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Consecutive neutral losses of H_2O and C_2H_4O from N-Terminal Thr–Thr and Thr–Ser in collision-induced dissociation of protonated peptides Position dependent water loss from single Thr or Ser

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Abstract

A two-step neutral loss from tryptic peptides containing Thr–Thr or Thr-Ser at their N-terminus is studied. This process also requires a 'mobile proton' (i.e., the number of charges on the peptide is greater than the number of basic amino acids) and leads to a net neutral loss of 62 Da. To elucidate this fragmentation a series of synthetic peptides containing threonine and serine were synthesized and their MS/MS spectra measured in ion trap and triple quadrupole instruments. Peptides composed of TTL_nK ($n = 2$–8) all show a significant loss of 62 Da when doubly protonated, but little such loss when singly protonated. Examination of the MS/MS spectra at different collision energies in a triple quadrupole mass spectrometer reveal that this loss takes place in two distinct steps: an initial water loss, followed by a loss of a 44 Da moiety at higher collision energies (20–30% higher). Corresponding losses in b/a ions show losses near the N-terminus and higher accuracy mass measurements indicate the loss to be C_2H_4O rather than CO_2. Further measurements show that doubly protonated TSL_nK peptides undergo similar processes but STL_nK and SSL_nK do not. Therefore, it is proposed that the 44 Da loss is a loss of C_2H_4O from the N-terminal threonine. Additional measurements on the loss of water from protonated peptides containing only one threonine or serine show a strong and unexpected dependence of the extent of water loss on the position of T or S within the peptide sequence. The most pronounced water loss is found in doubly protonated tryptic peptides containing T or S at the second or third position from the N-terminus, i.e., adjacent to the peptide bond that is most likely to cleave. It is, therefore, proposed that the initial loss of H_2O in the 62 Da loss process begins at the penultimate N-terminal threonine or serine. The origin of this positional sensitivity is not clear, though may be connected with the well-known relatively facile b_2 production in tryptic peptides.

Keywords: Peptides; Fragmentation; Proteomics; Electrospray; Cid

1. Introduction

Mass spectra from the collision-induced dissociation of protonated peptides are widely employed for identifying peptides, which in turn serve to identify the proteins from which they were derived. Current identification methods count fragment ions matching possible fragment ions from peptide bond cleavage, and make little use of relative ion abundances or unexpected fragments. In principle, these additional factors can increase the confidence of identification. This can be done most directly by matching reference spectra to spectra of previously identified peptides, a method that is attracting increased attention [1,2]. However, the quality of spectra in these libraries is a major concern since impurity peaks are commonly present in spectra and can prevent confident identification. Hence it is important to know whether unexplained peaks are due to unexpected fragmentation paths or impurity ions. Moreover, improved predictive ability can yield more reliable theoretical spectra for better matching observed spectra where library spectra are unavailable [3].

At present a large proportion of product ion abundance can be explained by well-known fragmentation paths. However, in the course of building a reference library of peptide fragmentation spectra [4] we have found various unexpected cleavages that could erroneously be labeled as impurity ions. In order to improve library annotation, enhance quality control and to aid

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in the prediction of fragmentation patterns we have been studying such reactions in some detail. A prior study was devoted to loss of water or ammonia from N-terminal glutamine [5]. The present study focuses on the loss of 62 Da from tryptic peptides (C-terminal lysine or arginine) having a threonine at the N-terminus adjacent to a serine or threonine residue and examines a series of threonine containing peptides to search for related reactions.

Our recent results on glutamine [5] show that in the absence of mobile protons (i.e., the number of protons is less than or equal to the number of bases (R, K, H) [6]), glutamine deamination is the most facile of all loss processes. For peptide ions with mobile protons, however, dehydration of N-terminal glutamine is the most facile. The data also show that serine and threonine, which bear a hydroxyl group on the side chain, exhibit water loss no greater than most other N-terminal residues. However, doubly protonated peptide ions containing N-terminal TT or TS show a significant fragment peak due to loss of 62 Da. In the present work we examine this loss in various synthetic peptides and as a function of collision voltage. We find it to be due to two consecutive losses at increasing collision voltage, first of water and then of a 44 Da moiety. The mechanism of this process is studied by using a selected series of synthetic peptides.

2. Experimental[1]

Some of the peptides were synthesized in a Protein Technologies Inc. (Tucson, AZ) PS3 peptide synthesizer and others in an AAPPTEC (Louisville, KY) APEX 396 synthesizer by using standard procedures. The peptides were dissolved in methanol/water (v/v, 1:1) containing 0.1% formic acid. For a few hydrophobic peptides with solubility too low to provide good quality spectra, methanol was replaced with 1-propanol and/or the fraction of water in the solvent mixture was decreased to 20%.

Electrospray ionization mass spectrometry was carried out with a Micromass (Waters Corp., Milford, Massachusetts) Quattro Micro triple quadrupole instrument. First the mass spectrum was measured at different cone voltages to determine the voltage at which the peptide ion peak is maximized. Then, selecting the precursor ion at that cone voltage into a collision cell (with 0.21 Pa (1.6 mTorr) Ar as collision gas), the MS/MS spectrum was measured at 20 different collision voltages. The range of collision voltage spanned from near zero up to a value where little precursor ion remained. The peak intensities of all the significant fragment ion peaks were calculated as a fraction from the total ion intensity and plotted as a function of collision voltage. MS^3 spectra were measured by using a high cone voltage so as to produce the initial fragment ion in the cone region and then this ion was selected for MS/MS measurement as above. Spectra were acquired in 'centroid' mode, whereby signals within each individual time interval in a given spectrum were centered and integrated by the instrument data system. Typically, relative *m*/*z* values were within 0.2 of the theoretical *m*/*z* values throughout the *m*/*z* range of interest.

To examine the influence of type of collisional excitation, MS/MS spectra of some of the same peptides were also measured with an ion trap instrument (LTQ, Thermo Electron Corp., Waltham, Massachusetts). Samples were prepared in a manner similar to that described above. Tandem mass spectra were collected at collision energy setting of 35%.

3. Results and discussion

The main set of peptides synthesized has the sequence TTL_nK ($n = 2$–8). Leucine residues were used as representative medium size amino acid with no reactive functional groups. The MS/MS spectra of a representative doubly protonated peptide ion, recorded with the two types of mass spectrometers (Fig. 1), matched well at collision energies in the triple quadrupole that were low enough to prevent extensive secondary dissociation, but high enough to provide significant product ion intensities. The spectra clearly show the peaks due to neutral losses of 18 Da and 62 Da. When the spectrum is recorded at different collision

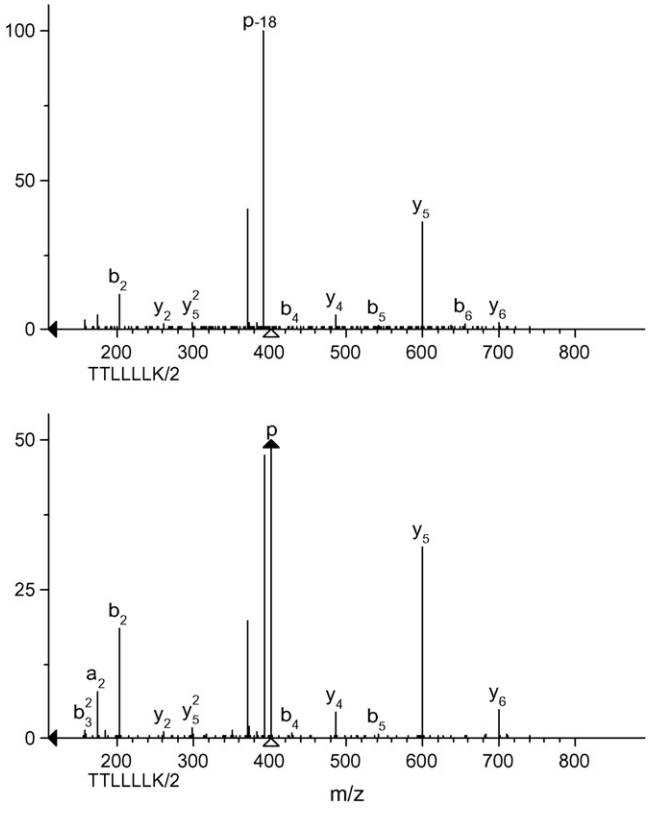


Fig. 1. Comparison of the MS/MS spectrum of the doubly protonated TTLLLLK ion measured in the ion-trap mass spectrometer (top) with a spectrum measured in the triple quadrupole instrument at an intermediate collision voltage (13 V) (bottom). While the precursor peak is completely depleted in the ion-trap spectrum, a large fraction of it still remains in the triple quadrupole spectrum, as is evident from the different intensity scales (*y* axis). The unlabeled intense peak at *m*/*z* 370.5 corresponds to the $(MH_2\text{-}62)^{2+}$ ion.

[1] Certain commercial equipment, instruments, or materials are identified in this document. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the products identified are necessarily the best available for the purpose.

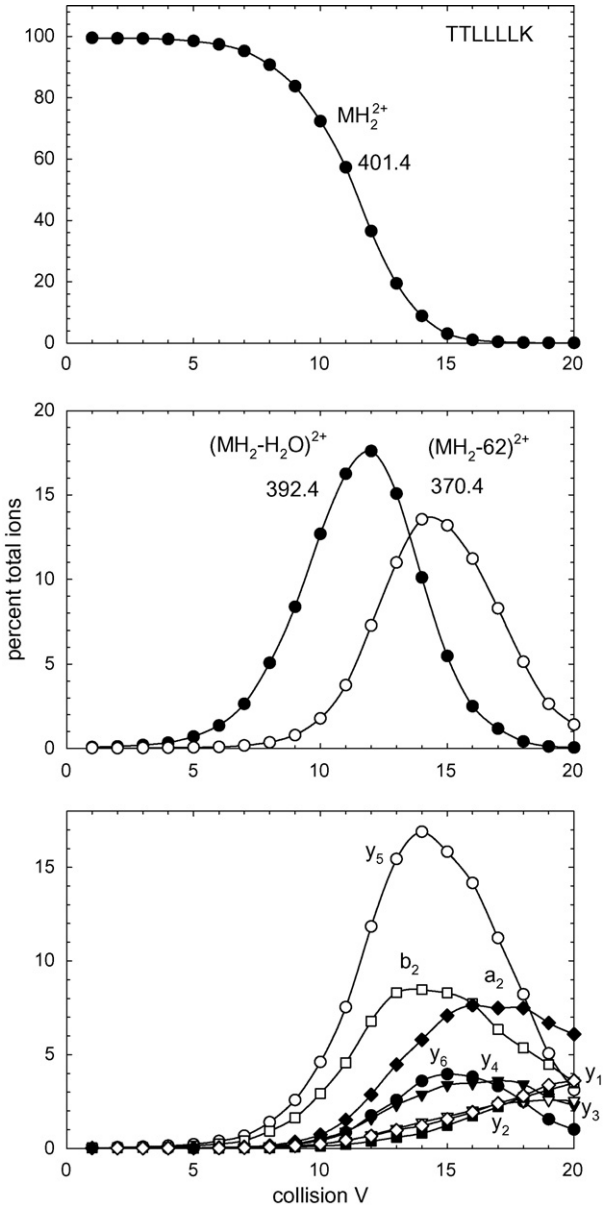


Fig. 2. Decay of the precursor peak and formation of fragment peaks as a function of collision voltage in the triple quadrupole MS/MS spectrum of doubly protonated TTLLLLK ion.

voltages in the triple quadrupole instrument we find two consecutive neutral loss processes at increasing voltage (Fig. 2). A very facile neutral loss of water occurs immediately upon decomposition of the precursor ion. A neutral loss of a 44 Da moiety occurs only at higher collision voltage. The other major fragmentations occurring in parallel with the latter process are mainly those forming the b_2 ion and the corresponding y ion. All these fragments show peak intensities that increase with collision voltage, reach a certain maximum level, and then decrease at higher voltage due to subsequent decomposition to smaller fragments, some of which are also shown in Fig. 2.

The results for all peptide ions of the TTL_nK series examined are qualitatively very similar. A comparison of all of these ions in a condensed form is presented in Table 1, where the maximum level reached and the collision voltage at which it is reached is listed for the two peaks resulting from the neutral losses. It is seen that the peak intensities reach a level of about 15.5% and 12.5% for the $(MH_2\text{-}H_2O)^{2+}$ and $(MH_2\text{-}62)^{2+}$, respectively, when $n = 2$–5. When n is increased from 5 to 8 the levels decrease gradually to 5% and 1.7%, respectively (Table 1). The collision voltages at which these levels are reached are always higher for the $(MH_2\text{-}62)^{2+}$ ions than for the $(MH_2\text{-}H_2O)^{2+}$ ion and in both cases they increase with the mass of the peptide. The values of $E_{1/2}$ given in the Table are the collision voltages at which the sum of the intensities of all fragment ions is equal to the remaining intensity of the precursor ion. These values increase linearly with peptide mass as discussed before [5,7].

The facile water loss is most likely from one of the terminal threonines (see below). The secondary loss of the 44 Da moiety may be either from the same region or the loss of CO_2 from the C-terminus. To distinguish between these two possibilities we measured MS^3 spectra of the $(MH_2\text{-}62)^{2+}$ ions. We increased the cone voltage to produce these fragment ions in the cone region and then selected them into the collision cell for subsequent fragmentation. The MS^3 spectra show the presence of the y ions expected from the precursor (MH_2^{2+}) but the a^+ and b^+ ions of the precursor were replaced by $(a\text{-}62)^+$ and $(b\text{-}62)^+$ ions. An example of such an MS^3 spectrum is shown in Fig. 3. These results indicate that the neutral loss is occurring at the N-terminal TT site. We propose that the 44 amu moiety is C_2H_4O, probably acetaldehyde.

By exact mass measurements, it should be possible to distinguish between loss of 44 Da due to CO_2 (43.9898 Da) or C_2H_4O (44.0262 Da). This was achieved by using an LTQ FT ultra hybrid mass spectrometer (Thermo Electron Corp., Waltham, Massachusetts) (infused with Nanomate, measured in the FT mode, resolution set to 50,000) (at the laboratory of Dr. Sonja Hess at NIH). The doubly protonated peptide TTLLLLK has $m/z = 401.27$. This precursor ion forms product ions with a peak at $m/z = 392.2709$ due to water loss and a peak at $m/z = 370.2576$ due to the second loss. The difference between these two peaks corresponds to 44.0266 Da, very close to the value for C_2H_4O and clearly higher than the value expected for loss of CO_2.

Further study of the neutral loss mechanism was undertaken by examining additional peptides as summarized in Table 1. Inserting an additional basic residue into TTL_nK, e.g., TTLLK-LLLLK, prevents formation of the $(MH_2\text{-}62)^{2+}$ ion from the doubly protonated peptide and only when this is triply protonated does it undergo the same neutral loss, now exhibited as $(MH_3\text{-}62)^{3+}$ ion (i.e., at m/z of precursor ion −20.7 instead of −31). This confirms the need of a mobile proton being available at the TT site for this reaction. The results for doubly protonated TTLLLLR are very similar to those for TTLLLLK, with only a slight decrease in the maximum levels of the neutral loss peaks. The singly protonated ions show a larger difference due to the higher basicity of R versus K [8,9], which is expressed in an increase in the $E_{1/2}$ value from 31.6 to 40.0 (data not shown in Table). When three or four threonines are present at the N-terminus the results are similar to those with

Table 1
Neutral losses and other major fragments in the MS/MS spectra of doubly protonated peptide ions containing TT or TS at the N-Terminus (MH_2^{2+})[a]

Peptide (M)	*m/z*	$E_{1/2}$	$(MH_2\text{-}H_2O)^{2+}$ (%)	coll (V)	$(MH_2\text{-}62)^{2+}$ (%)	coll (V)	Other major peaks
TTLLK	288.3	8.4	15.1	9	13.3	12	b_2, y_3
TTLLLK	344.9	9.9	14.5	10	10.4	13	b_2, y_4
TTLLLLK	401.5	11.1	17.6	12	13.5	14	b_2, y_5
TTLLLLLK	458.0	11.6	14.8	12	12.3	15	b_2, y_6
TTLLLLLLK	514.6	12.7	10.9	14	7.8	17	b_2, y_7
TTLLLLLLLK	570.9	14.6	7.5	15	4.2	19	b_2, y_8
TTLLLLLLLLK	627.7	15.2	5.0	17	1.7	21	b_2, y_7, y_8, y_9
TTLLKLLLLK	578.5	21.5	1.0	21	~0		y_8^{2+}, y_1, b_2
TTLLKLLLLK(3+)[b]	386.1	10.7	16.8	11	15.6	13	y_8^{2+}
TTLLLLR	415.3	10.5	13.7	11	10.8	14	b_2, y_5
TTTLLK	338.7	9.5	12.2[c]	10	8.5	12	y_4, y_5
TTTTLK	332.7	8.7	12.2[d]	9	7.7	12	y_4, y_5
TTPLLLK	393.4	9.0	19.3	10	13.2	13	b_2, y_5
TTLLLPLLLK	562.9	13.7	8.7	15	5.4	19	b_2, y_8, y_7
LTTLLLLLK	514.4	11.7	5.4[e]	13	~0.6	15	b_2, y_7, y_5
LLTTLLLK	457.9	11.3	26.3	13	~0		b_2, y_6
TSLLLK	337.8	10.1	7.4	10	6.2	12	b_2, y_4
STLLLK	337.8	9.3	20.0	11	~0		b_2, y_4
SSLLLK	330.8	9.5	12	11	~0		b_2, y_4

[a] The *m/z* values are experimental. The $E_{1/2}$ values are the collision voltage at which the sum of intensities of fragment peaks is equal to the remaining intensity of the precursor peak. The values for $(MH_2\text{-}H_2O)^{2+}$ and $(MH_2\text{-}62)^{2+}$ are percent of total intensity at the maximum level reached and coll is the collision voltage at which that level is reached.

[b] Results for triply protonated TTLLKLLLLK.

[c] There was also a peak due to loss of 2 water molecules, maximum level 9.3% at 11 V.

[d] There was also a peak due to loss of 2 water molecules, maximum level 8.5% at 10 V.

[e] There was also a peak due to loss of 2 water molecules, maximum level 20% at 14 V.

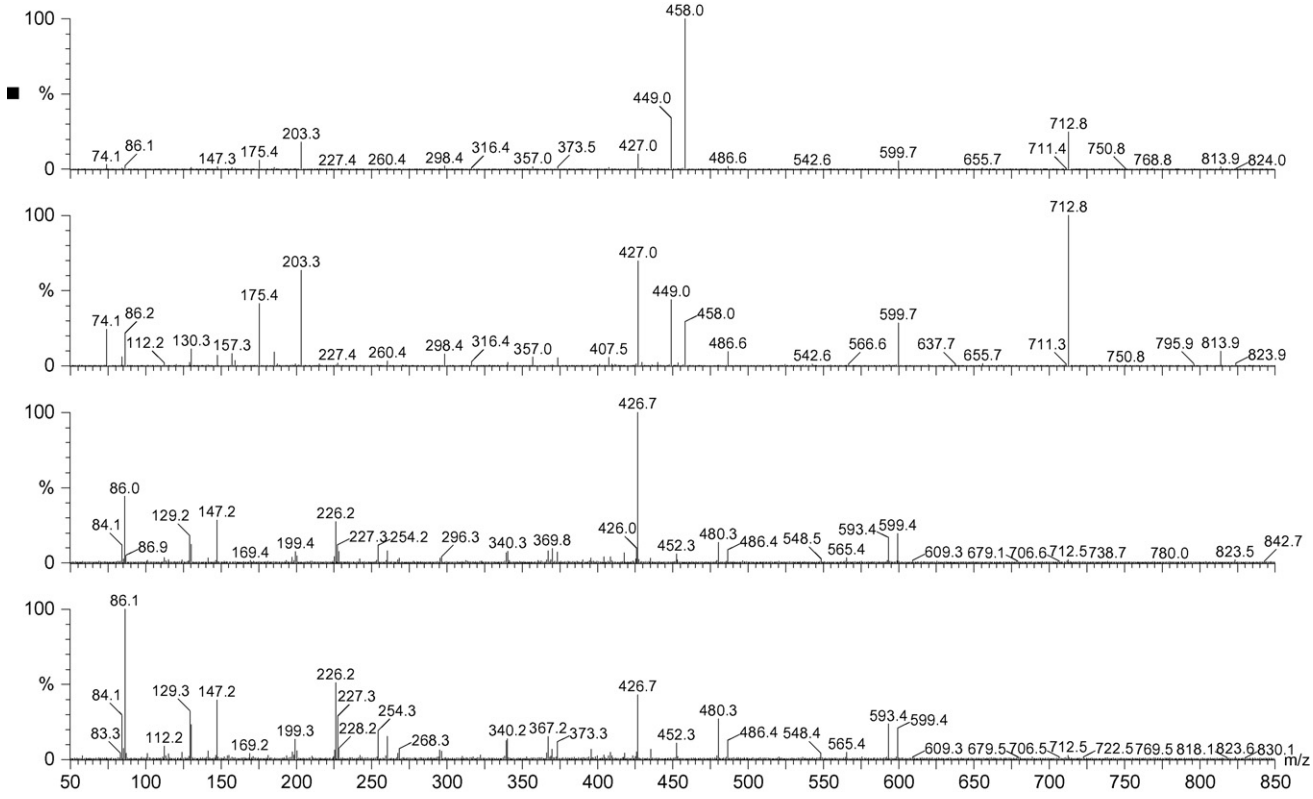


Fig. 3. Comparison of MS^2 spectra of doubly protonated TTLLLLLK ion (top two spectra, collision $V = 12$, 15) and MS^3 spectra of its $(MH_2\text{-}62)^{2+}$ fragment (bottom two spectra, collision $V = 18$, 19.5). The MS^2 spectra show the precursor peak MH_2^{2+} at *m/z* 458, the $(MH_2\text{-}H_2O)^{2+}$ peak at *m/z* 449 and the $(MH_2\text{-}62)^{2+}$ peak at *m/z* 427; the other major peaks are y_6 (712), b_2 (203), a_2 (175), y_5 (599), and y_4 (486). The MS^3 spectra show the precursor peak at *m/z* 427 and the product peaks: y_5 (599), y_4 (486), y_3 (373), y_2 (260), and y_1 (147), and peaks at 593 (b_6-62), 480 (b_5-62), 452 (a_5-62), 367 (b_4-62), 340 (a_4-62), 254 (b_3-62), and 226 (a_3-62) (the a and b ions are those expected from the original precursor at *m/z* 458).

two threonines, but in addition to the $(MH_2\text{-}H_2O)^{2+}$ and the $(MH_2\text{-}62)^{2+}$ ions there are also intense peaks due to loss of two water molecules.

The non-tryptic peptide TTLLLLL (not shown in Table 1) has a mobile proton even when singly protonated. Nevertheless, neutral loss of 62 Da was not detected with either the singly or doubly protonated ions of this peptide. The singly protonated ion undergoes ready loss of water and then loss of the terminal L to form the b_6 ion; at progressively higher energies the lower b ions are progressively formed. Doubly protonated TTLLLLL undergoes ready loss of water and concurrently fragments into y_1 and b_6 ions. This suggests that the path leading to 62 Da loss is not competitive with loss of C-terminal leucine.

To compare the extent of neutral loss at increasing collision voltage with the extent of backbone cleavage at the imino site of proline, which is known to be one of the most facile cleavages, we examined two peptides containing proline. TTPLLLK, by comparison with TTLLLLK, shows a slightly lower $E_{1/2}$ value, very slightly higher water loss but no discernible difference in the level of the $(MH_2\text{-}62)^{2+}$ ion. The additional predominant fragment ions are, as expected, b_2 and y_5. TTL_3PL_3K, by comparison with TTL_7K, gives slightly higher levels of neutral losses but the other predominant fragment ions are, in order of importance, y_8, y_6, y_7, b_2, and only then y_5. Although formation of the y_5 ion is found to be more facile in TTL_3PL_3K than in TTL_7K, due to the presence of P, this process is less facile than the water loss and the fragmentation to b_2 and y ions.

The results for LTTLLLLLK and LLTTLLLK demonstrate that, although TT at these positions undergo considerable water loss, the peak due to the $(MH_2\text{-}62)^{2+}$ ion is barely detected. This stresses the importance of the TT being at the N-terminus for the $(MH_2\text{-}62)^{2+}$ ion to be produced. The extent of water loss also depends on position (see below); the first of these two peptides shows loss of two water molecules in two distinct steps, while the second peptide shows only one loss but one that reaches a higher percentage level.

Replacing one threonine with serine at the N-terminal TT group has a major effect, which depends on the position. Diprotonated TSLLLK (Fig. 4) gives about half of the maximum intensities of the $(MH_2\text{-}H_2O)^{2+}$ and $(MH_2\text{-}62)^{2+}$ fragment ions as compared with TTLLLK. On the other hand, when T and S are placed in reverse order, i.e., STLLLK, the diprotonated ion exhibits increased loss of water but no formation of the $(MH_2\text{-}62)^{2+}$ ion. SSLLLK also shows water loss only. These results suggest that the 44 Da moiety lost in the second step is derived from the terminal T. The absence of this process when only one T is present (see below) suggests the involvement of the OH group of the second amino acid. If the terminal threonine loses a C_2H_4O moiety, serine at that position may lose a CH_2O moiety, i.e., 30 Da instead of 44 Da. We searched for a 30 Da loss in the MS/MS spectra of the N-terminal ST- and SS-peptides but found only very small peaks, <1% maximum intensity. Apparently, loss of formaldehyde from the terminal serine is much less favorable than loss of acetaldehyde from threonine. This is probably due to the electron donating effect of the additional methyl group in threonine, i.e., the difference between primary and secondary alcohols, which also makes water loss from serine in protonated peptides to be less favorable than water loss from threonine (see below).

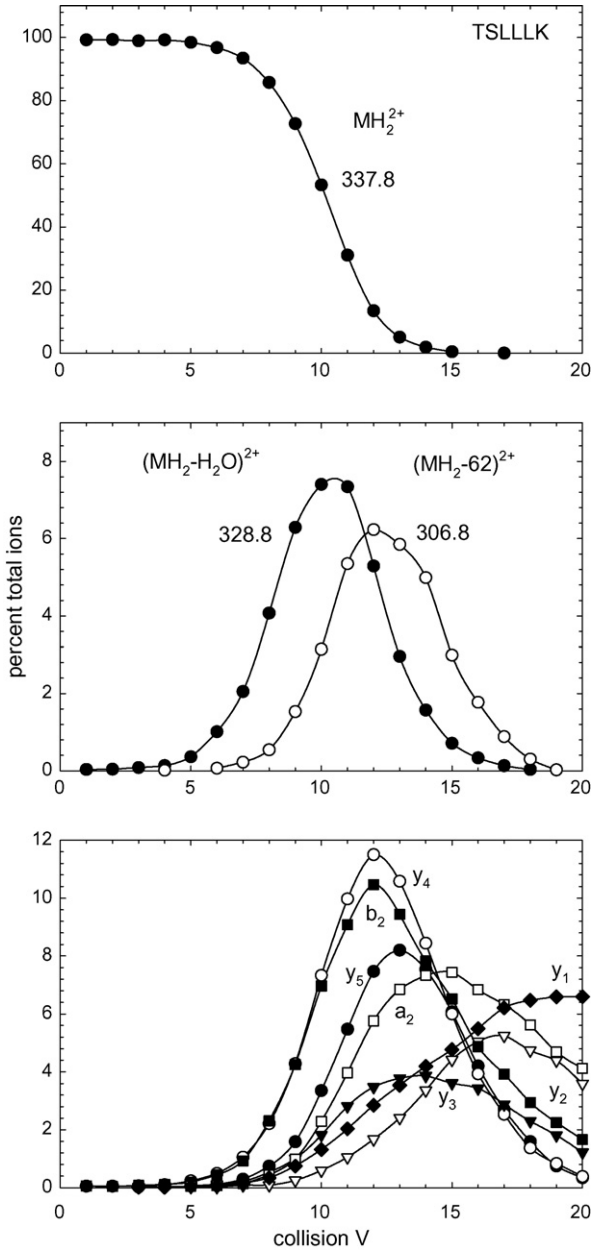


Fig. 4. Decay of the precursor peak and formation of fragment peaks as a function of collision voltage in the triple quadrupole MS/MS spectrum of doubly protonated TSLLLK ion.

The peptides in Table 1 were also examined as singly protonated ions and found to undergo very little loss of water and of 62 Da. The only peptide exhibiting >1% water loss is TTTTLK (1.3% maximum), which has four threonines. In the series TTL_nK, the maximum level of water loss decreased from 0.8% for the shortest peptide to almost 0% for the longest. Neutral loss of the 62 Da moiety was observable with the small peptides, 0.8% with TTLLK, decreasing to <0.1% with TTLLLLK, and decreasing further in the longer peptides. The other ions detected in the MS/MS spectra are mainly the b ions and a few weaker y ions. Intense y ions were observed with proline con-

Table 2
Neutral Loss of Water from Singly (MH^+) and Doubly Protonated (MH_2^{2+}) Peptide Ions Containing Threonine or Serine[a]

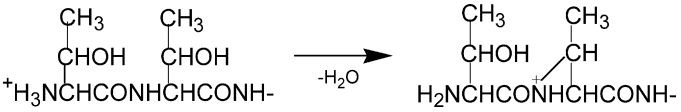
Peptide (M)	MH^+				MH_2^{2+}			
	m/z	$E_{1/2}$	$-H_2O$ (%)	coll (V)	*m/z*	$E_{1/2}$	$-H_2O$ (%)	coll (V)
TLLLLLK	813.6	32.8	0.2	32	407.3	11.7	2.0	13
LTLLLLK	813.6	32.8	0.4	34	407.3	11.9	11.1	12
LLTLLLK	813.6	32.4	0.5	33	407.5	11.0	17.6	12
LLLTLLK	813.6	33.0	0.6	30	407.3	11.7	1.2	12
LLLLTLK	813.6	33.5	1.9	33	407.5	11.6	0.55	12
LLLLLTK	813.6	32.0	2.1	33	407.5	11.5	0.53	12
SLLLLLK	799.6	31.7	~0.1	~32	400.3	10.6	1.2	12
LSLLLLK	799.6	32.1	0.4	32	400.3	11.7	3.7	12
LLSLLLK	799.7	33.2	~0.3	32	400.5	11.5	7.5	12
LLLSLLK	799.6	32.9	0.5	32	400.3	11.3	0.4	12
LLLLSLK	799.7	33.0	0.8	32	400.5	11.4	0.23	12
LLLLLSK	799.7	31.2	0.8	32	400.5	11.6	0.25	13
TLLLLLLLK	1039.8	40.4	~0.1	41	520.4	12.5	0.9	15
LTLLLLLLK	1039.8	40.5	~0.1	41	520.4	12.8	7.0	14
LLTLLLLLK	1039.8	40.0	~0.2	41	520.4	11.9	25.7	14
LLLTLLLLK	1039.8	39.8	0.4	39	520.4	12.8	5.4	14
LLLLTLLLK	1039.8	39.5	0.7	39	520.4	13.2	0.9	14
LLLLLTLLK	1039.8	39.8	1.5	39	520.4	12.5	0.9	13
LLLLLLTLK	1039.8	39.8	1.5	39	520.4	12.3	1.1	13
LLLLLLLTK	1039.8	39.4	4.1	39	520.4	12.5	2.1	13
TLLLLK	700.5	27.6	~0.2	~28	350.8	10.1	1.7	11

[a] The *m/z* values are experimental. The $E_{1/2}$ values are the collision voltage V at which the sum of intensities of fragment peaks is equal to the remaining intensity of the precursor peak. The values for $-H_2O$ are percent of total intensity at the maximum level reached and coll is the collision voltage at which that level is reached.

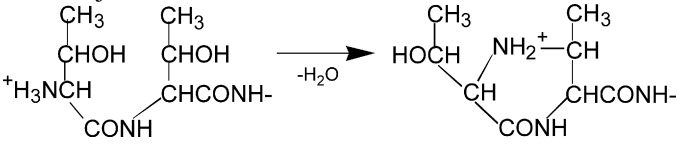
taining peptides, as expected. The short TT-peptides also gave intense peaks of the $(b_2\text{-}H_2O)^+$ and $(b_3\text{-}H_2O)^+$ ions due to water loss from threonines in the b ions.

To better understand the water loss processes from serine and threonine we studied peptides which contain only one such amino acid at different positions. The results (Table 2) show the strong dependence of the extent of water loss on the position of threonine or serine within the peptide ions. In singly protonated peptides the extent of water loss is very small and becomes significant only when the T or S are adjacent to the C-terminal K, i.e., near the positive charge. In doubly protonated peptide ions, T and S exhibit a small extent of water loss when located at the N-terminus, but much higher levels when they are located at the second and third positions from the N-terminus; further away - the level drops gradually to <1%. It has been observed that doubly protonated peptide ions tend to fragment between these positions, leading to b_2^+ ions and the complementary y^+ ions (see, e.g., Table 1). Presumably, the intermediate leading to peptide bond cleavage at this site also permits facile water loss as a competing process prior to full cleavage, possibly aided by increased charge density at the site.

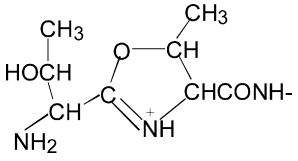
On the basis of the above results, showing that loss of water is more facile when S or T are in the second or third position in the peptide sequence rather than in the N-terminal position, it is proposed that the first loss of water from TT- and TS-peptides occurs from the second amino acid and not from the terminal amino acid. Loss of water from serine and threonine has been proposed to form an aziridine ring [10,11] involving C_α, C_β, and the N of the amino group. However, if the amino group is not terminal, i.e., if water is lost from the amino acid adjacent to the N-terminal acid, the expected aziridine ring would involve the N of the peptide bond, probably a less favorable process than in the free amino acids.



Instead, a six-membered ring may be formed by attack of the terminal positively-charged amino group on the CHOH moiety of the adjacent S or T.



Another possible intermediate (J.K. Merle, private communication) is a five-membered ring formed by attack of the carbonyl group on the CHOH moiety.



These intermediates may eliminate CH_3CHO from the terminal threonine to form the observed ions.

It is worth noting that serine and threonine in SM and TM dipeptides in aqueous solutions have been found to lose formaldehyde and acetaldehyde, respectively, via an oxidative mechanism mediated by the adjacent methionine radical cation [12]. Although the mechanism is very different from that operating in our gas-phase protonated peptides, it demonstrates the lability of the C–C bond being broken in both processes.

4. Summary and conclusions

Protonated peptide ions containing Thr or Ser undergo neutral loss of water to an extent that is strongly dependent on the availability of mobile protons and on the position of these amino acids in the peptide sequence. In the absence of mobile protons, e.g., singly protonated tryptic peptides, significant water loss is found only when T or S are very close to the C-terminus, which is where the proton is mostly localized. In the presence of mobile protons, very facile water loss occurs from T or S located at the second and third positions from the N-terminus. Protonated peptides containing N-terminal TT or TS, but not ST or SS, and having a mobile proton, exhibit an intense peak due to neutral loss of a 62 Da moiety. This product ion is formed via two consecutive neutral losses, first of water and then of C_2H_4O, and its intensity decreases with peptide length. It is clearly observable with peptide ions containing up to 11 amino acids synthesized in the present work. These fragment ions are also found among library spectra, though become hard to find at greater peptide lengths. In this work we also find a strong dependence of water loss from Thr and Ser near the N-terminus in peptides with mobile protons, but contrary to expectations, observe significant water loss only when the residue is at the second and third position. We speculate that this is somehow connected to the well known facile loss of b_2 ions in certain doubly charged peptide ions. These neutral losses can be used as additional markers to help in peptide identification.

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References

[1] H. Lam, E.W. Deutsch, J.S. Eddes, J.K. Eng, N. King, S.E. Stein, R. Aebersold, Proteomics 7 (2007) 655.
[2] R. Craig, J.C. Cortens, D. Fenyo, R.C. Beavis, J. Proteome Res. 2006 (5) (1843).
[3] Z. Zhang, Anal. Chem. 76 (2004) 3908.
[4] S. Stein, L. Kilpatrick, P. Neta, J. Roth, X. Yang, ASMS Abstract, June 2006.
[5] P. Neta, Q.-L. Pu, L. Kilpatrick, X. Yang, S.E. Stein, J. Am. Soc. Mass Spectrom. 18 (2007) 27.
[6] E.A. Kapp, F. Schutz, G.E. Reid, J.S. Eddes, R.L. Moritz, R.A.J. O'Hair, T.P. Speed, R.J. Simpson, Anal. Chem. 75 (2003) 6251.
[7] I. Haller, U.A. Mirza, B.T. Chait, J. Am. Soc. Mass Spectrom. 7 (1996) 677.
[8] E.P.L. Hunter, S.G. Lias, J. Phys. Chem. Ref. Data 27 (1998) 413.
[9] C. Bleiholder, S. Suhai, B. Paizs, J. Am. Soc. Mass Spectrom. 17 (2006) 1275.
[10] S.V. Serafin, K. Zhang, L. Aurelio, A.B. Hughes, T.H. Morton, Org. Lett. 6 (2004) 1561.
[11] G.E. Reid, R.J. Simpson, R.A.J. O'Hair, J. Am. Soc. Mass Spectrom. 11 (2000) 1047.
[12] C. Schoneich, F. Zhao, K.P. Madden, K. Bobrowski, J. Am. Chem. Soc. 116 (1994) 4641.