Catalysis and Inhibition of Ligand Substitution in Palladium(II) Square-Planar Complexes: Effects of DNA

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Abstract: The kinetics of substitution, by thiourea, of ethylenediamine (en) or *N*,*N*′-dimethylethylenediamine (Me_2en) coordinated to palladium(II) in the complexes $[Pd(4,4'\text{-}R_2bpy)(en)](PF_6)_2$ (bpy = 2,2′-bipyridine; R = H or Me), $[Pd(en)_2](PF_6)_2$ and $[Pd(Me_2en)_2](PF_6)_2$ have been studied at 25 °C, pH 7 and various ionic strength values, in the presence of calf thymus DNA. The rate of the reaction in water depends on ionic strength, pH, and nucleophile concentration; at fixed pH and ionic strength the k_{obsd} values are correlated to the square of the thiourea concentration. This rate law is not altered by the presence of DNA, but the rate of reaction is influenced, depending on the nature of ancillary ligand, L−L, bound to palladium. DNA inhibits the substitution process when L−L is bpy or 4,4′-Me_2bpy and catalyzes the same reaction when L−L is en or Me_2en. These opposite kinetic effects can be related to the noncovalent interactions of the various complexes with the DNA double helix. Inhibition of the reactivity of the complexes $[Pd(4,4'\text{-}R_2bpy)(en)]^{2+}$ is due to protection of the reaction center from nucleophile attack by DNA. Acceleration of the reaction when L−L is en or Me_2en is related to the dependence of the rate of reaction on pH. If, due to the higher activity of water under the electric field of phosphate groups, hydronium ion concentration on DNA surface is higher than in the bulk solution, the enzyme-like dependence of the rate of reaction on [DNA] is due to progressive accumulation of the complexes around the double helix. Regardless of the complexes' nature, the rate constant values obtained in DNA at pH 7 correspond to values determined in water at pH 5. This pH value on the DNA surface, lower by about two units with respect to the bulk solution, is in good agreement with theoretical predictions. Acceleration of ethylenediamine substitution has been observed for all of the complexes studied in the presence of sodium polyvinylsulfonate.

Introduction

Host−guest interactions in supramolecular systems alter some of the relevant guest's chemical properties. The most significant example is the substrate−enzyme[1] interaction that can easily enhance the reactivity of the former by a billion times. In principle, however, any supramolecular interaction modifies the chemical properties of both partners with resulting kinetic effects. In the presence of micellar aggregates[2] or polyelectrolytes,[3] the alteration of apparent rate constants of chemical reactions is due to the host's capability to act as a "microreactor", compartmentalizing and concentrating, or separating and diluting reactants. Rate effects, commonly observed in cyclodextrins,[4] can be related to steric and electronic factors due to inclusion of the guest within the cyclodextrin cavity.

Double-helical DNA also influences the rate of chemical reactions occurring within its domain; DNA has been reported to mediate electron transfer (photoinduced) between metal complexes[5] and to catalyze or inhibit reactions of small molecules such as diol epoxides[6] and simple coordination compounds.[7] These kinetic effects have been attributed to noncovalent interactions with the double helix. Duplex DNA can interact with small molecules in a variety of modes[8] that

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range from simple electrostatic binding to intercalation,[9] a type of interaction in which the guest molecule is rigidly held between two adjacent nucleobases. Therefore, DNA can either cause partitioning effects and exert major steric and electronic influences on small molecules interacting with it.

In this paper, we present an example in which the reactivity of a series of closely related substances can either be enhanced or inhibited depending on the type and extent of interaction with double-helical DNA. In particular, we report a kinetic study of the substitution of ethylenediamine (en) or *N*,*N*′-dimethylethylenediamine (Me_2en) by thiourea in the palladium(II) complexes $[Pd(4,4'\text{-}R_2bpy)(en)](PF_6)_2$ (R = H or Me), $[Pd(en)_2](PF_6)_2$, and $[Pd(Me_2en)_2](PF_6)_2$, in water and in the presence of calf thymus DNA. The kinetics have been followed in water at 25 °C, variable pH, and variable ionic strength, or in the presence of DNA at 25 °C, pH 7, and variable ionic strength. The rate of substitution of en has also been determined at 25 °C, pH 7, and 0.022 M ionic strength in the presence of sodium polyvinylsulfonate (PVS).

Experimental Section

Materials. (i) Complexes. The complexes $[Pd(4,4'\text{-}R_2bpy)(en)](PF_6)_2$ (R = H or Me), $[Pd(en)_2](PF_6)_2$ and $[Pd(Me_2en)_2](PF_6)_2$ were prepared as described in the literature[10] or by similar methods. 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 ϵ_{260} nm value[12] of 1.31×10^4 M^{-1} cm^{-1}.

(iii) PVS. Polyvinylsulfonate (sodium salt) was purchased from Aldrich. The molecular weight of the polymer, as derived from viscosimetric measurements, was about 6×10^4 (~450 residues).

$(NH_2)_2CS$, NaCl, and other chemicals were of reagent grade and were used without further purification.

Methods. All experiments were carried out at 25 °C, controlled pH in a phosphate buffer ($[KH_2PO_4]/[Na_2HPO_4]$) with enough NaCl 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.

Kinetics. The kinetics of ligand substitution, by thiourea, in the reported complexes was followed spectrophotometrically, in the range 300−400 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 syringes of stopped-flow contained respectively the complex and the nucleophile or the mixture nucleophile−DNA. The ionic strength (NaCl) and the pH (phosphate buffer) of both solutions were the same. The absorbance changes were displayed on a computer interfaced with the spectrophotometer, and pseudo-first-order rate constants k_{obsd} were obtained from a 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%.

Fitting to kinetic equations was performed using a Quantum Soft proFit 5.1.2 program.

Results

The complexes follow Beer's law and their absorption spectra do not change with hydronium ion concentration over a large pH range (2−10). Substitution of en, or Me_2en, by thiourea occurs according to the general scheme:

$$[Pd(L{-}L)(en)]^{2+} + 2\ tu \rightarrow [Pd(L{-}L)(tu)_2]^{2+} + en \quad (1)$$

$$[Pd(Me_2en)_2]^{2+} + 2\ tu \rightarrow [Pd(Me_2en)(tu)_2]^{2+} + Me_2en \quad (2)$$

(L−L = bpy, 4,4′-Me_2bpy or en). The process, which takes place in one observable step, can be easily monitored spectrophotometrically in the UV−vis region. The rate of reaction depends on ionic strength, pH, and thiourea concentration. Keeping both ionic strength and pH constant, under pseudo-first-order conditions with respect to the complex, the rate of reaction is related to thiourea concentration according to eq 3:

$$k_{obsd} = k[tu]^2 \quad (3)$$

A good linear trend with small intercept, over the whole concentration range investigated, is obtained on plotting the k_{obsd} values against the square of the thiourea concentration (Figure 1).

Dependence on ionic strength is unexpected on the basis of reaction stoichiometry; reporting the k_{obsd} values, obtained at fixed pH and thiourea concentration, versus ionic strength, according to the Brønsted−Bijerrum−Christiansen equation:[13]

$$\log k_{obsd} = \log k_0 + \frac{1.02 z_1 z_2 \sqrt{I}}{1 + \sqrt{I}} \quad (4)$$

(k_{obsd} is the observed rate constant; k_0 is the rate constant for infinite dilution; z_1 and z_2 are the charges of the two reactants) linear plots are obtained whose slopes are very close to the value of 2 (see inset of Figure 1).

In addition, at given ionic strength and thiourea concentration the rate of reaction increases on increasing $[H_3O^+]$ tending to a limiting value (Figure 2).

A possible mechanism for the reactions, which accounts satisfactorily for the dependence of the observed rate constants on $[H_3O^+]$, thiourea concentration, and ionic strength, is shown in Scheme 1.

This scheme is similar to those generally accepted for displacement of chelate groups from square-planar complexes.[14] It is assumed that displacement of the second nitrogen atom occurs only after protonation at the free site of ethylenediamine; the direct displacement of the unprotonated intermediate, by a second thiourea molecule, has been ruled out on the basis that the observed rate constants tend to zero as the hydronium ion concentration decreases. Also, the customary solvolytic path,[15] whose contribution to overall rate is undetectable, has not been

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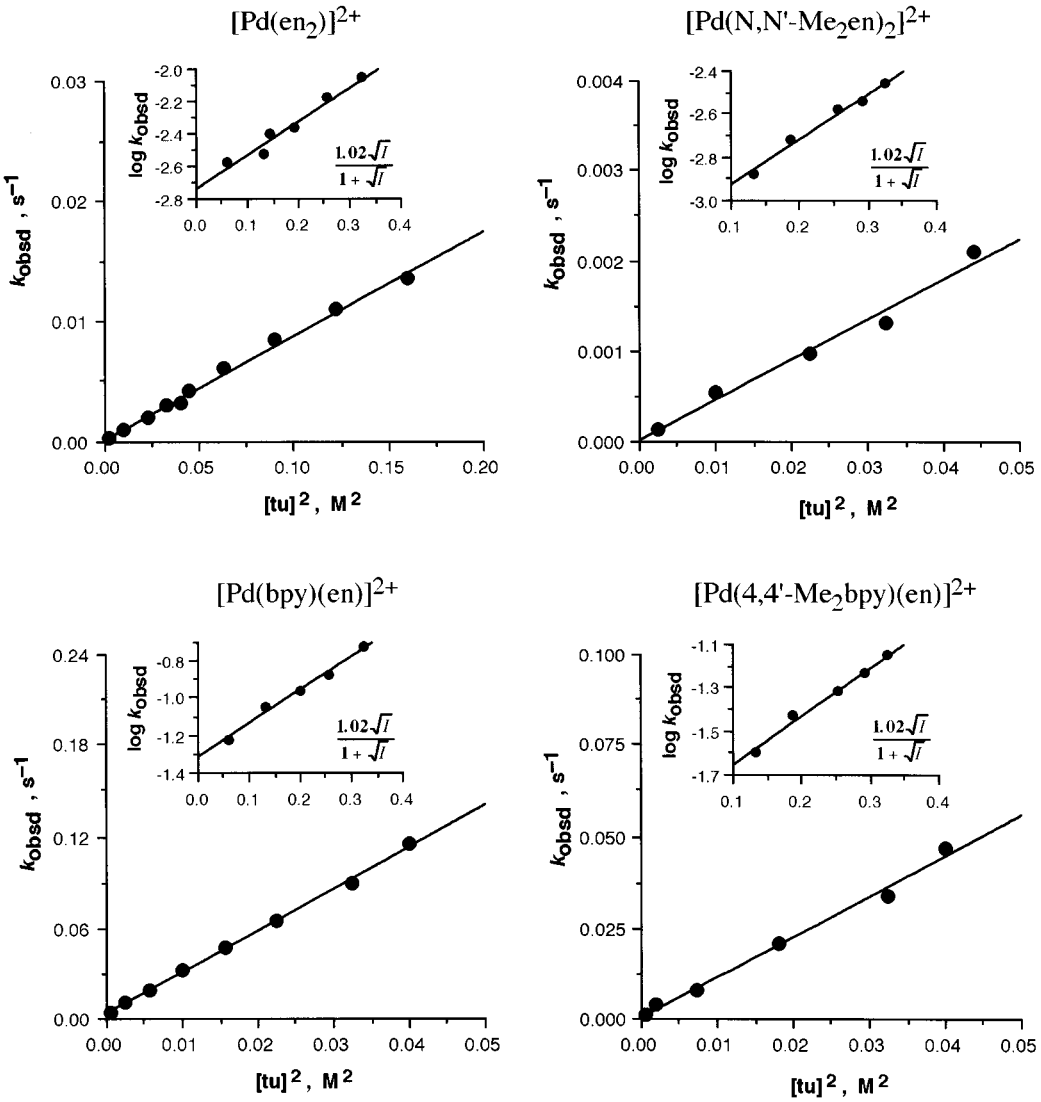


Figure 1. Plot of k_{obsd} vs $[tu]^2$ for reaction of the complexes with thiourea in water at 25 °C, pH 7, and 0.022 M ionic strength. Inset shows the dependence of rate constants on ionic strength for the same systems.

included in the scheme. From Scheme 1 eq 5 may be deduced,

$$k_{obsd} = \frac{k_1 K_1 K_2 [H^+][tu]^2}{1 + [tu](K_1 + K_1 K_2 [H^+])} \quad (5)$$

where:

$$K_1 = \frac{[\text{Int. 1}]}{[\text{Complex}][tu]} \quad (6)$$

$$K_2 = \frac{[\text{Int. 2}]}{[\text{Int. 1}][H^+]} \quad (7)$$

This equation is similar to the empirical expression 3. Even using a large thiourea concentration we could not detect intermediate **1**, suggesting that K_1 is very small. Under our experimental conditions of low acidity, the denominator can be reasonably approximated as unity.

The curves shown in Figure 2 were calculated according to eq 5 using an estimated value for k_1 obtained from the limiting k_{obsd} values.

Rate law 3 also holds in the presence of calf thymus DNA, suggesting that the substitution mechanism is the same as in

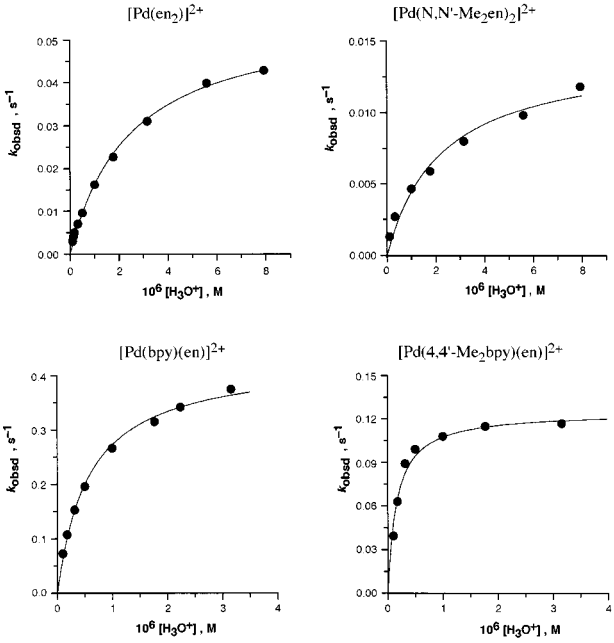
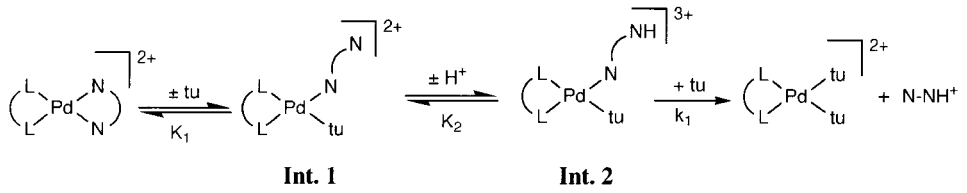


Figure 2. Effect of $[H_3O^+]$ on k_{obsd} for reaction of the complexes with thiourea in water at 25 °C and 0.022 M ionic strength.

Scheme 1



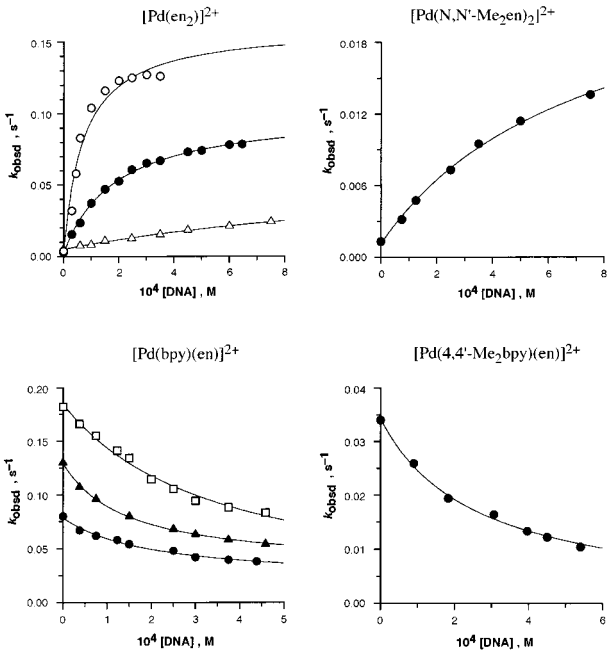


Figure 3. Influence of DNA on k_{obsd} for reaction of the complexes with thiourea at 25 °C, pH 7 at different ionic strength values: ○, I = 0.0055 M; ●, I = 0.022 M; △, I = 0.066 M; ▲, I = 0.11 M; □, I = 0.22 M.

water; however, DNA induces opposite changes in the rate of the reactions. Using the same experimental conditions, while the substitution of ethylenediamine in $[Pd(4,4'\text{-}R_2bpy)(en)]^{2+}$ is inhibited, the same reaction in $[Pd(en)_2]^{2+}$ and $[Pd(Me_2en)_2]^{2+}$ is accelerated.

In Figure 3, the k_{obsd} values, at fixed ionic strength and at a given $[H_3O^+]$ and thiourea concentration, are plotted versus [DNA]. There is a systematic decrease of the rate constants for the complexes $[Pd(4,4'\text{-}R_2bpy)(en)]^{2+}$ on increasing DNA concentration until a limiting value is reached. Conversely, the rate constants, k_{obsd}, for $[Pd(en)_2]^{2+}$ and $[Pd(Me_2en)_2]^{2+}$, obtained under the same experimental conditions, increase on increasing [DNA] exhibiting saturation.

DNA also changes the ionic strength dependence of the reactions. While in water the rate constants for the complexes $[Pd(en)_2]^{2+}$ and $[Pd(Me_2en)_2]^{2+}$ increase on increasing ionic strength; however, when DNA is present, it strongly decreases. In addition, both the sensitivity to the DNA-catalyzing effect and the limiting rate value decrease as the ionic strength is raised. For the complex $[Pd(4,4'\text{-}R_2bpy)(en)]^{2+}$, both the rate constant in water and that in DNA increase on increasing ionic strength, and the inhibiting effect increases also.

The reactions have been studied also in polyvinylsulfonate at pH 7 and 0.022 M ionic strength. Also this polyanion affects the rate of ethylenediamine substitution. However, only catalysis is observed for all of the complexes studied, including $[Pd(4,4'\text{-}R_2bpy)(en)]^{2+}$; saturation kinetics are reported in Figure 4.

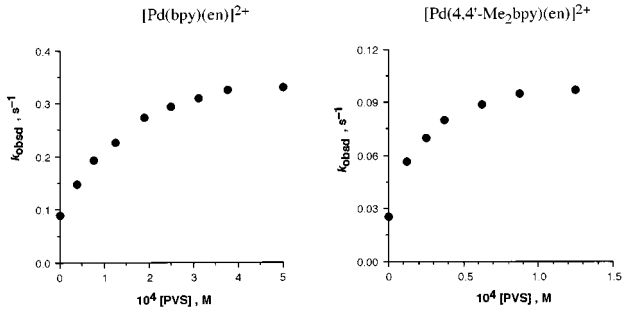


Figure 4. Influence of PVS on k_{obsd} for reaction of the complexes $[Pd(4,4'\text{-}R_2bpy)(en)]^{2+}$ (R = H or Me) with thiourea at 25 °C, pH 7, and 0.022 M ionic strength.

Discussion

Rate effects reported in this paper are dependent on the binding mode of the complexes with DNA. While catalysis is observed for $[Pd(en)_2]^{2+}$ and $[Pd(Me_2en)_2]^{2+}$ which bind superficially either simply electrostatically or in the groove,[16] the reaction of $[Pd(4,4'\text{-}R_2bpy)(en)]^{2+}$ (R = H or Me), which intercalate[17] into the base pairs, is inhibited.

Acceleration of ethylenediamine substitution, by DNA, appears to be related to the pH dependence of the rate of reaction. The catalytic effect of DNA increases, in fact, on increasing pH dependence of the various reactions in water. Both the sensitivity of k_{obsd} to H_3O^+ and to DNA are higher for $[Pd(en)_2]^{2+}$ than for $[Pd(Me_2en)_2]^{2+}$ (Figures 2 and 3). The apparent change in the rate constant for en substitution with respect to the same reaction, in the absence of DNA, could result from a change in the hydronium ion local concentration. Various researchers[18] have proposed that the effective pK_a of local water molecules around the DNA surface is increased by the large negative electrostatic potential generated by the phosphate groups of DNA, so that the hydronium ion concentration is higher than in the bulk solution. Lamm and Pack,[19] mapping out the hydronium concentration in the vicinity of DNA, within the Poisson−Boltzmann approximation, have estimated pH values about 2 units lower than in the bulk solution. The enzyme-like dependence of the rate of the reactions on DNA concentration observed can be, therefore, related to partitioning of the cationic complexes in the solution; on increasing [DNA], increasing amounts of the complexes move from the bulk solution to the double-helical domain where the pH is lower and the reaction faster. Limiting rate values are reached when the complexes are totally distributed around DNA. The limiting

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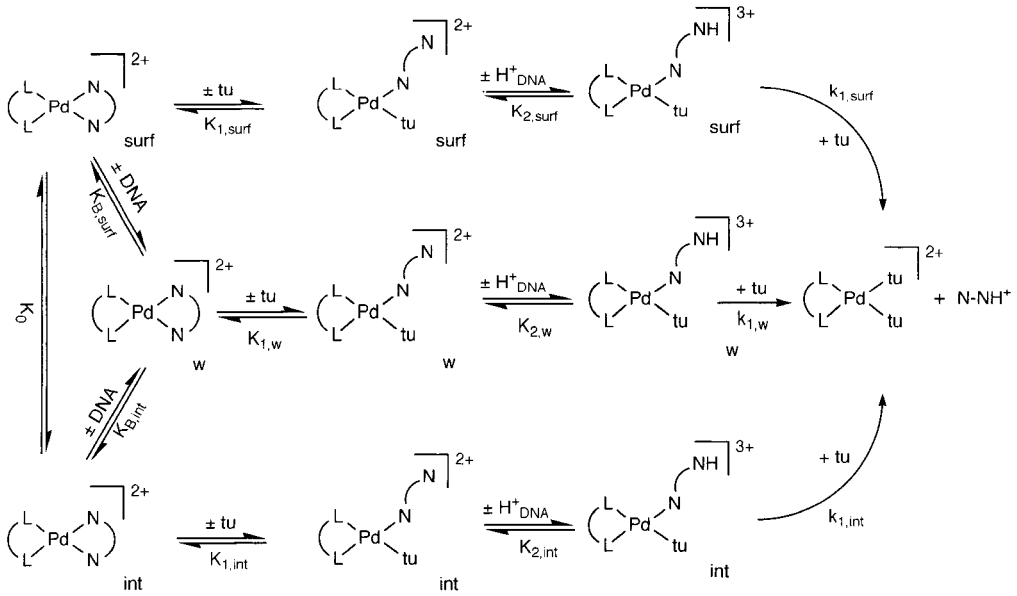
Scheme 2



values relative to solution buffered at pH 7 are comparable, both for $[Pd(en)_2]^{2+}$ and $[Pd(Me_2en)_2]^{2+}$, to those obtained in the absence of DNA at a pH close to 5. Such a value for the region around DNA, at 0.022 M ionic strength, is in good agreement with the theoretical prediction of Lamm.

The situation is more complicated for the intercalating complexes $[Pd(4,4'\text{-}R_2bpy)(en)]^{2+}$. Ligand substitution at palladium(II) tetracoordinated complexes is an associative process that occurs by attack of the nucleophile, at the metal, above or below the square plane. The nucleobases, in which a complex is sandwiched, shield the reaction center, preventing entry of the nucleophile. This steric protection leads to inhibition of the substitution process.[7b,20] Only the free portion of the complex can react; as experimentally observed at large [DNA] and low ionic strength, when the substance is almost totally intercalated, the reaction can be hardly observed. For the complexes $[Pd(4,4'\text{-}R_2bpy)(en)]^{2+}$ the increase in rate of substitution due to local pH influence is counterbalanced by the inhibition of the same process as a consequence of intercalation. Probably because of their strong intercalating ability,[17b] large amounts of the complexes partitioned in DNA domain are intercalated, and the observed DNA overall effect is of inhibition. The larger inhibiting effect for $[Pd(4,4'\text{-}Me_2bpy)(en)]^{2+}$ reflects its higher affinity for DNA with respect to that of $[Pd(bpy)(en)]^{2+}$. However, in principle, for weak intercalators increase in the rate of en substitution could prevail, and catalysis could be observed. A reaction scheme that reasonably accounts for the experimental findings is outlined in Scheme 2.

The scheme implies distribution of the complex between the bulk solution ("w") and DNA domain; in the latter region the complex is further partitioned between DNA core ("int" intercalated) and external DNA region ("surf" externally bound). The complex localized in the three environments reacts with thiourea, according to the mechanism already proposed in water. $K_{1,w}$, $K_{1,surf}$, $K_{1,int}$, $K_{2,w}$, $K_{2,surf}$ and $K_{2,int}$ are the equilibrium constants for the formation of the intermediates **1** and **2** respectively in the bulk solution, DNA surface and DNA core. $K_{B,surf}$ and $K_{B,int}$ are the binding constants of the complex on DNA surface and within nucleobases. K_0 is relative to the equilibrium between the complex intercalated and the complex in proximity of the double helix:

$$K_0 = \frac{K_{B,int}}{K_{B,surf}} \tag{8}$$

Equilibria among the intermediates present in the three environments have been neglected for simplicity. The rate law for such a scheme is:

$$k_{obsd} = \frac{a + b[DNA]}{1 + c[DNA] + d} \tag{9}$$

where:

$$a = k_{1,w}K_{1,w}K_{2,w}[tu]^2[H^+_w] \tag{10}$$

$$b = k_{1,int}K_{1,int}K_{2,int}K_{B,int}[tu]^2[H^+_{DNA}] + k_{1,surf}K_{1,surf}K_{2,surf}K_{B,surf}[tu]^2[H^+_{DNA}] \tag{11}$$

$$c = K_{B,surf} + K_{B,int} + [tu](K_{1,surf}K_{B,surf} + K_{1,int}K_{B,int} + K_{1,surf}K_{2,surf}K_{B,surf}[H^+_{DNA}] + K_{1,int}K_{2,int}K_{B,int}[H^+_{DNA}]) \tag{12}$$

$$d = [tu](K_{1,w} + K_{1,w}K_{2,w}[H^+_W]) \tag{13}$$

At constant $[H^+]$ and [tu], *a*, *b*, *c*, and *d* are constants. In the absence of DNA eq 9 is equivalent to eq 5:

$$k_{obsd} = \frac{a}{1 + d} \tag{14}$$

At large [DNA] values, when $b[DNA] \gg a$ and $c[DNA] \gg (1 + d)$, k_{obsd} becomes independent of DNA concentration:

$$k_{obsd} = \frac{b}{c} \tag{15}$$

The k_{obsd} value can either be lower or higher than the corresponding value in the absence of DNA and, consequently, both inhibition and catalysis can be observed. A detailed analysis of the ratio *b*/*c* is difficult due to the complexity of these terms. However, some considerations can be made.

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For nonintercalating complexes:

$$\frac{b}{c} = \frac{k_{1,\mathrm{surf}}K_{1,\mathrm{surf}}K_{2,\mathrm{surf}}[\mathrm{tu}]^2[\mathrm{H}^+_{\mathrm{DNA}}]}{1 + [\mathrm{tu}](K_{1,\mathrm{surf}} + K_{1,\mathrm{surf}}K_{2,\mathrm{surf}}[\mathrm{H}^+_{\mathrm{DNA}}])} \qquad (16)$$

As the rate and equilibrium constant values on DNA surface should be the same as those in the bulk solution, b/c differs from $a/(1 + d)$ only by hydrogen concentration (see equation). As a consequence, to observe catalysis:

$$[\mathrm{H}^+_{\mathrm{DNA}}] > [\mathrm{H}^+_{\mathrm{w}}]$$

For intercalating complexes a similar expression can be obtained:[21]

$$\frac{b}{c} = \frac{k_{1,\mathrm{surf}}K_{1,\mathrm{surf}}K_{2,\mathrm{surf}}[\mathrm{tu}]^2[\mathrm{H}^+_{\mathrm{DNA}}]}{1 + [\mathrm{tu}](K_{1,\mathrm{surf}} + K_{1,\mathrm{surf}}K_{2,\mathrm{surf}}[\mathrm{H}^+_{\mathrm{DNA}}]) + \dfrac{K_{\mathrm{B,int}}}{K_{\mathrm{B,surf}}}} \qquad (17)$$

Here, however, due to the presence of the term $K_0 = K_{\mathrm{B,int}}/K_{\mathrm{B,surf}}$, b/c is largely variable as a function of intercalating avidity of the substance. When the intercalating constant is very small, b/c may be still larger than the corresponding value in water, and catalysis still occurs. However, if $K_{\mathrm{B,int}}$ is large enough, as it happens for the complexes studied, inhibition is observed.

The curves shown in Figure 3 were obtained by fitting the experimental data according to eq 9. An initial approximation for a and d was obtained using the parameters $k_{1,\mathrm{w}}$, $K_{1,\mathrm{w}}$, and $K_{2,\mathrm{w}}$ previously derived from the fitting of eq 5.

Dependence of the rate of reactions on the ionic strength is in agreement with the scheme proposed. Both catalysis and inhibition are a consequence of the interaction of the complexes with DNA and occur within its domain. Due to competition for the anionic phosphate groups between the complexes and the other cations present in solution, ionic strength destabilizes[22] noncovalent interactions between cationic species and DNA. At higher ionic strength the number of complex cations around DNA is lower, and so is the catalytic effect. Likewise, a lower value of limiting rate and, therefore, of hydronium concentration at higher ionic strength, could be due to less efficient competition of this species with the other cations.

Inhibition, as already reported for other systems,[7b] should be less effective at higher ionic strength. The composite nature of the kinetic effect caused by DNA on the intercalated complex might account for the apparent increase of inhibition with ionic strength. Catalysis reduces the inhibition effect which intercalation alone would produce. If the catalytic effect is more sensitive to ionic strength than the inhibition effect, the decrease in rate due to intercalation is compensated more effectively at lower ionic strength.

Catalysis by DNA has already been reported for some diol epoxide hydrolysis[6] and adenine base alkylation by cyclopropylpyrrolindoles.[23] Here, however, unlike for our complexes where intercalation causes inhibition, this type of interaction leads to an increase in the rate of reaction. The reason for this has been attributed to the catalytic effect of hydronium ion on the reactions. According to computational studies,[18,24] pH in DNA minor groove where the compounds intercalate is much lower than in the bulk solution.

The catalytic effect is not exclusive of DNA; hydrolysis of benzo[*a*]pyrene-*cis*-7,8-diol 9,10-epoxide has been reported[6d] to be catalyzed both by double-helical DNA and single-stranded poly(G) and poly(A) nucleic acids. The data reported in this work show that acceleration of ethylenediamine substitution is observed also in the presence of the sodium polyvinylsulfonate. The catalytic effect is exerted also on the bipyridine complexes whose reactivity is inhibited by double-helical DNA (Figure 4). Polyvinylsulfonate has a single-stranded, random-coil structure;[25] the presence of negative charges at fixed distances produces a large negative electrostatic potential at its surface which may well decrease the pK_a of local water molecules.

In conclusion, DNA accelerates acid-catalyzed ligand substitution of square-planar complexes. If the complexes intercalate into the double helix, the increase in rate is more or less counterbalanced by the steric inhibition due to shielding of the metal by the nucleobases. The overall kinetic effect depends on the relative distribution of the complex between the external region and the core of the DNA. Inhibition is peculiar of DNA because it originates from intercalation into DNA base pairs. Catalysis appears to be a more general phenomenon which occurs at negatively charged surfaces and results from a decrease in local pH around polyanionic macromolecules.

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Supporting Information Available: Observed first-order rate constants for reaction of the complexes with thiourea as a function of thiourea concentration, pH, and ionic strength (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) As we have assumed that the intercalated complex is inaccessible to the nucleophile, all the terms including rate and equilibrium constants for intercalated species are negligible.

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