Interaction of a Luminescent Platinum(II) Complex of Substituted 2,2′-Bipyridine with DNA. Spectroscopic and Photophysical Studies

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Intercalation of the $[Pt^{II}(5,5'\text{-}Me_2bpy)(4\text{-}ampy)_2]^{2+}$ (5,5′-Me_2bpy = 5,5′-dimethyl-2,2′-bipyridine, 4-ampy = 4-aminopyridine) complex into calf-thymus DNA is accompanied by an observed hypochromicity in the UV–VIS spectral changes and an increase in both the intensity and lifetime of the emission of the platinum complex.

The design of specific probes for nucleic acid structure through binding studies of organic and inorganic molecules with deoxyribonucleic acid (DNA) has been an area of considerable interest.[1,2] When a small molecule binds to DNA, there could exist a series of modes of weak interactions, such as the π-stacking interaction associated with the intercalated small molecule and the base pairs, and H-bonding and van der Waals interactions of the externally bound complex with DNA in a groove of the DNA duplex.[3] Studies on such binding interactions may be valuable for probing DNA structure. In this context, we are studying square-planar platinum(II) complexes containing π-aromatic diimine ligands. These classes of complexes are well-known metallointercalators for DNA.[4] Furthermore they usually have long-lived emissions in solution,[5,6] suggesting that the photoluminescence can be used as a spectroscopic probe for DNA binding studies. Herein we report that the interaction between a water-soluble Pt^{II} complex and DNA can be probed by both absorption and emission spectroscopy.

The $[Pt^{II}(5,5'\text{-}Me_2bpy)(4\text{-}ampy)_2](CF_3SO_3)_2$, (5,5′-$Me_2$bpy = 5,5′-dimethyl-2,2′-bipyridine, 4-ampy = 4-aminopyridine), is prepared as follows: a yellow suspension of $[Pt^{II}(5,5'\text{-}Me_2bpy)Cl_2]$[7] and $AgCF_3SO_3$ in acetonitrile is heated under reflux with stirring for 4 h, and the resulting solution filtered through celite to remove insoluble AgCl. To the yellow filtrate an excess of 4-ampy is added, and the mixture heated under reflux for 1 h. The pale yellow product is obtained by precipitation and recrystallised by diffusion of diethyl ether into an acetonitrile solution. The structure of the product has been determined by an X-ray crystal analysis.† A perspective drawing of the complex cation is shown in Fig. 1. The UV–VIS absorption spectrum of the complex in water displays three absorption peaks at λ/nm (ε_{max}): 325 (1.85×10^4), 261 (6.27×10^4) and 201 (5.41×10^4). Photoexcitation of the complex (2.44×10^{-5} mol dm^{-3}) at 325 nm in degassed water gave a vibronic structural emission with λ_{max} at 506, 471 and 533 nm and with a lifetime of 5.3 μs. With reference to previous studies,[5] the emission is likely to be MLCT [Pt → π* (bpy)] in nature.

Calf-thymus (ct) DNA (Sigma Chemical Co) was purified by the literature method.[8] The purity of the DNA after ethanol precipitation was checked by the ratio of the absorbances at 260 and 280 nm ($A_{260}:A_{280}$ = 1.9:1). Its concentration was determined spectrometrically by using an extinction coefficient of 6600 dm^3 mol^{-1} cm^{-1} at 260 nm.[2] All experiments were conducted in an aerated Tris buffer (5 mmol dm^{-3} Tris, 50 mmol dm^{-3} NaCl, pH 7.2).

An absorption titration was performed, in which the platinum complex concentration was kept constant. As shown in Fig. 2, increasing the DNA concentration led to a corresponding decrease of the 325 nm band until a constant absorbance value was reached. The inset in Fig. 2 is a plot of A_0/A *vs.* [DNA]/[complex]. The hypochromicity at 325 nm was obtained as 40% in our experiment. In addition, a small red shift of λ_{max} (Δλ = 2 nm) and two isosbestic points at 304 and 342 nm were observed in the spectral changes. Such findings are consistent with the intercalation mode of binding, involving a strong π-stacking interaction between the Pt^{II}–5,5′-Me_2bpy moiety and the DNA base pairs. This is possible because the 5,5′-Me_2bpy ligand is planar, according to the crystal analysis of the complex. The two 4-ampy ligands could not intercalate into ct-DNA because of the noncoplanarity of the pyridine rings with

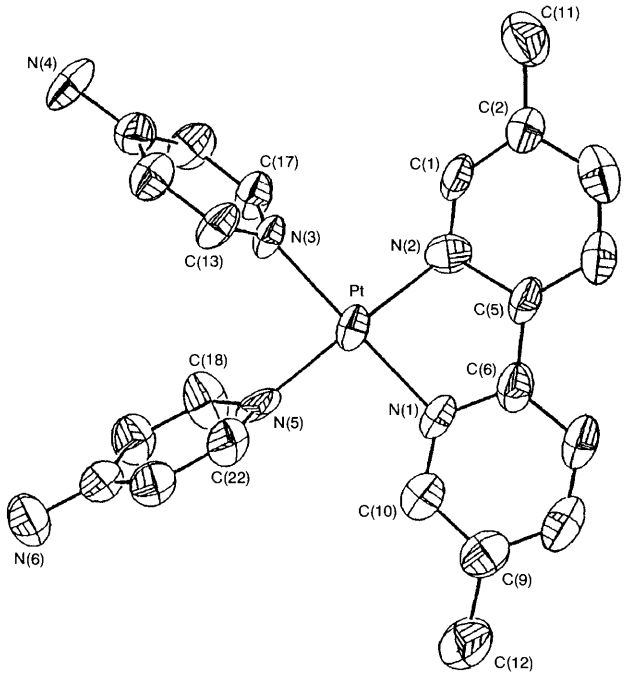

Fig. 1 Perspective drawing of $[Pt^{II}(5,5'\text{-}Me_2bpy)(4\text{-}ampy)_2](CF_3SO_3)_2$. Selected bond lengths (Å) and angles (°): Pt–N(1) 2.032(10), Pt–N(2) 1.980(10), Pt–N(3) 2.031(4), Pt–N(5) 1.990(10), N(1)–Pt–N(2) 83.4(4), N(1)–Pt–N(3) 178.5(4), N(3)–Pt–N(5) 86.7(4), N(2)–Pt–N(3) 95.2(4), N(1)–Pt–N(5) 94.8(4), Pt–N(2)–C(5) 111.5(8), N(2)–C(5)–C(6) 114.3(11).

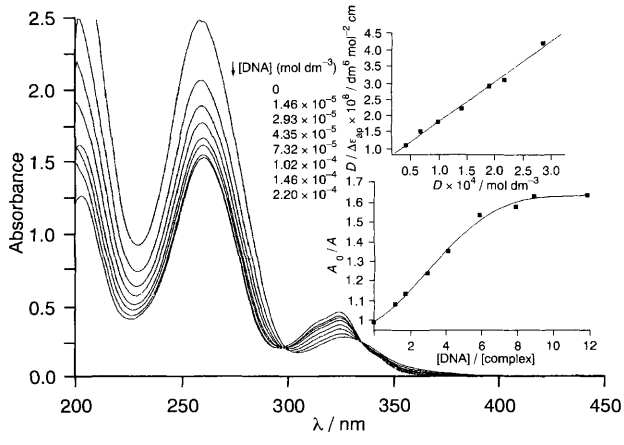

Fig. 2 UV–VIS spectra of $Pt^{II}(5,5'\text{-}Me_2bpy)(4\text{-}ampy)_2(CF_3SO_3)_2$ (2.44×10^{-5} mol dm^{-3}) in Tris buffer with increasing concentration of ct-DNA. Inset: plot of A_0/A *vs.* [DNA]/[complex] and plot of $D/\Delta\varepsilon_{ap}$ *vs.* D. Absorbance was monitored at 325 nm.

the Pt^{II}–5,5′-Me_2bpy plane. The intrinsic binding constant, K, of the platinum complex with ct-DNA was obtained from a plot of $D/\Delta\varepsilon_{ap}$ *vs.* D, according to eqn. (1),[2] where D is the

$$D/\Delta\varepsilon_{ap} = D/\Delta\varepsilon + 1/(\Delta\varepsilon \times K) \quad (1)$$

concentration of DNA, $\Delta\varepsilon_{ap} = |\varepsilon_a - \varepsilon_F|$ and $\Delta\varepsilon = |\varepsilon_b - \varepsilon_F|$,[1] $\varepsilon_a = A_{obs}/[complex]$, and ε_b and ε_F are the respective extinction coefficients of the complex in the presence and absence, respectively, of ct-DNA at 325 nm. As shown in Fig. 2, the plot is linear, and the $\Delta\varepsilon$ and K values are 8.7×10^3 $dm^3\ mol^{-1}\ cm^{-1}$ and $1.8 \times 10^4\ dm^3\ mol^{-1}$, respectively. Thus, ε_b is estimated to be $1.0 \times 10^4\ dm^3\ mol^{-1}\ cm^{-1}$. This value of ε_b is in excellent agreement with the value of $\varepsilon_b^{exp} = 1.1 \times 10^4\ dm^3\ mol^{-1}\ cm^{-1}$ determined from the absorbance of the solution at a [DNA] : [complex] ratio of 12 : 1.

An emission titration was also performed. Enhancement of the emission of the platinum complex with addition of ct-DNA was observed (Fig. 3). The emission intensity I, however, reaches saturation at a [DNA] : [complex] ratio of 9.02 : 1 (inset of Fig. 3). The maximum I/I_0 value is 9.3 (I_0 = intensity in the absence of ct-DNA). The data of the emission titration were also analysed with the McGhee–von Hippel equation [eqn. (2)],[2] where $r = (C_T - C_F)/[DNA]$, $C_F = C_T\ (I/I_0 - P)(1 - P)$, C_T is the total concentration of the complex, C_F is the

$$r/C_F = K_f(1 - nr)\{(1 - nr)/[1 - (n - 1)r]\}^{n-1} \quad (2)$$

concentration of the free complex, n is the binding site size in base pairs, K_f is the intrinsic binding constant and P is given by the y-intercept of the plot of I/I_0 *versus* 1/[DNA]. Fitting the experimental data gives $K_f = 1.7 \times 10^4\ dm^3\ mol^{-1}\ cm^{-1}$ and $n = 1.6$. The intrinsic binding constant K_f (1.7×10^4) obtained by this method is very similar to the value obtained from the absorption data (1.8×10^4). The n value could be estimated to about two and is close to the values reported for intercalators such as tetrakis(4-methylpyridyl)porphyrin and its copper complex.[9] This indicates that about two lattice residue sites are made inaccessible by the binding of a single Pt^{II}-(5,5′-Me_2bpy)(4-ampy)$_2^{2+}$ complex to ct-DNA. Binding of the complex to ct-DNA is also accompanied by an increase in the emission lifetime. Increasing the [DNA] : [complex] ratio from 0 to 11 : 1 leads to an increase in emission lifetime ([DNA]/[complex], τ/μs: 0, 0.62; 0.55, 4.3; 1.1, 4.4; 2.1, 4.7; 5.5, 5.2; 8.2, 5.8; 11, 5.9; 13, 5.9). Beyond the [DNA] : [complex] ratio of 11 : 1, little increase in lifetime was observed. The value of 5.9 μs is close to that of the lifetime of the platinum complex in the absence of ct-DNA and in degassed pure water (5.3 μs). As a matter of fact, the emission intensities of the platinum complex in the absence of ct-DNA in degassed pure water and the fully-bound complex by DNA in Tris buffer are also similar. Thus, as in Barton's previous works,[10,11] the enhancement in emission intensity and the increased lifetime may be attributed to the environmental changes and the decreased mobility of the Pt^{II}-5,5′-Me_2bpy moiety when it is intercalated into the base pairs of ct-DNA. The coordinated bipyridyl ring is protected from the buffer aqueous solution, leading to a decrease in the non-radiative decay rate constant of the excited state.

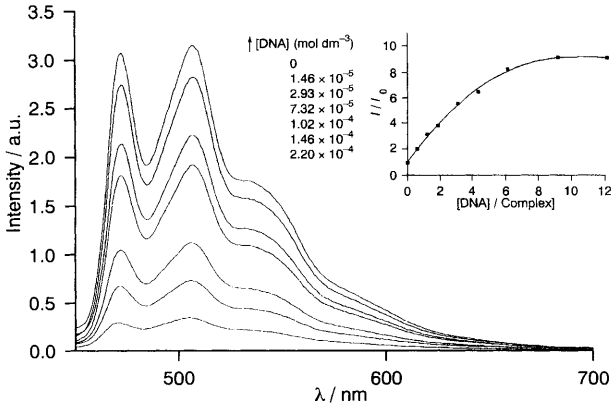

Fig. 3 Emission spectra of $Pt^{II}(Me_2bpy)(4\text{-ampy})_2(CF_3SO_3)_2$ (2.44×10^{-5} mol dm^{-3}) in Tris buffer with increasing concentration of ct-DNA, upon excitation at 325 nm. Inset: plot of the emission titration data, with excitation at 325 nm. Emission was monitored at 506 nm.

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† *Crystal data*: $[Pt^{II}(5,5'\text{-}Me_2bpy)(4\text{-ampy})_2](CF_3SO_3)_2{\cdot}C_2H_5OH$, $C_{26}H_{29}N_6F_6O_7S_2Pt$, M = 910.74, monoclinic, $P2_1/c$, a = 12.316(2), b = 9.9017(8), c = 28.268(5) Å, β = 101.62(1)°, V = 3376.8(8) Å^3, Z = 4, D_{calc} = 1.791 g cm^{-3}, μ(Cu-Kα) = 96.98 cm^{-1}, $F(000)$ = 1788, crystal dimensions 0.30 × 0.35 × 0.35 mm, no. of variables 434, no. of unique data measured 4234, no. of observed data with $I > 2\sigma(I)$ 3660, R = 0.060, R_w = 0.059, GOF = 5.4, weighting scheme $\omega^{-1} = \sigma^2(F)$. The residual extrema in the final difference map were 2.39 and −3.64 e Å^{-3}. Intensity data were collected on an Enraf-Nonius CAD 4 diffractometer with monochromated Cu-Kα radiation (1.5406 Å) at room temperature (298 K). The structure was solved by the Patterson method and refined by least squares analysis. All computations were performed using the NRCVAX program. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

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