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Investigation of peptide size, residue position, neighbor amino acid and side chain effect on macrocyclization of b_n ($n = 5$–7) ions

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ABSTRACT

A systematic study was carried out to examine the effects of the side chain, peptide size, residue position, and neighboring amino acid on the macrocyclization of *b* ions. The work utilized isomeric model peptides YAGFLV-NH_2, AGFLVY-NH_2, GFLVYA-NH_2, FLVYAG-NH_2, LVYAGF-NH_2, VYAGFL-NH_2, which all have the same amino acid sequence in cyclic form. The b_6 ions derived from all these isomeric peptides form the same macrocyclic structure due to the generation of the same amino acid sequence order upon cyclization. Hence, the MS/MS spectra and breakdown graphs of b_6 ions derived from these peptides are similar to each other. However, the relative intensities of the non-direct sequence ions in both the MS/MS spectra and breakdown graphs of the b_6 ions derived from FAYVGL-NH_2, GVYALF-NH_2 and VFYLAG-NH_2 show a different distribution from each other and the first series, even though they are all isomeric peptides. This could be due to the different amino acid sequence order in the cyclic forms of these peptides. It is clearly shown that the neighboring amino acid influences the selective opening of the macrocyclic form.

Additionally, XYAGFLV-NH_2 and YAGXFLV-NH_2 (where X = C, D, E, H, K, M, N, P, Q, S, T, and W are amino acid residues) were also studied in order to examine the influence of the peptide size, amino acid side chain, and position on the ring formation and cleavage of macrocyclic b_5, b_6 and b_7 ions. The results have clearly shown that b_6 and b_7 ions have a higher tendency of macrocyclization compared to b_5 ions with the exception of QYAGFLV-NH_2. Additionally, it was observed that selective ring opening is also dependent on the size of the *b* ions and the position of the amino acid residue. From our study of the macrocyclic b_6 ions of our model peptides, the Q, W, K, and M residues were found to be more favorable eliminations when compared to C, D, E, H, N, P, S, and T. Based on the results, no preferential cleavage order can be specified depending on the nature of amino acid side chain.

1. Introduction

Following the invention of soft ionization methods such as electrospray ionization (ESI) [1,2] and matrix-assisted laser desorption/ionization (MALDI) [3,4], tandem mass spectrometry (MS/MS) in combination with collision-induced dissociation (CID) [5] has been utilized as a routine means of protein sequencing in proteomics [6–8]. In low-energy CID, protonated peptides are cleaved at the amide bonds to generate a series of N-terminal *b* ions and/or C-terminal *y* ions [9,10] and later on, these ions can be used to sequence peptides produced from the enzymatic cleavage of unknown proteins. The structure of the *y* ions has been proven to be protonated truncated peptides [11–13] while a number of different structural forms have been established for the *b* fragment ions. Early studies [9,10] have suggested that the structure of the *b* ions was an acylium ion. Yalcin et al. [14] however, originally proposed that cyclization takes place via nucleophilic attack of adjacent carbonyl oxygen on the N-terminus to form a five-membered oxazolone ring structure of *b* ions.

Several studies have also supported this mechanism using infrared multiple photon dissociation (IRMPD) [15–19] and isotopic labeling techniques [20]. However, the existence of a strong nucleophile in the peptide backbone may cause side chain-specific cyclization reactions [21–28]. Diketopiperazine structures have also been postulated as structure for b_2 ions [12,13]. Recently, Wysocki et al. [29] reported that the b_2 ion obtained from HA has a combination of oxazolone and diketopiperazine structures. It could be said that the existence of two structures for b_2^+ fragments may not be general and may depend on the amino acid sequence.

Vachet et al. [30], and Yagüe et al. [31] reported that the internal amino acid residue eliminations are due to the intermolecular rearrangement and that this can make the interpretation of peptide MS/MS spectra more difficult. Since 2006, a large number of publications [32–41] have been published about the macrocyclization reactions of b_n ions ($n \geq 5$). They have shown that cyclization may occur through the attack of the free N-terminal amino group

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on the charged C-terminal oxazolone ring and that the subsequent opening of this macrocyclic ring at various amide bond positions could produce “non-direct” sequence ions.

Recently, the effect of peptide size [39] on the macrocyclization of *b* ions and the effect of amino acid nature [42] on the potential ring opening of this cyclic model at various amide bonds have been studied by Stipdonk et al. However, they have only considered a single isomeric series of the YAGFLVG model to monitor the macrocyclization of *b* ions, and focused only on the b_5 ions produced from YAXFLG (where X = G, K, E, D, N, Q) to obtain information about the side chain effect. In addition, Polfer et al. published a detailed study about b_2–b_8 ions to predict the influence of the peptide size on cyclization [43]. It was observed that the smaller *b* fragments (b_2, b_3) form an oxazolone structure, in contrast midsized ones (b_4–b_7) generate a combination of oxazolone and macrocylic structures.

In this work, we have carried out a more comprehensive systematic study to examine the effects of peptide size, residue position, neighboring amino acid and side chain on the macrocyclization and the potential ring opening of macrocyclic b_n (n = 5–7) ions. The study utilized two sets of model peptides. The first set was isomeric peptide series of YAGFLV-NH_2 to examine the neighboring amino acid effect on the ring cleavage of macrocyclic *b* ions. The second set was XYAGFLV-NH_2 and YAGXFLV-NH_2 where X denotes C, D, E, H, K, M, N, P, Q, S, T, and W amino acid residues. The position of the X amino acid residue was varied to examine the influence of the amino acid position and side chain on the ring formation and cleavage (b_5-X, b_6-X and b_7-X) of macrocyclic *b* ions. Moreover, the collision energy dependence of the preferential cleavage of macrocyclic *b* ions was also studied.

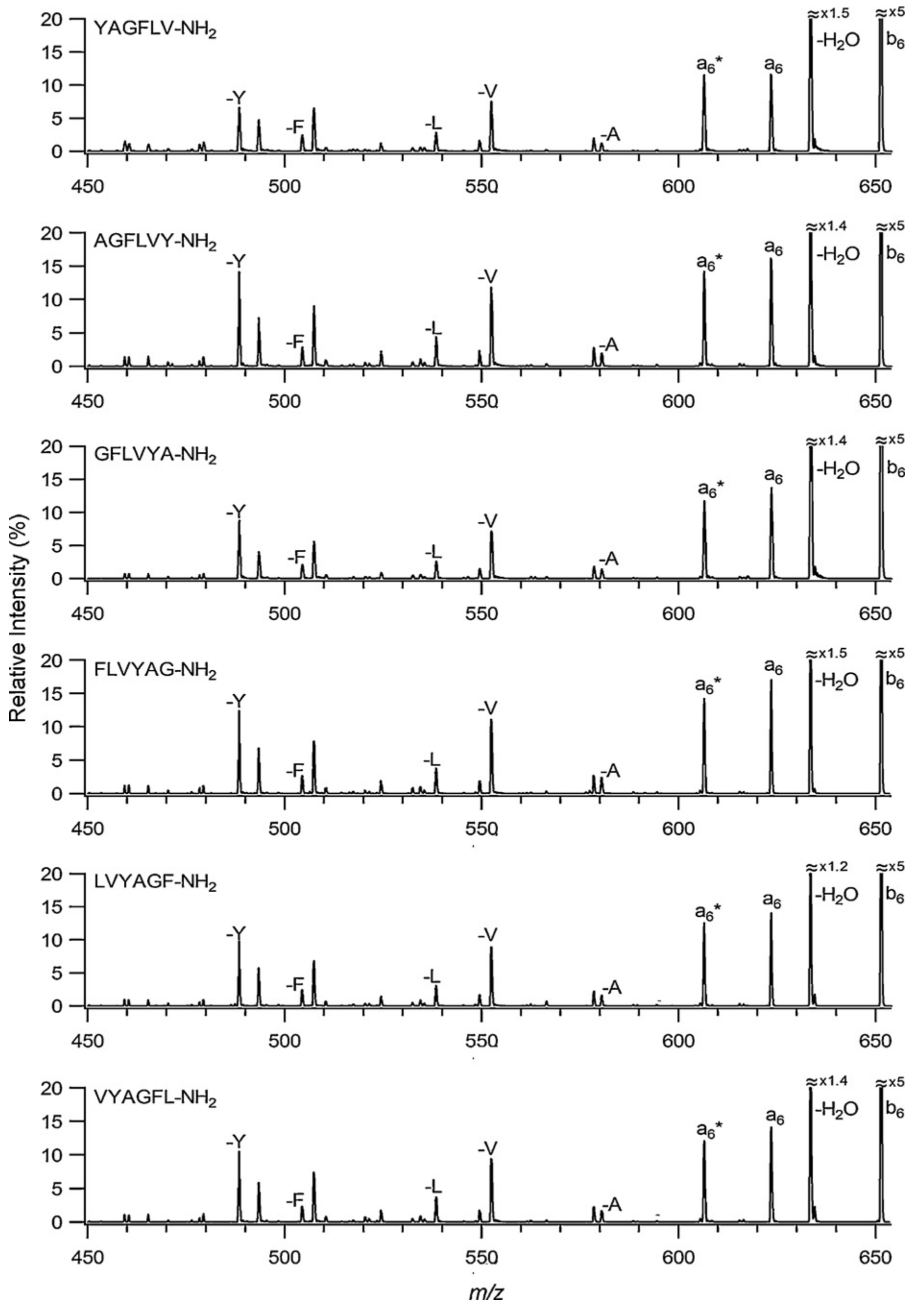

Fig. 1. CID spectra of b_6 ions derived from permuted isomers of YAGFLV-NH_2. Each precursor peptide sequence is shown with its related spectrum. Collision energy is 24 eV.

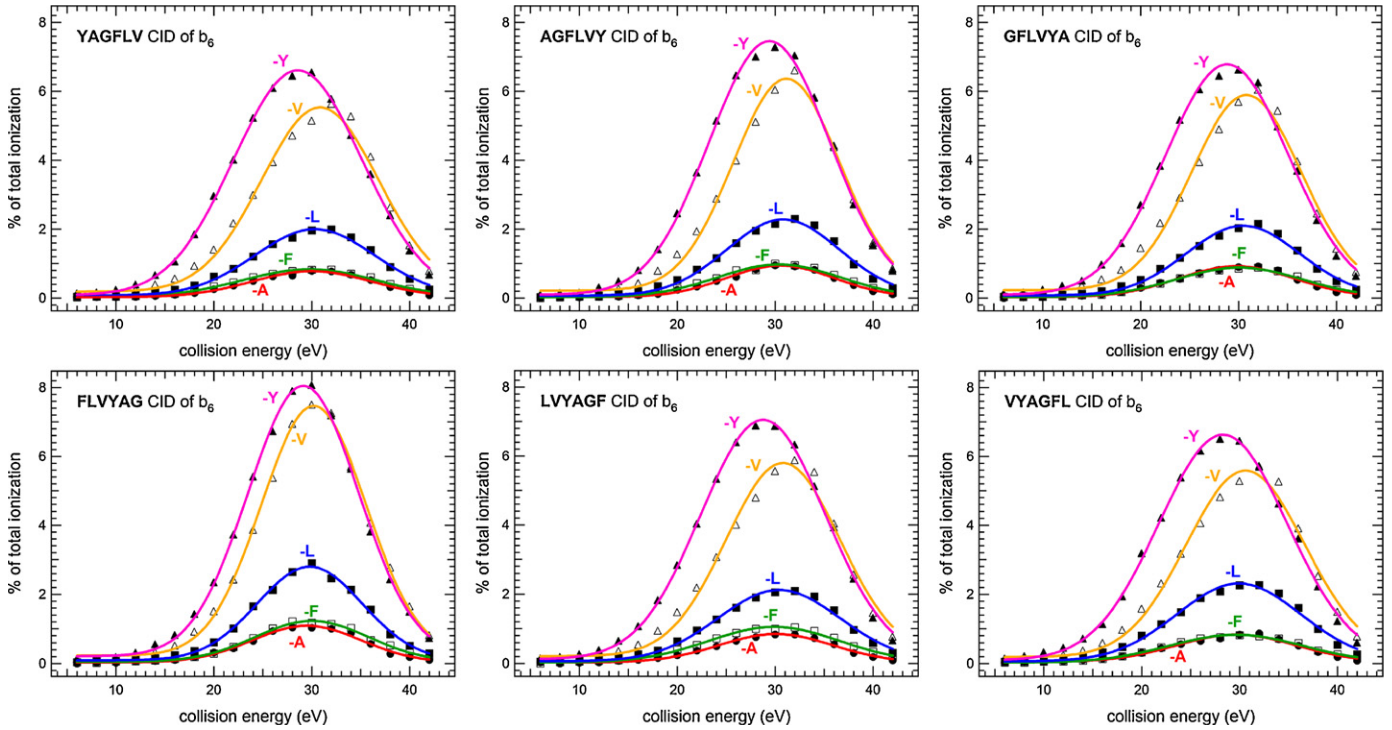

Fig. 2. Breakdown graphs for b_6 ions derived from permuted isomers of YAGFLV-NH_2. Nondirect sequence ions have shown only.

2. Experimental

2.1. Materials

All synthetic C-terminally amidated model peptides were obtained from GL Biochem (Shangai, China) and used as received with no further purification. HPLC grade methanol and formic acid were supplied by Merck (Darmstadt, Germany). The water used was ultrapure grade (Arium 611 UV, Sartorius AG, Goettingen, Germany). Stock solutions of peptides were prepared by dissolving solid material in a 1:1 (v/v) mixture of methanol and water to a concentration of 10^{-2} M. Peptide samples at micromolar concentration level were dissolved in 1:1 CH_3OH:H_2O containing 1% formic acid.

2.2. Mass spectrometry

All low-energy CID experiments were conducted on a hybrid triple quadrupole/linear ion trap 4000 QTRAP instrument (Applied Biosystems/MDS Sciex, Ontario, Canada) which is equipped with an electrospray ionization source. The peptide solutions were infused into the electrospray source at a 5 μL/min flow rate using a syringe pump from Harvard Apparatus (model 11 plus, Natick, MA, USA).

Nitrogen was used as nebulizer, curtain, and collision gas. The Q1 scan was applied first to adjust the declustering potential for the generation of the most intense ion signal with the best Gaussian distribution peak shape, which was then followed by enhanced product ion (EPI) scan for MS/MS experiments of *b* ion fragments in the collision cell. The EPI scan studies were operated using the following 4000 QTRAP instrumental parameters: curtain gas (CUR), 10.00 arbitrary units (arb); ion spray voltage (IS), 5500.00; temperature (TEM), 0.00 °C; ion source gas 1 (GS1), 19.00; ion source gas 2 (GS2), 0.00; interface heater (ihe), On; collision gas (CAD), 6.00 arb; collision energy spread (CES), 0.00; collision energy (CE), 6.0–42.0 eV (with increments of 2.0 eV), quadrupole 1 ion energy (IE1), 0.800; Q1 resolution, unit; detector channel electron multiplier (CEM), 2200.0; scan rate, 1000 Da/s; linear ion trap (LIT) fill time, 20 ms. All MS/MS data were collected as a sum of the 50 multichannel analysis (MCA) scans in centroid mode. Analyst 1.5 software was used for the instrument control, data acquisition and processing.

3. Results and discussion

3.1. Influence of neighbor amino acid on preferential opening of macrocyclic b ion

The MS/MS spectra of b_6 (651 *m*/*z*) ions derived from YAGFLV-NH_2, AGFLVY-NH_2, GFLVYA-NH_2, FLVYAG-NH_2, LVYAGF-NH_2, and VYAGFL-NH_2 are presented in Fig. 1. They produce both direct and nondirect sequence ions in agreement with the literature studies [32,34,35,38,39], and display nearly an identical fragmentation pattern. This is because the b_6 ions derived from the isomeric peptides form the same macrocyclic structure due to the generation of the same amino acid sequence order upon cyclization. The main product ions include H_2O elimination (633 *m*/*z*), a_6 ion (623 *m*/*z*), and $a_6{}^*$ ion (606 *m*/*z*). Additionally, all the spectra show significant nondirect sequence ions as the eliminations of Y (488 *m*/*z*), A (580 *m*/*z*), F (504 *m*/*z*), L (538 *m*/*z*), and V (552 *m*/*z*). It was noticed that in previous studies the MS/MS experiments concerning the macrocyclization of *b* ions were performed at a single collision energy value. Therefore, the collision energy dependence of the preferential cleavage of macrocyclic *b* ions was also investigated. Fig. 2 shows the breakdown graph of these isomeric peptides considering nondirect sequence ions only. However, it should be noted that some of the non-direct sequence amino acid eliminations might overlap with the direct sequence ions (e.g., b_6-V from YAGFLV might overlap with the b_5 ion). As one can easily see, they all follow nearly the same dissociation pattern regardless of the peptide sequence. Interestingly, the elimination tendency of each internal amino acid residue from the b_6 ion is same for all model peptides. A decreasing trend was shown in the order of Y > V > L > F ∼ A. The internal elimination of tyrosine was observed to be the most dominant event (∼7% of total ion current at maxima)

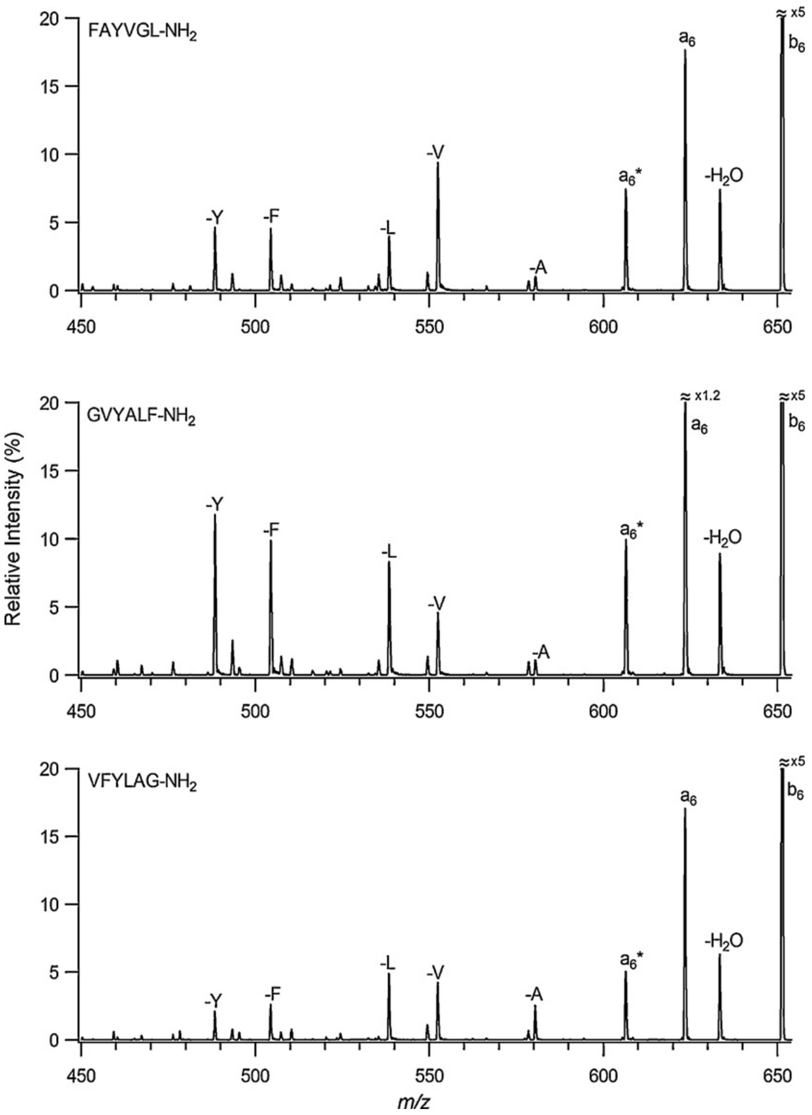

Fig. 3. CID spectra of b_6 ions of derived from FAYVGL-NH$_2$, GVYALF-NH$_2$, and VFYLAG-NH$_2$. Collision energy is 24 eV.

compared to the other eliminations. The results suggested that the ring-opening from the A—Y bond forming AGFLVY$_{oxa}$ linear hexapeptide isomer is energetically the most favored pathway and is in excellent agreement with the work previously published by Harrison et al. [34], which through scanning the potential energy surfaces, found that the energetically most favored ring-opening transition structure is AGFLY$_{oxa}$. However, the relative intensities of nondirect sequence ions in both the MS/MS spectra (as shown in Fig. 3) and breakdown graphs (as shown in Fig. 4) of b_6 ions derived from FAYVGL-NH$_2$, GVYALF-NH$_2$ and VFYLAG-NH$_2$ show different distributions with each other. As can be seen in Fig. 3, the distribution of the non-sequence ion intensities are also different from the other series shown in Fig. 1 even though they are all isomeric peptides. This could be due to the formation of a different

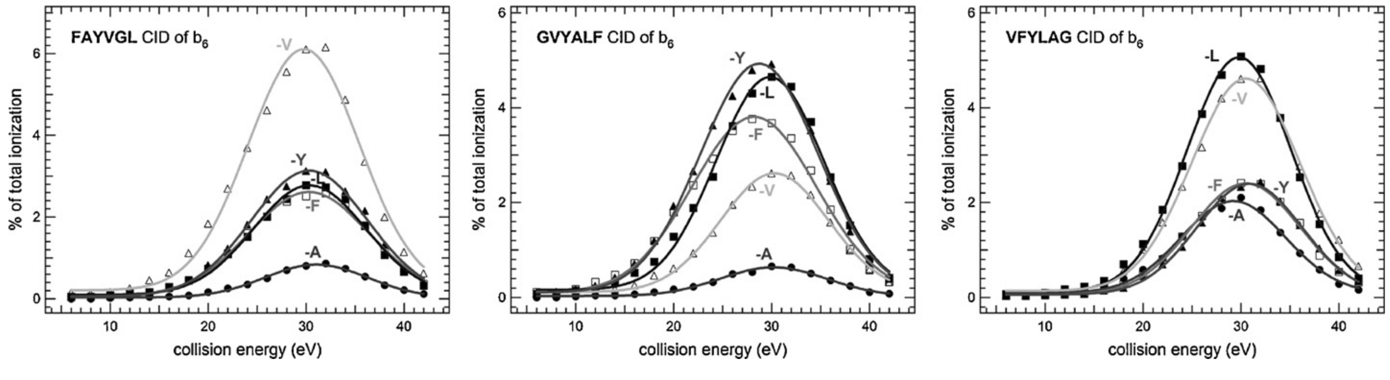

Fig. 4. Breakdown graphs for b_6 ions derived from FAYVGL-NH$_2$, GVYALF-NH$_2$, and VFYLAG-NH$_2$. Nondirect sequence ions have shown only.

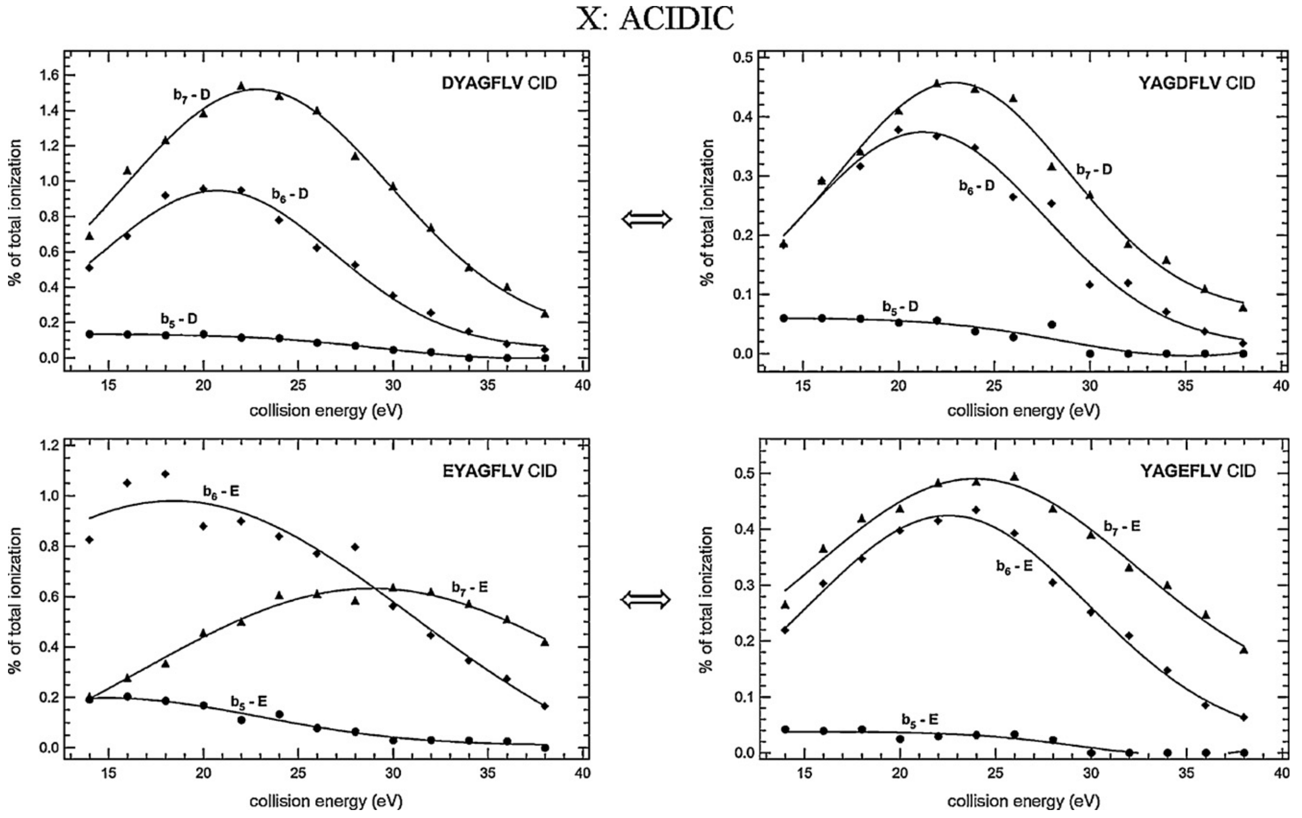

Fig. 5. Breakdown graphs for elimination of X from XYAGFLV-NH$_2$ and YAGXFLV-NH$_2$, where X is aspartic acid, D, and glutamic acid, E.

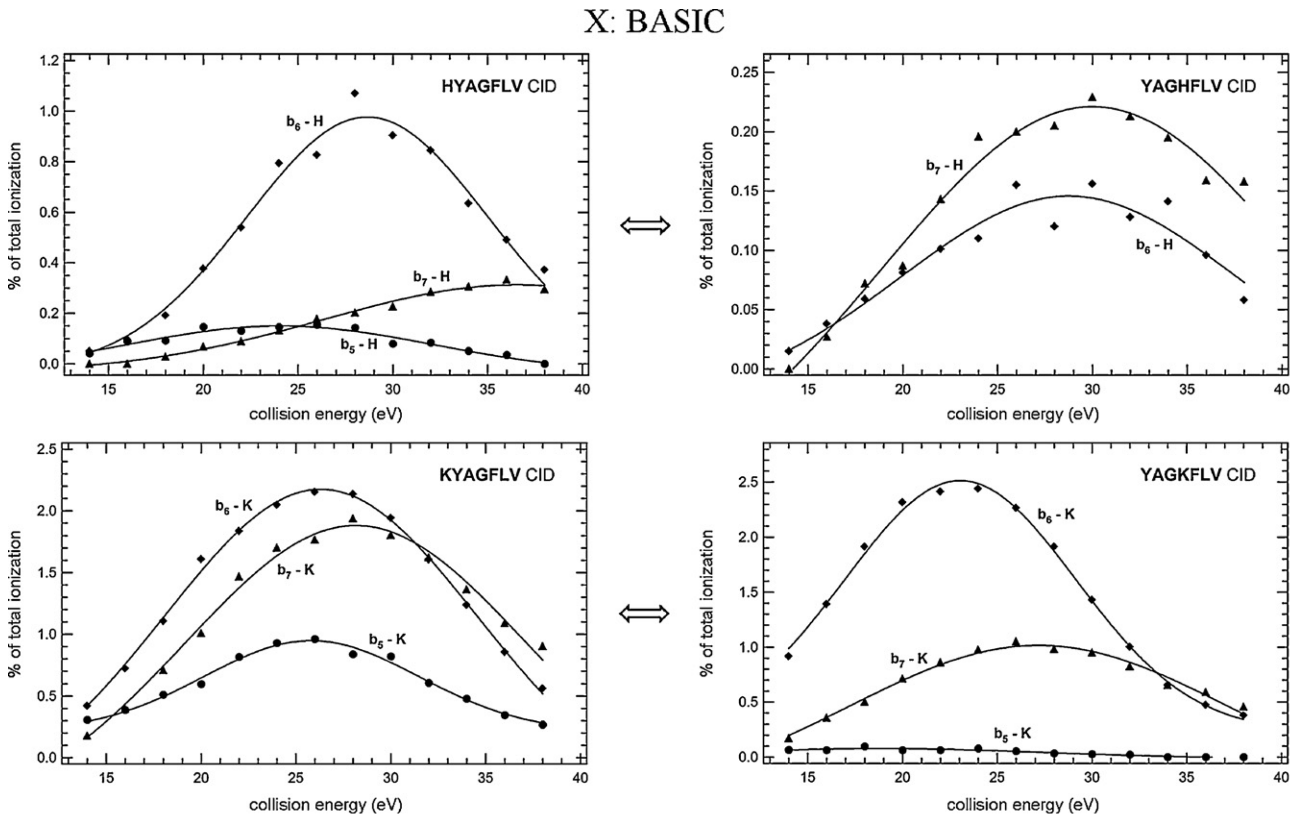

Fig. 6. Breakdown graphs for elimination of X from XYAGFLV-NH$_2$ and YAGXFLV-NH$_2$, where X is histidine, H, and lysine, K.

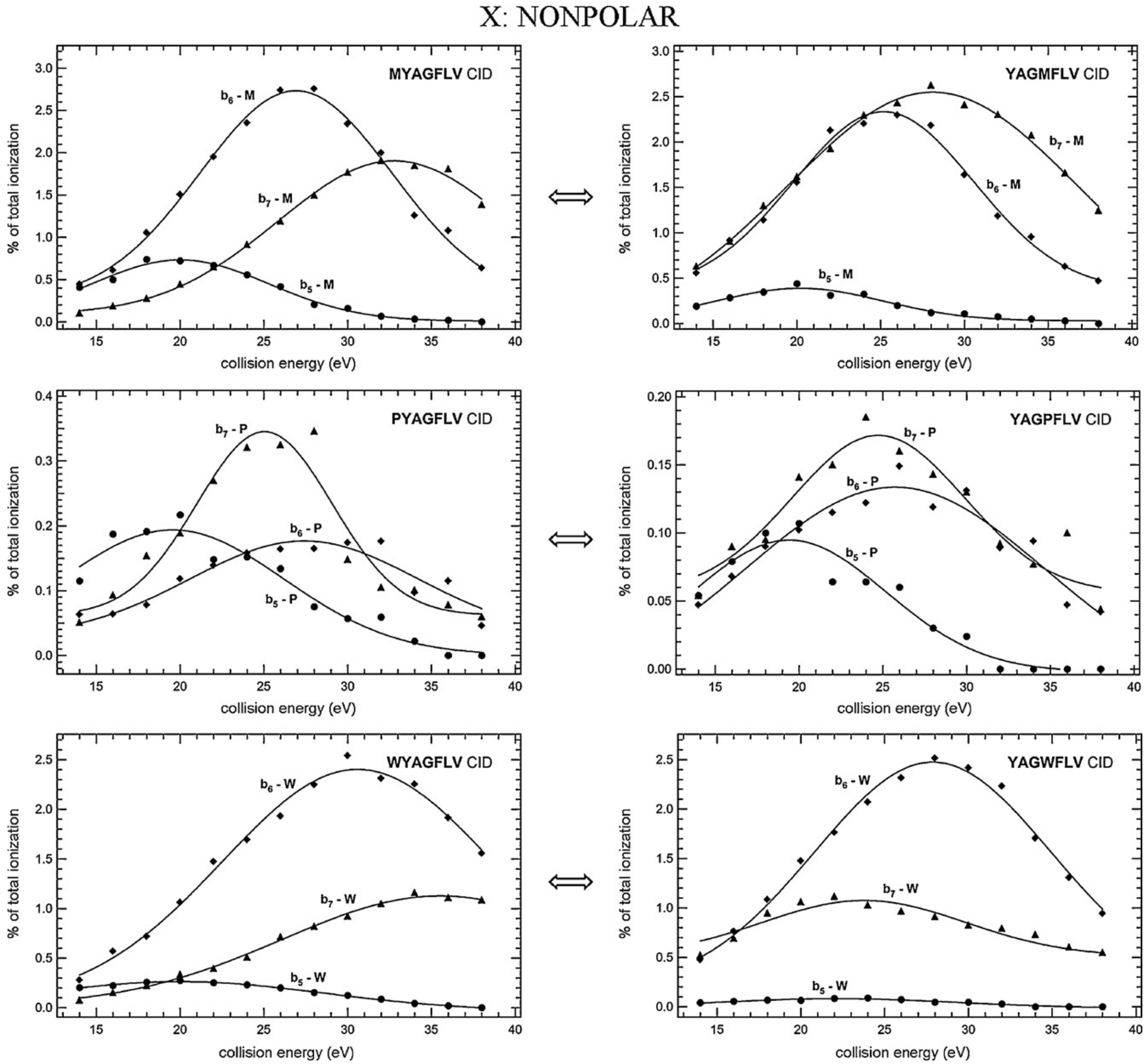

Fig. 7. Breakdown graphs for elimination of X from XYAGFLV-NH_2 and YAGXFLV-NH_2, where X is methionine, M, proline, P, and tryptophan, W.

amino acid sequence order in the cyclic forms of these peptides. It was clearly shown that the neighboring amino acid has influenced the selective opening of the macrocyclic b_6 ion. The breakdown graphs of these peptides (as shown in Fig. 4) have totally different distributions when compared to each other and the results which are shown in Fig. 2. Based on these findings, the single residue elimination orders are observed as V > Y > L ~ F > A, Y > L > F > V > A, and L > V > F ~ Y > A for FAYVGL-NH_2, GVYALF-NH_2, and VFYLAG-NH_2, respectively. Nevertheless, alanine has the lowest tendency to be eliminated from the macrocycle regardless of the sequence.

3.2. Influence of b ion size, amino acid side chain, and position on preferential opening of macrocyclic b ion

Recently, Stipdonk et al. have demonstrated that the preferential opening of the macrocyclic *b* ion structure is directly affected by the peptide size [39] and the nature of the amino acid side chain [41]. They have performed a CID of the b_5 ion derived from YAXFLG model sequence (where X is G, K, E, D, N, Q), and compared the peak intensities related to the loss of X from the b_5 ion. Using this approach, the b_5-X trend is found to follow the order of Q > K > D > N ~ E (no G elimination observed). However, they have only considered a single *b* ion size (b_5), a single residue position, and a limited number of amino acid residues. In this study, on the other hand, we have extended this approach by examining the effect of the amino acid side chain using a variety of residues, changing the residue position, and investigating the *b* ion size (b_5–b_7) on the ring formation and cleavage (b_5-X, b_6-X, and b_7-X) of macrocyclic *b* ions. For this purpose, the peptide series have been designed as XYAGFLV-NH_2 and YAGXFLV-NH_2 where X denotes C, D, E, H, K, M, N, P, Q, S, T, and W amino acid residues. The model peptides are divided into four main groups based on the side-chain nature of X residue such as acidic (D, E), basic (H, K), nonpolar (M, P, W), and polar (C, N, Q, S, T) groups. The collision energy dependence of the preferential cleavage of acidic, basic, nonpolar and polar peptide series are shown in Figs. 5–8, respectively. As is apparent in the graphs, the N-terminal position and the central position of all the residues mentioned above were considered. The results clearly show that b_6 and b_7 ions have a higher tendency toward macrocyclization compared to the b_5 ions with the

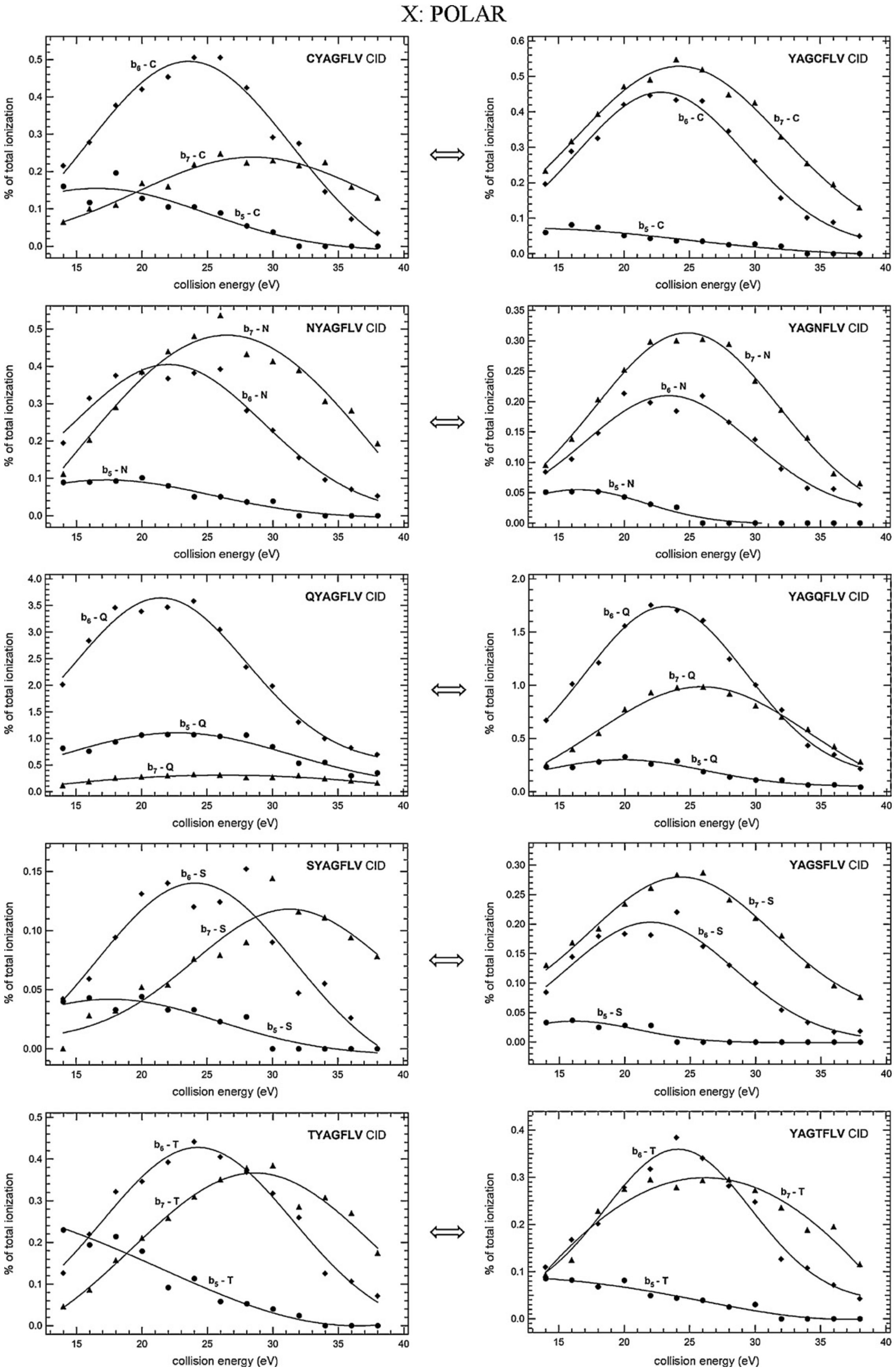

Fig. 8. Breakdown graphs for elimination of X from XYAGFLV-NH$_2$ and YAGXFLV-NH$_2$, where X is cysteine, C, asparagine, N, glutamine, Q, serine, S, and threonine, T.

exception of QYAGFLV-NH_2 which shows a higher tendency toward macrocyclization of the b_5 ion compared to that of the b_7 ion where b_5-Q > b_7-Q. There is no such an observation for asparagine although its side chain is similar to glutamine. This interesting behavior of glutamine is presently under investigation in our laboratory. In addition, it has been observed that the selective ring opening is also dependent on the size of the *b* ions and the position of the amino acid residue. It was shown that the elimination of N-terminally positioned aspartic acid or glutamic acid from b_6 or b_7 is favored compared to the central position case. Both, b_6-X and b_7-X (where X = D or E) showed similar distributions except for EYAGFLV-NH_2 (shown in Fig. 5).

The N-terminal and central position of the lysine residue showed a similar distribution when comparing to b_6-K profiles. In contrast, the b_7-K elimination from the N-terminal and the central position did not show a similar distribution (shown in Fig. 6). The preferential cleavage of b_5-K showed different distribution when N-terminal position is compared to the central position. In Fig. 6, it is shown that the b_6-H cleavages from the N-terminal and central position are similar. By contrast, the b_7-H cleavage from the N-terminal and central position showed a different distribution. In addition, the b_5-H cleavage from N-terminal position is low abundant and is in agreement with the report by Harrison et al. [44]. However, no acceptable signal was observed for central position.

Among the nonpolar residues, preferential cleavage of the proline residue from b_5, b_6, and b_7 showed similar distributions no matter where the position of the proline residue is (shown in Fig. 7). However, N-terminal and central position of the methionine showed a similar distribution when considering b_6 ions; although, b_7 showed a different distribution. The major difference observed for tryptophan is the preferential cleavage from b_7-W for the N-terminal and central positions.

Among the polar residues, they all showed a similar distribution for b_5, b_6 and b_7 when compared the N-terminal to central position (exception of QYAGFLV-NH_2). It should be also mentioned that some internal amino acid elimination from b_6 or b_7 might show slight changes in terms of intensity distribution in breakdown graph such as, cysteine, serine (shown in Fig. 8).

Finally, among the all amino acid residues, Q, W, K, and M were found to be more favored eliminations from macrocyclic b_6 ion. Different from Stipdonk et al. [39,41], our results have shown that no preferential cleavage order can be specified depending on the nature of amino acid side chain.

4. Conclusion

This systematic study of isomeric peptides clearly indicates that b_6 and b_7 ions have a higher tendency toward macrocyclization when compared to b_5 ions with the exception of QYAGFLV-NH_2 showing a higher tendency toward macrocyclization of the b_5 ion compared to that of the b_7 ion where b_5-Q > b_7-Q. We have also observed that the distribution of the nondirect sequence ion intensities of the first series (YAGFLV-NH_2, AGFLVY-NH_2, GFLVYA-NH_2, FLVYAG-NH_2, LVYAGF-NH_2, and VYAGFL-NH_2) are different from that of second series (FAYVGL-NH_2, GVYALF-NH_2, and VFYLAG-NH_2) although they are all isomeric peptides. This could be likely due to the change of the amino acid sequence order in the cyclic forms of these peptides, and it has clearly been shown that the neighboring amino acid influences the selective opening of the macrocyclic b_6 ion. The selective ring opening is also dependent on the size of the *b* ions and the position of the amino acid residue. It has also been found that no preferential cleavage order can be specified depending on the nature of amino acid side chain. Finally, Q, W, K, and M were found to be more favored eliminations from the macrocyclic b_6 ion compared to the b_5 ion.

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