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Novel DNA Sensor for Guanine Content

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The bifunctional complex $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$, **1**, formed with a $[Ru(TAP)_2Phen]^{2+}$ metallic unit linked to a quinoline moiety, and $[Ru(TAP)_2Phen]^{2+}$, **2**, as reference, have been tested as photoprobes of DNA. Interestingly, **1** exhibits an emission enhancement of a factor of 16–17 upon binding to calf thymus DNA. Moreover, this emission is modulated by the nucleic base content of the polynucleotide. It varies by almost an order of magnitude from a polynucleotide containing 100% of G–C to a guanine-free nucleic acid where the excited-state lifetime reaches about 2 μs. The origins of these interesting properties are analyzed by comparing **1** with reference **2** in the presence of different polynucleotides.

Introduction

In the past decades, Ru(II) complexes have been the subject of numerous research works because of their properties as photoprobes and photoreagents of DNA.[1−4] Ru(II) polypyridyl complexes exhibit luminescence which is extremely sensitive to the microenvironment.[5] Complexes such as $[Ru(Phen)_2DPPZ]^{2+}$ (DPPZ = dipyrido[3,2-a:2′,3′-c] phenazine) or $[Ru(Phen)_2PHEHAT]^{2+}$ (PHEHAT = 1,10-phenanthrolino [5,6-b] 1,4,5,8,9,12-hexaazatriphenylene) do not luminesce in aqueous solution, but their emission is switched on when they intercalate a portion of their extended aromatic ligand into the stacking of DNA bases.[6−8] The recognition of particular structures of DNA by Ru(II) or Rh(III) polypyridyl complexes has been reported only in a few cases.[9]

In our research group, a number of Ru(II) complexes capable of photo-oxidizing the guanine bases of DNA have been developed.[2,10−12] Some monometallic complexes based on TAP (1,4,5,8-tetraazaphenanthrene) and HAT (1,4,5,8,9,-12-hexaazatriphenylene) ligands belong to this category, but they exhibit a weak affinity for DNA.[13] One of the strategies adopted to overcome this drawback consists of functionalizing the complex with an organic unit which interacts also with DNA.[14−17] The photophysical properties of one of these

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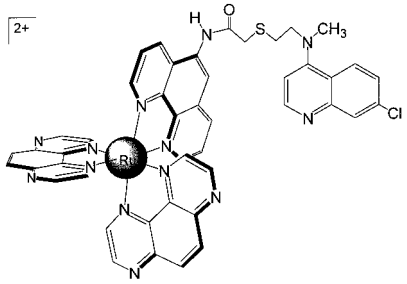

Figure 1. $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ (**1**) (TAP = 1,4,5,8-tetraazaphenanthrene, POQ-Nmet = 5-[4-[*N*-methyl-N−(7-chloro-quinolin-4-yl)amino]-2-thia-buthylcarboxamido]-1,10-phenanthroline).

so-called "bifunctional" complexes, $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$, composed of a $[Ru(TAP)_2Phen]^{2+}$ unit linked to an *N*-methylaminoquinoline moiety by a seven atom chain, have been carefully examined in previous studies.[14,15] The following results had been obtained. The emission of this complex depends very much on the pH. The luminescence of the metallic unit (or the complex without the quinoline unit) is partially inhibited in a phosphate buffer at pH lower than 7.5 and in HCl solutions by protonation of the excited state. Therefore, the metallic species in these pH conditions has a weak luminescence. Moreover, in acid conditions at pH 4.5, the quinoline unit (pK_a of 6.1) is also protonated, and in this condition, the luminescence of the metallic unit is exactly the same as that of the complex without quinoline at the same pH. At pH 7.5, the nonprotonated quinoline unit affects very much the luminescence of the metallic moiety. Indeed, in those conditions, the emission of the metallic unit is quenched at 97% by an intramolecular photoelectron transfer from the organic to the metallic moiety. Therefore, this bifunctional complex is quasi nonluminescent at pH 7.5 or higher, hence, in a pH region where its behavior in the presence of DNA is examined.

The goal of the present study is to examine whether the luminescence of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ (Figure 1, **1**) would be switched on upon interaction with DNA, as reported for the Ru−DPPZ complexes, but obviously for other reasons. We show that it is indeed the case for $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$. Moreover, this complex and the reference $[Ru(TAP)_2Phen]^{2+}$ exhibit a luminescence sensitivity to the guanine content of DNA.

Results

The interaction of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ (**1**) and $[Ru(TAP)_2Phen]^{2+}$ (**2**) with CT (calf thymus)-DNA and other polynucleotides was studied by absorption and steady-state and time-resolved emission spectroscopy. The titrations of the complexes by DNA were carried out at a constant concentration of complex, varying the P/D concentration ratio (P = polynucleotide; D = dye = complex). For these experiments, the absorption (*A*) and emission intensity (*I*) were measured by comparison to the corresponding values of a buffered solution containing the complex in the absence of DNA (A_0 and I_0). For **1**, plateau values were reached for *A* and *I* with the addition of nucleic acid ($A = A_\infty$, $I = I_\infty$). This means that for the DNA concentrations at the plateau, the whole amount of complex is bound to DNA. Therefore, the relative absorbance A_∞/A_0 (and $\epsilon_\infty/\epsilon_0$) and the relative emission intensity I_∞/I_0 quantify the spectroscopic effect in absorption and emission for a complete binding of the complex to DNA. The concentration of **1** (from 1 to 10 μM) does not influence the I_∞/I_0 value in the presence of CT-DNA, and in order to avoid a high loading of the nucleic acids, a concentration of 6 μM was used. For **2**, plateaus are not always reached, but in all the cases, the titrations provide an approximation of A_∞/A_0 and I_∞/I_0 within a 10% margin. Because of the dependence of the spectroscopic properties on pH (quinoline and metallic units), the studies were performed in acidic (pH 4.5) and neutral media. A pH of 7.5 was chosen in addition to 7.0, as the quinoline unit of **1** is completely deprotonated only at pH 7.5. All measurements were performed in air-equilibrated solutions.

Table 1. Absorption Data for Titrations with CT-DNA, for $[Ru(TAP)_2Phen]^{2+}$ and $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$[a]

	$\lambda = 464$ nm		$\lambda = 358$ nm	
complex	pH	$\epsilon_\infty/\epsilon_0$ (%H)	$\Delta\epsilon$ (10^3 M^{-1} cm^{-1})	ϵ_∞ (10^3 M^{-1} cm^{-1})
$[Ru(TAP)_2Phen]^{2+}$ [b]	4.5, 7.0, 7.5	0.94 (6)	+0.2	5.4
$[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ [c]	4.5	0.88 (12)	−5.1	15.3
	7.0	0.89 (11)	+5.3	15.4
	7.5	0.89 (11)	+3.2	12.5

[a] ϵ_∞ = absorption coefficient in the presence of an excess of CT-DNA; ϵ_0 = absorption coefficient in the absence of CT-DNA;[14] $\epsilon_\infty/\epsilon_0 = A_\infty/A_0$ (A = absorbance); %H (in parentheses) = percentage of hypochromicity; $\Delta\epsilon = \epsilon_\infty - \epsilon_0$; P/D = ratio of equivalent phosphate (or base) and complex concentrations (D = dye). [b] P/D = 250. [c] P/D = 125 or 150.

(1) Effect of CT-DNA on the Reference Complex $[Ru(TAP)_2Phen]^{2+}$ (2). The changes of absorption of $[Ru(TAP)_2Phen]^{2+}$ in the presence of CT-DNA were examined at pH 4.5, 7.0, and 7.5 (Table 1). As observed with many complexes upon addition of CT-DNA,[6,18] a hypo- and bathochromic effect was detected on the MLCT absorption band (isosbestic point at 478 nm) with an $\epsilon_\infty/\epsilon_0$ value of 0.94 at 464 nm (Table 1). Hence, the hypochromicity (6%) is rather weak.

The relative emission intensity for $[Ru(TAP)_2Phen]^{2+}$ (Table 2) decreases in the presence of CT-DNA. This luminescence quenching has been demonstrated to originate from an electron transfer from the guanine bases of DNA to the excited complex.[11,19] The ratio I_∞/I_0 reaches values of 0.26 at pH 4.5 (Figure 2), 0.35 at pH 7.0 (Figure 3), and 0.34 at pH 7.5 (Figure 4). The luminescence quenching at the three pH values contrasts with the behavior of the majority of Ru(II) polypyridyl complexes for which I/I_0 is

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Table 2. Relative Emission Intensities (I_∞/I_0) from Titrations with Various Polynucleotides (pNu) for $[Ru(TAP)_2Phen]^{2+}$ and $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$

		$[Ru(TAP)_2Phen]^{2+}$		$[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$	
pNu	% AT	pH[a]	I_∞/I_0[b]	pH[a]	I_∞/I_0[b]
$[poly(dA\text{-}dT)]_2$	100	7.0	1.77	7.5	63
CP-DNA	74	7.5	0.75	7.5	24.2
CT-DNA	58	7.0	0.35	7.5	16.5[c]
		7.5	0.34		
HS-DNA	58	7.5	0.32	7.5	15.6
SS-CT-DNA	58	7.0	0.35[d]	7.5	11.5
$[poly(dG-dC)]_2$	0	7.0	0.08	7.5	9.8[d]
CT-ADN	58	4.5	0.26	4.5	0.29

[a] pH 7.5: [phosphate buffer] = 10 mM. pH 7.0: [TRIS−HCl buffer] = 10 mM. pH 4.5: [phosphate buffer] = 10 mM. For all the experiments, [complex] = 6×10^{-6} M. [b] I_∞ = luminescence intensity at the plateau value (or close to the plateau for $[Ru(TAP)_2Phen]^{2+}$); I_0 = luminescence intensity in the absence of nucleic acid; λ(excitation) = 478 nm (isosbestic point); λ(emission) = 645 nm. Error: I_∞/I_0 for $[Ru(TAP)_2Phen]^{2+} \approx 3\%$; I_∞/I_0 for $[Ru(TAP)_2POQ\text{-}Nmet]^{2+} \approx 5\%$. [c] $I_\infty/I_0 = 0.42$ (58% quenching) when I_0 is the emission intensity of $[Ru(TAP)_2Phen]^{2+}$ in the absence of CT-DNA (I_∞ = the plateau emission of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$). [d] With denatured CT-DNA (SS-CT-DNA), the plateau value is not reached. Therefore, I_∞/I_0 is < 0.35. The emission quenching is thus slightly more important for SS-CT-DNA.

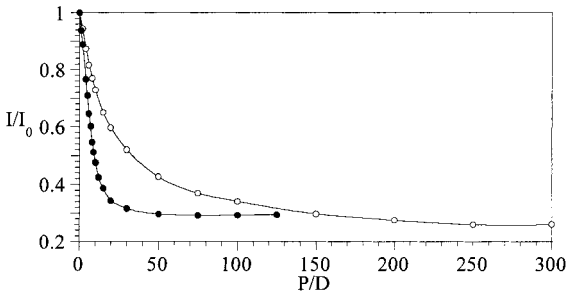

Figure 2. Relative emission intensity I/I_0 for $[Ru(TAP)_2Phen]^{2+}$ (open circles) and for $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ (filled circles) as a function of different P/D ratios of CT-DNA at pH 4.5 (10 mM buffer). I_0 = emission intensity in the absence of DNA, D = 6.0×10^{-6} M, λ_{exc} = 478 nm, λ_{em} = 645 nm, air-equilibrated solutions.

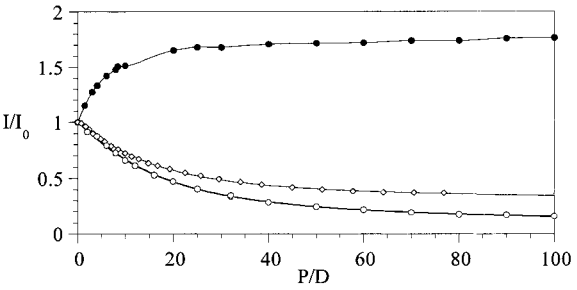

Figure 3. Relative emission intensities I/I_0 of $[Ru(TAP)_2Phen]^{2+}$ in the presence of $[poly(dA-dT)]_2$ (●), CT-DNA (◇), and $[poly(dG-dC)]_2$ (○) at pH 7.0 (10 mM buffer) for different P/D ratios. D = 6×10^{-6} M.

enhanced by addition of CT-DNA. This is the case, for example, for $[Ru(Phen)_3]^{2+}$ or $[Ru(Phen)_2DPPZ]^{2+}$.[1,6,18,20]

(2) Effect of CT-DNA on the Bifunctional Complex $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$.(A) Absorption. In the presence of CT-DNA, a 12−11% hypochromic effect on the MLCT absorption band is produced at 464 nm at pH 4.5 (P/D = 125) and pH 7.5 (P/D = 150) (Table 1, Figure 5). The hypochromicity is, thus, more important than for $[Ru(TAP)_2Phen]^{2+}$ (6%) and the previously studied bifunctional complex $[Ru(BPY)_2POQ\text{-}Nmet]^{2+}$ (3%).[16] The absorption of the organic moiety between 330 and 390 nm cannot be separated from that of the metallic unit of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$; however, like for the monofunctional complex (Table 1), the absorption variation of the metallic unit is probably very small. Therefore, the important pH dependent changes of absorption ($\Delta\epsilon$) in that wavelength range (Table 1 and Figure 5) are attributed to the organic unit. Around 350 nm, at pH 4.5, DNA induces an hypochromic effect ($\Delta\epsilon = -5.1\ 10^3\ M^{-1}\ s^{-1}$), and the λ_{max} is shifted from 354 to 358 nm. In contrast, in the same wavelength region, at pH 7.5 and 7.0, DNA causes an hyperchromic effect (Table 1), and the λ_{max} is again shifted to 358 nm. This wavelength corresponds to the λ_{max} of absorption of the protonated quinoline. Moreover, Table 1 shows that, at pH 4.5 and 7.0, the extinction coefficients at 358 nm are the same when the whole amount of complex is bound to DNA (plateau value, ϵ_∞).

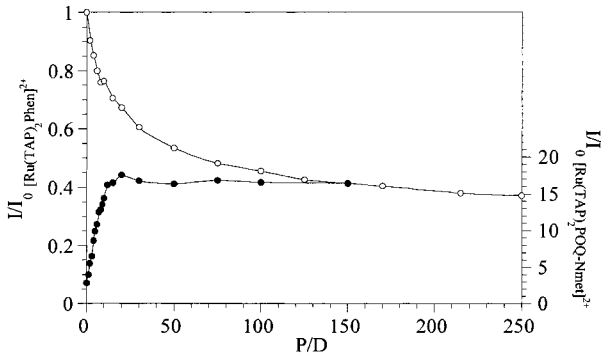

Figure 4. Relative emission intensity I/I_0 as a function of different P/D ratios of CT-DNA at pH 7.5 (10 mM buffer); D = 6×10^{-6} M. Left scale: Emission intensity of $[Ru(TAP)_2Phen]^{2+}$ (○) and of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ (●), both with I_0 of $[Ru(TAP)_2Phen]^{2+}$. Right scale: Emission intensity of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ with I_0 of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ (●).

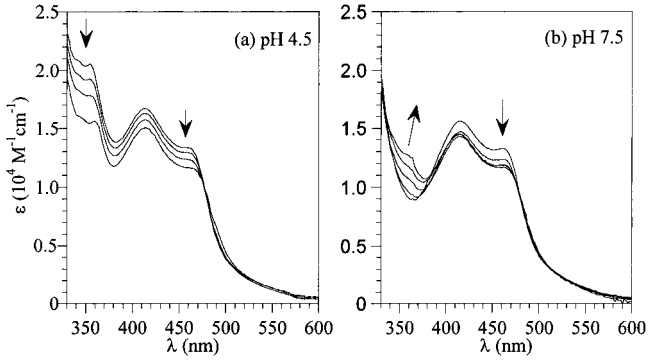

Figure 5. Absorption spectra of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ in the presence of CT-DNA, at different P/D ratios (P = equivalent phosphate or base concentration of DNA; D = dye = [complex] = 6.0×10^{-6} M). (a) At pH 4.5, the P/D ratios are the following: 0, 2, 4, and 125. (b) At pH 7.5, the P/D ratios are the following: 0, 2, 4, 8, and 150. The arrows show the change of the absorption with the addition of CT-DNA.

(B) Emission. In acid conditions, the I_0 value in the absence of DNA for $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ is equal to that of the monofunctional analogue $[Ru(TAP)_2Phen]^{2+}$. Therefore, with the addition of DNA in acid solutions, the corresponding calculated ratio I/I_0 for $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ is independent of the reference chosen (I_0 of

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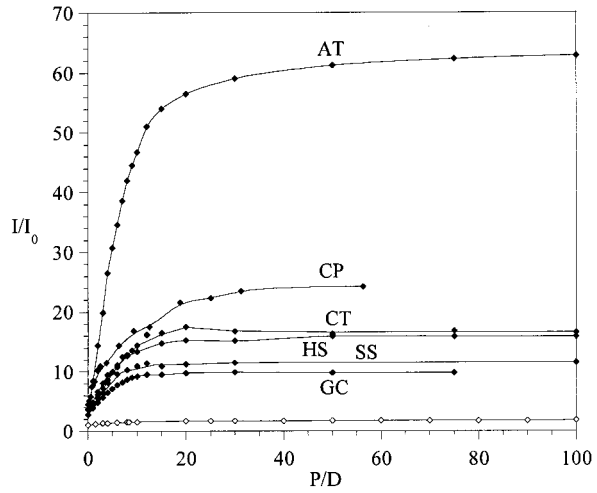

Figure 6. Relative emission intensities I/I_0 of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ (◆) in the presence of (from top to bottom) $[poly(dA-dT)]_2$, CP-DNA, CT-DNA, HS-DNA, SS-CT-DNA, and $[poly(dG-dC)]_2$ at pH 7.5 (10 mM buffer) for different P/D ratios. $D = 6 \times 10^{-6}$ M. I_0 corresponds to the emission of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ in the absence of nucleic acids. In the case of CP-DNA, I/I_0 is plotted as a function of $^1/_6 \times$ P/D (see also Table 1). Lowest curve: I/I_0 for $[Ru(TAP)_2Phen]^{2+}$ (◇) in the presence of $[poly(dA-dT)]_2$ (pH 7.0; I_∞/I_0=1.8).

$[Ru(TAP)_2Phen]^{2+}$, I_0^{Phen}, or I_0 of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$, $I_0^{POQ-Nmet}$). At pH 4.5, the emission of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ (**1**) is strongly inhibited by the addition of CT-DNA (Figure 2). The ratio I_∞/I_0 (0.29, Table 2) is comparable to the I_∞/I_0 value of the monofunctional analogue (**2**) (0.26, Table 2). The similarity of quenching by CT-DNA between both complexes exists also for the luminescence quenching by the guanosine-5′-monophosphate. Indeed, the emission quenching constant (k_q) for $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ (**1**) by GMP determined from a Stern−Volmer plot in lifetimes or in intensities (1.3×10^9 M^{-1} s^{-1} in acid conditions) is similar to that determined for $[Ru(TAP)_2Phen]^{2+}$ (**2**) in the same conditions (1.0×10^9 M^{-1} s^{-1}). The luminescence quenching by DNA or GMP for **1** is thus not affected by the quinoline functionalization. However, for **1** at pH 4.5, I_∞/I_0 is reached at a lower P/D ratio (P/D ≈ 50 for **1**, instead of >200 for **2**, Figure 2).

At pH 7.5, the calculated ratio I/I_0 for $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ (**1**) can be very different according to the reference I_0 which is chosen. Indeed, I_0 for $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ is much lower in neutral or basic solution than I_0 for reference **2**, due to the intramolecular photoelectron-transfer quenching in **1**. Therefore, when I_0 is chosen as the emission of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ (**1**) in the absence of DNA ($I_0^{POQ-Nmet}$) (Figure 4, right *Y* axis, filled circles, and Figure 6), the emission of **1** at pH 7.5 is tremendously increased by a factor of 16.5 by addition of CT-DNA (Table 2). This enhancement contrasts with the inhibition of luminescence of complex **1** in acid conditions (Figure 2) and with the quenching of the monofunctional analogue (**2**) at all the pHs (Figures 2−4). Moreover, this enhancement factor of 16.5 is also much higher than what has been usually measured for polypyridyl Ru(II) complexes ($I_\infty/I_0 \approx 2$ for $[Ru(Phen)_3]^{2+}$)[20] and exceeds largely the I_∞/I_0 ratio reached with the similar bifunctional complex $[Ru(BPY)_2POQ\text{-}Nmet]^{2+}$ ($I_\infty/I_0 = 1.4$).[16] This particularity of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ arises thus essentially from its very weak emission in the absence of DNA at pH 7.5 (weak $I_0^{POQ-Nmet}$). Indeed, when the reference I_0 of the ratio I_∞/I_0 for the bifunctional complex is chosen as I_0 of the monofunctional analogue **2** (i.e., I_0^{Phen}), although there is also an increase of the ratio I/I_0 with the DNA addition, this ratio always remains smaller than 1 (left *Y* axis of Figure 4, filled circles, Table 2, footnote c). Interestingly, the I_∞/I_0 value which is reached in this way is comparable to I_∞/I_0 for $[Ru(TAP)_2Phen]^{2+}$ (Figure 4, left *Y* axis, open circles). This clearly indicates that, at pH 7.5, the excited metallic unit of the bifunctional complex in interaction with CT-DNA is quenched like monofunctional analogue **2** at the same pH, by an electron transfer from the guanines of DNA. The presence of such a charge-transfer process with $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ is supported by laser flash photolysis experiments at pH 7.5 (not shown), where a weak transient absorption spectrum centered at 510 nm appears after pulsed laser excitation of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ or $[Ru(TAP)_2Phen]^{2+}$ in the presence of CT-DNA.[21] This transient is characteristic of monoreduced complex.[22] The 58% total quenching of the excited metallic unit leading to the electron abstraction from a guanine (Table 2, footnote c) is nevertheless weaker than the 97% intramolecular quenching of the excited complex without DNA at pH 7.5. This explains the increase in emission of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ upon binding to CT-DNA either with I_∞/I_0 of 16.5 when its luminescence is compared normally to its emission in the absence of DNA or with I_∞/I_0 of 0.42 when its luminescence is compared to the emission of $[Ru(TAP)_2Phen]^{2+}$ at the same pH.

(3) Effect of Various Polynucleotides. (A) Steady State Emission. For the majority of Ru(II) complexes, the emission intensities do not vary much according to different polynucleotide sequences. These metallic compounds are therefore considered insufficiently selective photoprobes of DNA.[3,23] The case of a Pt complex has been reported, where a more substantial sequence-dependent emission intensity is due to a specific photoreaction with the guanine bases of the nucleic acids.[24] Considering that the TAP complexes photoreact with the guanines, a sequence dependent photochemical behavior could thus be expected. Therefore, the bifunctional and monofunctional analogues of this study were tested with different polynucleotides containing various percentages of guanine base.

These different natural and synthetic polynucleotides did not differently influence the absorption spectra of $[Ru(TAP)_2Phen]^{2+}$ or $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$. In contrast, Table 2 and Figures 3 and 6 show that the relative emission intensities I_∞/I_0 are differently affected by different polynucleotides; in those cases, the I_0 values always refer to the corresponding complex in the absence of polynucleotide. The

(21) For **1**, this transient most likely does not originate from an intramolecular electron transfer. Indeed, no transient is detected for **1** in solution in the absence of DNA in these experimental conditions (see ref 14).

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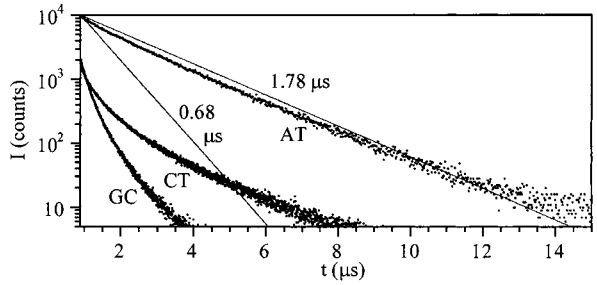

Figure 7. Luminescence decays of excited $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ after pulsed irradiation in the presence of the highest concentrations of $[poly(dA-dT)]_2$, CT-DNA, and $[poly(dG-dC)]_2$. The air-equilibrated solutions are buffered at pH 7.5. The decays have been normalized so that the ratios of the underlying areas equal the ratios of the averaged lifetimes. Straight lines: calculated decays for lifetimes of 0.68 μs and 1.78 μs (without convolution or background).

ratio I_∞/I_0 increases with the percentage of A−T content (or decreases with the percentage of G−C content).

With $[Ru(TAP)_2Phen]^{2+}$, I_∞/I_0 reaches 1.77 with $[poly(dA-dT)]_2$, that is, with a polynucleotide containing 100% A−T, and drops to 0.08 with $[poly(dG-dC)]_2$ (Table 2 and Figure 3). Intermediate ratios are observed with CT- and HS-DNA (58% A−T, 42% G−C base pairs) and with CP-DNA (74% A−T, 26% G−C base pairs).

$[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ can be regarded as a better probe for the different polynucleotides. With $[poly(dA-dT)]_2$, the emission of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ increases by almost 2 orders of magnitude: I_∞/I_0 reaches a value of 63 (Table 2, Figure 6). In contrast, the ratio I_∞/I_0 is only 10 in the presence of $[poly(dG-dC)]_2$ (Table 2, Figure 6). Again, intermediate values of I_∞/I_0 are observed with CT-, HS-, and CP-DNA, depending on the base pair content. The relation between the ratio I_∞/I_0 and the percentage of A−T base pairs is, however, not linear. The changes are small for the A−T poor nucleic acids, whereas the variations are more important when the A−T base pairs content exceeds 50%.

Moreover, for both complexes, the ratio I_∞/I_0 for partially denatured CT-DNA (SS-CT-DNA) is not exactly the same as for normal CT-DNA (Table 2, footnote d). This indicates that not only the base content but also the double strand character of the polynucleotides influences the emission intensity.

(B) Time-Resolved Emission. The luminescence decays after pulsed excitation of $[Ru(TAP)_2Phen]^{2+}$ and $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ (Figure 7 for **1**) were measured in the presence of the highest concentrations of $[poly(dA-dT)]_2$, $[poly(dG-dC)]_2$, and CT-DNA (same conditions as in Figures 4 and 6).

In the presence of $[poly(dA-dT)]_2$, the emission of the two complexes decays according to a biexponential function. For $[Ru(TAP)_2Phen]^{2+}$, one lifetime equals 0.66 μs, which is close to the excited-state lifetime of the complex in the absence of polynucleotide (0.70 μs); the second lifetime, which has the higher contribution to the emission (fraction of total emission intensity = 87%), is 1.73 μs. For $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$, the two lifetimes are, respectively, 0.70 μs (13%) and 1.83 μs (87%). Thus, the bifunctional complex has the same decay characteristics as the monofunctional analogue in the presence of $[poly(dA-dT)]_2$. However, for the bifunctional complex, none of the two lifetimes is equal to that of the excited complex in the absence of polynucleotide at pH 7.5, that is, 17 ns.

In the presence of $[poly(dG-dC)]_2$ and CT-DNA, the decays contain more than three components (Figure 7 for $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$). The errors associated with the resulting parameters from the fitting of these decay curves are thus important and do not allow a correct determination of the lifetime components. Consequently, we have simply compared the experimental decays in the presence of $[poly(dG-dC)]_2$ and CT-DNA to calculated monoexponential decays corresponding to lifetimes of 0.68 and 1.78 μs, respectively (Figure 7 for $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$). These lifetime values are average values for the short and long decay components found with $[poly(dA-dT)]_2$ and the mono- or bifunctional complex; hence, 0.68 and 1.78 μs are taken as arbitrary reference values. In Figure 7, for CT-DNA and $[poly(dG-dC)]_2$, it is seen from this comparison that the experimental curves essentially have components faster than 0.68 μs and that, with CT-DNA, one component is approximately equal to 1.78 μs. The same behaviors were observed with $[Ru(TAP)_2Phen]^{2+}$.

Discussion

The most striking result in this work is the sensitivity of the relative emission I_∞/I_0 of the bifunctional complex to the adenine−thymine content of the nucleic acid (or guanine−cytosine content in the reverse way). This base sensitivity is much more important than that of the well-known DNA "light switch" $[Ru(Phen)_2DPPZ]^{2+}$ for which the intensity I_∞ in the presence of $[poly(dA-dT)]_2$ is only twice as high as I_∞ in the presence of $[poly(dG-dC)]_2$ ($I_0 \sim 0$).[25] The factors responsible for the interesting base content sensitivity for $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ are stressed later.

(1) Modes of Binding of the Bifunctional Complex. The variation of the spectroscopic properties of the two units of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ with the addition of the different nucleic acids indicates that they both interact with the polynucleotides. Taking into account the length of the linking chain between the two units, we have to assume that, when one unit interacts in a groove of the double helix, the other unit interacts either in the same groove or in the sugar−phosphate backbone. This case has already been discussed for the bifunctional analogue $[Ru(BPY)_2POQ\text{-}Nmet]^{2+}$ [16] and is similar to that of dinuclear Ru(II) complexes discussed in the literature, where two identical $[Ru(Phen)_3]^{2+}$ units are linked by a flexible chain.[26]

(A) Organic Unit. At pH 4.5, the hypo- and bathochromic effect on the quinoline unit absorption (λ = 358 nm) by addition of CT-DNA is typical of a groove binding or an intercalation of the organic moiety between two base pairs. The absorption maxima indicate that the quinoline unit is protonated in solution and when bound to CT-DNA. At a

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bulk pH of 7.0, when the quinoline moiety is mostly nonprotonated in solution, its interaction with CT-DNA or other nucleic acids induces an hyper- and bathochromic effect on the absorption band ($\lambda = 358$ nm), leading to a spectrum identical to that recorded in the presence of DNA at pH 4.5. This suggests that the organic unit, when interacting with the nucleic bases, is in a protonated form, because of the acid microenvironment inside the polynucleotide. It has been estimated that, for a bulk pH of 7.0 and 5.7, the local calculated "pH" inside the CT-DNA corresponds to "5.5" and "4.2", respectively.[27] Thus, if the bulk pH is 7.0, and the local pH "5.5", the quinoline unit is completely protonated upon binding, as indicated by the spectrum which is identical to that at pH 4.5 with DNA. If the bulk pH is 7.5, and the local pH about "6.0", the quinoline unit is not completely protonated. In agreement with this, the quinoline moiety absorption with CT-DNA at pH 7.5 is weaker than with CT-DNA at pH 4.5.

(B) Metallic Unit. The metallic unit of the bifunctional complex and the monofunctional complex exhibit both by addition of nucleic acid (i) a typical hypo- and bathochromic effect of the MLCT absorption band and (ii) the existence of emitting species with similar excited-state lifetimes.

For $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ in the presence of $[poly(dA-dT)]_2$, a fraction of the excited metallic unit binds externally to the sugar−phosphate backbone. This is inferred from the existence of a luminescence lifetime of 0.70 μs which is the same as τ for excited $[Ru(TAP)_2Phen]^{2+}$ bound externally. The remaining fraction of the emission (87%) originates from protected species with longer excited-state lifetimes. The lifetimes and contributions are the same for the mono- and bifunctional complexes, showing that the interaction of the metallic unit in $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ with $[poly(dA-dT)]_2$ is not disturbed by the presence of the organic moiety. In the presence of $poly[(dG-dC)]_2$, the excited states of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ (Figure 7) are quenched by the guanines. In the presence of CT-DNA, most emission components of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ are short-lived and correspond to quenched species. A small fraction of the excited species are long-lived and are assigned to entities that are protected inside A−T grooves of the double helix. The same conclusions can be drawn for the reference, **2**, given that its luminescence decays are very similar to those of **1**.

(2) Binding Affinities. The titration curves of the bifunctional complex **1** by the different polynucleotides (Figure 6) do not exhibit tremendous differences of the P/D values at which half of the luminescence intensity enhancement is reached. This suggests a similar binding affinity for the different polynucleotides, hence, no strong binding selectivity.[28] In contrast, the comparison of the titrations by CT-DNA for the mono- and bifunctional complex indicates a difference of binding affinity. At both pH 4.5 and 7.5 (Figures 2 and 4), the bifunctional complex is completely bound at concentrations of CT-DNA lower than for the monofunctional analogue. Hence, $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ has a higher affinity for CT-DNA. The affinity constants were estimated by applying the McGhee−Von Hippel theory at pH 4.5. As described in the Experimental Section, the data needed were retrieved from the titration curves of both complexes by CT-DNA at pH 4.5. They yield values of $\approx 5 \times 10^5$ M^{-1} for $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ and $\approx 10^4$ M^{-1} for $[Ru(TAP)_2Phen]^{2+}$, respectively. It is probable that the protonation of the quinoline unit upon binding to DNA increases the electrostatic interaction between the triply charged complex and the polyanionic phosphate backbone. As shown by a detailed study of the interaction of an analogue of the present bifunctional complex, that is, $[Ru(BPY)_2POQ\text{-}Nmet]^{2+}$,[16] the nonelectrostatic interaction also plays a role in this type of complex.

(3) Light Switch Behavior of the Bifunctional Complex. The interaction of the bifunctional complex with a polynucleotide such as $[poly(dA-dT)]_2$ induces a restoration of luminescence of the metallic unit which is otherwise quenched in solution at pH 7.5. This is confirmed by the absence of short excited-state lifetimes (<0.7 μs) when the whole amount of complex is bound to $[poly(dA-dT)]_2$. The intramolecular photoelectron-transfer quenching for the complex in bulk solution disappears on DNA because of the partial protonation of the quinoline[14] and probably also because of a separation of the two units when in interaction with the polynucleotides.[15] This makes this complex a good DNA "light switch".

(4) Base Content Photoprobing by the Mono- and Bifunctional Complex. This study also shows clearly that the I_∞/I_0 ratio for both complexes varies with the base content of nucleic acids (Figures 3 and 6, Table 2). The change of the global emission intensity is probably caused mainly by the groove bound excited species that, depending on the sequence, have different distributions of emission lifetimes, and, thus, different luminescence decay profiles. The variety of lifetimes originates, of course, from the heterogeneity of the sites to which the complex binds. A linear relation between the intensity of emission and the percentage of A−T base pairs would mean that only one single base pair influences the lifetime of an excited state, which is not the case. Indeed, for the bifunctional complex, the I_∞/I_0 value of 16 with CT-DNA (58% of A−T) is not merely an average value between that with $[poly(dA-dT)]_2$ ($I_\infty/I_0 = 63$) and that with $[poly(dG-dC)]_2$ ($I_\infty/I_0 = 10$) but is much closer to that of $[poly(dG-dC)]_2$. Previous studies have shown that the Ru(II) polypyridyl complexes cover in the average about three or four base pairs,[29] we can thus assume that this number of bases remains the same for the luminophore of the bifunctional complex. Moreover, as the complexes can diffuse along a DNA groove with a diffusion constant of

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(28) When applicable, the McGhee−Von Hippel treatment yields similar binding constants for $[Ru(TAP)2POQ\text{-}Nmet]^{2+}$ in the presence of the different examined nucleic acids. Binding competition experiments between $[poly(dA-dT)]_2$ and $[poly(dG-dC)]_2$ also show the absence of selectivity. The complex binds less strongly to CP-DNA because of the presence of sodium chloride in solution.

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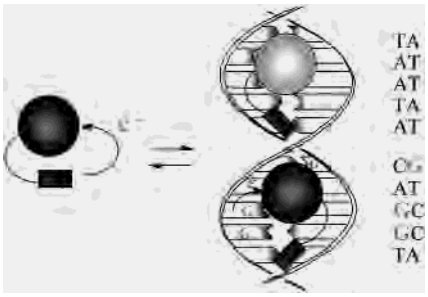

Figure 8. Schematic representation of the sequence-selective "light switch" behavior of $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$, in the presence of a double helix, with an arbitrary chosen sequence of bases. Dark sphere: metallic unit quenched by electron transfer. Bright sphere: luminescent metallic unit. Gray rectangle: quinoline unit.

the order of 10^{-8} cm^2 s^{-1},[30] the excited complexes probe certainly several base pairs during their excited states and have therefore different luminescence lifetimes and emission intensities. This variety of lifetimes can also be explained differently. It has been shown with an oligonucleotide chemically tethered $Ru(TAP)_2(L)^{2+}$ complex (L = ligand) that for a photoinduced electron transfer to take place, the complex must be in direct contact with a guanine.[31] In such conditions, the different electron-transfer rates may originate from different geometries of encounter complex−guanine (the complex is not an intercalating agent) and different ionization potentials of the guanines that depend on the sequence. Two extreme situations can be envisioned: (i) complexes groove-bound in an A−T sequence with a long emission lifetime and intense emission and (ii) complexes bound to G−C sequences with a short luminescence lifetime and weak emission attributed to the quenching by the guanines by electron transfer. Of course, all the intermediate cases exist and lead to a variety of short luminescence decay components. These different lifetimes and especially their varying proportions explain the dependence of I_∞/I_0 on the base content. Table 2 indicates also that with partially denatured CT-DNA (SS-CT-DNA) which contains 40% of nonhybridized single strand DNA portions, the quenching seems more important than with normal CT-DNA. This could be explained by the fact that the portions remaining hybridized in denatured DNA, to which the complexes essentially bind, are richer in G−C bases.

Conclusions

The photoreactive complexes based on TAP ligands in this work are interesting luminescent photoprobes of DNA. The bifunctional complex $[Ru(TAP)_2POQ\text{-}Nmet]^{2+}$ turns out to be the most interesting candidate because of its large luminescence enhancements with nucleic acids that depend on the base content (Figure 8). Its luminescence is switched on when it interacts with DNA; in some cases, it behaves as a "light switch", and the emission becomes 100 times more intense than in the bulk solution with a lifetime of ≈ 2 μs. This latter value is long compared to most Ru(II) "light switch" probes known in the literature such as $[Ru(Phen)_2DPPZ]^{2+}$. The most striking advantage over other probes is the *selectivity* of luminescence: long-lived excited species with adenine−thymine-rich sites and short-lived ones with G−C sites (Figure 8). The monofunctional analogue exhibits comparable characteristics but has a lower affinity for DNA and emits in bulk solution.

Experimental Section

Synthesis. The syntheses and characterizations of the Ru(II) complexes have been described elsewhere.[15]

Chemicals. The phosphate buffers were adjusted to the correct pH (at room temperature) by mixing equimolar solutions of Na_2HPO_4 and NaH_2PO_4 (Merck p.a., in MilliQ water). The Tris buffers were prepared by adding concentrated HCl to aqueous solutions of tris(hydroxymethyl)aminomethane (Aldrich p.a.). The solutions of GMP were prepared by mixing the disodic (Aldrich) and diacid forms (Sigma) of GMP.

The different DNAs used are the following: CT-DNA = calf thymus DNA (Pharmacia); HS-DNA = herring testis DNA (Sigma). CT- and HS-DNA were purified by exhaustive dialysis against a phosphate buffer solution and afterward against water. Concentrations were determined spectrometrically with $\epsilon_{260} = 6.6 \times 10^3$ M(base)$^{-1}$ cm^{-1}. Additional DNAs include the following: SS-CT-DNA = partially denatured CT-DNA (40% single stranded and 60% double stranded) by heating at 95 °C for 15 min followed by rapid freezing; CP-DNA = chlostridium perfringens DNA (Sigma, used as such); $[poly(dA-dT)]_2$ (Aldrich, 10 units purified solutions used as such, $\epsilon_{260} = 6.6 \times 10^3$ M(base)$^{-1}$ cm^{-1}); $[poly(dG-dC)]_2$ (Aldrich, 10 units purified solutions, $\epsilon_{257} = 8.5 \times 10^3$ M(base)$^{-1}$ cm^{-1}).

The solutions for the titrations were prepared by starting with the highest concentration of nucleic acid, which was progressively diluted with a solution containing no nucleic acid. The total volume and the concentrations of complex and buffer were kept constant. The steady-state and time-resolved luminescence measurements were performed in a cell holder thermostated at 25 °C after 15 min of stirring. The emission intensity of an aqueous solution of $[Ru(TAP)_2Phen]^{2+}$ (6×10^{-6} M in 10 mM buffer) was measured prior to each titration presented in the results section.

Absorption and Emission Spectroscopy. Absorption spectra were recorded on a Cary 219 or an HP 8452A UV−vis diode array spectrometer. The emission spectra were obtained with an Edinburgh Instruments spectrofluorimeter equipped with a Hamamatsu R-955S red sensitive photomultiplier tube or with a Shimadzu RF-5001 PC spectrofluorimeter equipped with a Hamamatsu R-928 red sensitive photomultiplier tube and were corrected for the instrument response.

The emission lifetimes were measured by time-resolved single photon counting (SPC) with an FL-900 Edinburgh Instruments spectrometer (Edinburgh, UK) equipped with a nitrogen-filled discharge lamp (gas pressure between 0.4 and 0.45 bar, 1.3 mm gap, and 4.9 kV between electrodes, operating at 30 kHz) and a Peltier-cooled Hamamatsu R-955S photomultiplier tube. The emission decays were analyzed with the Edinburgh Instruments software, based on nonlinear least-squares regressions using a modified Marquardt's algorithm. Typically, a time resolution of 19.5296 ns/channel (1000 channels) was used in the case of mono- or biexponential decays, and a resolution of 4.8824 ns/channel (4000 channels) was used when short lifetime components were observed. In both cases, 10 000−20 000 counts were accumulated in the peak

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channel (no scattering of the 379 nm excitation beam was observed, using a UV cut off filter and 300 mm focal monochromators).

Laser flash photolysis experiments were carried out using a modified Applied Photophysics laser kinetic spectrometer equipped with a Hamamatsu R-928 photomultiplier tube. The excitation source is composed of a frequency doubled neodymium YAG laser (Continuum NY 61-10) coupled with a dye laser (Continuum ND60; dye DCM; $\lambda_{exc} = 640$ nm) and with the mixing option (Continuum UVX), producing a 400 nm beam (10 ns pulse width, maximum of 27 mJ per pulse). Limitations on the lower accessible time scale are due to the response time of the detection system (minimum RC time constant ~12 ns).

The McGhee−Von Hippel analysis was carried out using the following equation:[32]

$$\frac{\nu}{c_f} = K_{aff}\frac{(1 - n\nu)^n}{(1 - (n-1)\nu)^{n-1}}$$

where ν is the binding ratio c_b/[DNA] ([DNA] = P/2, is the concentration of DNA expressed in base pairs bp), K_{aff} is the binding constant for the experimental conditions used, and n is the size of a binding site (expressed in base pairs). To use this equation, the concentrations of bound (c_b) and free (c_f) complex had to be determined for each concentration of DNA by using the titration curves and the following relation:

$$I/I_0 = 1 + (I_\infty/I_0 + 1)c_b/c_t$$

where c_t is the total complex concentration, I_∞, the emission when all the complex is bound, and I_0, the emission in the absence of DNA.

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