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Adduct formation by photo-induced electron transfer between photo-oxidising Ru(II) complexes and tryptophan†

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The ^{3}MLCT excited state of Ru(II) complexes with 1,4,5,8-tetraazaphenanthrene (TAP) ligands in the presence of tryptophan gives rise to an electron transfer process from the amino acid with subsequent formation of an adduct between the two partners, strongly influenced by oxygen.

DNA has been studied for more than half a century with a view to reaching different goals, more particularly in order to develop probes, reagents, or drugs targeting DNA. In this context, polyazaaromatic Ru(II) complexes have revealed themselves as very attractive photo-reagents. These metallic compounds are quite inert in the ground state but become reactive under visible irradiation.[1] Their photoreactivity can be tuned by the ligands. More particularly, our team has demonstrated that a photo-induced electron transfer (PET) takes place from the guanine base of mononucleotides or DNA to oxidizing polyazaaromatic complexes. This process is followed by the formation of a covalent bond between the electron donor and acceptor which leads to an adduct of the metallic complex on the nucleobase.[2] Such photo-adduct formations can be used to induce photocrosslinks between two oligonucleotide strands when one of the strands is chemically derivatized by the photoreactive complex and the complementary strand contains a guanine base in the vicinity of the tethered complex.[3] The appearance of these oligonucleotides photocrosslinks motivated us to investigate possible formation of other photo-adducts with amino acids in order to enlarge the possibilities of photocrosslinks between biomolecules[4] *via* metallic compounds.

Thus in this work, after a fast screening of the different amino acids with photo-oxidizing complexes ($Ru(TAP)_3^{2+}$ and $Ru(TAP)_2phen^{2+}$ (phen = 1,10-phenanthroline)) in order to test the existence of a possible photoreaction, we present the study of their photoreactivity with the amino acid tryptophan.

First, the behaviour of $Ru(TAP)_3^{2+}$, under irradiation in an aerated solution (10^{-5} M in a 10 mM TRIS buffer), is compared in the absence and in the presence of amino-acid (10^{-3} M). No photoreactivity is observed in the presence of most of them. With tryptophan (Trp) however, a strong hyperchromic effect appears with the irradiation time (Fig. 1) as compared to a bathochromic shift with appearance of an absorption band around 500 nm in the absence of Trp, attributed to a photodechelation process (loss of a ligand from $Ru(TAP)_3^{2+}$) (Fig. 1, *inset*). The photoreactivity with Trp is confirmed by the emission quenching of $Ru(TAP)_3^{2+}$ by increasing concentrations of Trp. Stern–Volmer plots give a quenching rate constant almost diffusion-limited: $k_q = 4.7 \times 10^9$ $M^{-1}{\cdot}s^{-1}$.

The nature of the photoreaction occurring between the complex and Trp is revealed by laser flash photolysis experiments. The $Ru(TAP)_3^{2+}$ is excited at 355 nm by a laser pulse in the presence of the amino-acid in aerated and deoxygenated solutions. The recorded transient species exhibits in both cases the differential absorption spectrum typical of the reduced complex (Fig. 2) which has been well characterised spectroscopically and kinetically.[1] This

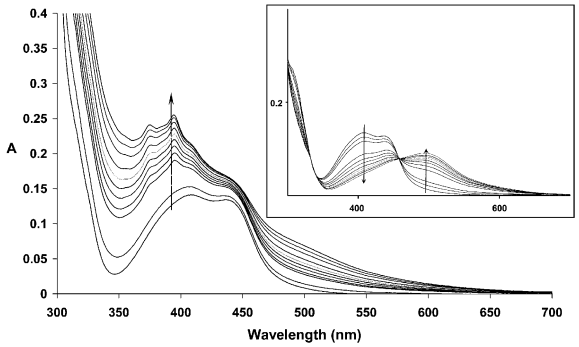

Fig. 1 Variation of the absorption spectra of $Ru(TAP)_3^{2+}$ (10^{-5} M) in presence of Trp (10^{-3} M) in 10 mM TRIS buffer (pH 7) with irradiation time (0 to 30 min). *Inset* variation of the absorption spectra of $Ru(TAP)_3^{2+}$ (10^{-5} M) alone in 10 mM TRIS buffer with irradiation time (0 to 90 min), same scale.

indicates the existence of a PET from the Trp to the excited $Ru(TAP)_3^{2+}$ (eqn. (1)), thermodynamically possible according to the redox potential values (E^+/E_{Trp} = +0.78 V *vs* SCE at pH 7, E^{2+*}/E^+_{Ru} = +1.32 V *vs* SCE).[1,5]

$$Ru(TAP)_3^{2+*} + Trp \rightarrow Ru(TAP)_3^{1+} + Trp^{\cdot+} \qquad (1)$$

Trp can thus behave as an electron donor like GMP *versus* the excited complex. On the other hand, the hyperchromic effect observed in the absorption spectra upon continuous irradiation with Trp could be due to the formation of an adduct induced by this PET. This hypothesis was checked by further continuous irradiation experiments of the Ru complex with Trp in deaerated solution. In such conditions however, the absorption spectra do not exhibit a hyperchromic effect as in Fig. 1, but a slight decrease of the MLCT band accompanied by an increase of absorption in the 500 nm region.† Although at first sight it would seem to correspond to a photodechelation, this is actually not the case since isosbestic points other than those characteristic of photodechelation are

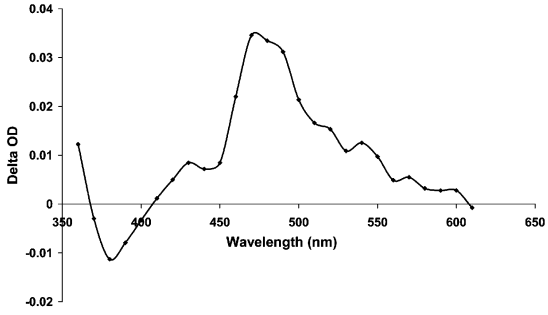

Fig. 2 Transient differential absorption spectrum of a solution containing $Ru(TAP)_3^{2+}$ (10^{-4} M) and Trp (10^{-2} M) illuminated with a pulsed laser and observed 10 μs after the pulse.

† Electronic supplementary information (ESI) available: supplementary figures 1–8. See http://www.rsc.org/suppdata/cc/b4/b411686k/

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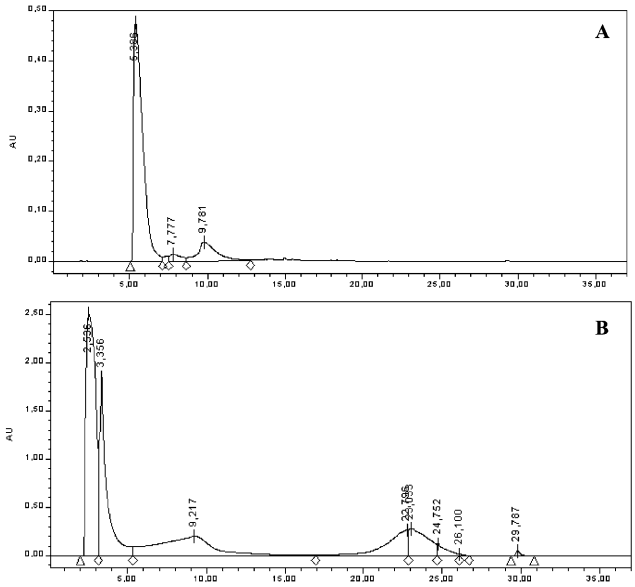

Fig. 3 HPLC chromatograms at 280 nm of A) an aerated solution containing $Ru(TAP)_3^{2+}$ (10^{-5} M) and Trp (10^{-3} M), B) the same solution illuminated for 2 h and after removal of the Trp remaining in solution.

observed. This result underlines the importance of the presence of oxygen in the photochemical reaction. Although O_2 can react with the tryptophanyl radical cation, the estimated rate constant is smaller than 10^6 $M^{-1}\cdot s^{-1}$, and no products have been identified.[6] This reaction seems therefore unlikely, but on the other hand, Ru(II) complexes in the excited state can deactivate by energy transfer to oxygen (eqn. (2)) and generate singlet oxygen, which reacts efficiently with Trp ($k = 7 \times 10^7$ $M^{-1}\cdot s^{-1}$) to form *N*-formylkynurenine (N-FK) and 3a-hydroxypyrrolidinoindole (HPI)[7]† (eqn. (3)).

$$Ru(TAP)_3^{2+*} + {}^3O_2 \rightarrow Ru(TAP)_3^{2+} + {}^1O_2 \quad (2)$$

$$^1O_2 + Trp \rightarrow N\text{-}FK + HPI \quad (3)$$

The possible formation of these products in the presence of O_2 was checked by HPLC equipped with a PDA detector. With a C18 column and using a H_2O–CF_3COOH/CH_3CN–CF_3COOH gradient, Trp and $Ru(TAP)_3^{2+}$ give retention times of $t_R = 5.4$ min and $t_R = 9.8$ min respectively (Fig. 3A). After irradiation for 2 hours in the presence of O_2 and removal of the remaining Trp, the injected mixture shows a different pattern of peaks (Fig. 3B). In addition to the non-transformed $Ru(TAP)_3^{2+}$, organic products ($\lambda_{max} = 295$ and 320 nm) with a $t_R = 2.5$ and 3.4 min respectively are detected as well as Ru(II) photoproducts ($\lambda_{max} = 410$ nm) with a $t_R = 23$ min which could correspond to a modified $Ru(TAP)_3^{2+}$.

The products of low t_R were identified by analysing an illuminated solution in a test experiment in which, instead of $Ru(TAP)_3^{2+}$, $Ru(bpy)_3^{2+}$ was used as O_2 photosensitizer. Indeed, this complex is not able to induce a PET because of its weak photo-oxidising power, but it is a strong $^1O_2$ generator. The chromatogram of the illuminated mixture reveals in addition to the peaks corresponding to $Ru(bpy)_3^{2+}$ and Trp peaks at $t_R = 2.5$ and 3.4 min.† An ESMS analysis of the solution shows that they correspond to a $m/z = 221.0$ and 237.0, in agreement with the mass of HPI and N-FK respectively. This confirms the existence of the energy transfer from excited $Ru(bpy)_3^{2+}$ (and by extension from $Ru(TAP)_3^{2+*}$) to molecular oxygen to produce singlet oxygen which reacts with Trp to form HPI and N-FK. Moreover, this experiment with $Ru(bpy)_3^{2+}$ does not reveal the presence of metallic photoproducts with a longer t_R. This indicates the need for an electron transfer to generate another process which would lead to the modified Ru(II) complex with a longer t_R. This latter photoproduct could originate from a PET (as evidenced by laser flash photolysis) either from Trp or from N-FK and/or HPI, to excited $Ru(TAP)_3^{2+}$. Deaerating the solution containing $Ru(TAP)_3^{2+}$ and Trp should prevent the production of singlet oxygen and therefore the formation of N-FK and HPI. Indeed, the HPLC analysis of such an illuminated solution does not reveal the presence of these products, *however* the peak at $t_R = 23$ min is still present, which proves that oxygen is not needed to produce this Ru photoproduct. The ESMS analysis of this photo-compound exhibits indeed an isotopic distribution characteristic of a Ru complex but the amount of which is too small to analyse precisely the mass.[8] Moreover, due to photodechelation of $Ru(TAP)_3^{2+}$, as outlined above, the presence of side-products complicates the analysis of the system. Therefore the study of $Ru(TAP)_3^{2+}$ has been replaced by that of $Ru(TAP)_2phen^{2+}$ which is photostable but slightly less oxidizing.[9] It turned out that $Ru(TAP)_2phen^{2+}$ exhibits the same behaviour as $Ru(TAP)_3^{2+}$ in the presence of Trp ($k_q = 3 \times 10^9$ $M^{-1}\cdot s^{-1}$) in aerated and deaerated solutions.† The metallic photoproduct has a mass which corresponds to $[Ru(TAP)_2phen + Trp - 2H)]^{2+}$ with an isotopic distribution characteristic of a Ru complex.† The MS/MS analysis provides more information with peaks at *m/z* values of 402 and 387 due to the loss of CO_2 and $NH_2CHCOOH$ respectively. This photo-product corresponds thus to an adduct of the complex to Trp with a chemical bond between a TAP ligand and the indole moiety of the Trp.

In conclusion, we have shown by laser flash photolysis, that the adduct formed between Trp and a photo-oxidising Ru(II) complex containing three or two TAP ligands originates from a photo-electron transfer. The hyperchromic effect observed by UV-Vis spectroscopy as a function of the irradiation time in the presence of oxygen is mainly due to the appearance of N-FK and HPI, while the photo-adduct is responsible for an absorption slightly more bathochromic than that of the starting complex. This photoreaction appears as very promising for a wide range of applications to peptides and proteins.

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8 The HPLC peak at $t_R = 23$ min gives a ESMS $m/z = 498.8$ (calc. = 499.0) which would correspond to $[(Ru(TAP)_3^{2+} + Trp - 2H) + TFA^- + Cl^- + H^+]^{1+}$, *i.e.* a photo-adduct between the complex and Trp. TFA^- = trifluoroacetate.
9 $E^{2+*}/E^+ = +1.06$ V *vs* SCE; according to this potential value, the photo-electron transfer between Trp and the excited complex is still possible.