Inorg. Chem. **2006**, *45*, 230–235

DNA Interaction of Platinum(II) Complexes with 1,10-Phenanthroline and Extended Phenanthrolines

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Received June 1, 2005

The interaction with DNA of the platinum(II) square planar complexes $[Pt(N–N)(py)_2]^{2+}$ (N–N = 1,10-phenanthroline (phen), dipyrido[3,2-*d*:2′,3′-*f*]quinoxaline (dpq), dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz), benzodipyrido[*b*:3,2-*h*:2′3′-*f*]phenazine (bdppz)) has been investigated by means of absorption, circular and linear dichroism spectroscopy, DNA melting, and viscosity. In the presence of excess [DNA] all the complexes intercalate to the double helix. For those with the most extended phenanthrolines the binding mode depends on the [DNA]/[complex] ratio (*q*); at low *q* values the substances bind externally to DNA probably self-aggregating along the double helix. When the DNA concentration is large enough, the aggregate breaks up and the complex intercalates within the nucleobases. The complexes self-aggregate, without added DNA, in the presence of a large salt concentration.

Introduction

The interactions between DNA and octahedral complexes with rigid bidentate ligands, such as 1,10-phenanthroline or 2,2′-bipyridyl, has been a widely investigated[1] subject due to their potential application in the molecular recognition of nucleic acids. Iron(II),[2] ruthenium(II),[3] and rhodium(III)[4] complexes with 1,10-phenanthroline or similar ligands have been reported to show enantioselectivity toward B DNA, one of the isomers, Λ or Δ, being more effective in binding to the double-helix. It has been assumed that these complexes interact by intercalation[5] within the biopolymer base pairs, but this binding mode has been challenged[6] and remains somehow controversial. For the homologous complexes with the ligands dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz) and benzodipyrido[*b*:3,2-*h*:2′,3′-*f*]phenazine (bdppz), however, intercalation has been proved unambiguously.[5c,7] These planar aromatic ligands are extended enough to protrude partially into adjacent nucleobase pairs and stack with them. Octahedral complexes of dppz and bdppz bind avidly to the double helix, and large binding constant values have been reported.[7a,8] Very little has been done on the interaction of these ligands coordinated to metals forming square-planar complexes. Due to the absence of significant steric clashes of the ancillary ligands with the DNA sugar phosphate backbone, intercalation of these substances is expected to be very effective. Nonetheless a paper[9] concerning platinum(II) complexes with the dppz ligand has quoted for $[Pt(dppz)(4\text{-}NH_2py)_2]^{2+}$ a binding constant value lower than those of octahedral complexes with the same ligands[5c,6,8] and at most comparable with that of square complexes with ligands of

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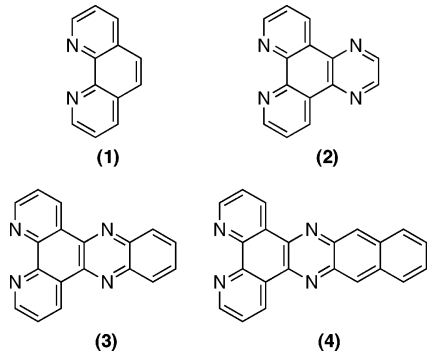
10.1021/ic050880o CCC: $33.50
Published on Web 12/08/2005

Chart 1. Formulas of the Ligands Used



smaller extension and all bearing a doubly positive charge.[10] This surprising observation has prompted us to perform an investigation of the interaction of platinum(II) complexes of 1,10-phenanthroline and extended phenantrolines to test if the ligand aromatic surface affects the binding avidity of the substances. To this end we have studied the interaction of the complexes $[Pt(phen)(py)_2]^{2+}$, $[Pt(dpq)(py)_2]^{2+}$, $[Pt(dppz)(py)_2]^{2+}$, and $[Pt(bdppz)(py)_2]^{2+}$ (phen = 1,10-phenanthroline (**1**); dpq = dipyrido[3,2-*d*:2′,3′-*f*]quinoxaline (**2**); dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine (**3**); bdppz = benzodipyrido-[*b*:3,2-*h*:2′,3′-*j*]phenazine (**4**); Chart 1) with calf thymus DNA by using a variety of techniques including absorption, circular and linear dichroism, DNA melting, and viscosity.

Experimental Section

Chemicals. Calf thymus DNA was purchased from Sigma Chemical Co. and purified as previously described.[11] Its concentration, expressed in base pairs, was determined spectrophotometrically using the molar absorptivity 1.31×10^4 M^{-1} cm^{-1} (260 nm).[12]

NaCl, $NaClO_4$, and other chemicals were of reagent grade and were used without further purification.

$[Pt(N-N)(py)_2](PF_6)_2$ complexes (N−N = 1,10-phenanthroline (Aldrich), dpq,[13] dppz,[14] bdppz[7b]) were synthesized by suspending $[Pt(N-N)Cl_2]$[15] in water and adding pyridine in large excess. The mixture was then heated to boiling and kept refluxing until almost complete dissolution of the suspension. The traces of solid material were eliminated by filtration, and the final products were precipitated with NH_4PF_6.

All the complexes were characterized by elemental analysis and ^1H and ^{13}C NMR.

Methods. All experiments were carried out at 25 °C and pH 7, in 1×10^{-3} M phosphate buffer and enough NaCl to give the desired ionic strength. pH was measured with a Radiometer PHM 62. Absorption spectra were recorded using a Cintra 20 GBC spectrophotometer. CD and LD spectra were recorded on a Jasco J-820 spectropolarimeter. ^1H and ^{13}C spectra were recorded on a Bruker ARX-300 spectrometer.

Thermal Denaturation Experiments. The thermal denaturation temperature of complex−DNA mixtures (1:10) was determined in 1×10^{-3} M phosphate buffer (pH 7) containing 7.8×10^{-6} M complex and 2×10^{-3} M NaCl. Melting curves were recorded at 260 nm on a Cintra 20 GBC spectrophotometer interfaced with a 486 IBM computer. The temperature was increased at a rate of 0.5 °C/min by using a PTP-1 Peltier system.

Viscometry. A Cannon-Ubbelhode semi-microdilution viscometer (series no. 75, Cannon Instruments Co.), thermostatically maintained at 25 °C in a water bath, was used for viscosity measurements. The viscometer contained 2 mL of sonicated DNA solution (approximately 600 base pairs) in 1×10^{-3} M phosphate buffer (pH 7) and 1×10^{-2} M NaCl. The complex solution ($(0.7-2.7) \times 10^{-4}$ M), containing also DNA (6.0×10^{-4} M) at the same concentration as that in the viscometer, was delivered in increments of 90−150 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 $\ln \eta/\eta^\circ$ against $\ln(1 + r)$ (η is the reduced viscosity of the DNA solution in the presence of complex; η° the reduced viscosity of the DNA solution in the absence of complex; r is $[complex]_{bound}/[DNA]_{tot}$). Under experimental conditions of low ionic strength and large [DNA]/[complex] ratio the complex is totally bound and $[complex]_{bound}$ coincides with $[complex]_{tot}$.

Linear Dichroism. Linear dichroism (LD) spectra were recorded on a Jasco J-820 spectropolarimeter, equipped with an Oxley prism to convert the circularly polarized light into linearly polarized light.[16] The DNA was oriented by a flow gradient[17] in a Couette cell with rotating inner cylinder, manufactured by Crystal Precision Optics (Rugby, Warwickshire, U.K.). The DNA used for LD measurements was sonicated by means of a 130 W ultrasonic processor for 1 min at 6 W.

Binding Constants Determinations. Spectrophotometric titrations were performed by adding to a complex solution $[(0.7-1.1) \times 10^{-4}$ M] successive aliquots of DNA, containing also the complex, in a 10 mm stoppered quartz cell and recording the spectrum after each addition. The data were analyzed by a nonlinear-least-squares fitting program, applied to the McGhee and von Hippel equation.[18] The binding constant K_B was determined by the program, using the extinction coefficient of the compounds, the free complex concentration, and the ratio of bound complex/mol of DNA. Extinction coefficients for bound compounds were determined by Beer's law plots in the presence of a large excess of DNA.

Results and Discussion

The presence of noncovalent interactions between a small molecule and DNA can be easily established by the analysis of various physical properties of the biopolymer. Any type of weak interaction modifies, reversibly, the double helix

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

complex	ΔT_m	complex	ΔT_m
$[Pt(phen)(py)_2]^{2+}$	12.3(±0.5)	$[Pt(bdppz)(py)_2]^{2+}$	8.4(±0.5)
$[Pt(dpq)(py)_2]^{2+}$	12.2(±0.5)	$[Pt(bpy)(py)_2]^{2+}$	10.5(±0.5)
$[Pt(dppz)(py)_2]^{2+}$	11.4(±0.5)	$[Pt(en)(py)_2]^{2+}$	2.9(±0.5)

structure; the structural changes are reflected in measurable properties. The DNA thermal denaturation temperature is one of these. With the exception of metal ions that bind strongly to nucleobase ethero-atoms,[19] upon interaction with a cationic species the double helix stability increases[20] and so does the DNA melting temperature. Although the increase in denaturation temperature is not specific of any particular type of noncovalent interaction, ΔT_m values may give some indications on the binding mode. Large increases in melting temperature are observed, in fact, only for the strongest type of interaction, i.e., intercalation.

Table 1 reports the increase of DNA melting temperature in the presence of the complexes under study and of the complexes $[Pt(bpy)(py)_2]^{2+}$ and $[Pt(en)(py)_2]^{2+}$. The latter substance, which cannot intercalate, has been used by Barton[21] et al. as a reference of a nonintercalating metal complex. The increase in melting temperature of the complexes studied is much larger than that for $[Pt(en)(py)_2]^{2+}$ and comparable to that of $[Pt(bpy)(py)_2]^{2+}$ whose intercalating properties are well-established.[10b] These results suggest that the complexes intercalate to the double helix.

Hydrodynamic properties and especially viscosity can give better indications on the binding mode of a small molecule to DNA. While intercalation results in a substantial change of the double helix, the other types of interaction produce only subtle changes in the structure and the DNA remains essentially in an unperturbed B DNA form. In particular, the former type of interaction causes lengthening and stiffening of the helix which results in an increase of the DNA viscosity.[22] For the complexes with phenanthroline and extended phenanthrolines an increase in viscosity comparable to that of $[Pt(bpy)(py)_2]^{2+}$ is observed. On the contrary for $[Pt(en)(py)_2]^{2+}$, which cannot intercalate, the viscosity value remains almost constant (Figure 1).

Again, these results strongly suggest that, in the presence of excess DNA as requested by viscosity experiments, all the complexes are intercalated. However, absorbance and circular dichroism measurements show that in the case of the complexes with the most elongated ligands $[Pt(bdppz)(py)_2]^{2+}$ and $[Pt(dppz)(py)_2]^{2+}$ the spectral changes, and so most likely the binding modes depend on the [DNA]/[complex] ratio (q). The solubility of the complexes in water is very low and decreases significantly from $[Pt(phen)(py)_2]^{2+}$ to $[Pt(bdppz)(py)_2]^{2+}$ with the extension of the aromatic ligand N−N. Nonetheless, due to the high values of absorptivity coefficients, absorption is a suitable technique to monitor the interaction of the substances with DNA.

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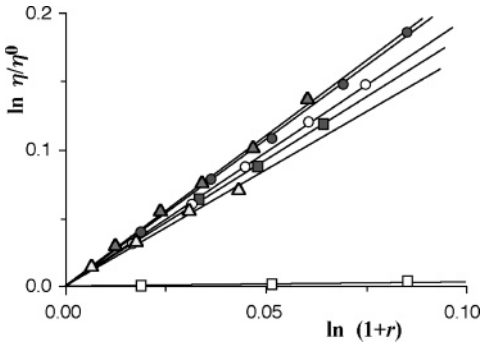

Figure 1. Viscometric titration of DNA (6.0×10^{-4} M) with (●) $[Pt(phen)(py)_2]^{2+}$, (○) $[Pt(dpq)(py)_2]^{2+}$, (▲) $[Pt(dppz)(py)_2]^{2+}$, (△) $[Pt(bdppz)(py)_2]^{2+}$, (■) $[Pt(bpy)(py)_2]^{2+}$, and (□) $[Pt(en)(py)_2]^{2+}$ at $T = 25$ °C and pH 7 (phosphate buffer, 1×10^{-3} M; NaCl, 1.0×10^{-2} M). η = reduced viscosity of the DNA solution in the presence of complex, η_0 = reduced viscosity of the DNA solution in the absence of complex, and $r = [complex]_{bound}/[DNA]_{tot}$.

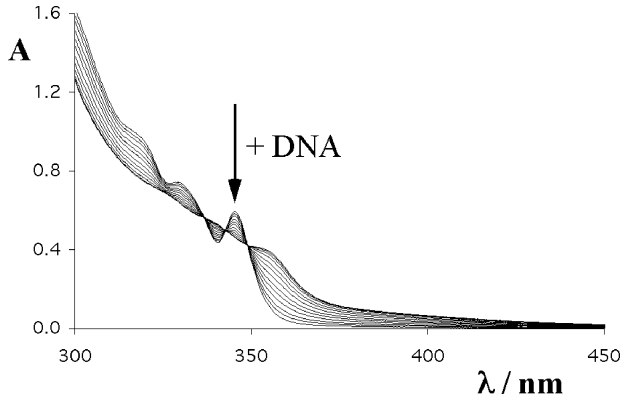

Figure 2. Spectrophotometric titration of $[Pt(dpq)(py)_2]^{2+}$ (1.11×10^{-4} M) with DNA at $T = 25$ °C and pH 7 (phosphate buffer, 1×10^{-3} M; NaCl, 2.1×10^{-2} M; [DNA] = (0−3.19) $\times 10^{-4}$ M).

Intercalation to DNA causes fast spectral variations of the absorption spectrum of the interacting substance characterized by hypochromism and shift to the red of the maxima. In the presence of excess DNA all the complexes here studied show these features. For $[Pt(phen)(py)_2]^{2+}$ and $[Pt(dpq)(py)_2]^{2+}$ hypochromism and shift to longer wavelength is progressive and concomitant as shown by the isosbestic points well maintained until the end of titration (Figure 2).

For $[Pt(bdppz)(py)_2]^{2+}$ the spectral variations occur in two steps as a function of the [DNA]/[complex] ratio (q) (Figure 3). At low biopolymer concentration ($q \leq 1$) hypochromism is observed with a very small bathocromic shift; when [DNA]/[complex] ratio increases, the maxima move to longer wavelengths and their intensity increase.

The complex $[Pt(dppz)(py)_2]^{2+}$ seems to show a similar behavior (Figure 4); again at low q values the spectrum undergoes mainly hypochromism. Only at higher [DNA]/[complex] ratios a consistent red shift occurs.

In the case of $[Pt(bdppz)(py)_2]^{2+}$ the presence of two different types of interaction as a function of [DNA]/[complex] ratio is clearly shown also by circular dichroism. Although the complexes here studied are all achiral species, they acquire an induced CD signal when binding to DNA. The CD is induced by the interaction between the bound complex and the chiral polyanion and appears in the

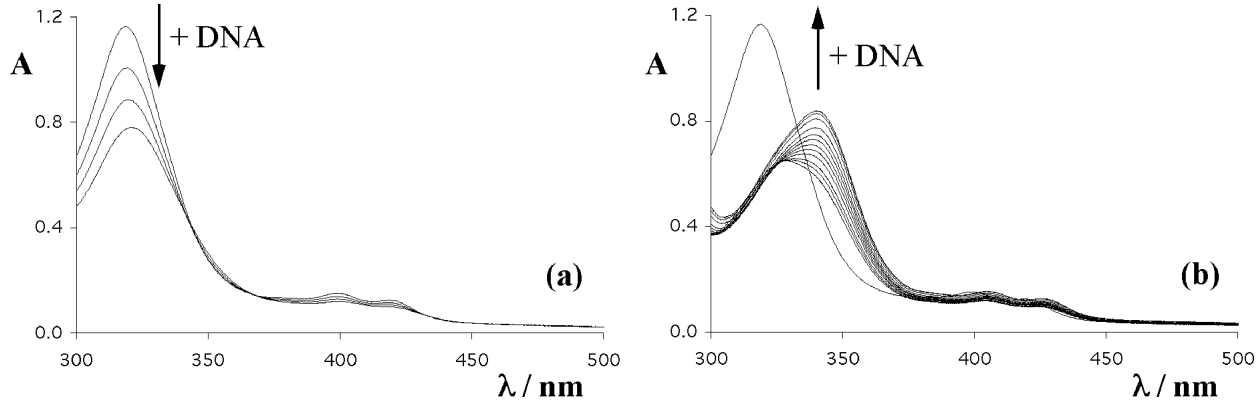

Figure 3. Spectrophotometric titration, at $T = 25$ °C and pH 7 (phosphate buffer, 1×10^{-3} M; NaCl, 2.1×10^{-2} M), of $[Pt(bdppz)(py)_2]^{2+}$ (2.2×10^{-5} M) with DNA at (a) [DNA]/[complex] < 1 and (b) [DNA]/[complex] ≥ 1.8 ([DNA] = $(0-2.1) \times 10^{-4}$ M).

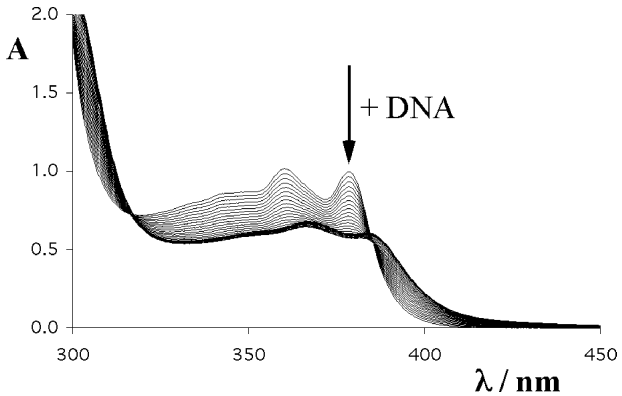

Figure 4. Spectrophotometric titration of $[Pt(dppz)(py)_2]^{2+}$ (7.3×10^{-5} M) with DNA at $T = 25$ °C and pH 7 (phosphate buffer, 1×10^{-3} M; NaCl, 2.1×10^{-2} M; [DNA] = $(0-3.81) \times 10^{-4}$ M).

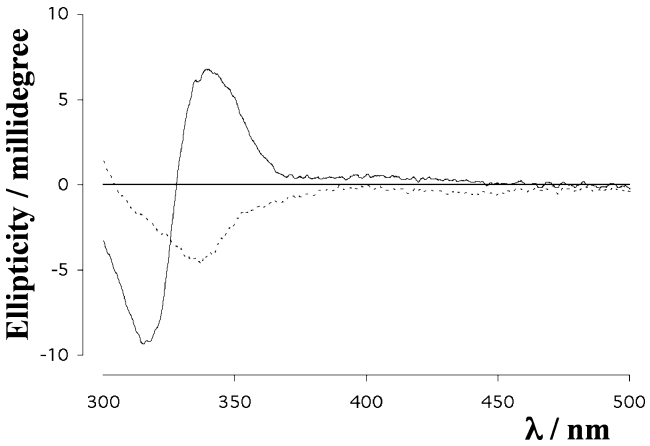

Figure 5. CD spectra, at $T = 25$ °C and pH 7 (phosphate buffer, 1×10^{-3} M; NaCl, 2.1×10^{-2} M), of $[Pt(bdppz)(py)_2]^{2+}$ (2.2×10^{-5} M) with DNA at (—) [DNA]/[complex] ≈ 0.7 and (---) [DNA]/[complex] ≈ 4.0.

wavelength range where the complex−DNA supramolecular species absorbs. The CD signal is dependent on the position and the orientation with respect to the DNA base pairs, and in principle it can be used to establish the binding mode of the complexes. However, so far, circular dichroism has been exploited routinely to distinguish between external binding and intercalation only for porphyrins.[23] More commonly the appearance of a CD signal is taken simply as an unambiguous proof of interaction with DNA. The presence of CD signals in the range where the bound complexes absorb again shows a peculiar behavior of $[Pt(bdppz)(py)_2]^{2+}$. At low [DNA]/[complex] ratio the induced CD signal shows a negative band at ≈315 nm and a positive one at ≈340 nm; when [DNA]/[complex] ratio exceeds 1, the two bands become smaller and smaller until they blend in one single negative band (Figure 5).

The different change in the spectroscopic properties of $[Pt(bdppz)(py)_2]^{2+}$ at low and high [DNA]/[complex] ratio can be explained in terms of the coexistence of two binding modes of the complex. Spectroscopic changes at $q \leq 1$ can be attributed to external binding of the complex along DNA double helix, while the absorption changes at large excess of intercalation binding sites ($q \gg 1$) can be explained by the intercalation of the complex with the biopolymer base pairs. The latter binding mode is strongly supported by melting and viscosity experiments. Further support to intercalation, at large excess of binding sites, comes from linear dichroism. Linear dichroism, LD, is defined as the difference in absorbance of light linearly polarized parallel and perpendicular to a macroscopic axis of orientation. In the case of flow LD on DNA solutions, the parallel direction is the flow one and the DNA molecules are oriented along the double helix long axis. That means that the strong in-plane $\pi-\pi^*$ transitions of the nucleobasis, responsible of the strong absorption band at 260 nm, are polarized close to perpendicular to the flow direction (helix axis for B-form DNA). Reduced linear dichroism, LD^r, is obtained dividing the LD spectrum by the isotropic absorption spectrum. Assuming an angle of 90° between the $\pi-\pi^*$ transition moments of the nucleobases and the DNA long axis, the angle of the drug's transition moments with respect to the DNA helix can be so estimated. Figure 6 shows (a) the LD spectrum of $[Pt(bdppz)(py)_2]^{2+}$ in the presence of a 5-fold excess DNA compared with that of DNA alone and (b) the LD^r spectrum of the complex bound to DNA.

The evidence of a strong negative LD signal at ≈340 nm, in the ligand-centered transitions region, due to an intraligand $\pi-\pi^*$ transition polarized along the C_2 symmetry long axis of the complex,[7b] clearly shows that the bdppz ligand is not aligned along any groove of the double helix; if it was so, in fact, a positive LD would be expected. Moreover, the

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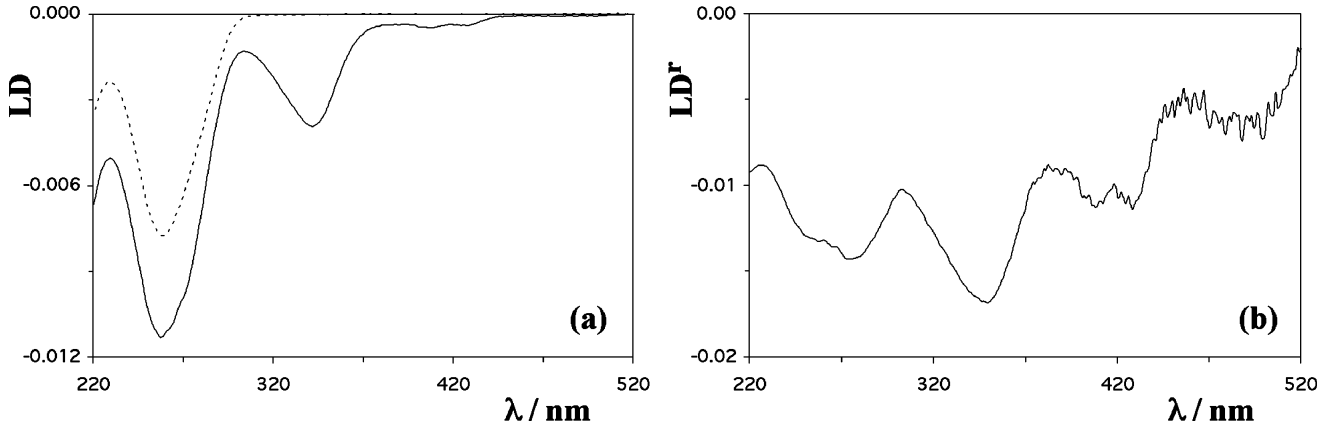

Figure 6. (a) LD spectra of [DNA] = 2×10^{-4} M (---) and of the DNA−[Pt(bdppz)$(py)_2]^{2+}$ mixture at [DNA]/[complex] ≈ 5 (—) and (b) LD^r spectrum of the same mixture (phosphate buffer (pH 7), 1×10^{-3} M; NaCl, 2.1×10^{-2} M).

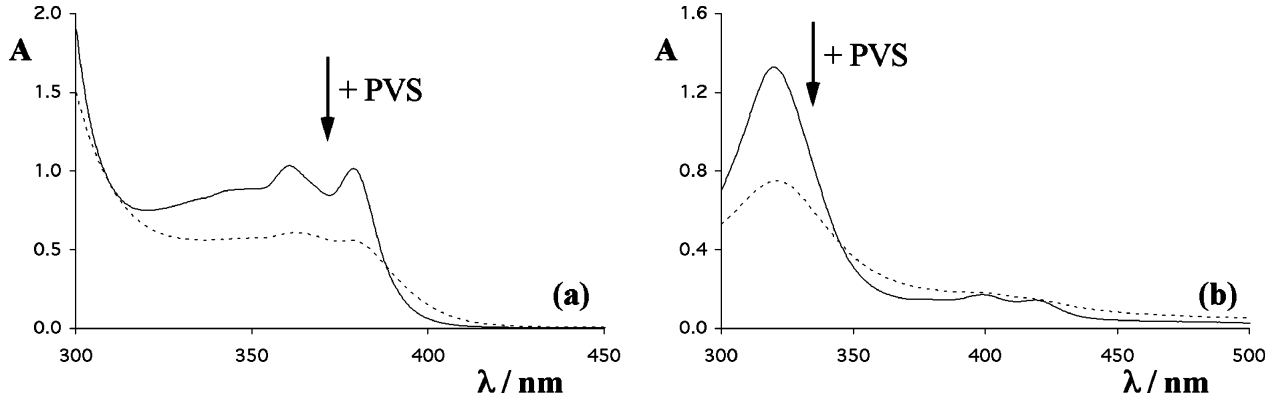

Figure 7. Spectral changes of (a) [Pt(dppz)$(py)_2]^{2+}$ (7.3×10^{-5} M) and (b) [Pt(bdppz)$(py)_2]^{2+}$ (2.5×10^{-5} M) with excess of PVS at $T = 25$ °C and pH 7 (phosphate buffer, 1×10^{-3} M; NaCl, 2.1×10^{-2} M).

circumstance that the LD^r band in the same region is larger than that in the DNA absorption one indicates an almost perpendicular orientation of the bdppz ligand respect to the biopolymer long axis and so its intercalation between DNA base pairs as suggested by the other experiments run in the presence of excess DNA.

External binding around DNA is usually described in terms of surface binding,[24] a type of interaction where a molecule of proper size and shape docks in one of the grooves of the biopolymer. Commonly surface binders are flexible molecules capable of exerting hydrogen bonds. For metal complexes bearing rigid aromatic rings this type of interaction is highly unlikely. A more realistic type of external binding is aggregation along the helix through stacking of the aromatic moiety; such an interaction, rather common for porphyrines and metalloporphyrines,[25] has been reported for a series of monocationic organometallic platinum complexes with terpyridines.[26] The possibility that the spectral variations observed at low [DNA] arise from self-staking of the aromatic moiety of the complexes is shown by experiments with sodium polyvinylsulfonate (PVS). In the presence of this single helix random coil polymer, hypochromism with no significant bathocromic shift (Figure 7) is observed for the complexes with the two most elongated ligands.

In the case of [Pt(dppz)$(py)_2]^{2+}$ and [Pt(bdppz)$(py)_2]^{2+}$ the spectral variations are similar to those observed at low [DNA]/[complex] ratio. The spectral changes induced by PVS, where neither intercalation nor stacking with the aromatic moiety of the complexes is possible, appear to arise from self-aggregation of the complexes by stacking of phenanthroline or extended phenanthroline. The tendency of the complexes to aggregate is shown both by solid-state studies and solution experiments. The complex [Pt(dppz)-Cl_2] forms a two-dimensional stacking structure[27] constructed with platinum−platinum and ligand $\pi-\pi$ interactions in the crystal where the complex cations are stacked in an antiparallel arrangement.

Absorption measurements of aqueous solutions of the complexes studied show that the peaks, as well as their apparent molar absorptivities, depend on the ionic strength; the presence of NaCl or $NaClO_4$ causes hypocromism and a small but significant shift to the blue of the absorption maxima (Figure 8).

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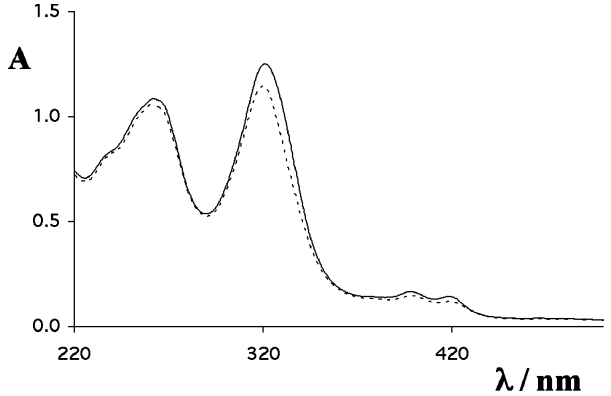

Figure 8. Spectral changes of $[Pt(bdppz)(py)_2]^{2+}$ (2.2×10^{-5} M) at $T =$ 25 °C and pH 7 (phosphate buffer, 1×10^{-3} M) (—) in aqueous solution and (---) in the presence of NaCl (1.0×10^{-1} M).

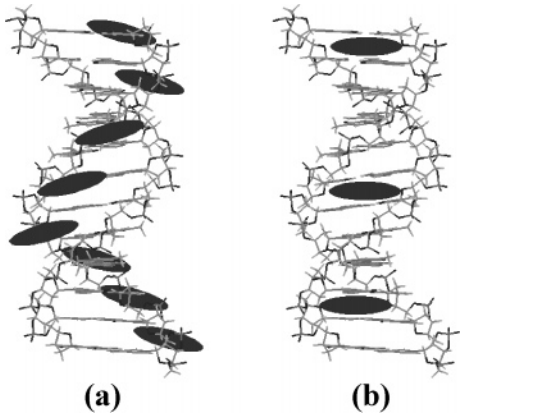

Figure 9. Pictorial representation of the complex molecules: (a) aggregated along the DNA double helix at low [DNA]/[complex]; (b) intercalated at high [DNA]/[complex] ratio.

This behavior can be explained by the formation of aggregated species in solution as a consequence of stacking between the planar aromatic moieties of the complexes. A dimerization model can account for the aggregation up to ionic strength values of 2.0×10^{-1} M,

$$2M \rightleftharpoons D$$

with the equilibrium constant $K_D = [D]/[M]^2$. The dimerization constants were obtained by a nonlinear fitting of the absorption data, using an equation previously reported by Pasternack[28] et al., in which the dimerization constant and the extinction coefficients of monomer and dimer were the parameters to be optimized. By the fitting of the data, the K_D values for $[Pt(bdppz)(py)_2]^{2+}$ and $[Pt(dppz)(py)_2]^{2+}$, at 25 °C, were respectively $(2.85 \pm 0.18) \times 10^4$ M^{-1} (at $I =$ 1.0×10^{-1} M) and $(1.30 \pm 0.07) \times 10^3$ M^{-1} (at $I = 2.0 \times$ 10^{-1} M).

In conclusion two binding modes characterize the interaction of these complexes with double-stranded DNA. At low [DNA]/[complex] ratio, when few intercalation binding sites are available, the complexes bind externally aggregating along the helix; as the [DNA]/[complex] ratio increases the aggregates break up and the complexes intercalate within the base pairs (Figure 9).

The overall behavior of the four substances is strongly influenced by their tendency to aggregate (through stacking of their aromatic moieties); for $[Pt(bdppz)(py)_2]^{2+}$, which exhibits the largest propensity to dimerize, the two binding modes appear to occur separately, at least at the extremes of the [DNA]/[complex] range. For $[Pt(dppz)(py)_2]^{2+}$ the two binding modes seem to coexist almost at any DNA concentration. For $[Pt(dpq)(py)_2]^{2+}$ and $[Pt(phen)(py)_2]^{2+}$, where the tendency to stack is much lower, aggregation along the helix is not significant and the binding equilibrium occurs essentially between free and intercalated form. For this reason, an evaluation of the binding costant value, K_B, for the interaction of the complexes with DNA could be done, through the McGhee−von Hippel equation, only for the complexes with the shortest ligands, phen and dpq ($K_B \approx$ $(2.3 \pm 0.2) \times 10^5$ M^{-1} and $(2.2 \pm 0.3) \times 10^5$ M^{-1}, respectively, at $I = 2.2 \times 10^{-2}$ M). Self-aggregation prevents even a rough rating of the K_B values for the complexes with dppz and bdppz; this circumstance could explain the binding constant value, lower than those of octahedral complexes with the same ligand, reported[9] for $[Pt(dppz)(4\text{-}NH_2py)_2]^{2+}$. Stacking surface is a major factor influencing the binding affinity of a small molecule;[8a,29] as a rule, on increasing extension of the aromatic planar surface the binding affinity for the biopolymer is enhanced. However, a large planar aromatic surface favors also self-aggregation so that under experimental conditions of low [DNA]/[complex] ratio this competing process may become dominant.

Acknowledgment. This work was supported by the MIUR (PRIN program 2003).

IC050880O

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