ is better than that of $[Ru(bpy)_4(dppz)]^{2+}$

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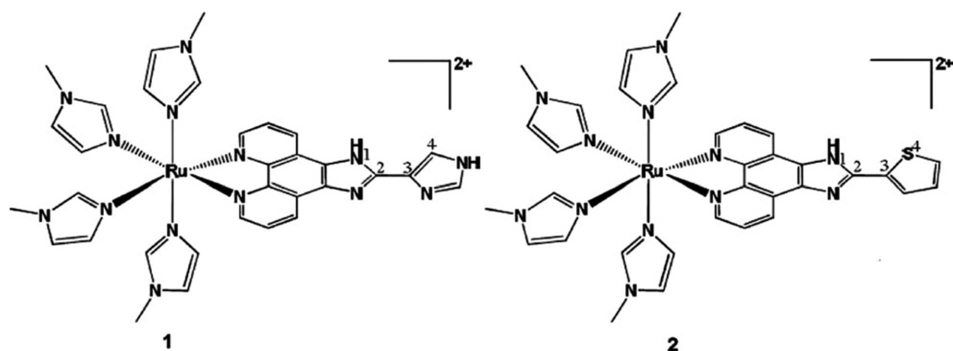

Figure 1. Structural diagram of $[Ru(MeIm)_4(iip)]^{2+}$ (**1**) and $[Ru(MeIm)_4(tip)]^{2+}$ (**2**).

emerged. Ru(II)-imidazole complexes and their derivatives have already been proven to possess antitumor [15–17] and immunosuppressive activity [18]. To more clearly understand the selectivity and efficiency of DNA recognized and cleaved by Ru(II) complexes, an appropriate intercalative ligand is helpful in distinguishing the small differences of the interaction of complexes with DNA.

In this article, two ruthenium complexes $[Ru(MeIm)_4(L)]^{2+}$ (L = iip or tip) (figure 1) were synthesized according to literature [19] and the interactions of these complexes with DNA as well as the related properties were experimentally explored.

2. Experimental

2.1. *Chemicals*

Buffers were prepared as follows: buffer A: 5 m mol L^{-1} *Tris*-HCl, 50 m mol L^{-1} NaCl, pH 7.2. buffer B: 1.5 m mol L^{-1} Na_2HPO_4, 0.5 m mol L^{-1} NaH_2PO_4, and 0.25 m mol L^{-1} Na_2EDTA.

Calf thymus DNA (CT-DNA) was obtained from Sigma (St. Louis, MO, USA). Solution of CT-DNA in buffer A gave a ratio of UV-Visible (UV-Vis) absorbance of 1.8–1.9 : 1 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein [20, 21]. The concentration of CT-DNA in nucleotide phosphate (NP or bases) was determined spectrophotometrically using a molar absorptivity of 6600 $(mol\ L^{-1})^{-1}\ cm^{-1}$ (260 nm) [22]. All reagents and solvents were purchased commercially and used without purification unless otherwise noted. Double distilled water was used to prepare buffers.

2.2. *Physical measurement*

Electrospray ionization mass spectrometry (ESI-MS) was recorded on a LQC system (Finnigan MAT, USA) using CH_3CN as mobile phase. The spray voltage, tube lens offset, capillary voltage, and capillary temperature were set at 4.50 kV, 30.00 V, 23.00 V, and 200°C, respectively, and the m/z values were quoted for the major peaks in the isotope distribution. Microanalysis (C, H, and N) were carried out with a Perkin Elmer

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240Q elemental analyzer. ^{1}H-NMR spectra were recorded on a Bruker AVANCE AV400 spectrometer with $(CD_3)_2SO$ as solvent at 400 MHz at room temperature. All chemical shifts are relative to tetramethylsilane. UV-Vis and emission spectra were measured on a Perkin Elmer Lambda-850 spectrophotometer and Ls55 spectrofluorophotometer at 25°C.

2.3. *DNA-binding experiments*

2.3.1. Electronic absorption titration. Absorption titrations of Ru(II) complexes in buffer A were performed by using a fixed Ru(II) complex concentration to which increments of DNA stock solution were added. Initially, 3000 μL solutions of the blank buffer and the Ru(II) complex ($20\,\mu\mathrm{mol\,L^{-1}}$) were placed in the reference and sample cuvettes (1 cm path length), respectively, and then the first spectrum was recorded from 200 to 650 nm. During the titration, an aliquot (3–10 μL) of DNA stock solution (concentration of $5\text{–}10\,\mathrm{m\,mol\,L^{-1}}$ in NP) was added to each cuvette to eliminate the absorbance of the DNA itself, and the solutions were mixed by repeated inversion. The solutions were allowed to incubate for 5 min before the absorption spectra were recorded. The titration processes were repeated until there was no change in the spectra for at least four titrations, indicating binding saturation had been achieved. The changes in the Ru(II) complex concentration due to dilution at the end of each titration were negligible. The intrinsic binding constants of both complexes, K_b, to DNA were obtained by monitoring the changes of the ^{1}MLCT absorbance for both complexes according to the following equation (1a) and (1b) [23–27]:

$$\left(\varepsilon_a - \varepsilon_f\right)/\left(\varepsilon_b - \varepsilon_f\right) = \left(b - \left(b^2 - 2K_b^2 C_t[DNA]/s\right)^{1/2}\right)2K_b C_t \tag{1a}$$

$$b = 1 + K_b C_t + K_b[DNA]/2s \tag{1b}$$

where ε_a is the extinction coefficient (A_{abs}/[M]) observed for the ^{1}MLCT absorption band at a given DNA concentration, ε_f and ε_b the extinction coefficient for the free Ru(II) complex and the extinction coefficient for the Ru(II) complex in the fully bound form, respectively, [DNA] the concentration of DNA in nucleotides, C_t the total Ru(II) complex concentration, K_b the equilibrium binding constant in $(\mathrm{mol\,L^{-1}})^{-1}$, and s the binding site size.

2.3.2. Competitive binding experiment. Experiments of DNA competitive binding with ethidium bromide (EB) were carried out in buffer A by keeping [DNA]/[EB] = 10 : 1 ([DNA] = $100\,\mu\mathrm{mol\,L^{-1}}$, [EB] = $10\,\mu\mathrm{mol\,L^{-1}}$) and varying the concentrations of the Ru(II) complex ([Ru] = $0\text{–}10\,\mu\mathrm{mol\,L^{-1}}$). Samples were excited at 340 nm and emission was observed between 530 and 670 nm. Control experiments were performed by keeping [EB] = $10\,\mu\mathrm{mol\,L^{-1}}$ (in the absence of DNA, [Ru] = $0\text{–}10\,\mu\mathrm{mol\,L^{-1}}$). The quenching constant K was calculated according to the classical Stern–Volmer equation (equation (2)) [28].

$$\frac{I_o}{I} = 1 + Kr \tag{2}$$

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where I_0 and I are the fluorescence intensities in the absence and presence of complex $[Ru(MeIm)_4(L)]^{2+}$ (L = iip or tip), respectively, and r the ratio of the total concentration of $[Ru(MeIm)_4(L)]^{2+}$ (L = iip or tip) to that of DNA.

2.4. *Circular dichroic spectra study*

Circular dichroic (CD) spectra of CT-DNA were measured on a JASCO J-810 spectropolarimeter at room temperature. The region from 220 to 620 nm was scanned for each sample in buffer A. CT-DNA was $2 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$ in NP and Ru(II) complexes were added to a ratio of [Ru]/[DNA] = 2 : 1. High-frequency noise was filtered out using JASCO Spectra Manager software. The CD spectral studies for each sample were repeated at least three times.

2.5. *Thermal denaturation*

Thermal denaturation studies were carried out on a Perkin Elmer Lambda-850 spectrophotometer equipped with a Peltier temperature controlling programmer PTP-6 (±0.1°C) in buffer B. With the use of the thermal melting program, the temperature of the cell containing the cuvette was ramped from 40 to 90°C. The absorbance at 260 nm was monitored by every 1°C for solutions of CT-DNA ($80\,\mu\mathrm{mol\,L^{-1}}$) in the absence and presence of the Ru(II) complex ($8\,\mu\mathrm{mol\,L^{-1}}$) at different concentrations. The melting temperature Tm, which is defined as the temperature where half of the total base pairs are unbonded, was determined from the midpoint of the melting curves. ΔT_m values were calculated by subtracting T_m of the DNA alone from that of the DNA-complex adduct.

2.6. *Viscosity studies*

Viscosity measurements were carried out using an Ubbelohde viscometer maintained at a constant temperature at 30.0 ± 0.1°C in a thermostatic bath. DNA samples approximately, 200 b.p. in average length, were prepared by sonication using buffer A in order to minimize complexities arising from DNA flexibility [29]. Flow time was measured with a digital stopwatch, and each sample was measured three times, and an average flow time was calculated. Data are presented as $(\eta/\eta^0)^{1/3}$ *versus* binding ratio, where η is the viscosity of DNA in the presence of complex and η^0 the viscosity of DNA alone.

3. Results and discussion

3.1. *Electronic absorption titration*

Electronic absorption spectroscopy is one of the most useful techniques to investigate the interactions of complexes with DNA [30]. Usually, complex binding to DNA leads to hypochromism and bathochromism, probably because the electronic structure of the complex is disturbed by DNA. The hypochromism or bathochromism depends on the

complex or the intercalation mode. It is generally accepted that the extent of the hypochromism in the UV-Vis band is consistent with the strength of intercalative interaction [24, 31, 32]. Absorption spectra of **1** and **2** in the absence and presence of CT-DNA (at a constant concentration of complexes, [Ru] = 20 μ mol L^{-1}) are given in figure 2(a) and (b).

Absorption spectra of **1** and **2** have some similar features, i.e., there are two distinct bands with comparable intensity from 230 to 650 nm. The first is a broad absorption centered at 400–600 nm, which is generally assigned to a singlet metal-to-ligand charge transfer (^{1}MLCT). The other band centered at 275 nm is attributed to intraligand (IL)

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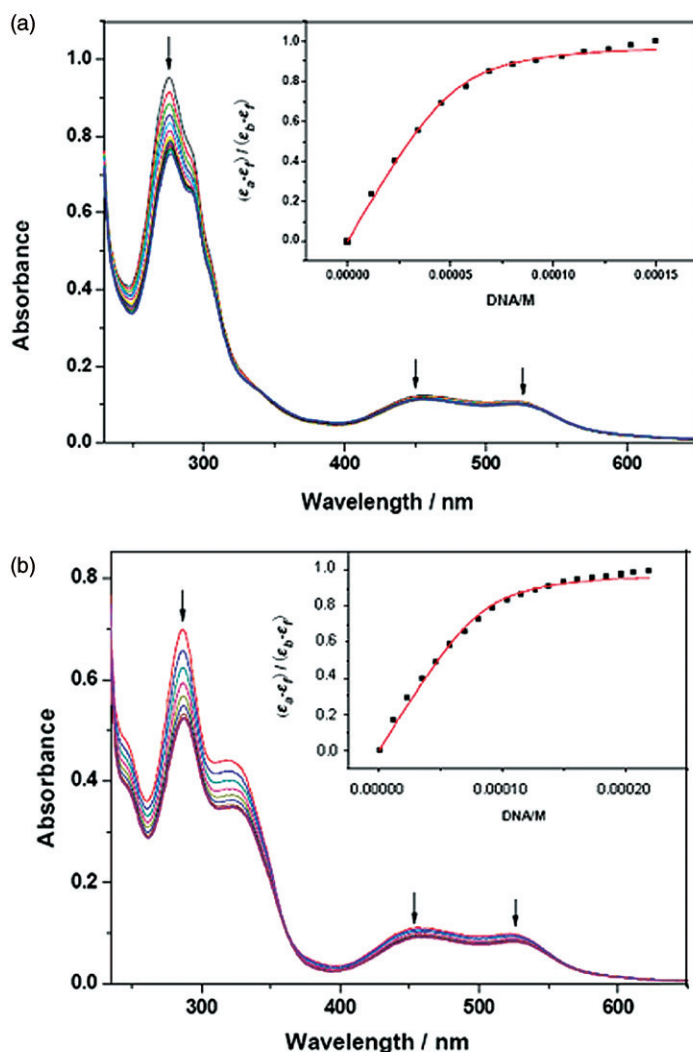

Figure 2. Absorption spectra of (a) **1** and (b) **2** in buffer A at 25°C in the presence of increasing amounts of CT-DNA. [Ru] = 20 μmol L^{-1}, [DNA] = 0–200 μmol L^{-1} from top to bottom. Arrows indicate the change in absorbance upon increasing the DNA concentration. Inset: plot of $(\varepsilon_a-\varepsilon_f)/(\varepsilon_b-\varepsilon_f)$ *vs*. [DNA] and the nonlinear fit for the titration of DNA to Ru(II) complexes.

Table 1. Absorption spectra (max/nm) and DNA-binding constants K_b of **1** and **2**.

Complex	λ_{max}	H (%)	$K_b/10^5$ (mol $L^{-1})^{-1}$	s
$[Ru(MeIm)_4(iip)]^{2+}$ (**1**)	523	6.3	6.1 ± 0.2	1.3
	456	7.5		
$[Ru(MeIm)_4(tip)]^{2+}$ (**2**)	276	21.2		
	523	23.8	7.2 ± 0.3	2.1
	456	25.2		
	287	32.1		

π–π^* transitions. Compound **2** has an obvious acromion centered at 330 nm, whereas **1** has none.

With increasing concentration of DNA, all the absorption bands show clear hypochromism, though no obvious red shift is observed. The hypochromism (H%), as defined by $H\% = 100\% \cdot (A_{free} - A_{bound})/A_{free}$, of metal-to-ligand charge transfer (^{1}MLCT) bands and intraligand (IL) bands of **1** were determined to be about 7.0% and 21.2%, respectively, with **2**, they were 24.0% and 32.1%, respectively. The change of absorption spectra is one of the most important evidences that the complex may bind to DNA through intercalation [33]. Obviously, the spectral characteristics suggest that strong π–π stacking occurred in the electrons between complexes and DNA. The percent hypochromism and K_b values of **1** and **2** in the presence of DNA at saturation are detailed in table 1.

The percent hypochromism of **2**, whether in the region of ^{1}MLCT band or IL band, is larger than that of **1**. The intrinsic binding constants K_b and s values of complexes follow the order of K_b(**2**) ($7.2 \pm 0.3 \times 10^5$) > K_b(**1**) ($6.1 \pm 0.2 \times 10^5$), s(**2**) > s(**1**). From reported results, such as $[Ru(bpy)_2(MCMIP)]^{2+}$ (3.92×10^4), $[Ru(phen)_2(MCMIP)]^{2+}$ (4.8×10^4) [4], $[Ru(dmb)_2(MCMIP)]^{2+}$ (2.25×10^4), $[Ru(dmp)_2(MCMIP)]^{2+}$ (5.42×10^4) [34], $[Ru(dmb)_2(dtni)]^{2+}$ (2.63×10^4), $[Ru(dmb)_2(dtni)]^{2+}$ (8.65×10^4) [35], $[Ru(dmb)_2(ITAP)]^{2+}$ (4.5×10^4) [36], $[Ru(bpy)_2(BFIP)]^{2+}$ ($4.6 \pm 0.1 \times 10^4$), $[Ru(dmb)_2(BFIP)]^{2+}$ ($3.2 \pm 0.3 \times 10^4$), $[Ru(phen)_2(BFIP)]^{2+}$ ($5.4 \pm 0.1 \times 10^4$) [37], and $[Ru(dmb)_2(ipdp)]^{2+}$ (7.18×10^3) [38], we can see that the K_b of **1** and **2** is larger than these complexes. The data obviously indicate that the DNA-binding affinity of **2** is stronger than that of **1**. As a result, we bring forward a hypothesis that the larger the hypochromism, the greater the DNA binding affinity of the complex [24, 31]. The distinction of DNA-binding affinity may be attributed to different structures between **1** and **2**, that is, the ligand iip of **1** is modified by imidazole while the ligand tip of **2** is modified by thiophene.

3.2. *Competitive binding experiment*

The molecular fluorophore EB emits intense fluorescence at 600 nm in the presence of CT-DNA due to its strong intercalation between the base pairs of DNA [39]. If a complex can replace EB from DNA-bound EB, the fluorescence of the solution will be quenched as free EB is quenched by surrounding water. No luminescence is observed for **1** and **2** in any solvent even in the presence of DNA. According to recent reports, $[Ru(dmb)_2(ITAP)]^{2+}$ [36], $[Ru(bpy)_2(BFIP)]^{2+}$, $[Ru(dmb)_2(BFIP)]^{2+}$ and $[Ru(phen)_2(BFIP)]^{2+}$ [37] emit luminescence in *tris* buffer (in the absence of DNA) at room temperature. Obviously, **1** and **2** do not have this characteristic. The control

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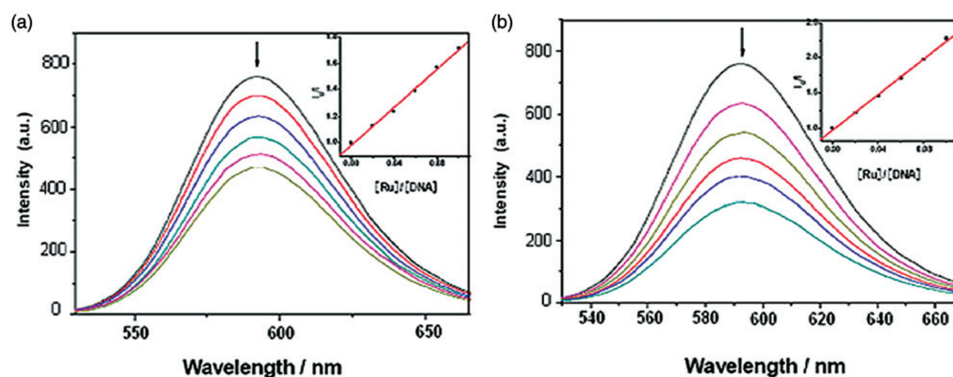

Figure 3. Changes in the emission spectra of DNA-bound EB in 5 m mol L^{-1} *Tris*-HCl buffer, pH = 7.2 ([EB] = 10 μmol L^{-1}, [DNA] = 100 μmol L^{-1}), with increasing concentrations of **1** (figure 3a) or **2** (figure 3b) from 0 to 10 μmol L^{-1}. Arrow shows the intensity change upon increasing concentrations of the complex. Inset: florescence quenching curve of DNA-bound EB by **1** or **2**, where I_0 and I are fluorescence intensities in the absence and the presence of the complexes.

experiments show that there is almost no change in the fluorescence intensity of free EB (in the absence of DNA) with increasing concentrations of **1** and **2**; consequently, competitive binding experiments using **1** and **2** as quenchers provide further information on the complex binding to DNA. The emission spectra of DNA-bound EB in the absence and the presence of **1** and **2** are given in figure 3.

A clear decrease in the emission intensity is observed with addition of **1** and **2** to DNA-bound EB solution, indicating intercalation of the complexes accompanied by release of EB. The fluorescence quenching curve (inset in figure 3) illustrates that the fluorescence quenching of DNA-bound EB is in good agreement with the linear Stern–Volmer equation [28]. The quenching constants K (the slope of quenching curve) follow the order $K(\mathbf{2}) > K(\mathbf{1})$, suggesting that **2** has a greater DNA-binding affinity than **1**. This trend is consistent with the absorption titration experiment. These results also indicate that **1** and **2** can replace EB from DNA-bound EB and intercalate into the DNA.

3.3. *CD spectra study*

CD spectra provide information about the chirality of spectroscopically active species in solution. The observed CD spectrum of CT-DNA contains a positive band at 275 nm due to base stacking and a negative band at 248 nm due to helicity, characteristic of DNA in the right-handed B form [26]. It is usually speculated that changed CD signals of DNA during interactions may be caused by structural distortion [40]. Thus, the CD spectral technique is used to evaluate the capability of some complexes for influencing the conformation of double helical structure of B DNA. It is commonly considered that classical intercalation can enhance base stacking, stabilize the helicity, and increase intensity of both bands, whereas simple groove binding and electrostatic interaction of small molecules show less or no perturbation on the base stacking and helicity bands [41].

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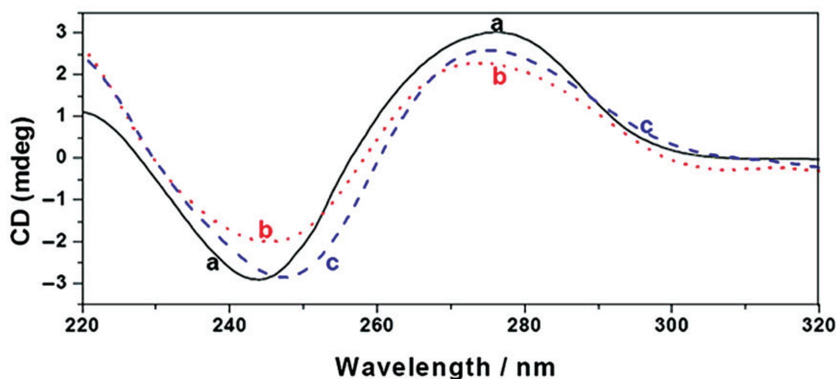

Figure 4. CD spectra of CT-DNA 240 μ mol L^{-1} in the absence (a ——) and presence of 30 μ mol L^{-1} **1** (b ······), and 30 μ mol L^{-1} **2** (c – – –).

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When **1** and **2** are incubated with CT-DNA at [Ru]/[DNA] = 1 : 8, the CD spectra of DNA in the presence of **1** (figure 4b) show a small intensity decrease at both positive and negative bands while no shift is observed. The intensity decrease of positive band shows that **1** affects the base stacking of CT-DNA, and the change of negative band suggests that it can induce uncoiling of CT-DNA [42]. The addition of **2** does not result in obvious CD spectra changes of CT-DNA (figure 4c), indicating that the structure of CT-DNA has not been greatly influenced.

3.4. *Thermal denaturation experiment*

Thermal denaturation for CT-DNA binding to the complex estimates the complexes' ability to stabilize the double-stranded structure of DNA [43]. It is well accepted that double-stranded DNA can dissociate to single strands gradually with increase of temperature of the solution, generating a hyperchromic effect in the absorption spectra of DNA bases (λ_{max} = 260 nm). Therefore, it is reasonable to monitor the absorbance of the DNA bases at 260 nm to determine the transition temperature of double strands to single strands [44, 45]. The interaction of small molecule with double helix DNA is known to increase the melting temperature (T_m) at which 50% of the double helix denatures into single-strand DNA [46, 47]. As a result, the thermal behavior of DNA in the presence of complexes can give insight into their conformation changes with temperature increase and offer some information concerning the interaction affinities of complexes for DNA.

The melting curves of CT-DNA in the absence and the presence of **1** and **2** are shown in figure 5. A T_m experiment carried out for CT-DNA (in buffer B, section 2) in the absence of any added complex shows a T_m of 60.0 ± 0.2°C under our experimental conditions. The addition of **1** and **2** results in the T_m of DNA increasing to 70.4 ± 0.2°C and 75.3 ± 0.2°C, respectively, at a concentration ratio [Ru]/[DNA] = 1 : 10. The ΔT_m values of **1** and **2** are determined as 10.4°C and 15.3°C, respectively, which are higher than those reported for $[Ru(NH_3)_4(dppz)]^{2+}$ (ΔT_m = 5.2°C) [26], $[Ru(MeIm)_4(dpq)]^{2+}$ (ΔT_m = 5.0°C) [22], $[Ru(MeIm)_4(dppz)]^{2+}$ (ΔT_m = 7.6°C) [7], $[Ru(dmb)_2(ITAP)]^{2+}$ (ΔT_m = 6.7°C) [36], $[Ru(bpy)_2(BFIP)]^{2+}$ (ΔT_m = 4.0°C), $[Ru(dmb)_2(BFIP)]^{2+}$

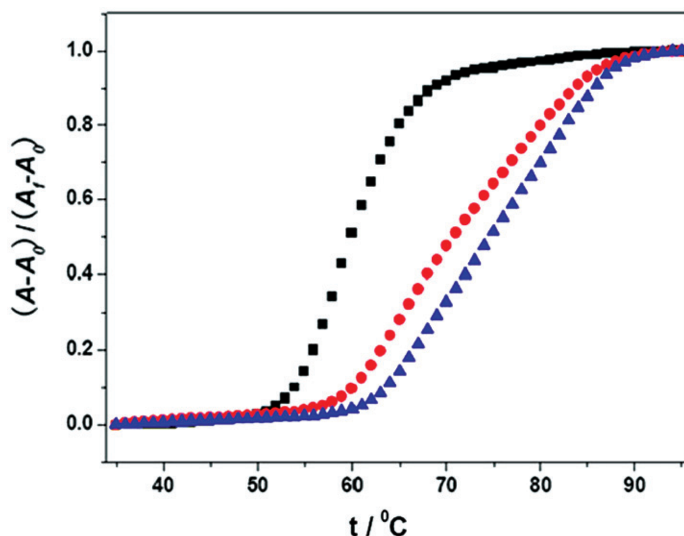

Figure 5. Melting curves of CT-DNA (100 m mol L^{-1}) at 260 nm in the absence (■) and the presence of **1** (•) or **2** (▲) at [Ru] = 10 mmol L^{-1}, where A_0, A_f and A are the absorption intensities at 35°C, 95°C and at a given temperature between 35°C and 95°C, respectively.

($\Delta T_m = 2.0$°C) and $[Ru(phen)_2(BFIP)]^{2+}$ ($\Delta T_m = 9.0$°C) [37]. These values fall in the range of typical classical intercalators [43, 44], indicating that the ligands (iip and tip) intercalate between the DNA base pairs strongly. Furthermore, the fact that the increase (13.5°C) in T_m of **2** is larger than that of **1** is also in good agreement with the trend discussed above.

3.5. *Viscosity studies*

Measuring the viscosity of DNA is regarded as the most critical test of a DNA binding model in solution and could provide strong arguments for intercalative binding [41, 48]. A classical intercalation model demands that the DNA helix lengthens as base pairs are separated to accommodate the binding ligand, which leads to an increase in the viscosity of DNA [49,50]. However, a partial and/or non-classical intercalation of ligand may bend (or link) the DNA helix, resulting in decreasing its effective length and, concomitantly, its viscosity [51,52]. In order to further elucidate the binding mode for the present complexes, viscosity measurements are carried out, keeping [DNA] = 0.5 m mol L^{-1} and various concentrations of the complexes.

As shown in figure 6, EB, a well-known DNA intercalator, increases the relative viscosity strongly by lengthening the DNA double helix through intercalation, whereas $[Ru(bpy)_3]^{2+}$, which binds to DNA in the electrostatic mode, exerts essentially no influence on DNA viscosity. With increasing amounts of **1** and **2**, the relative viscosity of DNA increases steadily, similar to the behavior of EB. The increased viscosity, which may depend on the binding affinity to DNA, decreases in the order EB > $[Ru(MeIm)_4(tip)]^{2+}$ (**2**) > $[Ru(MeIm)_4(iip)]^{2+}$ (**1**) > $[Ru(bpy)_3]^{2+}$. These experimental results suggest that **1** and **2** bind to DNA through classical intercalation, and the

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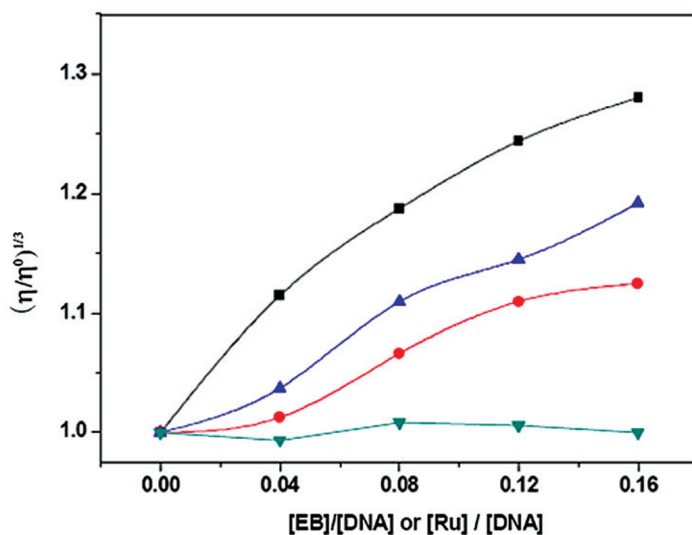

Figure 6. Effect of increasing amounts of EB (■), **1** (●), **2** (▲) and $[Ru(bpy)_3]^{2+}$ (▼) on the relative viscosities of CT-DNA at $30 \pm 0.1°C$; $[DNA] = 0.5\,mmol\,L^{-1}$.

binding affinity of **2** is stronger than that of **1**, consistent with the above experimental results.

4. Conclusions

The binding behavior of the complexes to CT-DNA were investigated by absorption, competitive binding, thermal denaturation, circular dichroism, and viscosity experiments. The results indicate that **1** and **2** can bind to DNA in an intercalative mode and the DNA-binding affinity of $[Ru(MeIm)_4(tip)]^{2+}$ ($K_b = (7.2 \pm 0.3) \times 10^5\,(mol\,L^{-1})^{-1}$) is greater than that of $[Ru(MeIm)_4(iip)]^{2+}$ ($K_b = (6.1 \pm 0.2) \times 10^5\,(mol\,L^{-1})^{-1}$). This difference in affinity may be due to the different structures of two complexes. We put forward a hypothesis – in **2**, the thiazole loop and the ip (ip = 1H-imidazo[4,5-f][1,10]phenanthroline) loop can approximately belong to the same plane, which could be beneficial for intercalating in the DNA. However, in the case of **1**, the imidazole loop and the ip (ip = 1H-imidazo[4,5-f][1,10]phenanthroline) loop are not in the same plane. This structural property may engender essential steric hindrance when inserting in DNA and the binding affinity of **2** is stronger than that of **1**.

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References

[1] J.K. Barton. *Science*, **233**, 727 (1986).
[2] K.E. Erkkila, D.T. Odom, J.K. Barton. *Chem. Rev.*, **99**, 2777 (1999).
[3] L.N. Ji, X.H. Zou, J.G. Liu. *Coord. Chem. Rev.*, **216**, 513 (2001).
[4] Y.J. Liu, H. Chao, Y.X. Yuan, H.J. Yu, L.N. Ji. *Inorg. Chim. Acta*, **359**, 3807 (2006).
[5] L. Mishra, A.K. Yadaw, S. Srivastava, A.B. Patel. *New J. Chem.*, **24**, 505 (2000).
[6] D.B. Hall, R.E. Holmlin, J.K. Barton. *Nature*, **382**, 731 (1996).
[7] J.G. Collins, A.D. Sleeman, J.R. Aldrich-Wright, I. Greguric, T.W. Hambley. *Inorg. Chem.*, **37**, 3133 (1998).
[8] W.J. Mei, J. Liu, K.C. Zheng, L.J. Lin, H. Chao, A.X. Li, F.C. Yun, L.N. Ji. *Dalton Trans.*, 1352 (2003).
[9] E. Tuite, P. Lincoln, B. Nordén. *J. Am. Chem. Soc.*, **119**, 239 (1997).
[10] X.W. Liu, J. Li, H. Deng, K.C. Zheng, Z.W. Mao, L.N. Ji. *Inorg. Chim. Acta*, **358**, 3311 (2005).
[11] X.W. Liu, J. Li, H. Li, K.C. Zheng, H. Chao, L.N. Ji. *J. Inorg. Biochem.*, **992**, 372 (2005).
[12] F. Gao, H. Chao, F. Zhou, Y.X. Yuan, B. Peng, L.N. Ji. *J. Inorg. Biochem.*, **100**, 1487 (2006).
[13] D. Lawrence, V.G. Vaidyanathan, B.U. Nair. *J. Inorg. Biochem.*, **100**, 1244 (2006).
[14] L.M. Chen, J. Liu, J.C. Chen, C.P. Tan, S. Shi, K.C. Zheng, L.N. Ji. *J. Inorg. Biochem.*, **102**, 330 (2008).
[15] A.H. Velders, A. Bergamo, E. Alessio, E. Zangrando, J.G. Haasnoot, C. Casarsa, M. Cocchietto, S. Zorzet, G. Sava. *J. Med. Chem.*, **47**, 1110 (2004).
[16] M. Ravera, S. Baracco, C. Cassino, P. Zanello, D. Osella. *Dalton Trans.*, 2347 (2004).
[17] G. Sava, K. Clerici, I. Capozzi, M. Cocchietto, R. Gagliardi, E. Alessio, G. Mestroni, A. Perbellini. *Anticancer Drugs*, **10**, 129 (1999).
[18] C.M. Bastos, K.A. Gordon, T.D. Ocain. *Bioorg. Med. Chem. Lett.*, **8**, 147 (1998).
[19] L.M. Chen, *Studies of Interactions of CT-DNA and Human Telomeric Sequence with Water-Soluble Ru-Imidazole Complexes*, **3**, 84 (in Chinese) (2008).
[20] C.L. Schildkraut, J. Marmur, P. Doty. *J. Mol. Biol.*, **3**, 595 (1961).
[21] J. Marmur, P. Doty. *J. Mol. Biol.*, **3**, 585 (1961).
[22] P.U. Maheswari, V. Rajendiran, M. Palaniandavar, R. Parthasarathi, V. Subramanian. *J. Inorg. Biochem.*, **100**, 3 (2006).
[23] F. Gao, H. Chao, F. Zhou, Y.X. Yuan, B. Peng, L.N. Ji. *J. Inorg. Biochem.*, **100**, 1487 (2006).
[24] X.W. Liu, J. Li, H. Li, K.C. Zheng, H. Chao, L.N. Ji. *J. Inorg. Biochem.*, **99**, 2372 (2005).
[25] P.U. Maheswari, M. Palaniandavar. *J. Inorg. Biochem.*, **98**, 219 (2004).
[26] R.B. Nair, E.S. Teng, S.L. Kirkland, C.J. Murphy. *Inorg. Chem.*, **37**, 139 (1998).
[27] M.T. Carter, M. Rodriguez, A.J. Bard. *J. Am. Chem. Soc.*, **111**, 8901 (1989).
[28] J.R. Lakowicz, G. Weber. *Biochemistry*, **12**, 4161 (1973).
[29] J.B. Chaires, N. Dattagupta, D.M. Crothers. *Biochemistry*, **21**, 3933 (1982).
[30] J.K. Barton, A. Danishefsky, J. Goldberg. *J. Am. Chem. Soc.*, **106**, 2172 (1984).
[31] X.W. Liu, J. Li, H. Deng, K.C. Zheng, Z.W. Mao, L.N. Ji. *Inorg. Chim. Acta*, **358**, 3311 (2005).
[32] A.M. Pyle, J.P. Rehmann, R. Meshoyrer, C.V. Kumar, N.J. Turro, J.K. Barton. *J. Am. Chem. Soc.*, **111**, 3051 (1989).
[33] S.A. Tysoe, R.J. Morgan, A.D. Baker, T.C. Strekas. *J. Phys. Chem.*, **97**, 1707 (1993).
[34] Y.J. Liu, J.F. He, J.H. Yao, W.J. Mei, F.H. Wu, L.X. He. *J. Coord. Chem.*, **62**, 665 (2009).
[35] Y. Liu, Y.J. Liu, J.H. Yao, W.J. Mei, F.H. Wu. *J. Coord. Chem.*, **62**, 1701 (2009).
[36] F.H. Wu, C.H. Zeng, Y.J. Liu, X.Y. Guan, L.X. He. *J. Coord. Chem.*, **62**, 3512 (2009).
[37] K.A. Kumar, K.L. Reddy, S. Satyanarayana. *J. Coord. Chem.*, **63**, 3676 (2010).
[38] Y.J. Liu, X.Y. Guan, X.Y. Wei, L.X. He, W.J. Mei, J.H. Yao. *Transition Met. Chem.*, **33**, 289 (2008).
[39] J.B. LePecq, C. Paoletti. *J. Mol. Biol.*, **27**, 87 (1967).
[40] V.I. Ivanov, L.E. Minchenkova, A.K. Schyolkina, A.I. Poletayev. *Biopolymers*, **12**, 89 (1973).
[41] B. Norden, F. Tjerneld. *Biopolymers*, **21**, 1713 (1982).
[42] Y. Zhao, W. He, P. Shi, J. Zhu, L. Qiu, L. Lin, Z. Guo. *Dalton Trans.*, 2617 (2006).
[43] J.M. Kelly, A.B. Tossi, D.J. McConnell, C. OhUigin. *Nucleic Acids Res.*, **13**, 6017 (1985).
[44] C.V. Kumar, E.H. Asuncion. *J. Am. Chem. Soc.*, **115**, 8547 (1993).
[45] M. Cory, D.D. McKee, J. Kagan, D.W. Henry, J.A. Miller. *J. Am. Chem. Soc.*, **107**, 2528 (1985).
[46] M. Cusumano, A. Giannetto. *J. Inorg. Biochem.*, **65**, 137 (1997).

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[47] G.A. Neyhart, N. Grover, S.R. Smith, W.A. Kalsbeck, T.A. Fairley, M. Cory, H.H. Thorp. *J. Am. Chem. Soc.*, **115**, 4423 (1993).
[48] L.S. Lerman. *J. Mol. Biol.*, **3**, 18 (1961).
[49] S. Satyanarayana, J.C. Dabrowiak, J.B. Chaires. *Biochemistry*, **32**, 2573 (1993).
[50] S. Satyanarayana, J.C. Dabrowiak, J.B. Chaires. *Biochemistry*, **31**, 9319 (1992).
[51] Y.J. Liu, H. Chao, L.F. Tan, Y.X. Yuan, W. Wei, L.N. Ji. *J. Am. Chem. Soc.*, **99**, 530 (2005).
[52] M.J. Waring. *J. Inorg. Biochem.*, **13**, 269 (1965).

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