Near-UV Photodissociation of Phosphopeptide Cation-Radicals

Christopher J. Shaffer, Kristína Slováková, František Tureček*

Department of Chemistry, Bagley Hall, Box 351700, University of Washington, Seattle, WA
98195-1700

Abstract

Laser photodissociation at 355 nm (near-UVPD) of phosphopeptide cation-radicals provides information on the position of the radical center and the phosphorylated serine residue. Cation-radicals of the *z* type are produced by electron transfer dissociation of peptide dications from pSAAAR, ApSAAR, AApSAR, and AAAPSR and found to absorb light at 355 nm to undergo photodissociation. Near-UVPD can induce backbone dissociation of the CO—NH bond next to the radical center on its *N*-terminal side concurrent with only minor radical scrambling and loss of the post-translational modification. The pSer residue effectively blocks backbone cleavage when the radical site is in its closest vicinity. Near-UVPD of loss-of-ammonia fragment ions produced by ETD results in backbone dissociations that distinguish isomeric ions differing in the site of ammonia loss. The experimental data are interpreted with the help of *ab initio* and density functional theory calculations of ion structures, transition states, and excitation energies.

Keywords: near-UV photodissociation; phosphopeptide ions; radicals; absorption spectra; dissociation mechanisms

Introduction

Dissociations of gas-phase ions formed by electrospray ionization of phosphorylated peptides have elicited much interest regarding the determination of peptide amino acid sequence and positions of phosphoester groups [1-3]. Collision-induced dissociation (CID) of phosphopeptide cations often results in phosphoric acid elimination that competes with proton-driven backbone dissociations, as studied under various collision energy regimes [4-9]. In addition, if both phosphorylated and non-phosphorylated serine and threonine residues are present in the peptide ion, collisional activation can induce phosphate group migration prior to dissociation and thus scramble the site positions of phosphorylation [10,11]. The scope of this effect has been debated in the literature [12,13]. Phosphoric acid elimination is also promoted by infrared photon absorption that targets the P=O [14] and O-H stretching vibrations [14-22]. Short-wavelength UV photodissociation has also been explored for phosphopeptide characterization [23-27]. The methods of choice for phosphopeptide analysis are based on radical dissociations that typically do not affect the phosphate groups and provide abundant sequence information. Both electron capture dissociation [28-33] and electron transfer dissociation (ETD) [34-37] have been used extensively for phosphopeptide sequencing. In particular, ETD has been explored and combined with CID in a parallel [38-41] or tandem fashion [42]. Other electron-involved ion activation methods use fast electron transfer from cesium atoms [43] and metastable atoms [44,45]. ETD of phosphopeptide ions largely preserves the phosphoester group in backbone fragments that carry partial sequence information. In particular, cation-radical fragments of the *z* type, that are produced by N—C_α backbone cleavages at positions *N*-terminal to the phosphorylated amino acid residue, often retain the phosphate group and can be further analyzed by tandem mass spectrometry (MSⁿ) methods.

Peptide cation-radicals of various types have been recently reported to undergo photodissociation at 355 nm, which we call near-UVPD [46-49]. Regular proteinogenic amino acid residues, as well as those post-translationally modified by phosphorylation, sulfation, and glycosylation are transparent at this wavelength [50, 51] and are thus not activated by photon absorption. Near-UVPD at 355 nm has been shown to involve electronic excitation in conjugated radical chromophores to trigger specific radical backbone dissociations of peptide cation-radicals, and that have been used for structure assignment [46-49]. Here, we report a near-UVPD study of isomeric cation-radicals of z type ions produced by ETD of phosphorylated peptides pSAAAR, ApSAAR, AApSAR, and AAAPSR and containing phosphoserine residues. We show that these z ions are efficiently photodissociated at 355 nm and the near-UVPD MS³ spectra provide structure information on the radical and phosphoserine position.

Experimental

The phosphorylated peptides used in this work were custom synthesized by GenScript (Piscataway, NJ, USA) at 98-99% purity [22, 52]. Electron-transfer dissociation and near-UV photodissociation mass spectra were measured on a modified LTQ-XL (ThermoElectron Fisher, San Jose, CA) linear ion trap mass spectrometer that was furnished with an EKSPLA NL 301 HT Nd-YAG laser providing the third harmonics line at 355 nm, as described previously [46]. Fragment ions were generated by ETD of doubly charged ions using fluoranthene anion radical as the electron donor at 100 ms ion-ion reaction time. The stability of the stored ions with respect to dissociation and ion-molecule reactions with background gases (O₂) was checked; for most ions these processes when combined amounted to <0.1% precursor ion intensity. Photodissociation of mass-selected ions was carried out by varying the number of laser pulses

over a 400 ms ion storage time The laser power was maintained at 15-20 mJ/pulse to secure single photon excitation.

Calculations

All electron structure calculations were performed with the Gaussian 09 suite of programs [53]. The precursor dications, (AAApSR + 2H)²⁺, (AApSAR + 2H)²⁺, (ApSAAR + 2H)²⁺, and (pSAAAR + 2H)²⁺, were global free-energy minima obtained by multistep conformational search and gradient optimization using the ConformSearch protocol [52, 54]. Initial structures of the z₄ ions were derived from the dication precursors by preserving the hydrogen bonding motifs of the polar groups, and the structures were fully optimized with hybrid density functional methods (B3LYP [55,56] and M06-2X [57]) with the 6-31+G(d,p) basis set. The calculations were run in a spin-unrestricted mode, and the optimized structures were confirmed as local energy minima or transition states by harmonic frequency analysis to have the appropriate number of imaginary frequencies. Excited state energies and transition moments were obtained by time-dependent DFT calculations [58] using the M06-2X, LC-BLYP [59], and ωB97XD [60,61] calculations, all run in a spin-unrestricted mode using the 6-311++G(2d,p) basis set. RRKM calculations were performed using a QCPE program [62] that was modified for larger molecular systems and recompiled for Windows, as reported previously [63].

Results and Discussion

UVPD and CID Spectra. Electron transfer to doubly charged AAApSR, AApSAR, ApSAAR, and pSAAAR ions (*m/z* 278.2) results in dissociations forming a complete z₁-z₄ series of C-terminal fragment ions [52]. The isomeric z₄ ions from ApSAAR, AApSAR, and AAApSR,

denoted respectively as $\bullet\text{pSAAR}^+$, $\bullet\text{ApSAR}^+$, and $\bullet\text{AApSR}^+$, appear at m/z 468. The z_4 ion from pSAAAR, denoted as $\bullet\text{AAAR}^+$, appears at a distinct m/z 372. This $\bullet\text{AAAR}^+$ ion was also generated by ETD of doubly charged AAAAR. The UVPD spectra of $\bullet\text{AApSR}^+$ showed an exponential depletion of the precursor m/z 468 ion ([Figure 1a](#) inset). The main dissociation products were the sequence fragment ion $(y_3 - 2H)^+$ (m/z 411) and its secondary dissociation product (m/z 313) due to elimination of phosphoric acid. Loss of phosphoric acid from the $\bullet\text{AApSR}^+$ ion formed the m/z 370 ion which further dissociated to the $(x_2 - H_3PO_4 - H)^+$ ion at m/z 270. The nature of these fragment ions was readily established from the dependence of their relative intensities on the number of laser pulses. [Figure S1](#) (Supplement) shows that the relative intensities of the m/z 411, 313, and 270 fragment ions increased with the number of laser pulses while the ratios of their intensities remained roughly constant. This indicated that these fragment ions were formed competitively, not consecutively, and did not absorb at 355 nm to be photodepleted with prolonged irradiation. Such a behavior is expected if these ions were even-electron species with no radical chromophore absorbing at 355 nm. In contrast, the relative intensity of the m/z 370 ion by loss of H_3PO_4 showed a maximum at 2 laser pulses and then decreased with continued pulses. This is typical of a reaction intermediate that is formed by UVPD and retains a chromophore absorbing at 355 nm, which is then depleted by subsequent laser pulses. The UVPD spectrum of the $\bullet\text{AApSR}^+$ ion is compared to the CID spectrum ([Figure 1b](#)). The latter shows a major loss of H_3PO_4 followed by loss of 100 Da, presumably the $\text{CH}_3\text{CH}_2\text{CONHCH}^\bullet(\text{CH}_3)$ radical (100 Da) from the Ala-Ala deaminated *N*-terminus. Our suggested mechanisms for the formation of the major fragment ions in the UVPD and CID spectra are sketched in [Scheme 1](#). This describes migration of the internal Ala H_α to the deaminated *N*-terminal C_α radical position to form an isomeric Ala C_α radical at an internal

position. Previous studies of z ions have established that hydrogen atom migrations of this kind are mildly exothermic because of captodative stabilization of the internal HN-C $_{\alpha}$ -CO radical [64, 65]. For steric reasons, the H $_{\alpha}$ atom migration first requires a *trans-cis* rotation of the radical amide group (not shown in [Scheme 1](#)), which is a facile process of a low activation energy promoted by the weakening of the amide C-N bond [66]. The H $_{\alpha}$ atom migration typically requires ≤ 100 kJ mol $^{-1}$ in the transition state relative to the *cis*-amide structure [66]. The radical-induced cleavage of the amide bond in the last step, resulting in elimination of CH $_3$ CH $_2$ CO radical, is an endothermic reaction requiring ≤ 140 kJ mol $^{-1}$ in the transition state. These energy estimates are corroborated by the more detailed analysis of the related $\bullet$ AAAR $^{+}$ ion, as described below.

The laser-induced dissociations depended on the position of the pSer residue in the peptide sequence. Moving pSer one position over to the middle of the chain, as in $\bullet$ ApSAR $^{+}$, resulted in substantial changes in fragmentation, particularly to the fragmentation of the phosphate-serine bond. [Figure 2a](#) inset shows that the m/z 468 precursor ion underwent a very efficient photodepletion by loss of H $_3$ PO $_4$ and backbone dissociations. The latter produce the ($x_2 - 2H$) $^{+}$, ($y_2 - 2H$) $^{+}$, z_2 , and z_1 fragment ions at m/z 272, 244, 230, and 159, respectively ([Figure 2a](#)). Herein, the ($y_3 - 2H$) $^{+}$ ion (m/z 411) is absent, suggesting that the backbone dissociations are outcompeted by elimination of phosphoric acid (m/z 370) which can also be followed by a subsequent dissociation forming the ($x_4 - H_3PO_4 - H$) $^{+}$ ion at m/z 341. A plot of the relative fragment ion intensities as a function of the number of laser pulses ([Figure S2](#), Supplement) shows the m/z 341, 272, and 244 peaks proportionately increasing, whereas the m/z 370 peak shows an early maximum at a single pulse irradiation and then decreases. This is consistent with the radical nature of the m/z 370 ion which has a chromophore absorbing at 355 nm and

undergoes photodissociation. In contrast to UVPD, the CID spectrum of $\bullet\text{ApSAR}^+$ shows a single dissociation channel by loss of H_3PO_4 while no backbone fragments are formed. ([Figure 2b](#)).

Substantial differences between UVPD and CID were also observed for $\bullet\text{pSAAR}^+$ in which the C_α radical center is on the phosphoserine residue. UVPD results in virtually no elimination of H_3PO_4 (m/z 370 is nearly absent), and most fragmentation results in backbone cleavages forming the $(y_3 - 2\text{H})$ and $(y_2 - 2\text{H})$ ions at m/z 315 and 244, respectively ([Figure 3a](#)). Photodepletion of $\bullet\text{pSAAR}^+$ showed >40% efficiency per laser pulse ([Figure 3a, inset](#)). The pulse-dependent formation of the major fragments showed proportionately increasing m/z 315, 371, and 244 peak relative intensities, indicating these belong to photostable even-electron ions ([Figure S3](#), Supplement). The $[m/z\ 315]/[m/z\ 244]$ ratio showed a slightly decreasing trend between 1 and 3 laser pulses, but then remained constant.

The formation of the $(y_3 - 2\text{H})^+$ and $(y_2 - 2\text{H})^+$ fragment ions can be depicted analogously to that shown in [Scheme 1](#). Presumably, it involves migration of an α -H from one of the Ala residues to the pSer α -position, creating new α -radicals on the internal Ala residues. This is followed by cleavage of the pSer-Ala and Ala-Ala amide bonds which is induced by the Ala α -radicals. In contrast to UVPD, CID of $\bullet\text{pSAAR}^+$ resulted in loss of water (m/z 450), CO_2 (m/z 424), phosphoric acid (m/z 370) and combinations thereof (m/z 352 and 316, [Figure 3b](#)).

UVPD and CID of the phosphoserine containing z_4 ions were compared with the spectra of the non-phosphate $\bullet\text{AAAR}^+$ ion at m/z 372 ([Figure 4](#)). In this case, UVPD resulted in competitive backbone amide cleavage between the Ala residues forming the $(y_3 - 2\text{H})^+$ and $(y_2 - 2\text{H})^+$ ions at m/z 315 and 244. CID showed more abundant loss of water (m/z 354) and CO_2 (m/z 328) which were analogous to the dissociations of $\bullet\text{pSAAR}^+$ ([Figure 3b](#)). The photodepletion

curve of the $\bullet\text{AAAR}^+$ ion (Figure 4a, inset) showed a 40% decrease of the precursor ion intensity per laser pulse.

Excited State Energies and Transition Intensities

To evaluate the photodissociation experiments we addressed the question of UV absorption wavelengths and intensities in the phosphoserine peptide cation-radicals using time-dependent DFT calculations. These were performed with the LC-BLYP, ωB97XD , and M06-2X hybrid functionals on fully optimized ion structures. The DFT methods used here have previously been benchmarked for vertical excitation energies and oscillator strengths for excited singlet [67] and doublet states [46, 68]. A complete set of excitation energies, resonant absorption wavelengths, and oscillator strengths for the systems we studied are compiled in Table S1 (Supplement). Select data are visualized in Figure 5. The calculated UV spectra indicate absorption bands with maxima near 355 nm, which are consistent with the experimental data. It should be noted that $\mathbf{z}$ ions are stored in the ion trap at ambient temperature (310 K) and their absorption bands are likely to be broadened to 20-30 nm by overlapping vibronic transitions [50,51]. Thus, a perfect match between the laser line wavelength and the absorption maximum is not necessary for photodissociation to proceed. The $\bullet\text{AAAR}^+$, $\bullet\text{ApSAR}^+$, and $\bullet\text{pSAAR}^+$ ions show weak transitions to the first excited ($\mathbf{A}$) state at the short-wavelength edge of the visible region (380-400 nm) which are absent for $\bullet\text{AApSR}^+$. These weak bands correspond to a combination of electron transitions to the singly-occupied π_z orbital (SOMO) which is delocalized over the C_α and amide C,O,N atoms (Figure S4). This excitation consists of a symmetry disallowed electron excitation from the π_{xy} orbital of the C-O group which is mixed with a small component of an allowed transition from a lower π_z orbital (MO95 for $\bullet\text{AAAR}^+$). The intense band at 338-359

nm is due to a symmetry allowed excitation from the doubly occupied π_z orbital (MO99 for $\bullet\text{AAAR}^+$) to the SOMO (excited B state). With $\bullet\text{AApSR}^+$, both long-wavelength excitations are blue-shifted by *ca* 30 nm. This may be associated with the different type of hydrogen bonding between the *N*-terminal amide and the phosphoester group, which has a more pronounced π -bonding character in $\bullet\text{AApSR}^+$ than in the other phosphopeptide z ions, as shown for optimized structures in [Figure S5](#).

Structures and Energies of z_4 Ions

The energetics of z_4 ion isomerizations and dissociations were investigated by electron structure calculations for the $\bullet\text{AAAR}^+$ ion as the simplest representative model. The relevant ions are sketched in the potential energy diagram ([Figure 6](#)), and the fully optimized ion structures are shown in [Schemes S1-S3](#) (Supplement). From the relative energies of the precursor structures, we assumed that the *trans*-Ala-1 radical (**1**) was the starting structure for the 0 kJ mol⁻¹ reference energy. Isomerization to the *cis*-Ala-1 radical (**2**) was 29 kJ mol⁻¹ endothermic and can be followed by Ala-2-H α migration forming the *cis*-Ala-2- α -radical (**3**) at 10 kJ mol⁻¹ relative to **1**. The transition state energy for the Ala-2-H α migration (**TS1**, 109 kJ mol⁻¹, [Table 1](#)) was very similar to TS energies for analogous H α migrations reported previously for $\bullet\text{ANAR}^+$, $\bullet\text{ASAR}^+$, and $\bullet\text{ATAR}^+$ peptide z ions (100-123 kJ mol⁻¹)[[48](#), [69](#)], indicating that these reactions share common features regarding electronic and steric factors affecting the TS energy. The subsequent CO-NH bond dissociation in the *cis*-Ala-2- α -radical **3** can proceed through **TS2** at 138 kJ mol⁻¹ relative to **1**. However, an alternative pathway for **3** is to isomerize by amide bond rotation to *trans*-Ala-2- α -radical (**4**, -29 kJ mol⁻¹ relative to **1**, [Scheme S2](#)); this amide rotation would proceed through a transition state of *ca.* 70-80 kJ mol⁻¹ energy relative to **3** [[66](#)]. *trans*-Ala-2- α -

radical **4** can undergo CO-NH bond dissociation through a lower energy **TS3** (127 and 146 kJ mol⁻¹ relative to **1** and **4**, respectively) to form a complex (**5**, 89 kJ mol⁻¹ relative to **1**) of the incipient (**y**₃ – 2H)⁺ (**6**) and CH₃CH₂CO radical fragments. The dissociation threshold for separated (**y**₃ – 2H)⁺ and CH₃CH₂CO[•] fragments was at 115 kJ mol⁻¹ relative to **1** ([Table 1](#)). The calculated TS and dissociation threshold energies indicate that the CO-NH bond dissociation is the highest-potential energy step for the formation of the (**y**₃ – 2H)⁺ fragment ions.

Dissociation Kinetics and Discussion of Reaction Mechanisms

UVPD of phosphopeptide z ions can be viewed as occurring on the potential energy surface of the excited **B** state or after vibronic transition to a vibrationally excited ground (**X**) electronic state. We investigated the unimolecular rate constants by RRKM calculations so as to determine whether activation by a 3.49 eV photon (355 nm) was sufficient to drive the CO-NH bond dissociation on the experimental time scale if the excited (**B**) electronic state of **1** underwent vibronic transition to a vibrationally excited ground state. The internal energy distribution in vibrationally hot **1** (*P(E)*) was obtained by convoluting the photon energy (3.49 eV, 337 kJ mol⁻¹) with the thermal energy distribution in the precursor ion that was anchored to its 298 K mean rovibrational enthalpy (61 kJ mol⁻¹). The resulting *P(E)* is shown in [Figure S6b](#). The RRKM rate constants for CO-NH bond dissociation indicate >90% conversion at ion internal energies >360 kJ mol⁻¹ ([Figure S6a](#)), assuming that internal energy is conserved in the dissociating ions for the 50 ms time period between laser pulses. However, [•]AAAR⁺ ions in the ion trap undergo collisions with the helium bath gas that can cool vibrationally excited ions and slow their dissociation. Collisional cooling occurs on the time scale of 20-30 ms with rates that are commensurate with those for dissociation [[70](#)]. This effect is illustrated by the calculated molar

fractions of surviving $\bullet\text{AAAR}^+$ on the time scale of 50 ms, yielding a 0.08 molar fraction of surviving precursor ions, and after shortening to effective 30 and 20 ms to give the respective surviving molar fractions of 0.16 and 0.25 (Figure S6c). According to this analysis, collisional cooling that would shorten the effective dissociation time to 20 ms would increase the branching ratio of the survival for photoexcited ions that underwent vibronic transition to the ground electronic state; nevertheless, substantial dissociation ($\geq 75\%$) is expected even under those conditions. Hence, RRKM analysis indicates that the observed dissociations of $\bullet\text{AAAR}^+$ are energetically possible after transition to the ground electronic state.

The branching ratio for dissociations proceeding directly from the **B** excited state compared to those occurring after conversion to the ground state is difficult to assess. Considering competing dissociations, formation upon UVPD of both the $(\text{y}_3 - 2\text{H})^+$ and $(\text{y}_2 - 2\text{H})^+$ ions is a three step reaction requiring amide rotation and hydrogen atom transfer onto the terminal Ala residue. The UVPD spectra show a $(\text{y}_2 - 2\text{H})^+ / (\text{y}_3 - 2\text{H})^+$ branching ratio of 0.26, favoring cleavage closer to the *N*-terminal residue. If proceeding from the **B** excited state, $(\text{y}_3 - 2\text{H})^+$ ions could be formed in a single step involving transfer of the Ala-2 α -H on the terminal amide oxygen and cleavage of the CO–NH bond, yielding a $\text{CH}_3\text{CH}=\text{C}^\bullet\text{-OH}$ enol radical as the neutral fragment. According to the calculated thermochemical threshold (243 kJ mol^{-1} relative to **1**, Table 1), this mechanism is energetically possible in the **B** state, while it is highly disfavored in the ground state.

Another peculiar feature of the UVPD spectra of $\bullet\text{AAAR}^+$ is the low relative intensity of the prominent CID ions due to loss of water and CO_2 . This is obvious from the UVPD spectrum obtained with a single laser pulse (Figure S7, Supplement) that avoids secondary depletion of primary cation-radical fragments by photodissociation upon repetitive laser pulses. The favorable

loss of water and CO₂ upon CID may be due to a different internal energy distribution as a result of the slow heating. Curiously though, the $(y_2 - 2H)^+/(y_3 - 2H)^+$ ratio upon CID (0.19) is not dramatically different from that in UVPD. This indicates that these dissociations occur in a fraction of $\bullet AAAR^+$ ions that have internal energies similar to those produced by UVPD. Note that the formation of $(y_2 - 2H)^+$ ions requires either an additional step of H-atom migration between the Ala-2 and Ala-3 alpha positions, or an eight-membered TS for H-atom migration between the Ala-1 and Ala-3 positions. The latter is expected to have a lower entropy compared to the H-atom migration leading to $(y_3 - 2H)^+$, and so the pertinent rate dissociation constants are expected to show different dependence on the ion internal energy.

Regarding phosphoric acid elimination and the ensuing fragment ions, we address the mechanism of backbone dissociation in the dephosphorylated ions from $\bullet AApSR^+$ where the CID spectrum ([Figure 1b](#)) shows the formation of the $(x_2 - H_3PO_4 - H)^+$ fragment ion. Elimination of phosphoric acid from pSer residues in even-electron peptide ions does not necessarily proceed as a 1,2-elimination to form dehydroalanine residues [10,11]. However, the formation of a dehydroalanine residue in the m/z 370 ion from $\bullet AApSR^+$ is consistent with the activation of the amide N-H bond for H-atom transfer forming a conjugated intermediate that readily undergoes C $_{\alpha}$ -CO bond cleavage, uniquely forming the $(x_2 - H_3PO_4 - H)^+$ fragment ion ([Scheme 2](#)). The favorability of this mechanism is described by electron structure calculations (for optimized structures see [Scheme S3](#)) which indicate that amide hydrogen transfer in the dephosphorylated $\bullet AApSR^+$ ion (**7**) is nearly thermoneutral, forming the amide *N*-centered radical **8** ([Table 1](#)). The TS for the amide H atom transfer (**TS4**) has a low energy ($E_{TS4} = 116$ kJ mol⁻¹ relative to **7**, [Table 1](#)). The C $_{\alpha}$ -CO cleavage in **8** is facilitated by the formation of an extensively π -conjugated O=C=N-C(=CH₂)-CONH system in the resulting $(x_2 - H_3PO_4 - H)$ ion

(**10**). Overall, this lowers the dissociation (78 kJ mol^{-1}) as well as the corresponding **TS5** energy ($E_{\text{TS5}} = 69 \text{ kJ mol}^{-1}$), both relative to **7**. Note that **TS5** is connected to a low-energy ion-molecule complex of the incipient fragments (**9**, 11 kJ mol^{-1} relative to **7**) which are bound by a strong hydrogen bond between the radical carbonyl of the neutral moiety (**11**) and the Arg charged group in ion **10** (Scheme S3).

Photodissociation of Other Phosphopeptide Cation-Radicals Formed by ETD

ETD provides a convenient means of generating different types of peptide cation-radicals that are amenable to near UV photodissociation [46]. In particular, ETD of the phosphopeptides studied here generates phosphoserine-containing $\bullet\text{pSR}^+$, $\bullet\text{ApSR}^+$, $\bullet\text{pSAR}^+$, $\bullet\text{pSAAAR}^+$, $\bullet\text{ApSAAAR}^+$, $\bullet\text{AApSAR}^+$, and $\bullet\text{AAApSR}^+$ fragment ions in addition to the above-described z_4 ions. The pentapeptide fragment ions are products of ETD-induced loss of ammonia which is of particular mechanistic interest [71]. Previous studies indicated that ETD loss of ammonia from Arg-C-terminated peptide ions can involve the *N*-terminal ammonium, forming z -type ions, as well as NH_3 from the protonated Arg residue [71, 72] forming isomers with a modified side chain [46]. Both of these species were found to undergo near-UVPD and could be readily distinguished by significant differences in their fragmentation patterns [46].

UVPD of $\bullet\text{pSAAAR}^+$ (m/z 539) generated two series of backbone fragment ions (Figure S8a, b). One series, m/z 173, 244, 315, and 386, consists of $(y_n - 2\text{H})^+$ fragment ions ($n = 1-4$) with an unmodified Arg residue. From previous results [a14], it is known that these ions are a strong signature for the presence of the z_5 isomer of $\bullet\text{pSAAAR}^+$ formed by ETD-elimination of the *N*-terminal ammonium. The other series consists of the m/z 158, 229, 300, and 371 fragment ions, labeled u_1 - u_4 to distinguish them from the usual peptide backbone fragments. These ions

have complementary backbone fragmentations but their mass shift of 15 Da signifies the presence of another $\bullet$ pSAAAR⁺ isomer formed during ETD via elimination of ammonia from the arginine side chain. The near-UVPD spectra confirm that the two suggested m/z 539 ions both contain a radical chromophore, as indicated by the complete photodepletion of the precursor ions after >5 laser pulses ([Figure S8b](#)). The ions of the ($y_n - 2H$) and u_n series persist over several laser pulses, indicating that they do not absorb light at 355 nm, in agreement with their proposed structure of even-electron ions. The relative intensities of the ($y_n - 2H$) and u_n ions depend on the backbone cleavage site. The integrated intensities for $n = 1-4$ showed a 63:37 ratio of ($y_n - 2H$) and u_n after 1 laser pulse that remained constant (64:36) all the way to 9 laser pulses. Hence, for ETD of (pSAAAR + 2H)²⁺ roughly two thirds of the ammonia molecules originate from the *N*-terminus and one third from the Arg side chain. Analogous analysis of ($y_n - 2H$) and u_n ion intensities for $\bullet$ ApSAAAR⁺ gave ratios ranging from 36:64 to 33:67 for 1 through 9 laser pulses, indicating a more prevalent loss of ammonia from the Arg side chain. This is illustrated by the ETD/UVPD spectra in [Figure S9](#). UVPD of the $\bullet$ AApSAR⁺ ion showed a dominant ($y_4 - 2H$) ion at m/z 482 whose assignment is corroborated by subsequent loss of H₃PO₄ (m/z 384). The u_n ions are also formed (m/z 158 and 229) indicating the presence of an Arg-modified isomer ([Figure S10](#)). Finally, UVPD of the $\bullet$ AAApSR⁺ ion also showed a dominant $y_4 - 2H$ ion at m/z 482 along with the ($y_1 - 2H$) ion at m/z 173. Ions of the u_n type are formed but at lower relative intensities (m/z 158) ([Figure S11](#)). The positional effects should most of all be noted in that the loss of H₃PO₄ was stronger, to absolutely dominant, as the serine residue was placed closer in relation to the initial site of the radical chromophore, much as it was observed for the z_4 ion series.

A peculiar feature of the UVPD spectra of the z_5 ions is the complete absence of ($y_n - 2H$) and u_n fragment ions from CO-NH dissociations at the *N*-terminal side of the pSer residue.

Such an effect could be expected if the pSer phosphate formed strong hydrogen bonds to its *N*-terminally neighboring amide. In this case, if a pSer C $_{\alpha}$ radical was formed, the radical CO-NH bond dissociation would be hampered by weakening the O=C....phosphate hydrogen bond in the intermediate and the transition state, because the incipient -C $^{\bullet}$ =O radical is a weaker electron donor than the amide group. Support of this hypothesis is discussed further below.

Excited Electronic States in z -Ions

The fact that the z -ions undergo photodissociation at 355 nm raises the question of excited electronic states involved in the process. This question was addressed by time-dependent density functional theory calculations of vertical excitation energies and transition moments for the z ions of interest. TD-DFT calculations for all the z_4 ions revealed vertical electronic transitions in the vicinity of the 355-nm laser line used in the photodissociation experiments. The calculations using different hybrid density functionals showed a spread of excitation energies that followed the order $\Delta E_{\text{exc}}(\text{LC-BLYP}) > \Delta E_{\text{exc}}(\text{M06-2X}) > \Delta E_{\text{exc}}(\omega\text{B97XD})$, leading to a blue shift in the LC-BLYP and M06-2X calculated absorption spectra relative to those from ωB97XD . The absorption wavelengths (λ_{max}) from M06-2X and ωB97XD differed by < 30 nm and, considering the thermal absorption band broadening, were generally consistent with the experimentally observed absorption at 355 nm. Regarding the ωB97XD λ_{max} , there is a 15-35 nm blue shift of the band for $^{\bullet}\text{ApSAR}^+$ ([Figure 5](#)) compared to the other z ions. This reflects a slight destabilization of the pertinent excited state for this excitation. The nature of this electronic excitation was studied for $^{\bullet}\text{AAAR}^+$ where it corresponds to a $\pi \rightarrow \pi^*$ electron transition. The ground electronic state shows unpaired electron density to be at the C $_{\alpha}$ carbon with delocalization into the adjacent amide π orbital. The excited state results from a promotion to the

π (SOMO) of an electron from an underlying π orbital (Figure S4). This creates a one-electron vacancy in a π orbital localized in the amide C=O group, decreasing the local electron density. In $\bullet\text{ApSR}^+$, the amide C=O group is engaged in a hydrogen bond to the pSer phosphate group (Figure S5), and lowering the electron density weakens the hydrogen bonding in the excited electronic state, raising its energy and causing the blue shift in the absorption spectrum.

The z_4 ions also differ in the calculated oscillator strength for the excitation near the 355 nm laser line, of which $\bullet\text{AApSR}^+$ is the lowest. The experimental data also show the lowest photodissociation cross section for this ion. This is illustrated by the exponents in the photodepletion curves that are products of the constant laser flux, path length, and structure-dependent absorption cross section. The combined value for $\bullet\text{AApSR}^+$ (0.332, Figure 1a), is substantially lower than those for the other z_4 ions (0.53-0.69, Figure 2a-4a). It should however be noted that the fine details of minor differences in absorption maximum wavelengths, band widths, and cross sections or equivalent oscillator strengths are difficult to precisely resolve on the basis of approximate TD-DFT calculations. Nevertheless, the agreement between the experimental data and the calculated parameters for electron excitation in the z ions is encouraging and provides semiquantitative interpretation of the photodissociation phenomena.

Conclusions

The experimental data and theoretical analysis regarding phosphopeptide cation-radicals allow us to arrive at the following conclusions. The z ions produced by ETD contain chromophores in the form of C_α -amide conjugated radicals that absorb photons at 355 nm to cause photodissociation. The fragmentations upon near-UVPD substantially differ from CID in that radical homolysis of bonds in the vicinity of the radical center predominates upon UVPD and thus localizes the

radical position. Backbone dissociations from UVPD can occur without concomitant dissociation of the phosphoserine bond, for which the likelihood is entirely dependent on positional effects. As these experiments are performed in tandem with ETD, these results further demonstrate the capability of the ETD/UVPD method to reveal greater sequence information when the initial radical induced dissociations are not fully cooperative.

Acknowledgments

This research received funding from the National Science Foundation Division of Chemistry (Grant CHE-1359810). F.T. thanks the Klaus and Mary Ann Saegebarth Endowment for support.

References

- [1] J. P. DeGnore, J. Qin, J. Am. Soc. Mass Spectrom. 9 (1998) 1175-1188.
- [2] J. D. Dunn, G. E. Reid, M. L. Bruening, Mass Spectrom. Rev. 29 (2009) 29-54.
- [3] P. J. Boersema, S. Mohammed, A. J. R. Heck, Mass Spectrom. 44 (2009) 861-878.
- [4] S. C. Moyer, R. J. Cotter, A. S. Woods, J. Am. Soc. Mass Spectrom. 13 (2002) 274-283.
- [5] Y. Zhang, S. B. Ficarro, S. Li, J. A. Marto, J. Am. Soc. Mass Spectrom. 20 (2009) 1425-1434.
- [6] P. M. Gehrig, B. Roschitzki, D. Rutishauser, S. Reiland, R. Schlapbach, Rapid Commun. Mass Spectrom. 23 (2009) 1435-1445.
- [7] S. Shimma, H. Nagao, A. E. Giannakopoulos, S. Hayakawa, K. Awazu, M. Toyoda, J. Mass Spectrom. 43 (2008) 535-537.
- [8] M. Chesnik, B. Halligan, M. Olivier, S. P. Mirza, Anal. Biochem. 418 (2011) 197-203.
- [9] J. Laskin, R. P. W. Kong, T. Song, I. K. Chu, Int. J. Mass Spectrom. 330-332 (2012) 295-301.
- [10] A. M. Palumbo, G. E. Reid, Anal. Chem. 80 (2008) 9735-9747.
- [11] L. Cui, I. Yapici, B. Borhan, G. E. Reid, J. Am. Soc. Mass Spectrom. 25 (2014) 141-148.
- [12] M. Aguiar, W. Haas, S. A. Beausoleil, J. Rush, S. P. Gygi, J. Proteome Res. 9 (2010) 3103-3107.

- [13] N. Mischerikow, A. F. M. Altelaar, J. D. Navarro, S. Mohammed, A. J. R. Heck, *Mol. Cell. Proteom.* 9 (2010) 2140-2148.
- [14] J. W. Flora, D. C. Muddiman, *Anal. Chem.* 73 (2001) 3305–3311.
- [15] J. W. Flora, D. C. Muddiman, *J. Am. Chem. Soc.* 124 (2002) 6546–6547.
- [16] J. W. Flora, D. C. Muddiman, *J. Am. Soc. Mass Spectrom.* 15 (2004) 121–127.
- [17] M. C. Crowe, J. S. Brodbelt, *J. Am. Soc. Mass Spectrom.* 15 (2004) 1581–1592.
- [18] M. C. Crowe, J. S. Brodbelt, *Anal. Chem.* 77 (2005) 5726–5734.
- [19] C. Correia, P. Balaj, D. Scuderi, P. Maitre, G. Ohanessian, *J. Am. Chem. Soc.* 130 (2008) 3359–3370.
- [20] C. N. Stedwell, A. L. Patrick, K. Gulyuz, N. C. Polfer, *Anal. Chem.* 84 (2012) 9907-9912.
- [21] A. L. Patrick, C. N. Stedwell, N. C. Polfer, *Anal. Chem.* 86 (2014) 5547-5552.
- [22] F. Tureček, C. L. Moss, I. Pikalov, R. Pepin, K. Golyuz, N. C. Polfer, M. F. Bush, J. Brown, K. Richardson, *Int. J. Mass Spectrom.* 354-355 (2013) 249-256.
- [23] C. L. Kalcic, T. C. Gunaratne, A. D. Jones, M. Dantus, G. E. Reid, *J. Am. Chem. Soc.* 131 (2009), 940-942.
- [24] J. H. Moon, Y. S. Shin, M. S. Kim, *Int. J. Mass Spectrom.* 288 (2009) 16-21.
- [25] T.-Y. Kim, J. P. Reilly, *J. Am. Soc. Mass Spectrom.* 20 (2009) 2334-2341.
- [26] Y. S. Shin, J. H. Moon, M. S. Kim, *J. Am. Soc. Mass Spectrom.* 21 (2010) 53-59.
- [27] S. A. Smith, C. L. Kalcic, K. A. Safran, P. M. Stemmer, M. Dantus, G. E. Reid, *J. Am. Soc. Mass Spectrom.* 21 (2010) 2031-2040.
- [28] A. Stensballe, O. N. Jensen, J. V. Olsen, K. F. Haselmann, R. A. Zubarev, *Rapid Commun. Mass Spectrom.* 14 (2000) 1793-1800.
- [29] S. D.-H. Shi, M. E. Hemling, S. A. Carr, D. M. Horn, I. Lindh, F. W. McLafferty, *Anal. Chem.* 73 (2001) 19-.
- [30] S. M. M. Sweet, A. J. Creese, H. J. Cooper, *Anal. Chem.* 78 (2006) 7563-7569.
- [31] J. Wiesner, T. Premisler, A. Sickmann, *Proteomics* 8 (2008) 4466-.
- [32] A. J. Creese, H. J. Cooper, *J. Am. Soc. Mass Spectrom.* 19 (2008) 1263-1274.
- [33] S. M. M. Sweet, C. M. Bailey, D. L. Cunningham, J. K. Heath, H. J. Cooper, *ol. Cell. Proteom.* 8 (2009) 904-912.
- [34] A. Chi, C. Huttenhower, L. Y. Geer, J. J. Coon, J. E. P. Syka, D. L. Bai, J. Shabanowitz, D. J. Burke, O. G. Troyanskaya, D. F. Hunt, *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 2193-2198.

- [35] M. Huzarska, I. Ugalde, D. A. Kaplan, R. Hartmer, M. L. Easterling, N. C. Polfer, *Anal. Chem.* 82 (2010) 2873-2878.
- [36] F. Kjeldsen, A. M. B. Giessing, C. R. Ingrell, O. N. Jensen, *Anal. Chem.* 79 (2007) 9243-9252.
- [37] A. J. Kleinnijenhuis, F. Kjeldsen, B. Kallipolitis, K. F. Haselmann, O. N. Jensen, *Anal. Chem.* 79 (2007) 7450-7456.
- [38] C.-W. Liu, C.-C.; Lai, J. *Am. Soc. Mass Spectrom.* 22 (2011) 57-66.
- [39] T. A. Hansen, M. Sylvester, O. N. Jensen, F. Kjeldsen, *Anal. Chem.* 84 (2012) 9694-9699.
- [40] C. K. Frese, H. Zhou, T. Taus, A. F. M. Altelaar, K. Mechtler, A. J. R. Heck, S. Mohammed, *J. Proteome Res.* 12 (2013) 1520-1525.
- [41] Y. Lu, X. Zhou, P. M. Stemmer, G. E. Reid, *J. Am. Soc. Mass Spectrom.* 23 (2012) 577-593.
- [42] A. R. Ledvina, C. M. Rose, G. C. McAlister, J. E. P. Syka, M. S. Westphall, J. Griep-Raming, J. C. Schwartz, J. J. Coon, *J. Am. Soc. Mass Spectrom.* 24 (2013) 1623-1633.
- [43] S. Hayakawa, M. Hashimoto, H. Nagao, K. Awazu, M. Toyoda, T. Ichihara, Y. Shigeri, *Rapid Commun. Mass Spectrom.* 22 (2008) 567-572.
- [44] S. L. Cook, C. M. Zimmermann, D. Singer, F. Fedorova, R. Hoffmann, G. P. Jackson, *J. Mass Spectrom.* 47 (2012) 786-794.
- [45] V. D. Berkout, V. M. Doroshenko, *Int. J. Mass Spectrom.* 278 (2008) 150-157.
- [46] C. J. Shaffer, A. Marek, R. Pepin, K. Slováková, F. Tureček, *J. Mass. Spectrom.* 50 (2015) 470-475.
- [47] A. Marek, C. J. Shaffer, R. Pepin, K. Slováková, K. J. Laszlo, M. F. Bush, F. Tureček, *J. Am. Soc. Mass Spectrom.* 26 (2015) 415-431.
- [48] H. T. H. Nguyen, C. J. Shaffer, A. R. Ledvina, J. J. Coon, F. Tureček, *Int. J. Mass Spectrom.* 2014, doi:10.1016/j.ijms.2014.06.028.
- [49] H. T. H. Nguyen, C. J. Shaffer, F. Tureček, *J. Phys. Chem. B* 119 (2015) 3948-3961.
- [50] C. S. Byskov, F. Jensen, T. J. D. Joergensen, S. B. Nielsen, *Phys. Chem. Chem. Phys.* 16 (2014) 15831-15838.
- [51] N. G. Hendricks, N. M. Lareau, S. M. Stow, J. A. McLean, R. R. Julian, *J. Am. Chem. Soc.* 136 (2014) 13363-13370.

- [52] C. L. Moss, T. W. Chung, J. A. Wyer, S. B. Nielsen, P. Hvelplund, F. Tureček, *J. Am. Soc. Mass Spectrom.* 22 (2011) 731-751.
- [53] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery Jr., J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, O. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Revision A. 02, Gaussian, Inc., Wallingford, CT, 2009.
- [54] R. Pepin, K. J. Laszlo, B. Peng, A. Marek, M. F. Bush, F. Tureček, *J. Phys. Chem. A* 118 (2014) 308-324.
- [55] A.D. Becke, *Journal of Chemical Physics* 98 (1993) 1372–1377.
- [56] A.D. Becke, *Journal of Chemical Physics* 98 (1993) 5648–5652.
- [57] Y. Zhao, D.G. Truhlar, *Theoretical Chemistry Accounts* 120 (2008) 215–241.
- [58] F. Furche, R. Ahlrichs, *Journal of Chemical Physics* 117 (2002) 7433–7447.
- [59] H. Iikura, T. Tsuneda, T. Yanai, K. Hirao, *J. Chem. Phys.* 115 (2001) 3540-3544.
- [60] J. D. Chai, M. Head-Gordon, *J. Chem. Phys.* 128 (2008) 084106/1-084106/15
- [61] J. D. Chai, M. Head-Gordon, *Phys. Chem. Chem. Phys.* 10 (2008) 6615-6620.
- [62] L. Zhu, W.L. Hase, Quantum Chemistry Program Exchange, Indiana University, Bloomington, 1994, Program No. QCPE 644.
- [63] A.J. Frank, M. Sadilek, J.G. Ferrier, F. Turecek, *J. Am. Chem. Soc.* 119 (1997) 12343–12353.
- [64] A. Rauk, D. Yu, J. Taylor, G. V. Shustov, D. A. Block, D. A. Armstrong, *Biochemistry* 38 (1999) 9089-9096.
- [65] A. C. Hopkinson, *Mass Spectrom. Rev.* 28 (2009) 655-671.
- [66] Chung, T. W.; Turecek, F. *J. Am. Soc. Mass Spectrom.* **2010**, 21, 1279-1295.
- [67] J. A. Sonk H. B. Schlegel, *J. Phys. Chem. A* 115 (2011) 11832-11840.

- [68] V. Riffet, D. Jacquemin, E. Cauet, G. Frison, J. Chem. Theory Comput. 10 (2014) 3308-3318.
- [69] A. R. Ledvina, J. J. Coon, F. Tureček, Int. J. Mass Spectrom. 377 (2014) 44-53.
- [70] R. Pepin, F. Tureček, J. Phys. Chem. B, 119 (2015) 2818-2826.
- [71] F. Turecek, R. R. Julian, Chem. Rev. 113 (2013) 6691-6733.
- [72] C. S. Jensen, J. A. Wyer, J. Houmoller, P. Hvelplund, S. B. Nielsen, Phys. Chem. Chem. Phys. 13 (2011) 18373-18378.

Table 1. Ion Energies.

Ion/Reaction	Relative Energy ^{a,b}				
	B3LYP ^c	PMP2 ^c	B3-PMP2 ^{c,d}	M06-2X ^c	ωB97XD ^c
1	0	0	0	0	0
2	31	28	29	25	27
3	10	10	10	6	5
4	-25	-14	-20	-18	-19
1 → TS1	112	106	109	121	110
1 → TS2	127	150	139	149	151
1 → TS3	115	138	127	139	140
5	85	93	89	104	110
1 → 6 + CH ₃ CH ₂ CO [•]	105	124	115	137	138
1 → 6 + CH ₃ C [•] =COH	225	262	243	250	261
7 → 8	13	-14	-1	-8	-5
7 → TS4	124	109	116	117	118
7 → TS5	76	61	69	71	78
7 → 9	6	16	11	15	30
7 → 10 + 11	59	97	78	102	103

^aIn units of kJ mol⁻¹.^bIncluding zero-point vibrational energy corrections.^cSingle point calculations with the 6-311++G(2d,p) basis set.^dFrom averaged B3LYP and spin-projected PMP2 single-point energies.

Figure Captions

Figure 1. (a) Single-laser pulse UVPD mass spectrum of the z_4 ion $^{\bullet}\text{AApSR}^+$ at m/z 468. Asterisks denote peaks due to laser desorption-ionization of surface-deposited fluoranthene in the CID source. Inset shows the relative intensity of the m/z 468 precursor ion as a function of the number of laser pulses. The blue dashed curve is the least-squares exponential fit to the experimental data with the indicated root-mean-square deviation (rmsd). (b) Reference CID mass spectrum of $^{\bullet}\text{AApSR}^+$ obtained at NCE = 11 and 500 ms excitation time.

Figure 2. (a) Single-laser pulse UVPD mass spectrum of the z_4 ion $^{\bullet}\text{ApSAR}^+$ at m/z 468. Asterisks denote peaks due to laser desorption-ionization of surface-deposited fluoranthene in the CID source. Inset shows the relative intensity of the m/z 468 precursor ion as a function of the number of laser pulses. The blue curve is the least-squares exponential fit to the experimental data with the indicated root-mean-square deviation (rmsd). (b) Reference CID mass spectrum of $^{\bullet}\text{ApSAR}^+$ obtained at NCE = 11 and 500 ms excitation time.

Figure 3. (a) Single-laser pulse UVPD mass spectrum of the z_4 ion $^{\bullet}\text{pSAAR}^+$ at m/z 468. Asterisks denote peaks due to laser desorption-ionization of surface-deposited fluoranthene in the CID source. Inset shows the relative intensity of the m/z 468 precursor ion as a function of the number of laser pulses. The blue curve is the least-squares exponential fit to the experimental data with the indicated root-mean-square deviation (rmsd). (b) Reference CID mass spectrum of $^{\bullet}\text{pSAAR}^+$ obtained at NCE = 11 and 500 ms excitation time.

Figure 4. (a) UVPD mass spectrum of the z_4 ion $^{\bullet}\text{AAAR}^+$ at m/z 468 after 5 laser pulses. Inset shows the relative intensity of the m/z 468 precursor ion as a function of the number of laser pulses. The blue curve is the least-squares exponential fit to the experimental data with the indicated root-mean-square deviation (rmsd). (b) Reference CID mass spectrum of $^{\bullet}\text{AAAR}^+$ obtained at NCE = 11 and 500 ms excitation time.

Figure 5. TD-DFT calculated transition wavelengths and oscillator strengths for peptide z_4 ions. The absorption bands for the M06-2X (blue lines) and ωB97XD -calculated transitions (brown lines) were artificially broadened to 20 nm full width half maximum by applying Lorentzian fits.

Figure 6. Potential energy diagram for hydrogen atom migrations and dissociations of $^{\bullet}\text{AAAR}^+$ ions. The energies are from combined B3LYP and PMP2/6-311++G(2d,p) single-point energy calculations and include zero-point vibrational corrections. For energies calculated at other levels of theory see Table 1.

Scheme 1. Proposed reaction scheme for backbone dissociations upon UVPD of $^{\bullet}\text{AApSR}^+$. The curled arrows indicate both electron and hydrogen atom migrations.

Scheme 2. Backbone dissociation after loss of H_3PO_4 from $^{\bullet}\text{AApSR}^+$. The curled arrows indicate both electron and hydrogen atom migrations.

Scheme 1

Scheme 2

Figure 1. (a) Single-laser pulse UVPD mass spectrum of the z_4 ion $\bullet\text{AApSR}^+$ at m/z 468. Asterisks denote peaks due to laser desorption-ionization of surface-deposited fluoranthene in the ETD CI source. Inset shows the relative intensity of the m/z 468 precursor ion as a function of the number of laser pulses. The blue dashed curve is the least-squares exponential fit to the experimental data with the indicated root-mean-square deviation (rmsd). (b) Reference CID mass spectrum of $\bullet\text{AApSR}^+$ obtained at NCE = 11 and 500 ms excitation time.

Figure 2. (a) Single-laser pulse UVPD mass spectrum of the z_4 ion $\bullet\text{ApSAR}^+$ at m/z 468. Asterisks denote peaks due to laser desorption-ionization of surface-deposited fluoranthene in the ETD CI source. Inset shows the relative intensity of the m/z 468 precursor ion as a function of the number of laser pulses. The blue curve is the least-squares exponential fit to the experimental data with the indicated root-mean-square deviation (rmsd). (b) Reference CID mass spectrum of $\bullet\text{ApSAR}^+$ obtained at NCE = 11 and 500 ms excitation time.

Figure 3. (a) Single-laser pulse UVPD mass spectrum of the z_4 ion $\bullet$ pSAAR $^+$ at m/z 468. Asterisks denote peaks due to laser desorption-ionization of surface-deposited fluoranthene in the ETD CI source. Inset shows the relative intensity of the m/z 468 precursor ion as a function of the number of laser pulses. The blue curve is the least-squares exponential fit to the experimental data with the indicated root-mean-square deviation (rmsd). (b) Reference CID mass spectrum of $\bullet$ pSAAR $^+$ obtained at NCE = 11 and 500 ms excitation time.

Figure 4. (a) UVPD mass spectrum of the z_4 ion $\bullet AAAR^+$ at m/z 468 after 5 laser pulses. Inset shows the relative intensity of the m/z 468 precursor ion as a function of the number of laser pulses. The blue curve is the least-squares exponential fit to the experimental data with the indicated root-mean-square deviation (rmsd). (b) Reference CID mass spectrum of $\bullet AAAR^+$ obtained at NCE = 11 and 500 ms excitation time.

Figure 5. TD-DFT calculated transition wavelengths and oscillator strengths for peptide z_4 ions. The absorption bands for the M06-2X (blue lines) and ω B97XD-calculated transitions (brown lines) were artificially broadened to 20 nm full width half maximum by applying Lorentzian fits.

Figure 6. Potential energy diagram for hydrogen atom migrations and dissociations of $\bullet\text{AAAR}^+$ ions. The energies are from combined B3LYP and PMP2/6-311++G(2d,p) single-point energy calculations and include zero-point vibrational corrections. For energies calculated at other levels of theory see Table 1.