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Communication

A Ru^{II}-TAP Complex, Photoreagent for Tryptophan-Containing Peptides: Structure of the Covalent Photoadduct

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We report the first structure determination of a covalent photoadduct between a Ru^{II}-tap complex and a tryptophan-containing peptide (AlaTrpAla) by mass spectrometry and NMR spectroscopy. Ru^{II}-tap complexes could thus be exploited as photodamaging agents of Trp-containing polypeptides or proteins.

There is an increasing interest for novel metal-based diagnostic or therapeutic agents in cellular biology over the last several years.[1] In this area, polyazaaromatic ruthenium(II) complexes are playing an important role as attractive photoprobes or photoreagents toward DNA and proteins.[2] Thus, the complexes containing at least two 1,4,5,8-tetraazaphenanthrene (tap) or 1,4,5,8,9,12-hexaazatriphenylene (hat) ligands are strong photooxidizing agents versus the guanine nucleobase. It has been shown that a photoinduced electron transfer (PET) from the guanine base to these particular complexes gives rise after several reaction steps to the formation of a covalent adduct.[3] Such photoreactions have been exploited to induce photo-cross-linkings between oligonucleotides.[4] Most interestingly, it has been found that a guanine-containing oligonucleotide probe linked to such a photoreactive complex (Ru-ODN) leads to its self-inhibition in the absence of its target and thus increases the specificity in gene-silencing applications.[5] Recently, a PET process has also been shown to occur between ruthenium(II) oxidizing complexes and tryptophan (Trp), leading to a covalent adduct as highlighted by mass spectrometry (MS) analyses.[6] This observation offers new interesting possibilities for photodamaging biomolecules. Unfortunately, up to now, the tentative determinations of the structure of those photoadducts have remained unsuccessful.

The present Communication reports elucidation of this structure in the case of $[Ru(tap)_3]^{2+}$ (**1**) and the Trp-containing tripeptide AlaTrpAla by MS and NMR spectroscopy.

The quenching of the excited state of **1** by AlaTrpAla, presumably by electron transfer, was confirmed by luminescence quenching experiments. The quenching rate constant measured at room temperature in a buffered solution (0.1 M Tris-HCl, pH 7), $k_q = 2.6 \times 10^9$ M^{-1} s^{-1}, was found to be close to the diffusion limit (Figure S1 in the Supporting Information). A solution (100 mL) of **1** (0.3 mM) and AlaTrpAla (1 mM) in water (0.6 mM Tris-HCl, pH 6) was illuminated for 2.5 h in a Pyrex photoreactor, under argon and continuous stirring, using a 250 W HPI-T metal halide lamp. Precipitation with NH_4PF_6, followed by purification by chromatography, was used to remove the excess of peptide and the unreacted ruthenium(II) complex. The isolated fraction was first analyzed by liquid chromatography (LC)−MS. The main peak observed at t_R = 4.6 min in the chromatogram features signals at m/z 496.1, 507.1, and 663.1 (Figure S2 in the Supporting Information). The ions detected at m/z 496.1 correspond to the expected covalent adduct $[Ru(tap)_3 + AlaTrpAla - 2H]^{2+}$ and the ions at m/z 507.1 to $[Ru(tap)_3 + AlaTrpAla - 2H - H^+ + Na^+]^{2+}$, whereas the singly charged ions at m/z 663.1 are due to a hitherto unidentified side product (the MSMS spectrum of

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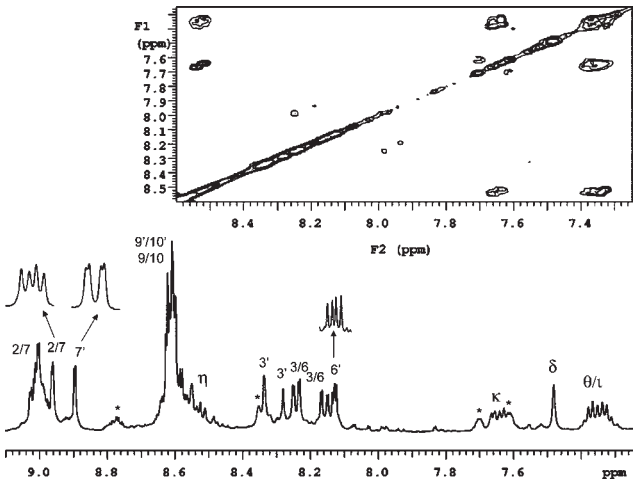

Figure 1. Regions of the 600 MHz ^{1}H and TOCSY NMR spectra of **2** in CD_3CN (see Figure 2 for signal labeling): (*) unidentified product (very low intensity signals are also observed between 10.1 and 9.1 ppm).

these ions shows the presence of a ruthenium atom and at least one tap ligand; Figure S3 in the Supporting Information). LC−MSMS analysis of the *m*/*z* 496.1 cations shows that various sets of peaks are present (Figure S4 in the Supporting Information). Among them, the signal at *m*/*z* 388.1 is structurally informative. Indeed, those doubly charged cations formally originate from a partial loss of the peptide backbone, i.e., minus the indole moiety. This observation indicates that the indole moiety of Trp is thus covalently linked to the complex.

After deuterium exchange, the sample was subjected to different NMR analyses in CD_3CN at 14.1 T (600 MHz for ^{1}H) and 298 K. The aromatic region of the 1D ^{1}H NMR spectrum shows many more resonances than the corresponding regions of the spectra recorded for $[Ru(tap)_3]^{2+}$ and AlaTrpAla (Figure 1). This is in agreement with the formation of a photoadduct because the symmetry of the starting complex is lost upon covalent binding to the peptide. It is worth pointing out that a racemic mixture of Λ-**1** and Δ-**1** was photoreacted with a L-amino acid AlaTrpAla peptide. As suggested by the resolution-enhanced regions of the ^{1}H NMR spectrum shown in Figure 1, it is likely that the sample contains a mixture of two diastereoisomers of the photoadduct.

The DOSY NMR spectrum reveals that the broad signals at 7.70 and 7.61 ppm belong to a minor compound (Figure S5 in the Supporting Information). This spectrum also provides further evidence for the existence of an adduct. Indeed, quasi identical diffusion coefficients were determined from all of the other signals of significant intensity that are observed between 9.1 and 7.3 ppm.

The dqf-COSY and TOCSY NMR spectra indicate that the ^{1}H signals observed at about 8.52, 7.64, 7.36, and 7.32 ppm form a four-spin system, which can be unambiguously assigned to the benzene ring of Trp (Figure 1). It can therefore be concluded that this part of the indole ring is not involved in the covalent bond in the adduct. ^{1}H−^{13}C HSQC and HMBC NMR spectra were used to confirm these assignments and reveal that the signal at 8.52 ppm corresponds to Trp-H_η (Figures 1 and 2). This proton is strongly downfield-shifted compared to the corresponding signal of AlaTrpAla (7.51 ppm in D_2O and 7.31 ppm in DMSO-d_6; Table S2 in the Supporting Information), which indicates that the chemical environment of this proton is considerably altered in **2**.

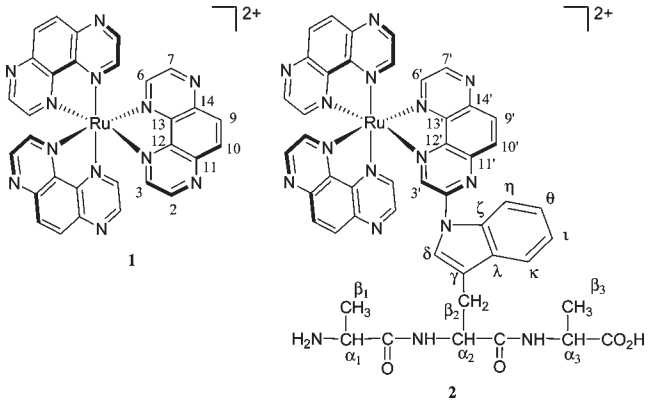

Figure 2. Structure and numbering of **1** and of the photoadduct with the tripeptide AlaTrpAla (**2**).

The HSQC and HMBC NMR spectra also indicate that the singlet signal at 7.48 ppm corresponds to Trp-H_δ. The observation of all of the aromatic ^{1}H signals of the indole ring, among which the strongly deshielded Trp-H_η, together with the structural information provided by MS, leads to the conclusion that the covalent bond between the ruthenium(II) complex and Trp involves the indole nitrogen atom. A ^{1}H NMR spectrum of **2** recorded in DMSO-d_6 confirms the absence of the NH indolic proton (Trp-H_ε), which is easily detected at 10.82 ppm with the unreacted peptide.

The ^{1}H and dqf-COSY NMR spectra of **2** also indicate various AX spin systems with a characteristic coupling constant of 2.8 Hz.[7] These doublet signals are observed between 9.1 and 8.1 ppm and correspond to tap-$H_{2,3}$ or tap-$H_{7,6}$ (tap-$H_{7',6'}$) pairs of vicinal protons. In agreement with the NMR spectra of **1**, the HSQC and HMBC NMR spectra of **2** show that the tap-$H_{2,7}$ (tap-$H_{2',7'}$) doublets are found between 9.1 and 8.8 ppm, while the tap-$H_{3,6}$ (tap-$H_{3',6'}$) doublets are between 8.4 and 8.1 ppm.[8] Interestingly, two singlet signals are detected at 8.34 and 8.28 ppm, i.e., in the tap-$H_{3,6}$ chemical shift region, and both of them are correlated in the HSQC NMR spectrum to ^{13}C at ∼143 ppm. These ^{13}C signals are high-field-shifted by more than 7 ppm compared to tap-$C_{3,6}$ of **1**, indicating structural alterations in the vicinity to these atoms. Furthermore, each of these singlet ^{1}H signals gives rise to an HMBC correlation with two quaternary aromatic ^{13}C, observed at 151.4 and 137.7 ppm ($C_{2'}$ and $C_{12'}$, respectively), while tap-$H_{3,6}$ of **1** gives rise to a single HMBC correlation with a quaternary aromatic ^{13}C (tap-$C_{12,13}$ at 142.8 ppm). These observations lead to the assignment of these singlet ^{1}H signals to tap-$H_{3'}$ of the two diastereomeric forms of the photoproduct. They indicate that the indole nitrogen atom of Trp is covalently bonded at position 2′, as shown in Figure 2. The HMBC correlation between Trp-H_δ and tap-$C_{2'}$ could not be observed. However, the ROESY NMR spectrum (Figure 3) provides further evidence for this structure. Indeed, a through-space dipolar interaction is observed between Trp-H_δ (singlet signal at 7.48 ppm) and tap-$H_{3'}$ (singlet signals at 8.28 and 8.34 ppm), confirming the proximity of these protons.

This ROESY correlation also affords useful information for the interpretation of the particular downfield shift

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(8) Signals of $H_{3,6}$ are also observed in ^{1}H and dqf-COSY NMR spectra at 8.55 and 8.60 ppm and have been assigned to the unidentified product observed in LC−MS.

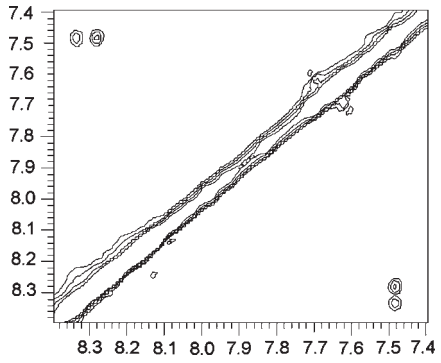

Figure 3. Region of the ROESY NMR spectrum of **2** in CD_3CN.

observed for Trp-H_η. Indeed, the proximity between Trp-H_δ and tap-$H_{3'}$ implies that Trp-H_η cannot be in the shielding cone of the other tap ligand but is probably in the deshielding region of the substituted tap (Figure 2). Trp-H_δ should also be in this deshielding region but, in contrast to Trp-H_η, it undergoes the shielding cone effect of the nearby tap. Moreover, a downfield shift of the indole protons is also in agreement with substitution of the indole (electron-rich heteroaromatic system) by a highly π-electron-deficient ligand such as the tap ligand.

The structure of **2** is also consistent with that of the photoadducts formed with the guanine nucleobase, which also involves the same position 2(7) of a tap or hat ligand,[3] as well as with recent photo-CIDNP (chemically induced dynamic nuclear polarization) experiments that reveal a high electron-spin density at these positions in monoreduced tap-containing ruthenium(II) complexes.[9]

In conclusion, MS and NMR spectroscopy were successfully used to unravel the structure of a new kind of covalent adduct, induced by a PET process between a Trp-containing peptide and **1**, which does not involve alteration of the complex coordination sphere. Elucidation of this structure constitutes a real milestone in the development of applications for Ru^{II}-tap complexes for photodamaging or photocross-linking Trp-containing polypeptides or proteins.

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Supporting Information Available: Experimental section, LC−MS results, peak attribution, and 2D NMR spectra (DOSY, dqf-COSY, TOCSY, HSQC, and HMBC), as well as chemical shift assignments for **1**, **2**, and AlaTrpAla. This material is available free of charge via the Internet at http://pubs.acs.org.

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