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Selective cleavage at internal lysine residues in protonated vs. metalated peptides

Michelle M. Kish, Chrys Wesdemiotis*

Department of Chemistry, The University of Akron, Akron, OH 44325, USA

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Dedicated to Professor Robert C. Dunbar on the occasion of his 60th birthday.

Abstract

The proton, lithium, sodium, potassium and silver ion (Cat^+) adducts of the peptides GGKAA and SIKVAV are formed by electrospray ionization and the unimolecular chemistry of these ions is examined by low-energy collisionally activated dissociation (CAD) in a quadrupole ion trap mass spectrometer. Comparison of the fragmentation characteristics of $[GGKAA + Cat]^+$ and $[SIKVAV + Cat]^+$ to those of $[YGGFL + Cat]^+$ reveals that an internal lysine residue enhances the cleavage of the amide bond in C-terminal position (i.e., Lys–Xxx). The amine group of the lysine side chain initiates this reaction, leading to b_n sequence ions with lactam structures, as corroborated by multiple-stage tandem mass spectrometry (MS^n) experiments. The selectivity for forming b-type ions at Lys–Xxx (vs. at other amide bonds) depends on Cat^+, increasing in the order $H^+ < K^+ < Ag^+ < Li^+ \approx Na^+$. Investigation of $[PTHIKWGD + Cat]^+$ further shows that, when both His and Lys residues are present in internal positions, Lys–Xxx bonds are cleaved more selectively than His–Xxx bonds if Cat = Na (or Li), whereas both these bonds break with competitive rates if Cat = Ag. These trends are attributed to the different binding properties of silver vs. alkali metal ions. The enhanced dissociation of Lys–Xxx bonds to b-type fragments should be particularly useful in the sequence analysis of non-tryptic as well as incomplete tryptic digests which may contain internal lysine residues.

Keywords: Protonated peptides; Metalated peptides; Internal lysine residues; Selective cleavage C-terminal to lysine

1. Introduction

Tandem mass spectrometry (MS/MS) methods are widely used for the sequence identification of both synthetic peptides and the peptides contained in proteolytic digests [1,2]. In the most common approach, protonated peptide ions formed by either electrospray ionization (ESI) [3] or matrix-assisted laser desorption ionization (MALDI) [4] are subjected to low-energy collisionally activated dissociation (CAD) to generate a series of fragment ions via cleavages of the backbone amide bonds [2]. The mass-to-charge ratios (m/z) of these fragment ion series reveal the arrangement of amino acid residues in the peptide. For an unequivocal sequence determination, complementary ion series (for example, N- and C-terminal ions) from cleavages of most (ideally all) peptide bonds are desired [1]. Research in several laboratories has shown that the amide bonds of peptide $[M + H]^+$ and $[M + nH]^{n+}$ ions cleave unselectively across the backbone, if a

* Corresponding author. Tel.: +1-330-972-7699; fax: +1-330-972-7370.
E-mail address: wesdemiotis@uakron.edu (C. Wesdemiotis).

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mobile proton is present, i.e., a proton that can be transferred upon CAD to the different backbone sites, where it can initiate scission of the C(=O)–N bonds to produce sequence-diagnostic fragments containing the N-terminus (b_n/a_n) or the C-terminus (y_n) [5–9]. Under the CAD conditions typically employed in peptide sequencing studies, only protons attached to an arginine residue are immobile [6]. Hence, unselective cleavages and complete (or almost complete) sequence ion series are obtained as long as the number of protons attached to a peptide exceeds the number of arginine residues [5–7].

Certain structural features have been found to promote unique fragmentation pathways in peptide ions, involving enhanced (or selective) cleavage at particular amino acid residues [6,7,10–18]. In these cases, the CAD spectra may be dominated by the products of these selective dissociations and generally do not contain contiguous fragment ion series. The most well documented selective cleavage is that taking place at the peptide bond C-terminal to an aspartic or glutamic acid residue in protonated or sodiated peptides that lack a mobile proton [6,7,10–16]. Enhanced cleavages have also been observed at the amide bond N-terminal to a proline residue in protonated peptides [17] and at the amide bond C-terminal to a histidine residue in peptides that are protonated [7] or complexed with Zn^{2+}, Co^{2+} or Ni^{2+} [18].

Selective cleavages are of interest because they generate simpler fragmentation patterns in CAD spectra, in analogy to enzymatic digests in solution which create simpler and more predictable peptide mixtures [12]. As has been emphasized by Wysocki and coworkers, understanding the structural requirements and mechanisms of selective cleavages also is necessary for the derivation of accurate sequencing algorithms [13–16]. This investigation reports a new selective cleavage, namely the preferred breakup of the C(=O)–N bond C-terminal to a lysine residue in protonated and metalated peptides. Such a cleavage would be useful for the identification of internal lysine residues in both non-tryptic digests and incomplete tryptic digests.

2. Experimental

All experiments were performed with a Bruker Esquire-LC ion trap mass spectrometer (Billerica, MA) equipped with an ESI ion source. The ESI solvent used was a 2:1 mixture of water-containing 0.1% trifluoroacetic acid and acetonitrile. Separate solutions of the peptides and lithium, sodium, potassium and silver trifluoroacetate were prepared by dissolving 1 mg of each compound in 1 mL solvent. A syringe pump was used to introduce the peptide solutions (for protonated ions) or mixtures of the peptide and metal salt solutions (for metalated ions) into the source at the rate of 300 μL/h. The entrance of the sampling capillary, which is directed at a right angle to the grounded spraying needle, was set at −4 kV. Nitrogen was used as the nebulizing gas (10 psi) and drying gas (8 L/min, 300 °C) and helium was used as the buffer gas in the trap. In the CAD experiments (MS^2, MS^3 or MS^4 mode), the excitation time was 40 ms and the resonance excitation amplitude (V_{p-p}) was adjusted with each isolated precursor ion until its intensity was reduced to 5 ± 2%; the selected V_{p-p} values ranged within 0.83 ± 0.17 V. Thirty scans were averaged per spectrum and the experiments were performed in triplicate. The peptides GGKAA, SIKVAV and PTHIKWGD were purchased from Bachem (King of Prussia, PA). Leucine enkephalin (YGGFL), pentaglycine (GGGGG), pentaalanine (AAAAA), the solvents and metal trifluoroacetate salts were purchased from Sigma–Aldrich (Milwaukee, WI). All materials were used in the condition received.

3. Results and discussion

The effect of an internal lysine residue on the fragmentation channels of peptide ions was examined for the H^+, Li^+, Na^+, K^+ and Ag^+ (Cat^+) adducts of the peptides YGGFL, GGKAA and SIKVAV. The fragmentation characteristics of $[YGGFL + Cat]^+$ in a quadrupole ion trap have been reported in detail in earlier studies [19,20] and are used here as a point of reference to determine any unique dissociation(s)

brought upon by the introduction of an internal Lys residue. Additionally, we studied PTHIKWGD which contains internal lysine and histidine residues. Since the side chain of His has been found to enhance the cleavage of the amide bond in C-terminal position to His (vide supra) [7,18], inclusion of PTHIKWGD allows for a comparison of Lys vs. His in their ability to facilitate breakup of adjacent amide bonds.

3.1. GGKAA vs. YGGFL

The CAD spectrum of protonated YGGFL (Fig. 1(a)), is dominated by the b_4/a_4 fragments arising from loss of the C-terminal leucine residue [19]. These ions are formed with substantially lower relative abundances from the alkali metal ion adducts of YGGFL, all of which mainly fragment to $[b_4+OH+X]^+$ (X = Li, Na, K), as attested in Fig. 1(b and c) by the CAD spectra of $[YGGFL+Na]^+$ and $[YGGFL+K]^+$, respectively [19–21]. The spectra of lithiated and sodiated YGGFL show essentially indistinguishable fragmentation patterns, which is also true for all other peptides studied here; for this reason, only the sodiated species are presented. The structure and formation mechanism of $[b_n + OH + X]^+$ ions have been investigated by many groups [22–27]. The current consensus is that such fragments are formed by a rearrangement at the C-terminus, which leads to a truncated peptide missing the C-terminal residue (Scheme 1). If sufficient internal energy is available, this reaction can take place anew to remove the new C-terminal residue [21]. With YGGFL, a repeated rearrangement according to Scheme 1 would lead to $[b_3 + OH + X]^+$; this fragment is indeed observed for X = Na (and Li), but not for X = K (cf. Fig. 1(b and c)). Evidently, the energy requirement of the consecutive dissociation $[b_4 + OH + X]^+ \rightarrow [b_3 + OH + X]^+$ lies between the YGGFL–Na^+ and YGGFL–K^+ binding energies, prohibiting this reaction in the less strongly bonded potassiated peptide [28]. Thus, a benefit of examining both Na^+ and K^+ adducts is that the branching ratios in the corresponding CAD spectra can be used to appraise the critical energies of competitive fragmentations. Silverated YGGFL undergoes a blend of the dissociations observed for protonated and alkali-metalated YGGFL (Fig. 1) [29]; the major products in its CAD spectrum are the truncated peptide $[b_4 + OH + Ag]^+$ (i.e. $[YGGF + Ag]^+$, cf. Scheme 1) and the silverated b_4/a_4 pair (i.e. $[b_4 - H + Ag]^+$ and $[a_4 - H + Ag]^+$; for brevity, these ions are abbreviated as b_4^*/a_4^*, the asterisk denoting that a proton has been replaced by a metal cation [30]).

The CAD spectrum of protonated GGKAA (Fig. 2(a)), contains an abundant b_4 sequence ion from the elimination of the C-terminal residue, in analogy to the CAD spectrum of protonated YGGFL (Fig. 1(a)); $[GGKAA + H]^+$ also forms a markedly stronger b_3 ion, however, the corresponding $[b_3]/[b_4]$ intensity ratios being 0.50 vs. 0.20 for protonated GGKAA and YGGFL, respectively. Similarly, $[GGKAA+X]^+$ (X = Li, Na, K) yield upon CAD significant b_3^* fragments (Fig. 2(b and c)), while $[YGGFL + X]^+$ do not produce any detectable b_3^* (Fig. 1(b and c)); in fact, the relative intensities of b_3^* from lithiated and sodiated GGKAA are almost as high as those of the dominant $[b_4 + OH + X]^+$ ion (part (b) of Fig. 2). The CAD spectrum of $[GGKAA + Ag]^+$ shows a substantially lower $[b_4 + OH + X]^+$ abundance vs. the CAD spectrum of $[YGGFL + Ag]^+$ (Fig. 2(d) vs. Fig. 1(d)), although both silverated peptides dissociate with high yield to b_4^*. However, unlike the Ag^+ adduct of YGGFL, that of GGKAA coproduces an equally abundant b_3^* fragment. Overall, a considerable portion of all GGKAA precursor ions studied generate upon CAD b_3-type ions via cleavage of the amide bond C-terminal to lysine, whereas the analogous cleavage is either absent or insignificant with the various YGGFL cations. The b_3-type fragments formed upon CAD of other pentapeptide ions without lysine residues, such as $[GGGGG + Cat]^+$ and $[AAAAA + Cat]^+$, also are of relatively low abundance or barely detectable (spectra not shown).

Attachment of a proton or metal cation (Lewis acid) to a backbone amide group significantly increases the electrophilic character of the amide C-atom. The b-type sequence ions are believed to arise by attack of such an activated amide C-atom by an N-terminally

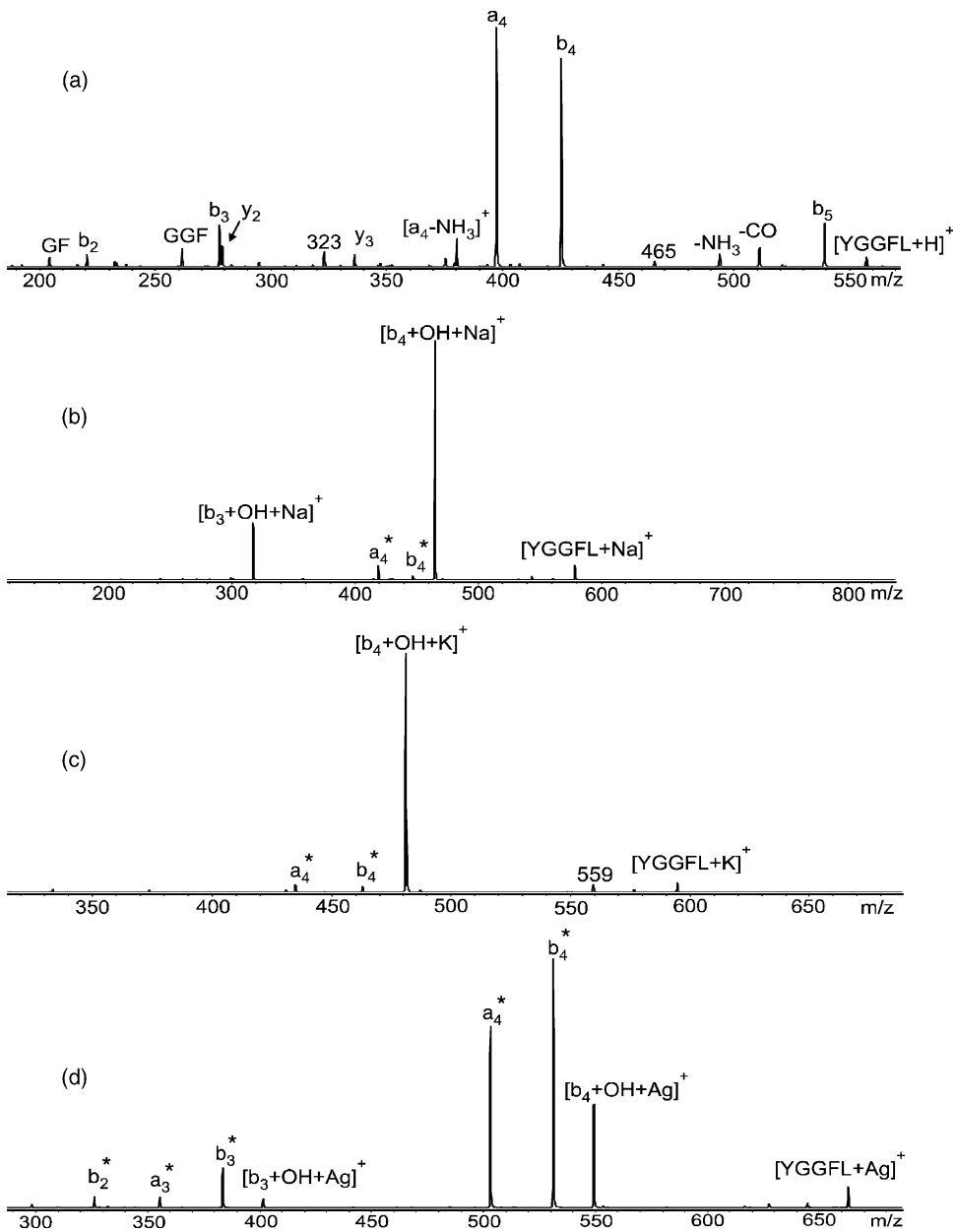
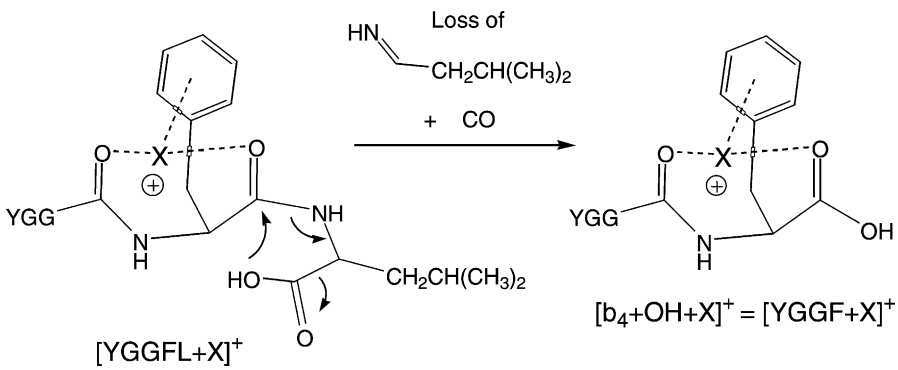


Fig. 1. CAD (MS^2) mass spectra of $[YGGFL + Cat]^+$ ions; Cat = (a) H, (b) Na, (c) K and (d) Ag. The spectrum of $[YGGFL + Li]^+$ is very similar to that of $[YGGFL + Na]^+$.

located nucleophile [2,9,31,32]. Most often, the attacking nucleophile is the carbonyl oxygen N-terminal to the reacting amide carbon, and the resulting product has the structure of an oxazolone [31,32]. Scheme 2 exemplifies this generally accepted mechanism for the b_4 (or b_4^*) fragment from protonated or metalated GGKAA ions. In the presence of side-chain functionalized amino acids, the attacking nucleophile



Scheme 1.

may alternatively be the functional group in the side chain N-terminal to the amide bond being broken. Such a neighboring group effect operates in the well-documented selective cleavage occurring C-terminal to aspartic and glutamic acid residues [10–16]. The enhanced cleavage observed here next to a lysine residue can similarly be rationalized by the involvement of lysine's side chain functionality (i.e., the amine group), as depicted in Scheme 3.

Corroborating evidence for the mechanistic proposals of Schemes 2 and 3 is provided by the MS^3 spectra of the b_3 and b_4 sequence ions from $[GGKAA+H]^+$; the protonated peptide was chosen for the MS^3 experiments, because it is formed with a quite high intensity upon ESI to permit the acquisition of useable multiple-stage tandem mass spectra. The CAD (MS^3) spectrum of b_4 from $[GGKAA+H]^+$ contains sizable b_3 and b_2 ions (cf. Fig. 3(a)). This feature, i.e., dissociation of b_n ions to smaller b-type ions is a typical property of oxazolone structures [32,33]. Other CAD channels that could be identified include the loss of water, which leads to the *m/z* 296 basepeak (vide infra) and the losses of Gly and GlyGly residues from the N-terminus of b_4, which lead to *m/z* 257 and 200, respectively. Most other CAD products observed can be accounted for by the consecutive elimination of H_2O, NH_3, CO and/or a combination thereof from the mentioned fragments. In sharp contrast to the CAD (MS^3) spectrum of b_4, that of the b_3 ion from $[GGKAA+H]^+$ (Fig. 3(b)), is much simpler, containing two major fragment ions generated via the losses of H_2O (*m/z* 225) and a GlyGly residue (*m/z* 129). The latter reaction dominates, suggesting that it yields a particularly stable product with low tendency for sequential fragmentation. These features are consistent with the b_3 structure shown in Scheme 3 which contains a protonated lactam ring. The protonated forms of cyclic amides are thermodynamically more stable than isomeric oxazolone structures [34], in accord with the lower fragmentation extent of b_3 vs. b_4. Further, cleavage of a GlyGly residue from the N-terminal segment of such a b_3 ion is readily feasible (cf. Scheme 4) and preserves the lactam connectivity, justifying the high yield of this pathway upon CAD.

We also conducted an MS^4 experiment on the b_3 fragment formed via the sequence $[GGKAA+H]^+ \rightarrow b_4 \rightarrow b_3$ (i.e., the b_3 ion in Fig. 3(a)). The resulting CAD spectrum (not shown) is identical within experimental error with the one shown in Fig. 3(b), revealing that the one-step reaction $[GGKAA+H]^+ \rightarrow b_3$ and the consecutive reactions $[GGKAA+H]^+ \rightarrow b_4 \rightarrow b_3$ produce the same b_3 fragment which, based on the forgoing arguments, has the lactam structure depicted in Schemes 3 and 4. Hence, the neighboring group effect of the lysine side chain operates upon the fragmentation of both Lys-containing precursor ions and Lys-containing b-type sequence ions.

It is noticed that the b_4 ion from $[GGKAA+H]^+$ forms a $[b_4-H_2O]^+$ fragment with a high yield but no detectable a_4 fragment (Figs. 3(a) and 2(a)). The

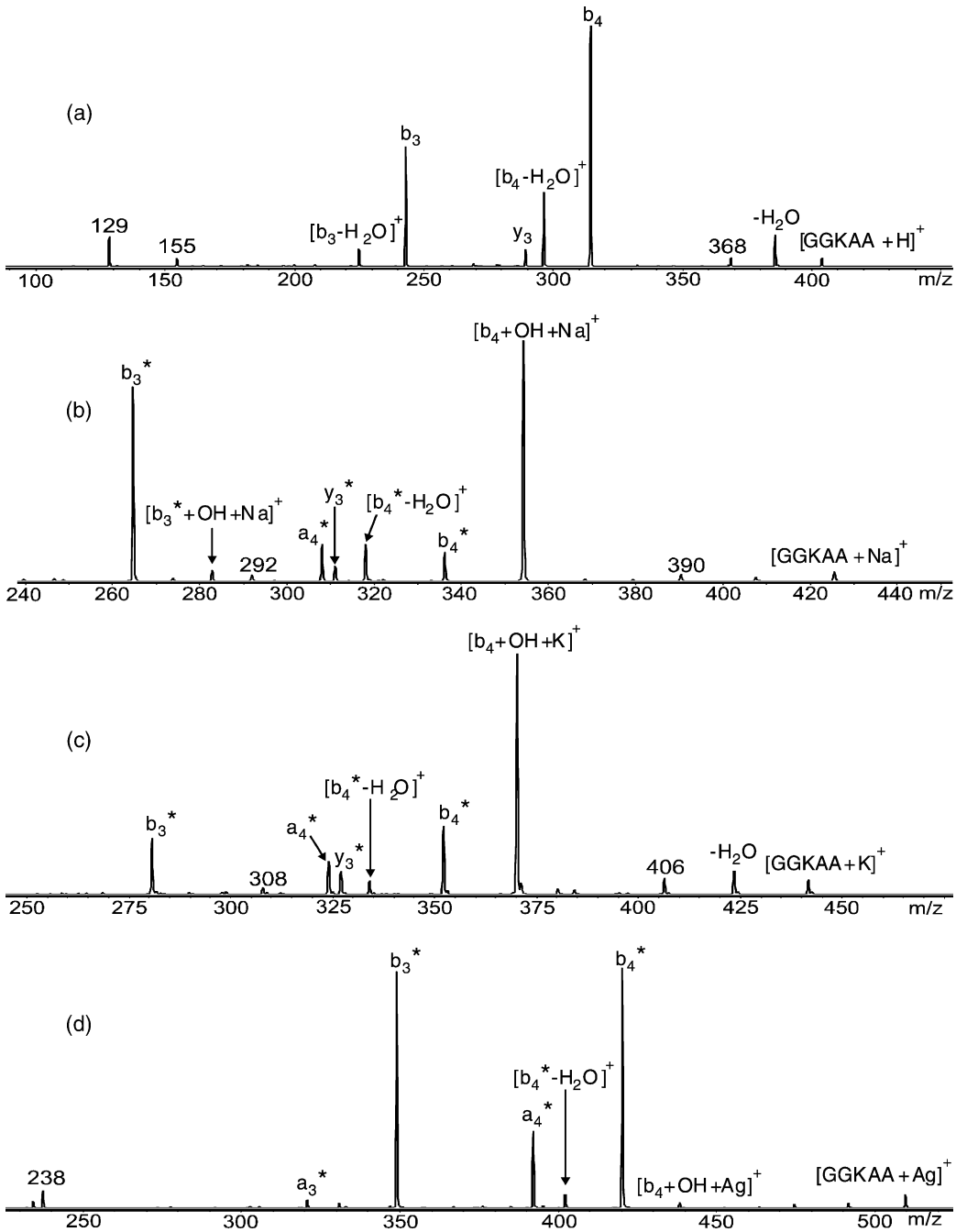
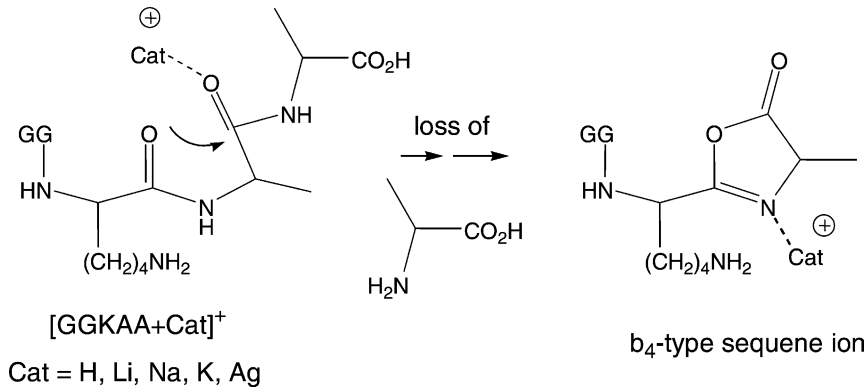


Fig. 2. CAD (MS^2) mass spectra of $[GGKAA + Cat]^+$ ions; Cat = (a) H, (b) Na, (c) K and (d) Ag. The spectrum of $[GGKAA + Li]^+$ is very similar to that of $[GGKAA + Na]^+$.

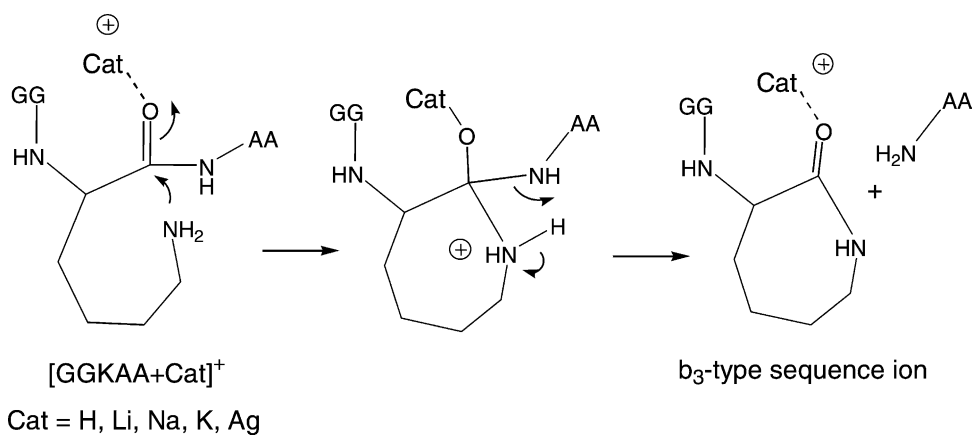


Scheme 2.

reverse is true for b_4 from $[YGGFL + H]^+$, which undergoes abundantly CO loss to form a_4 but does not lose H_2O to any measurable extent (Fig. 1(a)). The metalated GGKAA ions give rise to a_4^* (and occasionally a_3^*) fragments upon CAD, as do the corresponding YGGFL ions, see parts (b)–(d) Figs. 1–2; only the lysine-containing ions, however, coproduce detectable $[b_4^* - H_2O]^+$ fragments. The unique production of $[b_4/b_4^* - H_2O]^+$ from the GGKAA-derived b_4/b_4^* sequence ions is attributed to an involvement of the lysine side chain. Scheme 5 illustrates a possible structure of $[b_4/b_4^* - H_2O]^+$. Such a fragment is most efficiently formed from protonated GGKAA, where the competitive elimination of CO is completely suppressed.

3.2. SIKVAV

In SIKVAV, cleavage of the Lys–Val amide bond necessitates the loss of three amino acid residues from the C-terminus. The cleavage of that many C-terminal residues is either undetectable or barely above noise level in the variously cationized YGGFL and GGKAA precursors, as is evident from the relative abundances of b_2/b_2^* in the CAD spectra of Figs. 1–2. On the other hand, all cation adducts of SIKVAV besides $[SIKVAV+K]^+$ are found to produce significant b_3/b_3^* fragments upon CAD (Fig. 4), strongly suggesting that Lys promotes the cleavage of the amide bond at its C-terminal side. The participation of Lys in the formation of b_3/b_3^* is further supported by the CAD (MS^3)



Scheme 3.

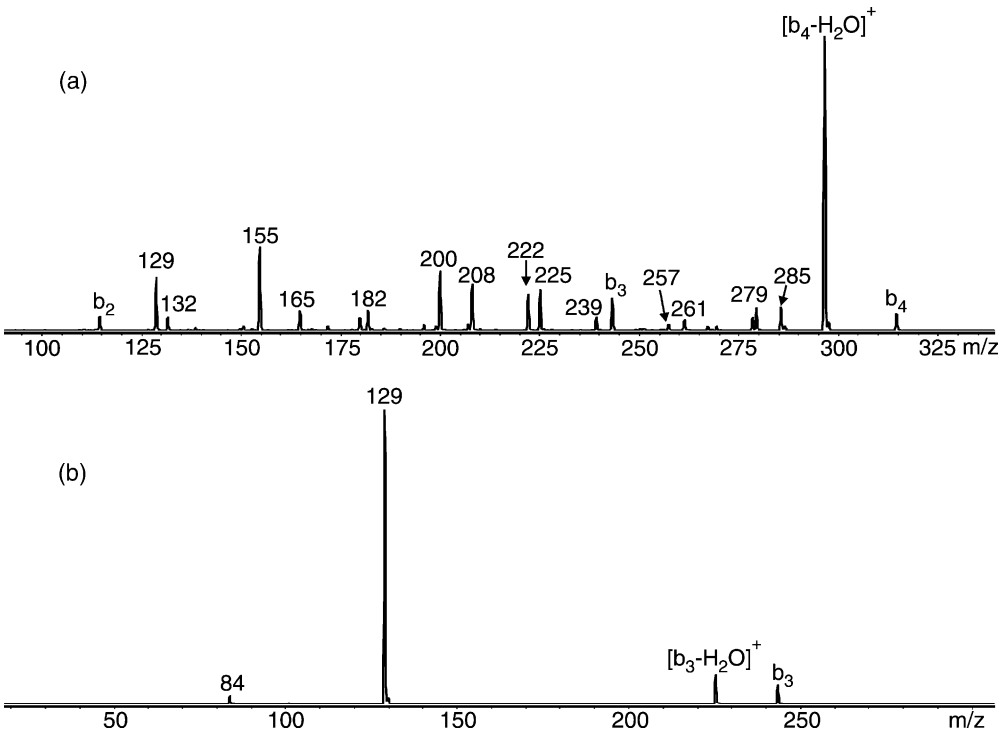
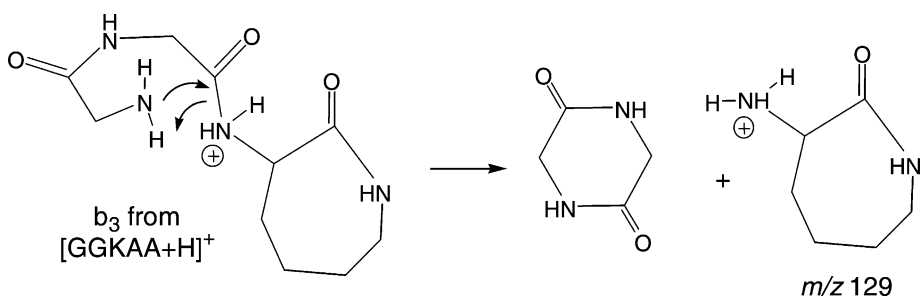
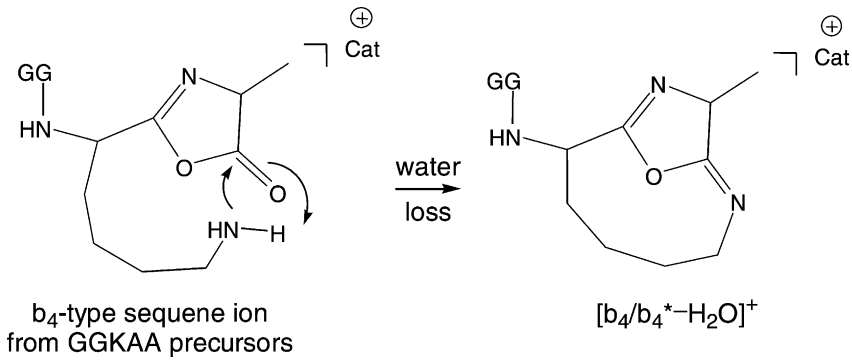


Fig. 3. CAD (MS^3) mass spectra of (a) b_4 and (b) b_3 sequence ion from $[GGKAA + H]^+$. (a) Likely compositions of the fragments from b_4: *m/z* 285, $[b_4 - CH_2NH]^+$; *m/z* 279, $[b_4 - H_2O - NH_3]^+$; *m/z* 261, $[b_3 + H_2O]^+$; *m/z* 257, $[b_4 - G]^+$; *m/z* 239, $[b_4 - H_2O - G]^+$; *m/z* 225, $[b_3 - H_2O]^+$; *m/z* 222, $[b_4 - H_2O - G - NH_3]^+$; *m/z* 208, $[b_3 - H_2O - NH_3]^+$; *m/z* 200, $[b_4 - GG]^+$; *m/z* 182, $[b_4 - H_2O - GG]^+$; *m/z* 155, $[b_4 - GG - NH_3 - CO]^+$; *m/z* 129, $[b_3 - GG]^+$ (see Scheme 4). (b) Likely compositions of the fragments from b_3: *m/z* 129, see Scheme 4; *m/z* 84, $NH_3 + CO$ loss from *m/z* 129.

fragmentation pattern of b_3 from $[SIKVAV + H]^+$, which echoes the fragmentation pattern encountered with b_3 from $[GGKAA + H]^+$ (cf. Figs. 5 and 3(b)); both spectra are dominated by *m/z* 129, which is diagnostic for the lactam structure that can emerge through attack of the Lys–Val amide bond by the amine substituent of the lysine side chain (see Scheme 3). Similarly, CAD (MS^3) of b_4 from $[SIKVAV + H]^+$ (spectrum not shown) leads to the characteristics seen upon collisional activation of b_4 from $[GGKAA + H]^+$,



Scheme 4.



Scheme 5.

including the formation of smaller b-type fragments (b_3 and b_2) and an abundant loss of H_2O (basepeak), i.e., to features that are consistent with an oxazolone structure (vide supra).

For $[SIKVAV + X]^+$, X = Li and Na, b_3^* is the most abundant b-type ion (more abundant than b_4^* or b_5^*), but $[b_5 + OH + X]^+$ is the most abundant CAD fragment; the latter fragment also dominates the CAD spectrum of potassiated SIKVAV. Replacement of the Li^+ or Na^+ charge site with K^+ reduces substantially the abundance of b_3^* vs. $[b_5 + OH + X]^+$ (cf. Fig. 4(b and c)). A similar trend is observed upon CAD of the GGKAA ions, where the abundance ratio of b_3^* to $[b_4 + OH + X]^+$ decreases from 0.80 to 0.23 when Li^+/Na^+ are exchanged with K^+ (cf. Fig. 2(b and c)). Since processes of higher critical energy are discriminated against in K^+ complexes (vide supra), our results point out that the cleavage of internal Lys–Xxx bonds in an alkali metalated peptide costs more energy than the rearrangement expelling the C-terminal residue to produce the truncated peptide $[b_n + OH + X]^+$. Alternatively, the reduced efficiency of Lys–Xxx bond cleavage in the potassiated peptides could originate from an unfavorable free energy of activation due to entropy effects.

Dissociation of the Lys–Val amide bond to produce b_3^* proceeds with relatively high yield in collisionally activated $[SIKVAV + Ag]^+$ (cf. Fig. 4(d)), although judging from the lower relative intensity of b_3^* vs. b_5^* in the CAD spectrum of the silverated precursor, the energetics (or entropy requirements) of b_3^* formation must be less favorable than those of b_5^* formation. Also for protonated SIKVAV, the b_3 fragment is less abundant than b_4 and b_5.

The fraction of b_3/b_3^* among all b_n/b_n^* ions observed in the collision-induced decomposition of $[SIKVAV + Cat]^+$ increases in the order: Cat = H^+ (9%) < K^+ (17%) < Ag^+ (29%) < Na^+ (52%). The corresponding order for the fraction of b_3/b_3^* among the b-type ions generated from $[GGKAA + Cat]^+$ is: Cat = H^+ (33%) < K^+ (45%) < Ag^+ (50%) < Na^+ (87%). The lower proportion for H^+ can be understood by considering that the side chain of Lys must contain a bare amine group for the enhanced cleavage at the Lys–Xxx amide bond to take place (cf. Scheme 3). Since Lys is one of the most basic amino acid residues [35,36], the amine group of its side chain is probably protonated and, hence, ineffective, in a large population of the $[GGKAA + H]^+$ and $[SIKVAV + H]^+$ precursor ions. CAD can move the proton to the backbone amide bonds [5–7], creating the free amine group needed to initiate the selective cleavage of Lys–Xxx (Scheme 3). In the metalated precursor ions, the lysine side chain presumably participates in the coordination of the metal ions. Coordinative H_2N–Cat^+ bonds are significantly weaker than covalent H_2N^+–H bonds, however [37]; consequently, the free NH_2 site can be generated more readily from metalated than protonated precursors, which explains the increased yield of Lys–Xxx bond cleavage from the former. Based on the peptides studied here, cationization with Na^+/Li^+, followed

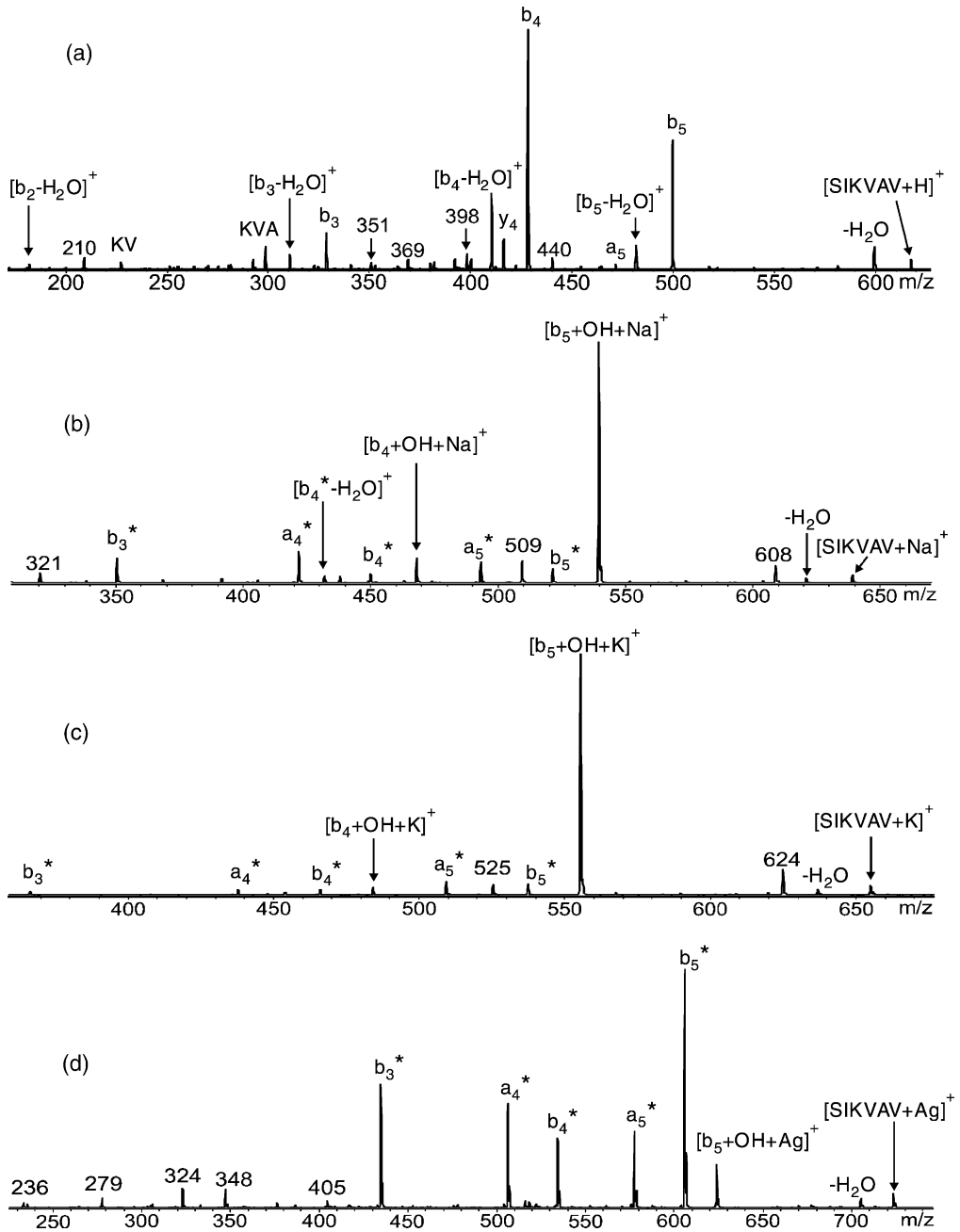


Fig. 4. CAD (MS^2) mass spectra of $[SIKVAV + Cat]^+$ ions; Cat = (a) H, (b) Na, (c) K and (d) Ag. The spectrum of $[SIKVAV + Li]^+$ is very similar to that of $[SIKVAV + Na]^+$.

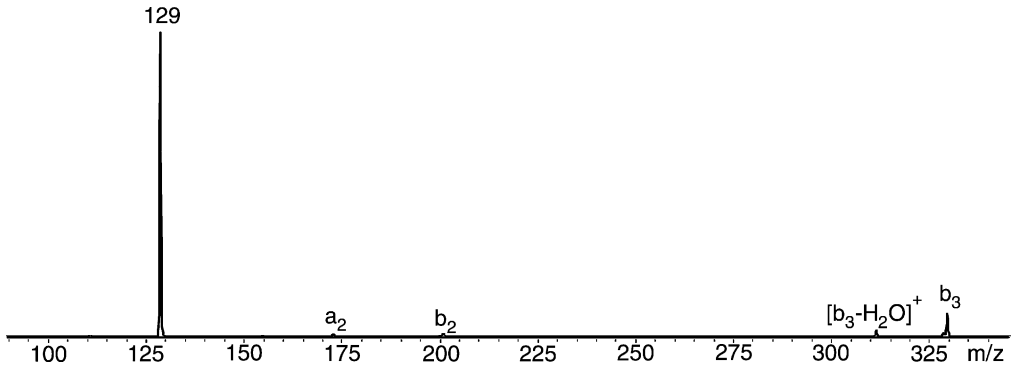


Fig. 5. CAD (MS^3) mass spectrum of the b_3 sequence ion from $[SIKVAV + H]^+$.

by cationization with Ag^+, appear to be most suitable for maximizing the efficiency of selective amide bond cleavage C-terminal to internal lysine residues.

3.3. PTHIKWGD

Fig. 6 displays the CAD spectra of $[PTHIKWGD + Na]^+$ and $[PTHIKWGD + Ag]^+$, i.e., of the metal ion adducts found to produce most efficiently b_n^* ions at the C-terminal side of internal Lys residues during low energy CAD in an ion trap (vide supra). The most prominent fragment in both spectra is the truncated peptide $[b_7 + OH + Cat]^+$, formed by rearrangement loss of the C-terminal Asp residue (115 u) according to Scheme 1. Interestingly, $[b_7 + OH + Na]^+$ undergoes consecutive elimination of its N-terminal ProThr and

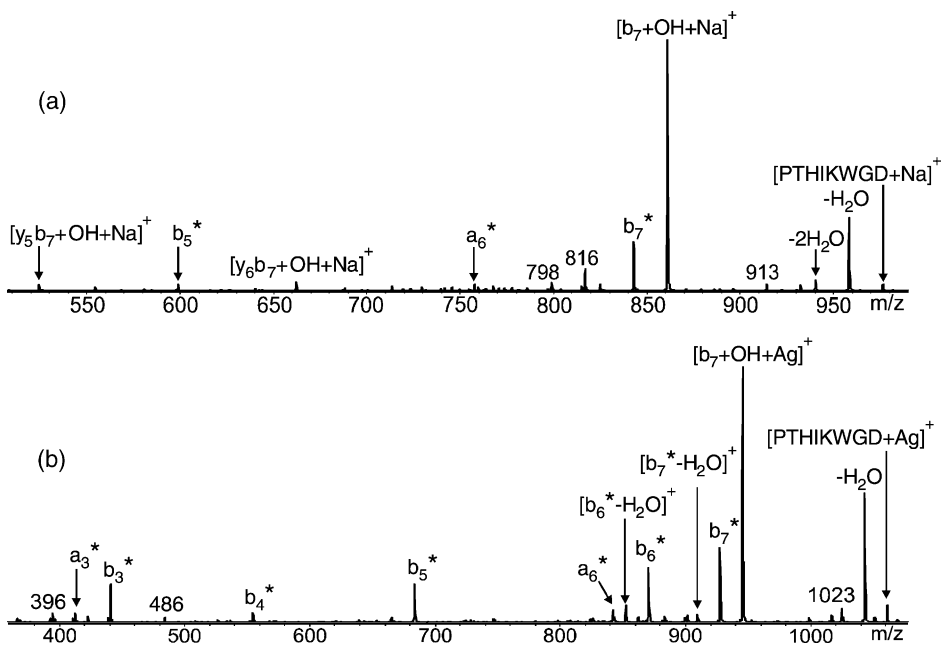


Fig. 6. CAD (MS^2) mass spectra of $[PTHIKWGD + Cat]^+$ ions; Cat = (a) Na and (b) Ag. The spectrum of $[PTHIKWGD + Li]^+$ is very similar to that of $[PTHIKWGD + Na]^+$.

ProThrHis residues, giving rise to the internal fragment ions $[y_6b_7+OH+Na]^+$ and $[y_5b_7+OH+Na]^+$, respectively, while these reactions do not occur with $[b_7 + OH + Ag]^+$. Both peptide precursor ions dissociate to b_7^*, which could arise via loss of water from $[b_7 + OH + Cat]^+$ and/or loss of aspartic acid (133 u) directly from the precursor ions. The only other b-type sequence ion observed from sodiated PTHIKWGD is b_5^*, formed by cleavage of the Lys–Trp amide bond. Hence, even in a peptide with several side-chain functionalized amino acids (i.e., PTHIKWGD), there is a preference for breakup of the amide bond C-terminal to a lysine residue upon CAD of the Na^+ adduct.

A less selective behavior is observed for the Ag^+ adduct (Fig. 6(b)), which shows an almost complete b_n^* series ($n = 3$–7). Nevertheless, it is noteworthy that the relative abundances of b_3^* and b_5^*, which are formed by cleavages of amide bonds C-terminal to His and Lys, respectively, are significantly higher than that of b_4^*, which cleaves an amide bond C-terminal to Ile; Hu and Loo [18] and Wysocki et al. [7] have reported that the imidazole group of histidine, which contains a nucleophilic site, can assist the cleavage of a C-terminally situated amide bond, like the amine group of lysine. The reason for the quite high relative abundance of b_6^* from $[PTHIKWGD+Ag]^+$ is unclear; perhaps, the His and/or Lys residues can facilitate the cleavage of C-terminally more distant bonds under certain conditions. On the other hand, the enhanced yield of b_7^* (from both peptide ions) is ascribed to the possibility of forming this ion via water loss from $[b_7 + OH + Cat]^+$.

It is proposed that the absence of b_3^* peaks in the CAD spectrum of $[PTHIKWGD + Na]^+$ is caused by the higher Na^+ binding energy of histidine vs. lysine [37], which makes it more difficult for the His side chain to detach from the metal ion so that it can assist in the cleavage of the His–Ile bond. In contrast, Ag^+ binds more strongly to Lys than His [29,38]; this difference in binding properties increases the likelihood that $[PTHIKWGD + Ag]^+$ will contain the free imidazole group needed to initiate adjacent amide bond cleavage, thus, permitting b_3^* formation to compete effectively with b_5^* formation in the silverated peptide.

4. Conclusions

The amine substituent in the side chain of an internal lysine residue is found to facilitate the cleavage of the C-terminally located peptide bond upon CAD in a quadrupole ion trap. This neighboring group effect leads to b-type sequence ions with a lactam structure, as affirmed by MS^n experiments. Comparison of the fragmentation patterns of protonated, alkali-metalated and silverated peptides reveals that the Lys–Xxx bond cleavage is most competitive in sodiated or lithiated peptides, in which the NH_2 nucleophile is unprotonated and not involved in strong coordinative bonding, so that it can readily attack the carbonyl group of the amide bond being broken.

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