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A mass spectrometric investigation of non-covalent interactions between ruthenium complexes and DNA†

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Electrospray ionisation mass spectrometry was used to investigate reactions between six ruthenium compounds and three different non self-complementary duplex oligonucleotides containing 16 base pairs. Each of the compounds studied formed non-covalent complexes containing between one and five ruthenium molecules bound to DNA. Competition experiments involving duplex 16mers and pairs of ruthenium compounds were used to determine the order of relative binding affinities of the metal compounds. Other competition experiments involving ruthenium compounds, and the organic DNA binding agents daunomycin and distamycin, provided information about the sites and modes of DNA binding of the ruthenium compounds.

Introduction

The discovery that $[Ru(phen)_3]^{2+}$ and related complexes can bind non-covalently in a shape-selective fashion to DNA has raised considerable interest in metal/DNA interactions,[1,2] and raised the prospect of designer molecules for a variety of applications. These include use as nucleic acid probes,[3,4] synthetic restriction enzymes,[2,5] and DNA repair agents.[2,6] Each of these applications requires reagents that can bind with high affinity and selectivity to specific DNA base sequences or structures.

Although square planar platinum(II) complexes were the first metal compounds shown to bind by intercalation to DNA,[7] most interest now focuses on metallointercalators containing ruthenium, rhodium or osmium. This is partially owing to the greater size of these octahedral molecules, which provides a greater number of intermolecular contacts with nucleic acids and therefore greater opportunities for selective molecular recognition. Additional interest arose from the discovery that $[Ru(phen)_3]^{2+}$ binds in an enantioselective fashion to B-DNA.[8] It was initially suggested that there were two binding modes for the enantiomers of $[Ru(phen)_3]^{2+}$, both of which involved the DNA major groove.[9] One of these binding modes was proposed to involve intercalation of a single phenanthroline ligand. However, later studies involving viscosity measurements suggested that neither enantiomer binds to DNA like a classical intercalator.[10] Debate over the mechanism of binding of both isomers of $[Ru(phen)_3]^{2+}$ continues, with some reports suggesting that they interact with the DNA minor groove,[11–15] and another providing evidence that the mechanism of binding is dependent on the concentration of the metal complex.[16]

There have now been many studies on the DNA binding properties of novel metallointercalators. Some of these studies have centred on complexes with the general formula $[Ru(phen)_2L]^{2+}$, where L is an extended planar aromatic ligand capable of intercalating deeply into the DNA base stack, and providing an overall stronger binding interaction than $[Ru(phen)_3]^{2+}$.[17–21] Fig. 1 illustrates the structures of six such complexes that were used in the present study. Ruthenium complexes containing the dppz ligand are generally regarded as having the greatest affinity for DNA of complexes of this type.[17] However, despite this potentially useful property, $[Ru(phen)_2(dppz)]^{2+}$ has been reported to display little DNA specificity, with only a slight binding preference for AT-rich DNA sequences.[18] Photophysical experiments involving both enantiomers of $[Ru(phen)_2(dppz)]^{2+}$ showed that the Δ isomer intercalates more deeply into the right handed helix of B-DNA than the Λ isomer, and consequently binds more tightly.[19] This trend has been observed with related complexes, illustrating the importance of chirality in molecular recognition.[1,2]

The complexes $[Ru(phen)_2(dpq)]^{2+}$ and $[Ru(phen)_2(dpqC)]^{2+}$ both contain an extended aromatic ligand similar to dppz, facilitating their intercalation into the DNA base stack. It was recently concluded on the basis of NMR spectroscopic evidence that $[Ru(phen)_2(dpq)]^{2+}$ binds by intercalation to the minor groove of DNA, and displays a preference for binding to purine–purine/pyrimidine–pyrimidine sequences.[20,21] Although a detailed binding study involving $[Ru(phen)_2(dpqC)]^{2+}$ was not performed, preliminary results were also consistent with this binding mode.

The binding of complexes such as those in Fig. 1 to DNA has been studied using many techniques, each with their own strengths. For example, circular dichroism spectroscopy provides information on the enantioselectivity of binding between chiral metallointercalators and DNA, but does not provide great detail about specific intermolecular interactions. On the other hand, NMR spectroscopy provides a large amount of information about such interactions, and provides evidence for the location of DNA binding sites. However, the complexity of NMR spectra has meant that this technique has been generally restricted to relatively short oligonucleotides, which may not have structures representative of cellular DNA. A further drawback of NMR spectroscopy is that it cannot always provide the same level of information about paramagnetic metal complexes as it can for diamagnetic complexes.

One technique that can be applied to all metal complexes, but has so far received little attention for studying their non-covalent interactions with DNA, is electrospray ionisation mass spectrometry (ESI-MS). ESI-MS is now routinely used to characterise biopolymers such as proteins and nucleic acids.[22–24] Furthermore it has also been used to study the formation of non-covalent complexes between biopolymers,[25,26] and between small organic molecules and biopolymers.[26–29] Recently the ability of ESI-MS to rapidly assess whether metal complexes act as minor groove binders or intercalators towards DNA was demonstrated.[30] The metal complexes

† Electronic supplementary information (ESI) available: Table S1: Assignments for ions observed in ESI mass spectra of reaction mixtures containing ruthenium compounds and DNA. Table S2: Assignments for ions observed in ESI mass spectra of reaction mixtures containing either daunomycin or distamycin, DNA and ruthenium compounds. See http://www.rsc.org/suppdata/dt/b4/b406889k/

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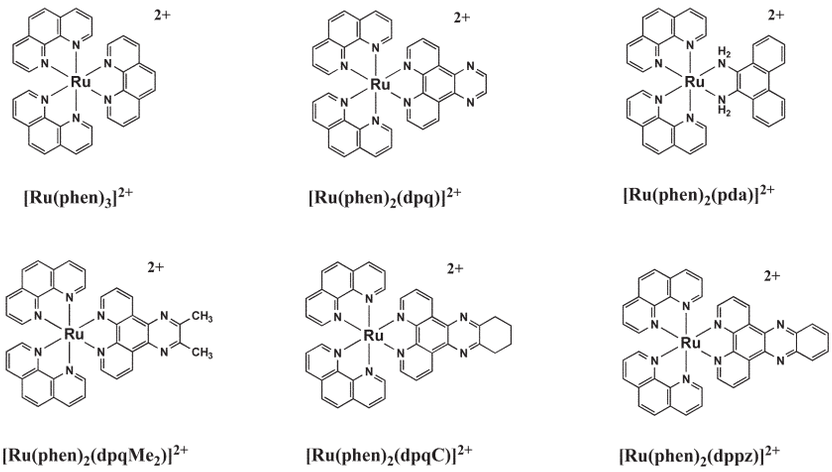

Fig. 1 Structures of the ruthenium compounds used in this study.

studied included several porphyrin derivatives, and a ruthenium(II) complex containing both dppz and a macrocyclic thioether ligand.

In this paper we present results obtained from a detailed investigation into the binding of the six compounds shown in Fig. 1 to the duplex 16 base pair oligonucleotides D1, D2 and D3. Each of the three duplexes has the same four base pairs at each end, but they differ in the identity of the central eight base pairs.

D1 d(CCTCGGCCGGCCGACC/GGTCGGCCGGCCGAGG)
D2 d(CCTCATGGCCATGACC/GGTCATGGCCATGAGG)
D3 d(CCTCAAAATTTTGACC/GGTCAAAATTTTGAGG)

The greater GC content of D1 and D2 was expected to enhance binding by ruthenium compounds that can intercalate,[31] while D3 contains AT base sequences that were expected to be favourable for compounds that bind along the DNA minor groove.[32] Experiments were also performed in which individual ruthenium compounds were allowed to compete for sites on DNA with either distamycin or daunomycin. These were conducted in order to further elucidate the sites and/or modes of DNA interaction of the ruthenium compounds. A preliminary account of some of this work has already been published.[33]

Experimental

Materials

$[Ru(phen)_3]Cl_2$ was obtained from Aldrich, while all other ruthenium compounds were prepared by literature methods.[20,34,35] Daunomycin and distamycin were obtained from Sigma. Oligonucleotides were obtained from Geneworks, Adelaide, South Australia as "trityl-on" derivatives, and deprotected and purified using procedures previously reported.[36] The concentrations of oligonucleotides were estimated by measurement of UV absorbance at 260 nm using values of ε_{260} for adenine, guanine, cytosine and thymine of 15200, 12010, 7050 and 8400 M^{-1} cm^{-1}, respectively.[37] Stock solutions (1.0 mM) of individual ruthenium compounds, daunomycin and distamycin, were prepared by dissolving the appropriate amount of compound in 0.1 M ammonium acetate (NH_4OAc), pH 8.5.

Double-stranded DNA (dsDNA) was prepared by heating complementary strands (0.4–2.0 mM) in 0.1 M aqueous ammonium acetate solution, pH 8.5, to 20 °C higher than the melting temperature for ~15 min, and then annealing by cooling slowly overnight. Complexes of dsDNA with individual ruthenium compounds were prepared by annealing samples of DNA in 0.1 M ammonium acetate, pH 8.5, and then adding the required amount of ruthenium compound. The same procedure was used for competition experiments, except that two ruthenium compounds were added after the annealing process was completed. The ratio of both ruthenium compounds relative to dsDNA in competition experiments was 3 : 3 : 1. A second type of competition experiment was performed in which ruthenium compounds were allowed to compete for sites on DNA with either daunomycin or distamycin. These experiments were performed by first annealing dsDNA overnight, and then adding the required amount of either a ruthenium compound, or one of the organic drugs, and allowing the solution to stand for 1 h. After this period of time an appropriate quantity of the second drug was then added, and ESI mass spectra obtained after 1 h further standing.

All mass spectra were acquired using a Micromass (Wyntheshawe, UK) Qtof2 mass spectrometer with a Z-spray probe. The mass analyser had an *m*/*z* (mass-to-charge ratio) range of 10000. All samples were diluted with 100 mM ammonium acetate, pH 8.5, giving a final concentration of dsDNA of 10 μM. These were injected directly using a Harvard model 22 syringe pump (Natick, MA, USA) at a flow rate of 10 μL min^{-1}. Negative ion ESI mass spectra were acquired using a probe tip potential of 2500 V, a cone voltage of either 40 or 50 V, and the source block and desolvation temperatures set to 60 and 80 °C, respectively. The transport and aperture were set to 2.0 and 13.0, respectively. In most experiments, spectra were acquired over the range *m*/*z* 400–3000. Typically 50–70 scans were summed to obtain representative spectra. The data were calibrated against a standard CsI solution (750 μM) over the same *m*/*z* range.

Results and discussion

Reactions between ruthenium complexes and individual duplex 16mers

ESI mass spectra were obtained of reaction mixtures containing metal : duplex DNA ratios ranging from 1 : 1 up to a maximum of 6 : 1. Under the experimental conditions, the most abundant ions assigned to complexes formed between ruthenium compounds and duplex DNA were 5− and 6− ions, with 7− ions also seen in some spectra. Assignments for all ions observed are available in the electronic supplementary information.† Fig. 2 shows the negative ion ESI mass spectra of reaction mixtures containing a 6 : 1 ratio of different ruthenium compounds and D2. Analysis of the spectra suggests that the ruthenium compounds vary significantly in their ability to form non-covalent complexes with duplex DNA. For example, Fig. 2(a)–(c) show an ion of high abundance at *m*/*z* 1626.4, and an ion of low abundance at *m*/*z* 1952.0, in spectra of reaction mixtures containing $[Ru(phen)_3]^{2+}$, $[Ru(phen)_2(pda)]^{2+}$ or $[Ru(phen)_2(dpq)]^{2+}$. These ions are from free duplex DNA ($[D2 - 6H]^{6-}$ and $[D2 - 5H]^{5-}$, respectively) and were absent from spectra of reaction mixtures containing $[Ru(phen)_2(dpqC)]^{2+}$, $[Ru(phen)_2(dppz)]^{2+}$ or $[Ru(phen)_2(dpqMe_2)]^{2+}$. This suggests that the latter three ruthenium compounds have a significantly greater affinity towards D2. It should be noted that the term "greater affinity" is used here to mean that under the same experimental conditions, for one specific metal compound a greater number of molecules can bind to a particular DNA sequence than for another metal compound. Since the DNA used in these experiments was 16 base pairs long, it is expected that there will be more than one drug binding site, especially if the binding mode is intercalation. These binding experiments cannot provide information on the magnitude of the binding constant for a single binding site. For example, it can-

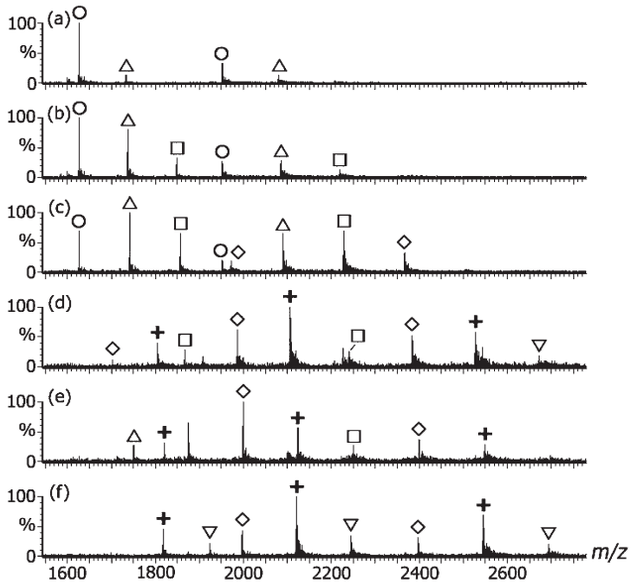

Fig. 2 Negative ion ESI mass spectra of solutions containing a 6 : 1 ratio of ruthenium compound and duplex D2: (a) $[Ru(phen)_3]^{2+}$; (b) $[Ru(phen)_2(pda)]^{2+}$; (c) $[Ru(phen)_2(dpq)]^{2+}$; (d) $[Ru(phen)_2(dpqMe_2)]^{2+}$; (e) $[Ru(phen)_2(dpqC)]^{2+}$; (f) $[Ru(phen)_2(dppz)]^{2+}$: ○ dsDNA; △ dsDNA + $[Ru(phen)_2(L)]^{2+}$; □ dsDNA + 2$[Ru(phen)_2(L)]^{2+}$; ◇ dsDNA + 3$[Ru(phen)_2(L)]^{2+}$; ✚ dsDNA + 4$[Ru(phen)_2(L)]^{2+}$; ▽ dsDNA + 5$[Ru(phen)_2(L)]^{2+}$.

not be ruled out when only one molecule of a particular ruthenium compound binds to D2, that the binding constant for this one binding site is not higher than for the more numerous binding sites used by a second ruthenium compound.

In addition to ions assigned to free DNA, Fig. 2(a) also contained ions of low abundance assigned to a non-covalent complex containing one $[Ru(phen)_3]^{2+}$ bound to D2. When $[Ru(phen)_2(pda)]^{2+}$ or $[Ru(phen)_2(dpq)]^{2+}$ was present in the reaction mixture, the abundance of ions assigned to non-covalent complexes containing one ruthenium molecule bound to D2 was considerably greater (Fig. 2(b) and (c)). These spectra also contained ions assigned to non-covalent complexes containing two ruthenium molecules bound to D2. This suggests that both $[Ru(phen)_2(pda)]^{2+}$ and $[Ru(phen)_2(dpq)]^{2+}$ have a greater affinity for D2 than $[Ru(phen)_3]^{2+}$. Although there have been no binding constants reported for any of the above three ruthenium compounds and D2, values of 0.7×10^3 M^{-1} and 5.9×10^4 M^{-1} have been reported for $[Ru(bipy)_3]^{2+}$ and $[Ru(bipy)_2(dpq)]^{2+}$, respectively, with calf thymus DNA.[38,39] These results are therefore consistent with those presented here, and with those of an NMR study which concluded that $[Ru(phen)_2(dpq)]^{2+}$ can more fully intercalate into the duplex $d(GTCGAC)_2$ than $[Ru(phen)_3]^{2+}$.[20] In addition the greater abundances of ions assigned to non-covalent complexes in Fig. 2(c) compared to Fig. 2(b), suggests that $[Ru(phen)_2(dpq)]^{2+}$ has a slightly greater affinity for D2 than $[Ru(phen)_2(pda)]^{2+}$. This is also supported by the presence of an ion of medium abundance at *m*/*z* 2367.3 in Fig. 2(c), which is assigned to a non-covalent complex containing three $[Ru(phen)_2(dpq)]^{2+}$ bound to D2.

The importance of hydrophobic interactions between ruthenium compounds and DNA is highlighted by comparison of the spectra of reaction mixtures containing $[Ru(phen)_2(dpq)]^{2+}$ and D2, and $[Ru(phen)_2(dpqMe_2)]^{2+}$ and D2. Fig. 2(d) shows that the most abundant ion present in the spectrum of the latter reaction mixture was assigned to a non-covalent complex containing four ruthenium molecules bound to D2. Ions of medium abundance assigned to non-covalent complexes containing three and five ruthenium molecules bound to D2 were also present, while ions assigned to free D2 or complexes containing only one or two ruthenium molecules were either absent or of low abundance. In contrast, the most abundant ion in the spectrum of the reaction mixture containing $[Ru(phen)_2(dpq)]^{2+}$ and D2 (Fig. 2(c)) was assigned to a non-covalent complex containing only one ruthenium molecule bound to D2. Ions assigned to non-covalent complexes containing two and three ruthenium molecules bound to D2 were also present. However, these were not of high abundance, and there was no evidence for non-covalent complexes containing greater numbers of ruthenium molecules. The only difference between the ruthenium compounds in these reaction mixtures is the presence of two methyl groups on the leading edge of the $dpqMe_2$ ligand. While these would not be expected to greatly increase the ability of $[Ru(phen)_2(dpqMe_2)]^{2+}$ to intercalate into DNA compared to $[Ru(phen)_2(dpq)]^{2+}$, they might participate in additional hydrophobic interactions with other non-polar groups in the interior of the DNA base stack.

The most abundant ion present in Fig. 2(e) is that at *m*/*z* 1999.4, which is assigned to a non-covalent complex containing three $[Ru(phen)_2(dpqC)]^{2+}$ bound to D2. Ions assigned to a complex containing four ruthenium molecules bound to D2 are also present but at lower abundance, and there are no ions assigned to a complex containing five ruthenium molecules bound to D2. In contrast Fig. 2(f) shows ions assigned to non-covalent complexes containing four and five $[Ru(phen)_2(dppz)]^{2+}$ bound to D2, with the ion at *m*/*z* 2121.4 assigned to $[4Ru(phen)_2(dppz) + D2 - 14H]^{6-}$ the most abundant in the spectrum. Both ruthenium compounds therefore show a greater ability to form non-covalent complexes containing larger numbers of ruthenium molecules than $[Ru(phen)_2(dpq)]^{2+}$. This can be attributed to the greater intercalating ability of the dpqC and dppz ligands compared to dpq, as a result of their more extended polycyclic structures. It also appears that the completely aromatic dppz ligand confers on $[Ru(phen)_2(dppz)]^{2+}$ a greater ability to bind to D2 than $[Ru(phen)_2(dpqC)]^{2+}$, presumably as a result of enhanced intercalation *via* the more planar dppz ligand.

We have previously shown that a convenient method for illustrating differences in affinities of different ruthenium compounds towards DNA is by plotting the relative abundances of ions assigned to different non-covalent complexes.[33] Relative abundances are obtained by dividing the combined intensity of 5−, 6− and 7− ions assigned to a specific non-covalent complex, by the combined intensity of all 5−, 6− and 7− ions. The relative abundances of non-covalent complexes formed in reaction mixtures containing a 6 : 1 ratio of ruthenium complex and D2 are shown graphically in Fig. 3. This figure shows that the relative abundance of ions containing four and five ruthenium molecules bound to D2 is highest for $[Ru(phen)_2(dppz)]^{2+}$, making it the most reactive ruthenium compound towards this DNA duplex. $[Ru(phen)_2(dpqMe_2)]^{2+}$ also formed ions containing four and five ruthenium molecules bound to D2, but with lower relative abundances than $[Ru(phen)_2(dppz)]^{2+}$. This suggests that $[Ru(phen)_2(dpqMe_2)]^{2+}$ has the second highest affinity towards D2. Fig. 3 shows that the relative abundances of ions assigned to non-covalent complexes containing three and four ruthenium molecules was significantly greater for $[Ru(phen)_2(dpqC)]^{2+}$ than the remaining three ruthenium compounds, showing that it has the next highest affinity. Overall Fig. 3 suggests that the relative affinities of the ruthenium compounds towards D2 follow the sequence: $[Ru(phen)_2(dppz)]^{2+}$ > $[Ru(phen)_2(dpqMe_2)]^{2+}$ > $[Ru(phen)_2(dpqC)]^{2+}$ > $[Ru(phen)_2(dpq)]^{2+}$ > $[Ru(phen)_2(pda)]^{2+}$ > $[Ru(phen)_3]^{2+}$. Similar trends in relative affinity were observed for the ruthenium compounds towards D1 and D3 when relative abundance data from reaction mixtures containing these duplexes were examined.

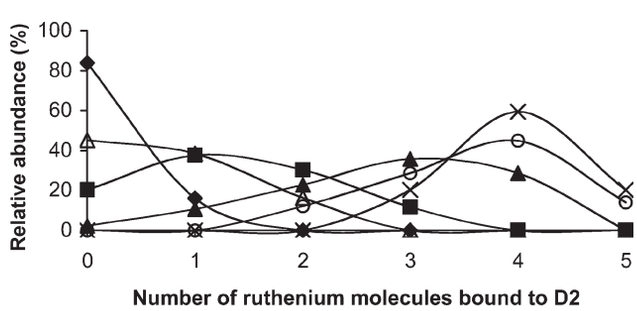

Fig. 3 Relative abundances of non-covalent complexes in reaction mixtures containing a 6 : 1 ratio of ruthenium compound and duplex D2: ◆ $[Ru(phen)_3]^{2+}$; ■ $[Ru(phen)_2(dpq)]^{2+}$; ▲ $[Ru(phen)_2(dpqC)]^{2+}$, ✕ $[Ru(phen)_2(dppz)]^{2+}$, ○ $[Ru(phen)_2(dpqMe_2)]^{2+}$; △ $[Ru(phen)_2(pda)]^{2+}$.

Competition experiments involving ruthenium compounds

In order to obtain further information about relative DNA binding affinities, a series of competition experiments were performed involving pairs of ruthenium complexes and D1, D2 or D3. In each reaction mixture the ratio of ruthenium complexes to duplex DNA was 3 : 3 : 1. Overall the results obtained with the different DNA duplexes were very similar, and support the trends in relative affinity noted above. In each experiment where $[Ru(phen)_3]^{2+}$ was involved, the ESI mass spectrum was dominated by ions assigned to non-covalent complexes containing one or more molecules of the second ruthenium compound bound to DNA. Ions assigned to non-covalent complexes containing $[Ru(phen)_3]^{2+}$ molecules were of very low abundance, confirming that $[Ru(phen)_3]^{2+}$ has the lowest affinity towards each of the DNA duplexes examined. ESI mass spectra of all other reaction mixtures were generally far more complex, owing to the presence of ions assigned to non-covalent complexes containing one or more of either or both ruthenium molecules.

On the basis of preliminary ESI mass spectra, it was concluded that $[Ru(phen)_2(dpq)]^{2+}$ and $[Ru(phen)_2(pda)]^{2+}$ both had DNA affinities greater than that displayed by $[Ru(phen)_3]^{2+}$, but less than that of the remaining three ruthenium compounds. The latter statement was supported by the results of experiments in which either $[Ru(phen)_2(dpq)]^{2+}$ or $[Ru(phen)_2(pda)]^{2+}$, was allowed to compete with $[Ru(phen)_2(dpqC)]^{2+}$, $[Ru(phen)_2(dppz)]^{2+}$ or $[Ru(phen)_2(dpqMe_2)]^{2+}$ for binding sites on D2. For example, Fig. 4(a) shows the ESI mass spectrum of a reaction mixture containing D1, $[Ru(phen)_2(pda)]^{2+}$ and $[Ru(phen)_2(dpqC)]^{2+}$. Ions at *m*/*z* 1751.4, 1875.8, 2000.2, 2101.7 and 2250.9 are assigned to non-covalent complexes containing one or more $[Ru(phen)_2(dpqC)]^{2+}$ bound to D1, and have greater abundance than ions at *m*/*z* 1738.3, 1849.3, 2086.1 and 2219.5, which are assigned to non-covalent complexes containing one or more $[Ru(phen)_2(pda)]^{2+}$. Fig. 4(a) also contains several ions, including those at *m*/*z* 1862.5 and 2235.3, which are assigned to non-covalent complexes containing both ruthenium molecules bound to D1.

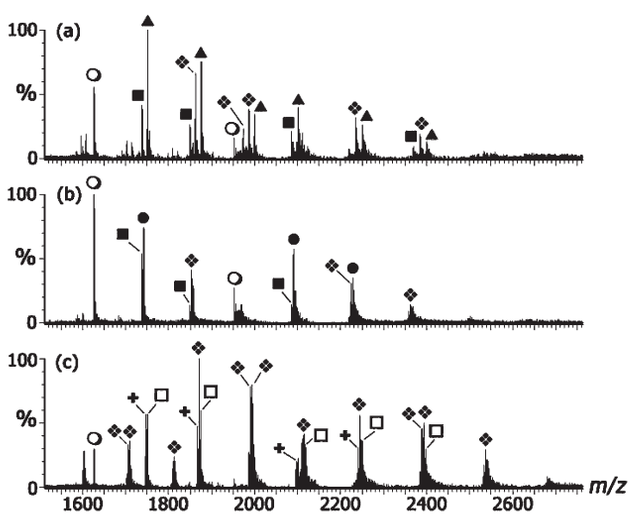

Fig. 4 Negative ion ESI mass spectra of solutions containing a 3 : 3 : 1 ratio of two ruthenium compounds and D1 : (a) solution containing $[Ru(phen)_2(pda)]^{2+}$ and $[Ru(phen)_2(dpqC)]^{2+}$; (b) solution containing $[Ru(phen)_2(pda)]^{2+}$ and $[Ru(phen)_2(dpq)]^{2+}$; (c) solution containing $[Ru(phen)_2(dppz)]^{2+}$ and $[Ru(phen)_2(dpqMe_2)]^{2+}$: ○ dsDNA; ▲ dsDNA + x[$Ru(phen)_2(dpqC)]^{2+}$, x = 1–3; ■ dsDNA + x[$Ru(phen)_2(pda)]^{2+}$, x = 1–2; ● dsDNA + x[$Ru(phen)_2(dpq)]^{2+}$, x = 1–2; □ dsDNA + x[$Ru(phen)_2(dppz)]^{2+}$, x = 1–4; ✚ dsDNA + x[$Ru(phen)_2(dpqMe_2)]^{2+}$, x = 1–2; ❖ dsDNA + one or more of both ruthenium molecules.

Fig. 4(b) shows the mass spectrum of the competition reaction mixture containing D1, $[Ru(phen)_2(dpq)]^{2+}$ and $[Ru(phen)_2(pda)]^{2+}$. Ions assigned to non-covalent complexes containing one and two $[Ru(phen)_2(dpq)]^{2+}$ molecules are present in higher abundance than the corresponding ions containing $[Ru(phen)_2(pda)]^{2+}$ molecules. This result supports the conclusion that $[Ru(phen)_2(dpq)]^{2+}$ has a slightly greater DNA affinity than $[Ru(phen)_2(pda)]^{2+}$. When the duplex present in the competition reaction mixture was either D2 or D3, ions assigned to non-covalent complexes containing $[Ru(phen)_2(dpq)]^{2+}$ were even more dominant, providing further support for this conclusion.

Competition experiments performed using $[Ru(phen)_2(dpqC)]^{2+}$, $[Ru(phen)_2(dpqMe_2)]^{2+}$ and $[Ru(phen)_2(dppz)]^{2+}$ supported the view that these three compounds have very similar DNA affinities. For example, Fig. 4(c) shows the ESI mass spectrum of a solution containing D1, $[Ru(phen)_2(dpqMe_2)]^{2+}$ and $[Ru(phen)_2(dppz)]^{2+}$. Ions assigned to non-covalent complexes containing one, two and three ruthenium molecules are present for both compounds in very similar abundances. The most abundant ions in the spectrum are those at *m*/*z* 1870.9, 1990.3 and 1994.6. The first of these is assigned to an ion that contains one molecule of both ruthenium compounds. The second ion contains one $[Ru(phen)_2(dppz)]^{2+}$ and two $[Ru(phen)_2(dpqMe_2)]^{2+}$ bound to D1, while the third ion contains two $[Ru(phen)_2(dppz)]^{2+}$ and one $[Ru(phen)_2(dpqMe_2)]^{2+}$ bound to D1. The similar abundances of the last two ions reinforces the conclusion that these ruthenium compounds have similar DNA affinities.

The results of competition experiments showed that the DNA affinity of the ruthenium compounds followed the sequence: $[Ru(phen)_2(dppz)]^{2+} \sim [Ru(phen)_2(dpqMe_2)]^{2+} \sim [Ru(phen)_2(dpqC)]^{2+} > [Ru(phen)_2(dpq)]^{2+} > [Ru(phen)_2(pda)]^{2+} > [Ru(phen)_3]^{2+}$. Binding constants for both enantiomers of $[Ru(phen)_2(dppz)]^{2+}$ and calf thymus DNA are more than one hundred times greater than for both enantiomers of $[Ru(phen)_3]^{2+}$, which is consistent with the above order.[40] While thermodynamic data for the binding of the other ruthenium compounds to DNA have not been reported, binding constants are known for complexes of the series $[Ru(bipy)_2L]^{2+}$, where L = bipy, dpq, dpqC and dppz, with calf-thymus DNA.[38,39] The binding constant for $[Ru(bipy)_2(dppz)]^{2+}$ ($K_b = 8.8 \times 10^6$ M^{-1}) is much greater than for either $[Ru(bipy)_2(dpq)]^{2+}$ ($K_b = 5.9 \times 10^4$ M^{-1}) or $[Ru(bipy)_2(dpqC)]^{2+}$ ($K_b = 8.5 \times 10^4$ M^{-1}), which are in turn significantly greater than that for $[Ru(bipy)_3]^{2+}$ ($K_b = 0.07 \times 10^4$ M^{-1}) The trend in binding constants for the series $[Ru(bipy)_2L]^{2+}$ is therefore qualitatively similar to the order of relative DNA affinities determined here for the series $[Ru(phen)_2L]^{2+}$ using ESI-MS.

Saturation experiments

Our preliminary experiments provided evidence that some ruthenium compounds could form non-covalent complexes with D2 containing up to five ruthenium molecules. This observation raises the question of whether the ions observed reflect true non-covalent complexes present in solution, or are a result of non-specific gas-phase associations. A second question that arises is exactly how many ruthenium molecules can bind to a duplex 16mer in solution. In order to address both these questions, ESI mass spectra were obtained using reaction mixtures containing up to a 20 : 1 ratio of $[Ru(phen)_2(dpqC)]Cl_2$: D2. Fig. 5 shows the variations in relative abundances of non-covalent complexes present in these spectra. The relative abundance of ions assigned to free D2 decreased rapidly as the Ru : DNA ratio was increased, reflecting the high affinity of $[Ru(phen)_2(dpqC)]^{2+}$ towards D2. At the same time the relative abundance of non-covalent complexes containing one and two $[Ru(phen)_2(dpqC)]^{2+}$ bound to D2 increased to a maximum value at relatively low Ru : D2 ratios, and then decreased. Fig. 5 also shows that the relative abundance of ions containing three and four $[Ru(phen)_2(dpqC)]^{2+}$ bound to DNA also reached maximum values, but at higher Ru : DNA ratios, while the relative abundance of ions containing five $[Ru(phen)_2(dpqC)]^{2+}$ bound to D2 was still increasing when the Ru : D2 ratio was 20 : 1. The changes in relative abundances illustrated in Fig. 5 resemble strongly the variations in concentrations expected for metal complexes involved in stepwise formation equilibria. This is consistent with the conclusion that that there is little non-specific association of the positively charged metal complex with negatively charged DNA, and is consistent with the proposal that ESI mass spectra faithfully reflect solution equilibria.

DNA selectivity

The variations in DNA affinity discussed earlier are consistent with intercalation playing a major role in non-covalent complex

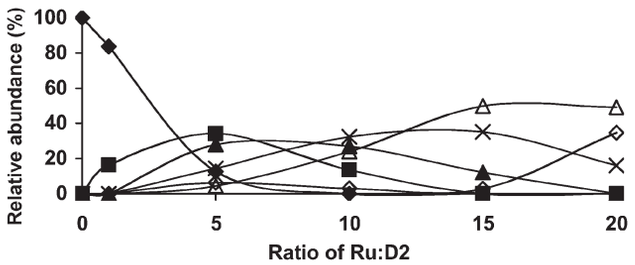

Fig. 5 Relative abundances of ions assigned to non-covalent complexes present in ESI spectra of reaction mixtures containing $[Ru(phen)_2(dpqC)]Cl_2$ and D2: ◆ 0 Ru bound; ■ 1 Ru bound; ▲ 2 Ru bound; ✕ 3 Ru bound; △ 4 Ru bound; ◇ 5 Ru bound.

formation for most of the ruthenium compounds examined. As intercalation is known to be enhanced with DNA duplexes having a high GC content,[31] it was expected that the ruthenium compounds would show lower affinity towards D3. Previously we showed that $[Ru(phen)_2(dpq)]^{2+}$ displayed a greater ability to form non-covalent complexes with D2 compared to D1 and D3.[33] Since D2 does not have the highest GC content of the duplexes examined, this suggests either that intercalation does not totally dominate interactions between this ruthenium compound and DNA, or that the increase in GC content on going from D2 to D1 does not necessarily result in more, or stronger, non-covalent interactions.

Most of the other ruthenium compounds examined here also exhibited greater affinity towards D2 compared to the other two duplexes. For example, Fig. 6 displays relative abundance data for ions present in reaction mixtures containing a 6 : 1 ratio of $[Ru(phen)_2(dpqMe_2)]^{2+}$ and each of the three duplexes. It can be clearly seen that the relative abundances of ions containing four and five $[Ru(phen)_2(dpqMe_2)]^{2+}$ molecules are significantly greater for D2 than either D1 or D3. Similar results were obtained when relative abundance data were plotted for reaction mixtures containing $[Ru(phen)_2(pda)]^{2+}$, $[Ru(phen)_2(dpqC)]^{2+}$ or $[Ru(phen)_2(dppz)]^{2+}$. For $[Ru(phen)_3]^{2+}$ there was very little difference between the relative abundance of ions containing the same number of ruthenium molecules, but different DNA duplexes. This suggests that $[Ru(phen)_3]^{2+}$ has the lowest DNA selectivity of the ruthenium compounds examined. An explanation for the greater affinity of most of these ruthenium compounds towards D2 awaits computer modelling of these interactions, or determination of structures using NMR spectroscopy or X-ray crystallography. These experiments will be challenging, since ESI mass spectra indicate that mixtures of non-covalent complexes with a range of stoichiometries are usually formed when ruthenium compounds and DNA are combined.

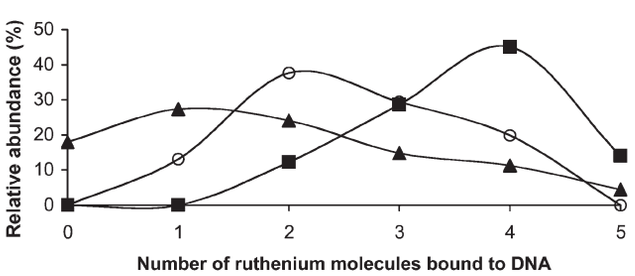

Fig. 6 Relative abundances of non-covalent complexes in reaction mixtures containing a 6 : 1 ratio of $[Ru(phen)_2(dpqMe_2)]^{2+}$ and D1–D3: ○ ruthenium molecules bound to D1; ■ ruthenium molecules bound to D2; ▲ ruthenium molecules bound to D3.

Competition experiments involving ruthenium compounds and either daunomycin or distamycin

Graphical analysis of ESI mass spectral data from reaction mixtures containing the same ruthenium compound, but different DNA duplexes, provided evidence that most ruthenium compounds displayed higher affinity towards duplex D2. In an attempt to obtain information about the DNA binding modes of the ruthenium compounds, and their preferred binding sites, a series of competition experiments were performed using organic drugs with well characterised intercalating or minor groove binding properties. In these experiments an excess of the organic drug was mixed with duplex DNA, and then a ruthenium compound added. The rationale behind these experiments was that the organic intercalator or minor groove-binding agent would prevent subseqent binding by ruthenium compounds that interact with DNA in the same fashion and/or at the same bases sequences.

The organic drugs chosen for these experiments were daunomycin and distamycin. Daunomycin contains an anthraquinone ring system that intercalates into DNA, and a sugar residue that participates in interactions with the minor groove. Daunomycin has been shown to bind preferentially to right-handed B-form DNA, and to display increasing binding affinity towards DNA with greater GC content.[41–44] Footprinting titration experiments provided evidence that triplet sequences containing GC or CG base pairs with either an A or T at the 5′ end were preferred binding sites,[43] while X-ray crystallography has provided direct evidence for the intercalation of daunomycin between GC base pairs.[45] Distamycin is a tripeptide containing three *N*-methylpyrrole units linked together to give a molecule with a curved shape that facilitates binding to the minor groove of DNA. Many studies have shown that distamycin binds to AT-rich DNA sequences containing at least four or five base pairs.[46–49] In addition it has been shown that the minor groove can expand to accommodate two distamycin molecules lying side by side.[50,51] The formation of such complexes was found to vary from strongly cooperative to anti-cooperative depending on whether the individual DNA strands contained alternating AT sequences or regions containing exclusively A or T sequences.[52]

Prior to competition experiments, ESI-MS was used to monitor the titration of D2 and D3 with daunomycin and distamycin, respectively. This was performed to determine how much organic drug must be added to occupy all intercalation or minor groove binding sites. Using this method it was shown that all binding sites became saturated at a organic drug : duplex ratio of 10 : 1.[53] Fig. 7(a) shows the ESI mass spectrum of a solution containing a 10 : 1 ratio of daunomycin : D2, while Figs. 7(b)–7(d) show spectra of identical solutions after $[Ru(phen)_3]^{2+}$, $[Ru(phen)_2(dpq)]^{2+}$ and $[Ru(phen)_2(dppz)]^{2+}$, respectively, were added. Assignments for all major ions observed in these experiments may be found in the supplementary material.† The two most abundant ions in Fig. 7(a) are those at *m*/*z* 2066.0 and 2153.9, assigned to non-covalent complexes containing five and six daunomycin molecules, respectively, bound to D2. Additional ions arising from non-covalent complexes containing different numbers of daunomycin bound to D2 are also present. These include ions at *m*/*z* 2241.9 and 2329.9 corresponding to non-covalent complexes containing seven and eight daunomycin molecules, respectively. This suggests that it is possible for D2 to readily bind six daunomycin molecules, and up to eight in total. Such a conclusion is consistent with previous studies which showed for a variety of DNA molecules that the size of the average binding site for daunomycin is two base pairs.[44]

Fig. 7(b) shows the effect of adding 30 equivalents of $[Ru(phen)_3]^{2+}$ to a solution containing D2 and 10 equivalents of daunomycin. The majority of non-covalent complexes present in solution prior to addition of $[Ru(phen)_3]^{2+}$ are either absent or considerably reduced in abundance. Many new ions are now present, the most abundant of which are those at *m*/*z* 2085.0 and 2172.6. These are assigned to $[D2 + 4daunomycin + Ru(phen)_3 - 8H]^{6-}$ and $[D2 + 5daunomycin + Ru(phen)_3 - 8H]^{6-}$, respectively. There are several possible pathways that may have led to the formation of these complexes. One pathway involves addition of $[Ru(phen)_3]^{2+}$ to ions containing four and five daunomycin already bound to D2. This would suggest that $[Ru(phen)_3]^{2+}$ has the ability to bind to different sites on D2 than daunomycin. However, the relatively high abundance of $[D2 + 4daunomycin + Ru(phen)_3 - 8H]^{6-}$ in Fig. 7(b), compared to the medium abundance of $[D2 + 4daunomycin - 8H]^{6-}$ in Fig. 7(a), suggests that this is not the major pathway for formation of the former ion. An alternative pathway for the formation of $[D2 + 4daunomycin + Ru(phen)_3 - 8H]^{6-}$ and $[D2 + 5daunomycin + Ru(phen)_3 - 8H]^{6-}$, is the displacement of one daunomycin from $[D2 + 5daunomycin - 8H]^{6-}$ and $[D2 + 6daunomycin - 8H]^{6-}$, by

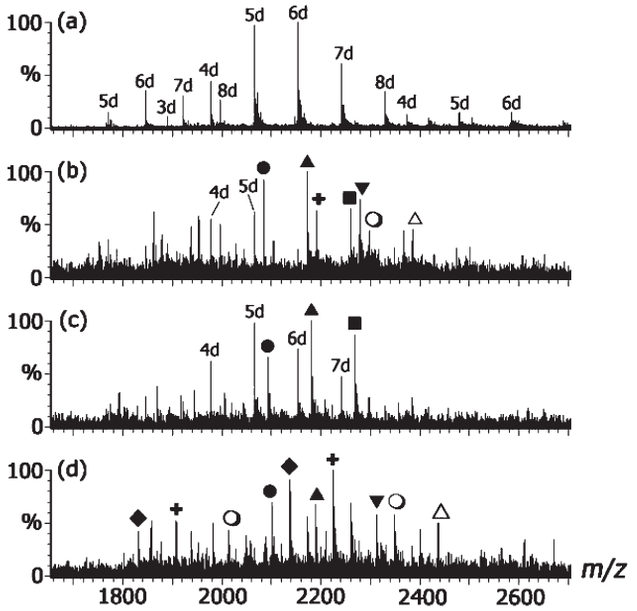

Fig. 7 Negative ion ESI mass spectra of solutions containing D2 and: (a) 10 equivalents of daunomycin; (b) 10 equivalents of daunomycin and 30 equivalents of $[Ru(phen)_3]^{2+}$; (c) 10 equivalents of daunomycin and 6 equivalents of $[Ru(phen)_2(dpq)]^{2+}$; (d) 10 equivalents of daunomycin and 6 equivalents of $[Ru(phen)_2(dppz)]^{2+}$: 3d = dsDNA + 3daunomycin; 4d = dsDNA + 4daunomycin; 5d = dsDNA + 5daunomycin; 6d = dsDNA + 6daunomycin; 7d = dsDNA + 7daunomycin; 8d = dsDNA + 8daunomycin; ● dsDNA + 4daunomycin + $[Ru(phen)_2(L)]^{2+}$; ▲ dsDNA + 5daunomycin + $[Ru(phen)_2(L)]^{2+}$; ■ dsDNA + 6daunomycin + $[Ru(phen)_2(L)]^{2+}$; ◆ dsDNA + 3daunomycin + $2[Ru(phen)_2(L)]^{2+}$; ✚ dsDNA + 4daunomycin + $2[Ru(phen)_2(L)]^{2+}$; ▼ dsDNA + 5daunomycin + $2[Ru(phen)_2(L)]^{2+}$; ○ dsDNA + 4daunomycin + $3[Ru(phen)_2(L)]^{2+}$; △ dsDNA + 5daunomycin + $3[Ru(phen)_2(L)]^{2+}$.

$[Ru(phen)_3]^{2+}$. At first glance this pathway would appear to be unlikely in view of the greater binding constants reported for binding of daunomycin to calf-thymus DNA, compared to those for binding of $[Ru(phen)_3]^{2+}$.[10] However, the concentration of $[Ru(phen)_3]^{2+}$ in the solution containing D2 is much greater than that of daunomycin, and not all of the daunomycin bound to D2 may be bound to high affinity sites. The similarity in abundance of ions assigned to [D2 + 5daunomycin − 8H]$^{6-}$ and [D2 + 6daunomycin − 8H]$^{6-}$ in Fig. 7(a), compared to those assigned to [D2 + 4daunomycin + $Ru(phen)_3$ − 8H]$^{6-}$ and [D2 + 5daunomycin + $Ru(phen)_3$ − 8H]$^{6-}$ in Fig. 7(b), provides support for the displacement mechanism. Additional support is provided by the similarity in relative abundance of other ions in Fig. 7(b) with those of potential precursor ions in Fig. 7(a). For example, Fig. 7(a) contains an ion of medium abundance at *m*/*z* 2241.9 assigned to [D2 + 7daunomycin − 8H]$^{6-}$. Displacement of one or two daunomycin from this non-covalent complex would give rise to the ions at *m*/*z* 2260.6 and 2279.3 in Fig. 7(b). These are assigned to [D2 + 6daunomycin + $Ru(phen)_3$ − 8H]$^{6-}$ and [D2 + 5daunomycin + $2Ru(phen)_3$ − 10H]$^{6-}$, respectively.

Inspection of Fig. 7(b) reveals that the two most extensively complexed DNA ions are those of low abundance at *m*/*z* 2367.4 and 2385.9. These are assigned to [D2 + 6daunomycin + $2Ru(phen)_3$ − 10H]$^{6-}$ and [D2 + 5daunomycin + $3Ru(phen)_3$ − 12H]$^{6-}$, respectively. Both ions contain a total of eight molecules non-covalently bound to D2, which is the same as the maximum number of daunomycin bound to D2 in Fig. 7(a). This suggests that $[Ru(phen)_3]^{2+}$ cannot find alternative binding sites on D2 molecules that are already heavily complexed by daunomycin, but can instead displace some daunomycin molecules to give mixed non-covalent complexes.

The same conclusion is reached after examination of spectra obtained after adding 6 equivalents of $[Ru(phen)_2(dpq)]^{2+}$, $[Ru(phen)_2(dpqC)]^{2+}$, $[Ru(phen)_2(dppz)]^{2+}$ or $[Ru(phen)_2(dpqMe_2)]^{2+}$, to solutions already containing D2 and 10 equivalents of daunomycin. In all cases there was no evidence for ions assigned to complexes containing more than a total of eight daunomycin and ruthenium molecules. For example, Fig. 7(c) contains ions assigned to non-covalent complexes containing four, five or six daunomycin as well as one $[Ru(phen)_2(dpq)]^{2+}$ bound to D2. In addition, ions assigned to non-covalent complexes containing four, five, six and seven daunomycin bound to D2 are present in medium to high abundance. Overall the degree of displacement of daunomycin by ruthenium molecules in this system appears to have been less than with $[Ru(phen)_3]^{2+}$. This is attributable to the lower ratio (6 : 1) of Ru : DNA used when the ruthenium compound was $[Ru(phen)_2(dpq)]^{2+}$, compared to 30 : 1 for $[Ru(phen)_3]^{2+}$.

The spectra of solutions containing 6 equivalents of $[Ru(phen)_2(dpqC)]^{2+}$, $[Ru(phen)_2(dppz)]^{2+}$ or $[Ru(phen)_2(dpqMe_2)]^{2+}$, as well as 10 equivalents of daunomycin and D2, were very similar to each other, and reflect the significantly greater DNA affinity of these ruthenium compounds compared to $[Ru(phen)_3]^{2+}$ and $[Ru(phen)_2(dpq)]^{2+}$. For example, Fig. 7(d) shows the ESI mass spectrum of a solution containing a 1 : 10 : 6 ratio of D2, daunomycin and $[Ru(phen)_2(dppz)]^{2+}$. Ions assigned to non-covalent complexes containing only daunomycin bound to D2 are not present. The two most abundant ions are those at *m*/*z* 2137.0 and 2224.9. These are assigned to [D2 + 3daunomycin + $2Ru(phen)_2(dppz)$ − 10H]$^{6-}$ and [D2 + 4daunomycin + $2Ru(phen)_2(dppz)$ − 10H]$^{6-}$, respectively. The most likely mechanism of formation of these complexes is displacement of two daunomycin molecules from non-covalent complexes containing five and six daunomycin bound to D2. Further evidence for the greater reactivity of $[Ru(phen)_2(dppz)]^{2+}$ compared to $[Ru(phen)_3]^{2+}$ and $[Ru(phen)_2(dpq)]^{2+}$, is provided by the observation of ions of low to medium abundance at *m*/*z* 1938.5, 2261.0, 2013.8, 2349.1, and 2437.5. These are assigned to non-covalent complexes containing three, four or five daunomycin as well as three ruthenium molecules bound to D2. It is important to note that spectra of competition solutions containing duanomycin and $[Ru(phen)_2(dpqC)]^{2+}$, $[Ru(phen)_2(dppz)]^{2+}$ or $[Ru(phen)_2(dpqMe_2)]^{2+}$, did not contain ions containing more than a total of eight daunomycin and ruthenium molecules. This observation, combined with the displacement of daunomycin by each of the ruthenium compounds, suggests that the latter share at least some of the same binding sites as daunomycin.

D3 was chosen for competition experiments involving distamycin because the latter is known to prefer to bind to DNA rich in AT base pairs. The ESI mass spectrum of a solution containing D3 and 10 equivalents of distamycin is shown in Fig. 8(a). Ions of medium to high abundance assigned to non-covalent complexes containing between four and seven distamycin bound to D3 are present, with those containing six distamycin the most abundant. This number of bound distamycin molecules is reasonable since D3 contains two AAAA/TTTTT base sequences which should be suitable for distamycin binding. In addition it has been shown that two distamycin molecules can lie side by side in the minor groove of DNA containing suitable AT base sequences.[46–51] It is probable that four distamycin are tightly bound in their preferred binding sites in the minor groove of D3, with two others less tightly bound somewhere else along the minor groove.

The ESI mass spectrum obtained after addition of 30 equivalents of $[Ru(phen)_3]^{2+}$ to a solution already containing 10 equivalents of distamycin and D3, is shown in Fig. 8(b). Most of the ions that were present in Fig. 8(a) are absent, with the only significant exception being the ion at *m*/*z* 1946.7, assigned to [D3 + 4distamycin − 6H]$^{6-}$. The most abundant ion in Fig. 8(b) is that at *m*/*z* 2053.5, assigned to [D3 + 4distamycin + $Ru(phen)_3$ − 8H]$^{6-}$. The 7− ion of this complex is present in medium abundance at *m*/*z* 1760.0, while the next most abundant ion is that at *m*/*z* 2160.2, assigned to [D3 + 4distamycin + $2Ru(phen)_3$ − 10H]$^{6-}$. No ions attributable to non-covalent complexes containing greater numbers of distamycin and ruthenium complexes bound to D3 were observed. This suggests that a major mechanism of formation of these mixed ions is by displacement of one or more distamycin from ions such as [D3 + 6distamycin − 6H]$^{6-}$ and [D3 + 7distamycin − 6H]$^{6-}$. Furthermore the absence of ions containing five distamycin and one or two ruthenium molecules bound to D3, suggests that one $[Ru(phen)_3]^{2+}$ is capable of displacing two distamycin molecules. These results therefore suggest that $[Ru(phen)_3]^{2+}$ can displace distamycin from

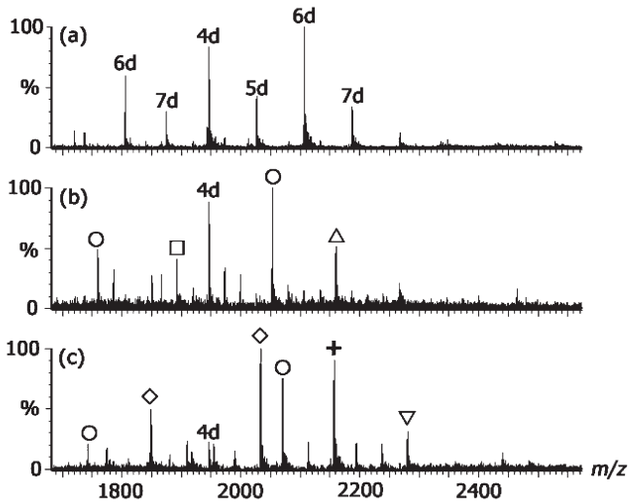

Fig. 8 Negative ion ESI mass spectra of solutions containing D3 and: (a) 10 equivalents of distamycin; (b) 10 equivalents of distamycin and 30 equivalents of $[Ru(phen)_3]^{2+}$; (c) 10 equivalents of distamycin and 6 equivalents of $[Ru(phen)_2(dppz)]^{2+}$. 4d = dsDNA + 4distamycin; 5d = dsDNA + 5distamycin; 6d = dsDNA + 6distamycin; 7d = dsDNA + 7distamycin; ○ dsDNA + 4distamycin + $[Ru(phen)_2(L)]^{2+}$; △ dsDNA + 4distamycin + 2$[Ru(phen)_2(L)]^{2+}$; □ dsDNA + 2 distamycin + $[Ru(phen)_2(L)]^{2+}$; ◇ dsDNA + 2distamycin + 2$[Ru(phen)_2(L)]^{2+}$; ✚ dsDNA + 2distamycin + 3$[Ru(phen)_2(L)]^{2+}$; ▽ dsDNA + 2distamycin + 4$[Ru(phen)_2(L)]^{2+}$.

some of its binding sites in D3. However, the results do not provide unequivocal evidence that $[Ru(phen)_3]^{2+}$ is binding to the minor groove of DNA, since the fifth and sixth distamycin molecules in ions such as $[D3 + 6distamycin - 6H]^{6-}$ may not have been binding as true minor groove binders. Evidence that $[Ru(phen)_3]^{2+}$ does at least in some circumstances bind to the minor groove of D3 is provided by the ion of medium abundance at *m*/*z* 1893.0, which is assigned to $[D3 + 2distamycin + Ru(phen)_3 - 8H]^{6-}$. Since there were no ions assigned to a non-covalent complex containing two distamycin bound to D3 in Fig. 8(a), this was probably formed by displacement of two distamycin from $[D3 + 4distamycin - 6H]^{6-}$ by one $[Ru(phen)_3]^{2+}$.

Stronger evidence for ruthenium molecules binding to the minor groove of DNA was provided by studying the effect of adding six equivalents of either $[Ru(phen)_2(dpqC)]^{2+}$ or $[Ru(phen)_2(dppz)]^{2+}$, to solutions already containing D3 and 10 equivalents of distamycin. The spectrum of the solution containing $[Ru(phen)_2(dppz)]^{2+}$ is shown in Fig. 8(c), which contains no ions containing only distamycin molecules bound to D3, apart from that at *m*/*z* 1946.7, assigned to $[D3 + 4distamycin - 6H]^{6-}$. An ion of medium abundance at *m*/*z* 2070.0 is assigned to $[D3 + 4distamycin + Ru(phen)_2(dppz) - 8H]^{6-}$, most likely formed by displacement of the fifth and sixth distamycin molecules bound to D3 in ions such as $[D3 + 6distamycin - 6H]^{6-}$. The two most abundant ions in the spectrum are those at *m*/*z* 2034.0 and 2157.2, which are assigned to $[D3 + 2distamycin + 2Ru(phen)_2(dppz) - 10H]^{6-}$ and $[D3 + 2distamycin + 3Ru(phen)_2(dppz) - 12H]^{6-}$, respectively. These as well as other ions at *m*/*z* 1848.8 ($[D3 + 2distamycin + 2Ru(phen)_2(dppz) - 11H]^{7-}$) and 2280.6 ($[D3 + 2distamycin + 4Ru(phen)_2(dppz) - 14H]^{6-}$), were probably formed in reactions where three or four distamycin were displaced from ions such as $[D3 + 6distamycin - 6H]^{6-}$, $[D3 + 6distamycin - 7H]^{7-}$ and $[D3 + 7distamycin - 6H]^{6-}$. While the first two of the distamycin molecules displaced may have been only weakly bound to D3, there is little doubt that the third and fourth were more tightly bound in the AT rich regions of this duplex. This shows that $[Ru(phen)_2(dppz)]^{2+}$ is not only capable of binding to the DNA minor groove, but also has a comparable binding affinity to distamycin.

Conclusion

An enormous variety of chemical, biochemical and spectroscopic techniques have been used to probe the binding interactions of metallointercalators with DNA. Despite this, the exact nature of their binding to DNA is, in many instances, still far from resolved. This is a result of a combination of factors, including difficulties associated with identifying binding sites on large, complex molecules such as calf-thymus DNA. In addition, many spectroscopic techniques are not well suited to analysing solutions containing mixtures of non-covalent complexes. This highlights one of the most important attributes of ESI-MS for examining such systems. Our results show that ESI-MS can readily provide information on the number, relative amounts, and stoichiometry, of non-covalent complexes present in solutions containing up to five metal/DNA complexes. No other technique can afford this information as readily, owing to the extremely sensitive nature of ESI-MS, and simplicity of the resulting spectra. These attributes combined with speed of analysis, make ESI-MS an attractive choice for large-scale screening of the DNA binding properties of metal complexes.

For the same reasons ESI-MS is also well suited to performing DNA competition experiments involving metal complexes and organic drugs. Our results from competition experiments involving distamycin and daunomycin provided evidence that some of the ruthenium compounds studied probably intercalate into DNA from the minor groove. However, there are alternative explanations for the observed results that do not rely on the ruthenium compounds directly displacing the organic drugs from their DNA binding sites. For example, the binding of a ruthenium compound elsewhere along the DNA duplex might induce a change in DNA conformation that results in the organic drug binding less tightly. It is also possible that electrostatic interactions between positively charged ruthenium and distamycin molecules located in close proximity to each other could result in displacement of some of the distamycin molecules. This displacement could occur either in solution or in the mass spectrometer.

Most of the ruthenium compounds examined here showed a greater ability to form non-covalent complexes with D2 than either D1 or D3. This was most evident with those ruthenium compounds with greater DNA binding affinity, and suggests that D2 has either a greater number of binding sites and/or its binding sites are more attractive to these compounds. This was somewhat surprising, as it was expected that the greater GC content of duplex D1 would have proved more attractive to compounds that bind predominantly by intercalation. One possible explanation is that D1 does contain a greater number of potential binding sites, but their close proximity to each other results in unfavourable steric and/or electrostatic interactions between ruthenium compounds. On the other hand D2 contains a smaller number of suitable binding sites, which are positioned sufficiently far apart to prevent unfavourable interactions.

A final question to be answered is where exactly do the ruthenium compounds bind to these DNA duplexes. This question cannot be resolved by ESI-MS alone, although clues might be obtained, for example, by performing additional binding studies using other 16mer duplexes with different base sequences. In addition, comparison of the results presented here with those obtained from binding studies involving smaller, related DNA duplexes might provide further insight. These studies are currently in progress, as well as other spectroscopic investigations to determine binding constants for the interactions of these ruthenium compounds with D1–D3.

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