International Journal of Mass Spectrometry 390 (2015) 71–80

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Near-UV photodissociation of phosphopeptide cation-radicals

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ARTICLE INFO

Article history:
Received 27 March 2015
Received in revised form 13 May 2015
Accepted 18 May 2015
Available online 6 June 2015

Keywords:
Near-UV photodissociation
Phosphopeptide ions
Radicals
Absorption spectra
Dissociation mechanisms

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.

1. 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 (MS^n) 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 MS^3 spectra provide structure information on the radical and phosphoserine position.

2. Experimental

The phosphorylated peptides used in this work were custom synthesized by GenScript (Piscataway, NJ, USA) at 98–99%

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http://dx.doi.org/10.1016/j.ijms.2015.05.009
1387-3806/

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 (O_2) 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.

3. 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 $\boldsymbol{z_4}$ 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].

4. Results and discussion

4.1. 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 $\boldsymbol{z_1}$–$\boldsymbol{z_4}$ series of C-terminal fragment ions [52]. The isomeric $\boldsymbol{z_4}$ ions from ApSAAR, AApSAR, and AAApSR denoted respectively as $^{\bullet}$pSAAR$^+$, $^{\bullet}$ApSAR$^+$, and $^{\bullet}$AApSR$^+$ appear at m/z 468. The $\boldsymbol{z_4}$ ion from pSAAAR, denoted as $^{\bullet}$AAAR$^+$, appears at a distinct m/z 372. This $^{\bullet}$AAAR$^+$ ion was also generated by ETD of doubly charged AAAAR. The UVPD spectra of $^{\bullet}$AApSR$^+$ showed an exponential depletion of the precursor m/z 468 ion (Fig. 1a inset). The main dissociation products were the sequence fragment ion $(\boldsymbol{y_3}-2H)^+$ (m/z 411) and its secondary dissociation product (m/z 313) due to elimination of phosphoric acid. Loss of phosphoric acid from the $^{\bullet}$AApSR$^+$ ion formed the m/z 370 ion which further dissociated to the $(\boldsymbol{x_2}-H_3PO_4-H)^+$ 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 $^{\bullet}$AApSR$^+$ ion is compared to the CID spectrum (Fig. 1b). The latter shows a major loss of H_3PO_4 followed by loss of 100 Da, presumably the $CH_3CH_2CONHCH^{\bullet}(CH_3)$ 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 $\boldsymbol{z}$ ions have established that hydrogen atom migrations of this kind are mildly exothermic because of captodative stabilization of the internal HN—C_α—CO radical [64,65]. For steric reasons, the H_α 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_α atom migration typically requires $\leq$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_3CH_2CO radical, is an endothermic reaction requiring $\leq$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. Fig. 2a inset shows that the m/z 468 precursor ion underwent a very efficient photodepletion by loss of H_3PO_4 and backbone dissociations. The latter produces the $(\boldsymbol{x_2}-2H)^+$, $(\boldsymbol{y_2}-2H)^+$, $\boldsymbol{z_2}$, and $\boldsymbol{z_1}$ fragment ions at m/z 272, 244, 230, and 159, respectively (Fig. 2a). Herein, the $(\boldsymbol{y_3}-2H)^+$ 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 $(\boldsymbol{x_4}-H_3PO_4-H)^+$ 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 $^{\bullet}$ApSAR$^+$ shows a single dissociation channel by loss of H_3PO_4 while no backbone fragments are formed (Fig. 2b).

Substantial differences between UVPD and CID were also observed for $^{\bullet}$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 $(\boldsymbol{y_3}-2H)$ and $(\boldsymbol{y_2}-2H)$ ions at m/z 315 and 244, respectively (Fig. 3a). Photodepletion of $^{\bullet}$pSAAR$^+$ showed >40% efficiency per laser pulse (Fig. 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 $(\boldsymbol{y_3}-2H)^+$ and $(\boldsymbol{y_2}-2H)^+$ 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