Stacking Surface Effect in the DNA Intercalation of Some Polypyridine Platinum(II) Complexes

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The interaction of the platinum(II) polypyridine complexes $[Pt(bipy)_2]^{2+}$, $[Pt(quaterpy)]^{2+}$, $[Pt(terpy)(n\text{-}Rpy)]^{2+}$ and $[Pt(bipy)(py)_2]^{2+}$ (bipy = 2,2′-bipyridine; terpy = 2,2′:6′,2″-terpyridine; quaterpy = 2,2′:6′,2″:6″,2‴-quaterpyridine; *n*-R = H, 2-CH_3, or 4-CH_3) with double-helix DNA has been studied with a variety of experimental techniques. Induced circular dichroism, strong hypochromism and red shifts of the absorption maxima of the complexes, increase in melting temperature and viscosity of DNA, and inhibition of the reaction of the complexes with thiourea in the presence of DNA, characterize the processes. Intercalation, implying the whole molecule or part of it, is the suggested binding mode. The binding constants, K_B, determined spectrophotometrically at 25 °C, pH 7, and $I = 0.15$ M, using the McGhee−von Hippel approach, increase in the order $[Pt(bipy)(py)_2]^{2+} < [Pt(terpy)(py)]^{2+} < [Pt(quaterpy)]^{2+}$, on increasing aromatic planar surface extension. The steric interference with double helix of the methyl group in $[Pt(terpy)(2\text{-}Mepy)]^{2+}$ destabilizes the interaction by reducing the stacking surface.

Introduction

Intercalation[1] of small molecules to DNA is a noncovalent interaction that depends on a number of factors[2] such as planarity, aromaticity, surface extension of the interacting moiety, etc. A convenient way to study these factors are to use platinum(II) intercalators.[3] These substances are inert toward possible competitive covalent processes and possess the geometry appropriate for the interaction; in addition, it is possible to tune to large extent their electronic and steric properties by suitable choice of the ligands. For instance, in a previous paper[4] we have studied the effect of the density charge of the stacking surface on the binding avidity for DNA using the complexes $[Pt(bipy)(4\text{-}Rpy)_2]^{2+}$ and $[Pt(4,4'\text{-}diphenylbipy)(4\text{-}Rpy)_2]^{2+}$ (bipy = 2,2′-bipyridine; R = H, CN, CH_3, NH_2) where the interacting part is constant and the σ-donor power of the pyridine spectator ligands changes gradually. In this paper we report the results of a study of the noncovalent interactions with DNA of the complexes $[Pt(bipy)_2]^{2+}$, $[Pt(quaterpy)]^{2+}$, $[Pt(terpy)(n\text{-}Rpy)]^{2+}$ (*n*-R = H, 2-CH_3, or 4-CH_3), and $[Pt(bipy)(py)_2]^{2+}$ (terpy = 2,2′:6′,2″-terpyridine; quaterpy = 2,2′:6′,2″:6″,2‴-quaterpyridine). All the substances bearing aromatic coplanar rings are potential DNA intercalators, and, in particular, this binding mode has been proved[4,5] for the latter complex. While the electrostatic contribution to the interaction of these dicationic complexes with the biopolymer is the same, the extension of the planar aromatic moiety varies systematically. Therefore, these complexes could be useful to assess the contribution of this structural feature to the intercalation process. To this purpose we have studied the interactions of the above-mentioned complexes with calf thymus (ct.) DNA, using a series of techniques, and determined the binding constants, K_B, at 25 °C, pH 7, and 0.15 M ionic strength.

Experimental Section

Materials. (i) Platinum(II) Complexes. $[Pt(bipy)_2](PF_6)_2$,[6] $[Pt(bipy)(py)_2](PF_6)_2$,[4] $[Pt(terpy)(n\text{-}Rpy)](BF_4)_2$[7] (*n*-R = H, 2-$CH_3$, or 4-$CH_3$), and $[Pt(quaterpy)](PF_6)_2$[8] were prepared as described in the literature. The substances were characterized by elemental analysis and ^{1}H NMR.

(ii) DNA. Calf thymus DNA was purchased from Sigma Chemical Co and purified as previously described.[9] DNA concentration, expressed in base pairs, was calculated spectrophotometrically using an ϵ_{260nm} value of 1.31×10^4 M^{-1} cm^{-1}.[10]

NaCl, $NaNO_3$, and other chemicals were of reagent grade and were used without further purification.

The complexes were directly dissolved in doubly distilled water containing 1.0×10^{-3} M phosphate buffer (pH 7) and enough NaCl or $NaNO_3$ to give the desired ionic strength. The concentrations were established on the basis of the molar absorptivities of the complexes. $NaNO_3$ was used only in the case of the substrates $[Pt(terpy)(n\text{-}Rpy)]^{2+}$, which undergo slow substitution of the coordinated pyridine by chloride. Sodium chloride (3×10^{-3} M) was, however, present in DNA solution, as this salt was used to purify by dialysis the biopolymer. Under these experimental conditions no significant pyridine replacement occurs during the time necessary to study the interaction process.

Methods. All experiments were carried out at 25 °C, pH 7, in a phosphate buffer and enough NaCl or $NaNO_3$ to give the desired ionic strength.

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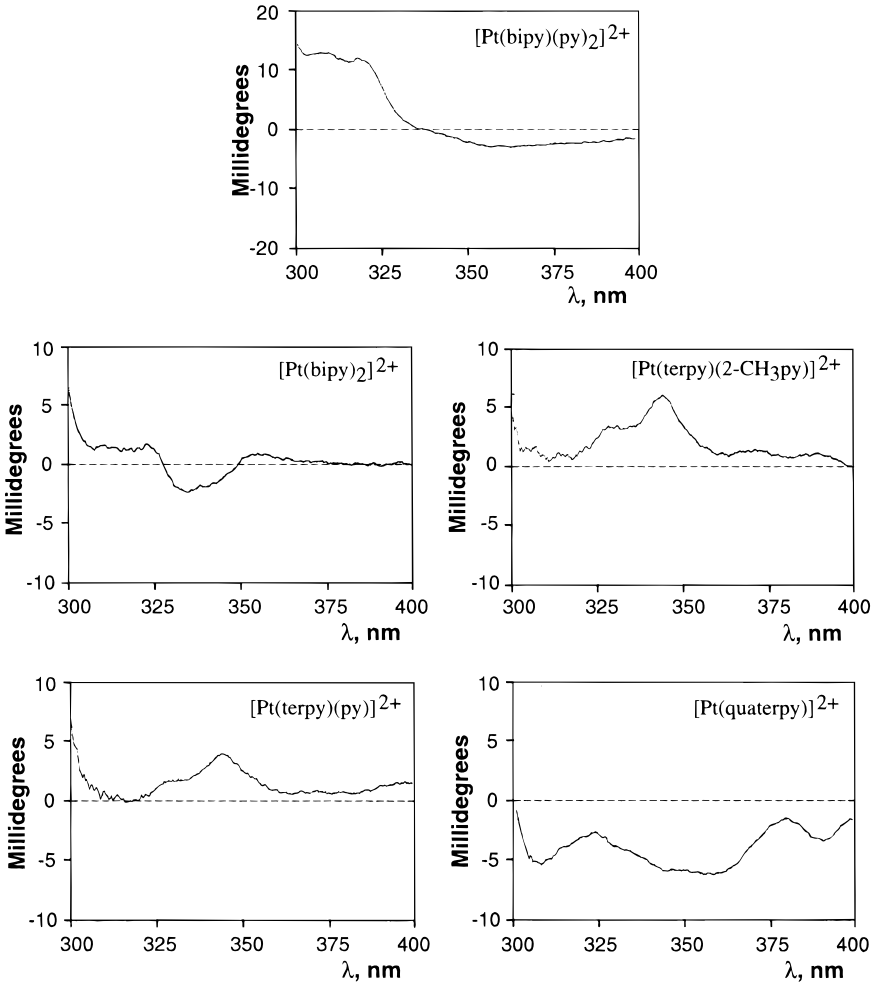

Figure 1. CD spectra of the complexes [(~5−8) × 10^{-5} M], in the presence of 10-fold excess DNA at 25 °C (phosphate buffer, 1.0 × 10^{-3} M; NaCl or $NaNO_3$, 1.5 × 10^{-1} M).

pH was measured with a Radiometer PHM 62.

Absorption spectra were obtained by using a Lambda 5 Perkin-Elmer spectrophotometer and 10-mm path length stoppered quartz cell.

^{1}H NMR spectra were recorded on a Bruker ARX-300 spectrometer.

CD spectra were recorded on a J-600 Jasco spectropolarimeter using 10-mm stoppered quartz cells.

Thermal Denaturation Experiments. The thermal denaturation temperature of complex−DNA mixtures (1:10) was determined in 1.0 × 10^{-3} M phosphate buffer (pH 7) containing 7.8 × 10^{-6} M complex and 2.0 × 10^{-3} M NaCl. Melting curves were recorded at 260 nm on a Lambda 3-B Perkin-Elmer spectrophotometer interfaced with a Macintosh LC computer. The temperature was increased at a rate of 0.5 °C/min by using a PTP-1 Peltier Perkin-Elmer temperature programmer.

Viscometry. A Cannon-Ubbelhode semi-microdilution viscometer (Series No 75, Cannon Instrument Co.), thermostatically maintained at 25 °C in a water bath, was used for viscosity measurements. The viscometer contained 2 mL of sonicated DNA solution (600 base pairs). The complex solution [(1.0−2.0) × 10^{-4} M], containing also DNA (4.0 × 10^{-4} M) at the same concentration as that in the viscometer, was delivered in increments of 50−80 μL from a micropipet. Solutions were freed of particulate material by passing them through Acrodisc CR PTFE syringe filters before use. Flow times were measured by hand with a digital stopwatch. Reduced viscosities were calculated by established methods and plotted as ln η/η_o against ln(1 + r) (η = intrinsic viscosity of the DNA solution in the presence of complex; η_o = intrinsic viscosity of the DNA solution in the absence of complex; r = $[complex]_{bound}/[DNA]_{tot}$). Under experimental conditions of low ionic strength and large [DNA]/[complex] ratio the complex is totally bound and $[complex]_{bound}$ coincides with $[complex]_{tot}$.

Binding Constants Determinations. Spectrophotometric titrations were performed, in the range 310−360 nm, by adding to a complex solution [(2.0−8.0) × 10^{-5} M] successive aliquots of DNA [(2.3−2.7) × 10^{-3} M], containing also the complex, in a 10 mm stoppered quartz cell and recording the spectrum after each addition. The data were analyzed by a non least-squares fitting program, applied to McGhee and von Hippel equation.[11] The binding constant K_B and the cooperativity parameter ω were determined by the program, using the molar absorptivities of the compounds, the free complex and DNA concentrations and the absorbance values after each DNA addition. Molar absorptivities for bound compounds were determined by Beer's law plots in the presence of a large excess of DNA.

Kinetics. The kinetics of ligand substitution, by thiourea, in the reported complexes were followed spectrophotometrically, in the range 320−360 nm, at 25 °C. In all cases at least a 10-fold excess of nucleophile was used to provide pseudo-first-order conditions and to force the reaction to completion. A Perkin-Elmer Lambda 5 spectrophotometer equipped with a SFA-11 Hi-Tech stopped-flow accessory was used to monitor the processes. The two syringes of stopped-flow contained respectively the complex and the nucleophile or the mixture nucleophile-DNA. The ionic strength of both solutions was 0.15 M (NaCl or $NaNO_3$) and the pH 7 (1 × 10^{-3} M phosphate buffer). The absorbance changes were displayed on a Macintosh 2 computer interfaced with the spectrophotometer and pseudo-first-order rate constants k_{obsd} were obtained from nonlinear least-squares fit of the experimental data to $A_t = A_\infty + (A_o - A_\infty)\exp(-k_{obsd}t)$, where A_o, A_∞, and k_{obsd} were the parameters to be optimized (A_o = absorbance after mixing of the reagents, A_∞ = absorbance at completion of reaction). The k_{obsd} values were reproducible to better than ±5%.

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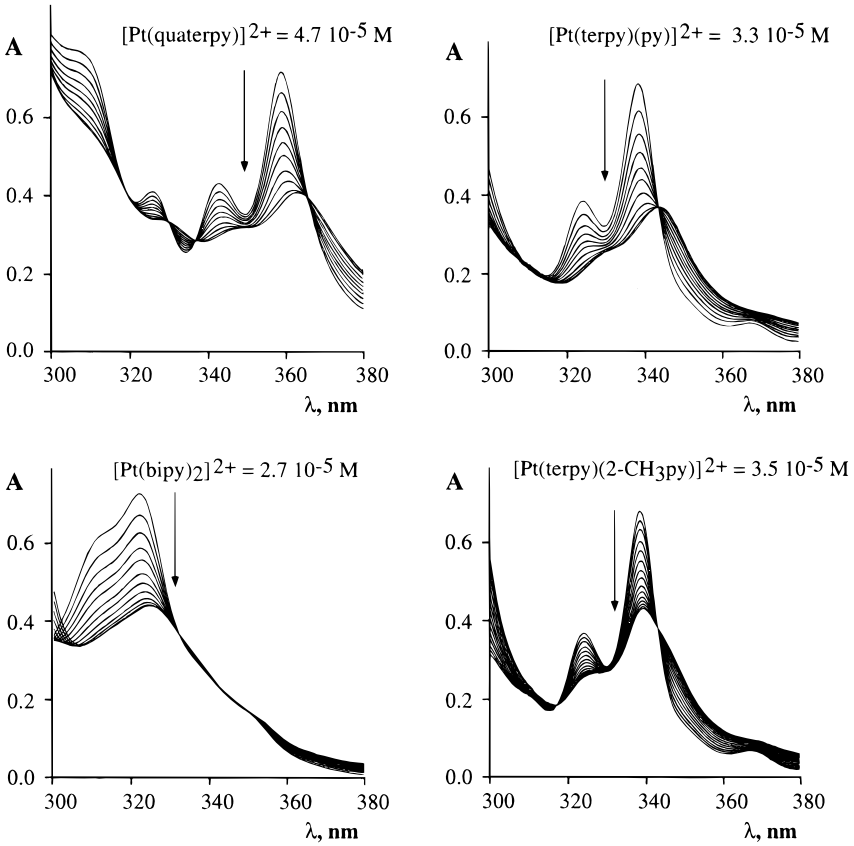

Figure 2. Spectrophotometric titration of the complexes with DNA (2.7×10^{-3} M) at 25 °C (phosphate buffer, 1.0×10^{-3} M; NaCl or $NaNO_3$, 1.5×10^{-1} M).

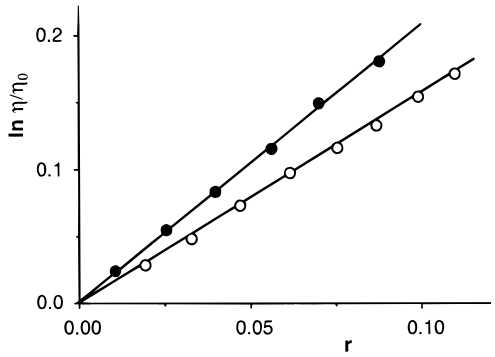

Figure 3. Viscometric titration of ct. DNA (4.0×10^{-4} M) with (○) [Pt(bipy)(en)]$^{2+}$ or (●) [Pt(quaterpy)]$^{2+}$, in 1.0×10^{-3} M phosphate buffer and 1.0×10^{-2} M NaCl; η = intrinsic viscosity of sonicated DNA in the presence of the complex; η_o = intrinsic viscosity of sonicated DNA in the absence of the complex; r = $[\text{complex}]_{\text{bound}}/[\text{DNA}]_{\text{tot}}$.

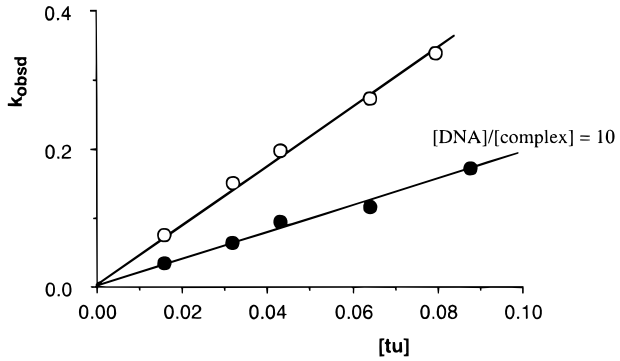

Figure 4. Plot of $k_{obsd}(s^{-1})$ vs [tu] for reaction of [Pt(terpy)(py)]$^{2+}$ with thiourea (T = 25 °C; phosphate buffer = 1.0×10^{-3} M, $NaNO_3$ = 1.5×10^{-1} M, [Complex] = 5×10^{-6} M), (○) in the absence and (●) in the presence of DNA.

Results

The terpyridine complexes, [Pt(terpy)(*n*-Rpy)]$^{2+}$, differently from the others, are not very stable in solution especially in the presence of nucleophiles stronger than water. Owing to the presence of the extensive π system of terpyridine that drains negative charge from platinum, the central metal is easily attacked[12] by nucleophiles with subsequent substitution of the coordinated pyridine. For these substances, $NaNO_3$, rather than NaCl, was used to adjust the ionic strength.

Evidence of strong interaction of the complexes with DNA comes from circular dichroism and absorption studies. For all the substances studied CD signals are observed (Figure 1) in the 300−400 nm region of the spectrum, where the bound complexes absorb. The signals are due to induced circular dichroism as a result of the rigid orientation assumed by the substance bound to the double helix.

In the presence of DNA [$(3.00-130) \times 10^{-5}$ M], the electronic spectra of the complexes change dramatically showing strong decrease in the peak intensities (hypochromicity) and red shifts of the bands. The spectral changes are characterized (Figure 2) by one or more isosbestic points well maintained till the end of the titrations of the complexes with DNA so one can rule out the presence of species other than the free and the intercalated complex. In addition, the spectral variations occur within the mixing time of the solutions and cannot be related to any process involving the first coordination sphere of the metal. The total reversibility of the reaction, which can be totally

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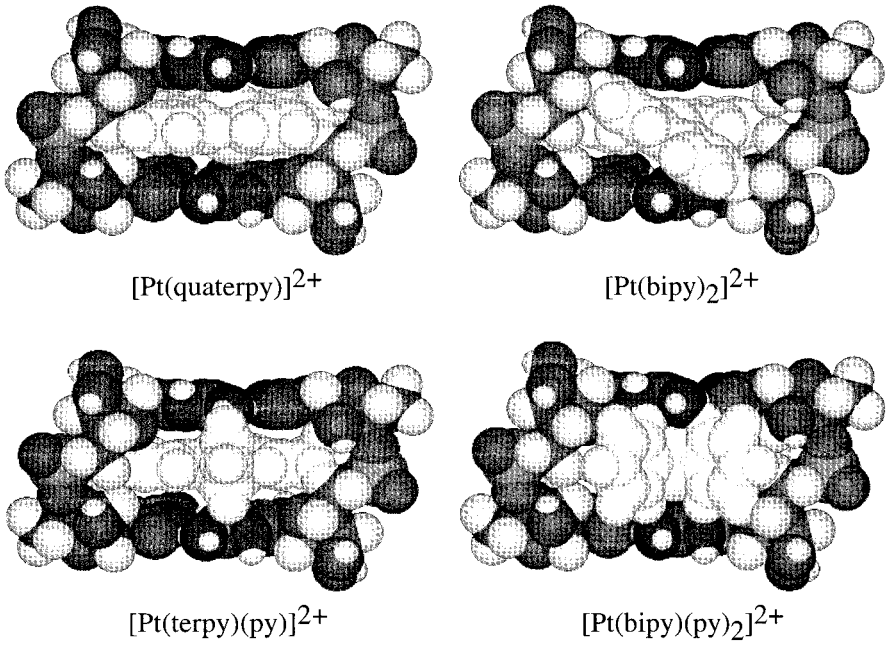

Figure 5. Pictorial representation of the intercalated complexes.

shifted to the left by addition of NaCl, confirms the noncovalent character of the process. Strong hypochromism, attributable to interaction between the electronic states of the binding chromophore and that of DNA bases, and red shifts of the bands are usually[13] observed for intercalation.

Intercalation can be proved by experimental techniques that show the changes induced by the process on the double helix. To accommodate the intercalator the DNA must partly unwind; this produces lengthening and contemporary stiffening that is reflected in an increase of DNA viscosity.[14] Furthermore the presence of an intercalator stabilizes the double helix and raises the temperature at which it unwinds. Therefore, an increase in thermal denaturation temperature and in viscosity of DNA suggests intercalative binding mode.

In the case of the terpyridine complexes complete DNA denaturation occurs in two steps. The denaturation temperature, for these complexes, was estimated using the first double-strand−single-strand transition; the second transition, already reported[15] for $[Pt(terpy)(4\text{-}CH_3py)]^{2+}$, is attributable, to our opinion, to the presence of new chemical species formed under prolonged heating, at high temperature of these unusually labile platinum(II) complexes. For all the other substrates only one transition, leading to complete denaturation, is observed. The increase in thermal denaturation temperature of DNA, in the presence of the complexes studied, is reported in Table 1. By sake of comparison, the increase of DNA melting temperature, in the presence of the known intercalator[16] $[Pt(bipy)(en)]^{2+}$ (en = ethylenediamine), has also been determined. The data clearly show that the duplex stabilization produced by the interaction with all the complexes here investigated is comparable to or larger than that observed for $[Pt(bipy)(en)]^{2+}$. Increase in the viscosity of DNA has been observed with all the complexes. Figure 3 shows that the increase in DNA viscosity due to interaction with $[Pt(quaterpy)]^{2+}$ is larger than for the reference intercalator $[Pt(bipy)(en)]^{2+}$.

Table 1. Increase in Melting Temperature of ct. DNA upon Interaction with the Complexes

complex	ΔT
$[Pt(bipy)(py)_2]^{2+}$ [a]	9.5 (±0.2)
$[Pt(bipy)_2]^{2+}$	9.7 (±0.4)
$[Pt(terpy)(py)]^{2+}$	12.2 (±0.3)
$[Pt(terpy)(2\text{-}CH_3py)]^{2+}$	12.3 (±0.6)
$[Pt(quaterpy)]^{2+}$	12.4 (±0.5)
$[Pt(bipy)(en)]^{2+}$	11.1 (±0.6)

[a] Value from ref 4a.

Finally indirect evidence[17] of intercalation comes from kinetic data for the reaction of the complexes with thiourea. As a result of intercalation the metal center of a complex becomes sterically protected by the nucleobases that prevent the entry of the reagent; in the presence of DNA the reactivity of an intercalating complex must, therefore, decrease. Any non-anionic reagent can be used to observe this kinetic effect; we have used thiourea, which being neutral (i) does not interact electrostatically with DNA, (ii) is not involved in proton transfer reactions and, in addition, (iii) is one of the most effective nucleophiles toward square planar complexes. For all the complexes used we have observed a decrease in the rate of the reaction with thiourea. This kinetic effect increases on increasing DNA concentration; in addition, at low ionic strength when the complex is almost totally intercalated, the reaction is completely inhibited. Figure 4 shows the decrease in rate observed for the complex $[Pt(terpy)(py)]^{2+}$. The reaction, which leads to the replacement of pyridine by thiourea (eq 1) occurs in one observable step.

$$[Pt(terpy)(py)]^{2+} + tu \rightarrow [Pt(terpy)(tu)]^{2+} + py \quad (1)$$

Under pseudo-first-order conditions with respect to the complex, the observed rate constant k_{obsd} is linearly correlated to the thiourea concentration:

$$k_{obsd} = k_2[tu] \quad (2)$$

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Table 2. Values of Binding Constants (K_B, M^{-1}) for the Interaction of the Complexes with ct. DNA, in Phosphate Buffer (1.0×10^{-3} M, pH = 7), $T = 25$ °C

complex	K_B, M^{-1}
$[Pt(bipy)(py)_2]^{2+}$ [a]	$(1.0 \pm 0.1) \times 10^4$
$[Pt(terpy)(py)]^{2+}$ [b]	$(3.5 \pm 0.3) \times 10^4$
$[Pt(quaterpy)]^{2+}$ [a]	$(2.2 \pm 0.2) \times 10^5$
$[Pt(terpy)(2\text{-}CH_3py)]^{2+}$ [b]	$(3.0 \pm 0.3) \times 10^3$
$[Pt(bipy)_2]^{2+}$ [a]	$(2.5 \pm 0.2) \times 10^4$
$[Pt(terpy)(4\text{-}CH_3py)]^{2+}$ [b]	$(5.3 \pm 0.5) \times 10^4$

[a] NaCl = 1.5×10^{-1} M. [b] $NaNO_3$ = 1.5×10^{-1} M.

In the presence of DNA, the same rate law is observed but the reaction is slower; the k_2 values, at 25 °C, are, in fact, 4.48 ± 0.14 and 2.39 ± 0.12, respectively.

The binding avidity of the substrates for DNA has been estimated by introducing the spectrophotometric titration data in the McGhee−von Hippel equation.[11] The binding constant values, K_B, are reported in Table 2.

Discussion

According to the experimental results reported intercalation seems to be the favored binding mode with DNA; however, owing to the molecular structure of the complexes, full intercalation is conceivable only for $[Pt(quaterpy)]^{2+}$. This cation is essentially planar[8] and exhibits only a slight twisting in the ligand frame. For the other complexes, intercalation implies, more likely, only part of the molecule. In $[Pt(bipy)(py)_2]^{2+}$ and $[Pt(terpy)(py)]^{2+}$ the pyridine ligands, which are skew[18,19] to the square plane, prevent the insertion of the whole cation between the nucleobases. Likewise, in $[Pt(bipy)_2]^{2+}$, the arrangement of the nitrogen atoms around the platinum atom is not planar but is distorted toward a tetrahedral configuration,[20] the dihedral angle between the bipyridine planes being about 23°. Although some twist about the torsional bonds joining the aromatic rings may complement or enhance the intrinsic propeller twist of DNA base pairs and, therefore, favor intercalation,[21] in this case deviation from planarity seems too large to allow stable interaction. More likely, intercalation occurs through only one bipyridine group and the remaining ligand in the groove. A pictorial representation of the binding mode of the various complexes is reported in Figure 5.

The K_B values, which vary by about 2 orders of magnitude along the series of complexes examined, are clearly dependent on the planar aromatic surface. The DNA binding affinity increases in the order $[Pt(bipy)(py)_2]^{2+} < [Pt(terpy)(py)]^{2+} < [Pt(quaterpy)]^{2+}$, on increasing extension of the aromatic planar surface. However, the K_B value on going from $[Pt(terpy)(py)]^{2+}$ to $[Pt(quaterpy)]^{2+}$ increases more than on going from $[Pt(bipy)(py)_2]^{2+}$ to $[Pt(terpy)(py)]^{2+}$. The strength of the interaction, depends, therefore, on the surface that can slide between the nucleobases and lead to stacking, rather than on the intrinsic extension of the planar aromatic surface. While there are no steric limitations to the intercalation of $[Pt(quaterpy)]^{2+}$, and the whole surface of this complex is potentially available for the interaction, for $[Pt(bipy)(py)_2]^{2+}$ and $[Pt(terpy)(py)]^{2+}$, the portion of the intrinsic planar aromatic moiety available for stacking with the nucleobases is limited by the steric interference of the pyridines out-of-plane with the double helix. This type of steric destabilization is very strong for $[Pt(terpy)(2\text{-}CH_3py)]^{2+}$. The DNA binding affinity of this complex is much lower than for $[Pt(terpy)(py)]^{2+}$ which has the identical planar aromatic surface; here the methyl group in 2 position at pyridine is an additional, strong obstacle to intercalation, and the stacking surface is further reduced. The destabilization is so large that the binding constant of this complex becomes lower than that of the bipyridine complex. The steric nature of this effect becomes evident if we compare the relative DNA binding affinity of the three terpyridine complexes; when the methyl group is placed in a position that does not interfere with the double helix, as in $[Pt(terpy)(4\text{-}CH_3py)]^{2+}$, the K_B value[22] is higher than for the complex with unsubstituted pyridine. In this case the effect of CH_3 is electronic in nature and should be due to the electrondonating properties of this group that enhance the electronic density of terpyridine. Previous studies[4] have shown that for this type of complexes the binding avidity increases on increasing electron density of the intercalating moiety. According to the intercalation model proposed, the methyl group should lie well outside the nucleobases and, therefore, no hydrophobic contribution to the interaction should be present.

Finally the data support the hypothesis that the complex $[Pt(bipy)_2]^{2+}$ intercalates partially through one of the bypyridine moieties. The DNA binding affinity of this substrate is much lower than for $[Pt(quaterpy)]^{2+}$ and is very close to that of $[Pt(bipy)(py)_2]^{2+}$.

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(22) Reference 15 reports two different K_B values for the interaction with AT-DNA. These values, determined at two different DNA/complex ratios, confirm the high DNA binding affinity of this substrate.