



RESEARCH ARTICLE

Formation and Fragmentation of Radical Peptide Anions: Insights from Vacuum Ultra Violet Spectroscopy

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Abstract

We have studied the photodissociation of gas-phase deprotonated caerulein anions by vacuum ultraviolet (VUV) photons in the 4.5 to 20 eV range, as provided by the DESIRS beamline at the synchrotron radiation facility SOLEIL (France). Caerulein is a sulphated peptide with three aromatic residues and nine amide bonds. Electron loss is found to be the major relaxation channel at every photon energy. However, an increase in the fragmentation efficiency (neutral losses and peptide backbone cleavages) as a function of the energy is also observed. The oxidized ions, generated by electron photodetachment were further isolated and activated by collision (CID) in a MS³ scheme. The branching ratios of the different fragments observed by CID as a function of the initial VUV photon energy are found to be independent of the initial photon energy. Thus, there is no memory effect of the initial excitation energy on the fragmentation channels of the oxidized species on the time scale of our tandem MS experiment. We also report photofragment yields as a function of photon energy for doubly deprotonated caerulein ions, for both closed-shell ($[M - 2H]^{2-}$) non-radical ions and open-shell ($[M - 3H]^{2-*$) radical ions. These latter ions are generated by electron photodetachment from $[M - 3H]^{3-}$ precursor ions. The detachment yield increases monotonically with the energy with the appearance of several absorption bands. Spectra for radical and non-radical ions are quite similar in terms of observed bands; however, the VUV fragmentation yield is enhanced by the presence of a radical in caerulein peptides.

Key words: Radicals, Photodissociation, VUV Spectroscopy, Damage, Gas Phase Peptides, Proteins

Introduction

Optical properties of proteins and radical proteins and their relaxation pathways in the VUV range is of

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fundamental interest to understand the response of biomolecules to radiation and oxidative stresses [1, 2], and may also have important applications in analytical sciences [3]. In particular, a better understanding of the different mechanisms involved between the initial excitation event and the experimentally observed fragmentation might allow the development of new strategies for peptide analysis and protein identification.

In UV photodissociation (UVPD, 200 nm < λ < 400 nm) and vacuum UV photodissociation (VUVPD, λ < 200 nm),

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the photon induces an electronic excitation of the peptide. After excitation, direct dissociation in excited states competes with internal conversion to the electronic ground state and with radiative de-excitation. In particular, in UVPD, specific fragmentation channels are detected, namely formation of radical cations following a hydrogen atom loss and C_{α} - C_{β} bond cleavage fragments [4, 5]. The above mentioned UV excitations rely on the existence of aromatic amino acids in the peptide sequence. Another approach, which can be generalized to any peptide whatever its sequence, is to use higher photon energy (in the VUV range) to excite not only the aromatic amino acids but also the peptide backbone amides (below 200 nm) [6]. In this aim, ArF (193 nm) and F₂ (157 nm) excimer lasers, which provide high photon fluences, short pulses duration, and high repetition rates, have been coupled to mass spectrometers [3]. Both 193 (6.4 eV) and 157 nm (7.9 eV) VUVPD methods are charge states dependent. That is, both yield mostly C_{α} -C bond cleavage for singly protonated species, but both generate very abundant b/y-type ions for multiply-charged precursors; 157 nm generally produces higher abundance x-ions compared with 193 nm, but both methods yield similar a-, v-, and w- products [7–10]. What is going on at much higher energy is an unexplored field.

Most of photodissociation experiments were first performed on peptide cations, while the interest in negative ions is growing due to the need of techniques suitable for acidic proteins, nucleic acids, or acidic sugars. This led to the development of negative electron transfer dissociation (NETD) [11–15], electron detachment dissociation (EDD) [16–18], and electron photodetachment dissociation (EPD) [19–22]. For peptide and protein polyanions, electron photodetachment is the main relaxation channel observed following UV excitation [23, 24]. For peptides that contain natural aromatic amino-acids (i.e., tryptophan, tyrosine and, in a lesser extent, phenylalanine), absorption at ~260 nm is mainly due to a resonant electronic excitation (π - π^* transition) [24, 25], followed by electronic reorganization and electron emission. This results in the production of radical species with low excess internal energy. The oxidized species can be isolated and further activated by collisions (activated-EPD) [19, 21]. In the case of peptides, the subsequent CID of the isolated radical anions results in intense backbone cleavages. Activated-EPD fragmentation patterns are characterized, for small peptides, by dominant backbone cleavage of C_{α} -C bonds giving *a* and *x*-type product ions [19]. For larger peptides, fragment ions originating from N- C_{α} and C_{α} -C backbone cleavages are also observed [21]. VUV excitation ($\lambda < 200$ nm) may open new pathways for electron loss and/or fast dissociation products [8]. The excitation of inner-valence electrons is possible and has still to be explored on fundamental and analytical points of view. Furthermore, as the photon energy increases, a large excess energy can be deposited in the oxidized product leading to the formation of hot radical ions that may relax by a subsequent fragmentation or second

electron emission without further excitation, which is not observed at low photon energy ($h\nu \sim 4.6$ eV). In particular, very recently the group of Brodbelt has implemented 193-nm ultraviolet photodissociation (UVPD) to sequence singly and multiply charged peptide anions. Upon dissociation by this method, *a*-*x*-type, followed by d and w side-chain loss ions, were the most prolific and abundant sequence ions, often yielding 100% sequence coverage [26].

In this paper, we aim at rationalizing the optical absorption and the relaxation processes that occur in peptide as well as in radical peptide anions following absorption of photons in the 4.5 to 20 eV VUV range, using the synchrotron radiation (SR). The present study focuses on caerulein peptide anions, which have been studied with a large array of dissociation techniques [16, 27–29]. Caerulein (pEQDY(SO₃H)TGWMDF-NH₂) is sulphated at the Tyr₄ residue. Additionally, it has one tryptophan and one phenylalanine aromatic residue as well as nine amide bonds. Caerulein is thus a model peptide for VUV photodissociation experiments since it possesses an extremely labile O-sulfation group and numerous aromatic amino-acids and amide bonds. In particular, we aim at studying the influence of the initial electronic excitation on electron detachment and peptide fragmentation patterns. We also question the influence of the initial excitation on the reactivity of oxidized product observed after electron photodetachment. High-energy activation of radical peptides following electron detachment is a totally unexplored field that may lead to new fragmentation patterns. In the last part of this article, we present the VUV spectroscopy of caerulein radical peptides obtained by a two-color experiment using a fixed-wavelength UV laser to photogenerate the radicals and the synchrotron radiation to record their VUV optical spectra.

Materials and Methods

Materials

Caerulein, melittin, and bovine insulin were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). Peptides were dissolved in 50/50 water/acetonitrile (vol/vol) at a concentration of 12 μ M and directly electrosprayed at a flow rate of 5 μ L/min for subsequent analysis in a negative-ion mode.

Mass Spectrometry and VUV Electron Photodetachment Dissociation

The experimental setup is based upon a linear ion-trap mass spectrometer (LTQ XL, Thermo Electron, San Jose, CA, USA) coupled to the DESIRS beamline [30] of the SOLEIL synchrotron radiation facility (France) to perform action spectroscopy in the VUV range (see Figure 1). VUVPD and action spectroscopy experiments were performed by illuminating trapped ions with the synchrotron radiation directly injected on the axis of the linear ion trap [31]. A

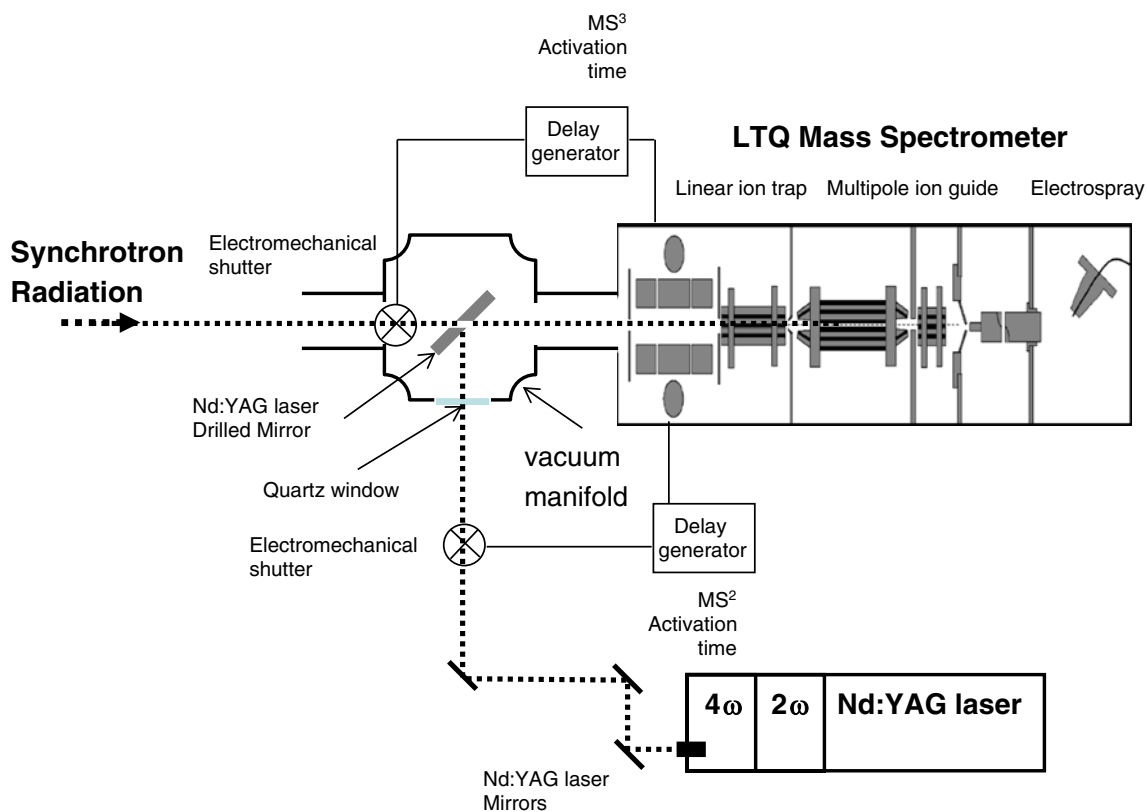


Figure 1. Experimental setup based upon a linear ion-trap mass spectrometer coupled to a UV laser and a VUV synchrotron source radiation beamline within a two-color scheme

differentially-pumped transfer line allows the coupling of the ion trap instrument to the ultra-high vacuum of the beamline. An MgF₂ windows (below 9 eV) and an Ar-filled gas filter (8–16 eV) [32] were used in order to suppress the high harmonics of the undulator that would be transmitted by the monochromator's grating, fixed at the first order, leading to a 12 meV bandwidth (at 10 eV) harmonic-free radiation that was sent onto the sample. In complement to VUV action spectroscopy, action spectroscopy between 4 and 5.6 eV was also performed using a quadrupole linear ion trap (LTQ; Thermo Fisher Scientific, San Jose, USA) coupled to a UV-Vis tunable laser OPO as described previously [21, 33]. CID experiments were performed with the LTQ apparatus.

To assess the reactivity and optical properties of radical peptide anions, we also combined a laser (Nd³⁺:YAG laser, time duration 5 ns, fourth harmonic $\lambda=266$ nm, repetition rate 20 Hz, 20 mJ/pulse), and the synchrotron source within a two-color scheme, as displayed in Figure 1. The two photon beams were spatially combined via a drilled-coated mirror located ~30 cm upstream of the ion trap entrance. The UV laser light was reflected at 90° by the coated mirror and injected collinearly to the axis of the ion trap. The SR beam was injected along the same ion trap axis passing through a 2 mm diameter hole drilled at the center of the coated mirror.

For VUV photodissociation experiments, the precursor parent ions were selected and irradiated by the SR for 400 ms. To characterize the reactivity and optical spectra of

radical species, we also report on activated-EPD experiments (MS³ experiments). For collisional activation of the radical species, the precursor parent ions were first selected (MS), irradiated by the UV laser with eight laser shots or by the SR, then the photogenerated radical ions were isolated during the MS² stage (which typically takes a few tens of milliseconds) and, after isolation, activated by collision. For VUV irradiation of oxidized product, the collisional activation was replaced by SR illumination. Two electromechanical shutters, electronically synchronized with the mass spectrometer, were placed along the laser and SR beams and allowed to inject the laser light and the SR beam on a given time sequence.

Results and Discussion

VUV Electron Detachment on Polypeptide Ions

Several peptides anions were subjected to VUV irradiation at 16 eV. Figure 2 displays mass spectra obtained following 400 ms of VUV irradiation at 16 eV for caerulein, melittin and insulin (sequence given in Supporting Information) polypeptide anions. For the three polypeptides, the main photon-induced process appears to be the production of the oxidized ions generated by electron photodetachment from the precursor ion. The electron photodetachment efficiency increases with the size of the polypeptide. This has to be

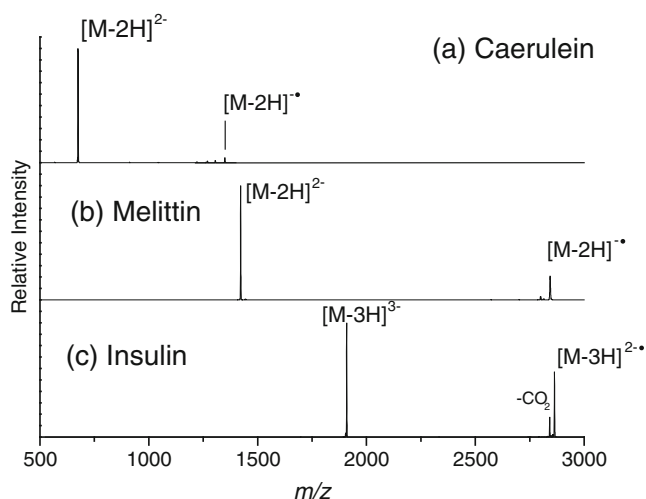


Figure 2. Mass spectra obtained after VUV irradiation at 16 eV of the doubly deprotonated caerulein (pEQDY(SO₃H)TGWMDF-NH₂) **(a)**, melittin (GIGAVLKVLTTGLPALIS-WIKRKRQQ-NH₂) **(b)**, and triply deprotonated insulin **(c)** polypeptides. The irradiation time was 400 ms

correlated to the increase in the density of states in the VUV range, due to the increase in the number of atoms in the system. In the following, we focus on the caerulein peptide. The mass spectra obtained following VUV irradiation at 6 and 19.8 eV of the doubly deprotonated caerulein peptide are shown in Figure 3a and b. The main photon-induced process appears to be the production of $[M-2H]^{\bullet}$ ions (m/z 1349) generated by electron photodetachment from the $[M-2H]^{2-}$ precursor ion. This fragmentation pattern is totally different from what is observed after collisional excitation of the close-shell ion (i.e., $[M-2H]^{2-}$), where the spectrum

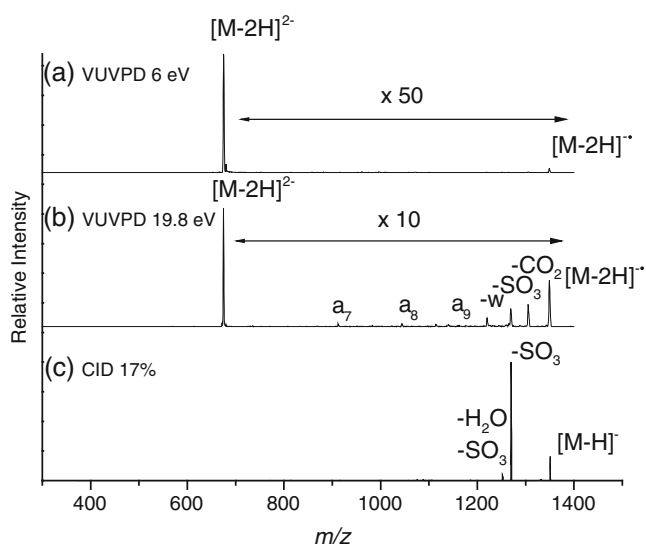
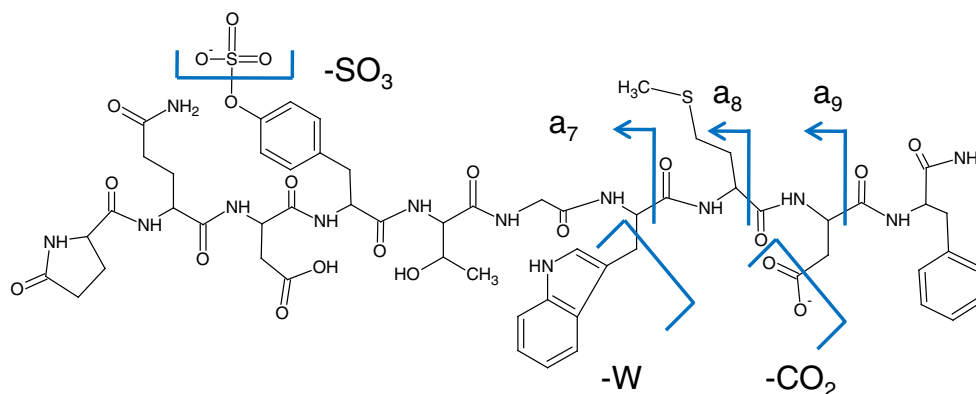


Figure 3. **(a)** and **(b)** VUV photodissociation spectra recorded for doubly deprotonated $[M-2H]^{2-}$ caerulein peptide anions respectively for photon energy of 6 and 19.8 eV. **(c)** CID spectrum of singly deprotonated $[M-H]^{\bullet}$ caerulein peptide anions at 17% of collision energy for 30 ms

(see Figure S1) is dominated by small neutral losses. While the electron photodetachment is the only observed channel at $h\nu=6$ eV, numerous fragment ions are observed at $h\nu\sim 20$ eV. Similar results are obtained with melittin polypeptide dianions (see Figure S2 in Supporting Information). For caerulein, these fragments are singly charged and correspond to (1) small neutral losses from the oxidized $[M-2H]^{\bullet}$ species, i.e., $-\text{CO}_2$ and $-\text{SO}_3$ groups, (2) a loss of 129 Da ($-\text{W}$), which corresponds to the side-chain loss of the neutral tryptophan radical, and (3) a -type product ion series (in particular a_7) due to the backbone cleavages (see Scheme 1). Most fragment ions retained the sulphate group.

The loss of 129 Da and the formation of a_7 ions may be both explained by the formation of a Trp^{\bullet} radical. The presence of the radical on the nitrogen atom of the indole residue increases the possibility to form a ions between the tryptophan and methionine residues [28, 34]. The most intense fragments discussed above (losses of CO_2 , SO_3 , 129 Da and a_7 fragment ions) are then thought to originate from a charge loss on the CO_2^- and SO_3^- followed either by loss of $-\text{CO}_2$ and $-\text{SO}_3$ molecules or by radical formation on tryptophan. The latter may be also due to the direct ionization of the tryptophan side chain which is possible at $h\nu=20$ eV. Similarly, backbone fragmentation may originate from direct ionization of peptide bonds as recently suggested by Breuker and colleagues in EDD experiments [34]. Figure 4 displays the branching ratios ($Br = \frac{F_i}{\sum_i F_i}$) of the different fragments as a function of the photon energy. As already mentioned, electron loss is the major relaxation channel over the studied photon energy range. Fragmentation efficiency globally increases as the photon energy increases. Moreover, the appearance energies for $[M-2H-\text{CO}_2]^-$, $[M-2H-\text{SO}_3]^-$, and $[M-2H-129]^-$ are different (i.e., 5.5, 7, and 9.5 eV, respectively). Backbone cleavages were only observed for photon energies above ~ 10 eV. Assuming that the electron is ejected from the same group (carboxylate group is the group for which the electron affinity ~ 3.25 eV [35] is the lowest) whatever the VUV photon, these thresholds may be directly related to the binding energy required to open the different fragmentation pathways. The electron would be ejected from the carboxylate group, and the CO_2 loss would be the lowest energy dissociation channel. On the other hand, SO_3 loss, Trp loss and peptide bond cleavage would require higher energies due to the need for radical migration. Another hypothesis is the possibility to detach electrons from different sites as the photon energy increases. The energy dependence of branching ratios after VUV irradiation may then be correlated to the ionization potential of different groups of the peptide chain. Indeed, while at 4.6 eV, a priori only the lowest bound electron localized on the carboxylate group can be photo-detached, different chemical groups can be ionized at higher energy. Indeed, the electron affinity of the sulfate group (EA (HSO_4^-)=4.7 eV [36]) is ~ 1.5 eV higher than the one of the



Scheme 1. Structure of caerulein (pEQDY(SO₃H)TGWMDF-NH₂). The fragments obtained following VUV irradiation of the doubly deprotonated caerulein peptide ions are displayed

carboxylate group (EA(CH₃CO₂⁻)=3.25 eV [35]). This is roughly the energy difference that is observed between the CO₂ and SO₃ loss appearance energies (i.e., 5.5 eV versus 7 eV). Thus, the difference between the CO₂ and SO₃ loss appearance energies could originate from a charge loss from CO₂⁻ or SO₃⁻. In the same way, further increase in energy would allow the ionization of the indole residue and of the peptide bond accounting for the tryptophan side chain and the formation of α -type product ion series. Indeed, ionization energies for tryptophan and aromatic amino-acid containing peptides were measured at 7.3 and 9.1 eV, respectively [37]. Fragmentation of the peptide would then occur close to the initial position of the radical owing to excess energy let in the peptide. Fragmentation at different parts of the peptide may also result from different electronic excitation on the peptides as a function of the photon energy (see VUV action spectroscopy on doubly deprotonated caerulein [M - 2H]²⁻ ions section), while in this case one would expect to observe resonances in the fragmentation channel instead of threshold (see Figure S4).

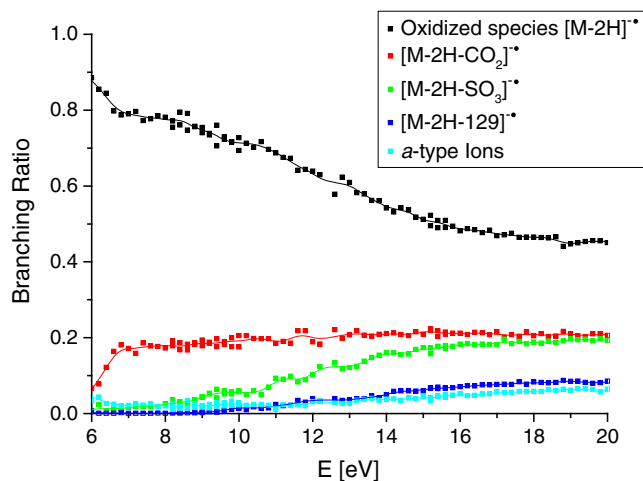


Figure 4. Branching ratios of the different fragments F_i as a function of the VUV photon energy for doubly deprotonated [M - 2H]²⁻ caerulein peptide anions

In order to get a deeper insight into these fragmentation processes, we performed MS³ experiments. In these experiments, [M - 2H]⁺ ions were generated by electron VUV-photodetachment of the [M - 2H]²⁻ precursor ions. The oxidized [M - 2H]⁺ ions were further isolated and activated by collision in a second stage. In the first set of experiments, we photodetached at 266 nm and varied the collision energy (see Supplement Material, Figure S3). All the fragments appear roughly at the same collision energy (from 15% of collision energy). In the second set of experiments, we set the collision energy at 12 % (in the second stage) and varied the energy of the initial photon (in the first stage). The branching ratios of the different fragments observed by CID as a function of the initial VUV photon energy are plotted in Figure 5. They appear to be independent of the initial photon

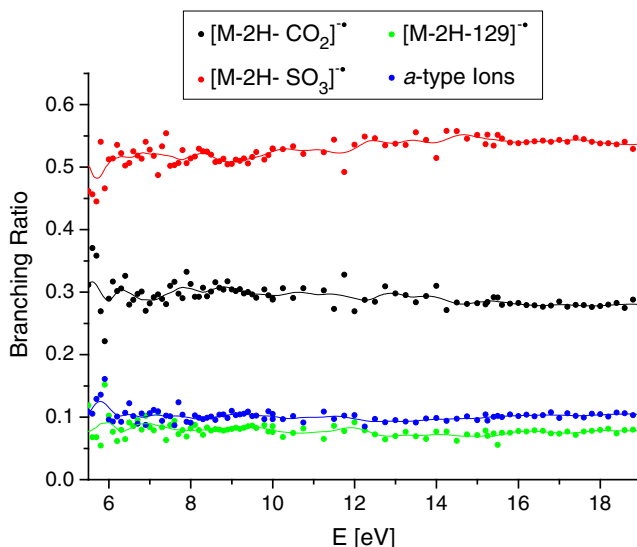


Figure 5. Branching ratios of the different fragments observed by CID of the oxidized [M - 2H]⁺ ions as a function of the initial VUV photon energy. MS² stage: VUV irradiation of [M - 2H]²⁻ ions for 400 ms, and MS³ stage: CID activation of the oxidized [M - 2H]⁺ ions at 12 % of collision energy for 30 ms

energy. In other words, here, there is no memory of the initial excitation energy in the fragmentation channels of the oxidized species on the time scale of the MS³ experiment. This result may be in favor of an electron expelled from the same site (i.e., CO₂ group), whatever the VUV initial photon energy absorbed by the precursor ion. The excess energy is distributed between the internal and the kinetic-energy-release of the fragments. However, we cannot exclude that in MS³ experiments, collisional excitation may allow radical migration before fragmentation, as recently evidenced [38].

VUV Action Spectroscopy on Doubly Deprotonated Caerulein [M – 2H]²⁻ Ions

To go further in the understanding of photon-induced processes of caerulein peptide, in this part, we explore its optical properties. Figure 6 presents the electron photodetachment efficiency as a function of the wavelength for the doubly deprotonated caerulein peptide anion. The yield of fragmentation (σ) was measured as a function of the photon energy [$\sigma = \ln(\{\text{parent} + \Sigma \text{frag}\} / \text{parent}) / \phi$], where ϕ is the photon flux, “parent” stands for the intensity of the parent peak and “ Σfrag ” represents the total intensity of the photofragment peaks). The detachment yield curve shows a strong dependence to the SR energy. Superimposed to a monotonical increase in the electron detachment yield with photon energy, several well-resolved bands are observed, in particular at 4.6, 5.7, and 6.7 eV. These bands may be attributed to the π - π^* bands for S₀→S₁, S₂, and S₃ transitions of phenylalanine, tyrosine, and tryptophan chromophores that are present in this peptide, even if at

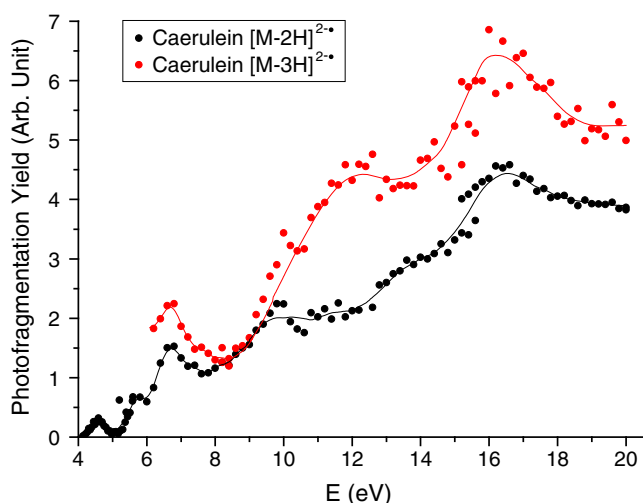


Figure 6. VUV action spectra of doubly deprotonated non-radical [M – 2H]²⁻ and radical [M – 3H]²⁻ caerulein peptide anions. The photon energy bandwidth is typically 12 meV at 10 eV. The action spectrum was recorded with the linear ion-trap LTQ XL mass spectrometer coupled to the DESIRS beamline of the SOLEIL synchrotron center, except between 4 and 5.6 eV, where the spectrum was recorded using the LTQ quadrupole linear ion trap coupled to a UV-Vis tunable laser OPO in Lyon

6.7 eV the peptide bonds play also a role [39]. For energy above 7 eV, the increase in absorption is due to excitation of other groups than aromatic rings. The 7.8 eV band was assigned to the absorption of the COOH group and the increase in absorption from 7.8 eV to 10.3 eV is associated with the excitation of σ electrons of the C–C and C–H bonds [40], as observed for polyamides [41]. The attribution and the nature of the bands observed at ~10 eV and ~16 eV, a nearly totally unexplored energy range, is challenging since many electrons in caerulein may contribute to the absorption in the 8–16 eV spectral range. We report here a benchmark spectrum that we hope will stimulate calculations in this energy range.

Comparison with Protonated Peptides

Here, we want to emphasize that the fragmentation channels observed for caerulein peptide dianions are drastically different from what is reported for protonated peptides. Using F2 lasers (7.9 eV) [8], Reilly and coworkers reported photodissociation of peptide backbone C _{α} –C bonds resulting in α - or x -ion fragments. They suggest that laser induced fragmentation occurs via a Rydberg excitation (and/or electron detachment mechanism) and subsequent C _{α} –C bond cleavage [9]. The ionization potentials of tryptophan and phenylalanine in the gas phase are, respectively, 7.4 and 8.65 eV [42]. Ionization thresholds for neutral polypeptides are, however, slightly higher in energy (around 9.8 eV) [43]. An increase in absorption above 8–10 eV may be associated with photofragmentation and photoionization processes. Very recently, Schlathöller and coworkers studied photodissociation of protonated leucine-enkephalin (leu-enk) in the VUV range of 8–40 eV. Different regimes of dissociation processes, below and above ionization energy (IE), have to be considered. For $h\nu < \text{IE}$, fragmentation is governed by IVR, while for $h\nu > \text{IE}$, the protonated peptide can be photoionized and a protonated leu-enk dication radical is formed, and the tyrosine side chain fragment in addition to immonium ions become the major fragments for photon energies exceeding 10 eV [44]. Note, however, that for multiply protonated proteins, photoionization is the main fragmentation pattern [31]. For peptide polyanions as discussed above, the oxidation of the precursor ion (electron loss) is the dominant relaxation pathway in the 4–20 eV energy range, even if fragment ions are intense at high energy.

VUV Action Spectroscopy on Radical Doubly Deprotonated Caerulein [M – 3H]²⁻ Ions

Besides the formation and fragmentation of radicals following VUV irradiation by electron photodetachment, it is fundamental to assess the effect of a VUV light on photogenerated radicals formed by environmental stress or radiation. Indeed, under VUV radiation, homolytic cleavages and ionization processes result in free-radical formation, which is one of the major causes of damage or degradation to DNA [45] or proteins [1] following VUV radiation absorption. VUV spectroscopy is a totally unexplored field.

This is in part due to the difficulty to isolate and characterize radicals under VUV irradiation in solution or in solids. By bringing a system into the gas phase, it is possible to eliminate the shielding of the radiations by the solvent as well as spurious intermolecular and/or solvent-molecule interactions. For this purpose, we recorded the VUV spectroscopy of radical doubly deprotonated caerulein $[M - 3H]^{2-\bullet}$ ions by a two-color experiment. We used a fixed-wavelength UV laser to photo-generate the caerulein $[M - 3H]^{2-\bullet}$ radicals (from the triply deprotonated $[M - 3H]^{3-}$ caerulein precursor ions) and the synchrotron radiation to record their VUV optical spectra by action spectroscopy. The electron loss (leading to $[M - 3H]^{2-}$) is the major fragmentation channel at every photon energy (as displayed in supplement material, Figure S4). This product was not observed after CID excitation of the oxidized product $[M - 3H]^{2-\bullet}$ (data not shown). Between 6 and 9 eV, abundant fragmentation arises from the side chain loss of tryptophan residue and the formation of a ions (and particularly a_7 ions). The fragmentation pathways strongly depend on the SR energy, with for example pronounced maxima observed at 12 eV for chain loss of tryptophan residue and a_7 product. This is different from what was found for the dianion (see Figure 4) and has to be correlated to the action spectrum of the $[M - 3H]^{2-\bullet}$ which is shown in Figure 6. Figure 6 compares the electron photodetachment efficiency as a function of the wavelength for the doubly negatively charged caerulein peptide anions in its radical ($[M - 3H]^{2-\bullet}$) and non-radical ($[M - 2H]^{2-}$) forms. The VUV action spectrum is enhanced by the presence of a radical in caerulein peptides, which may be due to a decrease in the stability of the radical anion as compared to the even electron species. Note that an enhancement in photofragmentation yields of radical species by one order of magnitude with respect to non-radical peptides was recently demonstrated for pentapeptide dianions [46]. Bands at 6.5 and 16.3 eV are observed for both species while the intense band at 12 eV is not observed for the non-radical species. While we recently explored the UV optical properties of radical peptides and proteins [47, 48], theoretically and experimentally very few is known on the nature of high energy absorption of radical peptides [46]. Maxima in the branching ratio corresponding to fragmentation (as opposed to electron loss) are correlated to the bands observed at 6 and 12 eV. The fragmentation is here directly related to the initial electronic excitation. In particular, intense transitions due mainly to $\pi-\pi^*$ transitions are predicted at ~ 6.7 eV for phenol and at ~ 6.1 eV for indole [46]. For photon energy higher than 6.2 eV, excitations are not only localized on the aromatic amino acid rings but also on the peptide backbone amides [6]. In this case, resonances in the fragmentation pathways are observed (Figure S4) while for VUV excitation of the non radical species only thresholds were observed (Figure 4).

Conclusion

In summary, the irradiation of vacuum ultraviolet (VUV) photons, with energies ranging from 4.5 to 20 eV, on

caerulein peptide polyanions leads mainly to an electron loss. Additionally, an increase in the fragmentation efficiency (neutral losses and peptide backbone cleavage) as a function of the energy is observed. The fragmentation pattern observed for the oxidized ions after activation by collisions are found independent of the initial photon energy on the time scale of the MS³ experiment. Thus, there is no memory effect of the initial excitation energy in the fragmentation channels of the oxidized species.

Electron photodetachment yield as a function of photon energy was used to record optical action spectra of deprotonated caerulein ions in the VUV range. Action spectra for both closed-shell ($[M - 2H]^{2-}$) non-radical ions and open-shell ($[M - 3H]^{2-\bullet}$) radical ions generated by electron photodetachment from $[M - 3H]^{3-}$ precursor ions) were recorded. The detachment yield increases with the photon energy. Several bands are superimposed to this general increase. The nature of the absorption bands in the VUV range is difficult to analyze since many electronic excitations may contribute to these bands. However, these bands constitute benchmarks for calculations. Spectra for radical and non-radical ions are very similar in terms of observed bands; however the VUV action spectrum is enhanced by the presence of a radical in caerulein peptides. This enhanced photofragmentation for radical species may be attributed to a decrease in binding energy for fragmentation channels.

These results are of great interest concerning the radiation damage to biomolecules. Indeed, the sign of the charge state of the peptide leads to drastic different relaxation channels, with in the case of polyanions a de-excitation that proceeds via a universal relaxation channel, i.e., electron loss, preventing the molecular anions from a major fragmentation.

Acknowledgments

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