International Journal of Mass Spectrometry 288 (2009) 16–21

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

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

Utility of reaction intermediate monitoring with photodissociation multi-stage (MS^n) time-of-flight mass spectrometry for mechanistic and structural studies: Phosphopeptides

Jeong Hee Moon [a], Young Sik Shin [b], Myung Soo Kim [b,*]

[a] Medical Proteomics Research Center, KRIBB, Daejeon 305-806, Republic of Korea
[b] Department of Chemistry, Seoul National University, San 56-1, Sillim-dong Gwanak-gu, Seoul 151-742, Republic of Korea

ARTICLE INFO

Article history:
Received 5 June 2009
Accepted 20 July 2009
Available online 25 July 2009

Keywords:
Reaction intermediate monitoring
Photodissociation
MS^n
Phosphopeptide

ABSTRACT

In tandem mass spectra of phosphopeptides, intact sequence ions are often missing or appear weakly. Instead, dephosphorylated sequence ions appear prominently. In this work, we used photodissociation (PD) multi-stage (MS^n) time-of-flight mass spectrometry that can monitor reaction intermediates with lifetime as short as 100 ns to study the formation of dephosphorylated sequence ions such as y_n-H_3PO_4. y_n-H_3PO_4 was found to be formed mainly by H_3PO_4 loss from y_n. For doubly phosphorylated peptides, y_n seemed to lose H_3PO_4 stepwise and form y_n-H_3PO_4 and y_n-$2H_3PO_4$. Even when y_n was absent in PD-MS^2 spectrum, its m/z could be predicted from those of y_n-H_3PO_4 and/or y_n-$2H_3PO_4$. Complete sequence coverage was possible when the data from PD-MS^2 and PD-MS^3 were combined, demonstrating the utility of transient ion detection by PD-MS^3 for structure analysis.

1. Introduction

It is well known that a mass spectral pattern is the result of competing and consecutive reactions of a primary ion formed by ionization [1]. The same applies to tandem mass spectrometry [2–4] that detects product ions formed from a selected precursor ion. A product ion formed from a precursor ion may not appear in tandem mass spectrum if the lifetime of the product ion is shorter than the operational time scale of the tandem mass spectrometer used. Instead, its consecutive dissociation product(s) may appear. Absence of important product ions in a spectrum due to their transient nature may cause some difficulty in mechanistic and structural studies.

Recently, we developed time-resolved ultraviolet photodissociation (UV-PD) tandem time-of-flight (TOF) mass spectrometry [5] for peptide ions formed by matrix-assisted laser desorption/ionization (MALDI). Photoexcitation was done inside a cell floated at high voltage. Let us suppose that the photoexcited precursor ion (m_1^+) can dissociate to a product ion (m_2^+) both inside and outside the cell. Due to the voltage on the cell, the kinetic energy of m_2^+ after the cell will be different depending on whether it is formed rapidly inside the cell (in-cell component, I) or slowly outside the cell (post-cell component, P). Hence, the two components are split in the voltage-on, or time-resolved, PD-tandem TOF spectrum. In actual experiments, we observed additional components due to the formation of the same ion (m_2^+) by consecutive reactions (consecutive components, C), $m_1^+ \rightarrow m_i^+ \rightarrow m_2^+$. Here, the photoexcited precursor ion dissociates rapidly to an intermediate ion (m_i^+) inside the cell. Then, m_i^+ further dissociates spontaneously to m_2^+ outside the cell. This is a form of MS^3 [6,7]. By using a cell with short length, it was possible to monitor m_i^+ formed rapidly inside the cell (within 100 ns after photoexcitation). More importantly, it was realized that reaction intermediate monitoring by PD-MS^3 would allow detection of transient species with a lifetime as short as 100 ns. Since only one activation step was used unlike in conventional MS^3 [8], the number of the second stage reactions ($m_i^+ \rightarrow m_2^+$) was limited. In addition, MS^3 for all m_2^+ was done simultaneously, viz. detection was fully multiplexed. This resulted in MS^3 sensitivity that was only a little poorer than MS^2. In a subsequent work, we built an improved instrument that could determine intermediate ion (m_i^+) masses within 4 Da [9].

Phosphorylation of serine (S), threonine (T), and tyrosine (Y) residues in a protein is the key mode of signal transduction and amplification for eukaryotes [10,11]. In tandem mass spectra of S/T-phosphorylated peptide ions [12,13] recorded by conventional techniques such as post-source decay (PSD) and collisionally activated dissociation (CAD), intact sequence ions such as b_n and y_n are often missing or appear weakly. Instead, sequence ions with phosphate loss such as y_n-H_3PO_4 and y_n-HPO_3 appear prominently. Whether dephosphorylated sequence ions such as y_n-H_3PO_4 would be formed from $[M+H]^+$ via $[M+H-H_3PO_4]^+$ or via y_n is an open question. For a peptide ion with S or T residues, a sequence ion such as

* Corresponding author. Tel.: +82 02 880 6652; fax: +82 02 889 1568.
E-mail address: myungsoo@snu.ac.kr (M.S. Kim).

1387-3806/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2009.07.008

y_n is often accompanied by a peak with H_2O loss. Hence, (y_n-H_3PO_4, y_n-HPO_3) pair may be confused with (y_n-H_2O, y_n) [12], an obstacle to structure determination. Let us suppose that y_n-H_3PO_4 is formed by $[M+H]^+ \rightarrow y_n \rightarrow y_n$-$H_3PO_4$. Identification of the intermediate ion, y_n, in this consecutive reaction—a task that requires three-stage tandem mass spectrometry (MS^3)—will prove that the final product ion in question (y_n-H_3PO_4 in the above case) is a dephosphorylated ion. This, in turn, will help to determine m/z of the intact sequence ion (y_n in the above case). However, it is difficult to detect an intermediate ion by conventional MS^3 techniques when its lifetime is shorter than the apparatus time scale.

In this work, dissociation of singly and doubly phosphorylated peptide ions with multiple S/T residues was investigated with PD-MS^3. Formation mechanisms for dephosphorylated sequence ions were determined by reaction intermediate monitoring. In particular, m/z information for intact sequence ions could be obtained even when they were absent in MS^2 spectrum.

2. Experimental

A schematic drawing of the homebuilt MALDI-tandem TOF equipped with a PD cell assembly used in this work is shown in Fig. 1. Details of the instrument and its operation were reported previously [9]. A brief description is as follows.

The apparatus consists of a MALDI source with delayed extraction, a linear reflectron, a voltage-floated PD cell, a quadratic reflectron, and the final detector. Two ion gates are installed on the ion trajectory, one in front of and the other after the linear reflectron. To guide the precursor ion beam, two deflectors are installed, one in front of and the other after the linear reflectron. The linear reflectron is installed simply for high-resolution selection of a precursor ion, viz. it is not the second-stage analyzer for tandem mass spectrometry. The PD cell assembly consists of four apertures E1–E4, with E1 and E4 grounded and E2 and E3 floated at the same high voltage. PD laser passes between E2 and E3. Time separation of the in-cell (I), post-cell (P), and consecutive (C) components of a product ion (m_2^+) occurs during their flight to the entrance of the quadratic reflectron. This reflectron time separates signals from different m_2^+.

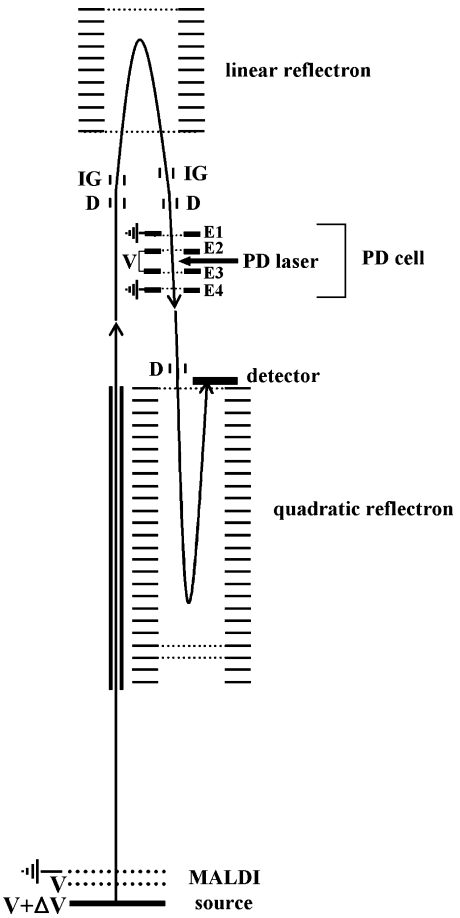

Fig. 1. A schematic drawing of PD-tandem TOF. A peptide ion pulse selected by the linear reflectron is irradiated by PD laser at the center of the voltage-floated PD cell consisting of four electrodes. Product ions formed inside and outside the cell and those formed via consecutive reactions are analyzed by the quadratic reflectron. D and IG indicate deflector and ion gate, respectively.

Details of the methods to record MALDI-TOF (MS), post-source decay (PSD, MS^2), PD (MS^2), and voltage-on PD (MS^3) spectra were reported previously [9]. A nitrogen laser is used for MALDI. Two ion gates are turned off to record MALDI-TOF spectra, and turned on for MS^2 and MS^3. In PD experiments, 193 nm pulse of an excimer laser is synchronized with the lowest mass isotopomer pulse of a precursor ion. The laser-off spectrum is subtracted from the laser-on spectrum to get PD spectrum.

Another MALDI-tandem TOF [14,15] equipped with only one reflectron that had been reported previously was used to record PSD spectra. A Q/TOF instrument (API QStar Pulsar I, Applied Biosystems, Carlsbad, CA, USA) was used to record low-energy CAD spectra for phosphopeptide ions generated by ESI.

2.1. Samples

All the phosphopeptides used in this work were purchased from Peptron (Daejeon, Korea). Other chemicals including the matrix, 2,5-dihydroxybenzoic acid (DHB), were purchased from Sigma (St. Louis, MO, USA). The method to prepare MALDI samples was the same as described previously [5].

3. Results and discussion

Regardless of the energy regime used to record tandem mass spectra in this work (low-energy regime: PSD and low-energy CAD, high energy regime: UV-PD), b and y type ions were major dissociation products from singly protonated peptides without an arginine residue. We will present our findings using phosphopeptides with lysine at the C-terminus as examples, a subset of tryptic peptides that are popularly used for protein sequencing. Results from the other subset, peptides with arginine at the C-terminus will be explained briefly thereafter.

3.1. Monophosphorylated peptides

PSD spectrum of $[ASSpSGK+H]^+$—pS denotes phosphorylated serine residue—recorded by MALDI-tandem TOF, its low-energy CAD spectrum recorded by ESI-Q/TOF, and 193 nm PD (voltage-off) spectrum are shown in Fig. 2. In PSD and CAD spectra, losses of H_2O, HPO_3, H_3PO_4, $H_3PO_4 + H_2O$, and $H_3PO_4 + 2H_2O$ from the precursor ion appear conspicuously. $[M+H-H_3PO_4]^+$ is more intense than $[M+H-HPO_3]^+$ in agreement with a previous report [12] on S/T-phosphorylated peptide ions. Similar neutral losses are also observed from the sequence ions b and y. b type product ions with H_3PO_4 and HPO_3 losses will be denoted b_n^* and b_n°, respectively. Similar notations will be used for y type ions also. The product ions appearing in PSD are listed in Table 1. Overall, y_n and related ions are more intense than b_n and related ions, probably due to the presence of lysine at the C-terminus. It is to be noted that y_n^* is observed for $n = 3$–5 and b_n^* for $n = 4$ and 5. That is, dephosphorylated sequence ions appear whenever the corresponding intact sequence ions contain a phosphate group, regardless of its position. The fact that y_3^* is the first of the y_n^* series, and b_4^* is the first of b_n^* also, is consistent

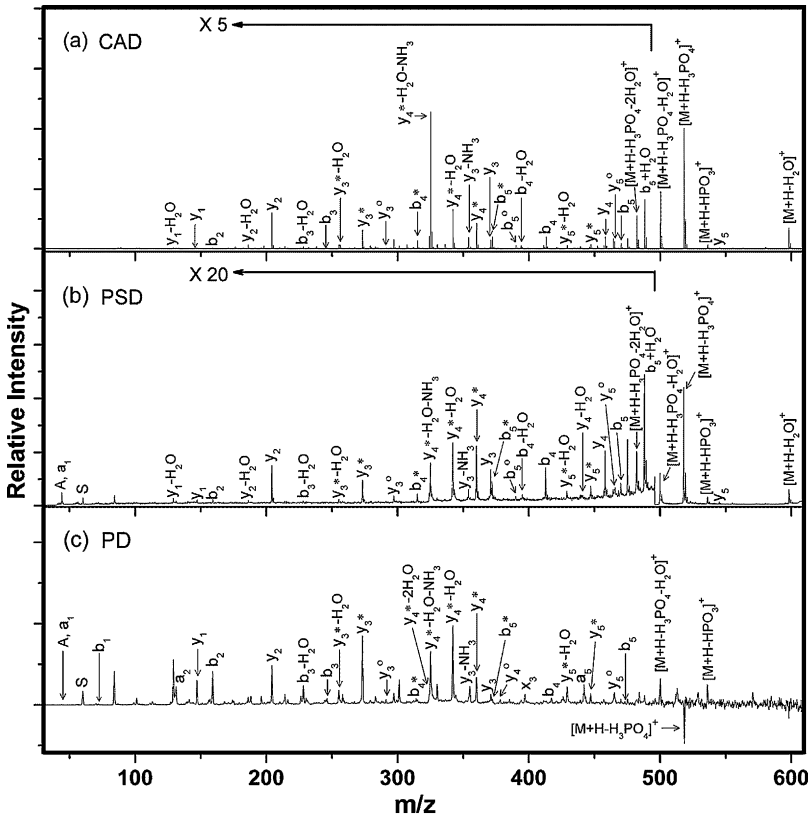

Fig. 2. (a) Low-energy CAD, (b) PSD, and (c) 193 nm PD (voltage-off) spectra of $[ASSpSGK+H]^+$. Precursor ions were generated by ESI in (a) and by DHB-MALDI in (b) and (c).

with the position of the phosphorylated residue. For all the phosphopeptide ions studied in this work, spectral patterns of CAD were similar to those of PSD and hence will not be discussed.

In 193 nm PD spectrum, the $[M+H-H_3PO_4]^+$ signal appears as negative. That is, its intensity in the presence of PD laser is weaker than in its absence (PSD). This happens [15,16] when loss of H_3PO_4 from the photoexcited precursor ion does not occur as preferentially as from the unexcited ion (in PSD) or when $[M+H-H_3PO_4]^+$ formed by PSD also undergoes efficient PD at 193 nm. Product ions formed by UV-PD are similar to those formed by PSD. They are listed in Table 1 also. One of the main differences between PSD and PD is in the relative intensities of (y_n, y_n^*) pairs. They are comparable in PSD except for $n=5$. In contrast, y_3 is weak and y_4 and y_5 are difficult to detect while y_3^*, y_4^*, and y_5^* are prominent in PD. Here again, it is to be noted that y_n^* appears whenever the corresponding y_n contains a phosphate group.

So far, it has been shown that the spectral patterns in PSD, low-energy CAD, and UV-PD are consistent with the sequence of the peptide and the position of the phosphorylated residue. Now, let us consider the situation from the analytical point of view. If the sequence of the corresponding unphosphorylated peptide is known already, phosphorylation at the third residue from the C-terminus can be readily identified from the presence of y_n^* ($n=3$–5), y_n° ($n=3$, 5), and y_n^*-H_2O ($n=3$–5). Otherwise, definite determination of the peptide sequence and phosphorylation site would be difficult due to the presence of many product ion peaks that cannot be easily assigned.

Some portions of 193 nm PD spectrum recorded with high voltage (5 kV) on the cell are shown in Fig. 3. In-cell, post-cell, and consecutive components are marked as I, P, and C, respectively. The intermediate ion assignment for each C based on its *m/z* determined by the method reported previously [9] is also shown in the figure. Let us first consider the product ion peak assigned to y_3^* in voltage-off PD. When high voltage was applied to the cell, this peak split into I, P, and four (or more) C components. The intermediate ions determined for the C components were y_3, y_5^*, $[M+H-H_3PO_4]^+$, and $[M+H-HPO_3]^+$. Among these, y_3 was the most prominent. That is, y_3^* was formed mainly by H_3PO_4 loss from y_3. Similarly, y_4

Table 1
Sequence ions, both intact and dephosphorylated, appearing in PSD and 193 nm PD spectra for phosphorylated peptide ions.[a].

Sample	PSD	PD
ASSpSGK	b_n ($n=2, 4, 5$), b_n^* ($n=4, 5$), b_n° ($n=5$), y_n ($n=1$–5), y_n^* ($n=3$–5), y_n° ($n=3$, 5), y_n^*-H_2O ($n=3$–5)	b_n ($n=1$–5), b_n^* ($n=4, 5$), y_n ($n=1$–3), y_n^* ($n=3$–5), y_n° ($n=3$–5), y_n^*-H_2O ($n=3$–5)
ASpSpSGK	b_n^* ($n=4, 5$), b_n° ($n=5$), y_n ($n=1$–5), y_n^* ($n=3$–5), y_n° ($n=4$), y_n^{**} ($n=4$), $y_n^{*\circ}$ ($n=4$)	b_n ($n=2$–4), b_n^* ($n=3$–5), b_n° ($n=5$), y_n ($n=1$–4), y_n^* ($n=3$–5), y_n° ($n=3$), y_n^{**} ($n=4, 5$), $y_n^{*\circ}$ ($n=4, 5$), $y_n^{\circ\circ}$ ($n=5$)
ApSSpSGK	b_n ($n=4$), b_n^* ($n=2, 4, 5$), b_n° ($n=5$), b_n^{**} ($n=4, 5$), y_n ($n=2, 3$), y_n^* ($n=3$–5), y_n^{**} ($n=5$), $y_n^{*\circ}$ ($n=5$)	b_n ($n=2, 3$), b_n^* ($n=2$–5), b_n° ($n=2, 4, 5$), b_n^{**} ($n=4, 5$), $b_n^{*\circ}$ ($n=4, 5$), y_n ($n=1$–4), y_n^* ($n=3$–5), y_n° ($n=3$), y_n^{**} ($n=5$), $y_n^{*\circ}$ ($n=5$)
VAAApSIR	a_n ($n=1, 2$), a_n^* ($n=6$), b_n ($n=1$–4, 6), b_n^* ($n=5, 6$), x_n ($n=1$), x_n^* ($n=3$), y_n ($n=1$–6), y_n^* ($n=3$–6), y_n° ($n=6$), y_n-NH_3 ($n=5$), y_n^*-NH_3 ($n=3$–6),	a_n ($n=1, 2$), a_n^* ($n=6$), b_n ($n=1$–3), b_n^* ($n=5$), x_n ($n=1$–6), x_n^* ($n=3$–6), y_n ($n=1$–5), y_n^* ($n=3$–6), y_n° ($n=3$), y_n^*-NH_3 ($n=3$–6), v_n ($n=2$, 4–6),

[a] Losses of HPO_3 and H_3PO_4 are denoted by the superscripts $^\circ$ and *, respectively.

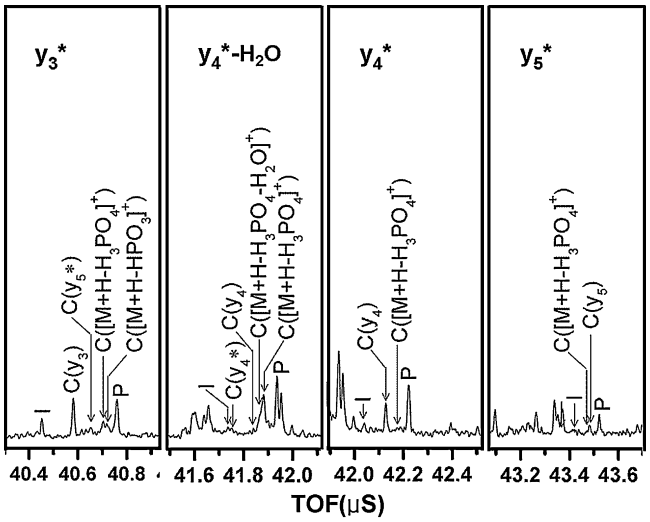

Fig. 3. Splitting patterns of y_3^*, y_4^*-H_2O, y_4^*, and y_5^* in 193 nm PD spectra of $[ASSpSGK+H]^+$ recorded with 5 kV on the cell. In-cell, post-cell, and consecutive components of each peak are marked as I, P, and C, respectively. The intermediate ion for each C component is written inside the parenthesis.

and y_5 were the most important intermediate ions for y_4^* and y_5^*, respectively. We also performed voltage-on PD experiments for several other peptide ions. In all the cases, $[M+H]^+ \rightarrow y_n \rightarrow y_n^*$ was the main channel for the consecutive formation of y_n^*, while $[M+H]^+ \rightarrow [M+H–H_3PO_4]^+ \rightarrow y_n^*$ was an important but minor channel. Evidence for H_3PO_4 loss could be found for $y_n^* - H_2O$ also. For example, y_4^*, y_4, $[M+H–H_3PO_4–H_2O]^+$, and $[M+H–H_3PO_4]^+$ were the intermediate ions for the consecutive formation of y_4^*-H_2O.

We have mentioned that the difficulty in detecting intact sequence ions by conventional tandem mass spectrometry is the main problem in its use for phosphopeptide analysis. In PD of the present sample, y_n ($n = 1$ and 2) appears prominently when it is unphosphorylated. When phosphorylated ($n \geq 3$), it becomes weak or difficult to detect. In its stead, y_n^* appears prominently. In fact, y_n^* ions, whenever they can appear, do appear prominently in PD of all the phosphopeptide ions studied in this work. That is, y_n together with y_n^* form a contiguous series. By utilizing information from PD-MS^3, this mixed series can be converted to a contiguous series of y_n, which can be useful for structure determination.

3.2. Doubly phosphorylated peptides

Investigations were made for several doubly phosphorylated peptides with multiple S/T to test the general applicability of the mechanistic results found for monophosphorylated peptides. PSD and voltage-off 193 nm PD spectra of $[ASpSpSGK+H]^+$ are shown in Fig. 4 together with voltage-on splitting patterns for some peaks in PD spectrum. Product ions appearing in these spectra are listed in Table 1. Due to double phosphorylation, a wider variety of neutral losses are observed than in the monophosphorylated cases, such as $H_3PO_4 + HPO_3$, $2H_3PO_4$, and $2H_3PO_4 + H_2O$. Product ions with $H_3PO_4 + HPO_3$, $2H_3PO_4$, and $2HPO_3$ losses from a sequence ion such as y_n are also observed. They are denoted as $y_n^{*\circ}$, y_n^{**}, and $y_n^{\circ\circ}$, respectively. The fact that y_n^* ions are observed for $n = 3$–5 and y_n^{**} for $n = 4$ and 5 is consistent with double phosphorylation, one at the third residue and the other at the fourth residue from the C-terminus.

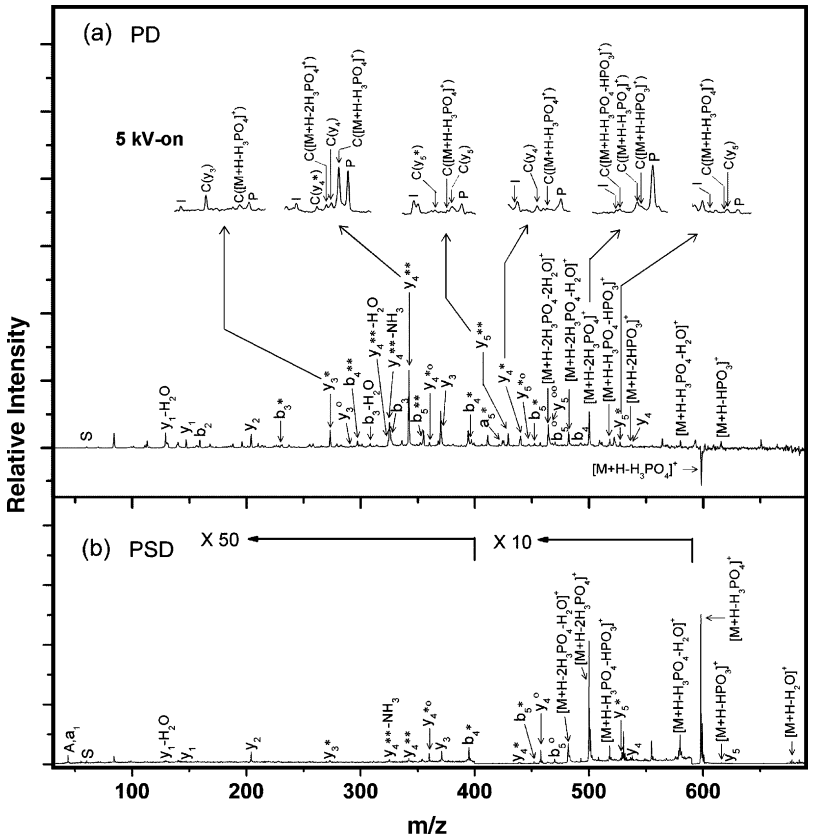

Fig. 4. (a) Voltage-off 193 nm PD and (b) PSD spectra of $[ASpSpSGK+H]^+$. Splitting patterns of y_3^*, y_4^{**}, y_5^{**}, y_4^*, y_5^*, and $[M+H–2H_3PO_4]^+$ in PD due to 5 kV on the cell are also shown in (a). In-cell, post-cell, and consecutive components of each peak are marked as I, P, and C, respectively. The intermediate ion for each C component is written inside the parenthesis.

Table 2
Important product ions formed by 193 nm PD of $[ASpSpSGK+H]^+$ and residue assignments based mass differences between intact sequence ions.

m/z	Character[a]	Δ(m/z)	Residue assignment
129.2	Loss of H_2O		
			K
147.2			
		56.9	
159.1			G
204.1			
273.2	Loss of H_3PO_4		
324–326	Composite peaks		
		167.0	$S + H_3PO_4 - H_2O$
342.1	Loss of $2H_3PO_4$		
368.0			
371.1[b]			
429.3	Loss of $2H_3PO_4$		
440.0	Loss of H_3PO_4		
464.3	$[M + H - 2H_3PO_4 - 2H_2O]^+$		
		166.8	$S + H_3PO_4 - H_2O$
482.2	$[M + H - 2H_3PO_4 - H_2O]^+$		
518.3	$[M + H - H_3PO_4 - HPO_3]^+$		
527.1	Loss of H_3PO_4		
537.9[c]			
580.0	$[M + H - H_3PO_4 - H_2O]^+$		
		87.2	S
616.0	$[M + H - HPO_3]^+$		
625.1[d]			
		71.1	A
696.2	$[M + H]^+$		

[a] Determined by PD-MS^3.
[b] Identified as an intact sequence ion based on 273.2 + 98.0 (H_3PO_4) = 371.2.
[c] Identified as an intact sequence ion based on 342.1 + 2 × 98.0 ($2H_3PO_4$) = 538.1 and 440.0 + 98.0 (H_3PO_4) = 538.0.
[d] Identified as an intact sequence ion based on 429.3 + 2 × 98.0 ($2H_3PO_4$) = 625.3 and 527.1 + 98.0 (H_3PO_4) = 625.1.

We also recorded PSD and PD spectra for $[ApSSpSGK+H]^+$ (spectra not shown). Neutral losses observed were similar to the previous case. Sequence ions, both intact and dephosphorylated, observed in these spectra are listed in Table 1. In both spectra, b_n^* and b_n^{**} start from $n = 2$ and 4, respectively. y_n^* starts from $n = 3$, while y_n^{**} occurs only for $n = 5$. These are consistent with double phosphorylation, one at the second residue and the other at the fourth residue from the N-terminus.

Voltage-on splitting patterns of some important product ions in 193 nm PD of $[ASpSpSGK+H]^+$ are shown in Fig. 4. First to be noted from the figure is that neutral loss peaks can be identified through reaction intermediate monitoring. For example, the peak at *m/z* 500.2 in voltage-off PD spectrum can be unequivocally identified as $[M+H-2H_3PO_4]^+$ based on the neutral losses of $2H_3PO_4$, $H_3PO_4 + HPO_3$, H_3PO_4, and HPO_3 involved in its consecutive formation from the precursor ion. Even more important is that dephosphorylated sequence ions can be readily identified. For y_n^* peaks, the intermediate ions marked in the figure are the same types as in the case of monophosphorylated peptides, viz. y_n and $[M+H-H_3PO_4]^+$. In particular, it is to be noted that y_5 is found as a precursor (intermediate) to y_5^*, even though y_5 itself is difficult to find in voltage-off PD spectrum. For y_n^{**} ($n = 4$, 5) ion, y_n^*, y_n, and $[M+H-H_3PO_4]^+$ are intermediate ions. That is, these peaks are mainly formed via H_3PO_4 loss from y_n^* and $2H_3PO_4$ loss from y_n, leading to their definite identification as product ions with $2H_3PO_4$ loss.

In PD spectrum of $[ASpSpSGK+H]^+$ shown in Fig. 4, y_n ($n = 1$ and 2) peaks are prominent only when they are unphosphorylated. In contrast, y_n^* ($n = 3$–5) appears when y_n contains one or two phosphate groups while y_n^{**} ($n = 4$, 5) appears only when y_n contains two phosphate groups. In addition, y_n^* is weaker than y_n^{**} whenever y_n^{**} can be formed. Hence, a contiguous, even though mixed, series of (y_n, y_n^*, y_n^{**}) can be formed from the prominent peaks in the spectrum. This can be converted to the contiguous y_n series by utilizing information from PD-MS^3. A method to utilize PD-MS^3 information for structure determination is summarized in Table 2. In this table, *m/z* values for all the prominent peaks in PD spectrum of $[ASpSpSGK+H]^+$ are listed. *m/z* values for intact sequence ions estimated from those with one or two H_3PO_4 losses are also included in the table even when they are difficult to find in PD spectrum. Then, *m/z* differences between adjacent intact sequence ions are calculated and used to identify the residues responsible for the differences. The correct structure of the peptide is obtained by connecting these residues, demonstrating the utility of PD-MS^3 in the structure determination of phosphopeptides.

3.3. Peptides with arginine at the C-terminus

In PSD spectrum of $[VAAApSIR+H]^+$ shown in Fig. 5, neutral losses of H_3PO_4 and HPO_3 occur prominently, with the relative intensities $[M+H-H_3PO_4]^+ > [M+H-HPO_3]^+$ as in the previous cases. Sequence ions, both intact and dephosphorylated, observed in PSD are listed in Table 1. y_n, y_n^*, and y_n° are important sequence ions in this case. The fact that y_n^* starts from $n = 3$ is consistent with phosphorylation at $n = 3$ (from the C-terminus).

Tandem mass spectral pattern for an unphosphorylated peptide with arginine at the C-terminus recorded in high energy regime is known to be dramatically different from those in the low-energy regime [17,18]. x, v, and w type product ions are dominant, while y type ions appear weakly. Homolytic C_α—CO cleavage [19] is thought to form $x_n + 1$ radical cation initially, which further dissociates to x_n, v_n, and w_n. In voltage-off PD spectrum of $[VAAApSIR+H]^+$ shown in Fig. 5, the neutral loss peaks $[M+H-H_3PO_4]^+$ and $[M+H-HPO_3]^+$ are very prominent, unlike in PD of phosphopeptide ions with lysine

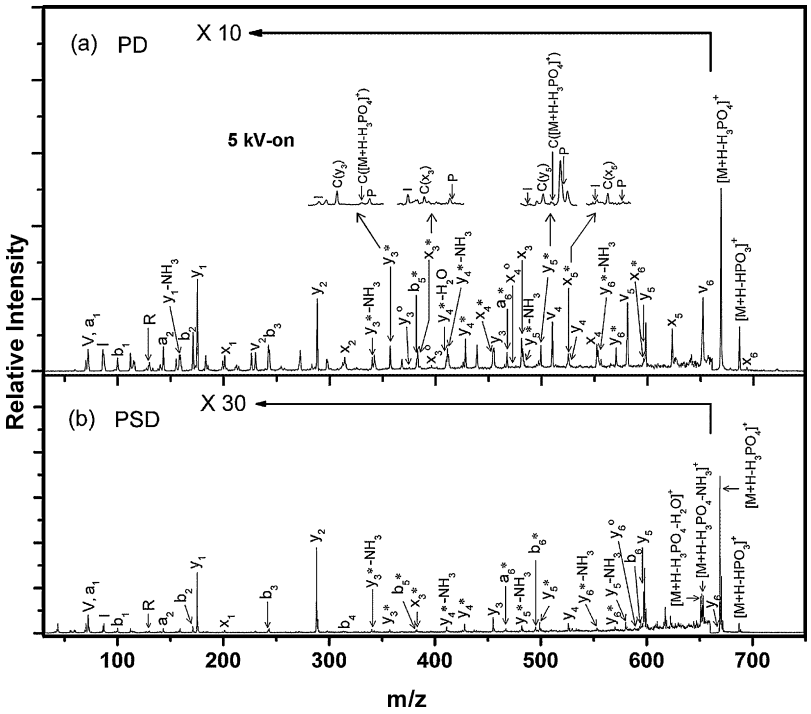

Fig. 5. (a) Voltage-off 193 nm PD and (b) PSD spectra of $[VAAApSIR+H]^+$. Splitting patterns of y_3^*, x_3^*, y_5^*, and x_5^* in PD due to 5 kV on the cell are also shown in (a). In-cell, post-cell, and consecutive components of each peak are marked as I, P, and C, respectively. The intermediate ion for each C component is written inside the parenthesis.

at the C-terminus. Sequence ions appearing in PD spectrum are listed in Table 1. As in PD of unphosphorylated peptide ions with arginine at the C-terminus, y_n peaks with large m/z are weak. On the other hand, y_n^* peaks, whenever they can appear, do appear prominently. Even though x_n^* peaks also appear, they are not as prominent as y_n^*. Voltage-on splitting patterns for some y_n^* and x_n^* are shown in Fig. 5. Both y_n and $[M+H-H_3PO_4]^+$ are the intermediate ions in consecutive formation of y_n^*, just as for peptide ions without an arginine residue. For x_n^*, x_n is the main intermediate ion. We have mentioned that $x_n + 1$ is thought to be the precursor to x_n. We could not confirm this from the voltage-on splitting patterns of x_n ions because of poor spectral resolution (several Da). Prominent y_n^* ions can be especially useful for structure analysis because identification of their nature (one H_3PO_4 loss) by PD-MS^3 leads to contiguous y_n series.

4. Conclusion

In tandem mass spectrometry of molecules with many labile bonds, an important product ion may further dissociate to smaller ions. Absence or weak appearance of the product ion due to its rapid dissociation can be an obstacle to mechanistic understanding of the dissociation process.

Tandem mass spectra of phosphopeptide ions are more complex than those of their unphosphorylated counterparts, which are attributed to the participation of the phosphate loss channels. However, mechanistic details of the process have been unknown. A more serious problem from the analytical point of view is that some of the intact sequence ions are missing or appear very weakly in the spectra. By reaction intermediate monitoring with PD-MS^3, we have found that dephosphorylated sequence ions such as y_n^* are formed mainly via their intact counterparts (y_n in this case). With the capability of PD-MS^3 to detect transient intermediate ions with lifetime as short as 100 ns, y_n could be detected even when it was absent in PD-MS^2 spectrum. Combining the information from PD-MS^3 and PD-MS^2, contiguous series of sequence ions could be obtained, which can be useful for phosphopeptide analysis.

Acknowledgments

This work was financially supported by Korea Research Foundation, Republic of Korea and by the Bio-signal Analysis Technology Innovation program (M10645010002-06N4501-00210) of the Ministry of Education, Science, and Technology, Republic of Korea. Y.S. Shin thanks the Ministry of Education, Science, and Technology, Republic of Korea, for Brain Korea 21 Fellowship.

References

[1] F.W. McLafferty, F. Tureček, Interpretation of Mass Spectra, fourth ed., University Science Books, Mill Valley, CA, 1993.
[2] K.L. Busch, G.L. Glish, S.A. McLuckey, Mass Spectrometry/Mass Spectrometry, VCH, Weinheim, 1988.
[3] P. Hernandez, M. Muller, R.D. Appel, Mass Spectrom. Rev. 25 (2006) 235.
[4] F.W. McLafferty, Tandem Mass Spectrometry, Wiley-Interscience, New York, 1983.
[5] S.H. Yoon, M.S. Kim, J. Am. Soc. Mass Spectrom. 18 (2007) 1729.
[6] J. Schwartz, A.P. Wade, C.G. Enke, R.G. Cooks, Anal. Chem. 62 (1990) 1809.
[7] K.D. Ballad, S.J. Gaskell, Int. J. Mass Spectrom. Ion Process. 111 (1991) 173.
[8] T. Solouki, L. PasaTolic, G.S. Jackson, S.G. Guan, A.G. Marshall, Anal. Chem. 68 (1996) 3718.
[9] Y.S. Shin, J.H. Moon, M.S. Kim, Anal. Chem. 80 (2008) 9700.
[10] T. Hunter, Cell 100 (2000) 113.
[11] J.M. Berg, J.L. Tymoczko, L. Stryer, Biochemistry, Freeman, 2002.
[12] R.S. Annan, S.A. Carr, Anal. Chem. 68 (1996) 3413.
[13] K.L. Bennett, A. Stensballe, A.V. Podtelejnikov, M. Moniatte, O.N. Jensen, J. Mass Spectrom. 37 (2002) 179.
[14] J.H. Moon, S.H. Yoon, M.S. Kim, Bull. Korean Chem. Soc. 26 (2005) 763.
[15] S.H. Yoon, J.H. Moon, K.M. Choi, M.S. Kim, Rapid Commun. Mass Spectrom. 20 (2006) 2201.
[16] J.Y. Oh, J.H. Moon, M.S. Kim, J. Am. Soc. Mass Spectrom. 15 (2004) 1248.
[17] K. Biemann, Methods in Enzymology, Mass Spectrometry, Academic Press, San Diego, CA, 1990, pp. 455–479.
[18] J.H. Moon, S.H. Yoon, M.S. Kim, Rapid Commun. Mass Spectrom. 19 (2005) 3248.
[19] M.S. Thomson, W. Cui, J.P. Reilly, Angew. Chem. Int. Ed. 43 (2004) 4791.