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Unambiguous Determination of Isobaric Histone Modifications by Reversed-Phase Retention Time and High-Mass Accuracy

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Abstract

Methylation and acetylation of lysines are crucial posttranslational modifications that regulate gene transcription and have been shown to be misregulated in many forms of cancers. Western blot, immunoprecipitation, and immunofluorescence are commonly used to characterize histone acetylation and methylation. However, these approaches are limited by the availability, site specificity, and cross-reactivity of antibodies. Mass spectrometry is emerging as an additional powerful tool for histone characterization. The isobaric nature of trimethylation and acetylation (TriMe = 42.0470 Da, Ac = 42.0106 Da) confounds histone characterization by means other than high-resolution / high-mass accuracy mass spectrometry. In this paper we adapted methodology that exploits difference in the relative retention time of acetylated and methylated peptides to unequivocally distinguish these two modifications even with low-mass accuracy mass spectrometers. The approach was tested on tryptic digest of *S. cerevisiae* histones. We found that acetylation resulted in increased retention in reversed-phase chromatography, while methylation, including trimethylation, showed little change in retention. For example, the acetylated forms of peptide ²⁷KSAPSTGGVKKPHR⁴⁰ eluted at 15.63 min whereas the methylated forms eluted at 13.89 min. In addition, the effect of acetylation was cumulative as observed in the case of peptide ⁹KSTGGKAPR¹⁷, whose un-, mono-, and diacetylated isoforms eluted at 7.43, 10.47, and 16.49 min, respectively. The modification patterns of the peptides in question were subsequently verified by high-mass accuracy tandem mass spectrometry.

Keywords

retention; high-mass accuracy; acetylation; methylation; trimethylation

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INTRODUCTION

Methylation and acetylation of lysines in histones are two crucial posttranslational modifications that regulate gene transcription [1] and have been shown to be misregulated in many forms of cancers [2;3]. In histone H3, K4, K9, K27, K36, and K79 can be methylated and is related to diverse transcription states [4]. Acetylation on histone H3 K9, K14, K18, K23, and K27 is generally associated with gene activation [5;6]. It is noteworthy that histone H3 K9 methylation (H3K9Me) participates in heterochromatin formation and gene silencing, whereas acetylation of histone H3 K9 (H3K9Ac) has been reported to be associated with gene activation [7]. The identification and quantitation of acetylation and methylation is therefore of high significance for understanding the role of histone modifications in gene regulation. The differentiation between trimethylation ($\Delta M = 42.0470$ Da) and acetylation ($\Delta M = 42.0106$ Da) is challenging since they are isobaric modifications that differ by 0.0364 Da, which requires a mass resolving power of 27,472 for a 1,000 Da peptide.

Traditional immunoassay methods, such as western blot and immunoprecipitation, are widely used to characterize histone modifications due to their high sensitivity. However, site specificity of antibodies is affected by adjacent modifications leading to poor specificity for epitopes with concomitant modifications [8;9;10]. In addition, these approaches are also limited by the availability of site specific antibodies for known modifications and not readily applicable for discovery and rapid characterization of novel modifications.

In recent years, mass spectrometry (MS) has been demonstrated to be very successful in the study of histone posttranslational modifications [11;12;13;14;15;16;17]. The high-mass resolving power and high-mass accuracy of FT-ICR mass spectrometers allow for distinction between acetylation and trimethylation by peptide mass fingerprinting [18]. Tandem mass spectrometry is also a powerful tool for distinguishing these two modifications. By collision induced dissociation acetylated peptides usually generate an ammonium ion at 126⁺ Da while trimethylated peptides generate a neutral loss of trimethyl amine (N(CH₃)₃) [19], therefore, acetylation and trimethylation can be distinguished by the presence of these diagnostic peaks. However, these peaks are not always observed, especially for low abundant species. Another approach to distinguish trimethylation and acetylation is to introduce heavy isotopes to the acetyl or methyl groups so that trimethylation and acetylation will no longer be isobaric and then can be distinguished easily by mass alone [20;21;22]. However, the labeling method is time consuming.

Liquid chromatography mass spectrometry (LC-MS) and LC tandem mass spectrometry (LC-MS/MS) are also important tools in the identification of lysine acetylation. For example, the unambiguous identification of lysine acetylation of in NAD-dependent histone deacetylase Sir2 was performed by use of LC-MS and LC-MS/MS [23;24]. Furthermore, relative retention in reversed-phase liquid chromatography (RPLC) has long been used as a supplement to MS for peptide identification [25;26;27;28;29]. A number of models have been proposed to calculate peptide hydrophobicities [30;31;32;33;34;35;36]. Posttranslational modification in turn can also significantly alter peptide hydrophobicity. For example, phosphorylation was believed to increase peptide hydrophobicity due to the addition of anionic/acidic phosphate groups, resulting in reduced retention. However, recent work by Steen and coworkers showed that this effect was not always born out. In some cases the increase of hydrophobicity was compensated for by charge neutralization when the peptide contains basic amino acids that are positively charged under standard LC-MS conditions [37]. In their study, the phosphorylated peptides eluted about the same time or later than the unmodified due to the presence of basic residues. Only peptides with a larger number of the phospho moieties than that of basic residues showed shorter elution times than their unmodified forms. Their work demonstrated that modifications that result in reduction of the net charge would reduce the overall hydrophobicity

and increase the retention time on RPLC. For modifications with no introduction of charges such as mono-, di-, and trimethylation, we would expect no significant change of retention time. However, modifications that alter the number of basic sites would see a change in relative retentions.

N-terminal acetylation of the alpha-amino group results in a substantial change in hydrophobicity and retention. Guo and Mant reported that N-terminal acetylated peptides eluted 1.5 ~ 3.5 min later than the isoforms of the same peptides with free N-terminus [32; 38]. Likewise, acetylation of lysine side-chains can neutralize the positive charge and lead to an increase in peptide hydrophobicity [39;40]. Thus acetylated peptides in general would be expected to elute later in reversed-phase chromatography than their unmodified isoforms. For example, Hunt showed that the peptides ${}^9\text{KSTGGK}_{\text{Ac}}\text{APR}^{17}$ and ${}^9\text{K}_{\text{Me}_3}\text{STGGKAPR}^{17}$ were separated by 17 min [15]. Hunt and coworkers have also reported the increase of histone peptide hydrophobicity by incubating histones with propionic anhydride before in-solution trypsin digestions. The formation of a propionyl amide effectively neutralizes the charges of unmodified or endogenously monomethylated lysine [41]. Propionic anhydride derivatization has also been applied to in-gel trypsin digestion of histones [42]. Acetic anhydride is another derivatization reagent of lysine, which will add one acetyl group to unmodified lysine residues resulting in an increased hydrophobicity of histone peptides [43].

Herein we report the general observation that acetylation of ϵ -amino groups of lysine results in shifted retention time whereas methylation does not. The combination of relative retention shifts with tandem mass spectrometry allows for the unequivocal determination of trimethylation vs. acetylation.

EXPERIMENTAL

YEAST HISTONE EXTRACTION AND H3 IN-GEL DIGESTION

S. cerevisiae strain BY4743 was obtained from Open Biosystems. Cell growth and histone purification were performed as previously described [44;45]. Histone H3 of *S. cerevisiae* was separated from other histones by use of SDS-PAGE with pre-cast 16.5% Tris-Tricine gels (BioRad Laboratories, Hercules, CA). H3 gel bands were in-gel digested with trypsin as previously described [29]. In brief, the H3 gel bands were excised into small pieces and washed twice (one hour each) with freshly made 50% methanol/5% acetic acid solution. The gel pieces were then dehydrated in 200 μl of acetonitrile for 5 min followed by a 5-min rehydration in 200 μl of 100 mM NH_4CO_3 . This dehydration-rehydration procedure was repeated once, followed by another 5-min rehydration in acetonitrile. 30 μl of freshly prepared trypsin (20 ng/ μl in 25 mM NH_4CO_3) were added and rehydrated on ice for 10 min, then digested at 37 $^\circ\text{C}$ for one hour. Tryptic digested peptides were extracted with 50% acetonitrile/5% formic acid three times and dried to about 10 μl in a vacuum concentrator.

NANO-LC-MS/MS

The digested peptides were subject to nano-LC-MS/MS analysis by use of either an LTQ FT-ICR mass spectrometer (Thermo Fisher, San Jose, CA) or an LCQ DECA XP+ ion trap mass spectrometer (Thermo Fisher) coupled with a Shimadzu LC 10ADvp capillary system (Columbia, MD, USA) [14;46]. Peptide separations were carried out with a commercial C_{18} column (5 cm, 5 μm , I.D. 75 μm , New Objective, MA) using a gradient and working conditions as previously described [47]. The peptides were separated using a 120-min gradient of mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile). Mobile phase B was increased linearly from 5 to 60% in 80 min, held at 60% for 5 min, then increased to 95% in 5 min, held for 5 min and then returned to 5% to equilibrate the column for 15 minutes. The column was washed between each run to minimize carryover. One

microliter of the digest was injected onto the column. The electrospray voltage was maintained at 1.3 kV and capillary temperature was set at 200 °C. The mass spectrometric detection range was 200–2000 (m/z). Three (LCQ) or five (LTQ-FT) data-dependent MS/MS scans with dynamic exclusion were carried out between each full MS scan. The product ion mass spectra were analyzed by use of in-house developed software, MassMatrix [48]. The search parameters included the following variable modifications: acetylation of lysine and N-terminus; methylation of lysine and arginine. Each of the tandem mass spectra matched by the database search was manually validated.

RESULTS AND DISCUSSION

POSTTRANSLATIONAL MODIFICATION PATTERNS OF YEAST HISTONE H3

Acetylation and trimethylation are isobaric posttranslational modifications that differ in mass by 0.0364 Da. High-resolution / high-mass accuracy mass spectrometers, such as FT-ICR or Orbitraps, can readily distinguish these modifications by mass alone. All initial experiments were carried out on a hybrid LTQ-FT in order to establish with high confidence the pattern of histone modifications for yeast histone H3. The full MS spectra were collected via the FT-ICR MS and product ion mass spectra were obtained in the LTQ. The high-mass accuracy precursor ion spectra were used to establish the presence of trimethylation vs. acetylation as described previously [18]. All peptide assignments were supported by MS/MS spectra with manually validated database search matches. The peptides identified with the LTQ-FT are listed in Table 1.

High-mass accuracy is a proven approach to discriminate between isobaric peptides. For example, a precursor ion was observed at $m/z = 358.7179$. Based on nominal mass the peptide could be either the acetylated or trimethylated peptides $^{18}\text{KQLASK}^{23}$ or $^{117}\text{VTIQKK}^{122}$. Based on accurate mass the trimethylated $^{18}\text{KQLASK}^{23}$ or $^{117}\text{VTIQKK}^{122}$ would have mass errors as high as 50 ppm. Such mass errors are highly improbable given a properly calibrated FT-ICR mass spectrometer. The more likely assignment is the acetylated peptide $^{18}\text{KQLASK}^{23}$ with a mass error of -4.04 ppm. The assignment of the backbone peptide sequence was corroborated by MS/MS. In this manner we confirmed K4, K36, and K79 were (tri)methylated and K9, K14, K18, K23, K27, and K56 were acetylated on yeast histone H3. As indicated in Table 1, each modification had a resulting error less than 6 ppm. These observed modification patterns determined by mass spectrometry are consistent with those obtained from other techniques [6;49;50;51;52;53].

In addition to the absolute mass, peptide mass shifts between different isoforms can also be used to corroborate modification identifications [54]. As shown in Figure 1, mass shifts of 42.0104 and 42.0096 Da between isoforms of peptide $^9\text{KSTGGKAPR}^{17}$ suggested K9 and K14 in the peptide were both acetylated rather than trimethylated. The tandem MS spectra shown in Figure 2 establish the predominant acetylation first occurring at K14 followed by K9. In the case of peptide $^{73}\text{EIAQDFKTDLR}^{83}$, a mass shift of 42.0450 Da (shown in Figure 3) between the isoforms suggested that the peptide was trimethylated. More importantly, the presence of isoforms at $m/z = 675.3560$, 682.3629, and 689.3711 correspond in mass to the mono-, di-, and trimethylated species. The tandem MS spectra shown in Figure 4 indicate K79 as the site of mono-, di-, and trimethylation. In the figure for trimethylation of K79, tandem mass spectra of the triply charged precursor ion at $m/z = 460.2520$ is shown instead of the doubly charged precursor ion at $m/z = 689.3711$ because the former showed better fragmentation.

In summary the data from accurate mass, relative mass shifts and tandem MS spectra support the conclusion that yeast histone H3 K4, K36 and K79 were methylated while H3 K9, K14, K18, K23, K27, and K56 were acetylated. These results, as summarized in Table 2, serve as

the well validated standards that will be used in our examination of relative retention time shifts between acetylation and trimethylation.

RETENTION TIME SHIFTS

As demonstrated above, accurate absolute mass and accurate relative mass shifts are powerful approaches to distinguish between trimethylation and acetylation. For data obtained on low-mass accuracy instruments additional corroborative data, such as the presence of supporting MS/MS reporter ions, are required. As previously reported [44], relative retention can also be used to distinguish between sequence assignments for isobaric peptides: $^{73}\text{EIAQDFKTDLR}^{83}$ vs. $^{28}\text{SAPSTGGVK}_{\text{Me}}\text{KPHR}^{40}$, $^{73}\text{EIAQDFK}_{\text{Me}}\text{TDLR}^{83}$ vs. $^{28}\text{SAPSTGGVK}_{\text{Me}2}\text{KPHR}^{40}$, and $^{73}\text{EIAQDFK}_{\text{Me}2}\text{TDLR}^{83}$ vs. $^{28}\text{SAPSTGGVK}_{\text{Me}3}\text{KPHR}^{40}$. This approach can also be extended to effectively distinguish between trimethylation and acetylation.

Acetylation of lysine effectively neutralizes the lysine's positive charge under acidic reversed-phase separation conditions. The change in charge results in increased retention time relative to the unmodified peptide isoform. This effect was observed for all acetylated peptide isoforms of yeast histone H3. As shown in Figure 2, unmodified fragment $^9\text{KSTGGKAPR}^{17}$ eluted at 7.4 min, whereas the K14 acetylated isoform eluted at 10.5 min and the K9 and K14 acetylated isoform eluted at 16.5 min. The effect of acetylation on retention time was also observed for peptides $^{18}\text{KQLASK}^{23}$, $^{10}\text{STGGKAPR}^{17}$, and $^{53}\text{RFQKSTELLIR}^{63}$ (listed in Table 1). From these peptides we conclude that the increase in retention is cumulative and of similar magnitude as N-terminal acetylation [32].

Unlike the addition of the acetyl group, the addition of methyl groups to lysine does not neutralize the lysine's charge. Therefore, it was expected that methylation would not dramatically alter retention time. As shown in Figure 4, the unmodified form of peptide $^{73}\text{EIAQDFKTDLR}^{83}$ eluted at 26.9 min whereas its mono-, di-, and trimethylated isoforms eluted at 27.2, 27.4, and 26.9 min, respectively. The similarity in retention time for the unmodified and methylated forms indicates that the addition of methyl groups to lysine has only a small effect on peptide retention time. The same effect was observed for the peptides $^3\text{TKQTAR}^8$ and $^{70}\text{LVREIAQDFKTDLR}^{83}$, which are also listed in Table 1.

Based on the above observation that acetylation has a significant effect on retention time while methylation has little, we hypothesize that a peptide subject to both acetylation and trimethylation would have its acetylated isoforms eluted several minutes later than the unmodified/methylated isoforms. Given this assertion, we propose that trimethylation and acetylation are easily distinguished based on relative retention times and supporting MS/MS. This hypothesis is supported by observations for the peptide $^{27}\text{KSAPSTGGVKKPHR}^{40}$ in which K27 and K36 are subject to concomitant acetylation and methylation, respectively. As seen in Figure 5, the methylated K36 isoforms eluted around 13.8 min whereas the isoforms in which K27 is acetylated (along with K36 methylations) eluted around 15.7 min. We should note that the unmodified form of this peptide was not observed. The top trace of Figure 5 corresponds to background noise. This assignment is further supported by the peptide assignments based on accurate mass in Table 1 and the mass spectra shown in Figure 6 and Figure 7. Thus the group of peptides eluting earlier are methylated isoforms of K36 and the later group is the same isoforms but with K27 acetylated. Figure 5 clearly shows the effect of acetylation vs. methylation of lysine on retention time. Given the supporting data of MS/MS and retention time it should be possible to unambiguously distinguish between these isobaric modifications without the need for accurate mass. To test this assertion the same analysis of acetylation and trimethylation was carried out on a common ion trap mass spectrometer.

UNAMBIGUOUS DETERMINATION OF ACETYLATION AND TRIMETHYLATION AT LOW-MASS ACCURACY

Results from the FT-ICR mass spectrometer demonstrated convincingly that acetylated isoforms elute later than the corresponding unmodified and methylated isoforms. This observation suggests that retention time can be used effectively to support MS/MS data for determining the presence of either acetylation or trimethylation. The nano-LC-MS/MS experiments were then performed on an LCQ DECA XP+ ion trap mass spectrometer.

To illustrate the power of the combined approach, peptide ²⁷KSAPSTGGVK³⁶, in which K27 and K36 are subject to acetylation or trimethylation, was examined. With the low-mass accuracy of the ion trap mass spectrometer it was impossible to conclude whether K27/K36 was either acetylated or trimethylated. However, based on the relative retention time shift we can confidently conclude that the isoform eluting at 10.8 min was acetylated not trimethylated because it eluted 4 min later than its unmodified form, Figure 8. Using retention time, we also identified the modification patterns of K18 and K23 in peptide ¹⁸KQLASKAAR²⁶. As shown in Table 3, the isoform of peptide ¹⁸KQLASKAAR²⁶ eluting at 25.5 min was believed to be the isoform with K18 acetylated not trimethylated because it eluted 5.9 min later than the other isoform. Methylated peptides showed similar retention times as expected (Table 3). Peptide ⁷³EIAQDFKTDLR⁸³ could be acetylated or trimethylated on K79. With the low-mass accuracy of ion traps, as with other peptides it is difficult to define the modification between acetylation and trimethylation. However, with retention time it is safe to conclude that K79 is subject to trimethylation instead of acetylation. As shown in Figure 9, the four isoforms of peptide ⁷³EIAQDFKTDLR⁸³ eluted within 0.5 min. The fourth isoform (the bottom panel) eluted closely with the unmodified form. This observation is consistent with that from high-mass accuracy mass spectrometer FT-ICR (Figure 4).

Based on our observations we conclude that relative retention time from RPLC plays a powerful supporting role in distinguishing between the isobaric modifications, acetylation and trimethylation, especially when the unmodified peptide is observed as a reference for RPLC retention time. The mono- and dimethylated isoforms can also be used as reference when the unmodified form is not present. Thus, using either the unmodified, mono- or dimethylated isoforms as references one can distinguish between trimethylation and acetylation (see Figure 5 for an example). In the case that no reference ion is observed, a high-mass accuracy mass spectrometer would be required.

CONCLUSION

The present study was undertaken to explore the feasibility of distinguishing acetylation from trimethylation of lysine by using retention time on RPLC. To address the question, histone H3 of *S. cerevisiae* was characterized by shotgun proteomics, nano-LC-MS/MS on FT-ICR and ion trap mass spectrometers. The data convincingly demonstrate acetylation resulted in substantial changes in retention time, whereas methylation not. Thus relative retention plays a powerful supporting role in distinguishing between these isobaric modifications.

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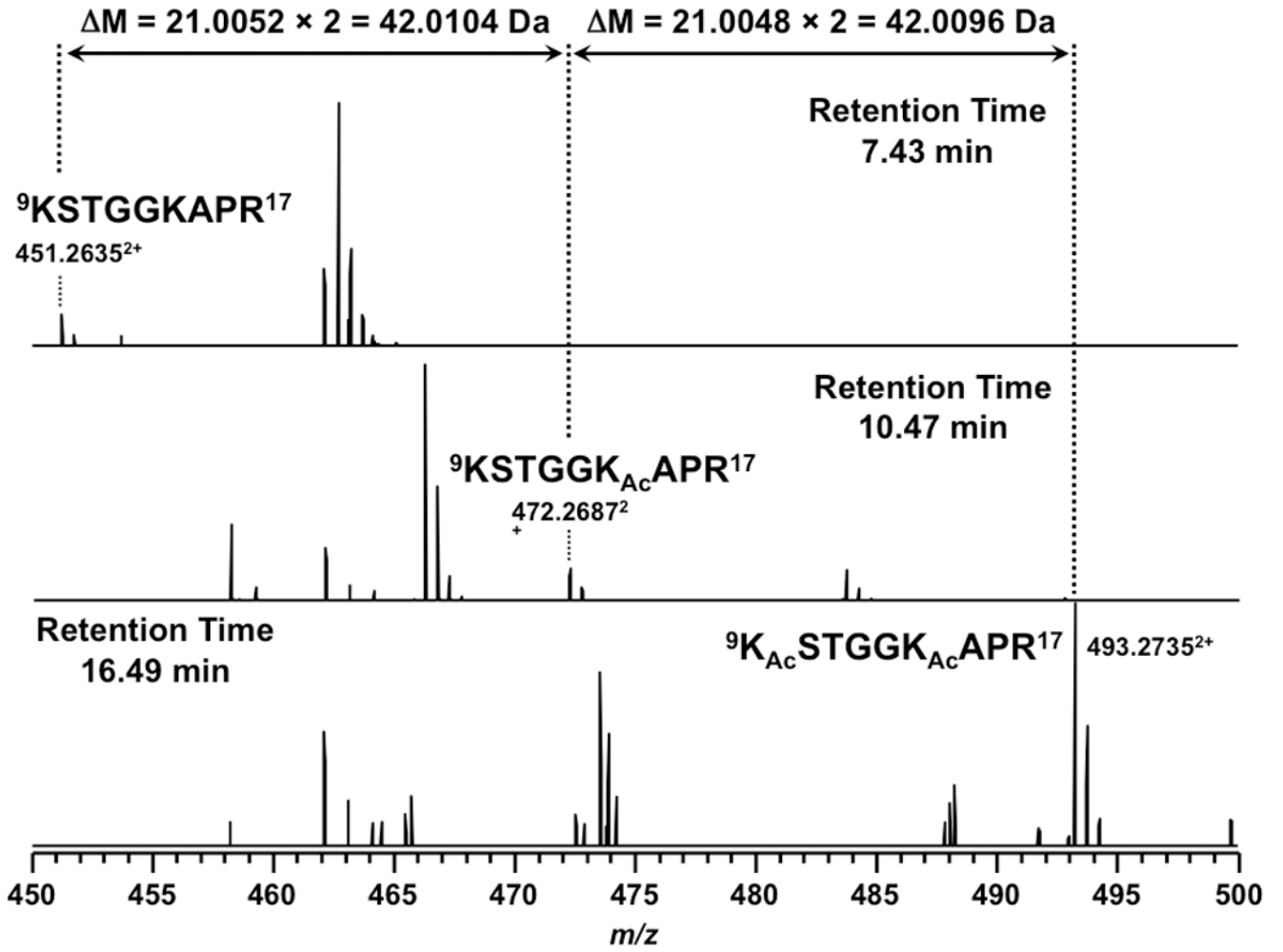


Figure 1.
 Full MS results of peptide ${}^9\text{KSTGGKAPR}^{17}$ from an LTQ FT-ICR mass spectrometer. The unmodified and modified isoforms are doubly charged.

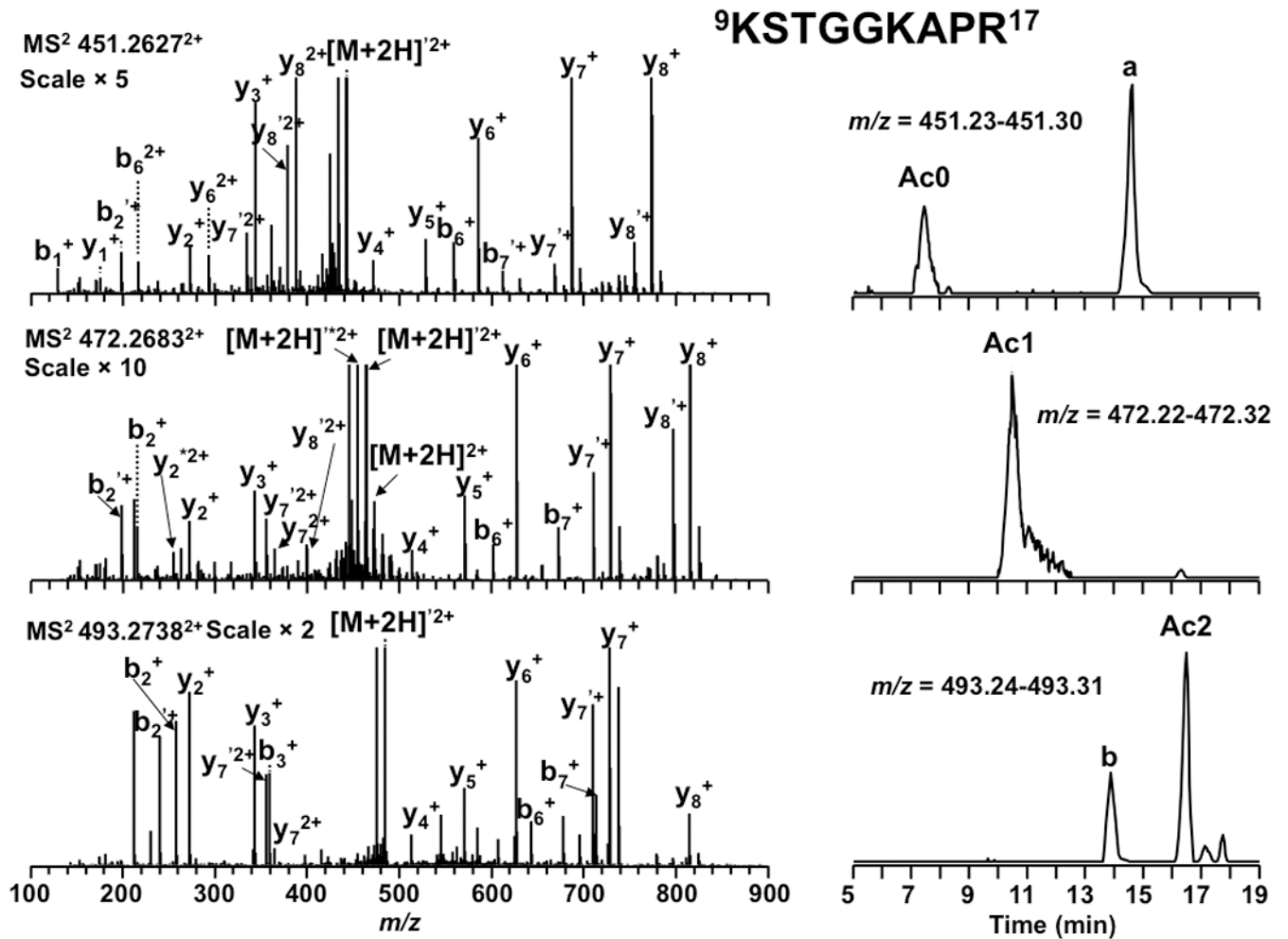


Figure 2.

Tandem MS results and LC-extracted ion chromatograms of peptide ⁹KSTGGKAPR¹⁷ from an LTQ FT-ICR mass spectrometer. Product ions resulting from neutral loss of H₂O or NH₃ are indicate by ' and * respectively. The peak labeled a was from the second isotopic peak of +3 charged peak at 450.5921 m/z and b was from an unidentified +3 charged peak at m/z 493.2911. Their MS/MS spectra were obtained but not matched by the database search. Lower charge states were not observed for peaks a and b.

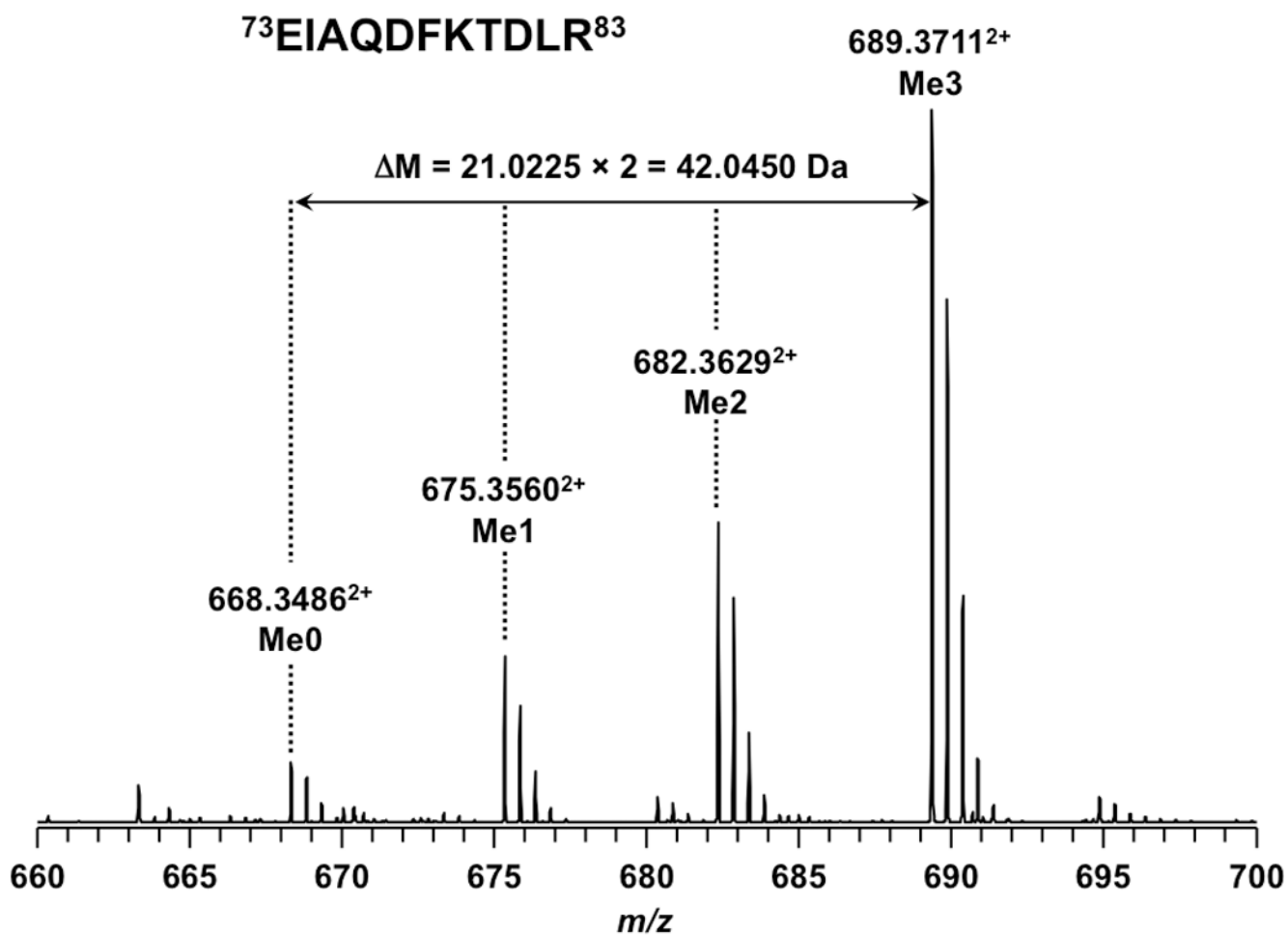


Figure 3. Full MS results of peptide $^{73}\text{EIAQDFKTDLR}^{83}$ from an LTQ FT-ICR mass spectrometer. The unmodified and modified isoforms are doubly charged.

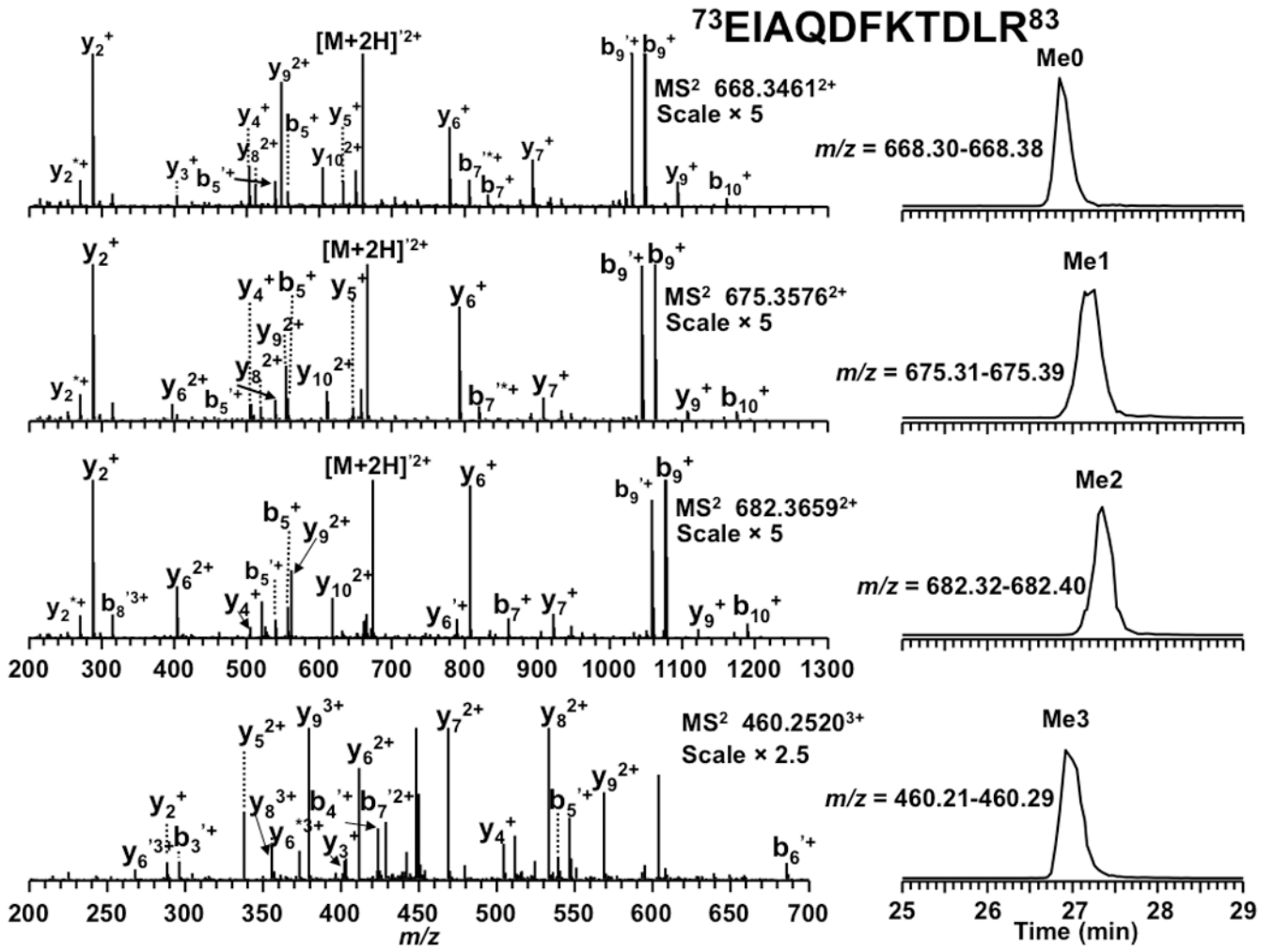


Figure 4. Tandem MS results and LC-extracted ion chromatograms of peptide ⁷³EIAQDFKTDLR⁸³ from an LTQ FT-ICR mass spectrometer. Product ions resulting from neutral loss of H₂O or NH₃ are indicate by ' and * respectively.

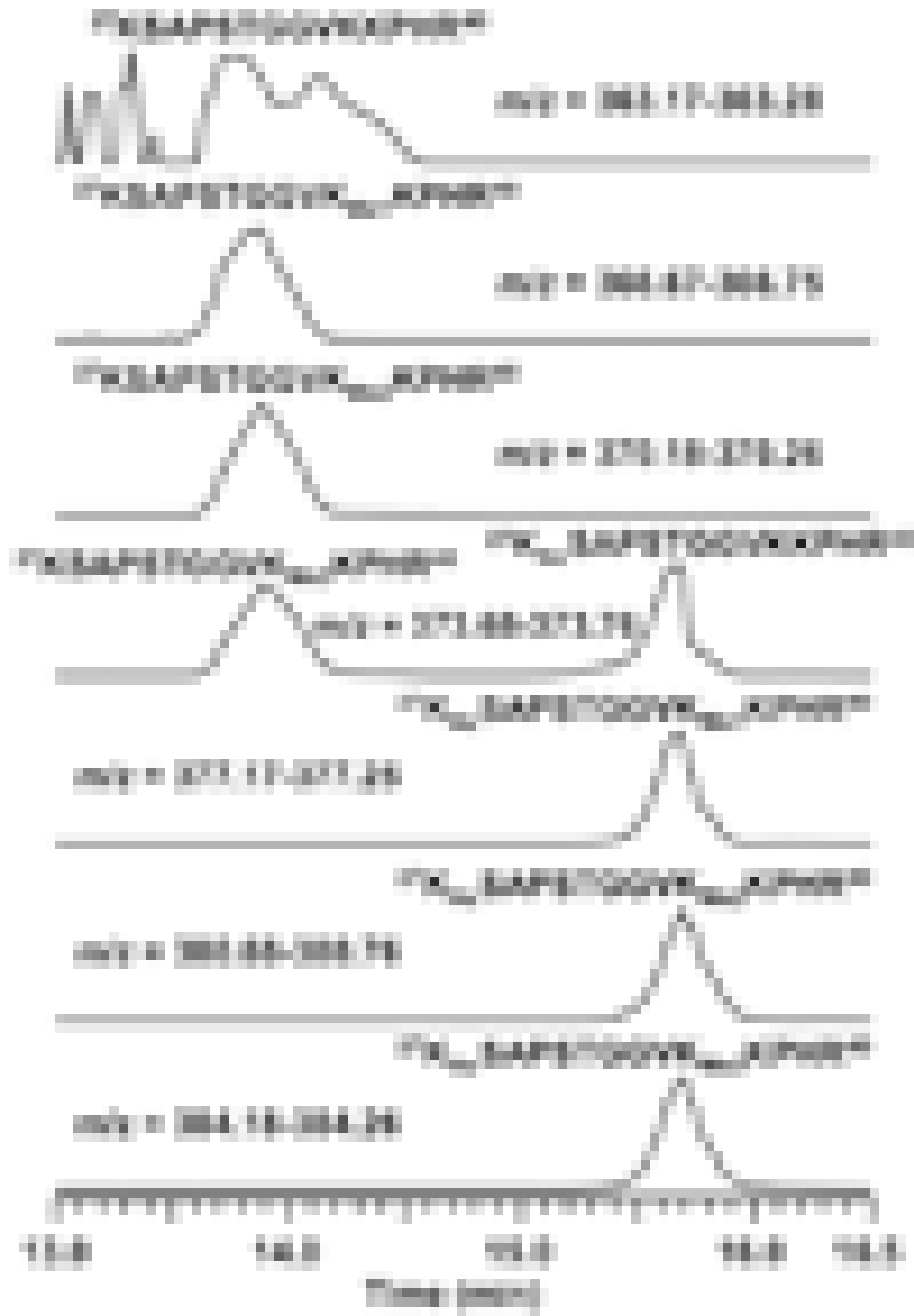


Figure 5. LC-extracted ion chromatograms of peptide $^{27}\text{KSAPSTGGVKKPHR}^{40}$ from an LTQ FT-ICR mass spectrometer.

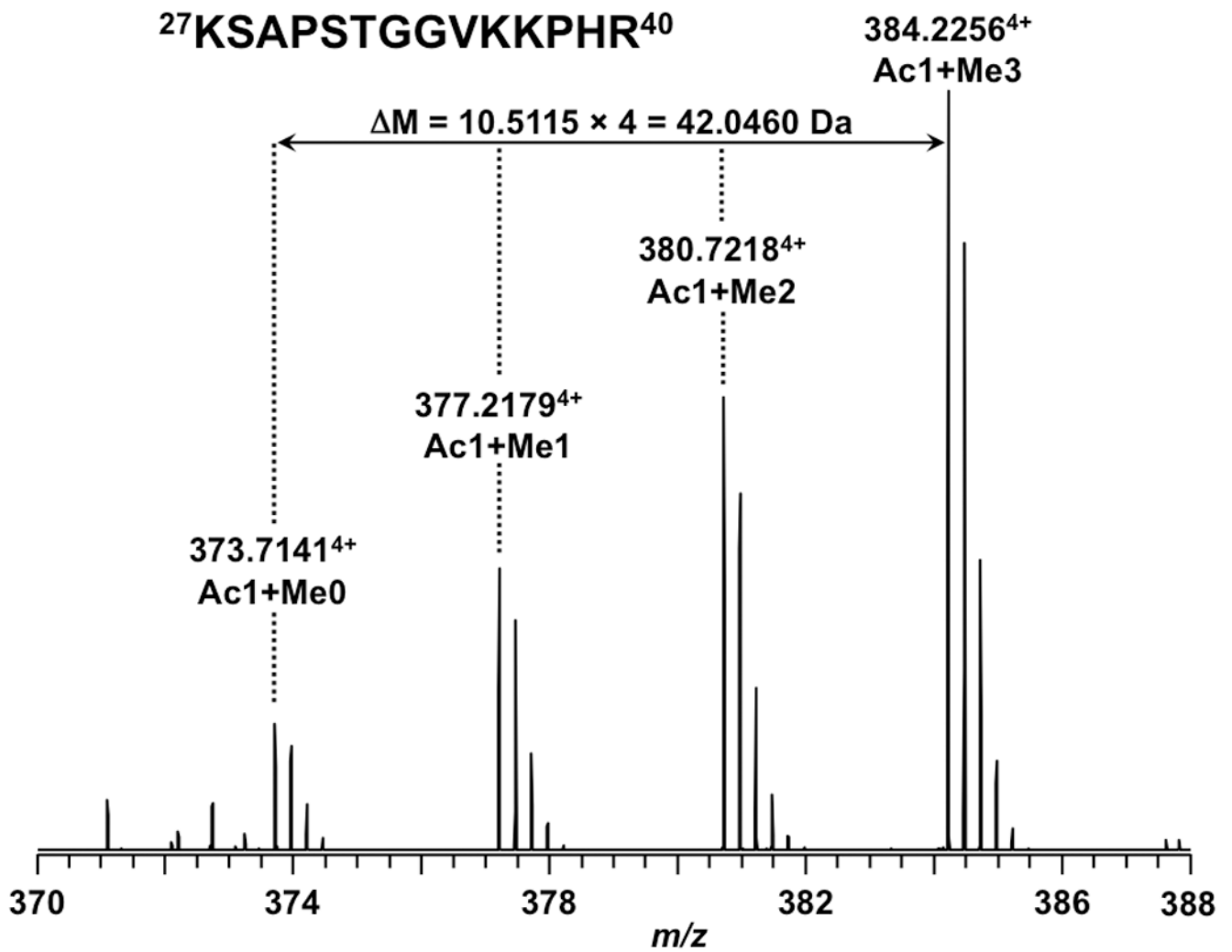
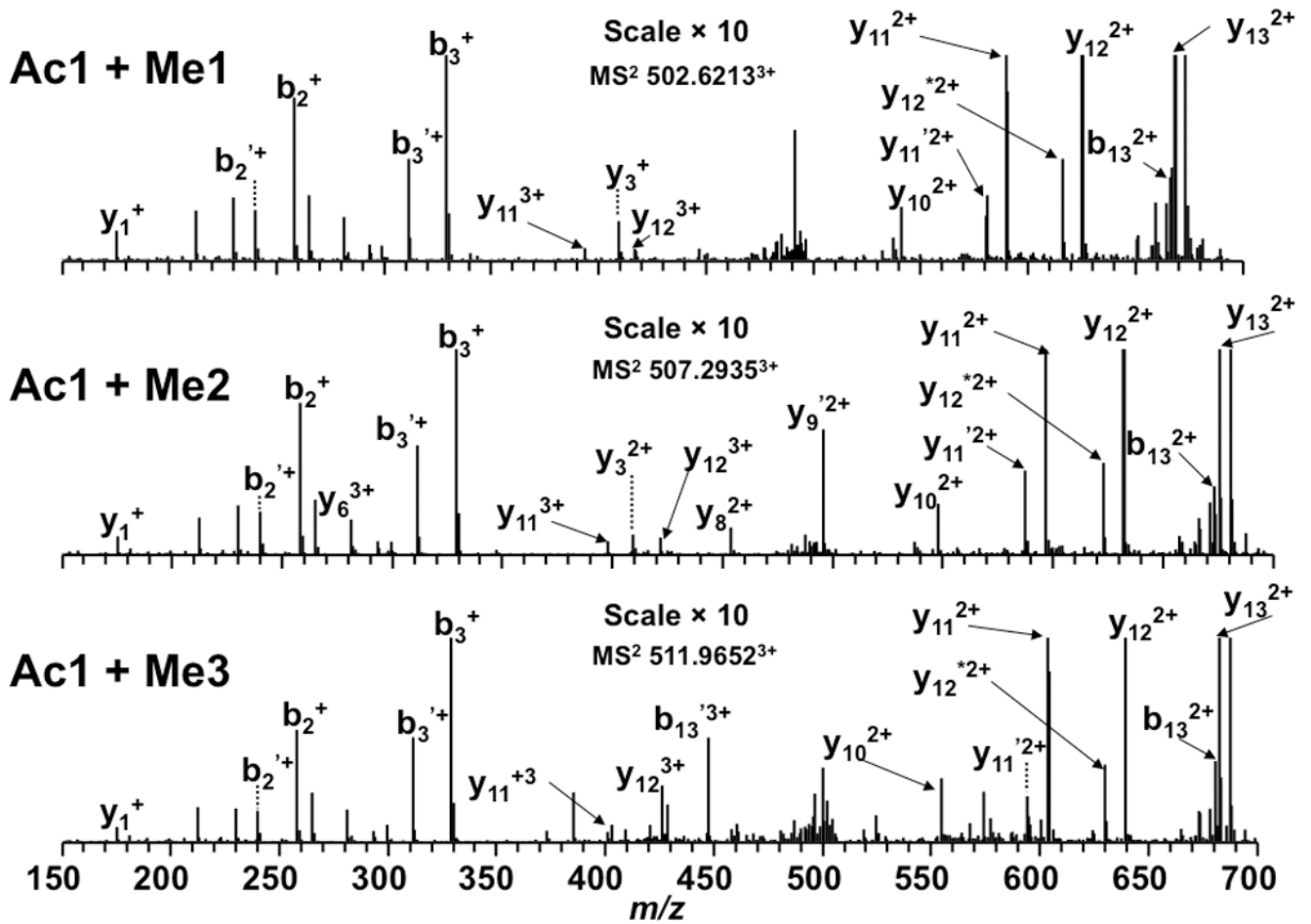


Figure 6. Full MS results of peptide $^{27}\text{KSAPSTGGVKKPHR}^{40}$ from an LTQ FT-ICR mass spectrometer. All the isoforms are +4 charged.

$^{27}\text{KSAPSTGGVKKPHR}^{40}$ **Figure 7.**

Tandem MS results of peptide $^{27}\text{KSAPSTGGVKKPHR}^{40}$ from an LTQ FT-ICR mass spectrometer. Product ions resulting from neutral loss of H_2O or NH_3 are indicated by ' and * respectively.

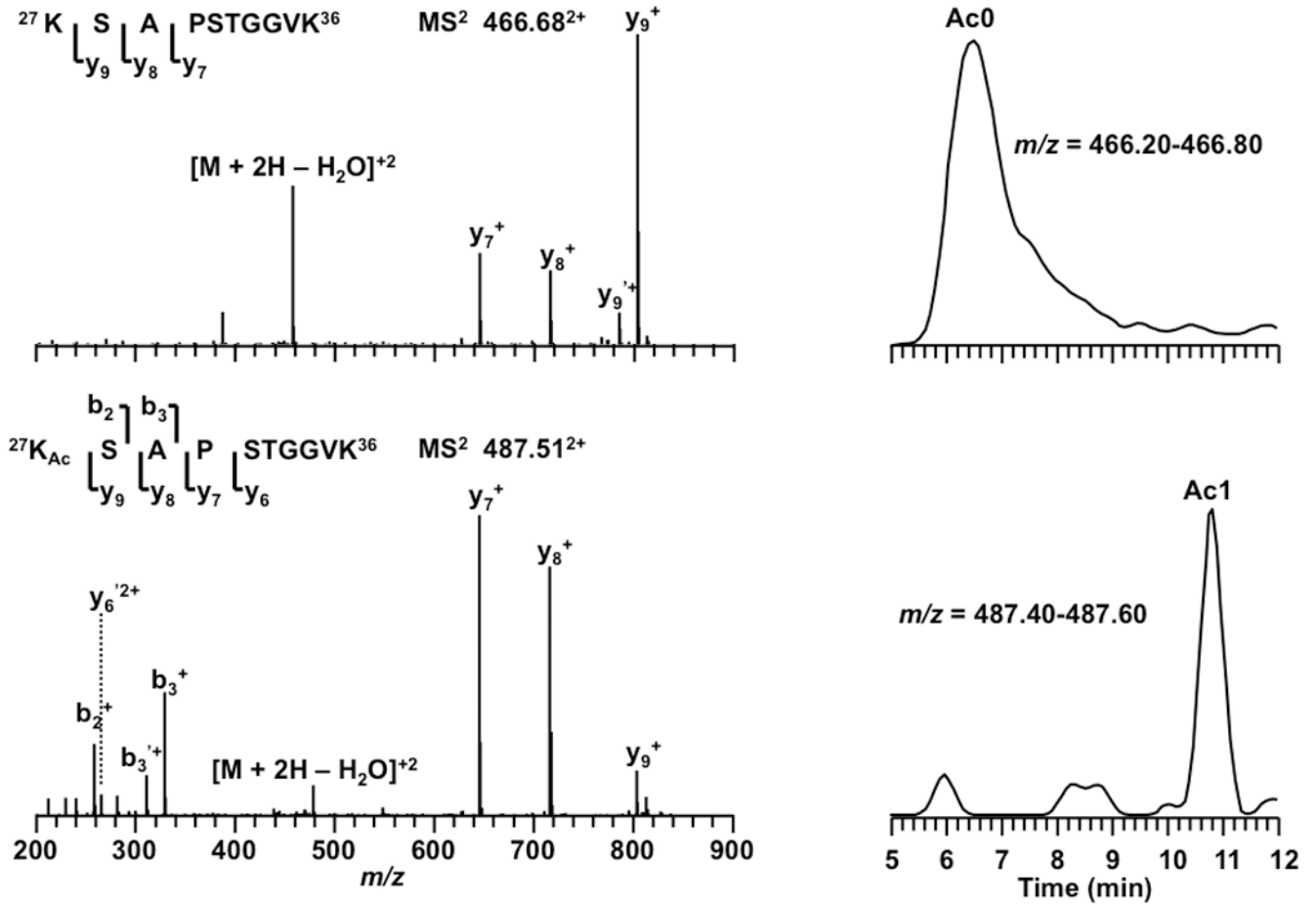


Figure 8. Tandem MS results and LC-extracted ion chromatograms of peptide ²⁷K_SA_PSTGGVK³⁶ from an LCQ DECA XP+ ion trap mass spectrometer. Product ions resulting from neutral loss of H₂O or NH₃ are indicated by ' and * respectively.

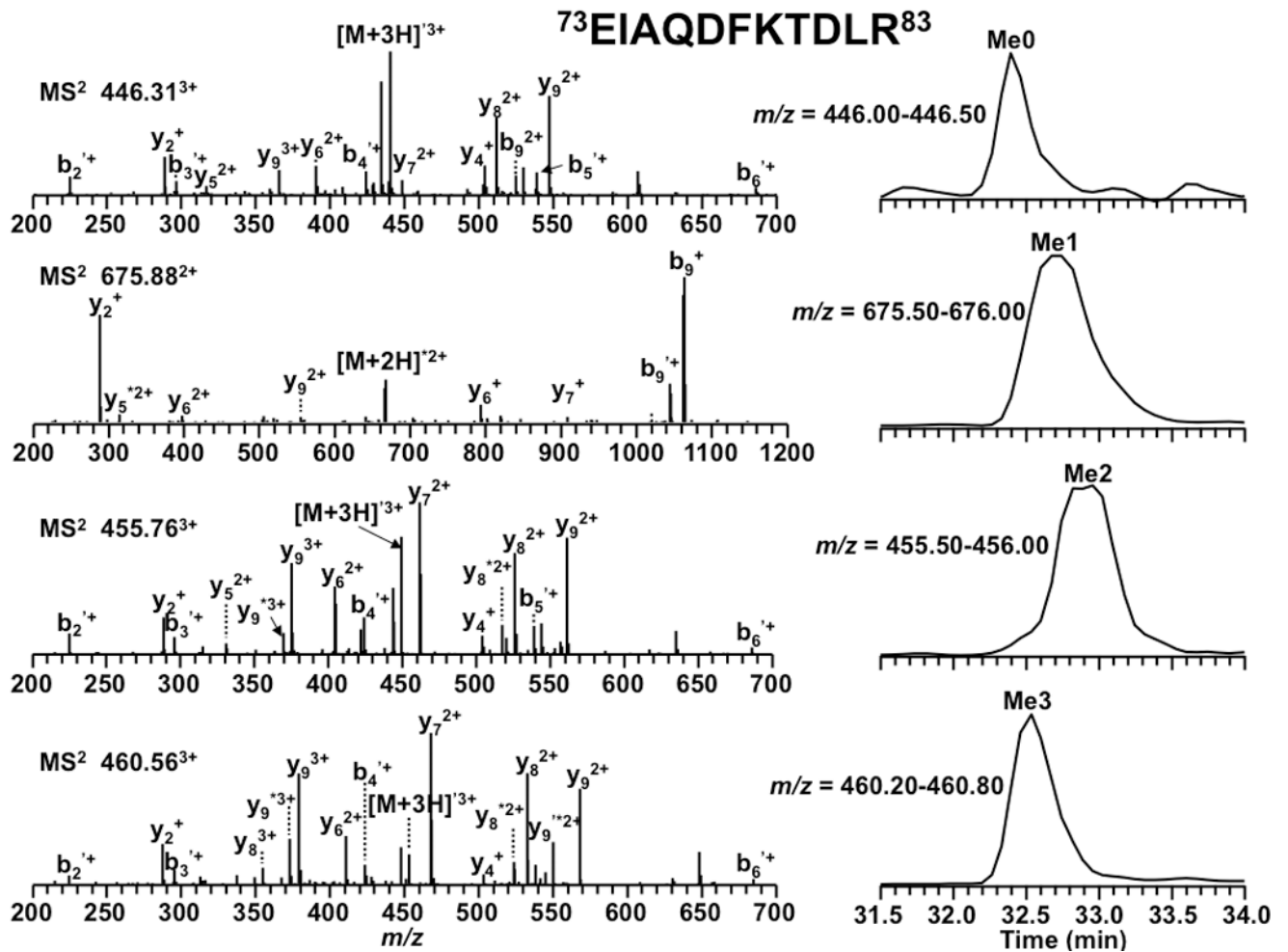


Figure 9. Tandem MS results and LC-extracted ion chromatograms of peptide ⁷³EIAQDFKTDLR⁸³ from an LCQ DECA XP+ ion trap mass spectrometer. Product ions resulting from neutral loss of H₂O or NH₃ are indicate by ' and * respectively.

Table 1

Peptides of yeast H3 detected by LTQ-FTMS (Me: Monomethylation; Me2: Dimethylation; Me3: Trimethylation; Ac: Acetylation; RT: Retention time)

Measured (m/z)	Calculated (m/z)	RT (min)	Sequence	Error (ppm)
337.7123 ²⁺	337.7141 ²⁺	8.16	¹⁸ KQLASK ²³	-5.33
358.7179 ²⁺	358.7194 ²⁺	16.15	¹⁸ K _{Ac} QLASK ²³	-4.18
	358.7375 ²⁺		¹⁸ K _{Me3} QLASK ²³	-54.64
	358.7375 ²⁺		¹¹⁷ VTIQKK ¹²²	-54.64
352.7056 ²⁺	352.7068 ²⁺	6.10	³ TKQTAR ⁸	-3.40
359.7131 ²⁺	359.7146 ²⁺	6.91	³ TK _{Me} QTAR ⁸	-4.17
366.7208 ²⁺	366.7224 ²⁺	7.13	³ TK _{Me2} QTAR ⁸	-4.36
373.7287 ²⁺	373.7303 ²⁺	6.99	³ TK _{Me3} QTAR ⁸	-4.28
	373.7121 ²⁺		³ TK _{Ac} QTAR ⁸	44.42
387.2156 ²⁺	387.2175 ²⁺	8.20	¹⁰ STGGKAPR ¹⁷	-4.91
408.2213 ²⁺	408.2228 ²⁺	15.48	¹⁰ STGGK _{Ac} APR ¹⁷	-3.67
	408.2410 ²⁺		¹⁰ STGGK _{Me3} APR ¹⁷	-48.26
451.2635 ²⁺	451.2650 ²⁺	7.43	⁹ KSTGGKAPR ¹⁷	-3.32
472.2687 ²⁺	472.2703 ²⁺	10.47	⁹ KSTGGK _{Ac} APR ¹⁷	-3.38
	472.2885 ²⁺		⁹ KSTGGK _{Me3} APR ¹⁷ / ⁹ K _{Me3} STGGKAPR ¹⁷	-41.92
493.2735 ²⁺	493.2756 ²⁺	16.49	⁹ K _{Ac} STGGK _{Ac} APR ¹⁷	-4.26
	493.2938 ²⁺		⁹ K _{Me3} STGGK _{Ac} APR ¹⁷ / ⁹ K _{Ac} STGGK _{Me3} APR ¹⁷	-41.15
	493.3119 ²⁺		⁹ K _{Me3} STGGK _{Me3} APR ¹⁷	-77.84
528.8105 ²⁺	528.8123 ²⁺	20.80	¹⁸ K _{Ac} QLASK _{Ac} AAR ²⁶	-3.40
	528.8305 ²⁺		¹⁸ K _{Ac} QLASK _{Me3} AAR ²⁶ / ¹⁸ K _{Me3} QLASK _{Ac} AAR ²⁶	-37.82
	528.8487 ²⁺		¹⁸ K _{Me3} QLASK _{Me3} AAR ²⁶	-72.23
366.7161 ⁴⁺	366.7172 ⁴⁺	13.89	²⁷ KSAPSTGGV _{Me} KPHR ⁴⁰	-3.00
370.2199 ⁴⁺	370.2211 ⁴⁺	13.89	²⁷ KSAPSTGGV _{Me2} KPHR ⁴⁰	-3.24
373.7237 ⁴⁺	373.7250 ⁴⁺	13.89	²⁷ KSAPSTGGV _{Me3} KPHR ⁴⁰	-3.48
	373.7159 ⁴⁺		²⁷ KSAPSTGGV _{Ac} KPHR ⁴⁰	20.87
373.7141 ⁴⁺	373.7159 ⁴⁺	15.63	²⁷ K _{Ac} SAPSTGGV _{Me} KPHR ⁴⁰	-4.82
	373.7250 ⁴⁺		²⁷ K _{Me3} SAPSTGGV _{Me} KPHR ⁴⁰	-29.17
377.2179 ⁴⁺	377.2198 ⁴⁺	15.63	²⁷ K _{Ac} SAPSTGGV _{Me} KPHR ⁴⁰	-5.04
380.7218 ⁴⁺	380.7237 ⁴⁺	15.63	²⁷ K _{Ac} SAPSTGGV _{Me2} KPHR ⁴⁰	-4.99
384.2256 ⁴⁺	384.2276 ⁴⁺	15.63	²⁷ K _{Ac} SAPSTGGV _{Me3} KPHR ⁴⁰	-5.21
	384.1936 ⁴⁺		²⁷ K _{Ac} SAPSTGGV _{Ac} KPHR ⁴⁰	83.29
497.9493 ³⁺	497.9519 ³⁺	15.63	²⁷ K _{Ac} SAPSTGGV _{Me} KPHR ⁴⁰	-5.22
	497.9859 ³⁺		²⁷ K _{Me3} SAPSTGGV _{Me} KPHR ⁴⁰	-73.50
502.6213 ³⁺	502.6237 ³⁺	15.63	²⁷ K _{Ac} SAPSTGGV _{Me} KPHR ⁴⁰	-4.77
507.2933 ³⁺	507.2956 ³⁺	15.73	²⁷ K _{Ac} SAPSTGGV _{Me2} KPHR ⁴⁰	-4.53
511.9651 ³⁺	511.9675 ³⁺	15.63	²⁷ K _{Ac} SAPSTGGV _{Me3} KPHR ⁴⁰	-4.69
695.9089 ²⁺	695.9126 ²⁺	27.47	⁵³ RFQKSTELLIR ⁶³	-5.32
716.9148 ²⁺	716.9178 ²⁺	29.35	⁵³ RFQK _{Ac} STELLIR ⁶³	-4.18
	716.9360 ²⁺		⁵³ RFQK _{Me3} STELLIR ⁶³	-29.57
638.8659 ²⁺	638.8671 ²⁺	32.58	⁵⁴ FQK _{Ac} STELLIR ⁶³	-1.88
	638.9011 ²⁺		⁵⁴ FQK _{Me3} STELLIR ⁶³	-55.09
568.6510 ³⁺	568.6533 ³⁺	29.92	⁷⁰ LVREIAQDFKTDLR ⁸³	-4.04
573.3231 ³⁺	573.3251 ³⁺	30.02	⁷⁰ LVREIAQDFK _{Me} TDLR ⁸³	-3.49
577.9946 ³⁺	577.9970 ³⁺	30.07	⁷⁰ LVREIAQDFK _{Me2} TDLR ⁸³	-4.15
582.6666 ³⁺	582.6689 ³⁺	29.92	⁷⁰ LVREIAQDFK _{Me3} TDLR ⁸³	-3.95
	582.6349 ³⁺		⁷⁰ LVREIAQDFK _{Ac} TDLR ⁸³	54.41
668.3486 ²⁺	668.3495 ²⁺	26.85	⁷³ EIAQDFKTDLR ⁸³	-1.35
	668.3789 ²⁺		²⁸ SAPSTGGV _{Me} KPHR ⁴⁰	-45.33
675.3560 ²⁺	675.3573 ²⁺	27.25	⁷³ EIAQDFK _{Me} TDLR ⁸³	-1.92
	675.3867 ²⁺		²⁸ SAPSTGGV _{Me2} KPHR ⁴⁰	-45.45
682.3629 ²⁺	682.3651 ²⁺	27.35	⁷³ EIAQDFK _{Me2} TDLR ⁸³	-3.22
	682.3763 ²⁺		²⁸ SAPSTGGV _{Ac} KPHR ⁴⁰	-19.64
689.3711 ²⁺	682.3945 ²⁺	26.92	²⁸ SAPSTGGV _{Me3} KPHR ⁴⁰	-46.31
	689.3729 ²⁺		⁷³ EIAQDFK _{Me3} TDLR ⁸³	-2.61
	689.3547 ²⁺		⁷³ EIAQDFK _{Ac} TDLR ⁸³	23.79
	689.3842 ²⁺		²⁸ SAPSTGGV _{Ac} K _{Me} PHR ⁴⁰	-19.00
	689.4024 ²⁺		²⁸ SAPSTGGV _{Me3} K _{Me} PHR ⁴⁰	-45.40
335.5339 ³⁺	335.5351 ³⁺	20.96	⁴¹ YKPGTVALR ⁴⁹	-3.58
534.3211 ³⁺	534.3233 ³⁺	31.44	⁵⁷ STELLIRKLPQR ⁶⁹	-4.12

Note: False assignments are italicized.

Table 2Posttranslational modification sites in histone H3 of *S. cerevisiae*

Residue	Posttranslational Modification
K4	Me1, Me2, Me3
K9	Ac
K14	Ac
K18	Ac
K23	Ac
K27	Ac
K36	Me1, Me2, Me3
K56	Ac
K79	Me1, Me2, Me3

Table 3

Peptides detected by LCQ DECA XP+ MS (Me: Monomethylation; Me2: Dimethylation; Me3: Trimethylation; Ac: Acetylation; RT: Retention time)

Measured (m/z)	Calculated (m/z)	RT (min)	Sequence
446.31 ³⁺	445.90 ³⁺	32.40	⁷³ EIAQDFKTDLR ⁸³
675.88 ²⁺	675.36 ²⁺	32.74	⁷³ EIAQDFK _{Me} TDLR ⁸³
455.76 ³⁺	455.25 ³⁺	32.89	⁷³ EIAQDFK _{Me2} TDLR ⁸³
460.56 ³⁺	459.92 ³⁺	32.54	⁷³ EIAQDFK _{Me3} TDLR ⁸³
669.07 ²⁺	668.38 ²⁺	15.38	²⁸ SAPSTGGVK _{Me} KPHR ⁴⁰
675.76 ²⁺	675.39 ²⁺	16.09	²⁸ SAPSTGGVK _{Me2} KPHR ⁴⁰
683.04 ²⁺	682.39 ²⁺	15.68	²⁸ SAPSTGGVK _{Me3} KPHR ⁴⁰
466.68 ²⁺	466.26 ²⁺	6.52	²⁷ KSAPSTGGVK ³⁶
487.51 ²⁺	487.27 ²⁺	10.83	²⁷ K _{Ac} SAPSTGGVK ³⁶
508.15 ²⁺	507.81 ²⁺	16.73	¹⁸ KQLASK _{Ac} AAR ²⁶
529.18 ²⁺	528.81 ²⁺	25.50	¹⁸ K _{Ac} QLASK _{Ac} AAR ²⁶
538.59 ³⁺	538.32 ³⁺	79.04	¹ _{Ac} SGRGKGGKGLGKGGAKR ¹⁷
828.09 ²⁺	827.98 ²⁺	82.17	¹ _{Ac} SGRGKGGKGLGKGGAK _{Ac} R ¹⁷