Inorg. Chem. **2007**, *46*, 7148–7153

Inorganic Chemistry

Article

DNA and RNA Noncovalent Interaction of Platinum(II) Polypyridine Complexes

Matteo Cusumano,* Maria Letizia Di Pietro,* Antonino Giannetto, and Pasquale Antonio Vainiglia

Dipartimento di Chimica Inorganica, Chimica Analitica e Chimica Fisica, University of Messina, 98166 Messina, Italy

Received March 14, 2007

A comparative investigation of the noncovalent interaction of the platinum(II) polypyridine complexes [Pt(dipy)(*n*-Rpy)$_2$]$^{2+}$ and [Pt(4,4′-Me$_2$dipy)(2-Rpy)$_2$]$^{2+}$ (dipy = 2,2′-dipyridine; Me = CH_3; n = 2–4; R = H or CH_3) with double-helical DNA (calf thymus) and RNA [poly(A)•poly(U)] has been conducted. With the exception of [Pt(dipy)(2-Mepy)$_2$]$^{2+}$, all of the complexes interact strongly, by intercalation, with both nucleic acids giving rise to large changes in the electronic spectra and induced circular dichroism signals; in addition, viscosity experiments on rodlike DNA and RNA show that both biopolymers elongate upon interaction with the complexes. The binding constant values, K_B, determined at 25 °C, indicate that, at 0.101 M ionic strength, the affinity for poly(A)•poly(U) is strongly dependent on the complexes nature, while for DNA it is leveled off. [Pt(dipy)(2-Mepy)$_2$]$^{2+}$ binds to DNA but does not interact appreciably with poly(A)•poly(U).

Introduction

The noncovalent interaction of small molecules with nucleic acids has been and continues to be the focus of considerable research.[1] Most of this interest is centered on the potential use of molecules binding to double-stranded DNA[2] as gene regulators and chemotherapeutic agents. Small molecules have also been used as structural and functional probes for nucleic acids; in particular, stable, inert, and water-soluble cationic complexes of transition-metal complexes[3] containing rigid aromatic rings have often been used for this purpose. Ruthenium(II)[4] octahedral and platinum(II)[5] square-planar complexes have proved to be especially valuable to the study of the basic features of nucleic acid binding modes. Most of these studies have involved double-stranded DNA; much less has been done with RNA. Although several investigations have shown that also double-stranded RNA can interact[6] noncovalently by intercalation and groove binding, very little is known on the properties that a small molecule must possess to bind efficiently to this biopolymer. Because of the serious diseases,[7] including AIDS, caused by RNA viruses, there is a considerable interest in developing substances that can bind to the RNA of these viruses and exert antiviral activity. Therefore, in order to design strong RNA binders, it is highly desirable to investigate those factors

* To whom correspondence should be addressed. E-mail: mldipietro@unime.it (M.L.D.), cusumano@unime.it (M.C.).

(1) Demeunynck, M., Bailly, C., Wilson, W. D., Eds. *Small Molecule DNA and RNA Binders: From Synthesis to Nucleic Acid Complexes*; Wiley-VCH: Weinheim, Germany, 2003; Vols. 1 and 2.
(2) (a) Waring, M. J. *Annu. Rev. Biochem*. **1981**, *50*, 159−192. (b) Blackburn, G. M., Gait, M. J. Eds. *Nucleic Acids in chemistry and biology*, 2nd ed.; Oxford University Press: Oxford, U.K., 1996.
(3) (a) Sundquist, W. I.; Lippard, S. J. *Coord. Chem. Rev*. **1990**, *100*, 293−322. (b) Erkkila, K. E.; Odom, T. D.; Barton, J. K. *Chem. Rev*. **1999**, *99*, 2777−2795. (c) Nordén, B.; Lincoln, P.; Åkerman, B.; Tuite, E. *Metal Ions in Biological Systems*; Sigel, A., Sigel, H., Eds.; Marcel Dekker: New York, 1996; Vol. 23, Chapter 7.
(4) (a) O'Donoghue, K.; Penedo, J. C.; Kelly, J. M.; Kruger, P. E. *J. Chem. Soc., Dalton Trans.* **2005**, *6*, 1123−1128. (b) Yavin, E.; Boal, A. K.; Stemp, E. D. A.; Boon, E. M.; Livingston, A. L.; O'Shea, V. L.; David, S. S.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A*. **2005**, *102*, 3546−3551. (c) Moon, S. J.; Moon Kim, J.; Choi Youn, J.; Kim, S. K.; Lee Seung, J.; Jang, H. G. *J. Inorg. Biochem*. **2005**, *99*, 994−1000. (d) Olofsson, J.; Wilhelmsson, L. M.; Lincoln, P. *J. Am. Chem. Soc*. **2004**, *126*, 15458−15465. (e) Urathamakul, T.; Beck, J. L.; Sheil, M. M.; Aldrich-Wright, J. R.; Ralph, S. F. *J. Chem. Soc., Dalton Trans.* **2004**, *17*, 2683−2690.
(5) (a) Cusumano, M.; Di Pietro, M. L.; Giannetto, A.; Vainiglia, P. A. *J. Inorg. Biochem.* **2005**, *99*, 560−565. (b) Che, C.-M.; Ma, D.-L. *Chem.*−*Eur. J.* **2003**, *9*, 6133−6144. (c) Chan, H.-L.; Ma, D.-L.; Yang, M.; Che, C.-M. *J. Biol. Inorg. Chem.* **2003**, *8*, 761−769. (d) Todd, J. A.; Rendina, L. M. *Inorg. Chem.* **2002**, *41*, 3331−3333. (e) Nikolis, N.; Methenitis, C.; Pneumatikakis, G. *J. Inorg. Biochem.* **2003**, *95*, 177−193. (f) Kurosaki, H.; Yamakawa, N.; Sumimoto, M.; Kimura, K.; Goto, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 825−828.
(6) (a) Barton, J. K.; Lippard, S. *Biochemistry* **1979**, *18*, 2661−2668. (b) Gorenstein, D. G.; Lai, K. *Biochemistry* **1989**, *28*, 2804−2812. (c) McConnaughie, A. W.; Spychala, J.; Zhao, M.; Boykin, D.; Wilson, W. D. *J. Med. Chem.* **1994**, *37*, 1063−1069. (d) Kagemoto, A.; Yoshii, A.; Kimura, S.; Baba, Y. *J. Phys. Chem.* **1994**, *98*, 5943−5952. (e) Chand, D. K.; Schneider, H.-J.; Aguilar, J. A.; Escarti, F.; Garcia-España, E.; Luis, S. V. *Inorg. Chim. Acta* **2001**, *316*, 71−78. (f) Sehlstedt, U.; Aich, P.; Bergman, J.; Vallberg, H.; Nordén, B.; Gräslund, A. *J. Mol. Biol.* **1998**, *278*, 31−56.

10.1021/ic700495x CCC: $37.00
© 2007 American Chemical Society
Published on Web 07/26/2007

that favor interaction with this nucleic acid. The structure of RNA differs[8] dramatically from that of DNA. The 2′-OH group of the ribose ring forces the sugar to adopt the 3′-endo conformation in duplex structures, and this results in an A-form double helix. Consequently, the narrow minor groove and wide major groove of B-form duplex DNA are replaced by a wide and very shallow minor groove and a deep and narrow major groove for double-helical RNA. On these grounds, the interaction of double-stranded DNA and RNA with small molecules may well be different.

In this paper, we report the results of a comparative study of the interaction of double-helical RNA [poly(A)·poly(U)] and DNA with a series of polypyridine platinum(II) complexes of the type $[Pt(dipy)(n\text{-}Rpy)_2]^{2+}$ and $[Pt(4,4'\text{-}Me_2\text{-}dipy)(2\text{-}Rpy)_2]^{2+}$ (dipy = 2,2′-dipyridine; Me = CH_3; n = 2−4; R = H or CH_3). Poly(A)·poly(U) is not simply a good model for natural RNA; it exhibits also an appreciable biological activity because it is capable of activating the synthesis of interferon in vivo.[9] Some of the complexes used are potent DNA intercalators via a dipyridyl ligand.[10] Probably owing to its reduced size, this ligand is capable of slipping easily between adjacent base pairs of the biopolymer, giving rise to efficient intercalative interaction. The presence of two *cis*-pyridine ligands skew to the square plane, and the resulting three-dimensional feature makes these complexes very suitable structural probes for nucleic acids.

Experimental Section

Chemicals. Calf thymus DNA and poly(A)·poly(U) were purchased from Sigma Chemical Co. DNA was purified as previously described;[11] poly(A)·poly(U) was dissolved as received in a phosphate buffer containing the desired amount of NaCl to adjust the ionic strength. Their concentration, expressed in base pairs, was determined spectrophotometrically using the molar absorptivities 1.31×10^4 M^{-1} cm^{-1} (260 nm)[12] and 1.40×10^4 M^{-1} cm^{-1} (257 nm)[13] for DNA and poly(A)·poly(U), respectively.

NaCl and other chemicals were of reagent-grade and were used without further purification.

The complexes were prepared by using the method reported in ref 10. To this end, solid $[Pt(dipy)Cl_2]$ or $[Pt(4,4'\text{-}Me_2dipy)Cl_2]$ was suspended in an aqueous solution of the appropriate pyridine and heated to boiling until dissolution of the solid. After filtration of the solution to remove a small amount of undissolved material, the complexes were precipitated by the addition of ammonium hexafluorophosphate, washed with water, and crystallized from methanol. Their purity was checked by elemental analysis and ^1H and ^{13}C NMR. The molar conductivity values of the complexes, in CH_3CN, ranged between 220 and 300 S cm^2 M^{-1}, as expected for 1:2 electrolytes.

Methods. All experiments were carried out at 25 °C and pH 7, in a 1×10^{-3} M phosphate buffer and enough NaCl to give the desired ionic strength (I).

The pH was measured with a PHM 62 radiometer.

Absorption spectra were recorded using a Cintra 20 GBC or a Lambda 16 Perkin-Elmer spectrophotometer.

^1H and ^{13}C NMR spectra were recorded on a Bruker ARX-300 spectrometer.

Circular dichroism (CD) spectra were recorded on a Jasco J-810 polarimeter.

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 a sonicated DNA or poly(A)·poly(U) solution, in a 1×10^{-3} M phosphate buffer (pH 7) and 0.021 or 0.1 M NaCl. The complex solution (2.5×10^{-4} M), containing also DNA (6.0×10^{-4} M) or poly(A)·poly(U) (8.0×10^{-4} M) at the same concentration as that in the viscometer, was delivered in increments of 90−180 μL from a micropipette. Solutions were freed of particulate material by passing them through nylon Acrodisc syringe filters before use. Flow times were measured by hand with a digital stopwatch. Reduced viscosities were calculated by established methods and plotted as $\ln \eta/\eta_0$ against $\ln(1 + r)$ for rodlike DNA (600 base pairs) or poly(A)·poly(U) (500 base pairs) (η = reduced viscosity of the biopolymer solution in the presence of the complex; η_0 = reduced viscosity of the biopolymer solution in the absence of the complex; $r = [\text{complex}]_{bound}/[\text{biopolymer}]_{tot}$).

Binding Constant Determination. Spectrophotometric titrations were performed by adding to a complex solution (3.75×10^{-5} M) successive aliquots of DNA or poly(A)·poly(U), 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.[14] 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 per mole of DNA. Extinction coefficients for bound compounds were determined by Beer's law plots in the presence of a large excess of DNA or poly(A)·poly(U).

Results

The complexes under study are very stable in aqueous solution in a wide pH range (3−11). The independence of the electronic spectra of the presence of large amounts of salt (NaCl or $NaClO_4 \approx 1$ M) shows that the substances do not have a significant propensity to self-aggregation. Marked changes in the chemicophysical properties of the complexes and of the two biopolymers, caused by the addition of DNA or poly(A)·poly(U) to the complex solutions, show their interaction. With the exception of $[Pt(dipy)(2\text{-}Mepy)_2]^{2+}$, the addition of DNA or RNA to the complexes at fixed ionic strength and pH 7 causes immediate spectral variations

(7) (a) Ehrenfeld, E.; Hund, T. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1075−1078. (b) Lower, R.; Boller, K.; Hasenmeier, B.; Kormacher, C.; Muller-Lantzsch, N.; Kurth. R. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 4480−4484. (c) DeClercq, E., Ed. *Design of Anti-AIDS Drugs*; Pharmacochemistry Library Series; Elsevier: New York, 1990; Vol. 14.

(8) (a) Dock-Bregeon, A. C.; Chevrier, B.; Podjarny, A.; Moras, D.; de Bear, J. S.; Gough, G. R.; Gilham, P. T.; Johnson, J. E. *Nature (London)* **1988**, *335*, 375−378. (b) Arnott, S. *Prog. Biophys. Mol. Biol.* **1970**, *21*, 265−319. (c) Holbrook, S. R.; Sussman, J. L.; Warrant, R. W.; Kim, S.-H. *J. Mol. Biol.* **1978**, *123*, 631−660.

(9) Hirabayashi, K.; Yano, J.; Inoue, T.; Yamaguchi, T.; Tanigawara, K.; Smyth, G. E.; Ishiyama, K.; Ohgi, T.; Kimura, K.; Irimura, T. *Cancer Res.* **1999**, *59*, 4325−4333.

(10) (a) Cusumano, M.; Di Pietro, M. L.; Giannetto, A. *Chem. Commun.* **1996**, 2527−2528. (b) Cusumano, M.; Di Pietro, M. L.; Giannetto, A.; Nicolǒ, F.; Rotondo, E. *Inorg. Chem.* **1998**, *37*, 563−568.

(11) Cusumano, M.; Giannetto, A. *J. Inorg. Biochem.* **1997**, *65*, 137−144.

(12) Wells, R. D.; Larson, J. E.; Grant, R. C.; Shortle, B. E.; Cantor, C. R. *J. Mol. Biol.* **1970**, *54*, 465−497.

(13) Chalikian, T. V.; Völker, J.; Plum, G. E.; Breslauer, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 7853−7858.

(14) McGhee, J. D.; Von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469−489.

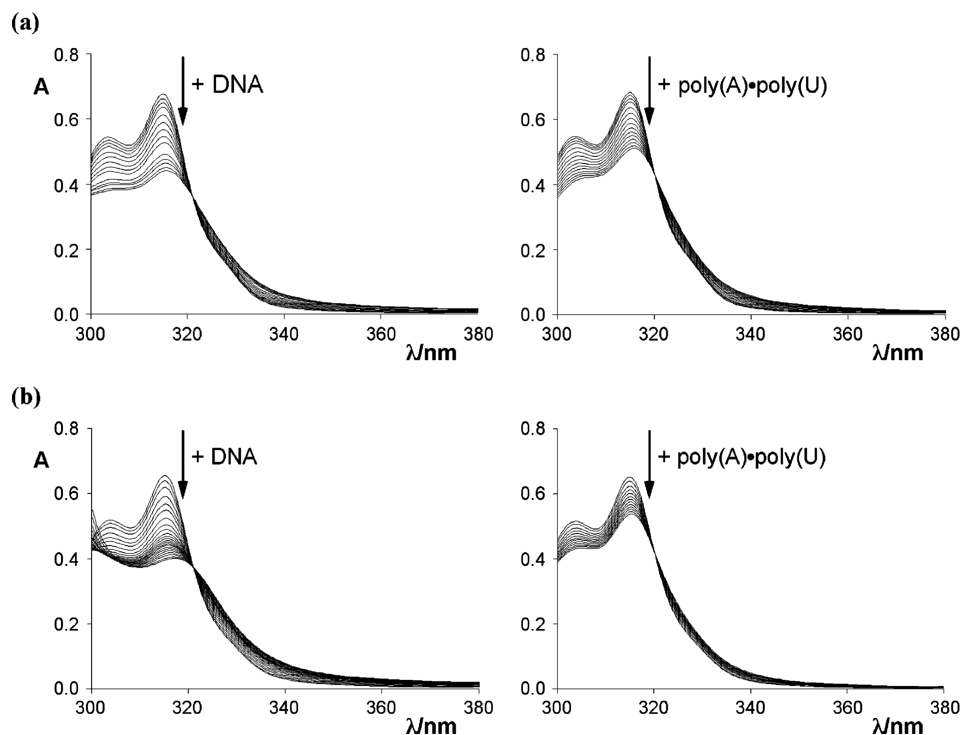

Figure 1. Spectrophotometric titrations, at T = 25 °C and pH 7 (phosphate buffer, 1 × 10^{-3} M; NaCl, 0.1 M), of (a) $[Pt(4,4'-Me_2dipy)(py)_2]^{2+}$ (3.75 × 10^{-5} M) and (b) $[Pt(4,4'-Me_2dipy)(2-Mepy)_2]^{2+}$ (3.75 × 10^{-5} M) with DNA and poly(A)·poly(U).

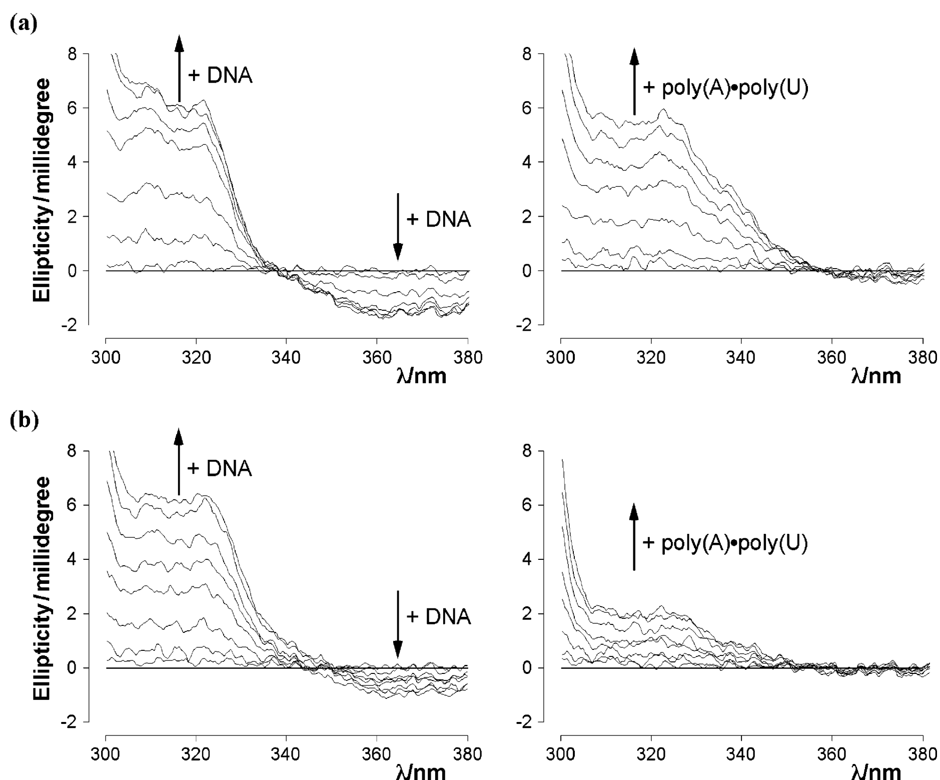

Figure 2. Spectropolarimetric titrations, at T = 25 °C and pH 7 (phosphate buffer, 1 × 10^{-3} M; NaCl, 0.1 M), of (a) $[Pt(dipy)(py)_2]^{2+}$ (3.75 × 10^{-5} M) and (b) $[Pt(dipy)(2-Mepy)_2]^{2+}$ (3.75 × 10^{-5} M) with DNA and poly(A)·poly(U).

characterized by strong hypochromism and a small shift to the red. Figure 1 reports the spectrophotometric titrations of $[Pt(4,4'-Me_2dipy)(py)_2]^{2+}$ and $[Pt(4,4'-Me_2dipy)(2-Mepy)_2]^{2+}$ with DNA and RNA. The spectral variations are very similar, suggesting a similar binding mode of the complexes with both nucleic acids.

The interaction with chiral DNA and RNA induces CD in all of the complexes. The CD spectra for the various substances show similar features (Figure 2); the signals that appear where the bound complexes absorb are characterized by a small, or very small, negative band in the 340−380 nm region and a positive one with a maximum at about 320

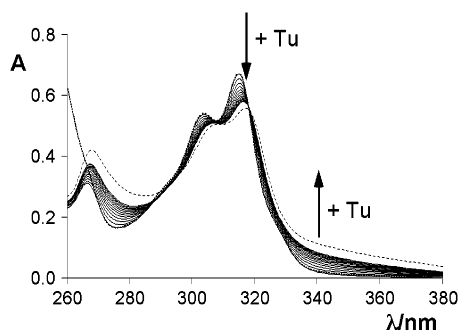

Figure 3. Spectral changes, at $T = 25$ °C and pH 7 (phosphate buffer, 1 × 10^{-3} M; NaCl, 0.021 M), of $[Pt(4,4'-Me_2dipy)(py)_2]^{2+}$ (3.75 × 10^{-5} M) with thiourea (0.01 M): (···) complex alone; (—) spectra of the mixture complex/thiourea registered every 5 min; (- - -) spectrum of the mixture registered after 3 h.

nm. The isodichroic point in DNA always occurs at a shorter wavelength than that in poly(A)·poly(U). The shift in the wavelength is smaller for the complexes bearing a 2-methylpyridine ligand; in addition, the CD signal for the interaction of $[Pt(dipy)(2\text{-}Mepy)_2]^{2+}$ with poly(A)·poly(U) is much smaller than that in any other case.

In principle, the RNA and DNA nucleobases can bind covalently to platinum by their nucleophilic sites. However, the inertness of the complexes used rules out this event. Thiourea, one of the strongest nucleophiles toward platinum(II), produces spectral changes upon reaction with the complexes (Figure 3) that occur much more slowly than the interaction of the same substances with poly(A)·poly(U) or DNA.

The noncovalent character of the processes is confirmed by their reversibility. Both the UV−vis and CD spectral variations can be fully reversed by the addition of sodium chloride or sodium perchlorate.

The viscosity of rodlike DNA and poly(A)·poly(U) solutions increases upon interaction with the complexes. Figure 4 shows the viscosity enhancement induced on both biopolymers by increasing amounts of $[Pt(dipy)(py)_2]^{2+}$ and $[Pt(dipy)(2\text{-}Mepy)_2]^{2+}$ at a 0.101 M ionic strength value and, for comparison, at $I = 2.2 \times 10^{-2}$ M on DNA. While for the former complex the increase of viscosity is comparable in all of the titrations, in the case of $[Pt(dipy)(2\text{-}Mepy)_2]^{2+}$ at high salt concentration there is a lower viscosity enhancement for both DNA and poly(A)·poly(U). As expected, the non-intercalator complex $[Pt(en)(py)_2]^{2+}$ does not produce any significant increase in the viscosity of both nucleic acids.

In order to obtain the binding constant values between the complexes and the two biopolymers, the results of the spectrophotometric titrations conducted at a 0.101 M ionic strength value have been reported in the form of Scatchard plots of r/m vs m, where r is the binding ratio $[complex]_{bound}/[DNA]_{tot}$ and m is the concentration of the unbound complex. The data were analyzed via the McGhee−Von Hippel equation[14] based on the nearest-neighbor exclusion model using a nonlinear least-squares program. The resulting binding constant values, K_B, are summarized in Table 1, together, for comparison, with those obtained for the interaction of the complexes with DNA at a 0.022 M ionic strength value.

Discussion

Inspection of Table 1 shows that the complexes interact preferentially with DNA. Almost in all cases are the binding constants with DNA larger than those with RNA. In addition, if we compare the data for the two biopolymers at the same ionic strength (0.101 M), we notice significant differences; while the K_B values for the interaction of the various complexes with DNA are similar, those relative to poly(A)·poly(U) are considerably dependent on the complex nature. Particularly relevant is the behavior of $[Pt(dipy)(2\text{-}Mepy)_2]^{2+}$; this complex does not show any significant electronic communication between its aromatic moiety and the RNA nucleobases, as shown by the absence of any noteworthy spectral change upon an increase in the amount of biopolymer, but interacts fairly to DNA behaving as an efficient spectroscopic probe capable of distinguishing between ribo- and deoxyribonucleotides. This appears to be a remarkable result because commonly nucleic acid binders display little selectivity[15] for DNA or RNA.

The data show also that the introduction of two methyl groups at the 4 and 4′ positions of 2,2′-dipyridine increase by about 1 order of magnitude the binding constant with RNA, while that with DNA remains almost constant. Again the presence of one methyl group at the 2 position of pyridine lowers substantially the affinity for RNA without altering that for DNA. By contrast, if we compare the binding constant values for RNA with the corresponding values for DNA at low ionic strength ($I = 0.022$ M), we notice that the affinity sequence for the two biopolymers is the same. The observed affinity dependence for RNA at $I = 0.101$ M and for DNA at $I = 0.022$ M along the series of complexes examined is in line with an intercalative binding mode.

The role of the methyl groups in the binding of the complexes to DNA and RNA is interesting. Methyl groups are known to exert a strong hydrophobic effect, increasing the affinity for the biopolymers; thus, the complexes methylated at dipyridine or at the 3 or 4 position of pyridines have DNA and RNA affinity larger than that for the corresponding unsubstituted substrates. However, when the complexes bear 2-methyl groups at pyridines, the affinity for the two biopolymers decreases, becoming lower than that for the unmethylated complexes. The position of these substituents on pyridines, confined out of the nucleobases because two pyridines bound to the central metal in a relative cis position do not lie in the plane and must assume a skew position,[16] can, in fact, reduce the portion of the intercalating moiety and so the binding affinity. $[Pt(dipy)(2\text{-}Mepy)_2]^{2+}$ exists[17] as a 1:1 mixture of "head-to-head" s-cis and "head-to-tail" s-trans rotamers (Figure 5), characterized by a very high activation energy of interchange. In both rotamers, the

(15) Wilson, W. D.; Ratmeyer, L.; Zhao, M.; Strekowski, L.; Boykin, D. *Biochemistry* **1993**, *32*, 4098−4104.
(16) (a) Colamarino, P.; Orioli, P. L. *J. Chem. Soc., Dalton Trans.* **1975**, 1656−1659. (b) Deacon, G. B.; Gatehouse, B. M.; Grayson, I. L.; Nesbit, M. C. *Polyhedron* **1984**, *6*, 753−755. (c) Kubiak, M.; Kuduk-Jaworska, J. *Acta Crystallogr.* **1986**, *C42*, 1703−1705. (d) Liu, H.-Q.; Peng, S.-M.; Che, C. M. *Chem. Commun.* **1995**, 509−510.
(17) Rotondo, E.; Bruschetta, G.; Bruno, G.; Rotondo, A.; Di Pietro, M. L; Cusumano, M. *Eur. J. Inorg. Chem.* **2003**, 2612−2618.

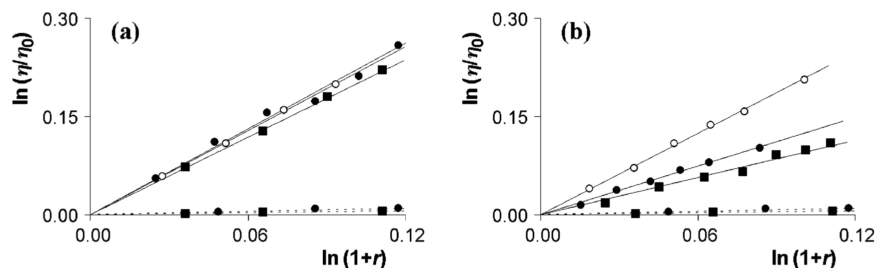

Figure 4. Viscometric titrations of sonicated DNA (6.0×10^{-4} M) and poly(A)·poly(U) (8.0×10^{-4} M) with (a) $[Pt(dipy)(py)_2]^{2+}$ and (b) $[Pt(dipy)(2\text{-}Mepy)_2]^{2+}$, at $T = 25$ °C and pH 7 (phosphate buffer, 1×10^{-3} M): (○) DNA at $I = 0.022$ M; (●) DNA at $I = 0.101$ M; (■) poly(A)·poly(U) at $I = 0.101$ M. In both figures are reported the results of the nucleic acid titrations with $[Pt(en)(py)_2]^{2+}$ (- - -).

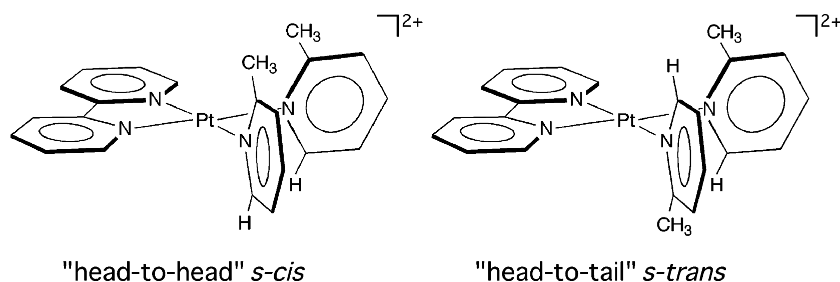

Figure 5. Representation of the two rotamers of $[Pt(dipy)(2\text{-}Mepy)_2]^{2+}$.

Table 1. Values of Binding Constants (K_B, M^{-1}) for the Interaction of the Complexes with DNA and Poly(A)·poly(U), at $T = 25$ °C in 1.0×10^{-3} M Phosphate Buffer (pH 7) at the Quoted Ionic Strength

	$10^{-3} K_B$, M^{-1}		
	DNA		poly(A)·poly(U)
complex	$I = 0.022$ M	$I = 0.101$ M	$I = 0.101$ M
$[Pt(dipy)(py)_2]^{2+}$	162[a] (±2)	31.0 (±0.9)	6.85 (±0.4)
$[Pt(dipy)(2\text{-}Mepy)_2]^{2+}$	20.5[a] (±0.2)	16.2 (±0.6)	very small
$[Pt(dipy)(3\text{-}Mepy)_2]^{2+}$	376 (±10)	52.5 (±0.9)	11.7 (±0.4)
$[Pt(dipy)(4\text{-}Mepy)_2]^{2+}$	270[a] (±7)	53.4 (±0.8)	10.5 (±0.4)
$[Pt(4,4'\text{-}Me_2dipy)(py)_2]^{2+}$	2630 (±50)	36.0 (±0.6)	45.0 (±0.8)
$[Pt(4,4'\text{-}Me_2dipy)(2\text{-}Mepy)_2]^{2+}$	150 (±8)	23.7 (±0.5)	5.77 (±0.5)

[a] Values from ref 10.

methyl substituents at pyridine, pointing at platinum, greatly reduce the portion of the aromatic dipyridine that can be inserted within the double helix.

It is tempting to attribute the abnormal behavior of DNA at high ionic strength to a binding mode other than intercalation. Aggregation along the DNA double helix through stacking of the aromatic moiety occurs commonly for porphyrins[18] and has been reported also for some organometallic platinum complexes[19] with terpyridines and, more recently, for some platinum(II) square-planar complexes[20] with very extended aromatic planar-ligand-like dipyrido[3,2-*a*:2′,3′-*c*]phenazine or benzodipyrido[*b*:3,2-*h*:2′3′-*j*]phenazine at a low [DNA]/[complex] ratio, when few intercalation binding sites are available. This binding mode, where the complex cations stack together through the aromatic moiety following the negative charges of a sugar phosphate backbone, should be less affected by the presence of substituents at the pyridine ring. Both intercalation and aggregation along the helix imply stacking of aromatic rings, which leads to hypochromism in the absorption maxima. Therefore, the spectral changes observed for interaction of the complexes with DNA and RNA are compatible with both types of interactions. However, the experimental data show also an increase in the viscosity of both polynucleotides upon interaction with all of the complexes. A significant increase in the viscosity of double-stranded DNA or RNA has been related[21] to elongation of the double helix consequent to intercalation. A key feature of the classic intercalation model[22] first proposed by Lerman is the lengthening and unwinding of the DNA helix as the base pairs are separated to accommodate the intercalator. By contrast, molecules that bind in the DNA groove cause less pronounced changes or no changes in the DNA solution viscosity. Therefore, the leveling off for the binding values of the complexes studied appears to be a feature[23] of the intercalation in DNA at high salt concentration.

(18) (a) Pasternack, R. F.; Bustamante, C.; Collings, P. J.; Giannetto, A.; Gibbs, E. J. *J. Am. Chem. Soc*. **1993**, *115*, 5393−5399. (b) Pasternack, R. F.; Schaefer, K. F. *Inorg. Chem.* **1994**, *33*, 2062−2065. (c) Hudson, B. P.; Sou, J.; Berger, D. J.; McMillin, D. R. *J. Am. Chem. Soc*. **1992**, *114*, 8997−9002. (d) Kano, K.; Fukuda, K.; Wakami, H.; Nishiyabu, R.; Pasternack, R. F. *J. Am. Chem. Soc*. **2000**, *122*, 7494−7502.
(19) (a) Arena, G.; Monsù Scolaro, L.; Pasternack, R. F.; Romeo, R. *Inorg. Chem.* **1995**, *34*, 2994−3002. (b) Casamento, M.; Arena, G. E.; Lo Passo, C.; Pernice, I.; Romeo, A.; Monsù Scolaro, L. *Inorg. Chim. Acta* **1998**, *275*, 242−249.
(20) Cusumano, M.; Di Pietro, M. L.; Giannetto, A. *Inorg. Chem.* **2006**, *45*, 230−235.
(21) Cohen, G.; Eisenberg, H. *Biopolymers* **1966**, *4*, 429−440.
(22) Lerman, L. S. *J. Mol. Biol*. **1961**, *3*, 18−30.
(23) Manning, G. S. *Q. Rev. Biophys*. **1978**, *11*, 179−246.

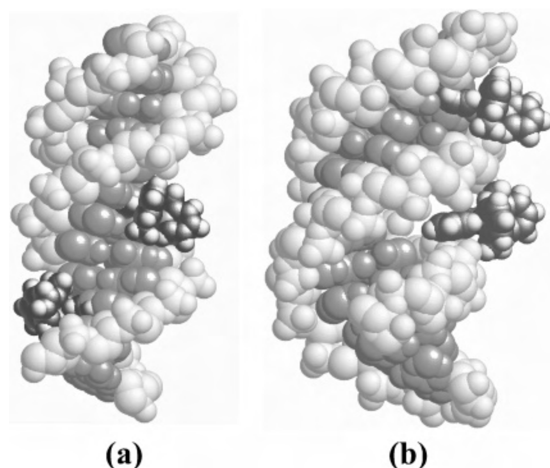

Figure 6. Pictorial representation for the interaction of $[Pt(dipy)(2\text{-}Mepy)_2]^{2+}$ with a double-helical: (a) DNA; (b) RNA.

In conclusion, with the exception of $[Pt(dipy)(2\text{-}Mepy)_2]^{2+}$, all of the complexes studied here intercalate between adjacent base pairs in both DNA and RNA. At high ionic strength, the leveling off of the binding constant values for the interaction of the substances with DNA, unlike RNA, is probably due to the different accessibility of the bases in the B and A forms of the double helix. The A form adopted by poly(A)•poly(U), in fact, probably makes the insertion of the complexes inside adjacent base pairs more difficult than that in the B form of DNA. This results in a different discriminant capability of the complexes toward DNA and RNA as a function of the complex nature, in spite of the leveling off effect of the high ionic strength values on the intercalation process. Moreover, this minor accessibility of the bases in the A form is probably at the origin of the absence of any significant spectral change for the interaction of $[Pt(dipy)(2\text{-}Mepy)_2]^{2+}$ with RNA, even though the same complex intercalates into a DNA double helix and the tendency to insert its aromatic moiety also between the RNA nucleobases is shown by the increase of the poly(A)•poly-(U) viscosity, suggesting its application as a spectroscopic probe capable of distinguishing between DNA and RNA (Figure 6).

Acknowledgment. This work was supported by funds from PRA (Progetto di Ricerca d'Ateneo) of University of Messina.

IC700495X