The dinuclear ruthenium(II) complex [{Ru(Phen)₂}₂(HAT)]⁴⁺ (HAT = 1,4,5,8,9,12-hexaazatriphenylene), a new photoreagent for nucleobases and photoprobe for denatured DNA

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The photophysical and photochemical properties of a dinuclear ruthenium(II) complex, [{Ru(Phen)₂}₂(HAT)]⁴⁺ (Phen = 1,10-phenanthroline, HAT = 1,4,5,8,9,12-hexaazatriphenylene), have been examined in the presence of mononucleotides and different polynucleotides. Characteristic new features, not observed with monometallic ruthenium(II) complexes, appear with this dimeric compound. First it forms strong ion pairs with the mononucleotides, adenosine- and guanosine-5′-monophosphate, detected from the absorption and emission characteristics under steady state and time-resolved conditions. Secondly, under steady state illumination, very weak luminescence enhancements are induced by the addition of double stranded calf thymus DNA (CT-DNA) whereas important increases of emission occur by the addition of denatured CT-DNA. The dinuclear species may thus be regarded as an excellent photoprobe for denatured DNA. On the other hand, the same photoreactivity with the nucleobases as that of the monometallic TAP (1,4,5,8-tetraazaphenanthrene) and HAT complexes has been retained by the dinuclear species, i.e. (i) a photoelectron transfer from guanosine monophosphate to the excited complex correlated with the formation of a photoproduct and (ii) a photoelectron transfer with DNA; in that case, however, this process is not systematically correlated with the formation of photoproduct in contrast to the monometallic species.

Introduction

During the last ten years, photophysics of several polypyridyl ruthenium(II) complexes in the presence of DNA has been examined in order to evaluate the possible use of these compounds as photoprobes of nucleic acids.[1–3] Some complexes have also been exploited for studies of photoelectron transfer processes on DNA, where a ruthenium(II) complex plays the role of the electron donor and a rhodium(III) complex is used as electron acceptor.[4] The examination of the electron transfer rates in the presence of DNA evidences different roles played by the DNA scaffolding. For example DNA concentrates the positively charged photoreactive species, on the double helix; this is accompanied by a slower electron transfer process due to slower diffusion;[5] it can also mediate the electron transfer process.[6]

In our laboratory we have tried for a few years to exploit the photoreactivity of some ruthenium(II) complexes towards certain DNA bases.[3,7] Recently, guanines belonging to specific DNA sequences have been targeted by the photoreaction.[8] We have shown that photoreactivity can be conferred upon the ruthenium(II) complex only when specific conditions are fulfilled.[2,9,10] First, the reaction has to be initiated by a photoelectron transfer from the nucleobase to the excited complex. Therefore the excited ³MLCT (metal to ligand charge transfer) state has to be sufficiently oxidizing. This condition is fulfilled when the complexes contain at least two TAP (1,4,5,8-tetraazaphenanthrene) or two HAT (1,4,5,8,9,12-hexaazatriphenylene) ligands. The guanines can then be photooxidized. Moreover, after the electron transfer process, followed by a proton transfer step, the radical formed on one TAP or HAT ligand of the complex recombines with the radical of the nucleobase. After rearomatization of the system, an adduct of the complex on the nucleobase is obtained; its structure has been determined.[11,12] The occurrence of such adducts can easily be detected from the irreversible change of the absorption spectrum of the complex as a function of its illumination time in the presence of guanosine monophosphate (GMP) or DNA.
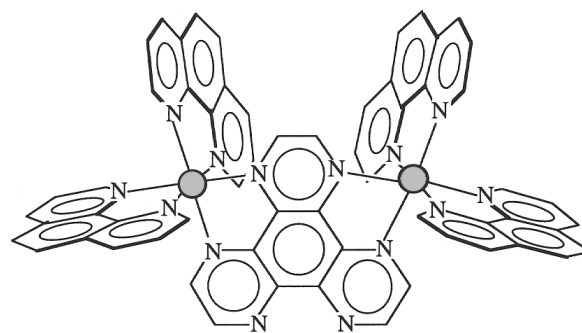
The goal of this paper is to examine whether this interesting photoreactivity can be extended to another HAT ruthenium(II) complex, the [{Ru(Phen)₂}₂(HAT)]⁴⁺, which is dinuclear and where the HAT bridges the two metal centres. The behaviour could be different to the previous ones because of the size of this compound. Its properties are thus studied in the presence of mononucleotides and different types of DNA, natural, denatured and synthetic.

Results and discussion

Luminescence in the presence of mononucleotides

The excited [{Ru(Phen)₂}₂(HAT)]⁴⁺ luminesces in aqueous solution from the ³MLCT excited state ($\lambda_{max} = 800 \pm 10$ nm) with a lifetime of 260 ns (Table 1); it is not quenched by oxygen.[13] In the pH domain investigated (7–9) the luminescence lifetime remains unchanged.

The emission intensities and lifetimes are affected by the addition of purine mononucleotides. With AMP, at pH 7 (or 9), a slight increase of emission intensity and lifetime is detected ($\tau_{with\ AMP}/\tau_{0without\ AMP} = 1.25$ for 0.02 mol l⁻¹ AMP, close to the plateau value reached with 0.1 mol l⁻¹ phosphate buffer, pH 7). This effect is accompanied by a slight change of the absorption spectrum of [{Ru(Phen)₂}₂(HAT)]⁴⁺ with AMP. These observ-

Table 1 Luminescence lifetimes for $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$ in the presence of different polynucleotides with a [DNA]:[complex] ratio = 40:1, a phosphate buffer 0.01 mol l^{-1}, at pH 7, in air at room temperature

Polynucleotide	τ_{short}/ns (%)	τ_{long}/ns (%)
ds CT-DNA	280 (77)	488 (23)
Denatured DNA	406 (66)	684 (34)
poly[d(A-T)]	252 (41)	486 (59)
poly[d(G-C)]	250 (55)	414 (45)
poly(dA)	280 (100)	
$[\{Ru(Phen)_2\}_2(HAT)]^{4+}$ (alone)	260 (100)	

The luminescence decays recorded at the emission maximum are analysed according to a biexponential function $I_{em}(t) = A_s\exp(-t/\tau_{short}) + A_l\exp(-t/\tau_{long})$; the preexponential factors represent the contributions of the different components just after the pulse (given in %).
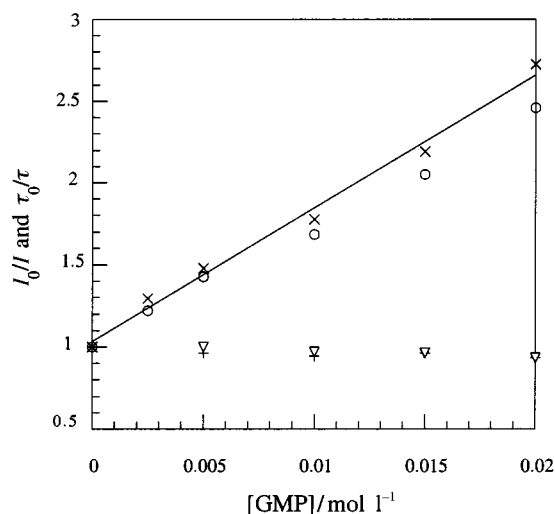

Fig. 1 Plots of I_0/I and τ_0/τ for $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$ as a function of GMP concentration (0.1 mol l^{-1} in phosphate buffer pH 7 and 9, under air), where I and τ are respectively the intensity and lifetime of emission in the presence of GMP, and I_0 and τ_0 in the absence of GMP. $\bigcirc$, I_0/I at pH 9; $\times$, τ_0/τ at pH 9; $+$, τ_0/τ at pH 7; $\bigtriangledown$, I_0/I at pH 7.

ations suggest that AMP and $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$ form ion pairs where the excited complex would be protected from the aqueous phase, inducing a slight increase of emission lifetime. This model could not be tested more quantitatively because of the complexity of the system due to the stereoisomers of the dinuclear complex.† We note that the capability to form ion pairs with mononucleotides had been observed with organic fluorescent dyes.[15]

With the addition of GMP the behaviour depends on the pH of the solution. At pH 7 slight enhancements of luminescence intensity and lifetime (a few percent) are observed (Fig. 1). These effects can also be correlated with a slight change of the absorption spectrum of the complex with GMP, and with a blue shift of the emission λ_{max} ($\approx$10 nm), again indicative of the formation of ion pairs. However with GMP the laser flash photolysis results (see further) indicate that a photoelectron transfer process takes place from the nucleobase to the excited complex. As there is no dynamic quenching of luminescence at pH 7 this charge transfer process would probably occur inside the ion pairs by static quenching.‡ At pH 9, where GMP starts deprotonating, which affects the redox potentials of guanine,

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† Two different excited state lifetimes have been found for the racemic mixture and the *meso* stereoisomer of $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$, 310 and 225 ns respectively, under air.[14] These two values cannot of course be distinguished when we use the natural mixture obtained after purification without stereoisomeric separation.

‡ Measurements with the pulsed laser equipment do not allow the detection of lifetimes shorter than or of the order of a few tens of nanoseconds.

there is a dynamic luminescence quenching by GMP (Fig. 1). The Stern–Volmer plots are approximately the same from measurements of luminescence lifetimes and intensities for low GMP concentrations. In this concentration range the quenching rate constant k_q is estimated to be 3×10^8 l mol^{-1} s^{-1}. For both types of measurements, the plot exhibits a downward curvature at GMP concentrations higher than 0.02 mol l^{-1} which is characteristic of a slight drop of k_q.[10] Indeed, although the buffer concentration is very high, for high quencher concentrations, the ionic strength is no longer constant and increases weakly, which leads to a slight k_q drop, thus a slight downward curvature. The dynamic quenching at pH 9, absent at pH 7, could originate from an increase of the exergonicity of the electron transfer process at pH 9 as compared to pH 7. The guanine is a weak base and has a pK_a for N^1–C^6–OH of 9.5–9.4.[16,17] Therefore, from pH 7 to 9, the guanine of GMP becomes partially deprotonated, which shifts the oxidation potential to a less positive value (from +1.29 to +1.17 V *vs*. SCE),[7] and consequently makes the free energy of the electron transfer $\Delta G°$ less positive (from 0.27 to 0.15 eV), increasing thus the driving force of the process. This would explain the occurrence of a dynamic quenching.

Laser flash photolysis in the presence of mono- and polynucleotides

With a reduction potential of +1.02 V *vs*. SCE for the excited dinuclear complex,[13] a photoelectron transfer from a guanine towards the excited bimetallic complex could take place on thermodynamic bases. Indeed from previous studies[2,7] we had concluded that with reduction potentials of the excited complexes of the order of +1V *vs*. SCE[7] an electron transfer from GMP can be induced. In agreement with this the laser flash photolysis results with $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$ show that a photoelectron transfer takes place indeed at pH 9 and 7 (see further, Fig. 2).

First, laser flash photolysis of $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$ in the absence of GMP[18] gives a differential transient absorption recorded 50 ns after the laser pulse with a positive absorption around 330 nm and a bleaching of the solution between 380 and 760 nm. The kinetic analysis of the recovery of the absorption at 590 nm according to a first order process leads to a lifetime of 300 ns in agreement (20% difference) with the luminescence lifetime of $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$.
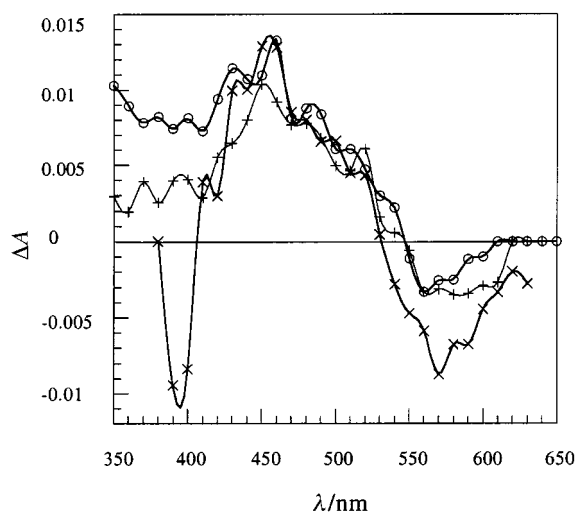

Fig. 2 Transient differential absorption spectra recorded 0.5 µs after the laser pulse for $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$ (5×10^{-5} mol l^{-1}) (Ar-saturated solution) with ($\times$) GMP (0.07 mol l^{-1}) at pH 7 phosphate buffer 0.1 mol l^{-1}; ($\bigcirc$) GMP (0.07 mol l^{-1}) at pH 9 phosphate buffer 0.1 mol l^{-1} and ($+$) in the presence of denatured CT-DNA phosphate buffer 0.01 mol l^{-1} at pH 7 ([DNA]/[complex] = 100; [DNA] is the equivalent concentration in bases).

Secondly, in the presence of GMP, the transient differential absorption recorded 0.5 µs after the laser pulse at pH 9 is shown in Fig. 2. The spectrum exhibits a positive differential absorption below 540 nm. In the 610–650 nm region there is no absorption and a bleaching is observed between 550 and 600 nm. A similar type of transient absorption is observed at pH 7 in the presence of GMP (except in the region below 400 nm, Fig. 2). With AMP no such long-lived transient absorption is detected. The comparison of the absorption spectra of the electrochemically reduced complex[18] with the differential absorption spectra obtained by laser flash photolysis with GMP at pH 7 and 9 indicates that the photoinduced transient differential absorption can be attributed to the reduced bimetallic complex. By comparison with the system "Ru(TAP)$_3^{2+}$ + GMP",[7] the electron transfer quantum yield at pH 9 is roughly estimated to be ≈0.1. Unfortunately, because the % of quenching is rather low the intensity of the transients is too weak to allow a quantitative kinetic analysis of the decay. However, qualitatively, under argon, two decay components can be detected at pH 7 and 9: one on a timescale of several hundreds of microseconds, and another on a much longer time domain. In the presence of air the first decay becomes faster (a few tens of µs). Therefore, the first decay under argon is probably associated with the reoxidation of the reduced dinuclear species by oxidized GMP. The effect of oxygen suggests that oxygen reacts faster than oxidized GMP with the monoreduced complex to regenerate the starting material; this was also observed with the system "Ru(TAP)$_3^{2+}$ + GMP". The origin of the second component is not quite clear. It may be possible that it corresponds to a stable photoproduct formed from the photoelectron transfer and in competition with the back electron transfer.

Laser flash photolysis has also been carried out in the presence of polynucleotides containing guanines, *i.e.* double stranded CT-DNA, poly[d(G-C)], and denatured CT-DNA. In each case a weak transient absorption is observed 0.5 µs after the laser pulse; an example is shown in Fig. 2 for [{Ru(Phen)$_2$}$_2$(HAT)]$^{4+}$ in the presence of denatured CT-DNA. A comparison of the transient obtained with denatured CT-DNA and GMP shows that the spectrum corresponds to the transient reduced complex. Again, because of the weak absorption, no kinetic analysis has been possible.

In conclusion, the pulsed laser results show that a photoelectron transfer occurs with the dinuclear complex and GMP or polynucleotides containing guanines.

Absorption and emission in the presence of polynucleotides

Absorption. For a ratio polynucleotide: [{Ru(Phen)$_2$}$_2$(HAT)]$^{4+}$ of 40:1 the addition of double stranded CT-DNA, denatured CT-DNA, double stranded poly[d(A-T)] and double stranded poly[d(G-C)] causes a slight hyperchromic effect on the MLCT absorption bands, different for each polynucleotide (not shown). These observations are thus different from the usual hypochromic and bathochromic effects associated with the interaction of mononuclear complexes with polynucleotides.[2] It has to be mentioned however that a slight bathochromic shift on the MLCT transition at 400 nm is detected, which probably corresponds to a Ru–Phen transition. In contrast, the single stranded poly(dA) has absolutely no effect on the absorption.

Steady state emission. The λ_{max} of emission of [{Ru(Phen)$_2$}$_2$(HAT)]$^{4+}$ is not much influenced by the addition of polynucleotide, except in the presence of denatured CT-DNA where there is an hypsochromic shift from ≈800 to ≈760 nm. The effect of addition of variable concentrations of different polynucleotides on the emission intensity at constant concentration of the dinuclear complex is presented in Figs. 3(a), 3(b), and 4. For comparison purposes, the study has also been carried out with mononuclear species such as [Ru(Phen)$_3$]$^{2+}$ and [Ru(Phen)$_2$-
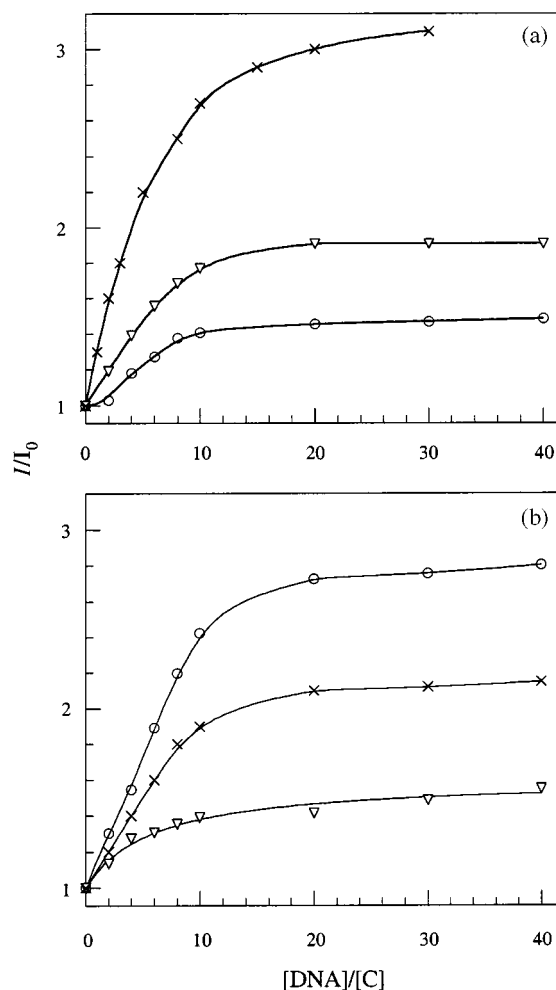

Fig. 3 Emission intensities of different complexes, (○) [{Ru(Phen)$_2$}$_2$-(HAT)]$^{4+}$, (▽) [Ru(Phen)$_3$]$^{2+}$, (×) [Ru(Phen)$_2$(HAT)]$^{2+}$, as a function of [DNA]:[complex] ratio ([DNA] is the equivalent concentration in bases), (a) with double stranded CT-DNA and (b) with denatured CT-DNA (phosphate buffer 0.01 mol l^{-1}, pH 7, complex concentration 1.5×10^{-5} mol l^{-1}).

(HAT)]$^{2+}$ under the same experimental conditions. In a first step, the effect of double stranded CT-DNA [Fig. 3(a)] has been examined by measuring the intensity ratio I/I_0 as a function of the ratio [DNA]:[complex] where I and I_0 correspond respectively to the luminescence intensity in the presence and in the absence of CT-DNA. The most important luminescence increase is observed for [Ru(Phen)$_2$(HAT)]$^{2+}$, and the less important one is found for the dinuclear complex. Fig. 4 illustrates on a magnified scale the weak effect of addition of different double stranded polynucleotides, poly[d(A-T)], poly[d(G-C)], and again CT-DNA, on the emission intensity of the bimetallic complex. These weak effects contrast with the situation with the mononuclear species. This difference suggests of course that the dinuclear complex does not interact well within the DNA grooves. This is understandable taking into account the dimensions of the dinuclear complex as compared to those of the DNA grooves. Very interestingly, if instead of double stranded CT-DNA denatured CT-DNA is added [{Ru(Phen)$_2$}$_2$(HAT)]$^{4+}$ leads to the most important emission increase [Fig. 3(b)]. This complex is thus much better protected from the aqueous environment by the hydrophobic bases of the denatured portions of CT-DNA where the two strands are separated than in the DNA grooves of a double helix. The presence of the double stranded portions (≈60%) in denatured DNA[19] is however needed to observe the important emission increase because a pure single stranded polynucleotide such as poly[d(A)] (Fig. 4) does not lead to significant luminescence increases. Thus the so-formed "pockets" inside
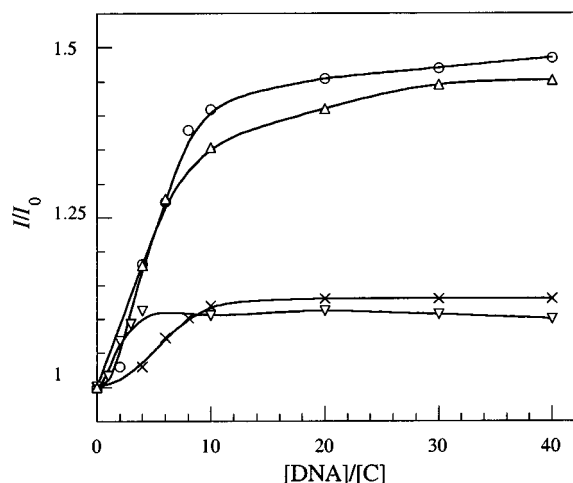
Fig. 4 Effect of different double stranded polynucleotides on the emission intensities of $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$ as a function of the [DNA]:[complex] ratio ([DNA] is the equivalent concentration in bases) in the presence of phosphate buffer 0.01 mol l^{-1} at pH 7, [complex] = 1.5×10^{-5} mol l^{-1}: $\bigcirc$, with CT-DNA [same as Fig. 3(a)]); $\triangledown$, with poly[d(G-C)]; $\triangle$, with poly[d(A-T)]. $\times$, Effect of single stranded poly[d(A)].

Fig. 5 Denaturation curves of poly[d(A-T)] alone ($\triangledown$) and in the presence of $[Ru(Phen)_3]^{2+}$ ($\bigcirc$), $[Ru(Phen)_2(HAT)]^{2+}$ ($\times$), and $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$ ($\square$), for a ratio [DNA]:[complex] = 10:1 ([DNA] is the equivalent concentration in bases), and a concentration in complex of 2×10^{-6} mol l^{-1}, phosphate buffer 0.01 mol l^{-1}, pH 7.

denatured DNA play the role of host *versus* the $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$ as guest.

Luminescence lifetimes. Under pulsed excitation the luminescence decays of $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$ with polynucleotides are biexponential, except with single stranded poly(dA) where the decay corresponds to a pure single exponential; the associated luminescence lifetimes are given in Table 1 (τ_{short} and τ_{long}). At a [DNA]:[complex] ratio of 40:1, for all the double stranded polynucleotides, the values of τ_{short} are approximately equal to 250–280 ns, *i.e.* the emission lifetime of $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$ in buffer solution. Thus with double stranded poly[d(A-T)], poly[d(G-C)] and CT-DNA one concludes that the short lifetime component corresponds, within the experimental error, to the lifetime of the excited complex which is not protected by the hydrophobic environment of the double helix. The excited state is probably in interaction with the polyphosphate backbone, thus in the ionic atmosphere surrounding the DNA. This is consistent with the fact that it has not been possible to extract the complex from the DNA under normal dialysis conditions (*i.e.* for a ratio [DNA]:[complex] of 40:1, 10 mmol l^{-1} phosphate buffer). Actually 10 mmol l^{-1} of MgCl$_2$ plus 50 mmol l^{-1} phosphate buffer were needed to extract 80% of the complex. Moreover the fact that τ_{long} in Table 1, for the double stranded DNAs, is less than a factor of two longer than the lifetime of the dinuclear species in buffer solution suggests again that the complex would be only partially protected by the nucleobases inside the helix and thus would be located closer to the polyphosphate backbone.

In contrast to this, it is interesting that, in the presence of denatured CT-DNA, the two luminescence lifetimes are both longer than in aqueous solution. Taking into account that, in denatured CT-DNA, approximatively 60% of the polynucleotide is present in the form of double stranded DNA, it may be possible that the shorter component is related to the lifetimes of the species in interaction with the double stranded portions, whereas the contribution of the longer species would originate from the excited complex interacting with the denatured portions (obviously if our assumptions are correct, the decays should correspond to more than two components but the experimental evidence for this, when the lifetimes are not extremely different, is not reliable). In the denatured portions the whole complex would be protected by the hydrophobic bases of the "DNA pockets". Again, in the absence of these "DNA pockets", thus in the presence of single stranded
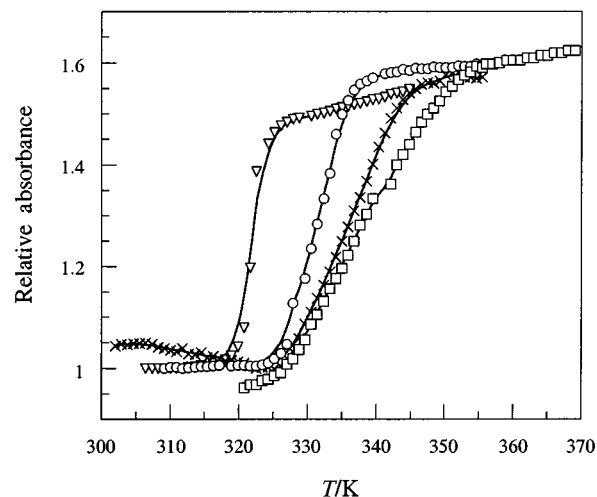
poly(dA) for example (100% single stranded polynucleotide) the luminescence of the complex is not affected at all, as indicated by the single excited state lifetime of $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$.

In conclusion, the luminescence decays data indicate that the dinuclear complex behaves as an excellent photoprobe of denatured DNA.

Effects of the dinuclear complex on the denaturation of poly[d(A-T)]

In order to evidence the interaction of the dinuclear complex with denatured polynucleotides by another method we have examined its effect on the denaturation curves of poly[d(A-T)]; for comparison purposes, we have included the corresponding data in the presence of two monometallic complexes, [Ru(Phen)$_2$(HAT)]$^{2+}$ and [Ru(Phen)$_3$]$^{2+}$. Fig. 5 shows that the dinuclear complex affects rather importantly the denaturation curve of the synthetic polynucleotide. The lower slope of the absorption *versus* temperature curves could indicate a multistep denaturation. This could originate from progressive changes of interaction of the compound when double stranded poly[d(A-T)] starts denaturing, *i.e.* when the complex can penetrate gradually inside the denatured DNA pockets, which stabilizes them accordingly.

Steady state photochemistry of $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$ with mononucleotides and polynucleotides; correlation with the photoelectron transfer process

In order to determine whether the photoinduced electron transfer observed with GMP and with polynucleotides containing guanines can be correlated with the formation of photoproducts (as found with the monometallic complexes[3,11,12]), steady state illuminations of $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$ have been carried out with GMP and with different polynucleotides. The formation of photoproduct has been monitored by UV-visible absorption spectroscopy as a function of the irradiation time.

Illumination in the presence of GMP at pH 7 and 9 (same pH as for the luminescence quenching studies) shows changes of the visible absorption spectrum of $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$ with the irradiation time (Fig. 6). These experiments demonstrate that a photoproduct is formed at both pH. This product is stable and thus does not correspond to a transient as in laser flash photolysis; it is indeed insensitive to oxygen. The HPLC analysis of illuminated $[\{Ru(Phen)_2\}_2(HAT)]^{4+}$ with GMP at pH 7 or 9 evidences formation of only one photoproduct.

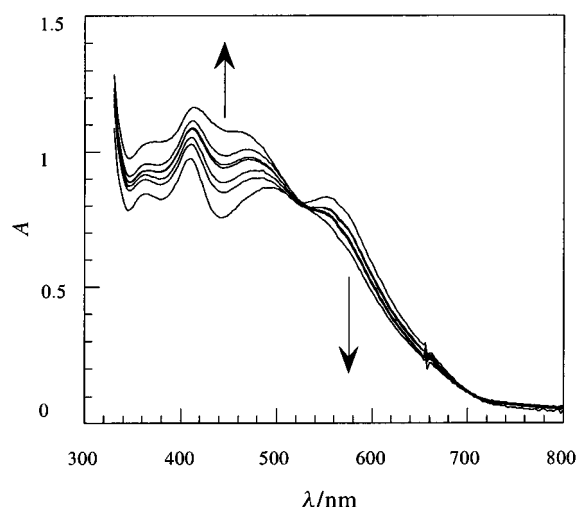**Fig. 6** Evolution of the absorption spectrum of [{Ru(Phen)$_2$}$_2$-(HAT)]$^{4+}$ 4×10^{-5} mol l^{-1} under continuous illumination in the presence of GMP (0.05 mol l^{-1}) and phosphate buffer 0.1 mol l^{-1} at pH 7, after 0, 15, 30, 60, 75, 90 and 120 min.

Preliminary results of characterization after isolation (^{31}P and ^{1}H NMR, ESMS) show that the photoproduct could correspond to a photoadduct of the guanosine monophosphate on the bridging HAT ligand.

When the illumination of [{Ru(Phen)$_2$}$_2$(HAT)]$^{4+}$ is performed in the presence of AMP instead of GMP at pH 7 no transformation of the absorption spectrum is detected. We can thus conclude that the formation of photoproduct is correlated with the photoelectron transfer process with GMP (no such process with AMP), as it had been concluded with the monometallic complexes. Interestingly, although photoproduct is formed with GMP, no phototransformation (no change of the absorption spectrum) of the starting material is detected with polynucleotides containing guanines, *i.e.* double stranded CT-DNA (for a [DNA]:[complex] ratio of 100:1), and this in spite of the presence of a photoelectron transfer with double stranded CT-DNA. This lack of photoproduct could originate from steric hindrances between [{Ru(Phen)$_2$}$_2$(HAT)]$^{4+}$ and the double helix which would prevent the production of photoproduct.

The steady state illuminations have been repeated several times with denatured CT-DNA. We have observed that for a few experiments a change of the absorption spectrum similar to that with GMP (Fig. 6) occurred. The single-stranded portions hosting the complex without steric hindrances could thus lead under those conditions to the formation of photoproduct, if guanines are present in those portions.

Conclusion

The results presented in this paper evidence important characteristics of the dinuclear HAT ruthenium(II) complex, which are quite different from the properties of monometallic compounds. First of all, the interaction with double stranded DNA is weak, in contrast to the situation with monometallic species. On the other hand the dinuclear species interacts well with denatured CT-DNA. This could offer the possibility to use this type of large polynuclear complex as a specific photoprobe of denatured DNA, or as a specific agent for the detection of deformed portions along double stranded DNA. In addition, the dinuclear species has retained the attractive properties of the TAP and HAT complexes, *i.e.* the photoreactivity with the guanine nucleobases. However photoproducts do not occur if the complex does not have free access to the guanines buried inside the double stranded DNA, as opposed to the free GMP or the more accessible guanines of denatured DNA. Thus a systematic investigation of the conditions of formation of photoproducts with the guanines of denatured DNA would offer the possibility to mark irreversibly these portions by the dinuclear complex under illumination. This could lead to a variety of applications in DNA studies, complementary to those found for artificial photonucleases, such as dinuclear Ru–phen complexes with an aliphatic linker,[20] or anthraquinone derivatives that target single-stranded regions of hairpin structures.[21]

Experimental

The synthesis and characterisation of [{Ru(Phen)$_2$}$_2$(HAT)]$^{4+}$ were described previously.[13] The guanosine- and adenosine-5′-monophosphate (Aldrich, Brussels, Belgium) were used without further purification as potassium salts. Calf thymus DNA ($\varepsilon_{260} = 6600$ l mol^{-1} cm^{-1}, Pharmacia) was dialysed extensively against a phosphate buffer solution and afterwards against water. Denatured CT-DNA was prepared by heating double stranded CT-DNA for 10 min at 90 °C, and by cooling rapidly the solution with liquid nitrogen; the sample was used immediately when it had reached the room temperature. The percentage of single stranded DNA was estimated from absorption spectroscopy[19] and corresponded generally to 40% ($\varepsilon_{260\ nm\ single\ stranded\ DNA} = 1.4\varepsilon_{260\ double\ stranded\ DNA}$). Synthetic polynucleotides (Pharmacia) [poly[d(A–T)], $\varepsilon_{262} = 6600$; poly-[d(G–C)], $\varepsilon_{260} = 6600$; poly(dA), $\varepsilon_{260} = 10000$ l mol^{-1} cm^{-1}] were used as received. The buffer solutions were prepared with KH$_2$PO$_4$ and K$_2$HPO$_4$ (Merck, Darmstadt, Germany); water was purified with a Millipore Milli-Q system. Absorption spectra were recorded on a Varian Cary 219 UV-VIS or an Hewlett-Packard (HP) 8452A diode array spectrophotometer, emission spectra on a Shimatsu spectrofluorimeter (RFPC 5001) equipped with a xenon lamp (250 W) as exciting source and an Hamamatsu R-928 photomultiplier.

The luminescence lifetimes were measured by using a modified Applied Photophysics laser kinetic spectrometer equipped with an Hamamatsu R-928 photomultiplier. The excitation source was a frequency doubled neodymium YAG laser (Continuum NY61-10) producing a 532 nm beam (10 ns pulse width, maximum 10 mJ per pulse). Signals were recorded with a digital oscilloscope (HP 54200A), connected through an IEEE interface to an HP 9816S computer and were averaged over at least 16 shots. A baseline correction was also introduced. Kinetic analyses of the traces were performed by non-linear least-squares regression[22,23] using a modified Marquardt's algorithm.[23] All the luminescence lifetimes in the presence of mono- or poly-nucleotides were carried out at room temperature in air saturated phosphate buffer solutions at pH 7 or 9 (0.1 mol l^{-1}).

Laser flash photolysis experiments were carried out using the pulsed Nd:YAG laser mentioned above (20 mJ per pulse) and a xenon lamp (250 W) as the monitoring source. Traces were recorded and transferred to the same analysis system as for the emission lifetimes.

Steady state illuminations in the presence of mono- and poly-nucleotides were carried out in a cylindrical cell (diameter 20 mm, optical pathlength 10 mm) with a 2000 W halogen lamp (Osram GY 16) and a UV cut off filter (NaNO$_2$, absorbance at 330 nm > 4).

The HPLC conditions for analysing the illuminated solutions of the dinuclear complex in the presence of GMP were as follows: gradient water–methanol (80:0 to 20:80) in 10 min on a micro-bondapak-C18 column (Waters), pH 2.5 (phosphoric acid 5×10^{-2} mol l^{-1}), rate 2 ml min^{-1}.

Acknowledgements

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