Relationship between binding affinity for calf-thymus DNA of $[Pt(2,2'\text{-bpy})(n\text{-Rpy})_2]^{2+}$ (n = 2,4) and basicity of coordinated pyridine

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The binding affinity for DNA of the complexes $[Pt(2,2'\text{-bpy})(n\text{-Rpy})_2]^{2+}$ (n = 2,4) systematically increases on increasing pK_a of the coordinated pyridine, with the exception of the 2-methyl-substituted derivative.

The interations between DNA and drugs[1] depend on properties such as charge, and electronic and steric characteristics of the interacting small molecule. A convenient way to study the influence of such properties on binding affinity for DNA is to use mixed-ligand metal complexes; for these substances it is possible to systematically vary one or more properties of the moiety involved in the interaction, without altering its chemical integrity, by simply changing the nature of the ancillary ligands. Here, we study the platinum(II) complexes $[Pt(bpy)(4\text{-}Rpy)_2][PF_6]_2$ and $[Pt(bpy)(2\text{-}Mepy)_2][PF_6]_2$ (bpy = 2,2'-bipyridyl, py = pyridine; R = CN, Cl, H, Ph, Me, NH_2) which differ only in the nature or position of substituents at the pyridines.

The complexes were prepared by heating under reflux an aqueous suspension of $[Pt(bpy)Cl_2]$ with an excess of the appropriate pyridine. After dissolution of the solid, NH_4PF_6 was added and the resulting complexes $[Pt(bpy)(Rpy)_2][PF_6]_2$ precipitated as yellow substances, which were recrystallized from water–acetone. Calf-thymus DNA (Sigma) was purified as previously described.[2]

When $[Pt(bpy)(py)_2]^{2+}$ is titrated with DNA (Fig. 1) the absorption maxima of the complex exhibits extensive hypochromism and a significant red shift. All the other complexes exhibit almost identical behaviour, usually indicative of intercalative binding.

Support for this type of interaction comes from viscometry and thermal denaturation experiments. Thus the average increase in melting temperature of DNA, as a result of interaction with the complexes (ΔT = 10 °C), is larger than for ethidum[3] (ΔT = 8.6 °C) and comparable with that relative to the known complex intercalator[4a,b] $[Pt(bpy)(en)]^{2+}$ (ΔT = 10.4 °C), under the same experimental conditions (1.0×10^{-3} mol dm^{-3} phosphate buffer–20.0×10^{-3} mol dm^{-3} NaCl). Increase in viscosity of rod-like DNA (600 base pairs), attributable to helix extension caused by intercalation, is observed upon interaction with all of the complexes studied. Fig. 2 compares the increase in viscosity for $[Pt(bpy)(py)_2]^{2+}$ and $[Pt(bpy)(en)]^{2+}$.

The experimental findings suggest partial intercalation of the complexes[5a,b] through stacking of the bipyridyl moieties, while the coordinated pyridine rings, oriented out of plane,[5b,6] do not insert into the base pairs.

Binding constants, K_B, were determined by analysing the absorption titration data with the McGhee–von Hippel[7] equation [eqn. (1)].

$$r/m = K(1 - nr)\{[(2\omega - 1)(1 - nr) + r - R]/[2(\omega - 1)(1 - nr)]\}^{n-1}\{[1 - (n - 1)r + R]/[2(1 - nr)]\}^2 \quad (1)$$

where $r = [\text{complex}]_{bound}/[DNA]_{tot}$, m = concentration of unbound complex, K_B = binding constant, $R = \{[1 - (n+1)r]^2 + 4\omega r(1 - nr)\}^{1/2}$, n = the number of consecutive sites made inaccessible to another ligand by binding of one ligand, ω = cooperativity parameter characterizing the interaction between immediately neighbouring bound ligands. Fitting the experimental data gives ω values always close to unity so that the process is non-cooperative. The number of excluded sites is *ca.* two for all the complexes.

The binding constant values, K_B, for interaction with DNA, vary *ca.* hundredfold along the series of complexes examined. The stability of the interaction products increase with increasing pK_a of the coordinated pyridines and a good linear relationship is obtained on plotting $\log K_B$ for the various complexes and the pK_a[8a,b] for the corresponding pyridines (Fig. 3). This relationship shows unambiguously that the binding affinity of the various intercalators is directly related to the charge density of the bipyridyl. On increasing the electron donor power of the substituents, the pyridine basicity increases as does the amount

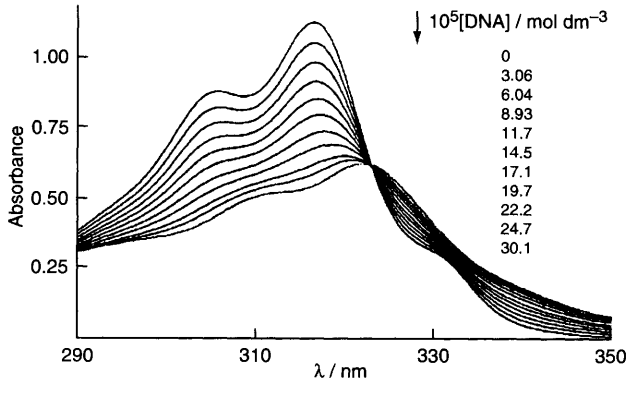

Fig. 1 Spectrophotometric titration (1 cm cell) of 6.76×10^{-3} mol dm^{-3} $[Pt(bpy)(py)_2]^{2+}$, with ct-DNA in 1.0×10^{-3} mol dm^{-3} phosphate buffer (pH 7)–20.0×10^{-3} mol dm^{-3} NaCl; T = 25 °C

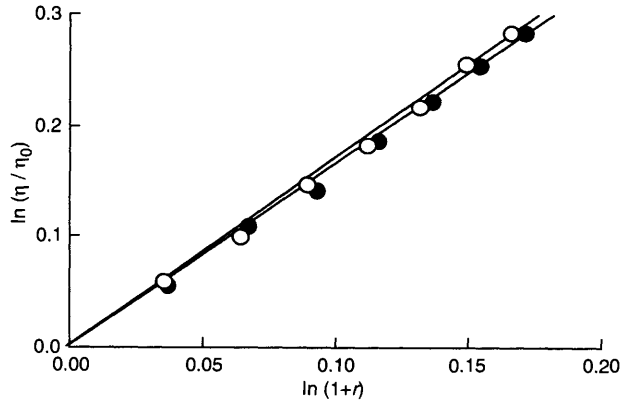

Fig. 2 Viscometric titration with ct-DNA of (●) $[Pt(bpy)(py)_2]^{2+}$ and (○) $[Pt(bpy)(en)]^{2+}$, in 1.0×10^{-3} mol dm^{-3} phosphate buffer (pH 7)–10.0×10^{-3} mol dm^{-3} sodium chloride; η = intrinsic viscosity of sonicated DNA in the absence of the complex; η_o = intrinsic viscosity of sonicated DNA in the presence of the complex; r = number of drug molecules bound to base pairs.

of negative charge transmitted, through the metal to the bipyridyl moiety. Variation in the nature of the substituents at pyridines also induces changes in the polar, hydrophobic and hydrogen bonding characteristics of the pyridines; the lack of dependence of the binding affinity for DNA on these properties rules out a direct interaction of these ligands with the biopolymer and indirectly confirms that bpy is the interacting moiety. The binding constant values for 2-Me-substituted pyridines, lower than expected on the basis of the pK_a values, reflect the steric hindrance of these ligands to intercalation. Owing to the perpendicular orientation of the two pyridine rings with respect to bipyridyl,[5b,6] the methyl substituents at the 2 position hamper the intercalation process.

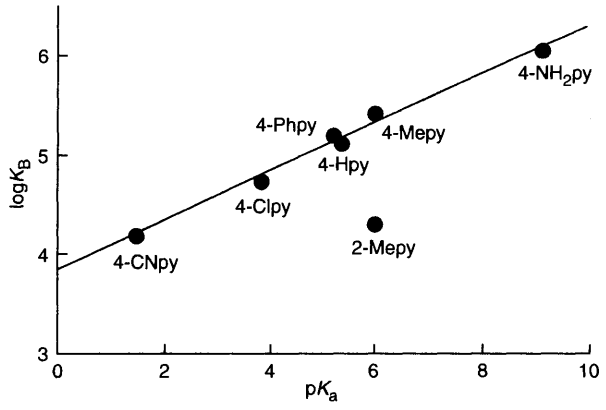

Fig. 3 Plot of $\log K_B$ for the various complexes against pK_a[8a,b] of coordinated pyridines

The complexes are all dicationic and, therefore, the electrostatic contributions to the interaction with DNA are the same; the binding constant values are, therefore, a measure of the relative stacking forces for the various substrates. The increase of the binding constants with increasing electron density of the bipyridyl suggests that stacking between the intercalated bpy and the nucleobases may be driven by London dispersion forces rather than by charge transfer[9] from the nucleobases to bipyridyl.

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