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Review Commentary

Mono- and polynuclear ruthenium(II) complexes, photoprobes and reagents for targeted DNA sites

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ABSTRACT: Several luminescent ruthenium(II) complexes were designed whose main characteristic is their photoreactivity towards mononucleotides and DNA. It was clearly demonstrated that this photoreactivity originates from a photoinduced electron transfer from a guanine to the excited complex. This process leads to the formation of an adduct which was characterized. The structure shows that the complex is anchored to the nucleotidic base via one of its polyazaaromatic ligands, thus marking irreversibly the DNA guanines. Interestingly, this property can be used in order to target, for example, (i) specific DNA sequences and (ii) particular DNA topologies. For each purpose a specific Ru(II) complex was designed. Synthetic oligonucleotides derivatized with mononuclear complexes were prepared to target and damage specific DNA sequences containing G sites. In these systems, it is shown that the DNA damage consists of an irreversible photo-crosslinking of the derivatized oligonucleotide with the complementary strand. In order to target portions of important deformation along double-stranded DNA, the dinuclear complex $[Ru(phen)_2]_2HAT^{4+}$ was prepared and studied. This complex is too large to penetrate inside the major or minor grooves of a DNA double helix, so that only single-stranded portions of denatured DNA are accessible to this compound. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: ruthenium (II) complexes; photoreactivity; targeted DNA sites

INTRODUCTION

DNA is a biological polyelectrolyte which has been extensively studied in biology and biochemistry.[1,2] However, although the knowledge in that area has progressed tremendously, there is an important need for methods and tools to determine local structures and topologies of DNA and relate them to their function. Some of the molecular tools developed in this field are based on polypyridyl metal complexes. The interest in such coordination compounds is due to their luminescence and photoreactivity in the presence of DNA.[3–7] The development of such molecules can also lead to new potential anti-tumour drugs based on metallic compounds, as is the case with the platinum(II) complexes. Despite the numerous studies on Pt(II) compounds in biological systems,[8–12] their toxicity has motivated researchers to investigate new metallic candidates for cancer therapy such as compounds based on ruthenium(II)[13] or ruthenium(III) ions. The latter have led to some clinical applications. Keppler[14] has shown that *trans*-imidazolium[tetrachlorobisimidazoleruthenate(III)] and analogues are active against different tumours, mainly by inhibiting the DNA replication.

On the other hand, recent studies have shown that tris(polypyridyl)ruthenium(II) complexes can penetrate cell membranes.[15] This offers an important advantage for clinical applications. In addition, in contrast to the Pt drugs which are active in the dark, the tris(polypyridyl)-ruthenium(II) complexes discussed in this paper would be active only under visible light. The formation of photoadducts of these coordination complexes with DNA could indeed interfere with the normal functions of DNA, for example by inhibiting the RNA polymerase.

When the metallic compounds are designed as novel DNA molecular tools or potential anti-tumour drugs, the main criteria for their preparation are their interaction and affinity for DNA, their photoreactivity towards nucleic acids and their interaction with special geometries or topologies of double-stranded DNA. This paper reviews the research performed with certain complexes which respond to these criteria and which could be used for possible applications in DNA studies.

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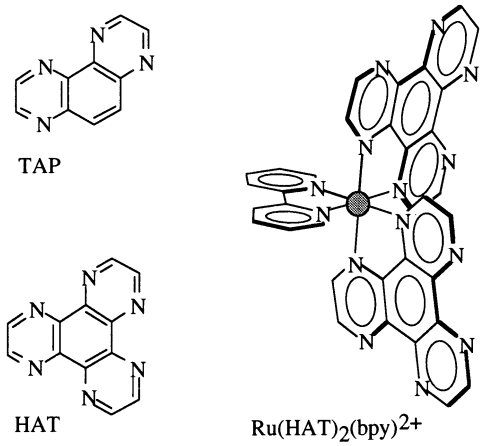


Figure 1. Structures of TAP (= 1,4,5,8-tetraazaphenanthrene), HAT (= 1,4,5,8,9,12-hexaazatriphenylene) and $Ru(HAT)_2(bpy)^{2+}$.

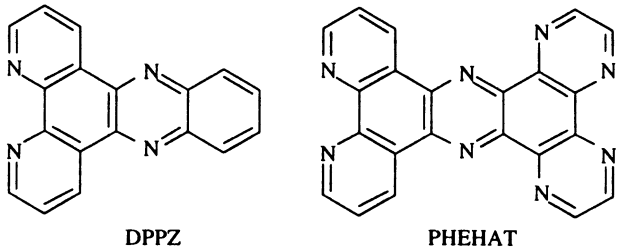


Figure 2. Structures of extended aromatic ligands: DPPZ = dipyrido[3,2-*a*:2′,3′-*c*]phenazine and PHEHAT = 1,10-phenanthrolino[5,6-*b*]1,4,5,8,9,12-hexaazatriphenylene.

INTERACTION AND AFFINITY

It has been shown that organic molecules and complexes interact with DNA according to different geometries.[1,16] As the tris(polypyridyl)ruthenium(II) complexes are positively charged, they can interact with the negatively charged phosphate backbone of the DNA helix. For example, $Ru(Me_2TAP)_3^{2+}$ (Me_2TAP = 2,7-dimethyl-1,4,5,8-tetraazaphenanthrene) has been shown to interact like Mg^{2+} with DNA and consequently electrostatic interactions have been proposed as dominating the association with DNA.[17] The absence of interaction with the nucleobases is attributed to steric hindrances between the methyl groups and the double helix backbone. Less sterically hindered molecular species may enter the minor or major DNA grooves. These include anti-cancer agents such as netropsin[18,19] and metallic complexes such as $Ru(TAP)_3^{2+}$ (TAP = 1,4,5,8-tetraazaphenanthrene)[20,21] and $Ru(TAP)_2(bpy/phen)^{2+}$ (bpy = 2,2′-bipyridine, phen = 1,10-phenanthroline)[22] (Fig. 1).

In many cases there are specific hydrogen bonding and van der Waals interactions between these molecules and DNA. A third possibility of binding is the intercalation of a planar portion of the molecule between the base pairs of DNA.[23] This induces a lengthening and unwinding of the double helix. A battery of methods[24–26] such as absorption, luminescence and proton NMR spectroscopy,[27–29] viscosity experiments,[25,26] circular and linear dichroism[30] and measurements of DNA unwinding[31,32] are needed to describe realistically the non-covalent binding of these compounds to DNA. For example, an intercalative mode of interaction has been unambiguously demonstrated for $Ru(phen)_2(DPPZ)^{2+}$ (DPPZ = dipyrido[3,2-*a*:2′,3′-*c*]phenazine),[33–37] $Ru(phen)_2(PHEHAT)^{2+}$ (PHEHAT = 1,10-phenanthrolino[5,6-*b*]-1,4,5,8,9,12-hexaazatriphenylene)[6,38] (Fig. 2) and $Ru(bpy)_2(TPPHZ)^{2+}$ (TPPHZ = tetrapyrido[3,2-*a*:2′,3′-*c*:3″,2″-*h*:2‴,3‴-*j*]phenazine)[39] behave similarly.

A complete intercalation of the extended aromatic ligand is, of course, prevented by the steric hindrance of the two ancillary ligands. Complexes with such extended aromatic ligands exhibit affinity constants for DNA (10^6–10^7 l mol^{-1})[34,36,38] much higher than the other complexes and even higher than ethidium bromide. This strong interaction results in important changes in the spectroscopic properties, making these compounds powerful spectroscopic probes for DNA. Complexes with less extended aromaticity such as $Ru(bpy/phen)_n(HAT)_{3-n}^{2+}$ (HAT = 1,4,5,8,9,12-hexaazatripenylene; n = 0, 1, 2)[40–43] and $Ru(bpy)_2(PPZ)^{2+}$ (PPZ = 4,7-phenanthrolino[5,6-*b*]-pyrazine)[44–47] also exhibit characteristics relevant to intercalation. For example, a clear hypochromic effect on the MLCT (metal to ligand charge transfer) transition in the presence of DNA[42,43,45,47] or a very slow mobility of the HAT complex along the double helix[48] can be mentioned. This illustrates clearly that changes in the chelating ligands may strongly modify the interaction with nucleic acids. An exhaustive list of complexes whose interaction with DNA has been examined can be found elsewhere.[49] It should also be noted that for metallic complexes, the binding modes may be more complicated than for purely organic molecules and neither intercalation nor a groove adsorption are unambiguous concepts but rather denomination for groups of binding modes with common features.

PHOTOREACTIVITY TOWARDS POLYNUCLEOTIDES

Photophysical properties

The understanding of the photoreactivity of tris(polypyridyl)ruthenium(II) complexes with species such as mononucleotides or DNA requires a good knowledge of their photophysics, which has been extensively examined.[50] The singlet MLCT excited state, populated by visible irradiation, deactivates rapidly by intersystem crossing to a manifold of four ^{3}MLCT states in Boltzmann equilibrium during the decay process. From

Table 1. Absorption and luminescence maxima of a series of complexes, along with oxidation (E_{ox}) and reduction potentials (E_{red}) and the corresponding reduction potentials in the excited ^{3}MLCT state (E_{red^*})

	H_2O		CH_3CN			
Complex	Absorption λ_{max} (nm)	Emission λ_{max} (nm)	E_{ox} (VVSSCE)	E_{red} (VVSSCE)	E_{red^*} (VVSSCE)	Ref.
$Ru(HAT)_3^{2+}$	410sh[a], 440	596	+2.07	−0.62	+1.46	52
$Ru(TAP)_3^{2+}$	408, 437	602	+1.94	−0.75	+1.32	51
$Ru(HAT)_2(phen)^{2+}$	408, 470	657	+1.86	−0.66	+1.23	55
$Ru(HAT)_2(bpy)^{2+}$	408sh, 472	661	+1.79	−0.76	+1.12	52
$Ru(TAP)_2(bpy)^{2+}$	412, 465	649	+1.70	−0.83	+1.06	55
$Ru(TAP)_2(phen)^{2+}$	410, 466	656	+1.73	−0.83	+1.06	55
$Ru(phen)_2(HAT)^{2+}$	430, 494sh	732	+1.53	−0.86	+0.87	38,52
$Ru(bpy)_2(HAT)^{2+}$	432, 484sh	742	+1.56	−0.84	+0.83	52
$Ru(bpy)_2(TAP)^{2+}$	439, 484sh	714	+1.51	−0.88	+0.86	51

[a] sh = Shoulder.

this ensemble of ^{3}MLCT states, considered as one unique state, the complex can deactivate by either radiative or radiationless deactivations, the latter generally controlling the lifetime of the ^{3}MLCT state, or by a conversion to the upper ^{3}MC (metal centred) state reached by thermal activation from the ^{3}MLCT state. Two different reactivities are associated with the two excited ^{3}MLCT and ^{3}MC states. In the former, the reactivity corresponds mostly to redox processes whereas the ^{3}MC state may photoreact via the loss of a ligand. As the photophysical characteristics of most of the Ru(II) complexes are such that they prevent an efficient chemistry from the ^{3}MC state, several studies have been focused on the ^{3}MLCT reactivity, in other words on photoredox processes. In addition, as no photoreactivity of Ru(II) complexes with DNA has been shown when the excited complex acts as the reductant, this review concerns Ru(II) complexes which are powerful oxidants in the ^{3}MLCT state and, therefore, are able to abstract an electron from rather poor reductants.

Detailed studies with HAT or TAP complexes with bpy or phen as ancillary ligands, rationalize the influence of the ligands on the spectroscopic and electrochemical properties of the corresponding complexes.[51,52]

The oxidation potential is shifted anodically by increasing the number of TAP or HAT ligands in the complex. In reduction, the addition of the first electron takes place on the ligand which exhibits the best π-acceptor ability: first HAT, then TAP and finally bpy or phen. As the orbitals involved in the electronic transitions are the same as those involved in electrochemistry, the energy of the transition (in absorption and in emission) is correlated with the difference between the first reduction and the first oxidation potential. Such a correlation allows one to estimate the redox potentials in the excited state from the potential in the ground state and the energy of the emission maximum. Table 1 shows that complexes containing two or three π-acceptor ligands are the most oxidizing in the excited state. It should be noted that HAT offers a supplementary advantage. This symmetric molecule is a trischelating ligand which can be used to design polymetallic complexes. Bi-, tri- and even heptametallic complexes have been prepared from Ru(II) and HAT.[52–54]. These polynuclear complexes exhibit very intense absorptions at wavelengths longer than their monometallic analogues. These compounds follow also the spectroelectrochemical correlation described above and are oxidants in their ^{3}MLCT state. They are therefore also good candidates as photoreagents of nucleic acids. Their photochemistry and special interactions will be discussed in the last section.

The modulation of the redox properties of the Ruthenium(II) complexes according to the type of ligand strongly influences their photoreactivity with the mono- and polynucleotides.

Evidence for electron transfers with nucleic acids

Generally, the interaction of a luminescent complex with a polynucleotide is accompanied by an increase in its luminescence intensity and lifetime, attributed to protection by the DNA microenvironment (rigidity, protection from water and from oxygen quenching). For the $Ru(bpy)_n(TAP/HAT)_{3-n}^{2+}$ ($n = 0, 1, 2, 3$) series, considerable changes in the emission are observed by increasing the DNA concentration (Fig. 3).

Depending on the combination of ligands in the complex, the emission shows two different behaviours.[21,42,55] If the complex contains less than two TAP or HAT oxidizing ligands, the luminescence increases upon addition of increasing amounts of DNA. This is attributed to the rigidity and hydrophobicity of the double helix environment, resulting in a decrease in the efficiency of the radiationless deactivation processes. In contrast, if the complex contains two or three π-acceptor ligands, the emission is inhibited by the DNA, and the correlation of this luminescence quenching with the redox potentials in the excited state suggests that the quenching results from the reduction of the excited complex by the most reducing base. This hypothesis has been supported by studies carried out with different polynucleotides. For all

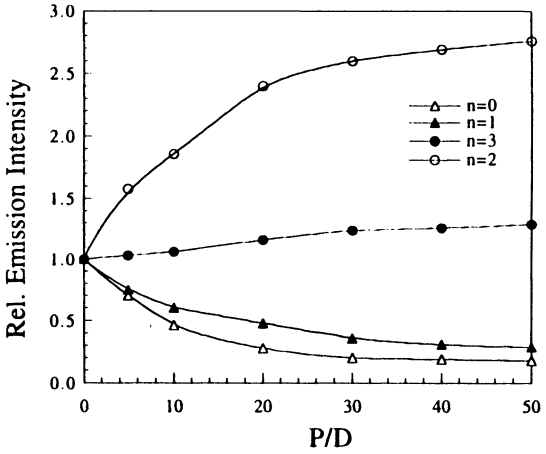


Figure 3. Effect of increasing ratio of [DNA] (equivalent in phosphate concentration)/[complex], i.e. P/D, on the emission intensity of the complexes at constant concentration, for $Ru(bpy)_n(TAP)_{3-n}^{2+}$, $n = 0, 1, 2, 3$) (adapted from Ref. 21).

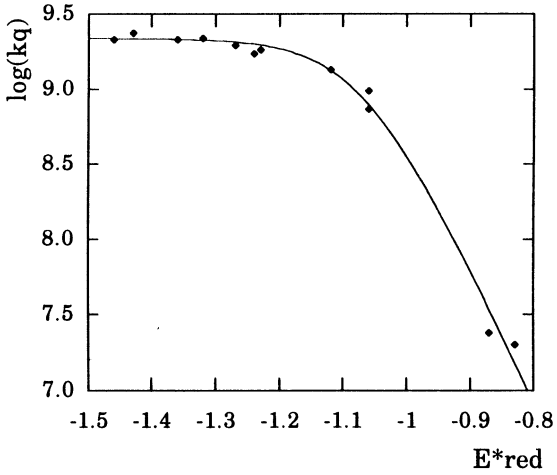


Figure 4. Plot of the logarithm of the luminescence quenching rate constants k_q, measured in the presence of GMP, versus the excited-state reduction potentials calculated for the complexes listed in Table 2 (adapted from Ref. 55).

the complexes containing at least two oxidizing ligands, whose luminescence is inhibited by DNA, [poly(dG–dC)]$_2$ also induces luminescence quenching. On the other hand, in the presence of [poly(dA–dT)]$_2$, the luminescence increases on adding the polynucleotide, except for the most oxidizing compound ($E^*_{red} \geq 1.4$ V vs VSCE). In that case, [poly(dA–dT)]$_2$ also induces a luminescence quenching. Other information in favour of the photoinduced electron transfer from the nucleobase to the excited complex has been gained from experiments performed with the mononucleotides. The emission of complexes containing TAP and HAT ligands is quenched in the presence of guanosine-5′-monophosphate (GMP)[56] The rate constant of these quenchings clearly depends on the $E_{Ru^{2+*}/Ru^{+}}$ value of the complexes. The plot of the logarithms of these constants as a function of the reduction potential of the excited complexes, gives a curve typical for a quenching by electron transfer (Fig. 4), where the plateau value corresponds to the most exergonic processes which are diffusion controlled.[55]

The emission of the most oxidizing complexes (containing three oxidizing ligands) is also quenched by adenosine-5′-monophosphate (AMP), but with lower rate constants, i.e. the emission quenching follows the redox potentials of the bases, as GMP is more easily oxidizable than AMP.[56]

Further evidence for the photoinduced electron transfer consists in detecting the monoreduced complex. This has been achieved by detailed flash photolysis studies, as both the monoreduced complex and oxidized guanine radical cation can be observed (Fig. 5).[56,55]

$$[Ru(TAP)_3]^{2+} + GMP \xrightarrow[k_q]{h\nu} [Ru(TAP)_2TAP^{-\cdot}]^{+} + GMP^{\cdot +}$$

Flash photolysis experiments performed with the series of TAP and HAT complexes and DNA lead to the same

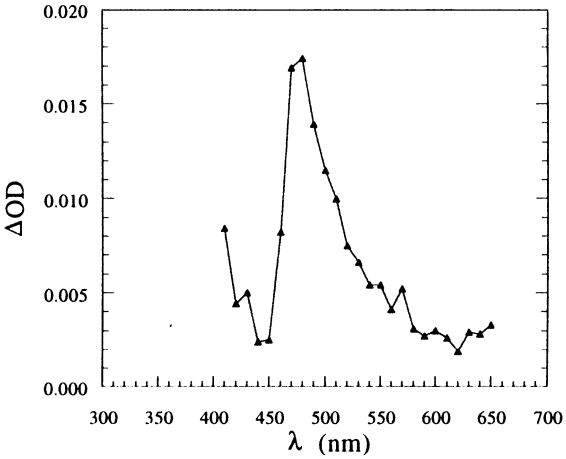


Figure 5. Differential transient absorption spectrum recorded by laser flash photolysis of $Ru(TAP)_3^{2+}$ in the presence of GMP (spectrum recorded 1 μs after the laser pulse) (adapted from Ref. 56).

conclusions, which demonstrate clearly the presence of a photoinduced electron transfer from the nucleobases to the excited complex.[55] The correlation of the luminescence quenching by the mono- and polynucleotides with the occurrence of a photoinduced electron transfer shown by flash photolysis has been observed for the whole series of TAP and HAT complexes.

The data, shown in Table 2, illustrate well that it is easy to modify the photophysical behaviour of a complex towards the polynucleotides by changing in a controlled fashion the nature of the ligands and their combination in the corresponding complex. As will be developed further, this photoinduced electron transfer with polynucleotides is correlated with an increased yield of strand cleavages and to the formation of adducts on DNA.

Table 2. Luminescence lifetimes and quenching rate constants (k_Q) by GMP and AMP in aqueous solution of 0.1 M phosphate buffer, (pH 7), [Ru] $\approx 5 \times 10^{-5}$ M, and the effect of increasing concentrations of various polynucleotides on the luminescence intensity at a fixed wavelength

		Quencher = GMP, E_{ox} = +0.92 VVSSCE		Quencher = AMP, E_{ox} = +1.32 VVSSCE				
Complex	τ_{buffer} (ns)	E_{red^*} (VVSSCE)	$k_Q \times 10^{-9}$ ($lmol^{-1}$ s^{-1})	$k_Q \times 10^{-9}$ ($lmol^{-1}$ s^{-1})	DNA	pd(GC)	pd(AT)	Ref.
$Ru(HAT)_3^{2+}$	176	1.46	2.16	0.87	↓		↓	42,55
$Ru(TAP)_3^{2+}$	231	1.32	2.20	0.12	↓	↓	↑	21,55
$Ru(HAT)_2(phen)^{2+}$	580	1.23	1.85		↓		↑	55
$Ru(HAT)_2(bpy)^{2+}$	519	1.12	1.36		↓		↑	42,55
$Ru(TAP)_2(bpy)^{2+}$	231	1.06	0.74		↓		↑	21,55
$Ru(TAP)_2(phen)^{2+}$	630	1.06	0.98		↓	↓	↑	55
$Ru(phen)_2(HAT)^{2+}$	108	0.87	0.024		↑		↑	38,55
$Ru(bpy)_2(HAT)^{2+}$	78	0.83	0.020		↑		↑	43,55
$Ru(bpy)_2(TAP)^{2+}$		0.86			↑		↑	21,42

Photochemical reactions

An interesting property of these (polypyridyl)ruthenium(II) complexes is the possibility of using them as DNA-modification agents and, by suitable derivatization of the complex, to direct the damage to targeted sites of DNA (see later).

Two interesting classes of reactions (DNA damages) are the strand photocleavages and the formation of covalent photoadducts between the complex and the polynucleotide.

Strand breaks. The induction of single strand breaks in DNA is commonly studied with plasmid DNA, where the conversion of the supercoiled closed circular form to its open circular form is readily monitored by gel electrophoresis. When Ru(II) complexes such as $Ru(phen)_3^{2+}$ or $Ru(bpy)_3^{2+}$ are irradiated with visible light, single strand cleavages are observed. These reactions proceed with relatively low quantum yields [$(1.2–6.6) \times 10^{-6}$] and have been shown to proceed via oxygen-dependent and oxygen-independent pathways.[57,58,59] In contrast, $Ru(TAP)_3^{2+}$, which has been shown by photophysical measurements to photo-oxidize DNA, is much more efficient at inducing single strand breaks.[21] Moreover, a correlation has been found between the photocleaving ability of the series $Ru(bpy)_n(TAP/HAT)_{3-n}^{2+}$ (n = 0, 1, 2, 3) and their oxidation power in the excited state.[21,55] This suggests that the guanine radical cation plays an essential role (possibly similar to that found with oxidizing radiation or high-power laser excitation) in the photocleavage processes. The generality of that reaction has been extended to complexes with intercalating ligands, by comparison of photochemical behaviours of $Ru(bpy)_2(DPPZ)^{2+}$ with the more strongly photo-oxidizing $Ru(BPZ/TAP)_2(DPPZ)^{2+}$.[15,60]

Formation of covalent photoadducts. While studies with plasmid DNA have indicated that the series of oxidizing complexes described above are more efficient to induce photocleavages than complexes such as $Ru(phen)_3^{2+}$ or $Ru(bpy)_3^{2+}$, experiments with ^{32}P-end-labelled oligonucleotides surprisingly have revealed that the dominant process is not a strand break, inducing the detection of short fragments, but the formation of adducts, inducing the detection of fragments having a lesser mobility than the starting oligonucleotide.[21] Adducts are formed with double- and single-stranded oligonucleotides, and the formation of photoadducts with DNA is readily monitored by spectroscopic and dialysis measurements. The visible light illumination of $Ru(TAP)_3^{2+}$ in the presence of DNA induces an important hyperchromic effect at *ca* 400 nm, different from the changes observed in the absence of polynucleotide. The irreversible covalent nature of the bond formed upon photolysis, between the complex and the polynucleotide, is shown by dialysis[61] (Fig. 6).

The absorption spectrum of a dialysed sample after photolysis in the presence of DNA shows that the complex is always present on DNA inside the membrane. Similar spectroscopic changes with $[poly(dG–dC)]_2$ but not with $[poly(dA–dT)]_2$ suggest that this adduct results from a reaction with guanine sites.[61] The same kinds of experiment were then been performed with GMP to gain information on the photoadduct.[61] The similar absorption changes combined with the results discussed above allow one to conclude that the photoadduct would result from the transfer of an electron from the guanine to the excited complex, followed by a proton transfer (both processes have been shown to occur by flash photolysis experiments[56]).

Large-scale photolysis experiments have been carried out with GMP and $Ru(TAP)_3^{2+}$ and GMP plus

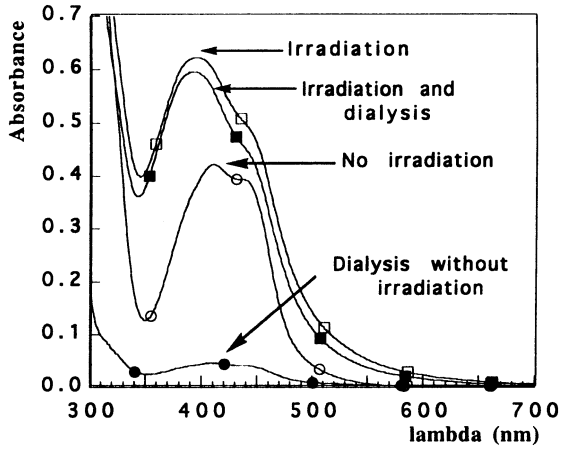


Figure 6. Changes in the absorption spectrum of $Ru(TAP)_3^{2+}$ in the presence of calf thymus DNA, (P/D = 50 in 10 mM phosphate buffer, pH 7) for different experimental conditions (adapted from Ref. 55).

$Ru(HAT)_2(bpy)^{2+}$ to determine the structure of such a photoadduct.[62] $Ru(HAT)_2(bpy)^{2+}$ has an oxidizing power similar to that of $Ru(TAP)_3^{2+}$ and, in addition, as one of the oxidizing ligands, is replaced by a better σ-donor, bpy, the resulting stabilization of the ^{3}MLCT state prevents access to the ^{3}MC state at room temperature and, consequently, prevents the formation of secondary photodechelation products which are observed with $Ru(TAP)_3^{2+}$.[63] The GMP adducts have been precipitated as PF_6 salts, purified by ion-exchange and high-performance liquid chromatography and characterized by electrospray mass spectrometry (ESMS) and nuclear magnetic resonance (NMR).[62] The phosphoribose can alternatively be removed by acid treatment. The structure of these photoadducts reveals the formation of a covalent bond between the exocyclic N-2 of the guanine and the carbon in the β-position to the chelating site. These products can be considered as resulting from a new mode of covalent binding of metal complexes to DNA, different from most other nucleic acid–metal adducts in which the base is directly attached to the metal centre. These adducts are proposed to result from the coupling of the protonated monoreduced complex with the deprotonated guanine radical, followed by rearomatization of the ligand by loss of two hydrogen atoms. Thus photolysis yields a compound in which the guanine is covalently linked to one ligand of the complex without affecting its coordination sphere, in other words without ligand substitution.

$$[Ru(TAP)_3]^{2+*} + GMP \longrightarrow [Ru(TAP)_2TAP^{-\cdot}]^{+} \cdots GMP^{+\cdot}\} \quad (2)$$

$$\{[Ru(TAP)_2TAP^{-\cdot}]^{+} \cdots GMP^{+\cdot}\} \rightarrow [Ru(TAP)_2TAPH^{\cdot}]^{2+} \cdots GMP(-H)^{\cdot}\} \quad (3)$$

$$\{[Ru(TAP)_2TAPH^{\cdot}]^{2+} \cdots GMP(-H)^{\cdot}\} \rightarrow [Ru(TAP)_2(2-GMP(-H)\text{-}TAPH)]^{2+} \quad (4)$$

$$[Ru(TAP)_2(2-GMP(-H)\text{-}TAPH)]^{2+} \rightarrow [Ru(TAP)_2(2-GMP(-H)\text{-}TAP(-H))]^{2+} \quad (5)$$

In the case of $Ru(HAT)_2(bpy)^{2+}$, two isomeric guanine adducts are formed, in both of which the guanine is bonded via its exocyclic amino group to one of the C atoms in the β-position to the chelated site[64] (see Fig. 7).

The formation of two isomers is, of course, due to the lower symmetry of the complex compared with that of

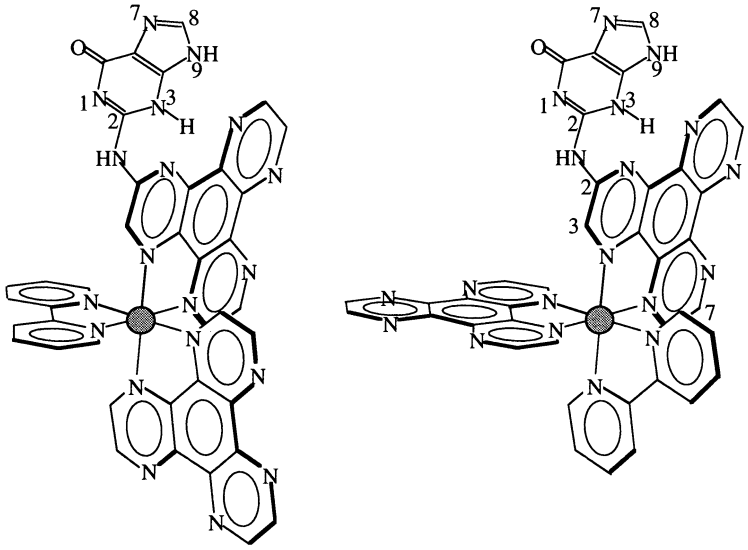


Figure 7. Structure of the photoadduct formed under irradiation of $Ru(HAT)_2(bpy)^{2+}$ and GMP, after HCl treatment to remove the ribose phosphate.

$Ru(TAP)_3^{2+}$. Recently, visible light irradiation of $Ru(TAP)_2(bpy)^{2+}$ in the presence of calf-thymus DNA has been carried out, followed by enzymatic digestion and acidic treatment of the modified DNA.[65] The NMR and ESMS studies indicated that $Ru(TAP)_2(bpy)^{2+}$ is again connected to the guanine exocyclic N-2 position at the carbon in the β-position to the chelated site of one of the TAP ligands. This connection would imply that the adduct is formed within the CT–DNA minor groove, where the NH_2 group is accessible. The formation of these adducts thus allows the generalization of this mechanism of photoaddition to a larger class of polypyrazinic complexes of Ru(II). The following sections illustrate strategies to direct these photoreactive compounds to targeted sequences of bases or to special topologies of DNA.

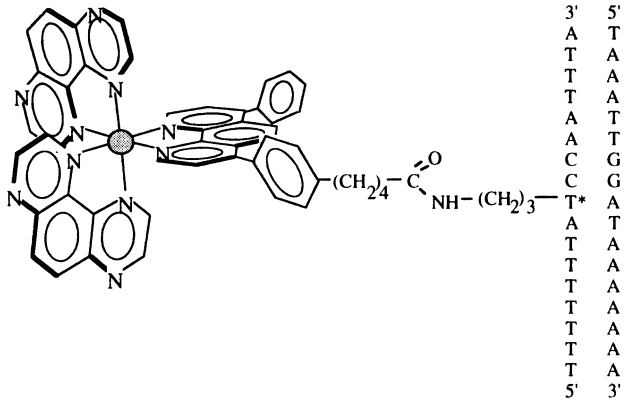


Figure 8. Structure of a Ru-derivatized oligonucleotide after hybridization with its complementary sequence.

DIRECTING THE PHOTOREACTIVE COMPLEX TO SPECIFIC TARGETED SEQUENCES OF BASES

Although several polypyridylruthenium(II) complexes are efficient photoactivatable reagents of nucleic acids and are able to form photoadducts with DNA, they do not exhibit interaction with specific DNA bases or sequences. Their only specificity originates from their photoreactivity exclusively versus the guanine bases. In order to use Ru(II) complexes for therapeutic applications, it is necessary to target particular nucleic acid sequences to damage only specific genes. To achieve this goal, we have developed systems where the photoreactivity of the complex is directed towards guanines belonging to specific sequences of bases. Our approach is based on the anti-sense or anti-gene strategy where in both cases the aim consists in inhibiting the expression (during the transcription or the translation processes) of the targeted sequence by a synthetic oligonucleotide. The inhibition effects of the interaction of this oligomer with double-stranded DNA or with messenger RNA should be increased by the occurrence of a photoreaction leading to an irreversible attachment of the synthetic oligonucleotide to its target sequence. Synthetic oligodeoxynucleotides functionalized with a photoreactive $Ru(TAP)_2dip^{2+}$ (dip = 4,7-diphenyl-1,10-phenanthroline) complex tethered to a thymine base in the middle of the probe sequence (Fig. 8) were therefore synthesized and their behaviours examined under illumination.[15] This approach should allow the irreversible photo-crosslinking of the Ru-derivatized oligonucleotide on the targeted sequence.

In contrast to most derivatizations where the active compound is anchored to a 3′- or 5′-terminal end,[66,67] our stategy[15] is based on the formation of a stable amide bond between the complex and the 5-position of a thymine in the middle of the synthetic oligonucleotide, both species (complex and thymine) being previously activated for their coupling reaction. Tethering the metal compound in the middle of the synthetic oligomer allows control on both sides of the anchored complex of the sequence and double-stranded structure; moreover, in this fashion one forces the complex to remain inside the major DNA groove. To prepare the metalated oligonucleotide,[15] called the conjugate, the thymidine is in a first step chemically activated at the 5-position and inserted into the synthetic oligonucleotide sequence. Subsequently, the Ru(II) complex is attached to the modified thymine of the oligonucleotide via the dip ligand previously activated. The so-obtained Ru-labeled single strand is then hybridized with its complementary sequence to form ruthenium tethered duplexes (Fig. 8). The luminescence properties of these metalated duplexes are examined by comparison with those of the Ru(II) complex attached to the corresponding single strand.

Five ruthenium-labelled 17-mer duplexes have been prepared[68,69] (Fig. 9). The three systems D1, D2 and D3 were designed in order to test the photoreactivity of the Ru-derivatized single-stranded oligomers towards the guanines in the complementary strands and facing the Ru complex labelled site. The D1 target sequence contains 6G whereas the target sequences of D2 and D3 contain only 2G towards the 5′-and the 3′-end, respectively. In the fourth system, D4, a four base pair mismatch was introduced near the Ru-modified site to examine the effect of such a mismatch. D5, with no guanine in its target sequence, was used as reference to verify the absence of photoreactivity in this case.

The ruthenium effect on the duplex formation and stability was examined from thermal denaturation curves measured by absorption spectroscopy. Comparisons of the resulting curves for the Ru-labelled duplexes with those of the unmetalated duplexes (used as references) show that the hybridization ability of the DNA strands is maintained in the labelled oligomer and that the attached complex induces a slight stabilization of the double-stranded oligonucleotides.

For each sequence, the emission quantum yields of the

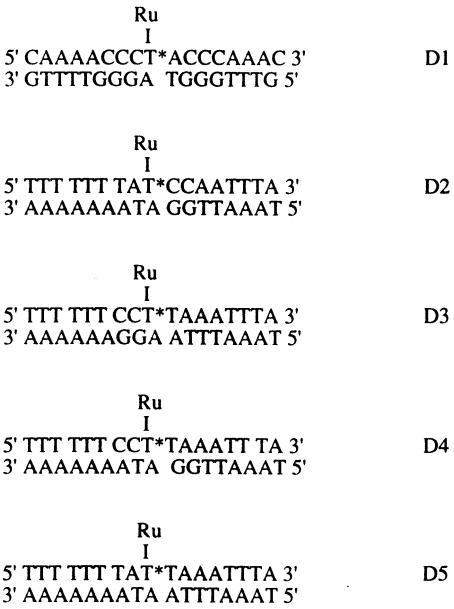


Figure 9. The five Ru-labeled 17-mer duplexes D1 and D5,[68] D2, D3 and D4.[69]

Ru(II) complex on the double-stranded oligonucleotide were measured and compared with those of its conjugate. Hybridization of the Ru-containing oligonucleotide of D1 with its complementary sequence induces an important decrease in emission intensity, as 85% of luminescence quenching of the complex is observed. The emission quantum yield of the duplex sequence containing 2G towards the 5′-position indicates a 50% inhibition upon duplex formation whereas with the other 2G sequence (without mismatch) only a 35% quenching is measured. In contrast, no detectable change in emission intensity was observed on addition of the mismatch-containing target strand to the conjugate D4. Similarly, for the duplex without guanine, no inhibition was measured. In contrast, hybridization of the Ru-labelled oligomer D5 to its complementary strand induces a slight increase in emission intensity.

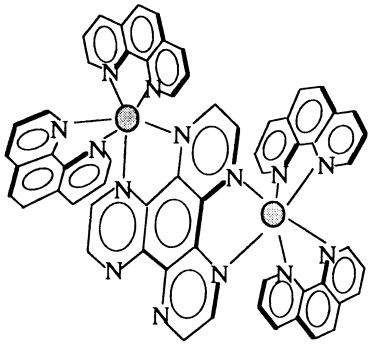
These luminescence data clearly show that the quenching process is due to the guanines contained in the target sequence and that its efficiency increases with increasing number of guanines. The attached complex can interact toward the 5′- or 3′- end of the complementary strand within the double-stranded DNA but the guanine position in the target sequence seems to play a role as the luminescence quenching by the 2G sequences (without mismatch) are not equivalent. Time-resolved luminescence studies have been performed with the free complex, the conjugates and the duplexes. Although a single exponential decay is obtained for the free complex, the decays are biexponential for all the metalated single strands. This is also the case for the duplex D5. The presence of a second lifetime twice as long as the normal luminescence lifetime of the free complex is due to a certain protection of the metal complex by the single strand itself or by the complementary strand in the duplex D5. In contrast, the non-single exponential decays obtained for the duplexes D1, D2 and D3 are dominated by a short component whose corresponding lifetime is about 10% of the value of the free complex. As these duplexes contain guanines in their complementary sequences, this short lifetime may be attributed to the quenching by these bases as observed from the experiments under continuous illumination. The question to be raised is to determine whether this quenching can be attributed to an electron transfer from the guanine bases to the attached excited complex. Based on the data in the previous sections, if this is the case, a photoadduct of the complex on these guanines should be observed, leading to irreversible photo-crosslinking of the two strands.

In order to check this possibility, continuous irradiation experiments were performed, followed by gel electrophoretic analyses and by absorption spectroscopic measurements. For this purpose, before the hybridization of the duplexes, the complementary strand of each conjugate was 5′ ^{32}P-end-labelled. After irradiation, the samples were analysed by electrophoresis through denaturing polyacrylamide gels at 50 °C. In the absence of photo-crosslinking, the bands on the gel should result from the migration of the target single complementary strand. For the illuminated duplex D5, the migration does correspond to the ^{32}P-labelled complementary oligonucleotide, in accord with the absence of luminesence quenching and thus absence of photo-crosslinking. These observations are also consistent with the spectroscopic results, which indicate the absence of photoproduct. In contrast, for the illuminated duplexes D2 and D3 (D1 was not tested) an additional band was detected and indicates the presence of an oligonucleotide containing a double number of bases. This clearly confirms an irreversible photo-crosslinking between the complex tethered to the probe sequence and a guanine of the target sequence. These results are also in agreement with the occurrence of a photoadduct detected by absorption spectroscopy.

This work thus constitutes an initial step in the design of new sequence-specific DNA photoreagents. Furthermore, this type of conjugate could be used for studying long-range transport (of electrons or holes) through the DNA double helix.

SPECIAL DNA GEOMETRIES OR TOPOLOGIES TARGETED BY THE COMPLEX

In order to improve the selectivity of the complex–DNA binding, other research studies have been focused on the design of complexes able to direct their interaction and photoreactivity to particular topologies of DNA. In that field, studies on the interactions and photoreactions of dinuclear Ru(II) complexes based on the bridging HAT ligand have evidenced interesting behaviours.



$[Ru(phen)_2]_2(HAT)^{4+}$

Figure 10. Structure of the bimetallic complex $[Ru(phen)_2]_2(HAT)^{4+}$.

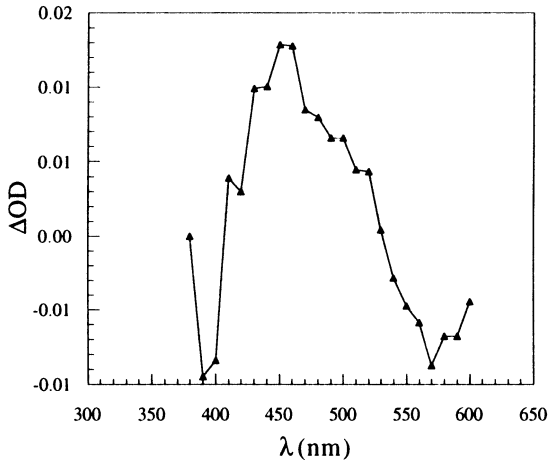


Figure 11. Laser flash photolysis of $[Ru(phen)_2]_2HAT^{4+}$ in the presence of GMP (spectrum recorded 2 μs after the laser pulse) (adapted from Ref. 70).

Interaction

$[Ru(phen)_2]_2HAT^{4+}$ (Fig. 10) has been shown to interact exclusively with denatured DNA.[70] In the presence of calf thymus double-stranded DNA, only weak emission increases are observed at constant concentration of the complex on increasing the DNA concentration, suggesting a poor protection of the excited complex by the double helix. This is attributed to the size of the dinuclear complex, which prevents its penetration inside the grooves of the normal DNA double helix. In contrast, this complex exhibits different behaviours in the presence of denatured DNA. This DNA contains 60% of normal double helix portions and 40% of single-stranded portions. With denatured DNA, the emission intensity of $[Ru(phen)_2]_2HAT^{4+}$ increases by a factor of 2.5,[70] suggesting that in that case, the denatured portions are accessible to the dinuclear complex and thus the protection from solvent quenching is efficient. In addition, the 4+ charge of the dinuclear complex induces a high affinity for the DNA strands. This complex therefore appears as a novel, interesting tool to be used for the detection of single-stranded DNA portions in irregular DNA structures.

Photoreactivity

As mentioned in previous sections, the bimetallic complexes are oxidants in the ^{3}MLCT state and are thus good candidates as photoreagents of nucleic acids. In order to establish the existence of a photoinduced electron transfer between the dinuclear complex and the nucleobases, flash photolysis experiments were carried out with GMP (see Fig. 11) and natural and denatured CT–DNA.

The transient spectra produced with $[Ru(phen)_2]_2HAT^{4+}$ and these nucleic acids show an absorption around 450 nm corresponding to the monoreduced HAT ligand. The comparison of the spectroelectrochemical and flash photolysis results leads to the conclusion that the monoreduced bimetallic complex is produced after the laser pulse, according to the following equation (for GMP):

$$\{[Ru(phen)_2]_2HAT\}^{4+} + GMP \xrightarrow[k_q]{h\nu} \{[Ru(phen)_2]_2HAT^{-\cdot}\}^{3+} + GMP^{+\cdot} \quad (6)$$

In correlation with this photoinduced electron transfer process, the formation of a photoadduct has been observed with GMP. However, with natural DNA, the photoelectron transfer does not lead to adduct formation. This probably originates from important steric hindrances which prevent good contact of the complex with the DNA bases. In contrast, the photoelectron transfer process leads in most cases to the formation of an adduct with denatured DNA. Under these conditions, the complex may probably approach more easily the guanine bases (when present) at the level of the denatured portions and thus produce the adduct. The luminescence quenching which should result from this reaction is probably largely compensated by the protection effect of the denatured portions, resulting in a luminescence enhancement of $[Ru(phen)_2]_2HAT^{4+}$ by increasing denatured DNA concentration.

In conclusion, it turns out that these bimetallic complexes could play the role of molecular tools and photoreagents to detect and target irregular DNA structures (single-stranded DNA, for example) along a double-stranded DNA. Recently, the pure stereoisomers of $[Ru(phen)_2]_2HAT^{4+}$ have been prepared and characterized by spectroscopic and electrochemical methods.[71] The behaviour of each of the three stereoisomers (ΔΔ, and Δ) with nucleic acids is currently being studied.

CONCLUSION

This paper has shown that, by changing the nature of the ligands and their combinations in the complex, it is easy to modify the photoredox properties of the resulting complex towards the nucleobases. The photoinduced electron transfer which occurs from the most reducible bases to the excited complex leads to DNA damage including strand breaks and the formation of covalent adducts. In the future it should be possible to increase the efficiency of these reactions by improving the design of the complexes in order to adapt them for clinical applications such as DNA-targeted phototherapies. For example, studies with derivatized oligonucleotides show that it is possible to increase the specificity of the photochemical processes, by controlling and directing the photoreactions on targeted DNA bases sequences. Another promising strategy is based on the use of polymetallic complexes to target specific DNA topologies such as single-stranded portions. These examples illustrate potential applications of these metallic complexes as molecular photoprobes and photoreagents of DNA.

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