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Probing DNA selectivity of ruthenium metallointercalators using ESI mass spectrometry†

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ESI mass spectra show that up to five ruthenium molecules can bind non-covalently to double stranded 16mer DNA, and provide information on the relative affinity and DNA sequence selectivity of different ruthenium complexes.

Metal compounds that bind non-covalently to DNA have attracted considerable interest.[1] This has been driven partly by a desire to understand these interactions, and partly by studies that have shown these compounds may be useful as nucleic acid probes,[2,3] synthetic restriction enzymes[1,4] or DNA repair agents.[1,5] In this paper we present recent results obtained using Electrospray Ionisation Mass Spectrometry (ESI-MS) to characterise non-covalent complexes formed between duplex 16mer oligonucleotides and racemic mixtures of the ruthenium compounds shown in Fig. 1.‡ ESI-MS provided information on the number, relative amounts, and metal/DNA stoichiometry of individual complexes. In addition ESI-MS was also used to determine the relative affinities of the ruthenium compounds for DNA, and obtain information about their DNA sequence selectivity. Although ESI-MS has been used widely to study non-covalent interactions between biomolecules,[6,7] or between biomolecules and small organic molecules,[7–10] there have been relatively few studies involving DNA and metal compounds.[11]

Each of the ruthenium compounds in Fig. 1 has similar charge, size and shape, and would therefore be expected to show a similar degree of electrostatic attraction towards the anionic phosphodiester backbone of DNA. Consequently any significant differences in DNA binding affinity between the ruthenium compounds must be a result of variations in their ability to act as minor groove binders or intercalators. The ability of these compounds to act as intercalators varies significantly as a consequence of the replacement of one phenanthroline ligand in $[Ru(phen)_3]^{2+}$ by larger ligands that can more readily insert into the DNA base stack. Three different non-self-complementary 16mer DNA duplexes, labelled D1, D2 and D3, with different numbers of GC and AT base pairs, were chosen for examination.§ The greater GC content of D1 and D2 was expected to enhance binding by ruthenium compounds that can intercalate,[12] while D3 contains an AT base sequence favourable to compounds that prefer to bind along the DNA minor groove.[13]

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

Fig. 2(a)–(d) shows ESI mass spectra of reaction mixtures containing different ratios of $[Ru(phen)_2(dppz)]^{2+}$ and D2.¶ When there was no metal in solution, the mass spectrum (Fig. 2(a)) contained ions at *m*/*z* 1626.5 and 1952.1, assigned to $[D2-6H]^{6-}$ and $[D2-5H]^{5-}$, respectively. When the ratio metal : D2 was 1.5 : 1 the ESI mass spectrum (Fig. 2(b)) showed ions of medium or high abundance attributable to unbound D2 and a complex in which one $[Ru(phen)_2(dppz)]^{2+}$ was bound to D2. Fig. 2(b) also shows ions of weak to medium abundance attributable to complexes containing two and three $[Ru(phen)_2(dppz)]^{2+}$ bound to D2. As the ratio metal : D2 was

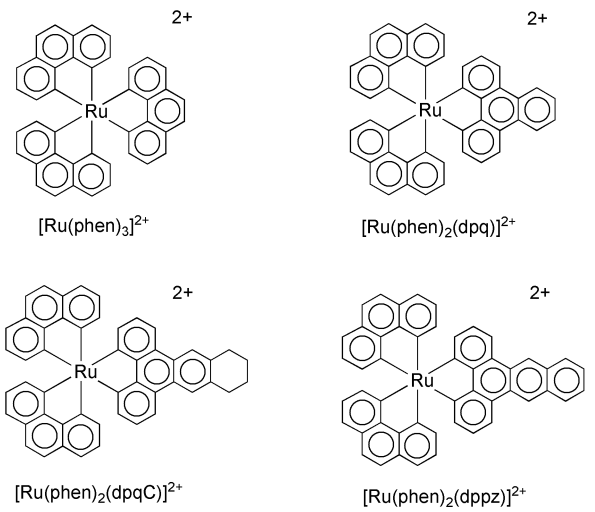

Fig. 1 Structures of the ruthenium compounds used.

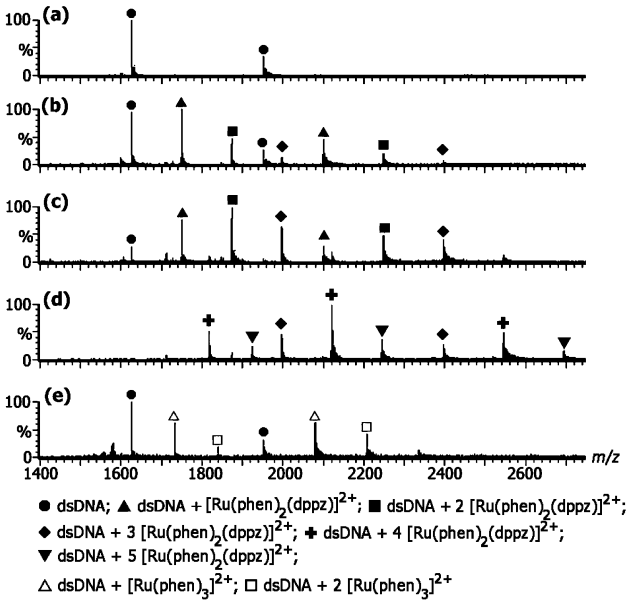

Fig. 2 Negative ion ESI mass spectra of reaction mixtures containing D2 and either $[Ru(phen)_2(dppz)]^{2+}$ or $[Ru(phen)_3]^{2+}$. (a) free D2. (b) $[Ru(phen)_2(dppz)]^{2+}$:D2 = 1.5 : 1. (c) $[Ru(phen)_2(dppz)]^{2+}$:D2 = 3 : 1. (d) $[Ru(phen)_2(dppz)]^{2+}$:D2 = 6 : 1. (e) $[Ru(phen)_3]^{2+}$:D2 = 20 : 1.

† Electronic supplementary information (ESI) available: table of mass spectral assignments. See http://www.rsc.org/suppdata/cc/b2/b212132h/

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increased the abundance of these ions also increased. When the ratio metal : D2 was 6 : 1 (maximum ratio permitted by the solubility of $[Ru(phen)_2(dppz)](PF_6)_2$) the most abundant ion in the spectrum (Fig. 2(d)) was from a complex containing four $[Ru(phen)_2(dppz)]^{2+}$ bound to D2. Also present in this spectrum were ions attributable to complexes containing three and five $[Ru(phen)_2(dppz)]^{2+}$ bound to D2.

Fig. 2(e) shows the spectrum of a solution containing a 20 : 1 ratio of $[Ru(phen)_3]^{2+}$ and D2. Despite the high ratio, the most abundant ion in the spectrum was assigned to free D2. The spectrum also contained ions of medium to high abundance from a complex containing one $[Ru(phen)_3]^{2+}$ bound to D2, and ions of low abundance from a complex containing two $[Ru(phen)_3]^{2+}$ bound to D2. These results are consistent with those obtained using other techniques, that showed that ruthenium(II) compounds containing the dppz ligand have the highest affinity for DNA of compounds of this type.[14] For example, the binding constants for association of Δ and Λ-$[Ru(phen)_2(dppz)]^{2+}$ with calf thymus DNA are 3.2×10^6 and 1.7×10^6 M^{-1}, respectively.[15] These values are significantly greater than those for binding of Δ and Λ-$[Ru(phen)_3]^{2+}$ to calf thymus DNA, which are 9×10^3 and 1.1×10^4 M^{-1}, respectively.[15] Further information concerning the relative affinities of all the compounds shown in Fig. 1 for D2 is presented in Fig. 3. This was constructed using relative abundances from spectra of reaction mixtures containing a 6 : 1 ratio of a single ruthenium compound and D2. Relative abundances were obtained by dividing the combined ion current for 5-, 6- and 7- ions assigned to a specific DNA complex, by the total ion current for all 5-, 6- and 7- ions.

Fig. 3 shows that $[Ru(phen)_2(dppz)]^{2+}$ and $[Ru(phen)_2(dpqC)]^{2+}$ formed the highest percentage of DNA complexes containing 4 and 5 molecules of ruthenium compound. This suggests that these complexes had the greatest affinity for D2. Comparison of the data for the other metal compounds shows that $[Ru(phen)_2(dpq)]^{2+}$ had the next highest binding affinity, and $[Ru(phen)_3]^{2+}$ the lowest affinity towards D2. By plotting relative abundances from reaction mixtures containing the same ruthenium compound, but different oligonucleotides, it was also possible to obtain information about DNA sequence selectivity. For example, Fig. 4 shows that $[Ru(phen)_2(dpq)]^{2+}$ formed a higher percentage of complexes containing 2, 3 or 4 molecules bound to DNA in experiments with D2, compared to experiments with the other two duplexes. This shows that this ruthenium compound displayed a preference for binding to DNA sequences in D2. Previous NMR studies showed that Δ-$[Ru(phen)_2(dppz)]^{2+}$ intercalates into DNA from the minor groove, particularly at purine–purine/pyrimidine–pyrimidine sequences.[16] One of the principal mechanisms of binding of this compound to D2 may therefore involve intercalation into the GGCC/GGCC sequence in the middle of the duplex. Intercalation was also expected to be favourable with D1 owing to its high GC content. The lower degree of interaction observed may be a result of closer proximity of binding sites in this duplex. Groove binding interactions between DNA and $[Ru(phen)_2(dpq)]^{2+}$ may also

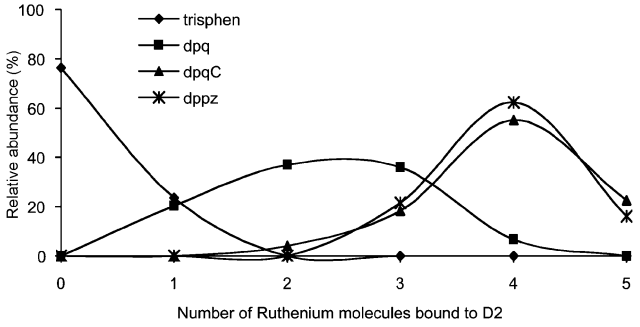

Fig. 3 Relative abundances of complexes in reaction mixtures containing a 6 : 1 ratio of ruthenium compound and duplex D2.

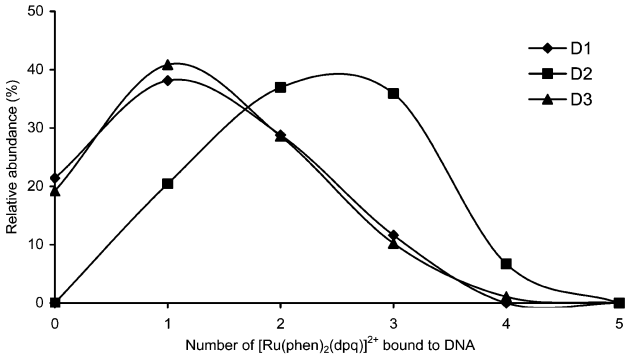

Fig. 4 Relative abundance of complexes in reaction mixtures containing a 6 : 1 ratio of $[Ru(phen)_2(dpq)]^{2+}$ and oligonucleotides.

occur, however the lower level of reactivity towards the AT rich duplex D3 suggests that these are less important than intercalation. We are currently investigating reactions with longer oligonucleotides, and competition reactions involving organic DNA binding agents, in order to learn more about the DNA selectivity of these ruthenium compounds.

Notes and references

‡ $[Ru(phen)_3]Cl_2$ was obtained from the Aldrich Chemical Co. $[Ru(phen)_2(dpq)]Cl_2$, $[Ru(phen)_2(dpqC)]Cl_2$ and $[Ru(phen)_2(dppz)]Cl_2$ were prepared using literature methods.[16,17]

§ Oligonucleotides were obtained from Geneworks, South Australia, as the 'trityl-on' derivatives. These were deprotected by acid treatment and purified by HPLC as previously described.[18]

¶ Reaction mixtures were prepared by first annealing complementary strands of DNA (1 mM), and then treating the resulting dsDNA with an appropriate amount of stock solution containing the desired ruthenium compound at 20 °C. The final concentration of dsDNA in reaction mixtures was 25 μM. Samples were diluted to 10 μM prior to analysis by ESI-MS. Negative ion ESI mass spectra were obtained using a Micromass Qtof2 mass spectrometer (cone voltage 50 V; desolvation temperature 80 °C). The solvent used for annealing, complex formation and obtaining ESI mass spectra was 0.1 M ammonium acetate, pH 8.5.

1 K. E. Erkkila, D. T. Odom and J. K. Barton, *Chem. Rev.*, 1999, **99**, 2777.
2 C. S. Chow and J. K. Barton, *J. Am. Chem. Soc.*, 1990, **112**, 2839.
3 A. Sitlani, C. M. Dupureur and J. K. Barton, *J. Am. Chem. Soc.*, 1993, **115**, 12589.
4 M. P. Fitzsimons and J. K. Barton, *J. Am. Chem. Soc.*, 1997, **119**, 3379.
5 P. J. Dandliker, R. E. Holmlin and J. K. Barton, *Science*, 1997, **275**, 1465.
6 J. Loo, *Mass Spectrom. Rev.*, 1997, **16**, 1.
7 J. L. Beck, M. L. Colgrave, S. F. Ralph and M. M. Sheil, *Mass Spectrom. Rev.*, 2001, **20**, 61.
8 Q. Gao, X. Cheng, R. D. Smith, C. F. Yang and I. H. Goldberg, *J. Mass Spectrom.*, 1996, **31**, 31.
9 A. Triolo, F. M. Arcamone, A. Raffaelli and P. Salvadori, *J. Mass Spectrom.*, 1997, **32**, 1186.
10 A. Kapur, J. L. Beck and M. M. Sheil, *Rapid Commun. Mass Spectrom.*, 1999, **13**, 2489.
11 K. X. Wan, T. Shibue and M. L. Gross, *J. Am. Chem. Soc.*, 2000, **122**, 300.
12 B. J. Geierstanger and D. E. Wemmer, *Annu. Rev. Biophys. Biomol. Struct.*, 1995, **24**, 463.
13 A. Pullman and B. Pullman, *Q. Rev. Biophys.*, 1981, **14**, 289.
14 C. M. Dupureur and J. K. Barton, *J. Am. Chem. Soc.*, 1994, **116**, 10286.
15 I. Haq, P. Lincoln, D. Suh, B. Norden, B. Z. Chowdhry and J. B. Chaires, *J. Am. Chem. Soc.*, 1995, **117**, 4788.
16 J. G. Collins, A. D. Sleeman, J. R. Aldrich-Wright, I. Greguric and T. W. Hambley, *Inorg. Chem.*, 1998, **37**, 3133.
17 C. M. Dupureur and J. K. Barton, *Inorg. Chem.*, 1997, **36**, 33.
18 G. Wickham, P. Iannitti, J. Boschenok and M. M. Sheil, *J. Mass Spectrom.*, 1995, **30**, S197.