Protein Radicals

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Spectroscopic Signatures of Peptides Containing Tryptophan Radical Cations**

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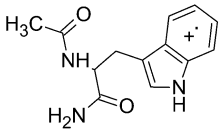
Radical centers present in peptides and proteins attract considerable interest for two major reasons: these centers play critical roles as intermediates in a variety of biological electron-transfer processes,[1] and they present a wealth of specific fragmentation pathways that are relevant to mass-spectrometry-based peptide sequencing in proteomics.[2] Charged or neutral radical centers may be generated on aromatic, sulfur-containing, and glycine residues by either radiative damage or oxidation involving a metal cofactor. Tryptophan radicals or, in particular, radical cations participate in electron transfer in cytochrome c peroxydase, DNA photolyase, galactose oxydase, and ribonucleotide reductase.[1c,3] Tryptophan also acts as a radical scavenger to protect proteins from oxidative damage.[4] The oxidation reaction of tryptophan is complex because the electron loss is often coupled with a proton transfer step.[5] The structure of the radical determines its reactivity and the resulting charge transfer. Reference spectroscopic data for the identification of tryptophan neutral radicals and tryptophan radical cations in proteins is thus of major importance.

The formation, transfer, and decay of transient radical species in proteins can be addressed in the condensed phase by a variety of spectroscopic techniques, including absorption, electron paramagnetic resonance, and vibrational spectroscopy.[6] However, direct observations of these species are hindered by their high and environment-sensitive reactivity. In this context, it has proven fruitful to adopt a gas-phase approach, in which stable radicals can be isolated and structural details can be obtained in the absence of any environmental effects. Stable radical ions are conveniently formed in vacuo by electron transfer, which occurs during collision-induced dissociation of ternary complexes composed of copper(II) ions, a ligand, and the amino acid or peptide of interest;[7] the radicals can then be manipulated with mass spectrometric techniques. IR spectroscopy has recently been used to demonstrate the structure and stability of the captodative form of histidine radical cations and the distonic form of cysteine radical cations.[8]

Herein, we address the localization of the radical in the complex environment of an oligopeptide, in which competitive radical centers may coexist.[9] The optical signature of a neutral radical tryptophan-containing peptide has already been reported.[10] We now present the optical signature associated with vibrational diagnostics of the tryptophan radical cation in a gas-phase peptide.

Capped tryptophan $AcTrpNH_2$ (Scheme 1) was used as a model for the smallest tryptophan-containing peptide. In Figure 1, the experimental vibrational and optical spectra are compared with the corresponding predicted spectra for the most stable structure (obtained by using density functional theory (DFT)). The vibrational spectrum of the radical cation was obtained in the spectral region of the NH stretching modes and shows a strong signal at 3460 cm^{-1} with two smaller features at 3435 cm^{-1} and around 3545 cm^{-1} (Figure 1 a). In contrast, the most intense feature of the protonated species (dotted line) appears at 3505 cm^{-1}. In both cases, the strong band can be readily attrib-



Scheme 1. Canonical π-radical form of tryptophan embedded in the $AcTrpNH_2^{\bullet+}$ radical cation.

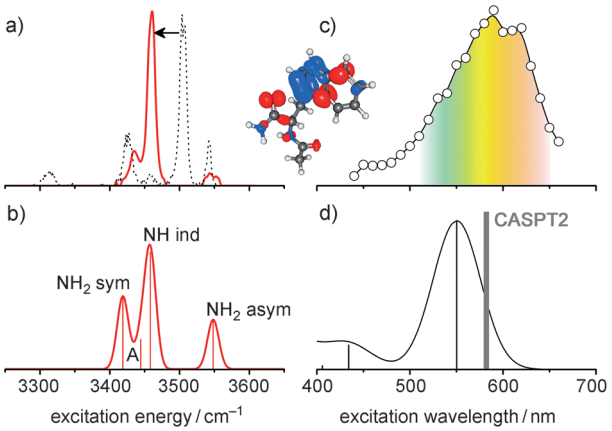


Figure 1. a) IRMPD spectra of $AcTrpNH_2^{\bullet+}$ (solid line) and $AcTrpNH_2{\cdot}H^+$ (dotted line). The arrow shows the red-shift of the indole NH signal. b) Theoretical vibrational spectrum of $AcTrpNH_2^{\bullet+}$. c) Visible photofragmentation spectrum of $AcTrpNH_2^{\bullet+}$. d) Theoretical TD-DFT spectrum of $AcTrpNH_2^{\bullet+}$ (thin bars and convolution) and CASPT2 calculated position for the leading transition (bold gray bar). Inset: differential electronic density of the transition (blue: increased density, red: decreased density). A = amide NH, sym = symmetric stretching mode, asym = asymmetric stretching mode.

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uted to the indole NH vibration and is diagnostic of whether the radical cation or the molecular indole are present, in agreement with previous observations on the indole cation and protonated tryptophan.[11] The canonical π form of the radical cation (shown in Scheme 1), in which the indole is not deprotonated and both the unpaired electron and the charge are formally on the indole group,[12] was shown to be particularly stable,[13] and was thus considered for our conformational search. The most stable structure adopts an extended backbone with torsion of the C_α–C bond, thus allowing a favorable interaction between the terminal C=O group and the radical indole. The corresponding vibrational spectrum shown in Figure 1b shows four signals that are in excellent agreement (after convolution) with the experimental pattern. Analysis of the four modes allows the following assignment: the asymmetric stretching mode of the NH_2 group at 3545 cm^{-1} (calcd: 3548 cm^{-1}), the stretching mode of the indole NH group at 3460 cm^{-1} (calcd: 3458 cm^{-1}) that masks the unresolved amide NH stretch, and the symmetric stretching mode of the NH_2 group at 3435 cm^{-1} (calcd: 3419 cm^{-1}). Two alternative conformations of the backbone (γ turn and β sheet like) found at higher energy are eliminated by their characteristic NH_2 and amide patterns (see Figure S1 in the Supporting Information). Remarkably, the spectra of all conformers show the indole NH signal at the same position, which thus appears to be a distinctive signature of the tryptophan radical cation and is essentially unaffected by the backbone conformation. The optical spectrum of the radical ion shows a broad and asymmetric absorption centered at 590 nm (Figure 1c). This wavelength constitutes a red-shift of more than 300 nm compared to molecular tryptophan (see, for example, Ref. [14] and the optical spectrum of protonated $AcTrpNH_2$ shown in Figure S2), and a red-shift of more than 100 nm compared to the neutral radical tryptophan reported previously.[10] Cam-B3LYP calculations predict a leading electronic transition at 550 nm for the low-energy conformer (Figure 1d), and a range from 550 to 600 nm for the higher-energy conformers (see Figure S1). This position was refined with the CASPT2 method (gray bar in Figure 1d). The prediction at 582 nm is in excellent agreement with the experimental measurements. Our calculations are consistent with previous results on indole radical containing molecules.[15] The optical spectrum shown in Figure 1 is also consistent with that of the tryptophan radical cation, both in solution[16] and embedded in proteins.[6a]

A larger peptide sequence was investigated, namely $AcGly_3TrpNH_2^{\bullet+}$; the IR spectra of $AcGly_3TrpNH_2$ in the radical cation and the protonated forms are presented in Figure 2a. The indole NH mode of the molecular form is apparent at 3510 cm^{-1} in the spectrum of the protonated ion, whereas it is clearly absent from this region in the spectrum of the radical species. A broader feature appears around 3480 cm^{-1} in both spectra, and is interpreted as an unresolved combination of the four amide NH peaks. The intense signal at 3440 cm^{-1}, which is visible only in the spectrum of the radical ion, is tentatively interpreted to correspond to the red-shifted indole NH signal. The optical spectrum of the radical ion is shown in Figure 2b, and displays a broad band with a maximum at 560 nm. Again, this result offers a consistent

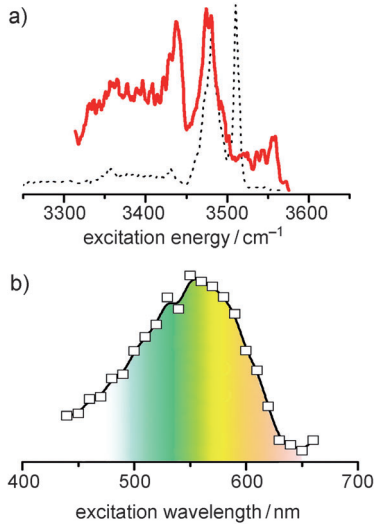

Figure 2. a) IRMPD spectra of $AcGly_3TrpNH_2^{\bullet+}$ (solid line) and $AcGly_3TrpNH_3^{+}$ (dotted line). b) Visible photofragmentation spectrum of $AcGly_3TrpNH_2^{\bullet+}$.

diagnostic of a peptide containing a tryptophan π-radical cation. Despite the 30 nm blue-shift observed with respect to $AcTrpNH_2^+$, the optical signature is incompatible with a neutral radical or molecular indole. The optical signature in the yellow region thus offers clear evidence for the presence of a tryptophan π-radical cation in the peptide, and is supported by the absence of the indole NH signal around 3500 cm^{-1}.

The absorption maximum at 560 nm is intermediate between the isolated $Trp^{\bullet}$ cation (590 nm) and the isolated neutral $Trp^{\bullet}$ radical (473 nm). Intramolecular interactions and solvent exposition modulate the exact nature of the radical in proteins,[6a,e,f] and thus the optical absorption, which consequently ranges between these two extremes. Here, intramolecular interactions account for the blue-shifted absorption of the larger peptide compared to that shown in Figure 1c.

We have reported the optical and vibrational spectra of the canonical π-cation form of the tryptophan residue embedded in small peptides. This work has shown that distinct spectroscopic signatures can be used to distinguish the radical cation from the molecular form. We demonstrated that UV/Vis photofragmentation spectroscopy can be used to distinguish between the nonradical, the neutral radical, and the π-radical cation forms of tryptophan within gas-phase oligopeptides. The optical diagnostic offers a reference signature for all three pure forms of tryptophan, that is, nonradical, neutral radical, and π-radical cation, with absorption maxima centered around 275 nm, 473 nm, and 590 nm, respectively. This result offers a benchmark spectrum for evaluating theoretical data and for monitoring the tryptophan radical activity in proteins.

Experimental Section

The solutions were prepared in a 1:1 water/methanol solution by mixing the peptide of interest, terpyridine, and copper(II) perchlo-

rate. Ternary complexes $[Cu^{II}(tpy)(M)]^{\bullet 2+}$ ($M = AcTrpNH_2$ and $AcGly_3TrpNH_2$) were formed in an electrospray source and isolated by mass spectrometry. The radical cations $M^{\bullet +}$ were obtained by fragmentation of the ternary complex and subsequently stored in an ion trap for further spectroscopic investigations. Electronic spectra were obtained in the 400–700 nm range by means of one-photon photofragmentation in a linear ion trap (Thermo Finnigan) coupled with a Nd:YAG pumped tunable UV/Vis OPO laser system (Continuum).[17] Vibrational spectra were obtained in the 3200–3600 cm^{-1} region by infrared multiple photon dissociation in a Fourier Transform Ion Cyclotron Resonance Spectrometer (Bruker 7T FT-ICR) coupled with a Nd:YAG pumped tunable IR OPO/OPA laser system (LaserVision).[18] In both cases, the intensity of parent ion and fragments ions after laser irradiation are monitored as a function of the excitation wavelength, and the photofragmentation yield is calculated for each wavelength with Equation (1):

$$\log((I_{\text{parent}} + \sum I_{\text{fragments}})/I_{\text{parent}})/\text{laser fluence} \quad (1)$$

A molecular dynamics conformational search was performed on $AcTrpNH^{\bullet +}$ with PM6-DH+ potential at a temperature of 2000 K and gave 100 conformers. These conformations were further optimized by density functional theory (DFT) with the B3LYP method and the 6–31+G* basis and the aug-cc-pvdz basis sets. The frequencies of the nine low energy conformers were calculated at the B3LYP/aug-cc-pvdz level of theory. A scaling factor of 0.959 was applied. The electronic spectra were predicted by time-dependent density functional theory (TD-DFT) with CAM-B3LYP/aug-cc-pvdz. The position of the leading transition in the red region of the visible range was refined with the CASPT2 method. The Gaussian 09 suite was used for DFT calculations,[19a] MOLPRO for CASPT2 calculations,[19b] and gabedit for data analysis.[20]

See the Supporting information for further details and references on the experimental and theoretical methods.

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