Binding of bimetallic 1,10-phenanthroline ruthenium(II) complexes to DNA

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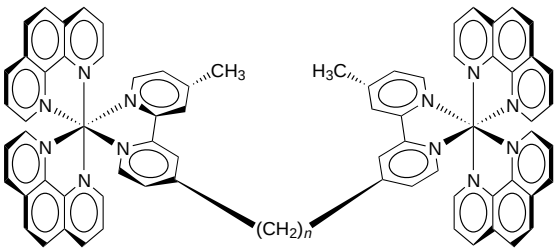
Letter

The binding of $[(phen)_2Ru^{II}(Mebipy)\text{-}(CH_2)_n\text{-}(bipyMe)Ru^{II}(phen)_2]^{4+}$ (Mebipy = 4-methyl-2,2′-bipyridyl-4′-, $n = 5$ **2a**, 7 **2b** or 10 **2c**) to double-stranded DNA is found to be sensitive to the linker chain length n and to show a DNA-induced stacking interaction at high binding concentrations (*e.g.* [Nu]/[Ru] = 1).

There is considerable interest in the DNA binding of metal polypyridyl complexes, because of potential applications as DNA probes[1,2] (including the study of electron transfer processes involving DNA[3]) and as possible antitumour agents.[4] A wide range of mononuclear complexes have been studied, with $[Ru(phen)_3]^{2+}$ (phen = 1,10-phenanthroline), in particular, being much investigated. The relatively weak binding ($K \approx 10^4$ dm^3 mol^{-1} basepair^{-1}) of this complex (for which the precise mode has been a matter of controversy[1,5]) and its displacement from DNA at even medium ionic strengths represent a serious limiting factor for the application of such systems, *e.g.* in *in vivo* conditions (150 mM Na^+). We[6] and others[7,8] have suggested that this problem might be overcome by the use of binuclear systems. To demonstrate this effect we initially chose to work with complexes of the type $[(bipy)_2Ru(Mebipy)\text{-}(CH_2)_n\text{-}(bipyMe)Ru(bipy)_2]^{4+}$, where Mebipy = 4-methyl-2,2′-bipyridyl-4′-, bipy = 2,2′-bipyridyl ($n = 5$ **1a** or 7 **1b**), as the parent complex $[Ru(bipy)_3]^{2+}$ is an extremely weak binder.[6] The bimetallic complexes proved to have much higher affinity for DNA. One might expect that the phenanthroline analogues **2** would bind even more strongly and we present here some DNA binding characteristics of these complexes. We also report that the linker chain length (n) is a crucial factor in determining the binding efficiency. For classic intercalators with polymethylene chains[9] it is known that the most effective binding occurs with $n > 8$, whereas for the bimetallic complexes **2** we show that the strongest binding occurs with a somewhat shorter chain length, consistent with the partial intercalation model proposed for $[Ru(phen)_3]^{2+}$.[5] It is also found that at low [Nu]/[Ru] ratios (*i.e.* the ratio of concentrations of DNA nucleotides to ruthenium centres) complexes **2a**–**2c** show unusual photophysical properties, consistent with DNA-induced interaction of the phenanthroline rings.



$[(phen)_2Ru^{II}(Mebipy)\text{-}(CH_2)_n\text{-}(bipyMe)Ru^{II}(phen)_2]^{4+}$
($n = 5$ **2a**, $n = 7$ **2b** or $n = 10$ **2c**)

The change in relative intensity of emission (I_{DNA}/I_{free}) with increasing DNA concentration, in aerated 10 mM phosphate buffer, is presented for the series of phen complexes over a range of [Nu]/[Ru] (0–40) in Fig. 1 and Table 1. Under conditions where 100% of the molecules are bound to DNA (*i.e.* in the plateau region of the plot), only the bimetallic heptane-linked complex **2b** exhibited a relative increase of emission intensity comparable to that for the monometallic ($I_{DNA}/I_{free} = 2.40$) and consistent with both centres being bound in a similar manner. (We anticipate that the mode of binding is similar to that of $[Ru(phen)_3]^{2+}$ which is partially intercalated between the base pairs leading to a kink in the DNA.[5]) The decane-linked complex **2c** exhibits an intensity increase only half of that found for **2b** (*i.e.* $I_{DNA}/I_{free} = 1.68$). The longer chain therefore seems to prevent interaction of the second centre with the base pairs of DNA. On the other hand, for **2a** ($n = 5$) where $I_{DNA}/I_{free} = 2.08$, it appears that the chain is too short to facilitate full interaction of the phen ligand on the second centre. In agreement with this the binding site sizes per complex determined using the McGhee von Hippel approach[10] (Table 1) are 3.2 base pairs for the monometallic complex $[Ru(phen)_2(Me_2bipy)]^{2+}$ (Me_2bipy = 4,4′-dimethyl-2,2′-bipyridyl), 8.8 base pairs for **2b** ($n = 7$), 6.4 for **2a** ($n = 5$) and 6.0 for **2c** ($n = 10$).

Table 1 Relative emission enhancements (I_{DNA}/I_{free}), binding constants (K) and site sizes (B_{ap}) measured in 10 mM potassium phosphate buffer, and fraction remaining bound upon addition of 500 mM NaCl for $[Ru(phen)_2(Me_2bipy)]^{2+}$ and bimetallic phenanthroline complexes **2a**–**2c**

Complex	I_{DNA}/I_{free} [a,b]	10^{-4} K [a,c] /mol dm^{-3} basepair^{-1}	B_{ap} [a,c] /basepair	Fraction bound [d] in 500 mM NaCl
$[Ru(phen)_2(Me_2bipy)]^{2+}$	2.40	7.5	3.2	0.00
2a ($n = 5$)	2.08	240	6.4	0.12
2b ($n = 7$)	2.36	360	8.8	0.21
2c ($n = 10$)	1.68	300	6.0	0.08

[a] [Ru centre] = 1.0×10^{-5} M in 10 mM potassium phosphate buffer, pH = 6.9. [b] Conditions were [Nu]/[Ru] = 80 and correspond to 100% bound ruthenium complexes. [c] These values were determined from the emission data using the McGhee von Hippel approach. [d] In buffer containing 10 mM phosphate and 500 mM NaCl with [Nu]/[Ru] = 40 for $[Ru(phen)_2(Me_2bipy)]^{2+}$ and 20 for bimetallic complexes **2a**–**2c**. The fraction bound is determined from the pre-exponential factor of the long-lived component of the emission decay and assumes that the short-lived component corresponds to ruthenium centres which are not bound to the DNA.

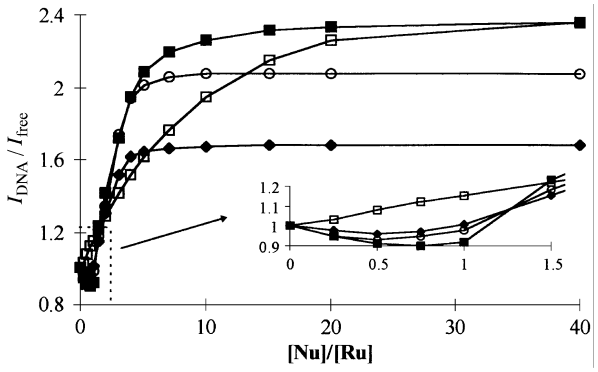


Fig. 1 Variation of relative emission intensity (at $\lambda = 610$ nm) upon addition of salmon sperm DNA to $[Ru(phen)_2(Me_2bipy)]^{2+}$ (□) and to the bimetallics $[(phen)_2Ru^{II}(Mebipy)\text{-}(CH_2)_n\text{-}(bipyMe)Ru^{II}(phen)_2]^{4+}$ (○) **2a**, $n = 5$; (■) **2b**, $n = 7$; (◆) **2c**, $n = 10$. [Ru centre] = 1.0×10^{-5} M in aerated 10 mM potassium phosphate buffer, pH = 6.9. Insert: expansion of effect at low [Nu]/[Ru] ratios ($0 \rightarrow 1.5$)

A serious drawback in the application of many mononuclear ruthernium complexes is their poor binding at high ionic strengths. In agreement with this, our steady-state and time-correlated emission studies show that $[Ru(phen)_2(Me_2bipy)]^{2+}$ which is 100% bound in 10 mM buffer is completely displaced from DNA upon addition of 250 mM NaCl. By contrast a significant fraction of the bimetallic complexes remain bound even in the presence of 500 mM NaCl, with **2b** showing a particularly high affinity (Table 1). This suggests that non-electrostatic binding is considerably more important for **2b** and therefore corroborates the results presented above.

An additional feature of the bimetallic complexes **2** is observed at high binding ratios (*i.e.* low [Nu]/[Ru] ratios). Careful examination of the effects of binding on the absorption spectra shows that when the absorbance ratio (A_{DNA}/A_{free}) is plotted as a function of [DNA] for both sets of bimetallic complexes **1** and **2** a two-stage [Nu]/[Ru]-dependent process is revealed, in contrast to that observed for the monometallic species $[Ru(L)_2(Me_2bipy)]^{2+}$ (Fig. 2). The emission behaviour at low [Nu]/[Ru] (0–1) ratios of the bimetallic phenanthroline complexes **2** however is strikingly different from that of the bipy analogues **1**. Thus whereas titration plots indicated that $[Ru(phen)_2(Me_2bipy)]^{2+}$ and the binuclear bipy complexes **1a** and **1b** each exhibit an increase in luminescence intensity upon addition of DNA, a pronounced decrease in the I_{DNA}/I_{free} of 5–10% was observed for each of **2a**–**2c** (inset in Fig. 1). The magnitude of this quenching process varies as a function of chain length according to $n = 7 > 5 > 10$. The time-resolved emission data for **2a** ($n = 5$) in deaerated solution at [Nu]/[Ru] = 1 indicated the presence of three components including a species with a lifetime significantly shorter ($\tau_{DNA} = 568$ ns) than that of the complex free in solution ($\tau_{free} = 1341$ ns). These results together with the absorption spectral data above correlate well with there being at equimolar concentrations of metal centre and nucleotide, a DNA-induced stacking conformation of the bimetallic phenanthroline complexes, which facilitates a partial emission quenching process. The fact that no emission quenching is observed for the bimetallic bipy complexes may be attributed to their less extended aromatic ligands.

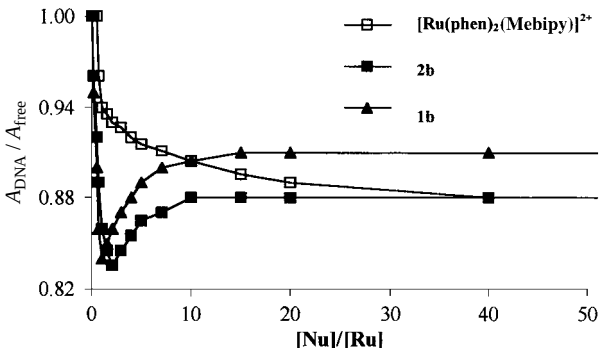


Fig. 2 Change in the absorbance upon addition of salmon sperm DNA to the ruthenium complex solutions in 10 mM phosphate buffer. (Absorbance measured at the λ_{max} for the unbound complex). [Ru centre] = 1.0×10^{-5} M in 10 mM potassium phosphate buffer, pH = 6.9

In conclusion we have shown that in designing bimetallic systems as DNA probes it is of great importance to optimize both the linker length and the nature of the other interacting ligand. With complexes **2a**–**2c** only the heptane-linker allows full interaction of both centres and this complex also shows the greatest resistance to increased ionic strength of the solution. The significant self-quenching observed for the phen complexes at low [Nu]/[Ru] ratios may also be an important factor.

Experimental

Synthesis of complexes

The ligands (Mebipy)-$(CH_2)_n$-(bipyMe) were prepared by a modified version of the procedure used by Furue *et al.*[11] Complexes $[L_2Ru(Mebipy)\text{-}(CH_2)_n\text{-}(bipyMe)RuL_2]^{4+}$ (L = phen or bipy) were prepared by reaction of RuL_2Cl_2 with the appropriate ligand. These products, which are mixtures of stereoisomers, were observed as single compounds on HPLC and TLC. ^1H NMR spectra were consistent with the proposed structures. An example of the preparative method is given below.

$[(phen)_2Ru(Mebipy)\text{-}(CH_2)_5\text{-}(bipyMe)Ru(phen)_2][PF_6]_4$ (2a). An aqueous methanol (1 : 1, v/v) solution (30 cm^3) of $Ru(phen)_2Cl_2$ (235 mg; 0.44 mmol) was refluxed for 1 h under nitrogen, and then an aqueous methanol (1 : 1, v/v) solution (25 cm^3) of (Mebipy)-$(CH_2)_5$-(bipyMe) (86 mg; 0.21 mmol) was added over a period of 30 min. The solution was refluxed for a further 23 h and monitored by UV/VIS spectroscopy. The complex **2a** was separated with an SP-Sephadex (C-25) cation exchange resin (40–120 μ) using 0.50 M aqueous NaCl as eluant and precipitated as its PF_6 salt using a saturated solution of KPF_6 (yield = 158 mg, 41%). Further purification was achieved by semi-preparative cationic exchange HPLC using an eluant of $CH_3CN : H_2O$ (80 : 20, v/v), 0.15 M KNO_3 and subsequent precipitation as its PF_6 salt.

Reactions with DNA

Salmon sperm DNA (Sigma) was purified to remove protein and dialysed into 10 mM potassium phosphate buffer (pH = 6.9). Experiments with DNA were carried out in 10 mM potassium phosphate buffer (pH = 6.9) using Millipore Milli-Q water, previously sterilized by autoclaving. The ruthenium complexes were converted to their chloride salts using Amberlite IRA 402 ion exchange resin. All ruthenium and DNA solutions were prepared fresh prior to each experiment. The ruthenium centre concentration was fixed at 10 μM, and DNA from a concentrated solution was added to achieve the desired nucleotide to ruthenium centre ratios ([Nu]/[Ru]) of 0–80.

Absorption spectra were recorded on a Pye-Unicam SP8-800 or SP8-200 UV/VIS spectrophotometer with data stored, manipulated and analysed using a PC computer. Steady-state emission spectra and luminescence intensity data were recorded on a Perkin-Elmer MPF-44B fluorescence

spectrophotometer and spectra were subsequently corrected. Luminescence lifetimes (τ) were measured using a time-correlated single photon counting apparatus (Edinburgh Instruments FL-900) with N_2 as the emission gas ($\lambda_{excit} = 359$ nm, frequency = 35 kHz) and using a gated delay generator. All solutions were thermostated to 25 °C.

Acknowledgements

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