Influence of DNA on the Rate of Ligand Substitution in Platinum(II) Terpyridine Complexes

Matteo Cusumano,* Maria Letizia Di Pietro, Antonino Giannetto, and Francesco Romano

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

Received June 25, 1999

The kinetics of substitution of pyridine or 2-methylpyridine, by iodide or thiourea, in the complexes [Pt(4′-R′terpy)(2-Rpy)]$(BF_4)_2$ (R′ = *o*-tolyl or H; R = H or CH_3) has been studied, at 25 °C, pH 7, and various ionic strength values, in the presence of and without calf thymus DNA. The reactions occur in one observable step, and plots of k_{obsd} against nucleophile concentration give straight lines with zero intercepts. DNA inhibits all the reactions studied without altering the rate law; the second-order rate constants k_2 decrease systematically on increasing DNA concentration and are larger at higher ionic strength values. Partitioning of the ionic reactants in solution on electrostatic grounds can account for this kinetic effect in the reaction with iodide. Iodide is kept off the double helix proximity while the dicationic complexes concentrate on it. The inhibiting effect observed for the uncharged reagent thiourea can be related to the specific binding mode of the complexes to DNA. The complexes studied are effective intercalators to double helix, and this type of interaction, which prevents attack of thiourea at platinum, decreases their actual concentration in solution. The inhibiting effect is larger for $[Pt(terpy)(py)]^{2+}$ that is a better intercalator. Likewise, the decrease in the rate of substitution of 2-Rpy, at a given [DNA] on decreasing ionic strength, is due to the influence of ionic strength on the complex−DNA interactions.

Introduction

Transition metal complexes can interact noncovalently[1] with nucleic acids by intercalation, groove binding, and external electrostatic binding. The recognition of double helix DNA, from a metal complex, is based on these supramolecular noncovalent interactions, and the design and construction of suitable transition metal complexes for use as structural probes for nucleic acids has been an area of active research[2] in the last 15 years.

Guest−host supramolecular interactions induce changes in electronic and steric properties that may result in profound modification in the reactivity of the species and sometimes also in the reaction mechanisms. It is well-known that reactions carried out in the presence of micelles[3] or polyelectrolytes[4] may be accelerated or inhibited by very large factors. Likewise the inclusion in cyclodextrins[5] alters, often to large extent, the reactivity of the interacting molecules. Also double helix DNA, thanks to its polyanionic nature and the variety of interactions with small cationic molecules, can produce kinetic effects on the species that bind noncovalently with it. Little work has been done,[6] however, in this field. In this paper we study the influence of DNA on the reactivity of a series of (terpyridine)platinum(II) complexes whose interaction with double helix DNA is well established.[7] The kinetic behavior of these substances has also been thoroughly examined.[8] We report here the results of a kinetic study of the reaction between the complexes [Pt(terpy)(*n*-Rpy)]$(BF_4)_2$ (*n*-R = H, 2-CH_3), and [Pt(4′-*o*-tolylterpy)(2-CH_3py)]$(BF_4)_2$ with thiourea or iodide, in the presence of calf thymus DNA, at 25 °C and various ionic strength values. The reactions are inhibited by the DNA, and the effect is related to the type and degree of interaction. In addition, we have studied the noncovalent interaction of the complex [Pt(4′-*o*-tolylterpy)(2-CH_3py)]$(BF_4)_2$ with calf thymus DNA and determined the binding constant, K_B, at 25 °C and various ionic strength values.

Experimental Section

Materials. (i) 4′-*o*-Tolyl-2,2′:6′,2″-terpyridine was synthesized with the method reported by Constable[9] for the corresponding 4′-phenyl derivative. To this end 2-acetylpyridine was added dropwise and with stirring to an *o*-tolylaldehyde emulsion in ethanol and aqueous solution of sodium hydroxide. The mixture was stirred for about 20 h at room temperature; the resulting white precipitate of 3-*o*-tolyl-1,5-bis(2-pyridyl)-1,5-pentadione, crystallized from ethanol, was dissolved in dimethylformammide, treated with ammonium acetate in large excess (1:50), and heated at 150 °C for 1 week. The mixture was evaporated

(1) (a) Nordén, B.; Lincoln, P.; Åkerman, B.; Tuite, E. *Metal Ions in Biological Systems;* Sigel, A., Sigel, H., Eds.; Marcel Dekker: New York, 1996; Vol. 33, Chapter 7. (b) Sundquist, W. I.; Lippard, S. J. *Coord. Chem. Rev.* **1990,** *100*, 293.
(2) Pyle, A. M.; Barton, J. K. *Progr. Inorg. Chem.* **1990**, *38*, 413. Dupureur, C. M.; Barton, J. K. *Inorg. Chem.* **1997**, *36*, 33.
(3) (a) Bunton, C. A.; Savelli G. *Adv. Phys. Org. Chem.* **1986**, *22*, 213. (b) Burgess, J.; Pelizzetti, E. *Gazz. Chim. Ital.* **1988**, *12*, 803. (c) Cusumano, M.; Giannetto, A.; Cavasino F. P.; Sbriziolo C. *Inorg. Chim. Acta* **1992**, *201*, 197.
(4) (a) Ise, N.; Matsui, F. *J. Am. Chem. Soc.* **1968**, 4242. (b) Morawetz, H.; Vogel, B. *J. Am. Chem. Soc.* **1969**, *91*, 563. (c) Morawetz, H. *Adv. Catal.* **1969**, *20*, 341.
(5) (a) Shortreed, M. E.; Wylie, R. S.; Macartney, D. H. *Inorg Chem.* **1993**, *32*, 1824. (b) Imonigie, J. A.; Macartney, D. H. *J. Chem. Soc., Dalton Trans.* **1993**, 891. (c) Granados, A.; de Rossi, R. H. *J. Am. Chem. Soc.* **1995**, *117*, 3690.
(6) Pasternack, R. F.; Gibbs, E. J.; Santucci R.; Shaertel, S.; Ellinas, P.; Sah, S. C. *Chem. Commun.* **1987**, 1771.
(7) (a) McCoubrey, A.; Latham, H. C.; Cook P. R.; Rodger, A.; Lowe, G. *FEBS Lett.* **1996**, *380*, 73. (b) Cusumano, M.; Di Pietro, M. L.; Giannetto, A. *Inorg. Chem.* **1999**, *38*, 1754.
(8) (a) Mureinik, R. J.; Bidini, M. *Inorg. Chim. Acta* **1978**, *29*, 37. (b) Cusumano, M.; Guglielmo, G.; Ricevuto, V. *Inorg. Chim. Acta* **1978**, *27*, 197.
(9) Constable, E. C.; Lewis, J.; Liptrot, M. C.; Raithby, P. R. *Inorg. Chim. Acta* **1990**, *178*, 47.

10.1021/ic990747u CCC: $19.00
Published on Web 12/17/1999

to dryness under reduced pressure, and 4′-*o*-tolyl-2,2′:6′,2″-terpyridine was crystallized from ethanol.

[Pt(terpy)(*n*-Rpy)]$(BF_4)_2$ (*n*-R = H, 2-CH_3) and [Pt(4′-*o*-tolylterpy)-(2-CH_3py)]$(BF_4)_2$ were prepared as described in the literature.[10] 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.[11] DNA concentration, expressed in base pairs, was calculated spectrophotometrically using an $\epsilon_{260\ nm}$ value of 1.31×10^4 M^{-1} cm^{-1}.[12] NaCl, $NaNO_3$, and other chemicals were of reagent grade and were used without further purification.

Methods. All experiments were carried out at 25 °C, pH 7, in a phosphate buffer 1×10^{-3} M ($[KH_2PO_4]/[Na_2HPO_4] = 1.61$) and enough NaCl or $NaNO_3$ to give the desired ionic strength.

pH was measured with a Radiometer PHM 62.

Absorption spectra were recorded using a Lambda 5 Perkin-Elmer spectrophotometer.

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

Thermal denaturation experiments were performed as previously described.[7b]

Also the binding constants for the interaction between CT DNA and [Pt(4′-*o*-tolylterpy)(2-CH_3py)]$^{2+}$ were determined spectrophotometrically, using the McGhee and von Hippel equation,[13] as previously described[7b] for the related complexes [Pt(terpy)(2-Rpy)]$^{2+}$ (R = H or CH_3).

Kinetics. The kinetics of ligand substitution, by thiourea, in the reported complexes was followed spectrophotometrically, in the range 320−400 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 stopped-flow syringes contained respectively the complex and the nucleophile or the mixture nucleophile−DNA. The ionic strength of both solutions was the same ($NaNO_3$) and the pH 7 (1×10^{-3} M phosphate buffer). The absorbance changes were displayed on a Macintosh SE/30 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 $A_t = A_\infty + (A_0 - A_\infty)\exp(-k_{obsd}t)$, where A_0, A_∞, and k_{obsd} were the parameters to be optimized (A_0 = absorbance after mixing of the reagents, A_∞ = absorbance at completion of reaction). The k_{obsd} values were reproducible to better than ±5%. The second-order rate constants, k_2, for the reactions in the presence and absence of DNA were obtained by least-squares analysis of the plots of k_{obsd} against [nucleophile].

Results

The complexes under study are very stable in aqueous solution; in the presence of chloride ions, however, slow substitution of pyridine occurs. To avoid this competing reaction, the ionic strength of the solutions was adjusted with sodium nitrate instead of sodium chloride.

In the presence of DNA, the electronic spectrum of [Pt(4′-*o*-tolylterpy)(2-CH_3py)]$^{2+}$ shows decrease in the peak intensities (hypochromicity) and red shifts of the bands (Figure 1). In addition, the thermal denaturation temperature of DNA, in the presence of [Pt(4′-*o*-tolylterpy)(2-CH_3py)]$^{2+}$, increases by over 10 °C as expected for a dicationic intercalator.[14] These findings suggest that, as already shown for [Pt(terpy)(*n*-Rpy)]$^{2+}$ (*n*-R = H, 2-CH_3), [Pt(4′-*o*-tolylterpy)(2-CH_3py)]$^{2+}$ intercalates to DNA. The presence of the *o*-tolyl substituent at terpyridine does not alter significantly the interaction of the complex. The binding constant K_B for [Pt(4′-*o*-tolylterpy)(2-CH_3py)]$^{2+}$ ($(3.3 \pm 0.4) \times 10^3$), determined spectrophotometrically using the McGhee−von Hippel approach at 25 °C and 0.158 M ionic strength, is similar to that reported for [Pt(terpy)(2-CH_3py)]$^{2+}$ ($(3.0 \pm 0.3) \times 10^3$).[7b]

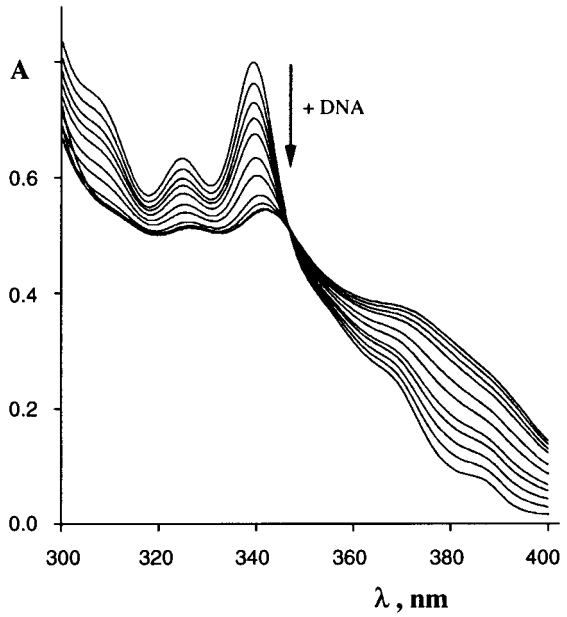


Figure 1. Spectrophotometric titration of [Pt(4′-*o*-tolylterpy)(2-CH_3py)]$^{2+}$ with DNA at 25 °C and pH 7 (phosphate buffer, 1.0×10^{-3} M; $NaNO_3$, 1.5×10^{-1} M).

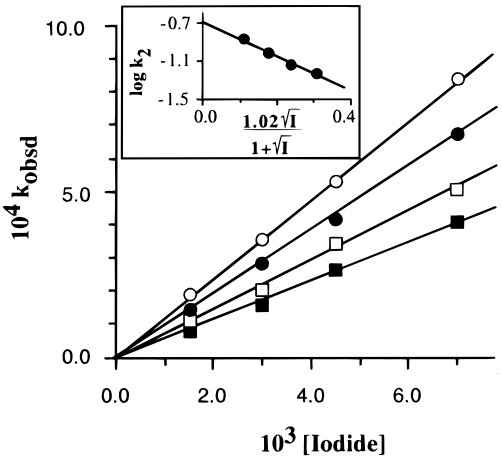


Figure 2. Plot of k_{obsd} (s^{-1}) for reaction of [Pt(terpy)(2-CH_3py)]$^{2+}$ with iodide vs [iodide] (T = 25 °C; pH = 7), at various ionic strength values: ○, I = 0.013 M; ●, I = 0.0395 M; □, I = 0.079 M; ■, I = 0.158 M. The inset shows the second-order dependence for the reaction on ionic strength, according to the Bronsted−Bijerrum−Christiansen equation.

The reaction between the complexes and the nucleophiles thiourea and iodide occurs according to the following scheme:

$$[Pt(4'\text{-}R'terpy)(2\text{-}Rpy)]^{2+} + Y^{n-} \rightarrow [Pt(4'\text{-}R'terpy)(Y)]^{(2-n)+} + 2\text{-}Rpy \quad (1)$$

Large spectral variations in the UV−visible region characterize the process that can be conveniently monitored spectrophotometrically; only one step of reaction is observed.

Under pseudo-first-order conditions with respect to the complex, the k_{obsd} values are linearly correlated to the nucleophile concentration:

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

(10) Lowe, G.; Vilaivan, T. *J. Chem. Res. Synop.* **1996**, 386.
(11) Cusumano, M.; Giannetto, A. *J. Inorg. Biochem.* **1997**, *65*, 137.
(12) Wells, R. D.; Larson, J. E.; Grant, R. C.; Shortle, B. E.; Cantor, C. R. *J. Mol. Biol.* **1970**, *54*, 465.
(13) McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469.
(14) Cusumano, M.; Di Pietro, M. L.; Giannetto, A.; Nicolò, F.; Rotondo, E. *Inorg. Chem.* **1998**, *37*, 563.

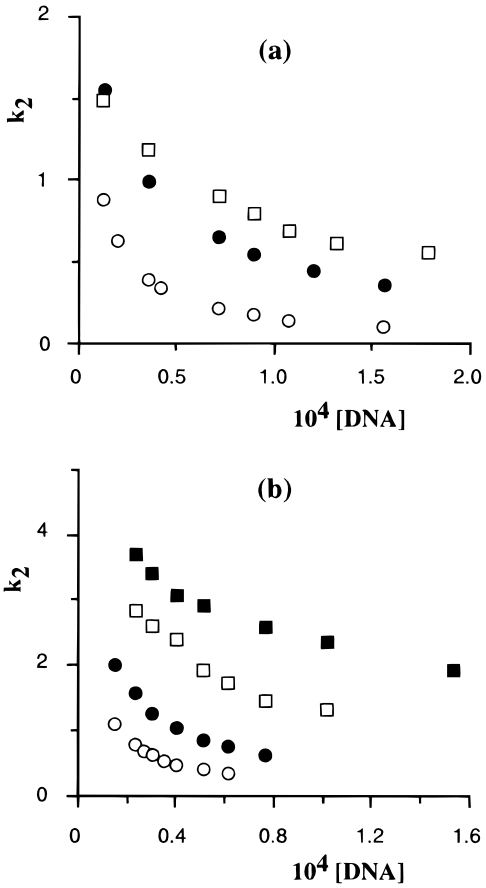


Figure 3. Plot of second-order rate constant, k_2 (M^{-1} s^{-1}), for reaction of $[Pt(terpy)(py)]^{2+}$ with (a) iodide and (b) thiourea vs [DNA], at various ionic strength values ($T = 25$ °C; pH = 7): ○, $I = 0.0395$ M; ●, $I = 0.079$ M; □, $I = 0.158$ M; ■, $I = 0.316$ M.

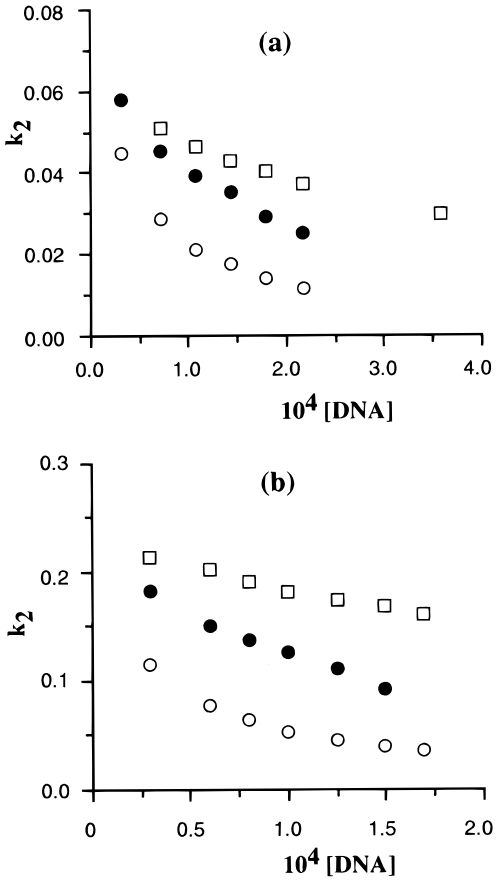


Figure 4. Plot of second-order rate constant, k_2 (M^{-1} s^{-1}), for reaction of $[Pt(terpy)(2\text{-}CH_3py)]^{2+}$ with (a) iodide and (b) thiourea vs [DNA], at various ionic strength values ($T = 25$ °C; pH = 7): ○, $I = 0.0395$ M; ●, $I = 0.079$ M; □, $I = 0.158$ M.

Here k_2 is the second-order rate constant for the bimolecular attack of the nucleophile at platinum (Figure 2). The absence in the rate law of a term independent of the nucleophile concentration shows that the solvolytic step does not contribute significantly to the overall rate of substitution. On the other hand, the circumstance that the term k_2 is overwhelming agrees with the much higher nucleophilic character[15] of iodide and thiourea respect to water.

The kinetic behavior exhibited by these complexes is that typical of ligand substitution at square complexes. So thiourea (Figures 3 and 4) is in all cases a better nucleophile than iodide; furthermore, in line with the associative character of the process, the complex $[Pt(terpy)(2\text{-}CH_3py)]^{2+}$, where the methyl group in the 2 position of pyridine shields the metal center, is much more inert than $[Pt(terpy)(py)]^{2+}$. The reactivity of [Pt(4′-*o*-tolylterpy)(2-CH_3py)]$^{2+}$ (Figure 5) is very similar to that of $[Pt(terpy)(2\text{-}CH_3py)]^{2+}$ and this shows that the introduction of the *o*-tolyl group neither alters the electronic properties of terpy nor interferes sterically with the reaction center.

As expected for reactions that occur between ions of opposite charge, the rate of the reactions of the complexes with iodide decreases on increasing ionic strength. Reporting the values of second-order rate constants against ionic strength, according to the Bronsted−Bijerrum−Christiansen equation[16] (eq 3), linear

$$\log k = \log k_0 + \frac{1.02 z_1 z_2 \sqrt{I}}{1 + \sqrt{I}} \qquad (3)$$

plots are obtained whose slopes (Figure 2) are very close to the theoretical value of −2. In eq 3, k is the specific rate constant, k_0 is the same for infinite dilution, and z_1 and z_2 are the charges on the two reactants.

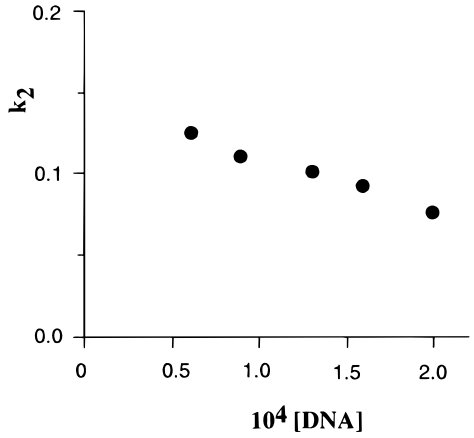


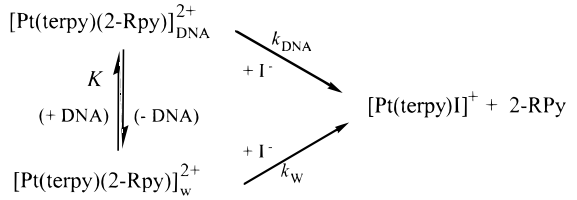
Figure 5. Plot of second-order rate constant, k_2 (M^{-1} s^{-1}), for reaction of [Pt(4′-*o*-tolylterpy)(2-CH_3py)]$^{2+}$ with thiourea vs [DNA], at 0.079 M ionic strength ($T = 25$ °C; pH = 7).

Neither the rate law nor the reactivity order of the various complexes with iodide and thiourea is altered by the presence

(15) (a) Basolo F.; Pearson, R. G. *Mechanisms of Inorganic Reactions*; John Wiley: New York, 1968. (b) Wilkins, R. G. *Kinetics and Mechanisms of Reactions of Transition Metal Complexes*; VCH: Weinheim, Germany, 1991.

(16) Frost, A. A.; Pearson, R. G. *Kinetics and Mechanisms*; John Wiley and Sons: New York, 1961; Chapter 7.

Scheme 1



of DNA, suggesting that the substitution mechanism is the same as in water. However, DNA inhibits all the reactions studied and changes their ionic strength dependence; the influence of ionic strength on the reactions with iodide becomes much larger than in water, and the rate of reaction of the complexes with the uncharged reagent thiourea decreases on increasing ionic strength. In Figures 3 and 4 the second-order rate constants k_2, obtained in the presence of DNA, are plotted against [DNA] at various ionic strength values. There is a systematic decrease of the rate constants on increasing DNA concentration over the whole [DNA] range investigated; at a given [DNA] the inhibiting effect on the reactions is larger at lower ionic strength.

Discussion

The inhibition of the reactions with iodide is easily interpretable considering the polyelectrolytic nature of DNA. The complexes, thanks to their double positive charge, tend to accumulate around DNA; the repulsion of phosphate groups prevents anions from approaching DNA surface. Therefore, the reaction between iodide and the cationic complexes here studied occurs only in the "bulk" solution. The reduced concentration of one of the reagents, i.e., the complex, accounts for the inhibition of the reaction by DNA. Scheme 1 presents a kinetic scheme adoptable for the reactions with iodide, in the presence of DNA, that is used for the reactions between cationic and anionic reactants in the presence of anionic micelles.[17]

In Scheme 1, k_w is the second-order rate constant for reaction 1 in the bulk solution ($k_w = k_2$ when [DNA] = 0) and k_{DNA} is the second-order rate constant for the same reaction in the DNA "pseudophase". K is the equilibrium constant for distribution of the complex between the DNA "pseudophase" and the bulk solution:

$$K = \frac{[[\text{Pt(terpy)(2-Rpy)}]_{\text{DNA}}{}^{2+}]}{[[\text{Pt(terpy)(2-Rpy)}]_{\text{w}}{}^{2+}][\text{DNA}]} \qquad (4)$$

By DNA "pseudophase" we mean DNA itself and a layer extending tens of hundreds of angstroms normal to the surface of the DNA−solution interface.[18] DNA "pseudophase" hosts all those cations that interact simply electrostatically, either territorially or site bound, or bind to DNA superficially or by intercalation.

According to Scheme 1 and by consideration that the distribution of the complex between the "pseudophase" DNA and the bulk solution is fast, the following expression correlating

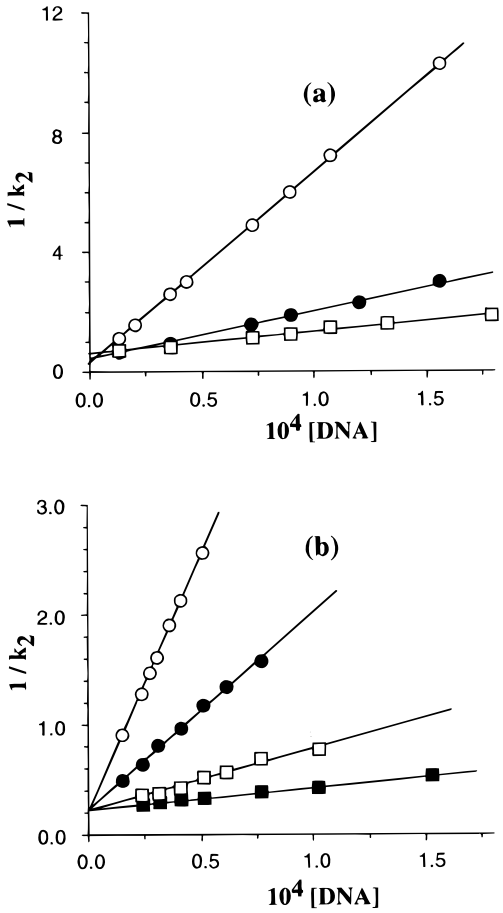


Figure 6. Plot of $1/k_2$, for reaction of $[Pt(terpy)(py)]^{2+}$ with (a) iodide and (b) thiourea vs [DNA], at various ionic strength values (T = 25 °C; pH = 7): ○, I = 0.0395 M; ●, I = 0.079 M; □, I = 0.158 M; ■, I = 0.316 M.

the second-order rate constants with [DNA] is obtained:

$$k_2 = \frac{k_{\text{DNA}}K[\text{DNA}] + k_{\text{W}}}{1 + K[\text{DNA}]} \qquad (5)$$

By the plotting of $1/k_2$ against [DNA] (Figures 6 and 7) a linear trend is obtained and the estimated values of k_w (1/intercept) agree well with those experimentally determined without DNA (Table 1). This shows that the reaction occurs predominantly in the bulk solution, and $k_{DNA}K$[DNA] = 0; therefore, eq 5 reduces to

$$k_2 = \frac{k_{\text{w}}}{1 + K[\text{DNA}]} \qquad (6)$$

Partitioning of the complexes, between DNA "pseudophase" and bulk solution, on purely electrostatic terms, cannot account for inhibition of the reactions with thiourea. This nucleophile is chargeless, and therefore, it is not confined in any particular region of the solution. The decrease in the rate of substitution of 2-Rpy, by thiourea, is attributed to the specific type of interaction of these complexes with DNA. The platinum(II) terpyridine complexes belong to a well-known family of metallointercalators studied by Lippard;[19] in particular it has been shown[7a,b] that $[Pt(terpy)(Rpy)]^{2+}$ (R = H, 4-CH_3, or 2-CH_3)

(17) (a) Martinek, K.; Yatsimirskii, A. K.; Levashov, A. V.; Berezin, V. I. *Micellization, Solubilization and Microemulsion*; Mittal, K. L., Ed.; Plenum Press: New York, 1977; Vol. 2, p 489. (b) Bunton, C. A. *Solution Chemistry of Surfactants*; Mittal, K. L., Ed.; Plenum Press: New York, 1979; Vol. 2, p 519.

(18) (a) Turro, N. J.; Barton, J. K.; Tomalia, D. A. *Acc. Chem. Res.* **1991**, *24*, 332. (b) Bunton, C. A.; Nome, F.; Quina, F. H.; Romsted, L. S. *Acc. Chem. Res*. **1991**, *24*, 3572.

(19) (a) Howe-Grant, M.; Wu, K. C.; Bauer, W. R.; Lippard, S. J. *Biochemistry* **1976**, *15*, 4339. (b) Wong, Y. S.; Lippard, S. J. *Chem. Commun.* **1977**, 824. Lippard, S. J. *Acc. Chem. Res*. **1978**, *11*, 211.

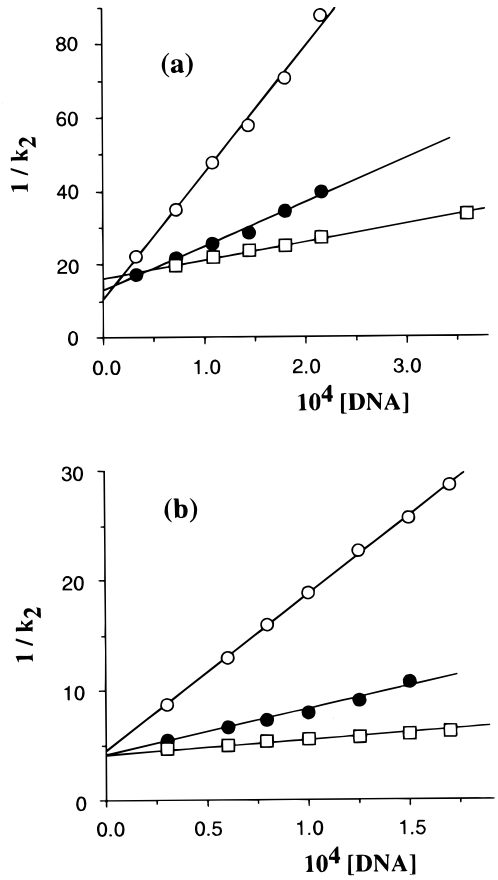
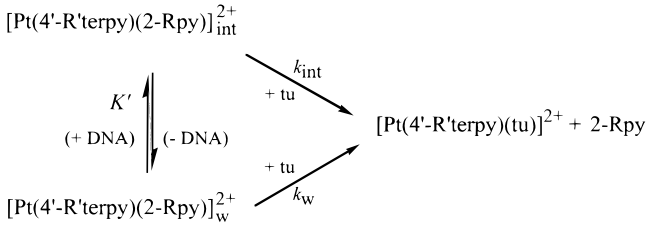


Figure 7. Plot of $1/k_2$, for reaction of $[Pt(terpy)(2\text{-}CH_3py)]^{2+}$ with (a) iodide and (b) thiourea vs [DNA], at various ionic strength values (T = 25 °C; pH = 7): ○, I = 0.0395 M; ●, I = 0.079 M; □, I = 0.158 M.

are potent DNA intercalators, although the pyridine groups that are skew to the terpyridine plane[20] prevent total intercalations of these cations. Intercalation consists of the insertion of a cation, or part of it, between two adjacent base pairs of DNA with the formation of ordered stacking of complex and nucleobases according to the next-neighbor exclusion principle. The steric implications of such a type of interaction are obvious. Shielding of the reaction center, by the nucleobases, renders an intercalated square-planar complex inert to substitution. In the presence of DNA, inhibition of the reactivity is, therefore, expected because of decreased free complex concentration. In the case of total intercalation the reaction does not occur at all. Intercalation of the terpyridine complexes here studied to DNA can account for the dependence of the second-order rate constant for reaction with thiourea, both on [DNA] and on ionic strength of the solutions. Taking into account that intercalation is much faster than any covalent process, a possible reaction scheme is that depicted as follows:



The reaction scheme and the relative rate law are formally identical to that proposed for the reactions with iodide:

$$k_2 = \frac{k_{int}K'[DNA] + k_w}{1 + K'[DNA]} \quad (7)$$

Here, however, k_{int} is the second-order rate constant for reaction of thiourea with the intercalated complex, k_w is the rate constant for reaction of thiourea with free complex, and K' refers to the equilibrium between free and intercalated form of the complex.

$$K' = \frac{[[Pt(4'\text{-}R'terpy)(2\text{-}Rpy)]_{int}^{2+}]}{[[Pt(4'\text{-}R'terpy)(2\text{-}Rpy)]_{w}^{2+}][DNA]} \quad (8)$$

The linear trend obtained by plotting $1/k_2$ against [DNA] (Figures 6 and 7) shows that the reaction does not occur with the intercalated complexes. So one can assume that $k_{int}K'$[DNA] is equal to zero and rate law (7) reduces to

$$k_2 = \frac{k_w}{1 + K'[DNA]} \quad (9)$$

The dramatic decrease in rate observed if the kinetics are performed at very low ionic strength, when the complexes are almost completely intercalated, strongly supports this assumption. The estimated values of k_w (1/intercept), which agree with those experimentally determined without DNA, also corroborate the validity of the proposed reaction mechanism. Finally, the slope of these plots gives the K' values (slope/intercept) reported in Table 1 together with the binding constants for intercalation of the complexes to DNA determined spectrophotometrically through the McGhee−von Hippel approach. The satisfactory agreement between the two sets of data supports the mechanism proposed to interpret the observed kinetic effect of DNA upon these reactions.

According to the interpretation given, the inhibiting effect of DNA depends on the intercalating ability of the complexes. So at a given [DNA], the difference in ($k_w - k_{int}$) (Figures 3 and 4) is larger for $[Pt(terpy)(py)]^{2+}$ which is a much better intercalator than $[Pt(terpy)(2\text{-}CH_3py)]^{2+}$.

The unexpected dependence of the rate of reaction between the complexes and the uncharged reagent thiourea on the ionic strength of the medium is due to influence of this parameter on the DNA−small molecule interactions. Ionic strength destabilizes[21] any type of noncovalent interaction between cationic species and DNA. This is due to competition of the interacting small molecule and the other cations present in solution for the anionic phosphate groups of DNA. In addition, ionic strength influences DNA conformation; increasing ionic strength reduces repulsion between phosphate groups allowing adjacent base pairs to get closer so disfavoring intercalation. In the case of the reactions with iodide the rate dependence on the ionic strength is the contribution of two terms. The process, which occurs between ions of opposite charge, is intrinsically dependent on the ionic strength, and the rate of reactions decreases on increasing this parameter. On the other hand, the ionic strength destabilizes the interaction between DNA and the complexes so that at high ionic concentration there is a smaller DNA inhibiting influence. This last effect is dominating; in the presence of the biopolymer, the rate of reaction increases as the ionic strength is raised. Finally it is interesting that the equilibrium constants of the various complexes for the reactions

(20) Roszak, A. W.; Clement, O.; Buncel, E. *Acta Crystallogr., Sect. C* **1996**, *52*, 11645.
(21) (a) Manning, G. S. *Acc. Chem. Res.* **1979**, *12*, 446. (b) Record, M. T., Jr.; Anderson, C. F.; Lohman, T. M. *Q. Rev. Biophys.* **1978,** *11*, 103.

Table 1. Comparison of the k_w and K_B Values, Estimated through Eqs 6 and 9, with Those Directly Determined ($T = 25$ °C, pH = 7 (Phosphate Buffer))

complex	Y	I (M)	1/intercept	k_w[a]	slope/intercept[b]	$K_{B,\,spect}$[d]
$[Pt(terpy)(py)]^{2+}$	iodide	0.0395	3.23	3.24	206 000	
		0.079	2.40	2.24	38 500	
		0.158	1.65	1.73	11 900	
	thiourea	0.0395	4.87	4.48	227 000	
		0.079	4.16		74 300	
		0.158	4.57		25 800	35000 ± 3000[c]
		0.316	4.21		7 960	
$[Pt(terpy)(2\text{-}CH_3py)]^{2+}$	iodide	0.0395	0.10	0.09	34 500	
		0.079	0.08	0.07	9 310	
		0.158	0.06	0.06	2 960	3000 ± 300[c]
	thiourea	0.0395	0.22	0.23	31 300	
		0.079	0.25		10 400	
		0.158	0.23		2 560	
$[Pt(4'\text{-}o\text{-}tolylterpy)(2\text{-}CH_3py)]^{2+}$	thiourea	0.079	0.17	0.17	5 840	9800 ± 1300

[a] Determined without DNA. [b] Intercept and slope refer to plots of Figures 6 and 7. [c] Values from ref 7b. [d] Determined spectrophotometrically through the McGhee−von Hippel approach.

with thiourea and iodide are similar. K and K' have a different meaning; while K refers to the equilibrium between the complex in the DNA domain and the free complex, K' refers to the equilibrium between the intercalated and nonintercalated complex. The analogy between the two sets of values suggests that most of the complex in the DNA domain is intercalated.

In conclusion, double helix DNA inhibits the reactions between cationic complexes and anionic nucleophiles. This kinetic effect is independent of the specific type of DNA−complex interaction and is due to partitioning of the complex between DNA domain and bulk solution. The inhibition of reactions between cationic complexes and uncharged (or cationic) nucleophiles is the consequence of the intercalation of the complexes to DNA that shields the metal center preventing entry of the nucleophile.

Acknowledgment. This work was supported by the MURST and CNR.

IC990747U