Communication

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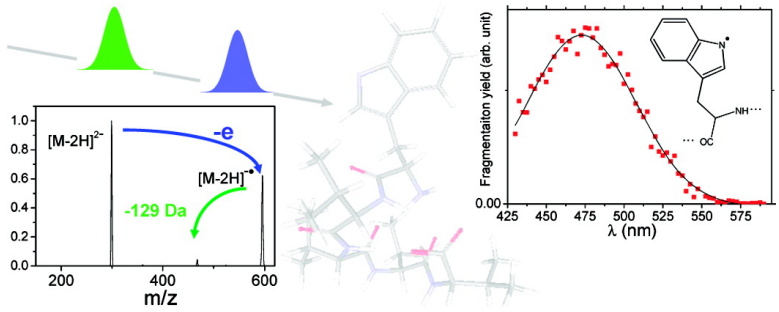

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Formation and Spectroscopy of a Tryptophan Radical Containing Peptide in the Gas Phase

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Phenoxyl and indolyl radicals are ubiquitous in biology. For example, indolyl derivatives are known to possess antioxidant properties as radical scavengers.[1] Tryptophan radicals participate in electron and radical transfers in proteins,[2−5] and they have been recognized in numerous enzymes as catalytically relevant redox agents.[4] Radical transfers occur either by electron transfer followed by proton release to create a neutral radical, or by H-atom transfer with the simultaneous transfer of electrons and protons. Distinguishing between neutral and cationic radical forms in a protein is crucial because the identity of the radical determines the details of its reactivity.[6] The combination of spin resonance spectroscopic techniques and density functional theory (DFT) theoretical results is currently the most advanced approach for elucidating the nature of radicals.[5,7,8] Optical properties have also undergone extensive investigation. For tryptophan, radical spectra of the relevant protonated and deprotonated forms in solution are obtained from pulse radiolysis experiments[9] and are usually used as reference spectra for characterizing tryptophan radicals in proteins.[2,10] However, it has been shown that electronic transitions in tryptophan radicals are highly environment-dependent (in particular for structurally defined environments).[8,11] Moreover, the difficulty of modeling the influence of noncovalent contacts on the spectra of these radicals may limit the interpretation of these experiments. By bringing a system to the gas phase, it is possible to eliminate nonexternal interactions and study well-defined systems. Gas-phase electronic spectroscopy measurements have succeeded in providing structural information on isolated and microhydrated tryptophan-containing molecules.[12,13] However, up to now these studies have been restricted to neutral and cationic species. The characterization of radical and charge transfers in the gas phase is still challenging and could provide the basis for building atomic models of functional molecules in action. In this Communication, we present the formation and optical spectrum of a long-lived neutral tryptophan radical ($Trp^{\bullet}$) containing peptides in the gas phase.

The experimental setup consists of an ion-trap mass spectrometer[13] coupled to a nanosecond pump−probe laser experiment. A Nd^{3+}:YAG laser (time duration 5 ns, fourth harmonic $\lambda = 266$ nm, repetition rate 20 Hz) was used to photogenerate the radical species. A UV−vis tunable OPO (time duration 7 ns) laser was used to fragment the radicals and record their optical spectrum. The pump and probe lasers were electronically synchronized with an adjustable delay. Before injection in the ion trap, the two beams were spatially combined and passed through three diaphragms and a mechanical shutter electronically synchronized with the mass spectrometer. A lens was used to focus the laser beams on the ion packet. To characterize the formation and then the optical spectra of radical species, MS^2 and MS^3 experiments were performed. In the MS^2 experiments, precursor ions were first isolated in the trap. After isolation, they were irradiated for one laser shot by one or both synchronized laser beams and the resulting mass spectrum was recorded. In the MS^3 experiments, the precursor parent ions were first selected and irradiated by the pump laser for five laser shots, then the photogenerated radical ions were isolated during the MS^3 stage (which typically takes a few dozen milliseconds) and, after isolation, again irradiated by the probe tunable OPO laser for three laser shots.

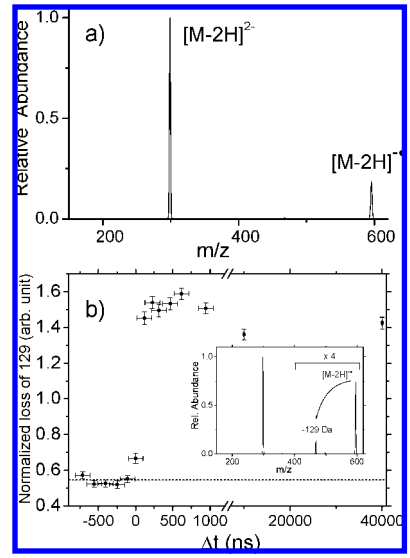

Figure 1. (a) MS^2 mass spectrum obtained after laser irradiation ($\lambda = 266$ nm) of the doubly deprotonated $[M - 2H]^{2-}$ WVVVV peptide precursor ions. (b) Normalized loss of 129 Da ($I_{[M-129]^-}/(I_{[M-129]^-} + I_{[M-2H]^{2-}} + I_{[M-2H]^{-\bullet}})$) measured after laser irradiation at 266 nm (pump) + 490 nm (probe) as a function of the pump−probe delay. The horizontal dashed line displays the loss of 129 Da observed with only the pump laser on ($\lambda = 266$ nm). Inset: MS^2 mass spectrum obtained after laser irradiation (266 + 490 nm, $\Delta t = 616$ ns) of the doubly deprotonated $[M - 2H]^{2-}$ WVVVV peptide precursor ions.

Tryptophan-based pentapeptide (WVVVV, W = tryptophan, V = valine) from Activotec was dissolved at a concentration of 200 μM in H_2O/CH_3CN (50/50, v/v). The pH was adjusted to 7.5 by adding KOH to the solution. Figure 1a shows the mass spectrum obtained following laser irradiation at 266 nm of the doubly deprotonated WVVVV peptide. The main fragment observed corresponds to the oxidized $[M - 2H]^{-\bullet}$ ions generated by electron detachment from the doubly deprotonated precursor ions. (The laser power dependencies of the fragmentation yields are given in the Supporting Information.) The C-terminal carboxyl group is a deprotonation site for the WVVVV peptide. Since the peptide has no acidic residue, the second deprotonation could occur on the peptide backbone or in the nitrogen of the indole ring. To investigate the latter hypothesis, we recorded the resonant detachment yield spectrum for $[M - 2H]^{2-}$ as a function of laser wavelength. The results are given in the Supporting Information. As recently discussed for tyrosine containing proteins,[14] this spectrum shows that the indole ring in tryptophan was deprotonated. Electron detachment from this deprotonated ring leads to the formation of an indolyl radical (see Scheme 1).

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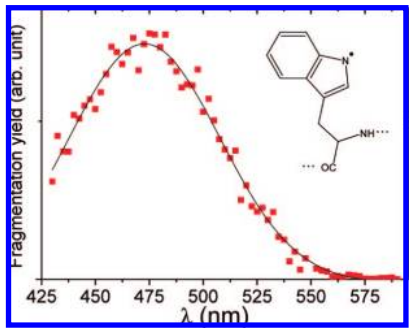
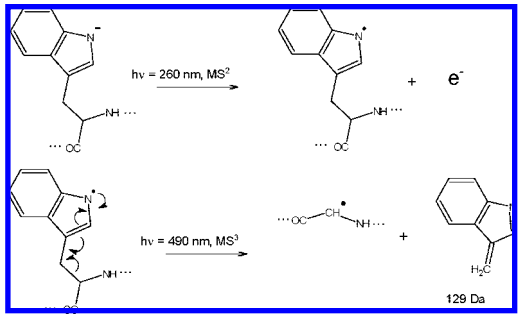


Figure 2. Experimental fragmentation yield of radical $[WVVVV\text{-}2H]^{-\bullet}$ pentapeptides.

Scheme 1. Photogeneration and Fragmentation of Trp• Radicals



The insert in Figure 1b displays the mass spectrum obtained after laser irradiation at 266 + 490 nm (Δt = 616 ns) of $[M - 2H]^{2-}$. A significant loss of 129 Da, caused by the probe laser at 490 nm was observed. The loss of 129 Da stemmed from the fragmentation of the $[M - 2H]^{-\bullet}$ and was due to the cleavage of the dehydrogenated tryptophan side chain (see scheme 1, the loss of 129 Da confirms the existence of an indolyl radical). To further confirm this, experiments were also performed with VVVVW peptides. Due to the presence of the negative charge at the carboxyl group of the C-terminal, the second deprotonation could not occur on the indole and no loss of 129 Da was observed.

In an initial set of spectroscopic experiments, the yield of 129 Da loss was recorded as a function of the delay between the pump and the probe lasers. The results are plotted in Figure 1b. For negative delays (visible light injected before UV light), no effect of the visible light was observed while for positive delays, a strong increase in the loss of 129 Da was observed. This was due to the absorption of the visible light by the radical produced by the first laser pulse. The dynamics observed in this figure shows that the loss of electrons leading to the Trp• radical is fast (less than 100 ns) and that the photogenerated radical is stable for long periods (at least 40 μs). In fact, the MS^3 experiments showed that radicals can be isolated for several tens of milliseconds.

In the second set of spectroscopic experiments, the long-lived radical ions were isolated and, after isolation, irradiated with the tunable visible laser (MS^3 experiments). The fragmentation spectrum of the radical was systematically recorded as a function of the second laser wavelength in the 430−590 nm range. The resulting experimental photofragmentation spectrum is shown in Figure 2. It displays a single absorption band centered at 473 nm with full width at half-maximum fwhm = 82 nm. The calculated absorption spectrum for the neutral indolyl radical displays a transition at 474.4 nm (see Figure S3), which is in good agreement with the experimental photodissociation spectrum. This band corresponds to a transition from an inner π orbital to the single occupied π HOMO.[15] The good agreement between the experimental photofragmentation spectrum and the calculated absorption spectrum indicates that the rest of the peptide has a weak influence on the optical properties of the radical. The present optical spectrum of Trp• in the gas phase is blue-shifted by ~25 nm compared to the spectra obtained for Trp• and indolyl radicals in water solution.[16] Such shifts could be explained by the relative change in the negative charge density on the nitrogen radical on hydration as observed for phenols and octupolar molecules.[17] Furthermore, the absorption spectra of photogenerated tryptophan radicals in a structurally defined protein environment exhibits a double-band pattern with maxima at 512 and 536 nm,[8] while a single band is observed here. This confirms the strong sensitivity of Trp• transitions to its local environment.

In conclusion, we have presented the generation of long-lived Trp• radical containing peptides in the gas phase by using electron photodetachment from doubly deprotonated peptides. The photofragmentation spectrum of the neutral tryptophan radical was recorded and constitutes a new benchmark for calculations and optical measurements. These results also open the path for studying the dynamics of radical initiation and electron and radical transfers in proteins in the gas phase.

Supporting Information Available: Electron photodetachment yield measured as a function of laser wavelength and power for $[M - 2H]^{2-}$ WVVVV ions; excited spectra calculated for neutral, deprotonated tryptophan, and neutral indolyl radical. This material is available free of charge via the Internet at http://pubs.acs.org.

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