Interaction of a series of bimetallic ruthenium(II) bipyridyl complexes with DNA

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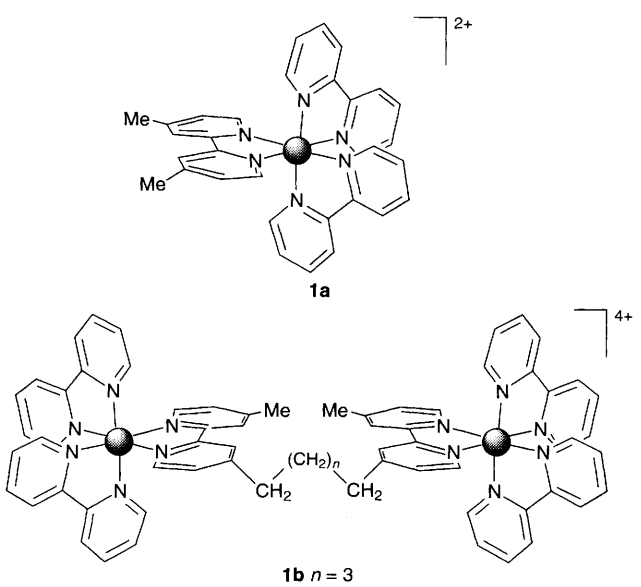
In contrast to $[Ru(bipy)_3]^{2+}$, the bimetallic ruthenium bipyridyl complexes $[(bipy)_2Ru^{II}\{Mebipy(CH_2)_nbipyMe\}Ru^{II}(bipy)_2]^{4+}$ (n = 5,7) bind more strongly to DNA and can photosensitise DNA strand breaks even at high ionic strengths.

The binding of ruthenium polypyridyl complexes to DNA is an area of considerable interest and current activity, with recent developments of these systems including 'light switches', stereospecific DNA agents and the formation of photo-adducts.[1–4] A particular emphasis recently has been on the use of complexes with extended aromatic ligands, so as to enhance non-covalent binding, especially through intercalation. Another approach to improve DNA interaction, previously used in organic systems,[5] is to prepare covalently linked bifunctional compounds.[6] We have used this approach, to investigate a series of ruthenium complexes based on the weakly interacting 2,2′-bipyridyl (bipy) ligand. The results show that these compounds exhibit useful behaviour as photophysical and photochemical probes for DNA. We report that these bimetallic complexes exhibit (*a*) much higher binding affinity (*b*) more efficient photocleavage properties and (*c*) less sensitivity to ionic strength than their monometallic analogue $[Ru(bipy)_2(Me_2bipy)]^{2+}$ (Me_2bipy = 4,4′-dimethyl-2,2′-bipyridyl).

Previous studies,[7,8] have clearly demonstrated that $[Ru(bipy)_3]^{2+}$ differs from complexes with polypyridyl ligands such as 1,10-phenanthroline (phen) in that it binds weakly to the DNA helix, and only at low ionic strengths (< 10 mmol dm^{-3} NaCl), probably by groove-binding. We now report on how the binding of the bimetallic derivatives of $[Ru(bipy)_3]^{2+}$ **1b** and **1c**† differs from that of the monometallic analogue $[Ru(bipy)_2(Me_2bipy)]^{2+}$ **1a**.



Steady-state emission studies, at low ionic strength, show that upon addition of salmon-sperm DNA to the ruthenium complex solutions, an emission intensity enhancement (I_{DNA}/I_{free}) and a red shift, are observed for both mono- (**1a**) and bimetallic (**1b**, **1c**) complexes. However, from the luminescence titration curve [Fig. 1(*a*)] we see that the strength of binding, indicated by the value $([Nu]/[Ru])_{1/2}$,‡ is much greater for the bimetallic complexes (**1b**, **1c**) and preliminary calculations using the McGhee–von Hippel method[9] indicates that the binding constants are two orders of magnitude greater. Comparison of the emission intensity enhancement for the bimetallic complexes (**1b**, **1c**) with that observed for $[Ru(bipy)_2(Me_2bipy)]^{2+}$ **1a** (I_{DNA}/I_{free} = 1.60 at saturation) leads to the conclusion that both metal centres of **1b** and **1c** are bound to DNA.§

Addition of sodium chloride to the monometallic complex **1a** in DNA solution results in strong quenching of the emission intensity as the binding is significantly diminished in the presence of salt. However, this effect is much less significant for the bimetallic complexes (**1b**, **1c**) as shown by the luminescence titration curve at 50 mmol dm^{-3} NaCl [Fig. 1(*b*)]. This shows that, even at high DNA concentrations, the binding to DNA remains very significant for the bimetallic complexes (**1b**, **1c**) while that for the monometallic complex (**1a**) is very weak. Indeed further experiments have shown that the bimetallic

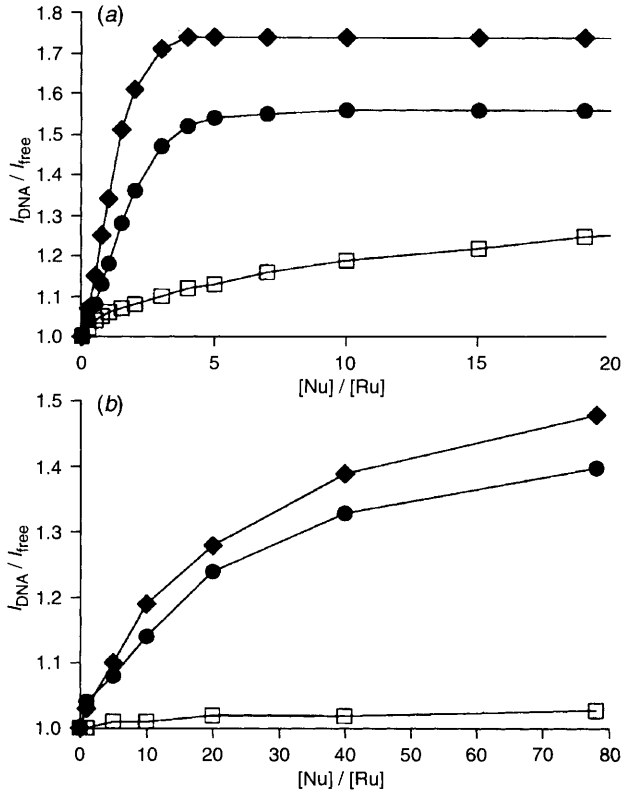


Fig. 1

compounds still bind significantly to the DNA helix at sodium chloride concentrations as high as 100 mmol dm^{-3}.

Upon photo-excitation, ruthenium complexes have been shown to induce single-strand breaks in DNA.[7,8,10] This can be most conveniently studied by monitoring the conversion of the covalently closed circular (ccc) form of pBR322 plasmid DNA to the relaxed open circular (oc) form. Previous studies in low ionic strength buffer have shown that $[Ru(phen)_3]^{2+}$ and $[Ru(bipy)_3]^{2+}$ are approximately equally effective.[7] The irradiation of plasmid DNA in the presence of the bimetallic complexes (**1b**, **1c**) and the monometallic analogue (**1a**) has been studied to determine the efficiency of these complexes as photocleavage agents. The results were analysed by gel electrophoresis and microdensitometric scanning. $[Ru(phen)_3]^{2+}$, extensively studied in the literature,[3] was used as a reference. There is evidence for single-strand breaks with all the complexes, under the conditions studied. For the bimetallic complex **1b** double-strand breaks, leading to the linearization of pBR322, were observed at irradiation times of 30 min in low ionic strength conditions. Studies showed that both bimetallic complexes (**1b**, **1c**) are equally effective as photocleavage agents and that they are both more effective (per ruthenium centre) than $[Ru(phen)_3]^{2+}$, in particular under high ionic strength conditions. At low ionic strengths, it can be seen that the monometallic complex **1a** induces conversion of 65% of the ccc to oc form after 30 min irradiation [Fig. 2(*a*)]. The bimetallic species **1b**, however, completely converts the plasmid to the oc form after only 10 min. At high ionic strengths (50 mmol dm^{-3} NaCl) this difference is more pronounced, where significant cleavage occurs only with the bimetallic complex **1b** [Fig. 2(*b*)]. Combined with the titration data above, this indicates that the enhanced binding, shown previously, potentiates photosensitised DNA cleavage. Irradiation, in the presence of the singlet-oxygen quencher sodium azide (20 mmol dm^{-3}),[11] produced a 50% decrease in cleavage for all the complexes, suggesting that the cleavage mechanism involves both type I (radical mediated)[10] and type II (singlet-oxygen mediated)[7,8] processes.

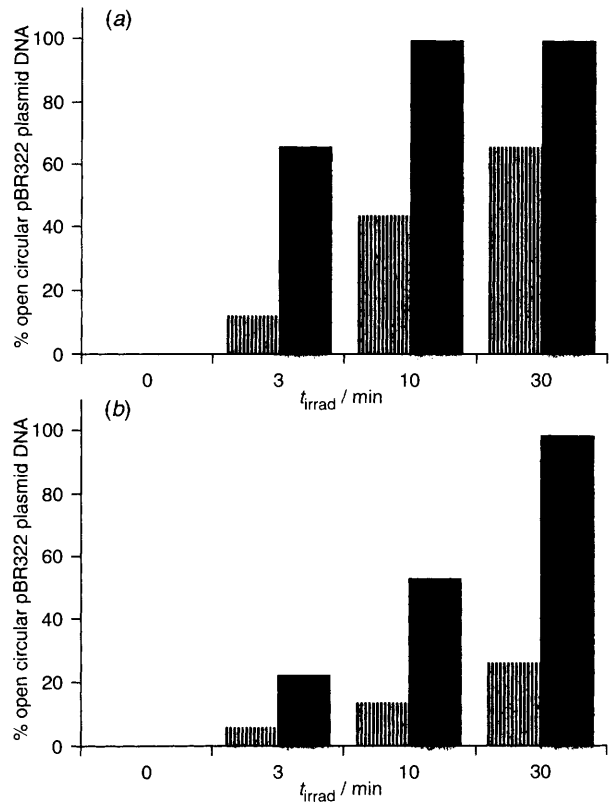


Fig. 2

In conclusion we have shown that these bimetallic complexes, under the conditions described, show (*a*) higher binding affinity, (*b*) more efficient photocleavage properties and (*c*) less sensitivity to ionic strength than their monometallic analogues.

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Footnotes

† The ligands were prepared by the method of Furue *et al.*[12] and the complexes, which are a mixture of stereoisomers, by reaction with the bis-ligand complex $[Ru(bipy)_2Cl_2]$.[13] Purification was performed on SP-Sephadex C-25. All of the compounds gave satisfactory microanalytical, spectral and ^1H NMR spectroscopic data in accord with their assigned structures.

‡ [Nu]/[Ru] is the ratio of concentrations of nucleotide to ruthenium centre. $([Nu]/[Ru]_{1/2}$ is the value, taken from the titration plot, where $[(I_{DNA}/I_{free}) - 1] = 1/2[(I_{DNA}/I_{free})_{max} - 1]$ is reached.

§ The different emission intensity enhancement $(I_{DNA}/I_{free})_{max}$ for **1b** compared to that for **1c** may be due to quenching of the excited state by the other ruthenium centre of the molecule in solution. This process is more important in the free pentane-linked species (**1b**) than in the heptane-linked complex (**1c**). This quenching process will be eliminated as binding to DNA causes separation of the metal centres. Lifetimes in degassed 10 mmol dm^{-3} potassium phosphate buffer: **1a**, 620 ns; **1b**, 520 ns; **1c**, 570 ns (±10 ns).

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