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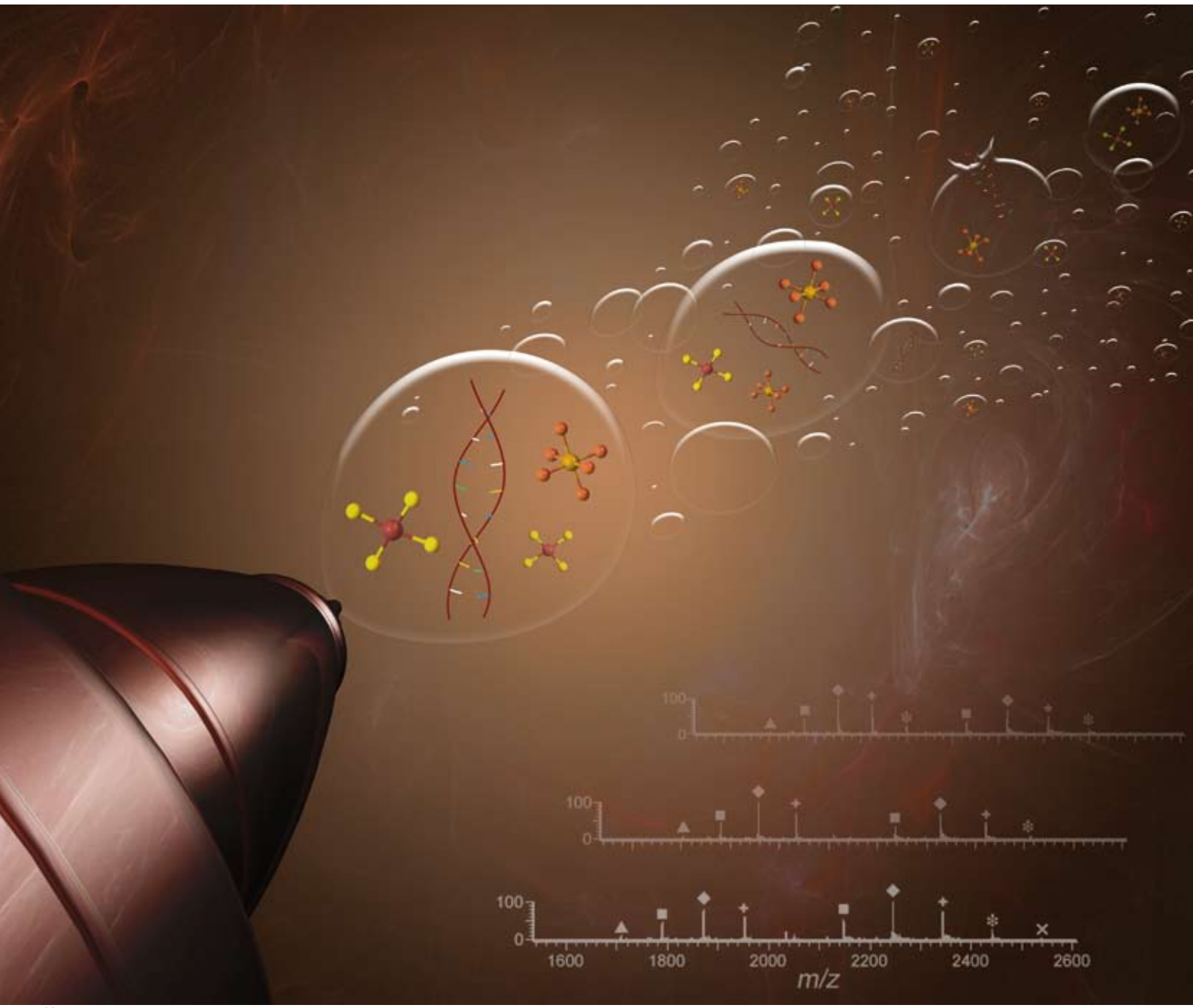

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A comparison of the binding of metal complexes to duplex and quadruplex DNA

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Electrospray ionisation mass spectrometry (ESI-MS) and circular dichroism (CD) spectroscopy were used to compare the binding of mononuclear nickel, ruthenium and platinum complexes to double stranded DNA (dsDNA) and quadruplex DNA (qDNA). CD studies provided evidence for the binding of intact complexes of all three metal ions to qDNA. ESI mass spectra of solutions containing platinum or ruthenium complexes and qDNA showed evidence for the formation of non-covalent complexes consisting of intact metal molecules bound to DNA. However, the corresponding spectra of solutions containing nickel complexes principally contained ions consisting of fragments of the initial nickel molecule bound to qDNA. In contrast ESI mass spectra of solutions containing nickel, ruthenium or platinum complexes and dsDNA only showed the presence of ions attributable to intact metal molecules bound to DNA. The fragmentation observed in mass spectral studies of solutions containing nickel complexes and qDNA is attributable to the lower thermodynamic stability of the former metal complexes relative to those containing platinum or ruthenium, as well as the slightly harsher instrumental conditions required to obtain spectra of qDNA. This conclusion is supported by the results of tandem mass spectral studies, which showed that ions consisting of intact nickel complexes bound to qDNA readily undergo fragmentation by loss of one of the ligands initially bound to the metal. The ESI-MS results also demonstrate that the binding affinity of each of the platinum and ruthenium complexes towards qDNA is significantly less than that towards dsDNA.

Introduction

Deoxyribonucleic acid (DNA) forms a variety of less common structures in addition to the well-known anti-parallel double helix. These include hairpins and Holliday structures, triplexes and several different types of quadruplexes.[1,2] The latter are formed by guanine rich DNA sequences such as telomeres, the dimerisation region of HIV, centromeres, a portion of the insulin regulatory gene, fragile X-syndrome repeat regions, and the upstream promoter regions of some oncogenes.[1,3] Telomeres are guanine rich sequences present at the end of chromosomes that do not code for specific proteins, but rather are thought to protect the chromosome against damage. These sequences are believed to form structures in which four co-planar guanines form a tetrad by means of Hoogstein hydrogen bonds, and the structure is further stabilised by interactions between the guanine residues and monovalent cations such as sodium or potassium. A variety of quadruplex structures have been proposed for oligomeric DNA containing guanine rich sequences.[2–6] The most widely examined is probably the intermolecular tetrameric structure formed by four parallel DNA strands with identical base sequences. However, other structures are possible, including parallel and antiparallel quadruplexes formed from two DNA strands, and an intramolecular quadruplex formed from a single DNA chain. In each of these structures three or four adjacent guanine tetrads form a stacked structure that is stabilised by π–π interactions.

Telomeres have recently attracted considerable attention as potential therapeutic targets, as a result of the differences between telomeres in normal and cancer cells. In normal somatic human cells, telomeres function to protect the ends of chromosomes, but undergo shortening every time the cell undergoes DNA replication. Eventually the telomeres become too short to protect the chromosome, leading to apoptosis and cell death. In contrast, many cancer cells are able to maintain the length of their telomere sequences by increasing the level of activity of the telomerase enzyme, rendering these cells immortal. Telomerase is expressed in 85% of cancer cells.[7] Consequently it is not surprising that a number of research groups are trying to develop small molecules that will selectively inhibit this particular enzyme.[3,8–14] In many cases this has involved the synthesis of small molecules that can stabilise or induce the formation of quadruplex structures in telomeric DNA, as telomerase requires single stranded DNA as a template. One of the most potent anti-telomerase compounds known is the natural product telomestatin.[8,9] This macrocyclic compound has been shown to inhibit telomerase at nanomolar concentrations, and presumably stabilises or induces formation of guanine quadruplexes through π–π stacking interactions between the thiazoline and oxazole rings of the natural product and the planar aromatic rings of the guanine bases. This discovery has encouraged the search for more potent and selective telomerase inhibitors from amongst other types of macrocyclic molecules,[10] including porphyrins.[11] However, there have also been many other

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reports of non-macrocyclic compounds which bind to or stabilise quadruplex DNA, and in some instances have also been shown to inhibit telomerase with a high degree of efficiency.[12–17] All of these compounds contain a central aromatic moiety that contains several aromatic rings, as well as two or more flexible arms bearing tertiary amines. It has been speculated that the function of the central portion of the molecule is to participate in π–π stacking interactions with the guanine tetrad,[12] and that the flexible arms of the molecules may interact with the quadruplex grooves.[18] In keeping with these design principles, Reed and co-workers recently prepared two square planar nickel(II) complexes of derivatised salphen ligands, and showed that they both had a significant ability to inhibit telomerase.[19] Furthermore these workers were able to use fluorescent resonance energy transfer experiments to show that their compounds exhibited considerable selectivity for quadruplex DNA over duplex DNA. This highlights the potential of metal complexes for development as anticancer agents based on their ability to selectively bind to telomeres and inhibit telomerase.

A variety of methods, including equilibrium dialysis, gel electrophoresis, circular dichroism spectroscopy, NMR spectroscopy and X-ray diffraction, have been used to examine how small molecules bind to quadruplex DNA. Many of the earliest investigations in this area used classical duplex DNA intercalators and groove binding agents. Not surprisingly, the ethidium ion was found to bind to quadruplex DNA by an intercalative mode.[20] However, the X-ray crystal structure of a complex of another well known intercalator, daunomycin, with $d(TGGGGT)_4$, showed that this drug binds *via* π–π interactions with the terminal G-tetrad at the 5′ end of the quadruplex, rather than by a classical intercalative mode.[21] This end stacking mode of interaction has been proposed for a variety of other compounds, including porphyrins, a polycyclic methylacridinium salt, and a quinobenzoxazine.[3,22–24] NMR spectroscopy was also used to show that the classical minor groove binding drug distamycin interacts with guanine quadruplexes formed at both ends of the sequence d(TAGGGTTA).[25]

Over the past two decades electrospray ionisation mass spectrometry (ESI-MS) has become a routine tool for the analysis of DNA and drug–DNA interactions.[26–29] More recently the attention of some groups has turned to the characterisation of drug complexes with quadruplex DNA.[30–33] One of the first of these studies examined the binding of three different compounds, including distamycin, to the parallel quadruplex $[d(T_2G_5T)]_4$.[30] ESI-MS was used to probe the specificity of the drugs for quadruplex *versus* double stranded DNA, whilst collision activated dissociation provided evidence for differences in the binding modes used by the compounds. Rosu and co-workers used ESI-MS to examine the formation of three different quadruplexes in ammonium acetate solution.[31] For the tetrameric quadruplex structure examined it was demonstrated that ammonium ions were specifically incorporated, and that these were not displaced after addition of a quadruplex-specific porphyrin molecule. This provided evidence that the porphyrin binds at the ends of the molecule, rather than by intercalation between the guanine tetrads. The preferential binding of the drug dicertalium to quadruplex DNA was shown by ESI-MS,[32] while in a later study the interactions of ethidium ion and a series of ethidium derivatives with different quadruplex molecules was examined using both ESI-MS and equilibrium dialysis.[33] Both techniques gave similar selectivity profiles, while ESI-MS showed for each of the drugs investigated that a maximum of 2 molecules could bind per quadruplex, which was interpreted as evidence for the presence of a single binding site at each end of each quadruplex. Rosu and co-workers have also recently examined the kinetics of binding of telomestatin to human telomeric DNA, and showed that complexation significantly retards unwinding and conversion of quadruplex DNA to the duplex form.[34]

In contrast to the above studies, there have been comparatively few reports of the interactions between metal complexes and quadruplex DNA,[19,35–37] and none that have examined these binding interactions using ESI-MS. This technique is eminently suitable for studying the non-covalent interactions of metal complexes with duplex DNA,[38–41] due partially to its high sensitivity, and the ease of sample preparation and spectral analysis. In this paper we present the results of a study into the ability of ESI-MS to examine the binding of different classes of metal complexes to quadruplex DNA. These interactions were also examined using circular dichroism spectroscopy, and are compared to those between the same complexes and duplex DNA.

Experimental

Materials

$[Ru(phen)_3]Cl_2$ was obtained from the Aldrich Chemical Company, while all other ruthenium and platinum complexes were prepared by literature methods.[42–44] The preparation of the nickel complexes will be described in a forthcoming publication.[41] $[Zn(phen)_3](ClO_4)_2$ and $[Fe(phen)_3](ClO_4)_2$ were prepared by reacting ethanolic solutions of $ZnSO_4 \cdot 7H_2O$ and $FeSO_4 \cdot 7H_2O$ with slightly greater than three equivalents of 1,10-phenanthroline for 30 minutes, and then adding an excess of $NaClO_4$. The structures of the different compounds used in this study are shown in Fig. 1. Single stranded oligonucleotides were obtained from Geneworks (South Australia) and purified using procedures previously reported.[45] 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.[46] Stock solutions of individual metal complexes were prepared by dissolving the appropriate amount of compound in 0.1 M ammonium acetate (NH_4OAc), pH 7.4 or 0.15 M ammonium acetate, pH 7.0. The former solutions were used in experiments with dsDNA while the latter were used with qDNA.

The double-stranded DNA molecule D2 was prepared by first mixing equimolar quantities of its component DNA single strands (both dissolved in 0.1 M NH_4OAc, pH 7.4) so that the final concentration of DNA was 1 mM. This solution was then heated to 20 °C higher than the melting temperature of D2 for 15 minutes, and subsequently allowed to cool slowly to room temperature. The quadruplex DNA molecule Q1 was prepared by an analogous procedure using the single precursor DNA molecule required, and 0.15 M NH_4OAc, pH 7.0. Solutions containing D2 or Q1 and metal complexes were prepared by first annealing samples of DNA using the above procedure, and then adding the required amount of stock metal solution. The sequences of D2 and Q1 are:

D2 (CCTCGGCCGGCCGACC/GGTCGGCCGGCCGAGG)

Q1 $(TTGGGGGT)_4$

All mass spectra were obtained using a Waters extended mass range Q-ToF Ultima mass spectrometer equipped with a Z-spray

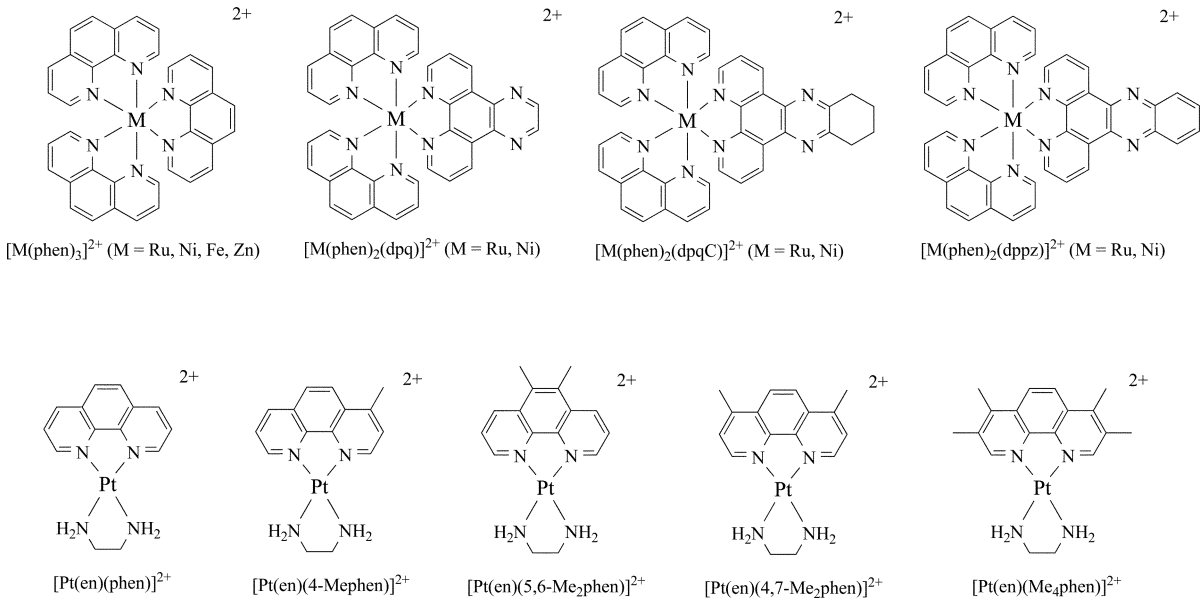

Fig. 1 Structures of the complexes used in this study.

probe and a mass analyser with an m/z range of 32000. All dsDNA and qDNA samples were diluted with 0.15 M NH_4OAc, pH 7.4, and 0.15 M NH_4OAc, pH 7.0, respectively giving a final concentration of DNA of 10 μM. These were injected using a Harvard model 22 syringe pump (Natick, MA, USA) at a flow rate of 10 μL min^{-1}. Negative ion ESI mass spectra of solutions containing dsDNA were obtained using a capillary tip potential of 2500 V, a cone voltage of 100 V, and a desolvation temperature of 100 °C. For samples containing qDNA the capillary tip potential was the same, while the cone voltage and desolvation temperature were 150 V and 150 °C, respectively. The transport and aperture were both set to 5, and the RF lens1 energy to 70 V, for both types of samples. Spectra were typically acquired over the m/z range 500–3000, with between 30 and 50 scans averaged to give the final spectrum. All data were calibrated against a standard CsI solution (750 μM) over the same m/z range.

Circular dichroism (CD) spectra (200–320 nm) were obtained using a Jasco J-810 spectropolarimeter and 0.1 cm pathlength quartz cell. Spectra were obtained using 0.15 M NH_4OAc solutions (pH 7.0) containing 0 : 1, 1 : 1, 4 : 1, 10 : 1, 20 : 1 and 40 : 1 ratios of metal complex and Q1. The concentration of Q1 in each case was 5 μM. Each solution containing Q1 and metal complex was allowed to stand at room temperature for 10 minutes prior to the CD spectrum being obtained.

Results and discussion

Interactions of ruthenium(II) complexes with duplex DNA and quadruplex DNA

In earlier work we used ESI-MS to examine the interactions of a series of mononuclear ruthenium complexes $[Ru(phen)_2L]^{2+}$ (phen = 1,10-phenanthroline, L = bidentate heterocyclic amine ligand) with different 16mer duplex DNA molecules.[38,39] These studies showed that in some instances solutions containing a 6 : 1 ratio of metal to DNA formed mixtures of non-covalent complexes with between 1 and 5 ruthenium molecules bound to DNA. Furthermore the spectra could be used to determine an order of relative binding affinities that matched closely to what was expected based on other spectroscopic methods. We therefore decided to use ESI-MS to investigate whether these ruthenium complexes displayed the same propensity to bind to quadruplex DNA.

Fig. 2 shows negative ion ESI mass spectra of free Q1 and solutions containing different ratios of $[Ru(phen)_2(dppz)]^{2+}$ and Q1. The spectrum of free Q1 (Fig. 2(a)) shows ions of high abundance at m/z 1674.8 and 2009.9 assigned to $[Q1 + 4NH_4^+ - 10H]^{6-}$ and $[Q1 + 4NH_4^+ - 9H]^{5-}$, respectively. The presence of monovalent ions such as Na^+, K^+ or NH_4^+ is known to play an important role in stabilising quadruplex DNA structures, and has been confirmed experimentally by a variety of techniques.[1]

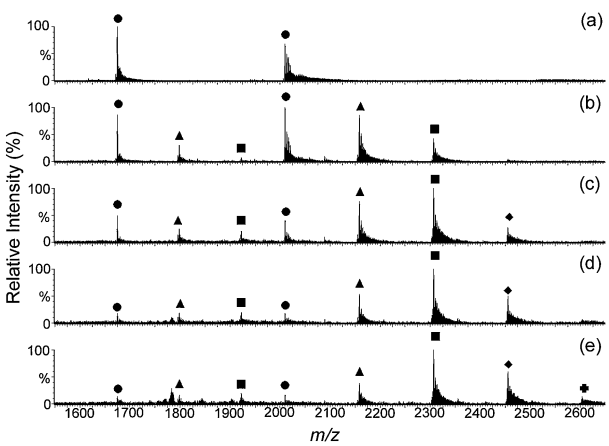

Fig. 2 Negative ion ESI mass spectra of free Q1 and solutions containing different ratios of $[Ru(phen)_2(dppz)]^{2+}$ and Q1: (a) free Q1; (b) Ru : Q1 = 10 : 1; (c) Ru : Q1 = 20 : 1; (d) Ru : Q1 = 30 : 1; (e) Ru : Q1 = 40 : 1; ● free Q1; ▲ Q1 + 1$[Ru(phen)_2(dppz)]^{2+}$; ■ Q1 + 2$[Ru(phen)_2(dppz)]^{2+}$; ◆ Q1 + 3$[Ru(phen)_2(dppz)]^{2+}$; + Q1 + 4$[Ru(phen)_2(dppz)]^{2+}$.

Typically the number of monovalent cations present is expected to be one less than the number of guanine tetrads.[31] In the case of Q1 four ammonium ions are therefore expected to be present, and were indeed always observed not only for ions assigned to free Q1, but also for all ions containing one or more metal complexes bound to Q1.

Addition of 10 equivalents of $[Ru(phen)_2(dppz)]^{2+}$ to Q1 resulted in the spectrum shown in Fig. 2(b), which shows new ions with 5− and 6− overall charges that are attributable to non-covalent complexes containing one and two ruthenium molecules bound to Q1. Increasing the ratio of Ru : Q1 further to 20 : 1 resulted in the abundance of these ions increasing and now dominating those attributable to free qDNA (Fig. 2(c)). An interesting observation was that the abundance of 5− ions attributable to specific non-covalent complexes was always considerably greater than that of the corresponding 6− ions for solutions containing $[Ru(phen)_2(dppz)]^{2+}$ or the other ruthenium complexes. Fig. 2(c) also contains 5− ions of low abundance attributable to non-covalent complexes consisting of 3 ruthenium molecules bound to Q1. When the Ru : qDNA ratio was increased to 30 : 1 the abundance of the latter ions increased relative to those assigned to the other non-covalent complexes (Fig. 2(d)), however there were only relatively minor changes in relative abundance of each of the other ions present. Furthermore the spectrum of this solution differs little from that of a solution containing a 40 : 1 ratio of Ru : qDNA (Fig. 2(e)), indicating that equilibrium was being approached. Fig. 2(d) and Fig. 2(e) both contained ions of very low abundance at $m/z \sim 2604$ that may be tentatively assigned to non-covalent qDNA complexes containing four ruthenium molecules. In addition the spectra of solutions containing high Ru : qDNA ratios also contained other ions of very low abundance at even higher m/z that can be assigned to the corresponding 4− ions of some of the non-covalent complexes discussed above.

The above results demonstrate first of all the ability of ESI-MS for detecting non-covalent complexes consisting of metal complexes bound to qDNA. In addition they also show that the binding affinity of $[Ru(phen)_2(dppz)]^{2+}$ towards qDNA is considerably less than that towards dsDNA. This is consistent with observations reported in an earlier study involving this ruthenium complex, which showed that it had only a small effect on the melting temperature of the human telomere sequence.[37] Evidence in support of this conclusion in the present study is provided by the observation that the most abundant ions in Fig. 2(d) were those attributable to non-covalent qDNA complexes containing just two ruthenium molecules. In contrast our previous work showed that the most abundant non-covalent ions present in the spectra of solutions containing a 6 : 1 ratio of $[Ru(phen)_2(dppz)]^{2+}$ and D2 contained four ruthenium molecules.[38,39] This difference suggests that the binding interactions between $[Ru(phen)_2(dppz)]^{2+}$ and the two types of DNA molecules differ significantly, a proposal that is supported by the results obtained with the other ruthenium complexes as well as those containing platinum.

Negative ion ESI mass spectra of solutions containing the same ratio of different ruthenium complexes and qDNA proved to be very similar to each other. For example, Fig. 3 shows the spectra of solutions containing a 40 : 1 ratio of different ruthenium complexes and qDNA. In each case the abundance of ions attributable to free qDNA is very low and the most abundant ions attributable to non-covalent complexes are those containing two ruthenium molecules. The spectra also all contain ions of medium–high abundance attributable to non-covalent complexes consisting of a single ruthenium molecule bound to qDNA, as well as ions that were generally of low abundance attributable to non-covalent complexes containing three ruthenium molecules. The results presented in Fig. 3 suggest that the binding affinities of these four ruthenium complexes towards qDNA are very similar. The slightly greater abundance of ions attributable to non-covalent complexes containing three $[Ru(phen)_2(dppz)]^{2+}$ bound to qDNA suggests that this compound may have a slightly higher binding affinity than the others. However, this is nowhere near as dramatic as the greater binding affinity exhibited by $[Ru(phen)_2(dppz)]^{2+}$ towards dsDNA found previously.[47–49] A further surprising aspect of the results shown in Fig. 3 is how similar the binding affinity of $[Ru(phen)_3]^{2+}$ towards qDNA is compared to that of the other ruthenium complexes. Overall the ESI-MS results suggest that a different binding mode may be operating between these ruthenium complexes and qDNA compared to that which occurs with dsDNA. Furthermore the presence of strongly intercalating ligands such as dppz in the metal's coordination sphere does little, if anything, to enhance overall binding affinity.

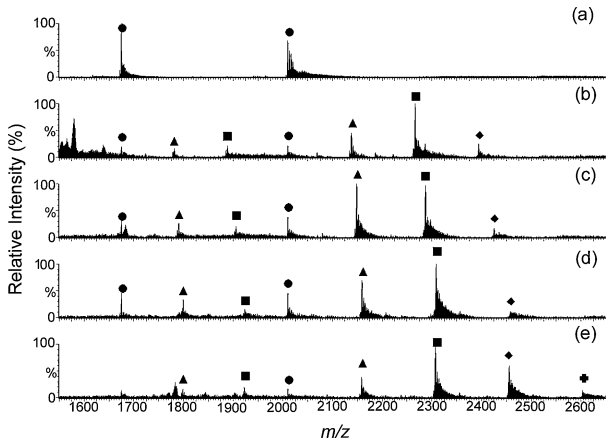

Fig. 3 Negative ion ESI mass spectra of free Q1 and solutions containing a 40 : 1 ratio of different ruthenium complexes and Q1: (a) free Q1; (b) Q1 + $[Ru(phen)_3]^{2+}$; (c) Q1 + $[Ru(phen)_2(dpq)]^{2+}$; (d) Q1 + $[Ru(phen)_2(dpqC)]^{2+}$; (e) Q1 + $[Ru(phen)_2(dppz)]^{2+}$; ● free Q1; ▲ Q1 + 1$[Ru(phen)_2L]^{2+}$; ■ Q1 + 2$[Ru(phen)_2L]^{2+}$; ◆ Q1 + 3$[Ru(phen)_2L]^{2+}$; + Q1 + 4$[Ru(phen)_2L]^{2+}$.

CD spectra were also obtained of solutions containing different ratios of ruthenium complexes and Q1 in an effort to corroborate the similar levels of binding affinity revealed by ESI-MS. Fig. 4 illustrates the results of CD titrations performed using all four ruthenium complexes. The spectrum of free Q1 is consistent with those reported previously for qDNA,[50–54] and shows subtle but distinct differences from that of dsDNA. In addition to positive CD signals centred at 265 and 210 nm, the spectrum of Q1 also contains a negative CD band at 242 nm. Addition of increasing amounts of ruthenium complex to Q1 was found to result in a significant decrease in ellipticity of each of the above CD bands. A similar decrease in molar ellipticity was observed for the lower energy CD band of the parallel four-stranded quadruplex formed from T_4G_4, upon addition of increasing amounts of porphyrins.[53] Examination of the data shown in Fig. 4 shows that addition of the four ruthenium complexes causes comparable decreases in ellipticity of each of the three CD signals, supporting the

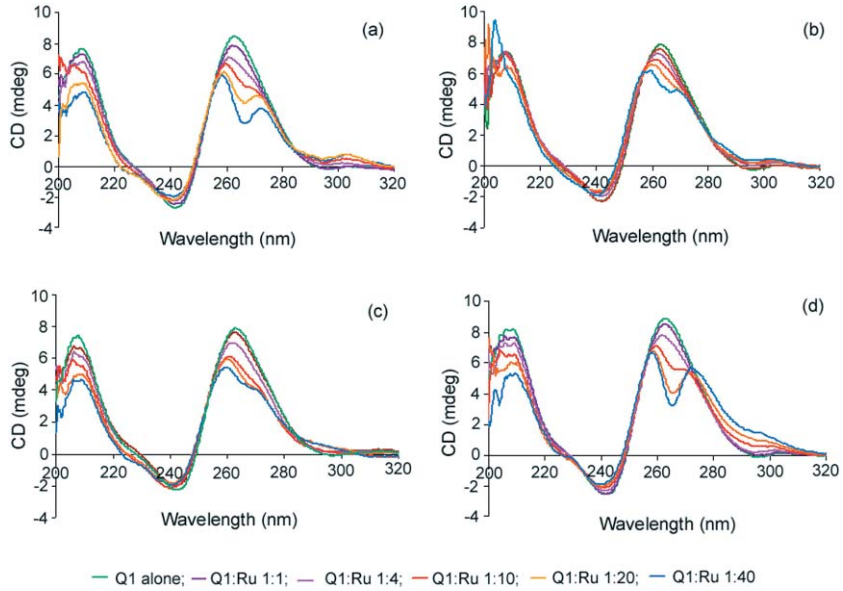

Fig. 4 Circular dichroism spectra of solutions containing different ratios of ruthenium complexes and Q1: (a) $[Ru(phen)_2(dpqC)]^{2+}$; (b) $[Ru(phen)_2(dpq)]^{2+}$; (c) $[Ru(phen)_2(dppz)]^{2+}$ and (d) $[Ru(phen)_3]^{2+}$.

conclusion based on ESI-MS results that these molecules have similar qDNA binding affinities. A further point of similarity is that the positive CD signal at 265 nm is found to split into two distinct bands at higher Ru : Q1 ratios. This was most clearly evident in the case of CD spectra of solutions containing $[Ru(phen)_3]^{2+}$, however spectra of each of the remaining solutions either showed two distinct peak maxima in this region or, in the case of $[Ru(phen)_2(dppz)]^{2+}$, a clearly discernible shoulder at higher wavelengths.

Interactions of platinum(II) complexes with duplex DNA and quadruplex DNA

The study of non-covalent interactions between platinum complexes and DNA can be traced back to the initial studies of Lippard and co-workers, who investigated the DNA binding of square planar platinum(II) complexes containing the terpyridine ligand.[55] Since then most studies of the binding of platinum complexes to DNA have focussed on those molecules capable of eliciting an antitumour response as a result of covalent binding. However, there have been several studies which showed that complexes of the type $[Pt(en)L]^{2+}$, where L is either phenanthroline or one of several methylated phenanthroline ligands, display cytotoxicity towards murine leukaemia L1210 cells that was found to be dependent on the number and position of methyl substituents.[44,56] Circular dichroism spectroscopy was used to investigate the interactions of these complexes with calf thymus DNA, and provided binding constants which suggested that their affinities for DNA were comparable to that of complexes such as $[Ru(phen)_2(dppz)]^{2+}$. No simple correlation was found between the extent of methylation of the phenanthroline ligand and the binding constants, or between the binding constants and cytotoxicity. In order to learn more about the potential mechanisms of cytotoxicity of these complexes we therefore decide to use ESI-MS to investigate their interactions with both dsDNA and qDNA.

Addition of an equimolar quantity of the platinum complexes shown in Fig. 1 to D2 resulted in the formation of significant amounts of non-covalent complexes containing one platinum molecule bound to DNA. In the case of both $[Pt(en)(Me_4phen)]^{2+}$ and $[Pt(en)(5,6\text{-}Me_2phen)]^{2+}$, ions attributable to these complexes were the most abundant observed in ESI mass spectra, which also showed ions assigned to non-covalent complexes containing two bound platinum molecules. Increasing the metal : D2 ratio resulted in the formation of non-covalent complexes containing even greater numbers of bound platinum molecules. For example, Fig. 5 shows the spectra of solutions containing a 5 : 1 ratio of different platinum complexes and D2. The spectrum of the solution containing $[Pt(en)(phen)]^{2+}$ (Fig. 5(b)) is dominated by ions attributable to non-covalent complexes containing one, two and three platinum molecules bound to DNA. Fig. 5(c) shows that the addition of $[Pt(en)(4,7\text{-}Me_2phen)]^{2+}$ to D2 resulted in the formation of many analogous non-covalent complexes. On closer inspection it can be seen that the relative abundance of ions containing one platinum molecule bound to D2 is lower in Fig. 5(c) than in Fig. 5(b). This result, together with the observation that Fig. 5(c) also contains ions assigned to non-covalent complexes containing four platinum molecules bound to D2, whose combined abundance is greater than that of the analogous ions in Fig. 5(b), suggests that the affinity of $[Pt(en)(4,7\text{-}Me_2phen)]^{2+}$ towards D2 is slightly greater than that of $[Pt(en)(phen)]^{2+}$.

ESI mass spectra of solutions containing the remaining three platinum complexes and D2 also showed ions consisting of four platinum molecules non-covalently bound to DNA, as well as ions attributable to more highly complexed DNA molecules (Fig. 5(d)–(f)). Overall the results indicate that the affinity of the platinum complexes towards duplex DNA is significantly affected by both the extent of methylation of the phenanthroline ligand, as well as by the position of the methyl substituents. Furthermore the mass spectra also clearly demonstrate the tremendous affinity that each of the platinum complexes display towards D2, despite the

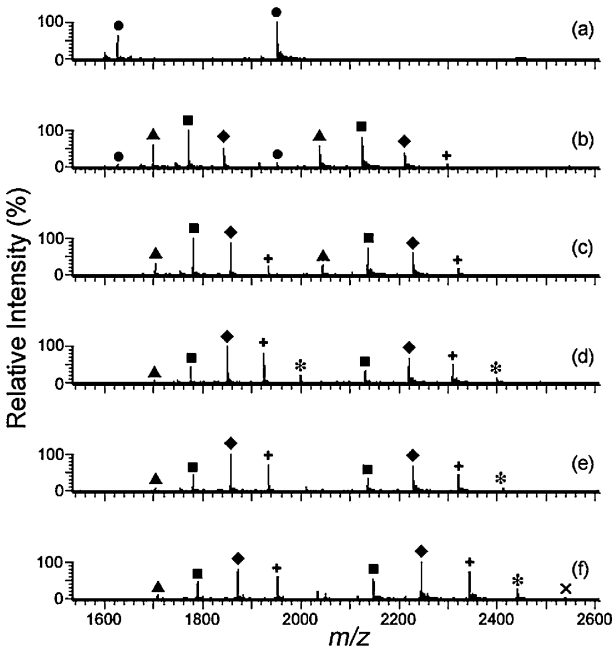

Fig. 5 Negative ion ESI mass spectra of free D2 and solutions containing a 5 : 1 ratio of different platinum complexes and D2: (a) free D2; (b) D2 + $[Pt(en)(phen)]^{2+}$; (c) D2 + $[Pt(en)(4,7\text{-}Me_2phen)]^{2+}$; (d) D2 + $[Pt(en)(4\text{-}Mephen)]^{2+}$; (e) D2 + $[Pt(en)(5,6\text{-}Me_2phen)]^{2+}$; (f) D2 + $[Pt(en)(Me_4phen)]^{2+}$; ● free D2; ▲ D2 + 1 $[Pt(en)L]^{2+}$; ■ D2 + 2 $[Pt(en)L]^{2+}$; ◆ D2 + 3 $[Pt(en)L]^{2+}$; + D2 + 4 $[Pt(en)L]^{2+}$; * D2 + 5 $[Pt(en)L]^{2+}$; × D2 + 6 $[Pt(en)L]^{2+}$.

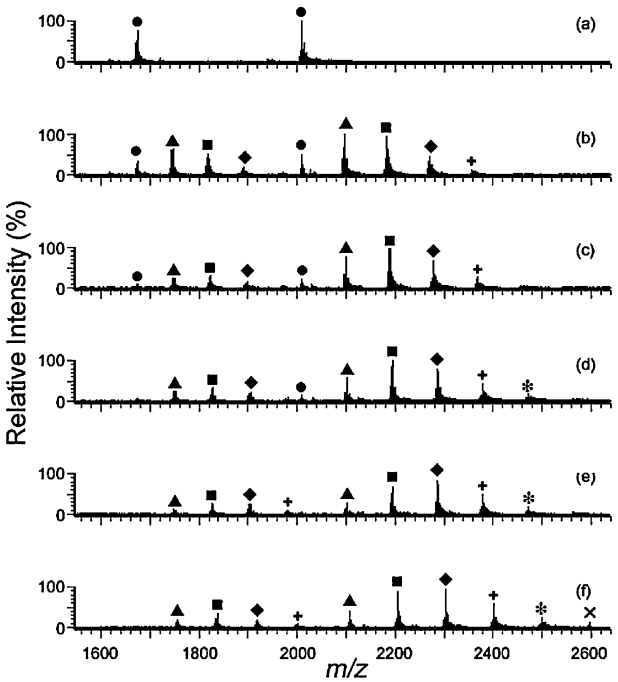

Fig. 6 Negative ion ESI mass spectra of free Q1 and solutions containing a 30 : 1 ratio of different platinum complexes and Q1: (a) free Q1; (b) Q1 + $[Pt(en)(phen)]^{2+}$; (c) Q1 + $[Pt(en)(4\text{-}Mephen)]^{2+}$; (d) Q1 + $[Pt(en)(5,6\text{-}Me_2phen)]^{2+}$; (e) Q1 + $[Pt(en)(4,7\text{-}Me_2phen)]^{2+}$; (f) Q1 + $[Pt(en)(Me_4phen)]^{2+}$; ● free Q1; ▲ Q1 + 1$[Pt(en)L]^{2+}$; ■ Q1 + 2$[Pt(en)L]^{2+}$; ◆ Q1 + 3$[Pt(en)L]^{2+}$; + Q1 + 4$[Pt(en)L]^{2+}$; * Q1 + 5$[Pt(en)L]^{2+}$; × Q1 + 6$[Pt(en)L]^{2+}$.

absence of a strongly intercalating ligand such as dppz in their structures. In fact when the metal : D2 ratio was increased further to 10 : 1, the mass spectra of solutions containing $[Pt(en)(4,7\text{-}Me_2phen)]^{2+}$, $[Pt(en)(5,6\text{-}Me_2phen)]^{2+}$ and $[Pt(en)(Me_4phen)]^{2+}$ all showed the presence of ions of medium to high abundance attributable to non-covalent complexes containing six or seven platinum molecules bound to DNA (results not shown). This further illustrates the favourable intercalating properties conferred on these metal complexes by their square planar geometry.

It was then decided to obtain ESI mass spectra of solutions containing different ratios of the platinum complexes and Q1, in order to determine whether these square planar molecules also have a high binding affinity towards quadruplex DNA. Fig. 6 shows the spectra of solutions containing a 30 : 1 ratio of the different platinum complexes and Q1. The lowest binding affinity towards qDNA was exhibited by $[Pt(en)(phen)]^{2+}$, which formed non-covalent complexes of high abundance containing one and two platinum molecules bound to Q1, and ions of low or medium abundance assigned to non-covalent complexes containing three and four bound platinum molecules (Fig. 6(b)). Replacement of the phenanthroline ligand in this complex by one of the methylated derivatives resulted in the relative abundance of these more highly substituted non-covalent complexes increasing, as well as the appearance of ions attributable to non-covalent complexes containing five and six bound platinum molecules in some instances. In the case of solutions containing $[Pt(en)(4,7\text{-}Me_2phen)]^{2+}$ (Fig. 6(e)) and $[Pt(en)(Me_4phen)]^{2+}$ (Fig. 6(f)) the most abundant ions observed were those attributable to non-covalent complexes containing three platinum molecules bound to Q1. It can therefore be concluded that at least some of the platinum molecules display a slightly higher binding affinity towards Q1 than the ruthenium complexes described above, since it will be recalled that the most abundant ions present in spectra of solutions containing a 40 : 1 ratio of the latter complexes and Q1 always contained only two metal molecules.

It is noteworthy that the least reactive platinum complex towards both duplex DNA and quadruplex DNA was $[Pt(en)(phen)]^{2+}$, while the most reactive was $[Pt(en)(Me_4phen)]^{2+}$. This highlights the important role that hydrophobic interactions can play in determining overall binding affinities towards DNA. It also appears that the binding of the platinum compounds to qDNA did not significantly affect the secondary structure of the latter, since this produced much smaller changes in CD signals (1–2 mdeg) than what was observed on addition of identical quantities of either ruthenium or nickel complexes (3–4 mdeg). This is most likely due to the greater ability of the larger octahedral metal compounds to induce distortions in the secondary structure of Q1 as a result of interactions involving the ancillary ligands.

Interactions of other metal complexes with duplex DNA and quadruplex DNA

The results described above, and our earlier studies,[39,40] demonstrate the potential of ESI-MS for detecting non-covalent complexes formed between ruthenium(II) or platinum(II) complexes with both dsDNA and qDNA. Complexes containing ruthenium(II) in particular have attracted considerable attention due

to their robustness and interesting spectroscopic properties, while platinum(II) complexes offer ease of synthesis together with kinetic stability. Studies of the interactions between complexes and DNA have not, however, been restricted to these metal ions, and complexes of numerous other metal ions including rhodium(III), iridium(III), zinc(II), copper(II), nickel(II), chromium(III), iron(II) and nickel(II) have also been examined.[47,57–65] In order to more fully analyse the potential of ESI-MS for examining interactions of metal complexes with DNA, we have commenced an examination of the interactions of both dsDNA and qDNA with complexes of some of these metal ions. In work to be published elsewhere,[41] ESI mass spectra were obtained of solutions containing the nickel(II) complexes $[Ni(phen)_2L]^{2+}$ (L = phen, dpq, dpqC and dppz) and D2. These studies demonstrated once again the ability of ESI-MS to detect non-covalent complexes containing intact metal complexes bound to DNA, and revealed a significant dependence of overall binding affinity on the identity of the unique ligand L.

Surprising results were obtained, however, when solutions containing the above nickel complexes and Q1 were analysed by ESI-MS. For example, Fig. 7 shows the mass spectra of solutions containing a 10 : 1 ratio of $[Ni(phen)_2(dpq)]^{2+}$ or $[Ni(phen)_2(dpqC)]^{2+}$ and Q1. Neither spectrum contains ions of medium or high abundance attributable to non-covalent complexes containing Q1 and one or more intact nickel molecules. Instead the most abundant ions, other than those assigned to free Q1, were those attributable to Q1 + $[Ni(phen)(dpq)]^{2+}$, and Q1 + $[Ni(phen)(dpqC)]^{2+}$. These were almost certainly formed by loss of a phenanthroline ligand from non-covalent complexes containing intact $[Ni(phen)_2(dpq)]^{2+}$ or $[Ni(phen)_2(dpqC)]^{2+}$ molecules bound to Q1, and suggest that significant levels of fragmentation had taken place in the mass spectrometer. Further evidence for this is provided by the observation of other ions of low to medium abundance, such as Q1 + $[Ni(phen)]^{2+}$ and Q1 + 2 $[Ni(phen)]^{2+}$, which are present in both spectra. Similar results were also obtained when ESI mass spectra were obtained of solutions containing $[Ni(phen)_3]^{2+}$ or $[Ni(phen)_2(dppz)]^{2+}$ and Q1, consistent with the conclusion

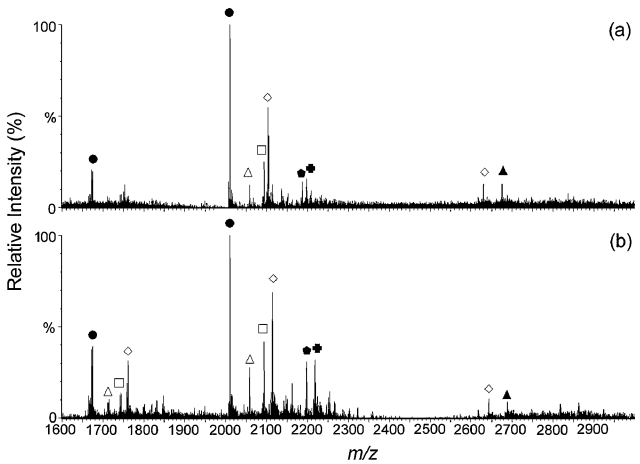

Fig. 7 Negative ion ESI mass spectra of solutions containing: (a) a 10 : 1 ratio of $[Ni(phen)_2(dpq)]^{2+}$ and Q1, and (b) a 10 : 1 ratio of $[Ni(phen)_2(dpqC)]^{2+}$ and Q1; ● free Q1; △ Q1 + $[Ni(phen)]^{2+}$; □ Q1 + $[Ni(phen)_2]^{2+}$; ◇ Q1 + $[Ni(phen)(L)]^{2+}$ (L = dpq or dpqC); ⬟ Q1 + $[Ni(phen)_2]^{2+}$ + $[Ni(phen)(L)]^{2+}$ (L = dpq or dpqC); + Q1 + 2$[Ni(phen)(L)]^{2+}$ (L = dpq or dpqC); ▲ Q1 + $[Ni(phen)_2(L)]^{2+}$ (L = dpq or dpqC).

that non-covalent complexes containing intact nickel molecules bound to Q1 are unstable in the mass spectrometer. In contrast, circular dichroism spectra of solutions containing the nickel complexes and Q1 strongly resembled those obtained from the corresponding ruthenium complexes, suggesting that intact non-covalent complexes containing nickel molecules bound to Q1 are formed, and that fragmentation only takes place inside the mass spectrometer.

The above results were unexpected in view of the absence of evidence for fragmentation occurring during mass spectral analysis of solutions containing any of the other metal complexes and dsDNA, or solutions containing ruthenium or platinum complexes and qDNA. However, it appears that the slightly lower thermodynamic stability of the nickel complexes, combined with the slightly harsher instrumental conditions required to obtain mass spectra of qDNA (cone voltage = 150 V instead of 100 V and desolvation temperature = 150 °C instead of 100 °C), are sufficient for fragmentation to occur. In order to obtain further evidence for this conclusion we performed a comparative tandem mass spectral analysis of solutions containing Q1 and either $[Ni(phen)_3]^{2+}$ or $[Ru(phen)_3]^{2+}$. Although mass spectra of solutions containing the former complex were dominated by ions containing only fragments of the initial $[Ni(phen)_3]^{2+}$ complex bound to Q1, ions of weak abundance attributable to a non-covalent complex containing one $[Ni(phen)_3]^{2+}$ and four ammonium ions bound to Q1 were present in the 4− region of the spectrum. Collision-induced dissociation (CID) of these ions revealed that the lowest energy pathway for fragmentation involved loss of a phenanthroline ligand. In contrast the corresponding non-covalent complex containing one $[Ru(phen)_3]^{2+}$ and four ammonium ions bound to Q1 only underwent fragmentation at much higher collision energies, and did so by a pathway that resulted in loss of an ammonium ion. Similar results were obtained when tandem MS experiments were performed using other ions present in these solutions or solutions containing different nickel and ruthenium complexes.

Overall the evidence supports the conclusion that it is the lower stability of Ni–N bonds that results in fragmentation of non-covalent complexes containing nickel molecules bound to Q1 inside the mass spectrometer. This conclusion has important implications concerning the analysis of non-covalent interactions between metal complexes and DNA, as it indicates that the initial coordination sphere of only the most thermodynamically stable metal ions may be able to survive the ESI process. Further evidence of this was obtained by examining the interactions of both $[Fe(phen)_3]^{2+}$ and $[Zn(phen)_3]^{2+}$ with D2. Despite the gentler ESI conditions used to examine solutions containing dsDNA, the mass spectra of both systems provided evidence that considerable fragmentation of non-covalent metal–DNA complexes had occurred. In the case of solutions containing $[Fe(phen)_3]^{2+}$, ions attributable to non-covalent complexes containing intact or fragmented iron molecules bound to D2 were of roughly comparable abundance. However, in the case of $[Zn(phen)_3]^{2+}$, the only ions that could be assigned to non-covalent complexes were those containing fragments of the initial zinc molecule bound to D2.

Conclusions

Our previous investigations of the interactions between mononuclear ruthenium(II) complexes and duplex DNA showed that

ESI-MS can provide an unparalleled level of detail concerning the number, relative amounts and stoichiometry of non-covalent complexes present.[38,39] Here we have shown that ESI-MS can also provide a comprehensive analysis of interactions involving ruthenium complexes and quadruplex DNA, or of platinum(II) complexes and either duplex or quadruplex DNA. However, results obtained with complexes containing other metal ions indicate that ESI-MS may be suitable for analysis of solutions containing only the thermodynamically most stable metal ions. For example, mass spectra of solutions containing nickel(II) complexes and qDNA contained significant quantities of ions consisting of fragments of the original nickel molecules bound to DNA. In contrast, mass spectra of solutions containing the same nickel(II) complexes and duplex DNA did not show evidence of fragmentation.[41] These observations may be explained by noting the less severe instrumental conditions required to obtain spectra of dsDNA, and by proposing that the thermodynamic stability of the nickel(II) complexes is just sufficient to withstand these conditions, but not those required to obtain spectra of solutions containing qDNA. Mass spectra of solutions containing zinc(II) or iron(II) complexes and dsDNA provided evidence that considerable fragmentation of the non-covalent complexes that would have formed occurred, suggesting that complexes of these two metal ions were the thermodynamically least stable of those investigated.

The mass spectral evidence presented here suggests that the ruthenium(II) complexes examined have similar binding affinities towards qDNA. This conclusion is supported by the results of an investigation into the effect of adding ruthenium complexes on the CD spectrum of Q1, and contrasts sharply with the widely differing and, in some cases much greater, binding affinities displayed by these complexes towards D2 found previously.[38,39] These results may be rationalised by noting that the secondary and tertiary structures of qDNA may not afford the same opportunities for intercalative interactions that dominate the non-covalent DNA binding interactions of octahedral metal complexes containing one strongly intercalating ligand such as dppz. This then hints at the possibility of different binding modes for ruthenium complexes with qDNA, as has been suggested previously for several other classes of DNA binding agents.[3,21–24]

ESI mass spectral studies of solutions containing platinum(II) complexes and dsDNA showed that the binding affinities of these complexes were just as, if not greater than, those of the ruthenium(II) complexes studied previously. This suggests that any potential decrease in overall binding affinity caused by the absence of a strongly intercalating ligand such as dppz amongst the platinum(II) complexes is more than offset by their square planar geometry. The latter enables efficient insertion of both unsubstituted phenanthroline and substituted phenanthroline ligands into the DNA base stack, without steric complications that are created by the ancillary ligands present in the coordination sphere of octahedral metal ions. Studies involving qDNA pointed to a lower overall binding affinity of the platinum(II) complexes compared to when duplex DNA is present. This is again consistent with the idea that metal complexes do not interact with qDNA *via* a standard intercalative binding mode. The introduction of methyl groups onto the phenanthroline ligand resulted in enhanced binding of the platinum(II) complexes with both dsDNA and qDNA, a fact that may prove useful when designing new anticancer drugs to target the telomeric regions of chromosomes.

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