ChemComm

Opposite influence of calf thymus DNA on the rate of substitution of ethylenediamine, by thiourea, in the complex cations $[Pd(bpy)(en)]^{2+}$ and $[Pd(bromazepam)(en)]^{2+}$ (bromazepam = 7-bromo-1,3-dihydro-5-(2-pyridyl)-2*H*-1,4-benzodiazepin-2-one)

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Received (in Cambridge, UK) 4th June 1999, Accepted 29th June 1999

Calf thymus DNA inhibits the substitution of ethylenediamine, by thiourea, in $[Pd(bpy)(en)]^{2+}$ and catalyses the same reaction in $[Pd(bromazepam)(en)]^{2+}$ (bromazepam = 7-bromo-1,3-dihydro-5-(2-pyridyl)-2*H*-1,4-benzodiazepin-2-one); this kinetic effect can be related to the different binding modes of the two complexes to the biopolymer.

Inclusion in receptors such as cyclodextrins[1] as well as interactions with micelles[2] or polyelectrolytes[3] alter, often to a large extent, the physical properties and the reactivity of the interacting molecules. DNA can also, thanks to a variety of non-covalent interactions with small molecules,[4] produce these effects.[5] We report here an example in which calf thymus DNA exerts an opposite influence on the reactivity of the two similar palladium(II) complexes $[Pd(bpy)(en)](PF_6)_2$ and $[Pd(bromazepam)(en)](BF_4)_2$ (bromazepam = 7-bromo-1,3-dihydro-5-(2-pyridyl)-2*H*-1,4-benzodiazepin-2-one). For comparison, we report also the effect of DNA on the reactivity of $[Pd(en)_2]Cl_2$ and $[Pd(Me_4en)(py)_2](PF_6)_2$.

$[Pd(bromazepam)(en)](BF_4)_2$ was obtained by treating $[Pd(en)Cl_2]$[6] with $AgBF_4$ (ratio 1 : 2) in acetone, and then adding the corresponding amount of bromazepam. $[Pd(Me_4en)(py)_2](PF_6)_2$ was prepared by heating $[Pd(Me_4en)Cl_2]$, suspended in water, in the presence of an excess of pyridine. After dissolution of the complex, the product was obtained as a yellow precipitate by addition of NH_4PF_6. $[Pd(Me_4en)Cl_2]$,[7] $[Pd(bpy)(en)](PF_6)_2$[8] and $[Pd(en)_2]Cl_2$[6] were prepared by literature methods. Calf thymus DNA (Sigma) was purified as previously described.[9]

The complexes $[Pd(N–N)(en)]^{2+}$ (N–N = bpy or bromazepam) react with thiourea according to reaction (1). The nature of

$$[Pd(N–N)(en)]^{2+} + 2\ tu \longrightarrow [Pd(N–N)(tu)_2]^{2+} + en \quad (1)$$

the reaction products, isolated from water, was established by elemental analysis and ^1H NMR. The kinetics of ligand substitution, by thiourea, were followed spectrophotometrically, in the range 310–370 nm, at 25 °C. Under pseudo-first-order conditions with respect to the complex, the rate of reaction (1), which occurs in one observable step, is related to thiourea concentration by law (2). A good linear trend with zero

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

intercept, over the whole thiourea concentration range investigated, is obtained on plotting the k_{obs} values against the square of the thiourea concentration $[tu]^2$. If the reaction is monitored in the presence of calf thymus DNA ([DNA]/[Complex] = 6), the same rate law is observed; however DNA induces opposite changes in the rate of the reactions. Fig. 1 shows that, using the same experimental conditions ($I = 2.2 \times 10^{-2}$ mol dm^{-3} by addition of NaCl or $NaNO_3$; pH = 7 by using phosphate buffer; T = 25 °C), while the substitution of ethylenediamine in $[Pd(bpy)(en)]^{2+}$ is inhibited, the same reaction in $[Pd(bromazepam)(en)]^{2+}$ is accelerated. This kinetic effect can be related to the mode of interaction of the complexes with DNA. $[Pd(bpy)(en)]^{2+}$ intercalates to the double helix.[9,10] $[Pd(bromazepam)(en)]^{2+}$ binds externally, either docking in one of the grooves or simply electrostatically. No changes in the absorption spectrum of the latter complex nor any increase in the DNA viscosity are observed upon interaction. In addition, the increase in thermal denaturation temperature of the double helix, in the presence of $[Pd(bromazepam)(en)]^{2+}$ (1.4 ± 0.2 °C),† is much smaller than expected for a dicationic intercalator.[9] Intercalation protects the reaction centre from nucleophile attack making $[Pd(bpy)(en)]^{2+}$ unreactive; only the non-intercalated portion of this complex participates in the reaction and the

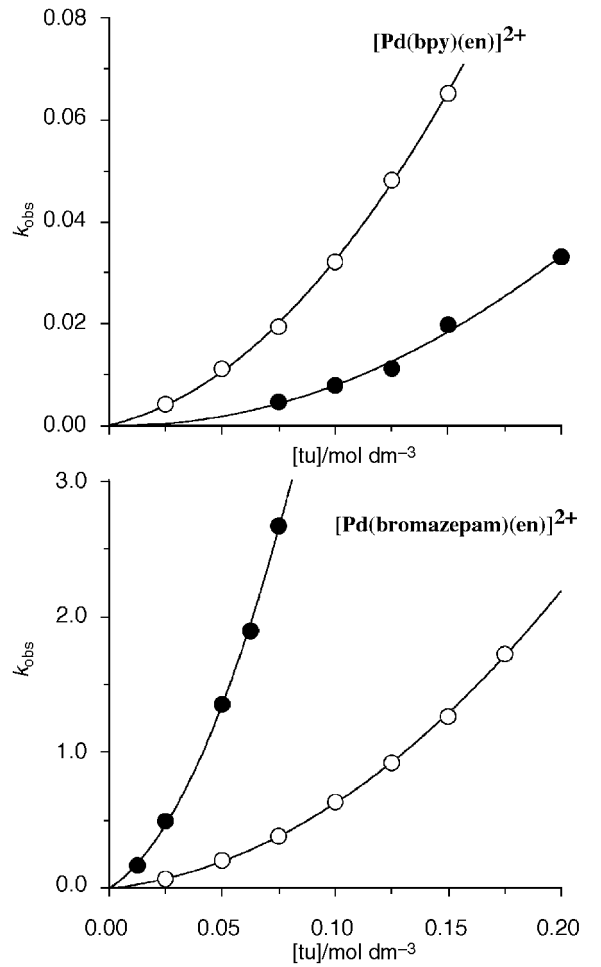

Fig. 1 Plot of k_{obs} against [tu] for reaction (1) (○) in the absence and (●) in the presence of DNA; 1.0×10^{-3} mol dm^{-3} phosphate buffer (pH 7) and 2.1×10^{-2} mol dm^{-3} NaCl or $NaNO_3$; T = 25 °C.

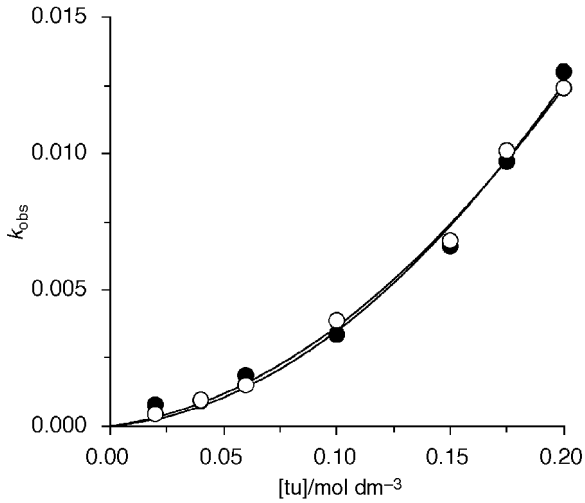

Fig. 2 Plot of k_{obs} against [tu] for reaction of $[Pd(Me_4en)(py)_2]^{2+}$ with thiourea (○) in the absence and (●) in the presence of DNA; 1.0×10^{-3} mol dm^{-3} phosphate buffer (pH 7) and 2.1×10^{-2} mol dm^{-3} NaCl or $NaNO_3$; T = 25 °C.

decreased concentration of one of the two reactants accounts for the decrease in the rate of substitution. In principle, an increase in the local concentration of the reagents can explain the catalytic effect of DNA; owing to its cationic nature [Pd(bromazepam)(en)]$^{2+}$ concentrates around DNA and if we assume that, for some reason, thiourea interacts with the biopolymer accumulating around it, enhanced concentration of the two reacting species would account for the observed acceleration in rate. More likely the observed catalytic effect is electronic in origin; the NH_2 hydrogen atoms of en are acidic[11] enough to interact with the DNA phosphate groups and so hydrogen bounding could stabilise the reaction intermediate with monodentate ethylenediamine. Kinetic data for the reaction of thiourea with $[Pd(en)_2]^{2+}$ and $[Pd(Me_4en)(py)_2]^{2+}$ corroborate this hypothesis. Both complexes bind externally to the double helix; however, while DNA catalyses the substitution of en, the rate of reaction for the latter complex, where no hydrogen atoms are available for interaction, is practically unaffected by the presence of the biopolymer (Fig. 2).

In conclusion, our data show that the observed kinetic effect of DNA on the reactivity of a small molecule can, in principle, be used as an indicator of its binding mode to the biopolymer.

Financial support from MURST and CNR is acknowledged.

Notes and references

† The thermal denaturation temperature of the complex–DNA mixture (1:10) was determined in 1.0×10^{-3} mol dm^{-3} phosphate buffer (pH 7) containing 7.8×10^{-6} mol dm^{-3} complex and 2.0×10^{-3} mol dm^{-3} NaCl.

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Communication 9/04471J