International Journal of Mass Spectrometry 281 (2009) 24–30

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

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

Study of acetylation on Ser/Thr/Tyr/Lys, and trimethylation on Lys using electrospray tandem mass spectrometry

Yan Li, Haydn L. Ball*

Protein Chemistry Technology Center, Department of Internal Medicine, University of Texas Southwestern Medical Center, 6001 Forest Park Rd., Dallas, TX 75390, USA

ARTICLE INFO

Article history:
Received 26 August 2008
Received in revised form 11 November 2008
Accepted 24 November 2008
Available online 6 December 2008

Keywords:
Post-translational modification
Fragmentation
Acetylation
Trimethylation
Fragmentation efficiency

ABSTRACT

Post-translational modifications (PTM) corresponding to a gain in mass of 42 Da are of increasing interest. It has been widely recognized that acetylation and trimethylation on Lys regulates gene transcription and silencing. In addition, it was recently discovered that acetylation of Ser and Thr residues on a signaling kinase can block its activation. In this paper, three series of model peptides were chemically synthesized to generate comparative MS data. Electrospray collision-induced dissociation tandem mass spectrometry was used to characterize the fragmentation pattern of acetylation on Ser, Thr, and Tyr residues. In separate experiments, the fragmentation pattern and efficiency were studied for acetylation and trimethylation on Lys. Our results confirmed those previously reported, that a characteristic immonium ion at m/z 126 corresponds to an acetylated Lys, and we further differentiated acetylation from trimethylation by their effects on peptide fragmentation efficiency. With the same primary sequence, a trimethylated peptide requires higher energy to fragment compared to the acetylated analogue. For peptides containing acetylated Ser, the y-60 and b-60 ions are commonly observed when the acetylation site is at, or close to, the C-terminus or N-terminus of the daughter ion, respectively; for acetylated Thr, in addition to y-60 and b-60 ions, y-42 ions are usually dominant. The loss of 42 Da and 60 Da can correspond to the loss of CH_2CO through deacetylation and CH_3COOH through β-elimination, respectively. Meanwhile, loss of 42 Da and 18 Da individually can also contribute to the loss of 60 Da. When peptide containing acetylated Tyr/Lys is fragmented, the acetyl group remains attached to their respective side-chains. The fragmentation pattern was similar whether the acetylation site was close to C-terminus or N-terminus of the peptide. This study provides a better understanding of the MSMS fragmentation character of peptides with acetylation on Ser, Thr, and Tyr, and the differences between acetylated and trimethylated Lys.

Published by Elsevier B.V.

1. Introduction

Protein acetylation is a form of post-translational modification (PTM) known to regulate many diverse biological processes [1–3]. The acetylation of protein amino terminii and the side-chain of Lys residues are well known phenomena. To date research has focused almost exclusively on acetylation on Lys. However, our recent study showed that mitogen-activated protein kinase kinase MAPKK6-J is acetylated on Ser207, Lys210 and Thr211 residues [4]. The acetylation on Ser207 and Thr211 residues caused by YopJ, one of the *Yersinia pestis* outer proteins, directly competed with phosphorylation, thus the enzymatic cascade critical for triggering an innate immune response is not activated. Later on, Mittal et al. [5] confirmed that YopJ is an acetyltransferase that catalyses O-acetylation of Ser and Thr residues. The direct competition of acetylation for phosphorylation may be a more common cellular strategy for regulation of flux through signal transduction pathways than previously thought. Given that this may be a widespread mode of biochemical regulation of endogenous processes, it is important to identify acetylation on the residues that are commonly phosphorylated, i.e., Ser, Thr, and Tyr. Mass spectrometry-based proteomics, as a powerful tool to map and identify PTM's, rely on the understanding of peptide fragmentation, which could be used to develop refined bioinformatic algorithms for MSMS-based proteomics [6]. In this study, we try to understand the effects of acetylation on the fragmentation of the peptides possessing this modification. We performed the fragmentation analysis on peptides acetylated on Ser, Thr, Tyr and Lys residues to understand the key identifying MSMS fragmentation features of these variants.

In addition to Lys acetylation, we also studied Lys trimethylation. It has been shown that like acetylation, trimethylation on Lys residues is a common PTM involved in several biological processes including transcription and gene regulation [7–9]. Trimethylation and acetylation have similiar mass differences, 42.0470 Da and 42.0106 Da, respectively. One challenge in analyzing the PTM's on Lys is to be able to differentiate between acetylation and trimethy-

* Corresponding author. Tel.: +1 214 6456296; fax: +1 214 6456298.
E-mail address: haydn.ball@utsouthwestern.edu (H.L. Ball).

1387-3806/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.ijms.2008.11.014

lation, especially when the mass spectrometer employed does not offer extremely high resolution capabilities. By studying the fragmentation pattern and identifying characteristic daughter ions of acetylated and trimethylated Lys, it offers the possibility to have greater confidence in distinguishing between these two PTM's. Zhang et al. [10] showed that an immonium ion at m/z 126 corresponds to an acetylated Lys and the neutral loss ion (MH^+-59) is unique for trimethylated Lys.

Other than identifying the m/z 126 immonium ion, we also investigated the influence of Lys acetylation and trimethylation on peptide fragmentation efficiency. The mobile proton model studies have shown that the energy required to drive a proton from a basic side-chain or the amino terminus depends on the amino acid composition and gas-phase basicity, such that the collision energy (CE) required decreases in the order of decreasing gas-phase basicity [11–13]. Acetylation and trimethylation alter the Lys gas-phase basicity. We believe the difference can help distinguish trimethylated peptides from acetylated peptides with the same primary sequence.

2. Experimental

2.1. Model peptides studied

First model peptide series was based on VDSVAKTIDA, corresponding to aa 205–214 region in the activation loop of MAPKK6 protein. Five forms of the sequence were synthesized: wild-type VDSVAKTIDA; VDSVAK(Ac)TIDA; VDSVAK(triMe)TIDA, VDS(Ac)VAKTIDA, and VDSVAKT(Ac)IDA.

Second model peptide series was: AAAHAAAK(Ac)VHV, AAAHAAAS(Ac)VHV, AAAHAAAT(Ac)VHV, and AAAHAAAY(Ac)VHV.

Third model peptide series was: AAAK(Ac)AAAHVHV, AAAS(Ac)AAAHVHV, and AAAY(Ac)AAAHVHV.

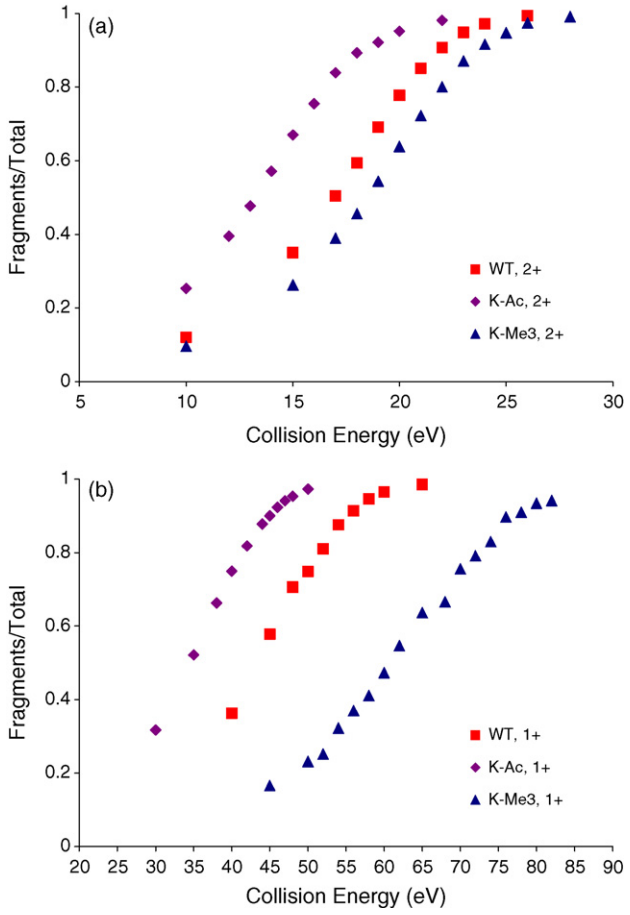

Fig. 1. Fragmentation efficiency curves. WT represents the wild-type VDSVAKTIDA; K-Ac represents VDSVAK(Ac)TIDA; K-Me3 represents VDSVAK(triMe)TIDA. (a) Parent ions were doubly charged; (b) parent ions were singly charged. Compared to the wild-type peptide, the acetylation on Lys significantly decreased the energy needed to fragment the peptide, while trimethylation on Lys had the opposite effect requiring more energy for fragmentation.

2.2. Peptide synthesis

Peptides were synthesized on an Applied Biosystems 433 automated peptide synthesizer (Foster City, CA), using optimized Fmoc chemistry [14]. Crude peptides were purified on a Waters 600 HPLC system (Milford, MA) using a Vydac C18 semi-preparative column (250 mm × 10 mm) at 3 ml min^{-1} and 0–100% B in 120 min, where A is water/0.045% TFA and B is acetonitrile/0.036% TFA. The purified peptides were characterized using either MALDI-MS or ESI-MS.

2.3. Mass spectrometry analysis

Model peptides were dissolved in acetonitrile/water/formic acid 50/50/0.1 to form a 3 μM solution. Samples were directly infused using Proxeon Biosystems nanospray emitters (Odense, Denmark) and spectra were collected on a Sciex QSTAR XL Q-TOF mass spectrometer (Foster City, CA). For the MSMS experiments, unit resolution window was used. The majority of parent ions passing through into the collision cell were ions with protonated monoisotopic mass (M), and a portion with carbon-13 isotopic mass ($M+1$). Parent ions with mass ($M+2$) or higher were not retained. The resolution window was kept the same for all MSMS spectra acquired in this experiment. The goal is to keep the MSMS spectra as clean as possible, whilst still being able to show the charge state of fragment ions.

3. Results and discussion

3.1. Fragmentation efficiency of model peptide VDSVAKTIDA with acetylated Lys and trimethylated Lys

Three model peptides were prepared for this experiment: VDSVAKTIDA, VDSVAK(triMe)TIDA, and VDSVAK(Ac)TIDA. Compared to the wild-type peptide, the acetylated peptide starts to fragment at lower CE, whilst the trimethylated peptide required higher CE to fragment. The fragment efficiency curves show the fraction of fragmentation as a function of CE [15]. In this study, the fraction of fragmentation is the ratio of (sum of relative peak intensity of all fragments in the MSMS spectrum) over (sum of relative peak intensity of all peaks in the MSMS spectrum), i.e., Σ fragments/Σ total. The fragment efficiency curves (Fig. 1) clearly showed that compared to the trimethylated form, the acetylated analogue fragmented to the same extent at much lower energy, around 5 eV lower for doubly charged peptides, and more than 20 eV lower for singly charged species. Although the proton affinity (PA) data of the wild-type and modified Lys side-chain has not been found reported, properties of related bases have been systematically studied [16–18]. The PA values of methylamine, N-methylacetamide, N-methylmethanamine and trimethylamine, as determined by Hunter and Lias [17], are listed in Table 1. When one of the amine H atoms of methylamine is replaced with a methyl group to become N-methylmethanamine, the PA value increases from 214.9 kcal/mol to 222.2 kcal/mol, whereas in the case of trimethylamine, the PA value further increases to 226.8 kcal/mol. Therefore it is reasonable to assume that PA of trimethylated Lys would increase compared to the original Lys, which results in a more localized proton. Compared to the wild-type peptide, more energy was needed to fragment the corresponding peptide with

Table 1
Proton affinity values of reference bases [17].

Molecule	Name	PA value
CH_3–NH_2	Methylamine	214.9 kcal/mol
CH_3CO–NH–CH_3	N-methylacetamide	212.4 kcal/mol
CH_3–NH–CH_3	N-methylmethanamine	222.2 kcal/mol
$N(CH_3)_3$	Trimethylamine	226.8 kcal/mol

trimethylated Lys. This result is in agreement with the mobile proton model [11–13], which states that energy is required to initiate proton transfer from the basic side-chain of the residues to the peptide backbone in order to induce dissociation. When an acetyl group is substituted in methylamine to form N-methylacetamide, the PA value decreases from 214.9 kcal/mol to 212.4 kcal/mol. Similarly, the PA value of the Lys side-chain would decrease after acetylation. In our experiment, given the same CE, VDSVAK(Ac)TIDA fragmented to a greater degree than VDSVAKTIDA. This result also suggests that acetylation on Lys decreases its proton affinity. Thus, less energy is required to drive the proton to the peptide backbone.

3.2. Fragmentation pattern of model peptide VDSVAKTIDA with acetylated and trimethylated Lys

As described in Section 2, only the parent ions with protonated monoisotopic mass M, and a small portion with carbon-13 isotopic mass ($M+1$) were chosen to be retained in the collision cell. The MSMS spectra were acquired under the condition that the value of Σ fragments/Σ total was 94–95%. The fragments of VDSVAK(Ac)TIDA and VDSVAK(triMe)TIDA showed several noticeable differences.

The MSMS spectrum of VDSVAK(triMe)TIDA (Fig. 2a) contained more doubly charged daughter ions than those of VDSVAK(Ac)TIDA (Fig. 2b). For clarity of the figure, not all doubly charged ions (with S/N ratio larger than 10) were labeled. Doubly charged ions with S/N ratio larger than 10 are shown in Table 2. The fragmentation efficiency data suggested that the order of proton affinity is

Table 2
Doubly charged fragment ions (with S/N ratio larger than 10) found in the MSMS spectrum of VDSVAK(triMe)TIDA (Fig. 2a), VDSVAK(Ac)TIDA (Fig. 2b), and wild-type VDSVAKTIDA (Fig. 2c). Assignment with * indicate ions possessing chemical modifications. "√" means that the peak was detected in the spectrum, while "–" means that it was not detected in the spectrum.

Ions ($z=2$)	Ion assignment	Fig. 2a	Fig. 2b	Fig. 2c
m/z 298.7	b6*-18	√	–	–
m/z 307.7	a6*	√	–	–
m/z 312.7	b6*-18	√	–	–
m/z 321.7	b6*	√	–	–
m/z 349.2	a7*-18	√	–	–
m/z 358.2	a7*	√	–	–
m/z 363.2	b7*-18	√	–	–
m/z 365.2	Unknown	√	–	–
m/z 370.2	Unknown	√	–	–
m/z 372.2	b7*	√	–	–
m/z 379.2	Unknown	√	–	–
m/z 405.8	a8*-18	√	–	√, a8-18 at m/z 384.7
m/z 414.8	a8*	√	–	√, a8 at m/z 393.7
m/z 419.8	b8*-18	√	–	–
m/z 423.8	y8*	√	–	√, y8 at m/z 402.7
m/z 428.8	b8*	√	√	–
m/z 463.3	a9*-18	√	–	√, a9-18 at m/z 442.3
m/z 472.3	a9*	√	–	√, a9 at m/z 451.3
m/z 477.3	b9*-18	√	√	√, b9-18 at m/z 456.3
m/z 481.3	y9*	√	–	–
m/z 486.3	b9*	√	√	√, b9 at m/z 465.3
m/z 512.8	b10*-18	√	–	√, b10-18 at m/z 491.8
m/z 521.8	b10*	√	√	√, b10 at m/z 500.8

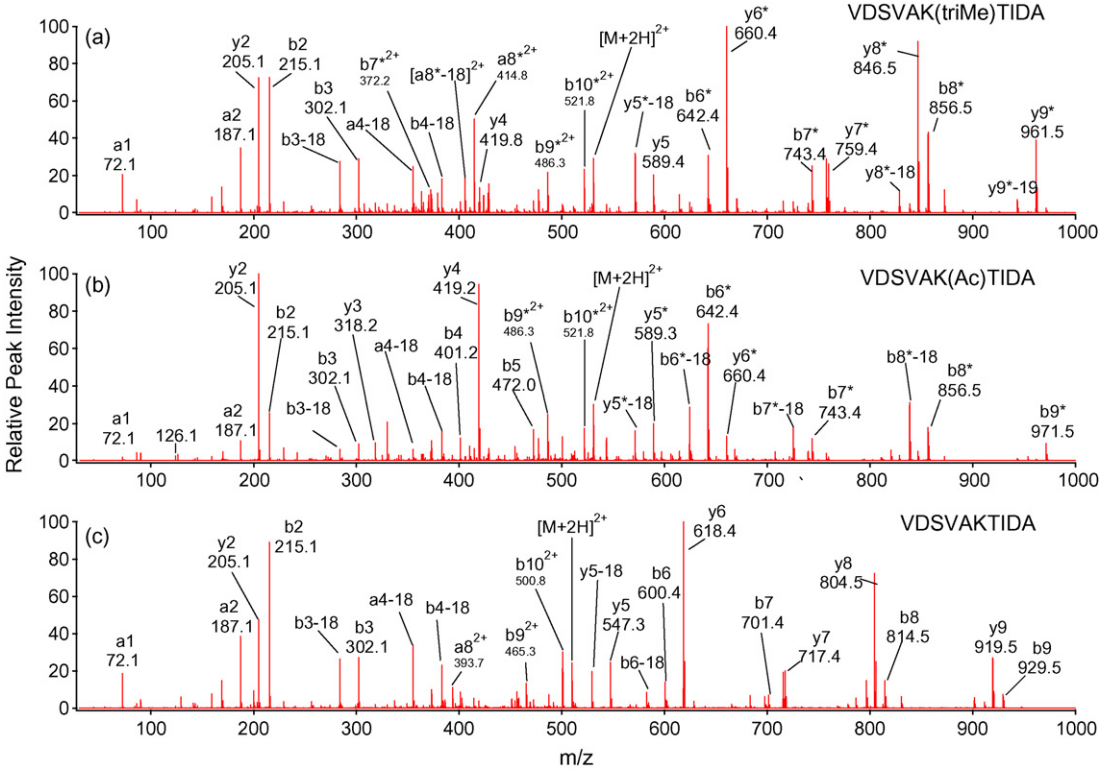

Fig. 2. MSMS spectra of three different forms of doubly charged model peptides VDSVAKTIDA. Peaks labeled with * indicate ions possessing chemical modifications. (a) MSMS spectrum of VDSVAK(triMe)TIDA acquired at CE 26 eV. More doubly charged daughter ions were observed. (b) MSMS spectrum of VDSVAK(Ac)TIDA acquired at CE 20 eV. The characteristic ion at m/z 126 was detected. At the m/z 500–1000 mass range, b ions were dominant. (c) MSMS spectrum of wild-type VDSVAKTIDA acquired at CE 24 eV. At the m/z 500–1000 mass range, y ions were dominant.

trimethylated Lys > wild-type Lys > acetylated Lys. This order is consistent with that observed from the MSMS spectra. Due to the increased proton affinity, it is relatively easy for the MSMS fragments that contain the trimethylated Lys to gain the extra proton, thus resulting in a doubly charged ion.

At the *m*/*z* 500–1000 mass range, where the fragments contain the Lys residue, y ions were dominant for VDSVAKTIDA (Fig. 2c); b ions were dominant for VDSVAK(Ac)TIDA (Fig. 2b); whilst for VDSVAK(triMe)TIDA, y ions were still dominant, and b ion intensity also increased (Fig. 2a). Based on the sequence of the peptide and the fact that the Lys residue is closer to the N-terminus, there is a possibility to form y ions over b ions in the case of wild-type VDSVAKTIDA (Fig. 2c). An increase in the PA of the Lys residue would strengthen the intensity of peaks which contain the Lys residue. This effect was observed in the dataset obtained (Fig. 2a). The fact that peak intensity of y6*-y9* in VDSVAK(Ac)TIDA dramatically weakened further suggests that PA of Lys residue decreases after it is acetylated.

An immonium ion at *m*/*z* 126 was only detected in VDSVAK(Ac)TIDA (Fig. 2b), in agreement with data reported by Zhang et al. [10]. They also showed that the neutral loss ions (MH^+-59) corresponded to a trimethylated Lys. In this study (y5-59), (y6-59), (b6-59), and (b7-59) were detected with S/N ratio greater than 10, only when parent ions were singly charged (data not shown), suggesting that the loss of the trimethylamine, $N(CH_3)_3$, requires more energy.

Since b ions can be N-terminally protonated, loss of H_2O for b ions, b-18, is commonly observed [6,19]. Several b-18 and y-18 ions were found in VDSVAKTIDA, VDSVAK(Ac)TIDA and VDSVAK(triMe)TIDA. A few b-36 ions with low intensity, not labeled in Fig. 2b, were found for VDSVAK(Ac)TIDA.

3.3. Fragmentation pattern of model peptide VDSVAKTIDA with acetylated Ser or Thr

In an earlier study [4], we showed that the acetylation on Ser and Thr residues directly competed with phosphorylation. Model peptides VDS(Ac)VAKTIDA and VDSVAKT(Ac)IDA were employed in the MSMS experiment in order to study the fragmentation pattern of the peptide when Ser or Thr was acetylated. The fragment efficiency curves of peptides VDS(Ac)VAKTIDA and VDSVAKT(Ac)IDA did not show much difference from one another (Fig. 3). They were also very similar to the curve obtained for the wild-type VDSVAKTIDA (Fig. 3). When CE was set at 25 eV, the Σ fragments/Σ total ratio was 97–98% for both VDS(Ac)VAKTIDA and VDSVAKT(Ac)IDA.

Unlike the MSMS spectrum of VDSVAK(Ac)TIDA (Fig. 2b), peaks with a neutral loss of 60 Da were found in the MSMS spectra of both VDS(Ac)VAKTIDA and VDSVAKT(Ac)IDA. For VDSVAKT(Ac)IDA (Fig. 4a), fragments that contain the acetyl group included y4-y9 and b7-b9, and were marked with *. The peak of y4* was barely detectable (S/N = 3). However, peaks corresponding to y5*, y6*, y7*, y8*, and y9* were strong. Among the y* ion series (y4*-60), (y5*-60), and (y6*-60) peak was detected with S/N ratio greater than 10. Among b* ion series (b7*-60) and (b8*-60) peaks had signals that were more than 10 times stronger than the noise. Similarly, for VDS(Ac)VAKTIDA (Fig. 4b), fragments that contain the acetylation started with y8 for y ions, and b3 for b ions. Although the S/N ratio were greater than 10 for (y8*-60) and (y9*-60), they were much weaker compared to that of y8* and y9*. Among b* ions (b3*-60), (b4*-60), (b7*-18) and (b8*-18) were detected with a strong signal.

So far, the data suggests that unlike acetylation on Lys, when acetylated Ser or Thr is fragmented, a 60 Da neutral loss is observed if the modification is at or close to either terminus of the daughter ion. This is similar to what happens in the case of phosphorylation, whereby Ser/Thr phosphorylation results in a mass increase of 80 Da, while a neutral loss of 98 Da is commonly detected during the fragmentation [20–24].

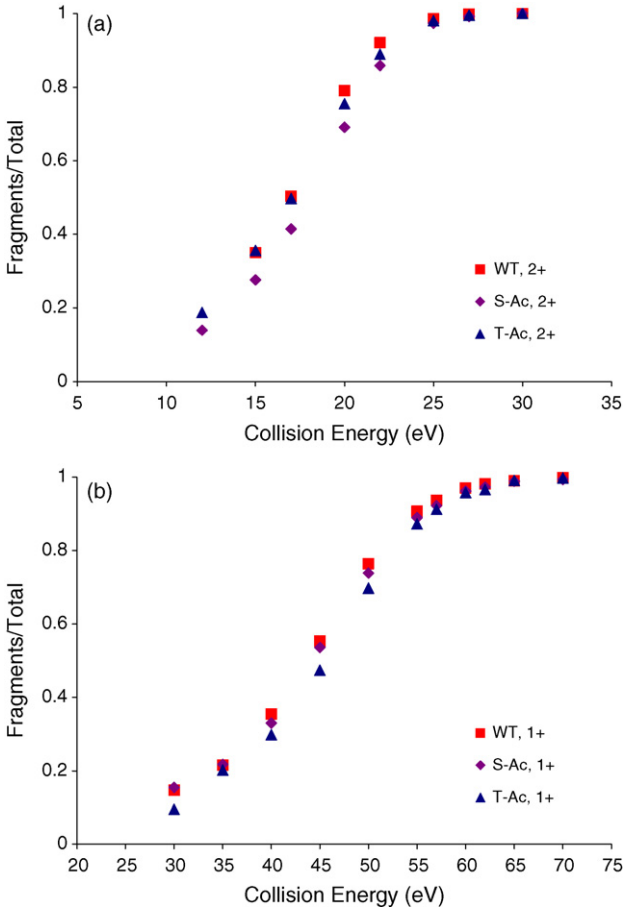

Fig. 3. Fragmentation efficiency curves. WT represents the wild-type VDSVAKTIDA; S-Ac represents VDS(Ac)VAKTIDA; T-Ac represents VDSVAKT(Ac)IDA. (a) Parent ions were doubly charged; (b) parent ions were singly charged. The acetylation on Ser and Thr did not show obvious effect on the energy needed to fragment the peptide.

3.4. Validating the fragmentation pattern with 2nd model peptide series

An acetylated form of Tyr has not been reported in vivo to date. However, it is conceivable that an acetylated form of Tyr could compete with phosphorylation events on this residue, and therefore represents a worthwhile target for study. In this experiment, four model peptides were synthesized and studied: AAAHAAAK(Ac)VHV, AAAHAAAS(Ac)VHV, AAAHAAAT(Ac)VHV, and AAAHAAAY(Ac)VHV.

For this series of peptides, under conditions where Σ fragments/Σ total ratio was within 94–95%, the intensity of fragments within the 600–900 Da mass range decreased dramatically, while the intensity of fragments within the 50–200 Da mass range increased significantly. Therefore to obtain a more balanced spectrum, CE was reduced for 2nd model peptide series in order to generate a Σ fragments/Σ total ratio of 90–91%. The CE used for AAAHAAAK(Ac)VHV, AAAHAAAS(Ac)VHV, AAAHAAAT(Ac)VHV, and AAAHAAAY(Ac)VHV was 31 eV, 29 eV, 29 eV, and 31 eV, respectively.

Again, *m*/*z* 126.1 was detected only for AAAHAAAK(Ac)VHV (Fig. 5a), which further confirmed that this is the characteristic ion for acetylated Lys. The neutral loss of 42 Da or 60 Da was

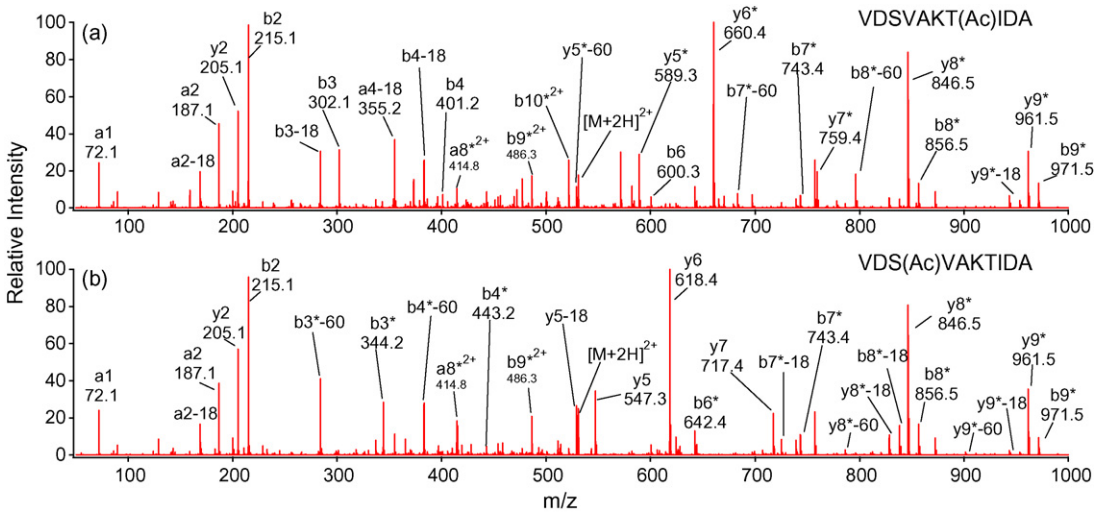

Fig. 4. MSMS spectra of two different forms of acetylated model peptides VDSVAKTIDA. Acetylated ions indicated by *. (a) MSMS spectrum of VDSVAKT(Ac)IDA acquired at CE 25 eV. Although peaks with the acetyl group remains attached were commonly detected, (y5*-60), (b7*-60) and (b8*-60) peaks were also observed. (b) MSMS spectrum of VDS(Ac)VAKTIDA acquired at CE 25 eV. (b3*-60), (b4*-60), (b7*-18), and (b8*-18) showed strong peak intensity compared to b3*, b4*, b7*, and b8*, respectively.

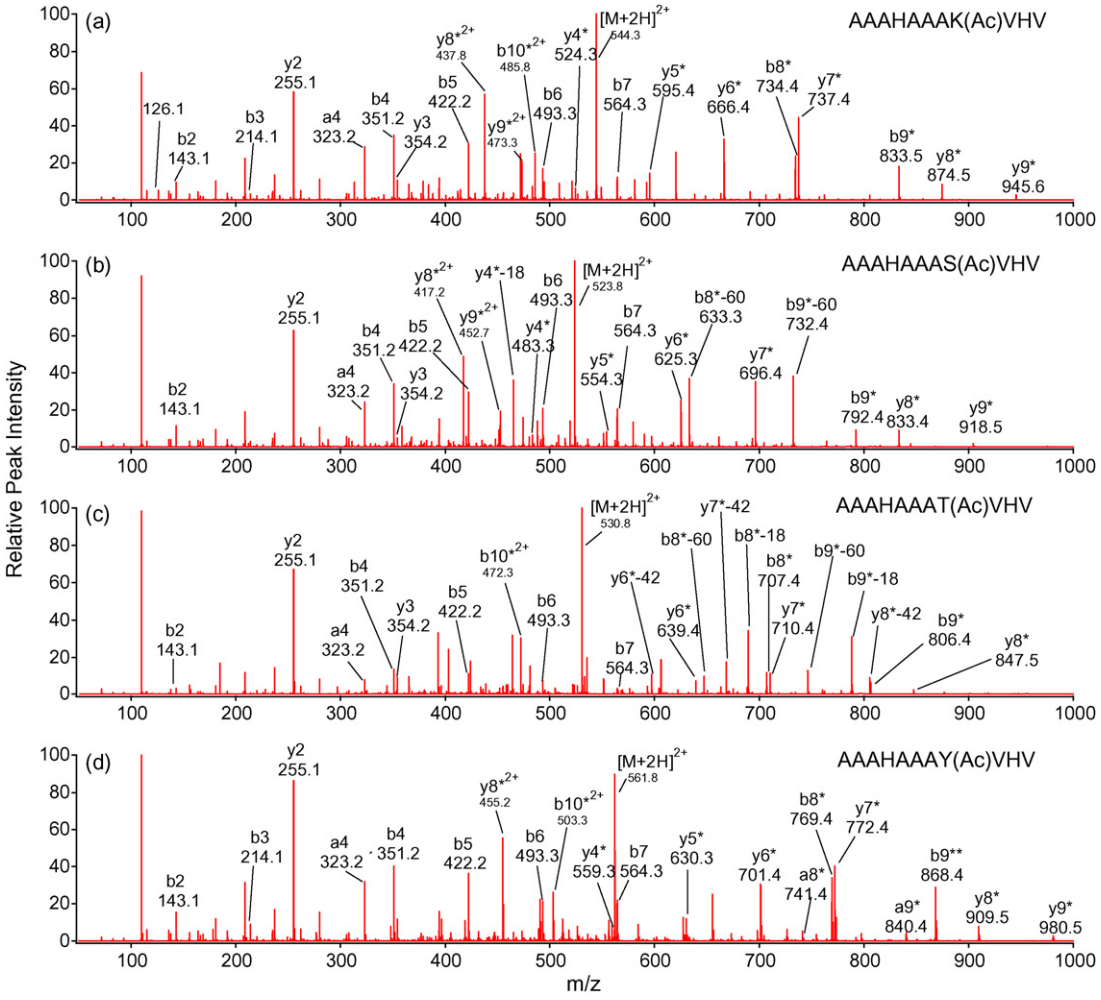

Fig. 5. MSMS spectra of 2nd model peptides series. Acetylated ions marked with *. (a) MSMS spectrum of AAAHAAAK(Ac)VHV, CE = 31 eV. Acetyl group remains attached to Lys residue during the fragmentation. (b) MSMS spectrum of AAAHAAAS(Ac)VHV, CE = 29 eV. The neutral loss of 60 Da and 18 Da were observed with strong signal. (c) AAAHAAAT(Ac)VHV, CE = 29 eV. Not only (b*-60), but (b*-18) and (b9*-18) had strong peak intensity. Also (y7*-42), (y8*-42), and (y9*-42) peaks were stronger than the corresponding y* peak. (d) AAAHAAAY(Ac)VHV, CE = 31 eV. The neutral loss of 18 Da, 42 Da or 60 Da was not detected for either b* ions or y* ions.

not detected for either b* ions or y* ions of AAAHAAAK(Ac)VHV, which confirmed that the acetylation on Lys was stable. For AAAHAAAS(Ac)VHV (Fig. 5b) (b8*-60) and (b9*-60) peaks were stronger than b8* and b9*; while y* ions were dominant compared to the (y*-18) forms; the neutral loss of 42 Da or 60 Da was not detected for y* ions. In the case of AAAHAAAT(Ac)VHV (Fig. 5c) (b8*-60) and (b9*-60) peaks were still stronger than b8* and b9*, but (b8*-18) and (b9*-18) peaks were even stronger. Surprisingly, (y7*-42), (y8*-42), and (y9*-42) peaks were stronger than the corresponding y* peak. This suggested that when Thr residue is acetylated, loss of the acetyl group can occur even when the Thr residue is not close to the terminus of the fragment. The neutral loss of 42 Da or 60 Da was not detected for either b* ions or y* ions of AAAHAAAY(Ac)VHV (Fig. 5d), suggesting that the acetylation on Tyr is more stable than the acetylation on Ser or Thr. This is consistent with the fact that phosphorylation on Ser/Thr was more labile than phosphorylation on Tyr [20,21].

3.5. Validating the fragmentation pattern and efficiency with 3rd model peptide series

The acetylation site was at the N-terminus of the peptide in 3rd model peptide series. MSMS experiments were performed on the 3rd model peptide series to determine if the location of the acetylation site affects the fragmentation pattern. Peptides used were AAAK(Ac)AAAHVHV, AAAS(Ac)AAAHVHV, and AAAY(Ac)AAAHVHV, and the CE used were 30 eV, 28 eV, and 30 eV, respectively, to give 90–91% fragmentation.

Like their counterparts where the site of acetylation was close to C-terminus of the peptide, the fragments of AAAK(Ac)AAAHVHV and AAAY(Ac)AAAHVHV did not show any sign of neutral loss of 42 Da or 60 Da (data not shown). For AAAS(Ac)AAAHVHV, intensity of (b4*-60) and (b5*-60) was 3–5 times stronger than that of b4* and b5*, respectively; intensity of y8* and y9* was 5–8-fold stronger than that of (y8*-60) and (y9*-60), respectively. The only detectable peak with a neutral loss of 42 Da was (y8*-42) at S/N = 5 (data not shown).

The fragmentation efficiency curves obtained with AAAHAAAK(Ac)VHV were compared to AAAHAAAK(triMe)VHV, and AAAK(Ac)AAAHVHV compared to AAAK(triMe)AAAHVHV (data not shown). The data further confirmed that trimethylation on Lys requires more energy to induce dissociation.

3.6. The neutral loss of 18 Da, 42 Da, and 60 Da

During the dissociation of peptide containing phosphorylated Ser/Thr, the fragments with both the neutral loss of 98 Da and 80 Da, corresponding to the loss of phosphoric acid ($-H_3PO_4$) and phosphate group ($-HPO_3$), respectively, were commonly detected [21,22]. The signal with neutral loss of 98 Da is usually more intense than that of the neutral loss of 80 Da [22]. For phosphorylated Tyr, the neutral loss of 98 Da is often absent. As demonstrated by Tholey et al. [23], the aliphatic phosphoester group can form a six-centered intermediate and eventually lose the H_3PO_4 group. The elimination of phosphoric acid from Tyr is not favored because the Tyr aromatic ring makes it impossible to form the six-centered transition state [23]. Using deuterated phosphotyrosine, Metzger et al. [24] confirmed that even when a mass loss of 98 Da was detected for phosphorylated Tyr, it was a result of the HPO_3 loss and water elimination from another residue in the peptide.

In the case of acetylated Ser/Thr, where the C=O group is analogous to the P=O in phosphate, the six-centered intermediate can be generated, lose acetic acid, and form a carbon–carbon double bond on the Ser/Thr side-chain. Thus the loss of 60 Da and 42 Da can correspond to the loss of CH_3COOH through β-elimination and CH_2CO through deacetylation, respectively. In many cases the fragmentation of peptide shows a combination of different pathways [6,23,24]. Since (y*-18) and (b*-18) were detected in this study, loss of 42 Da and 18 Da individually can also contribute to the loss of 60 Da. The question stands as to how these peptides lose 18 Da? There are three possible pathways reported for the loss of water from protonated peptides [25]: dehydration at the carboxy terminus of the peptide or side-chain carboxyl groups on Asp and Glu; from backbone amide oxygens, and from side-chains of Ser and Thr. In this study, there are no Asp or Glu residues in our model peptides. Loss of water from the C-terminal COOH group can contribute to the formation of (y*-18), but cannot explain the formation of (b*-18). The studies from Ballard et al. [25] and Reid et al. [26] showed that a proton can be moved to the Ser or Thr side-chain oxygen, then form a five-membered or six-membered ring with the adjacent C-terminal amide oxygen with the resulting elimination of a water molecule. However, this side-chain-backbone pathway would be blocked when Ser/Thr was acetylated. It is likely that the formation of [b*-18] ions is a result of the backbone–backbone reactions demonstrated by Reid et al. [27]. However, as they indicated, it is not clear if it is the carbonyl group on the N-terminal side attacking an O-protonated carbonyl group that is closer to the C terminus, or if it is the reverse situation.

4. Conclusion

ESI/MSMS has been used for the characterization of peptides with acetylated Ser, Thr or Tyr, and acetylated or trimethylated Lys. We demonstrated that higher energy is required to fragment the peptide if Lys residue is trimethylated; while lower energy is required if a Lys residue is acetylated. Acetylation on Ser/Thr residues does not dramatically change the energy needed to break the peptide. This study confirmed that m/z 126.1 is the characteristic ion for acetylated Lys. It was shown that y-60 and b-60 ions are commonly observed for acetylated Ser, when the acetylation site is at, or close to, either terminus of the daughter ion. For acetylated Thr, in addition to y-60 and b-60 ions, y-42 ions are usually dominant. In the case of acetylated Tyr/Lys, the acetyl group remains attached to these residues during the CID process. The fragmentation pattern was similar whether the acetylation site was close to the C-terminus or N-terminus of the peptide.

References

[1] A. Ruiz-Carrillo, L. Wangh, V. Allfrey, Science 190 (1975) 117.
[2] J. Boyes, P. Byfield, Y. Nakatan, V. Ogryzko, Nature 96 (1998) 594.
[3] M.A. Glozak, N. Sengupta, X. Zhang, E. Seto, Gene 363 (2005) 15.
[4] S. Mukherjee, G. Keitany, Y. Li, Y. Wang, H.L. Ball, E. Goldsmith, J. Orth, K. Yersinia, Science 312 (2006) 1211.
[5] R. Mittal, S.Y. Peak-Chew, H.T. McMahon, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 18574.
[6] B. Paizs, S. Suhai, Mass Spectrom. Rev. 24 (2005) 508.
[7] J.C. Rice, D.C. Allis, Curr. Opin. Cell Biol. 13 (2001) 263.
[8] B.M. Turner, Cell 111 (2002) 285.
[9] M. Lachner, T. Jenuwein, Curr. Opin. Cell Biol. 14 (2002) 286.
[10] K. Zhang, P.M. Yau, B. Chandrasekhar, R. New, R. Kondrat, B.S. Imai, M.E. Bradbury, Proteomics 4 (2004) 1.
[11] R. Ashok, A.R. Dongre, J.L. Jones, A. Somogyi, V.H. Wysocki, J. Am. Chem. Soc. 118 (1996) 8365.
[12] K.A. Cox, S.J. Gaskell, M. Morris, A. Whiting, J. Am. Soc. Mass Spectrom. 7 (1996) 522.
[13] X. Tang, R.K. Boyd, Rapid Commun. Mass Spectrom. 6 (1992) 651.
[14] H.L. Ball, P. Mascagni, Int. J. Peptide Protein Res. 48 (1996) 31.
[15] V.H. Wysocki, G. Tsaprailis, L.L. Smith, L.A. Breci, J. Mass Spectrom. 35 (2000) 1399.
[16] A. Baeten, F. De Proft, P. Geerlings, Chem. Phys. Lett. 235 (1995) 17.
[17] E.P. Hunter, S.G. Lias, J. Phys. Chem. Ref. Data 27 (1998) 413.
[18] S.G. Lias, J.F. Liebman, R.D. Levin, J. Phys. Chem. Ref. Data 13 (1984) 695.
[19] Q. Fu, L. Li, J. Mass Spectrom. 41 (2006) 1600.
[20] M. Mann, O.N. Jensen, Nat. Biotechnol. 21 (2003) 255.

[21] J.P. DeGnore, J. Qin, J. Am. Soc. Mass Spectrom. 11 (1998) 1175.
[22] R. Hoffmann, S. Metzger, B. Spengler, O. Otvos Jr., J. Mass Spectrom. 34 (1999) 1195.
[23] A. Tholey, J. Reed, W.D. Lehmann, J. Mass Spectrom. 34 (1999) 117.
[24] S. Metzger, R. Hoffmann, J. Mass Spectrom. 35 (2000) 1165.
[25] K.D. Ballard, S.J. Gaskell, J. Am. Soc. Mass Spectrom. 4 (1993) 477.
[26] G.E. Reid, R.J. Simpson, R.A.J. O'Hair, J. Am. Soc. Mass Spectrom. 11 (2000) 1047.
[27] G.E. Reid, R.J. Simpson, R.A.J. O'Hair, Int. J. Mass Spectrom. 190/191 (1999) 209.