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A spectroscopic investigation of the self-association and DNA binding properties of a series of ternary ruthenium(II) complexes

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Abstract

Six ruthenium(II) complexes of the general form *cis*-α-[Ru(*N_4-tetradentate*)(*N_2-bidentate*)]Cl_2 have been synthesized from the two related tetradentate ligands 1,6-di(2′-pyridyl)-2,5-dimethyl-2,5-diazahexane (*picenMe_2*) and 1,6-di(2′-pyridyl)-2,5-dibenzyl-2,5-diazahexane (*picenBz_2*) and the bidentate ligands 2,2′-bipyridine (*bipy*), 1,10-phenanthroline (*phen*) and dipyrido[3,2-f:2′3′-h]quinoxaline (*dpq*). Synthetic intermediate species of the general form *cis*-α-[Ru^{II}(*N_4-tetradentate*)(DMSO)Cl][PF_6] were isolated. The N_4-tetradentate ligand picenMe_2 formed only the *cis*-α stereoisomer, while *picenBz_2* formed both the *cis*-α and *cis*-β stereoisomers. These latter stereoisomers were resolved by fractional crystallisation. Dimer self-association constants, K_D, were estimated from the concentration dependence of the ^{1}H NMR shifts for some of these complexes in aqueous solutions at 25 °C. The values of K_D ranged from 0.6 to 7.9 M^{-1} and a relationship was observed between the aromatic surface area of the bidentate component and the degree to which self-association occurred, whereby a greater level of self-association correlates with a larger surface area for the bidentate ligand. Some of these complexes demonstrate an ability to bind to DNA that is consistent with intercalation of the bidentate molecular component between the base pairs of the DNA molecule. Using calf-thymus DNA, the equilibrium binding constants, K_B, were determined for some of the complexes using intrinsic methods and these ranged from 3.32 to 5.11 M^{-1}, the intercalating abilities of the different bidentate ligands being in the order *dpq* > *phen* > *bipy*. This relationship between aromatic surface area of the bidentate ligand and the degree of DNA binding activity is the same as that observed in the self-association study.

Keywords: Ruthenium complex; π–π interactions; DNA binding

1. Introduction

The three dimensional structure of DNA is strongly influenced by the vertical non-covalent π–π stacking interactions that exist between the nucleobase pairs of the DNA molecule [1]. Such π–π stacking interactions are also important in the packing of aromatic molecules in crystals [2,3] and their aggregation in solution [3–5]. Such interactions are involved in the intercalation into DNA of organic [6,7] and inorganic [8–14] molecules that posses a planar aromatic fragment.

Several studies have shown that there is a correlation between the ability of a compound to bind to DNA via an intercalative mechanism and its propensity to self-associate in solution via π–π interactions. The planar aromatic drugs proflavine and acridine orange, which are well known to be strong DNA intercalators, [6] self-associate in aqueous solutions via stacking interactions [7]. Stacking interactions in solution also occurs for inorganic complexes possessing a planar aromatic

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component such as for the square planar Pt(II) complex $[Pt(phen)(en)]^{2+}$ [15] and other related Pt(II) complexes [16]. The ternary Ru(II) complex $[Ru(phen)_3]^{2+}$ self-associates by a stacking interaction in aqueous solution, [17] but the mode by which this complex binds to DNA (either intercalative or electrostatic groove binding) is the subject of some debate [18–22].

For some time, we have been investigating the capacities of ternary cations of the general form $[Ru(N_4\text{-}tetradentate)(N_2\text{-}bidentate)]^{2+}$ to function as stereo- and enantio-discriminatory intercalating probes of DNA structures [9,10,23–26]. In these complexes, the tetradentate is chosen to have characteristics that allow it to govern its DNA binding via helical DNA groove interactions, thereby providing a molecular recognition function. The metal ion provides a substitutionally inert octahedral geometry and the bidentate serves as the intercalating chromophore [27]. We have shown using ^{1}H NMR spectroscopy that some complexes of the general form $[Ru(N_4\text{-}tetradentate)(N_2\text{-}bidentate)]^{2+}$ self-associate in aqueous solution in a manner consistent with the intermolecular π-stacking of the bidentate ligands [10,24,28]. Here, we report the synthesis, self-association and DNA binding properties of six Ru(II) complexes formed from combinations of the tetradentate ligands 1,6-di(2′-pyridyl)-2,5-dimethyl-2,5-diazahexane (*PicenMe*$_2$), 1,6-di(2′-pyridyl)-2,5-dibenzyl-2,5-diazahexane (*PicenBz*$_2$) and the bidentate ligands 2,2′-bipyridine (*bipy*), 1,10-phenanthroline (*phen*) and dipyrido[3,2-f:2′3′-h]quinoxaline (*dpq*) (structures in Fig. 1).

2. Experimental

2.1. Materials

Reagent grade chemicals for the synthesis of the Ru(II) complexes and calf-thymus DNA were obtained from the Sigma–Aldrich chemical company and were used without further purification.

2.2. Instrumentation

^{1}H nuclear magnetic resonance spectra were recorded at 400 MHz from D_2O solutions in 5 mm tubes using a Varian XL-400 spectrometer operating at 25 ± 0.2 °C. The chemical shifts were recorded in parts per million (ppm) relative to the central peak of the tetramethylammonium ion (TMA^+) triplet, taken as 3.1855 ppm [29]. TMA was used as an internal reference rather than (trimethylsilyl)propanesulfonate (TSP) as the later has been found to be unreliable in the presence of species with aromatic moieties [29]. Electronic absorbance spectra were recorded on a Varian Cary-1 Bio spectrophotometer.

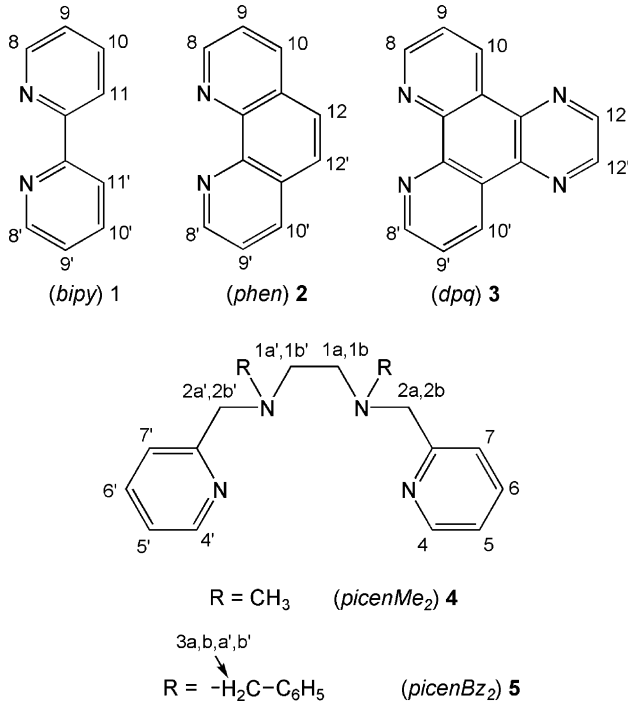

Fig. 1. The structures and proton numbering schemes for the bidentate ligands, **1** 2,2′-bipyridine (*bipy*), **2** 1,10-phenanthroline (*phen*) and **3** dipyrido[3,2-*f*:2′3′-h]quinoxaline (*dpq*) and the tetradentate ligands, **4** 1,6-di(2′-pyridyl)-2,5-dimethyl-2,5-diazahexane (*picenMe*$_2$) and **5** 1,6-di(2′-pyridyl)-2,5-dibenzyl-2,5-diazahexane (*picenBz*$_2$). The same numbering scheme used for **1**–**5** was also used for the C_2 symmetric *cis*-α-$[Ru^{II}(N_4\text{-}tetradentate)(N_2\text{-}bidentate)]^{2+}$ combinations of these ligands.

2.3. ^{1}H NMR studies of the self-association of ruthenium(II) complexes

The concentration dependence of the chemical shifts of the complexes studied were examined in D_2O at 25 ± 0.2 °C. The ^{1}H NMR spectra were recorded from freshly prepared solutions of the complexes at concentrations of 1, 3, 5, 10, 15, 20, 25, 35, 45 and 55 mM in D_2O containing the internal reference and NaCl (100 mM).

Assignments of the ^{1}H resonances for the complexes were made with the aid of previously published results [9,30] and COSY NMR experiments where necessary. All NMR data recorded for the concentration dependence studies were processed on an IBM compatible personal computer using the NMR Utility Transform Software (NUTS) package [31].

2.4. Studies of the binding of Ru(II) complexes to DNA

Spectroscopic titrations were carried out to determine the relative intrinsic binding constants, K_B, between the individual complexes and calf-thymus DNA. The concentration, expressed as base pairs, of a cacodylate (16 mM) buffered solution of calf-thymus DNA was