Inorganic Chemistry
Article

Inorg. Chem. **2010**, *49*, 10867–10874 **10867**
DOI: 10.1021/ic101214m

Photoinduced Electron Transfer from Tryptophan to Ru^{II}TAP Complexes: The Primary Process for Photo-Cross-Linking with Oligopeptides

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Received June 17, 2010

The photoreaction mechanism of $[Ru(TAP)_2(phen)]^{2+}$ and $[Ru(TAP)_3]^{2+}$ (TAP = 1,4,5,8-tetraazaphenanthrene) with tryptophan (Trp), *N*-acetyl-Trp, and Lys-Trp-Lys is examined. The existence of a photoelectron-transfer process from the amino acid unit is demonstrated by laser flash photolysis experiments. The back electron transfer (BET) from the reduced complex to the oxidized amino acid, occurring at the microsecond time scale, corresponds approximately to an equimolecular−bimolecular process; however, it is disturbed by another reaction, originating from the oxidized Trp. Moreover, in competition with the BET, the reduced and oxidized intermediates give rise to an adduct. The latter is clearly detected by gel electrophoresis experiments in denaturing conditions, with a system composed of an oligonucleotide derivatized at the 3′ end by the Ru^{II}TAP complex and hybridized with the complementary sequence functionalized at the 5′ end by the tripeptide Lys-Trp-Lys. Thus, upon illumination, a cross-linking between the two strands is observed, which originates from the presence of a Trp residue.

Introduction

For many years, ruthenium(II) complexes of polypyridyl ligands have received much attention because of their various redox and photophysical properties, making them attractive candidates for different applications.[1−6] More recently, their remarkable photophysical and photochemical properties have been exploited using biomolecules, more particularly with mono- and polynucleotides as well as with DNA.[7−10] We have shown that, in the presence of these nucleobases, the photoreactivity of Ru-TAP complexes (TAP = 1,4,5,8-tetraazaphenanthrene) is initiated by a primary photoelectron transfer (PET) process from a biomolecule containing a guanine (G) to the excited complex. However, this PET is exergonic only if the excited complex contains at least two TAP ligands, so that the oxidation power is sufficiently high for extracting the electron from the nucleobase.[11,12] This is the case for $[Ru(TAP)_2(phen)]^{2+}$ (phen = 1,10-phenanthroline) and $[Ru(TAP)_3]^{2+}$ (Figure 1), for which the PET process, kinetically coupled to a proton transfer, has been unambiguously demonstrated by laser flash photolysis with G-containing biomolecules, such as guanosine-5′-monophosphate (GMP) or polynucleotides.[13−15] The different elementary processes were analyzed using different time scales after the laser pulse, from the picosecond to the nano- and microsecond time domains. Thus, the back-electron-transfer (BET) process, which follows the PET, is close to a

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Published on Web 11/02/2010

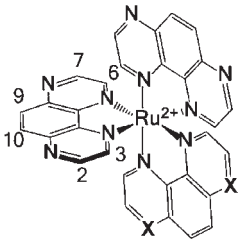

Figure 1. Structure of the ruthenium(II) complexes: X = N for $[Ru(TAP)_3]^{2+}$; X = CH for $[Ru(TAP)_2phen]^{2+}$.

process controlled by diffusion when the oxidized G species belongs to GMP and corresponds to a bimolecular−equimolecular process in the microsecond time domain. In contrast, the BET occurs in the nanosecond time domain when the oxidized G base belongs to a polynucleotide, with which the ruthenium(II) complex interacts. In this case, the BET corresponds to a first-order process because both the electron donor and acceptor remain fixed during the BET process. At the physiological pH, this BET is also coupled to a proton transfer, in competition with the bimolecular reaction of the radical ($TAP^{\bullet-}$ or $TAPH^{\bullet}$) of the reduced complex and the deprotonated radical cation of the G moiety, giving rise to a covalent bond between the electron donor (i.e., guanine) and acceptor (i.e., TAP ligand of the complex).[16] After rearomatization of the system, an adduct between the metallic complex and the nucleobase is formed. This PET-initiated reaction is particularly interesting because it provides access to irreversible cross-linking between the two strands of a duplex[17] or inside a single-stranded telomeric sequence,[18] and this is at the level of their G bases. Moreover, oligonucleotide (ODN) probes derivatized by such photoreactive complexes[19] have also been shown to be very attractive for gene-silencing applications. Indeed, they photo-cross-link specifically with their complementary targeted strand at a G base, or in the absence of their target, they give rise to self-inhibition if they contain a G in their own sequence.[20]

Recently, the existence of a PET with two amino acids as electron donors, tyrosine (Tyr) and tryptophan (Trp), has also been demonstrated. The case of Trp is particularly relevant because, like the guanine nucleobase, this amino acid also generates a photoadduct upon illumination of the ruthenium complex.[21] On the other hand, under pulsed UV irradiation of Trp alone, photoelectron ejections were detected and studied.[22] This process is accompanied by the production of numerous radical reactions, particularly in the presence of oxygen.[23−28] Some products resulting from this Trp oxidation have been determined.[29]

On the basis of these previous observations with Trp, the present work provides a mechanistic study of the photoreactions of two Ru-TAP complexes with Trp-containing biomolecules, i.e., Trp itself for comparison purposes, Trp derivatives, a Trp-containing tripeptide Lys-Trp-Lys, and a more sophisticated system in which the Lys-Trp-Lys tripeptide is chemically attached to an ODN. Investigation of the kinetics of the primary processes of the photoreactions in these systems by laser flash photolysis was performed with the aim of (i) determining the mechanism of the photoreactions and (ii) applying these reactions to designed biomolecular systems, in which photo-cross-linking should be generated via a Trp residue.

Experimental Section

1. Materials. $[Ru(TAP)_2(phen)]^{2+}$ and $[Ru(TAP)_3]^{2+}$ (with Cl^- as a counterion) were synthesized and purified as previously described.[30,31] Trp, *N*-acetyl-Trp, and Lys-Trp-Lys were purchased from Sigma-Aldrich and used without further purification. The buffered aqueous solutions were prepared with Millipore Milli-Q water and Tris-HCl buffer at pH 7. The pH values of the solutions were verified by measurements using a Consort P601 pH meter. The solutions were deoxygenated by bubbling argon for at least 30 min before the measurements.

The conjugates 3′Ru-ODN (with the Ru-TAP complex attached to the 3′ side of the ODN) and 5′Lys-Trp-Lys-ODN (with the tripeptide Lys-Trp-Lys attached to the 5′ end of the complementary ODN) were synthesized by using the oxime coupling reaction according to previously reported methods.[32,33] Briefly, the 3′ conjugate was prepared by reacting the ODN ($^{5'}$TAAATTTAATA-AAAAAAX$^{3'}$, in which X represents the aldehyde-containing linker) with a slight excess of the ruthenium complex bearing the oxyamino group on the phenanthroline ligand in a 0.4 M ammonium acetate buffer overnight at room temperature. In a similar manner, the 5′ conjugate functionalized with the Lys-Trp-Lys peptide was prepared by reacting the complementary ODN ($^{5'}$YTTTTTTTATTA-AATTTA$^{3'}$, in which Y represents the aldehyde-containing linker) with the tripeptide Lys-Trp-Lys containing the oxyamino group at the N-terminus in the same conditions as above. The two conjugates were purified by PAGE and desalted by size-exclusion chromatography. The 3′Ru-ODN was previously characterized by nano-electrospray ionization mass spectrometry (nano-ESI-MS).[19] The new ODN-5′Lys-Trp-Lys was also characterized by nano-ESI-MS (Figure S1 in the Supporting Information, SI).

2. Methods. The flash photolysis experiments were performed in a cross-beam configuration by using the excitation source composed of a frequency-tripled (355 nm) Nd:YAG Q-switched laser (Continuum Inc.) coupled with an optical parametric oscillator

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(Continuum Inc.) covering the wavelength region 410−2300 nm with a maximum pulse energy from 10 to 120 mJ depending on the wavelength. The average pulse duration was 5 ns. As a probe source, we used a 150 W, xenon arc lamp producing a continuous spectral distribution ranging from 190 to 2600 nm. The grating Czerny−Turner monochromator (Spectra Pro 2300i, Acton Research Corp.) was used for the spectral selection. The solutions were placed in a quartz cell of 10 mm optical path length for the analytical beam and 1 mL sample volume.

The transient absorption spectra were measured in the 300−750 nm spectral range with a gated intensified CCD camera (PI-MAX, 1024 × 256 pixels, Princeton Instruments) coupled to a monochromator (the detection gate was 2 ns). The time delays for probing, following the laser excitation pulse, were controlled by a programmable time generator (Princeton Instruments). For kinetic analyses, the transient absorption traces at selected individual wavelengths were transferred through the same optical system and were detected by a photomultiplier (R928, Hamamatsu). The signal was recorded with a digital oscilloscope (HP 54200A), connected through the IEEE488 interface to a personal computer, and was averaged over at least 16 shots; baseline corrections were also introduced. Kinetic analyses of the absorption traces were performed by nonlinear least-squares regression modified by Levenberg−Marquardt algorithms.[34] The ground-state absorption spectra were measured using a Perkin-Elmer (Lambda 40) UV/vis spectrometer.

The luminescence lifetimes were measured by the time-correlated single-photon-counting (TC-SPC) technique with an Edinburgh Instruments FL900 spectrometer equipped with a hypobaric nitrogen-filled discharge lamp pulsed at 30 kHz and a red-sensitive, Peltier-cooled photomultiplier (R928, Hamamatsu). The samples were thermostatted at 20 ± 2 °C with a Haake NB22 temperature controller. The data were collected by a multichannel analyzer (2048 channels) with a minimum number of counts in the first channel (t = 0) equal to 10^4. The resulting decays were deconvoluted for the instrumental response and fitted to exponential functions using the original manufacturer software package (Edinburgh Instruments). The reduced χ^2, weighted residuals and autocorrelation function were employed to judge the quality of the fits. The steady-state luminescence spectra and intensity measurements were recorded on a Shimadzu (RF5001PC) spectrofluorimeter.

For the gel electrophoresis experiments, the ODN-Ru strands were 5′-end-labeled by T4 polynucleotide kinase using γ-[^{32}P]-ATP 3000 Ci mmol^{-1} (Perkin-Elmer Life Sciences) at 37 °C for 25 min. The duplex solutions (6 μM) were prepared by mixing equimolar solutions of ODN-Ru and complementary ODN′-Lys-Trp-Lys single strands. The ODNs were hybridized by heating up to 90 °C for 10 min before slow cooling at room temperature overnight. The samples were illuminated with a 442 nm line of a He/Cd laser (Melles Griot He/Cd Laser Omnichrome LC-500). A volume of 10 μL of the sample was added to the loading buffer (95% formamide, 0.1% xylene cyanol, and 0.1% bromophenol blue). The mixture was loaded on polyacrylamide gel (20% with a 19:1 acrylamide/bisacrylamide ratio) containing 7 M urea in a TBE buffer (90 mM Tris-borate and 2 mM EDTA). After migration, the gel was exposed to a Phosphor Imager screen overnight at 4 °C. The screen was scanned with Phosphor Imager Storm 860 (Amersham Pharmacia Biotech) equipment. The reference ODN used for attribution of the different bands is constituted of a 17-mer strand with an attached ruthenium(II) photooxidant complex ([Ru(TAP)$_2$(DIP)]$^{2+}$; DIP = 4,7-diphenylphenanthroline with the linker on one of the phenyl groups[35]) and the complementary strand incorporating a G unit near the complex after hybridization of the two sequences (see further).

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Table 1. Luminescence Quenching Rate Constants (k_q) by Trp and Its Derivatives in Aqueous Solution[a]

	$k_q \times 10^9$ (M^{-1} s^{-1})	
amino acid	[Ru(TAP)$_3$]$^{2+}$ (τ_0 = 230 ns)	[Ru(TAP)$_2$(phen)]$^{2+}$ (τ_0 = 840 ns)
Trp	3.0	2.4
N-acetyl-Trp	5.9	5.2
Lys-Trp-Lys	1.3	1.1

[a] 0.1 M Tris-HCl buffer, pH 7, 10^{-5} M in ruthenium complex, and errors on the order of 10%. τ_0 is the luminescence lifetime of the free complex. From pH 7 to 8, the k_q values are the same within the experimental error.

The samples for the denaturation temperature measurements contained the ODNs (2 μM) in a buffered aqueous solution (Tris-HCl 10 mM) with NaCl salt (50 mM). The duplex solutions were heated at 85 °C for 10 min and cooled overnight at room temperature for homogeneous hybridization. The experiment was performed by absorption measurements using a Perkin-Elmer Lambda 35 spectrophotometer equipped with a Peltier temperature controller (PTP-1). The absorbance of the nucleotidic bases was monitored at 260 nm and recorded every 0.2 °C. All of the samples were heated and cooled (1 °C min^{-1}) at least three times. The denaturation temperatures were found as the inflection points of the calculated curves representing the fraction of denatured material versus temperature.

Results and Discussion

1. Luminescence Quenching in the Presence of Trp, *N*-Acetyl-Trp, and Lys-Trp-Lys. [Ru(TAP)$_2$(phen)]$^{2+}$ and [Ru(TAP)$_3$]$^{2+}$ luminesce from their ^{3}MLCT excited states with an intense and broad emission band with a maximum of around 600−650 nm in aqueous solution.[36] The luminescence lifetimes correspond to a few hundred nanoseconds (Table 1) and are quenched as a function of increasing concentrations of Trp, *N*-acetyl-Trp, and tripeptide, Lys-Trp-Lys (lysine does not lead to any quenching)[21] with quenching rate constants (k_q) close to the diffusional limit for both complexes (Table 1). For [Ru(TAP)$_3$]$^{2+}$, the values of k_q are a bit higher because of the higher oxidizing power of this complex.[10,11] Moreover, for Trp or *N*-acetyl-Trp, the k_q values are slightly higher than those for the tripeptide. The weaker quenching by Lys-Trp-Lys can be attributed to the presence of two positive charges (at pH 7) on the side chains of the two Lys peptides that introduce an electrostatic repulsion with the complex. The k_q values are the same under air and argon and do not depend on the pH value (from 7 to 8) within an experimental error of ±10%.

2. Laser Flash Photolysis of the Complexes in the Presence of Trp, *N*-Acetyl-Trp, and Lys-Trp-Lys. In order to show that the luminescence quenchings originate from a PET, a laser flash photolysis experiment was performed in the absence and presence of these three reducing agents. In the absence of amino acid, the differential transient absorptions of the triplet metal-to-ligand charge-transfer (^{3}MLCT) states of the two complexes were recorded at 100 ns after laser excitation (Figure 2). These spectra clearly exhibit a depletion (negative absorption) in the region of ground-state absorption of the complexes (i.e., 400−500 nm; see the absorption spectra in Figures S2 and S3 in the SI) and a positive band with a maximum at 350 nm, attributed to

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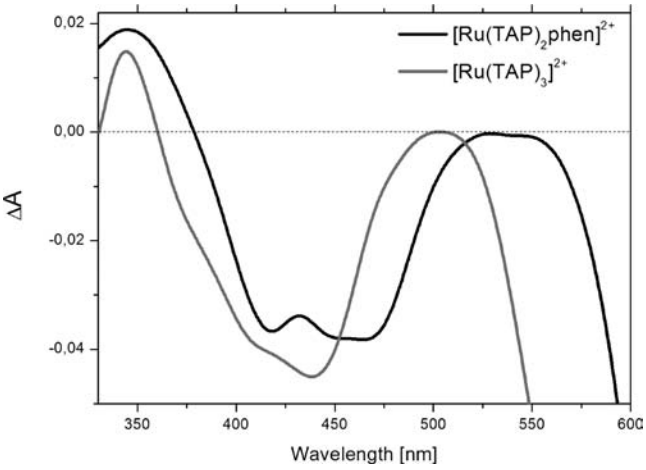

Figure 2. Differential transient absorptions of $[Ru(TAP)_2phen]^{2+}$ (black) and $[Ru(TAP)_3]^{2+}$ (gray) (10^{-5} M) in an aqueous argon-saturated solution (0.1 M Tris buffer, pH 7) recorded at 100 ns after the laser pulse. At wavelengths >500 and >550 nm for $[Ru(TAP)_3]^{2+}$ and $[Ru(TAP)_2phen]^{2+}$, respectively, the negative signals are due to the emission.

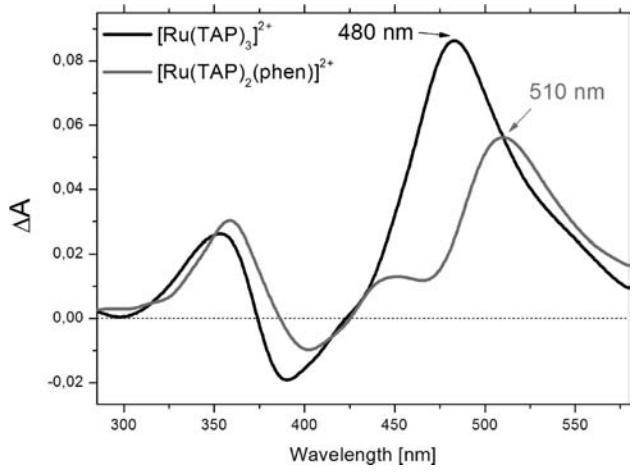

Figure 3. Differential transient absorption of $[Ru(TAP)_2phen]^{2+}$ (gray) and $[Ru(TAP)_3]^{2+}$ (black) (10^{-4} M) in the presence of Lys-Trp-Lys (10^{-2} M) in an aqueous argon-saturated solution (0.1 M Tris buffer, pH 7) recorded 1 μs after the laser pulse. Excitation at 410 nm.

absorption of the ^{3}MLCT states (T−T absorptions). These transients decay according to monomolecular processes (Figures S4 and S5 in the SI), with rate constants corresponding to the luminescence decays (only the short wavelength tail of the luminescence band is visible in Figure 2).

In the presence of tripeptide (10^{-2} M, 0.1 M Tris-HCl buffer, pH 7), for example, both excited-state complexes exhibit a differential absorption spectrum (sum of the absorptions of the intermediate species; Figure 3),[37] which is reminiscent of the reduced complexes $[Ru(TAP)_2(TAP^{\bullet -})]^+$ (λ_{max} = 480 nm) and $[Ru(TAP)(TAP^{\bullet -})(phen)]^+$ (λ_{max} = 505 nm) as determined by pulse radiolysis experiments.[11,14,38]

(37) It was observed that when the transient absorptions were recorded in the presence of oxygen 1 μs after the laser pulse, the intensity of the absorption at 510 nm was more important, most probably because of absorption of the reaction products of singlet oxygen with Trp.

(38) The λ_{max} values of absorption of these reduced complexes and their corresponding protonated species are approximately the same, but the absorption coefficients are different (see ref 14). At pH 7, there is a mixture of both species, probably more protonated for reduced $[Ru(TAP)_2(phen)]^{2+}$ than for reduced $[Ru(TAP)_3]^{2+}$ because of a higher pK_a value.

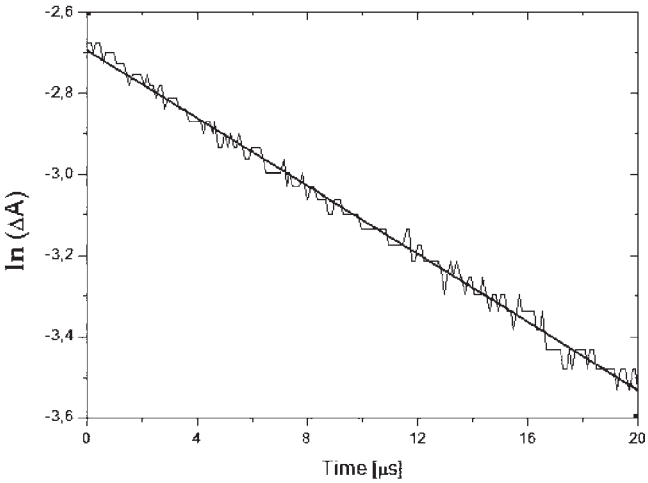

Figure 4. First-order kinetic analysis of transient absorption decay after the laser pulse for $[Ru(TAP)_2(phen)]^{2+}$ (10^{-4} M) photoreduced by Trp (10^{-2} M) in an aqueous dioxygen-saturated solution (0.1 M Tris buffer, pH 7.5) recorded at 510 nm. Excitation at 355 nm.

Thus, the strong positive band appearing after excitation (i.e., at 1 μs after complete decay of the excited state) originates from absorption of the monoreduced complex, at least partially, which indicates the existence of a photoinduced electron transfer from the Trp residue of Lys-Trp-Lys to the excited-state complex ($[Ru(TAP)_3]^{2+}$ or $[Ru(TAP)_2(phen)]^{2+}$). The transient differential absorptions of $[Ru(TAP)_2(phen)]^{2+}$ with Trp and *N*-acetyl-Trp are similar to those with Lys-Trp-Lys (Figure S6 in the SI).

Baugher and Grossweiner[26] have shown that the $Trp^{\bullet +}$ radical cation produced by flash photolysis in aqueous solution at pH 7 is rapidly deprotonated and the neutral $Trp(-H)^{\bullet}$ radical exhibits absorption with a maximum at 510 nm, thus in the same absorption region as the reduced complexes. The strong absorption band at 480−510 nm in Figure 3 thus contains two contributions, one from the monoreduced complex (protonated and nonprotonated)[38] and the other from the oxidized and deprotonated Trp.

$$[Ru(TAP)_2(phen)]^{2+*} + \text{Lys-Trp-Lys} \xrightarrow{H_2O/pH\ 7} [Ru(TAP)(TAP^{\bullet -})(phen)]^+ \text{ (and } [Ru(TAP)(TAPH^{\bullet})(phen)]^{2+}) + \text{Lys-Trp}(-H)^{\bullet}\text{-Lys} \quad \text{(I)}$$

These two absorptions are especially overlapped for $[Ru(TAP)_2(phen)]^{2+}$. We performed further experiments in order to get other arguments in favor of the PET.

Because the reduced complex (protonated and nonprotonated) is very sensitive to oxygen,[14] it should restore the starting complex according to process (II).[14]

$$[Ru(TAP)(TAP^{\bullet -})(phen)]^+ \text{ (and } [Ru(TAP)(TAPH^{\bullet})(phen)]^{2+}) + O_2 \xrightarrow{H_2O/pH\ 7} [Ru(TAP)_2(phen)]^{2+} + O_2^{\bullet -} \rightarrow \rightarrow \quad \text{(II)}$$

Therefore, we tested the behavior of the 450−500 nm transient for $[Ru(TAP)_2(phen)]^{2+}$ in the presence of oxygen. Under those conditions, with 1.25×10^{-3} M in dioxygen, the transient absorption at 510 nm decays in a time range of 20 μs according to a pseudo-first-order process (Figure 4), with a rate constant $k = 3.4 \times 10^7$ M^{-1} s^{-1}. This is in agreement with reaction (II) in which the dioxygen concentration

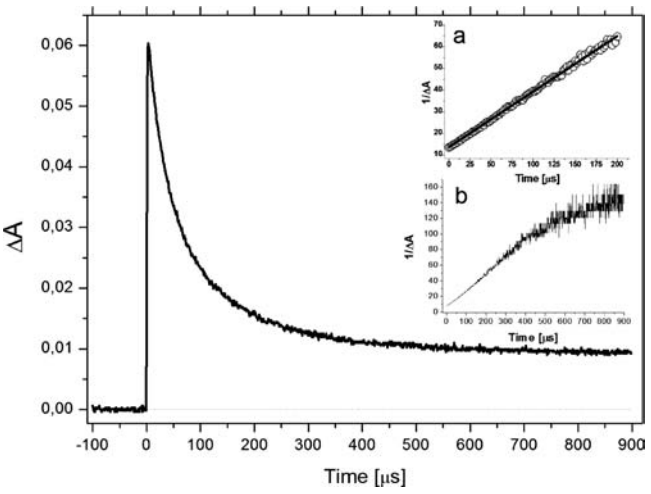

Figure 5. Transient absorption decay after the laser pulse for $[Ru(TAP)_2(phen)]^{2+}$ (10^{-4} M) in the presence of Lys-Trp-Lys (10^{-2} M) in an aqueous argon-saturated solution (0.1 M Tris buffer, pH 7) recorded at 510 nm. Inset: second-order kinetic analysis for the beginning of decay at 0−200 μs (a) and for the whole time scale of 0−900 μs (b).

is in excess and is considered as constant compared to that of the transients. The remaining tryptophanyl radical reacts probably with species produced from the peroxide to form products absorbing in the UV.[29] In contrast, in the absence of oxygen, these transients decay in a longer time scale corresponding to a few hundreds of microseconds (see, for example, Figure 5 for $[Ru(TAP)_2(phen)]^{2+}$ and the tripeptide) according to a bimolecular-equimolecular process, attributed to the BET with reprotonation of the Trp radical.[39]

$$[Ru(TAP)(TAP^{\bullet -})(phen)]^{+} \text{ (and } [Ru(TAP)(TAPH^{\bullet})(phen)]^{2+}) + Trp(-H)^{\bullet} \xrightarrow{H_2O/pH\ 7} [Ru(TAP)_2(phen)]^{2+} + Trp \qquad (III)$$

This conclusion is indeed in agreement with the linear relationship between the reciprocal of the absorption plotted as a function of time (inset of Figure 5, second-order kinetic analysis).[40] It must be noticed, however, that the fitting to this linear relationship is good for the beginning of the decay (first 200 μs; see inset a of Figure 5), but this is not the case in the longer time scale (inset b of Figure 5). Moreover, there is also a remaining absorption at least until a few seconds, which means that the initial compounds are not fully restored as a result of the BET. This behavior is also observed with Trp or *N*-acetyl-Trp as reductive quenchers.[41]

In spite of these difficulties, we determined $k_{BET}/\Delta\varepsilon$ (from the slope of the straight line in the inset of Figure 5) at different pH values and for $[Ru(TAP)_2(phen)]^{2+}$ (Table 2). The data indicate that, in spite of some probable variations of $\Delta\varepsilon$ with the pH, the BET is not very sensitive to the pH. Because the $\Delta\varepsilon$ values are not known for $[Ru(TAP)_2phen]^{2+}$ but can be determined for $[Ru(TAP)_3]^{2+}$ (the ε value for reduced $[Ru(TAP)_3]^{2+}$ was determined at different pHs in the past),[14] we can assess, in that case, the rate constant k_{BET} from slope $= k_{BET}/\Delta\varepsilon$ at pH 7. For this complex and Trp,

(39) In reaction (III), we assume that the reduced complex is protonated. There is probably a mixture of protonated and nonprotonated complexes.

(40) Connors, K. A. *Chemical Kinetics, the Study of Reaction Rates in Solution*; VCH Publishers: New York, 1991.

(41) Even when we artificially shift the zero level for the fitting procedure, the decay kinetics do not correspond to a perfect second-order process for the complete time domain.

Table 2. Slopes of the Straight Lines ($1/\Delta A$ vs Time) Corresponding to the Transient Absorption Decays at 510 nm for $[Ru(TAP)_2(phen)]^{2+}$ in the Presence of the Different Trp Derivatives and for the First 200 μs of the Decay[a]

	$k_{BET}/\Delta\varepsilon \times 10^6$ (cm s^{-1})		
amino acid	pH 6.0	pH 7.0	pH 8.0
Trp	3.1	3.0	3.3
N-acetyl Trp	2.4	2.8	3.2
Lys-Trp-Lys	2.3	2.8	3.2

[a] Errors on the order of 15−20%.

$k_{BET} = 3.8 \times 10^9$ M^{-1} s^{-1}; thus, this reaction is diffusion-controlled.

3. Possible Photoproducts Formed under Illumination. As was mentioned earlier, the transient species formed after pulsed laser excitation do not completely restore the initial compounds because of (i) reactions of the excited-state complex before its quenching by the reducing agent or (ii) possible reactions of the reduced complex and/or oxidized Trp residue. For example, a photoreaction (photosubstitution of a bidentate ligand) can take place from the ^{3}MC excited state,[36] as shown in Figure S2 in the SI, by the occurrence of a new red-shifted band around 500 nm after a steady illumination of the complexes in the absence of electron donor. This photodechelation and substitution is much more important for $[Ru(TAP)_3]^{2+}$ than for the bis-TAP complex.[36] However, this photoreaction is not sufficiently significant, especially in the presence of quenching by the Trp residue, to explain most of the residual absorption at 510 nm after the laser pulse (Figure 5). In the presence of amino acid, this residual absorption could also originate from a permanent absorption by a photoadduct of the Trp residue with the metallic complex as shown previously.[21] This photoadduct does indeed absorb in the 500 nm region (Figure S2 in the SI) after illumination of the complex in the presence of Trp (or tripeptide).

4. Luminescence Quenching of ODN-Ru by ODN′-Lys-Trp-Lys. On the basis of these data and thanks to this photoanchoring process of the metallic species to the amino acid residue, it should be possible to photo-cross-link two biomolecules. With the view of demonstrating this possibility, the behavior of an ODN attached to a $[Ru(TAP)_2(phen)]^{2+}$ complex at the 3′ position (ODN-Ru) was examined under illumination in the presence of the complementary sequence bearing the tripeptide Lys-Trp-Lys at the 5′ position (ODN′-Lys-Trp-Lys; Figure 6). In order to avoid photoreaction with a G base, neither of the two complementary ODN sequences contains a G unit. After hybridization of the two sequences, the duplex (ODN-Ru/ODN′-Lys-Trp-Lys) was studied. Luminescence of the single-stranded ODN-Ru, compared to that of the free complex, was first examined by SPC measurements (Table 3). If we compare the weight-average luminescence lifetimes (τ_{av}), it is noticed that the lifetime of the free complex $[Ru(TAP)_2(phen)]^{2+}$ (840 ns) becomes significantly longer when attached to the single ODN strand (ODN-Ru, 1200 ns). The excited-state complex is thus protected from the aqueous phase by the ODN, and therefore the efficiency of the nonradiative deactivation processes decreases. Actually, the luminescence decay exhibits a biexponential behavior, with lifetimes $\tau_1 = 887$ ns and $\tau_2 = 1598$ ns, which contribute more or less equally to the total decay and correspond to a lifetime similar to that of the free excited-state complex and a protected one, respectively.

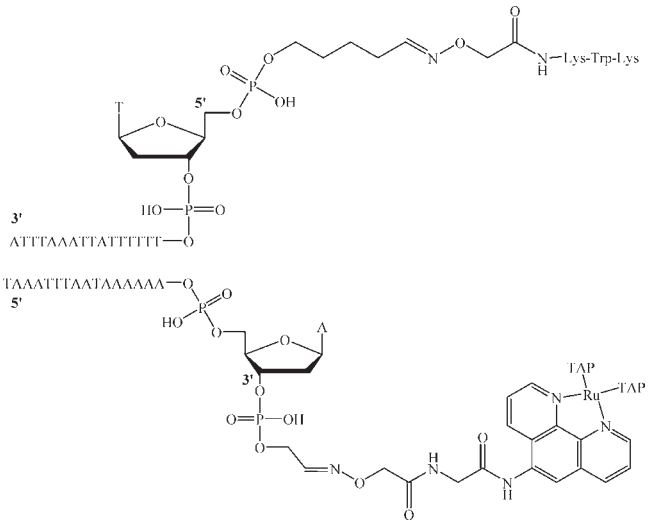

Figure 6. Structure of the ODN duplex derivatized by Lys-Trp-Lys at the 5′ position and $[Ru(TAP)_2(phen)]^{2+}$ at the 3′ position, i.e., Ru-ODN/ODN′-Lys-Trp-Lys.

Table 3. Luminescence Lifetimes of $[Ru(TAP)_2(phen)]^{2+}$Alone and Attached to the ODN in the Different Systems

structure	luminescence lifetimes (ns, A_i)	weight-average luminescence lifetime (ns)
$[Ru(TAP)_2(phen)]^{2+}$	τ_0 = 840 (100%)	
ODN-Ru	τ_1 = 887 (56%)	τ_{av} = 1200
	τ_2 = 1598 (44%)	
ODN-Ru/ODN′	τ_1 = 703 (33%)	τ_{av} = 1276
	τ_2 = 1558 (67%)	
ODN-Ru/ODN′-Lys-Trp-Lys	τ_1 = 710 (28%)	τ_{av} = 862
	τ_2 = 1561 (41%)	
	τ_3 = 76 (31%)	

The luminescence decays monitored at 650 nm have been analyzed according to a multiexponential function: $I_{em}(t) = \sum_i a_i \exp(-t/\tau_i)$ (i = 1, ...). In parentheses, the percentage of contribution of the different decay components to the initial emission is calculated according to $A_i = a_i/(\sum a_i)$. The weight-average luminescence lifetime is obtained according to $\tau_{av} = \sum a_j\tau_j/\sum a_i$.

Such a behavior observed previously[42] can probably be attributed to self-structuration of the single-stranded ODN-Ru. Indeed, in the duplex (ODN-Ru/ODN′), thus in a well-structured form, the emission lifetimes are similar, with, however, a more important contribution of the longer lifetime because of better protection. Finally, when the complementary strand contains the tethered tripeptide (ODN-Ru/ODN′-Lys-Trp-Lys), the luminescence decay has to be analyzed according to a triexponential function with a much shorter component (around 70 ns); the two other lifetimes are similar to those of the previous case and are due to nonprotected and protected excited-state complexes. Hence, we have to conclude that the shorter component should be attributed to the luminescence quenching induced by the PET from the Trp residue of ODN′-Lys-Trp-Lys to the attached excited-state complex $[Ru(TAP)_2(phen)]^{2+}$. The fact that, for the duplex ODN-Ru/ODN′-Lys-Trp-Lys, three types of excited-state geometries contribute more or less equally to the total decay suggests that the attached ruthenium complex is not completely embedded in only one microenvironment of the duplex. Thus, the linker of the complex allows it to move from one site of the duplex (close to the Trp, for example) to two other sites (nonprotected and protected by the ODN) in a time scale longer than the excited state. These movements should occur also just after the PET, leading, in part, to a solvated reduced complex and oxidized Trp, similarly to the production of these two intermediate species produced with the free complex and free tripeptide (see above). Therefore, we performed the same laser flash photolysis experiments as those above but with the system "ODN-Ru/ODN′-Lys-Trp-Lys" in the same time domain, with the view of observing the decay of the transients.

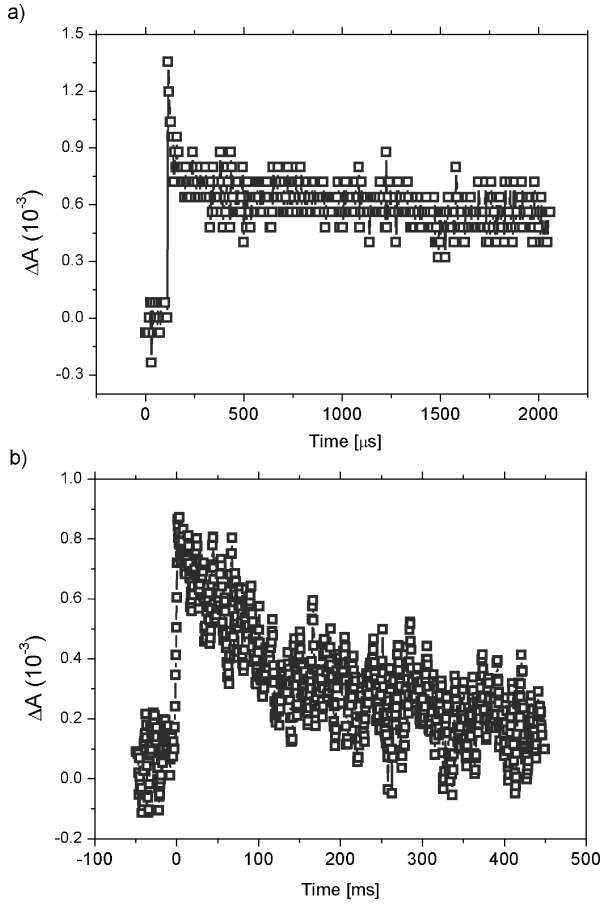

Figure 7. Transient absorption decays after the laser pulse for ODN-Ru/ODN′-Lys-Trp-Lys (10^{-6} M) in an aqueous argon-saturated solution recorded at 510 nm in two different time scales, 2 ms (a) and 500 ms (b). Excitation at 355 nm.

5. Laser Flash Photolysis of the Duplex Ru-ODN/ODN′-Lys-Trp-Lys. This duplex (Figure 6), for which the luminescence decay under pulsed illumination was analyzed in Table 3 (entry 4), was studied by laser flash photolysis. After pulsed laser excitation at 355 nm, the transient absorption of this derivatized duplex was measured at 510 nm. The decay of this positive signal due to the sum of the absorption of the reduced attached complex and oxidized attached Trp is shown in Figure 7a in the microsecond time domain. It is quite different from that of Figure 5 for the free species (not attached) in the same time scale. Indeed, the amplitude of the decay in Figure 5 is much more important ($^5/_6$ of the initial signal after the pulse) than that in Figure 7a ($^1/_2$ of the initial signal). Unfortunately, this weak decay, due to the small amount of duplex available, could not be analyzed kinetically and can probably be attributed to the

(42) Garcia-Fresnadillo, D.; Lentzen, O.; Ortmans, I.; Defrancq, E.; Kirsch-De Mesmaeker, A. *Dalton Trans.* **2005**, *5*, 852–856.

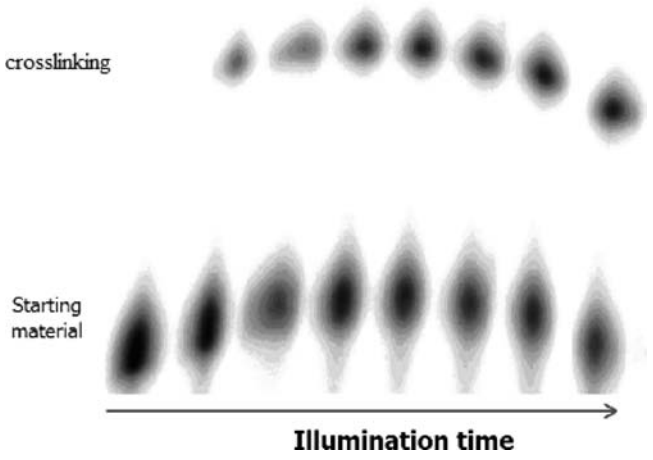

Figure 8. Denaturing gel electrophoresis for the photoreaction between ODN-Ru and ODN′-Lys-Trp-Lys (6 μM for each ODN) in aqueous buffer (10 mM Tris-HCl) with NaCl (50 mM), percent of photo-cross-linking for increasing illumination times with the He−Cd laser. From left to right: 0% (0 min), 15% (5 min), 22% (10 min), 28% (20 min), 33% (40 min), 39% (60 min), 39% (90 min), 41% (180 min).

BET from the attached reduced complex to the attached oxidized Trp. The remaining absorption after this decay in Figure 7a could be caused by the presence of a photoadduct. The latter could be formed from the ion pair and should indeed be more important with this duplex system than with the free species. However, this remaining absorption in the microsecond time window (Figure 7a) may not be attributed exclusively to the formation of a photoadduct. Indeed, as shown in Figure 7b, a part of this absorption decays in a much longer time scale, in the millisecond time domain. It might be due to some Trp radicals and a reduced complex that escape the BET, in agreement with our conclusions concerning deviation to the bimolecular-equimolecular process mentioned above for the free species. This "leak" reaction to the BET could be caused by a secondary reaction of the Trp radicals. Presently, it is difficult to attribute the origin of this slow 50−100 ms decay (Figure 7b).

6. Gel Electrophoresis of the Derivatized ODN Duplex. If the irreversible changes of the absorption spectra (figures in the SI) and permanent absorption in the laser flash photolysis experiments (as in Figure 7b) arise from the formation of the photoadduct of the ruthenium compound on the Trp residue, the illumination of the two complementary ODN strands with their anchored reactive partners should lead to photo-cross-linking. Such a process can be easily detected and quantified by polyacrylamide gel electrophoresis (PAGE) experiments under denaturing conditions by using radiolabeled ODNs for the ODN-Ru/ODN′-Lys-Trp-Lys duplex (the ODN-Ru is ^{32}P-labeled at the 5′ extremity). The proximity of the reacting species (complex and Trp) should favor indeed the covalent binding between the two partners. A denaturing PAGE analysis of this system for increasing illumination times is shown in Figure 8. The faster migrating spot originates from the labeled ODN-Ru, whereas the slower one is attributed to the formation of the photoadduct, thus giving rise to a photo-cross-linking between the two strands. Indeed, there is a significant difference in the electrophoretic mobility between a 17-mer strand (i.e., starting single-stranded ODN) and an irreversibly photo-cross-linked duplex corresponding to a 34-mer (Figure S7 in the SI).[43] After 40−60 min of illumination (Figure 8), the yield of photo-cross-linking reaches a plateau value of around 40%. There might be possible explanations for this limitation. The length and flexibility of the linker and the positive charge of the tripeptide is probably responsible for a lack of attraction between the electron donor and acceptor. Therefore, another reaction of the oxidized Trp residue might be at the origin of the plateau reached for the photo-cross-linking. Nevertheless, the PAGE results clearly demonstrate that the PET produces a photo-cross-linking and thus the formation of a covalent bond between the Trp residue and the complex (and not, for example, between Trp and A−T bases of the ODN).

(43) The difference in the mobility between a single-stranded 17-mer ODN and a 34-mer duplex irreversibly photo-cross-linked by a ruthenium complex has been shown in ref 35. In Figure S7 in the SI, we compare the migration of this reference system studied in the past with that of ODN-Ru/ODN′-Lys-Trp-Lys irreversibly photo-cross-linked.

Table 4. Denaturation Temperatures of the Different ODN Duplexes[a]

structure	temperature (°C)
ODN/ODN′	30
ODN-Ru/ODN′	32
ODN/ODN′- Lys-Trp-Lys	32
ODN-Ru/ODN′-Lys-Trp-Lys	33

[a] Errors about 1%.

7. Thermal Denaturation of the Derivatized ODN Duplex. A supplementary interesting experiment, which confirms the irreversible binding nature between the Ru-TAP complex and the amino acid, induced by illumination, is the investigation of the denaturation temperature of the duplex with the anchored species. We thus measured absorption of the ODNs at 260 nm for increasing temperatures. The denaturation temperature is determined as the inflection point of the sigmoid curve representing the fraction of denatured material (α) versus temperature. The parameter α was calculated for each temperature according to the equation

$$\varepsilon = (1-\alpha)\varepsilon_1 + \alpha\varepsilon_2$$

in which ε_1 and ε_2 are molar absorption coefficients of native and denatured materials, respectively. For the duplex ODN/ODN′, thus not derivatized, the separation into two single strands occurs at 30 °C (Table 4). For the duplexes ODN-Ru/ODN′ and ODN/ODN′-Lys-Trp-Lys as well as for the duplex ODN-Ru/ODN′-Lys-Trp-Lys, the differences of the denaturation temperatures are hardly higher than the experimental error (Table 4). However, an interesting observation is made from inspection of Figure 9 for the illuminated ODN-Ru/ODN′-Lys-Trp-Lys duplex. Two inflection points can be detected, the first one at 33 °C and the second one at 55 °C or higher, which indicates the presence of a stronger interaction between the two strands. The illuminated solution contains thus the intact duplex, which is denatured at about 33 °C, and the duplex that has photoreacted. The photo-induced covalent bond between both strands keeps them like the two arms of a tweezers. Such an effect of covalent binding in a duplex on its denaturation temperature has already been observed in the literature with cross-linked duplex DNA[44] and capped duplex DNA.[45]

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(45) Bannwarth, W.; Dorn, A.; Iaiza, P.; Pannekouke, X. *Helv. Chim. Acta* **1994**, *77*, 182–193.

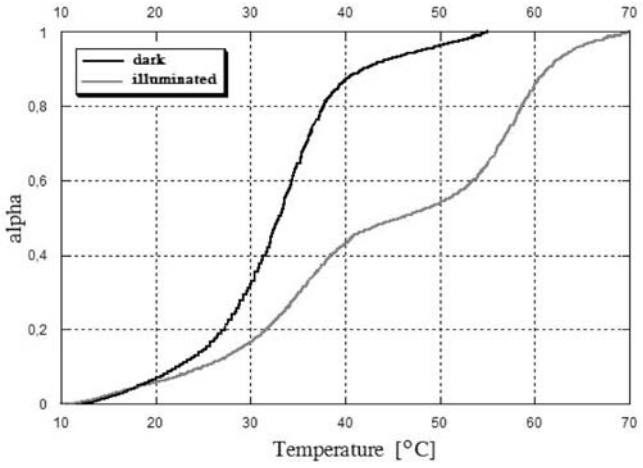

Figure 9. Denaturation curves of the ODN-Ru/ODN′-Lys-Trp-Lys duplex in the dark (black) and after an irradiation of 240 min with the He−Cd laser (gray).

Conclusion

With the three tryptophanyl derivatives, we have clearly demonstrated that the luminescence quenching of the two complexes originates from an electron transfer from the Trp residue to the excited-state complex. This PET is responsible for the formation of photoadducts or the occurrence of photo-cross-linkings depending on the system. It has been shown in the past, by measurements of the reduction potentials of the metallic complexes, that the ruthenium(II) compounds with at least two TAP ligands are strongly oxidizing in their ^{3}MLCT excited state.[11] Thus, with reduction potentials (vs SCE) of the excited-state complex of +1.06 V for $[Ru(TAP)_2(phen)]^{2+}$ and +1.32 V for $[Ru(TAP)_3]^{2+}$ (in MeCN) and with an oxidation potential of Trp of +0.78 V (vs SCE)[46,47] at pH 7, the PET process is thermodynamically possible with an exergonicity of −0.28 and −0.54 eV for $[Ru(TAP)_2(phen)]^{2+}$ and $[Ru(TAP)_3]^{2+}$, respectively. If we compare this PET process with the process previously studied with GMP, we can conclude that with GMP the PET becomes exergonic only if it is kinetically coupled to proton transfer,[15] whereas in the case of Trp as the electron donor, a kinetic coupling to proton transfer is not needed to make the reaction sufficiently exergonic. Therefore, the oxidized Trp probably gets deprotonated at pH 7 after and not during the PET process. The percentage of protonated reduced complex should be the same with GMP or Trp as the reducing agent at the same pH because it depends on the pK_a of the reduced Ru-TAP complex.

Laser flash photolysis results have shown that the electron-transfer process is followed by a BET from the Trp$^\bullet$ radical to the monoreduced complex (protonated and nonprotonated), which occurs in a microsecond time scale. We have found that this equimolecular-bimolecular process between these free species or inside the derivatized ODN duplex is accompanied by other reactions, for example, the formation of photoadducts. We have to mention, however, that, although photoadducts were also observed with GMP, their formation did not disturb the kinetics of the BET process[11] as in this case with Trp. Therefore, it might be possible that still other reactions due to the Trp radicals are in competition with the BET.

On the other hand, by gel electrophoresis and thermal denaturation experiments with illuminated derivatized ODN duplexes, we have clearly demonstrated the presence of a covalent bond between the two strands at the level of the Trp unit and the attached complex. This study thus opens the way to a wide range of applications with peptides and proteins.

Acknowledgment. The authors thank the Belgian Science Policy (Program IAP/PAI, P6/27) and FRFC-FNRS for financial support. J.G. is grateful to the FNRS for a Ph.D. grant, and M.R. is grateful to the IAP Program P6/27 for a postdoctoral grant. The authors also thank COST for their collaboration with the French research group who has gratefully given the team access to the Grenoble facilities of the Synthesis platform NanoBio program. The authors are grateful to P. Gerbaux (University of Mons) for his contribution to mass spectrometry.

Supporting Information Available: Characterization of ODN-5′Lys-Trp-Lys by mass spectrometry (Figure S1), absorption spectra of $[Ru(TAP)_3]^{2+}$ after illumination in the absence and presence of Trp (Figure S2), absorption spectra of $[Ru(TAP)_2(phen)]^{2+}$ after illumination in the absence and presence of Trp (Figure S3), transient absorption trace at 350 nm recorded for $[Ru(TAP)_2(phen)]^{2+}$ (Figure S4), absorption recovery at 460 nm for $[Ru(TAP)_2(phen)]^{2+}$ (Figure S5), transient absorption spectra of $[Ru(TAP)_2(phen)]^{2+}$ reduced by Trp and *N*-acetyl Trp (Figure S6), and the reference system for the attribution of the bands in gel electrophoresis (Figure S7). This material is available free of charge via the Internet at http://pubs.acs.org.

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