



## RESEARCH ARTICLE

# Differentiation of $\alpha$ - or $\beta$ -Aspartic Isomers in the Heptapeptides by the Fragments of $[M+Na]^+$ Using Ion Trap Tandem Mass Spectrometry

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**Abstract**

Electrospray ionization coupled with low energy collision induced dissociation (CID) in an ion trap mass spectrometer was used to examine the fragmentation patterns of the  $[M+Na]^+$  of eight pairs of heptapeptides containing  $\alpha$ - or  $\beta$ -Asp residues in second and sixth amino acid positions, respectively. Selective cleavages at the peptide backbone C-terminal to two Asp residues were observed, which generated a series of C-terminal  $y_5$  ions and N-terminal  $b_6$  ions. Two typical ions:  $[y_5 + Na - H]^+$  and  $[b_6 + Na + OH]^+$ , produced by  $\alpha$ -Asp containing peptides were noted to be much more abundant than those of the peptides with  $\beta$ -Asp, which could be used for distinction of the isomers in Asp2 and Asp6, respectively. In addition, a series of internal ions generated by simultaneous cleavages at Asp residues were detected. Competitive reactions of carboxylic groups occurred between Asp6 side chain and C-terminus. Formation mechanisms of most product ions are proposed. The results obtained in this work are significant since low energy CID has been demonstrated to be effective for the distinction of Asp isomers.

**Key words:** Low energy CID,  $\alpha$ - or  $\beta$ -Aspartic isomers, Fragments of  $[M+Na]^+$ , Fragmentation pathway

## Introduction

A beta-aspartic acid ( $\beta$ -Asp) residue is an abnormal peptide linkage that presents in a polypeptide by inserting the carboxylic group originally on the side chain of alpha-aspartic acid ( $\alpha$ -Asp) into the peptide backbone and leaving the  $\alpha$ -carboxylic group as the side chain. The  $\beta$ -Asp residue can be formed spontaneously through deamination reaction of asparagines and the isomerization of  $\alpha$ -Asp under physiologic conditions [1–3]. The generation of a  $\beta$ -Asp residue can cause structural changes to a protein and can lead to complete or partial loss of protein function and activity [4, 5]. Many researches have proven that some diseases such as Alzheimer's disease (AD) are closely associated with the formation of the  $\beta$ -Asp [6, 7], while

$\beta$ -Asp-containing peptides have also been developed as drugs for disease therapy [2].

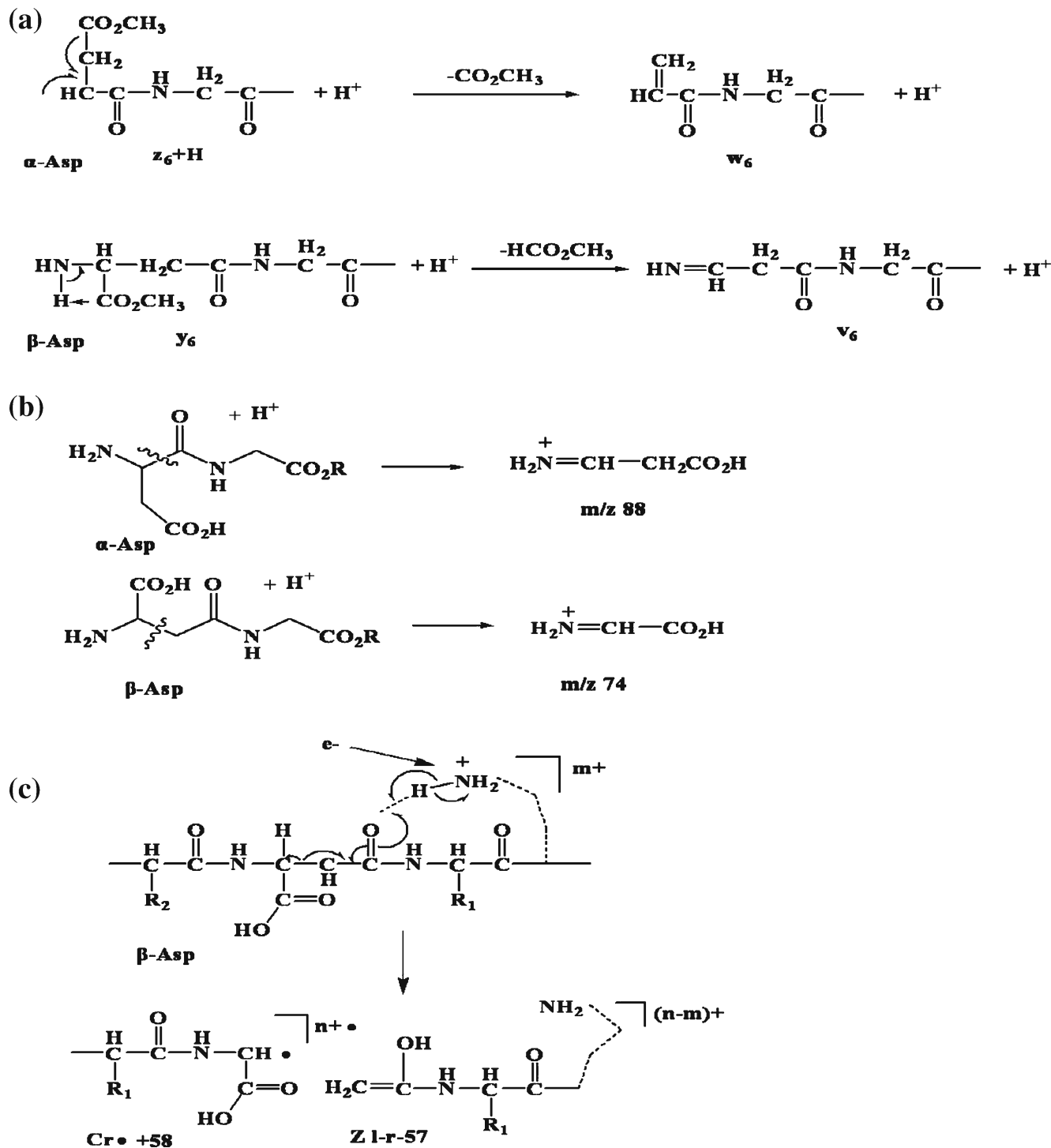
Various methods have been developed and applied to differentiate and quantify  $\alpha$ - and  $\beta$ -Asp isomers, including enzymatic methylation [8], Edman degradation [9], carboxypeptidase Y digestion [10], chromatography [11, 12], and mass spectrometry based techniques [13–22]. Mass spectrometry based techniques have demonstrated advantages in sensitivity, speed, and specificity for the analysis of the Asp isomers. Early work focused on the use of fast-atom bombardment (FAB) mass spectrometer coupled with high energy CID to characterize peptides containing Asp isomers. Thus, Carr and his co-workers successfully distinguished  $\alpha$ - and  $\beta$ -Asp isomers in tumor necrosis factor (TNF) via the formation of  $w_n$  and  $v_n$  ions ( $n$  for the position of Asp in peptide) produced by the cleavage of Asp side chain

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(Scheme 1a) [23]. Castet et al. also used high energy CID to generate immonium ions and the fragments from the side chain to distinguish  $\alpha$ - and  $\beta$ -Asp isomer in dipeptides (Scheme 1b) [17]. Recently, electron capture dissociation (ECD) [13–15] and electron-transfer dissociation (ETD) [16] have been used to examine the fragmentation reactions of  $\beta$ -Asp-containing peptides. Two diagnostic ions,  $C_n \bullet + 58$  and  $Z_{1-n} - 57$ , were observed in both ECD and ETD mass spectra

( $l$  is total number of amino acids) (Scheme 1c). These have been used to examine deamination reactions and for the unambiguous identification of Asp isomers in peptide mixture.

Although ECD, ETD, and high-energy CID have proven to be effective in the identification of Asp isomers in peptides, they are not as readily available as low-energy collision induced dissociation (CID) common to most mass



Scheme 1. Formation pathways of (a)  $w_n$  and  $v_n$  ions (b) immonium ions; (c)  $C_n \bullet + 58$  and  $Z_{1-n} - 57$  ions

spectrometers. Gonzalez and his co-workers used ESI coupled with low-energy CID to examine the fragmentation of protonated  $\beta$ -Asp-containing peptides. The structurally diagnostic ions  $[y''_{1-n+1} - 46]^+$  and  $[b_{n-1} + H_2O]^+$  were, however, influenced by the presence and position of basic amino acids [19]. Lehmann et al. distinguished the Asp isomers through determination of the relative abundance of Asp immonium ion ( $m/z$  88) and the relative abundance ratios of  $[b_n]^+$  and  $[y_{1-n}]^+$  ion that generated by the cleavages of peptide amide bonds adjacent to the Asp residue; the ratios of  $[b_n]^+/[y_{1-n}]^+$  for the  $\beta$ -Asp-containing peptides were smaller than that of the  $\alpha$ -Asp-containing peptides of the same sequence, and the relative abundance of the  $\beta$ -Asp immonium ion decreased. These changes are free from the existence of basic amino acids [20]. Similar studies in our laboratory showed that the  $[b_n]^+/[y_{1-n}]^+$  ratios were only related to the presence not the position of the Asp in the peptide, and the ratios of  $[M + H - H_2O]^+/[M + H]^+$  and  $[b_n - H_2O]^+/[b_n]^+$  could also be used for monitoring the presence of the Asp isomer [22]. Despite these promising reports, there is still a need to develop an effective and reliable low energy CID method to distinguish Asp isomers.

Previous studies had showed that fragmentation pattern of sodium associated peptide  $[M+Na]^+$  was significantly different from the pattern of protonated peptide  $[M + H]^+$  [24–29]. Selective cleavages occurred in amide bonds of the peptide adjacent to Asp residue for Asp-containing peptides [30]. The presence of a fixed positive charge or a sodium ion in the peptide promoted this selective reaction [31–33]. Various mechanisms had been proposed to explain the formation of product ions formed via CID of  $[M+Na]^+$  of peptides [24–38]. For example, a sodium ion associated with the C-terminal carboxylic group has been proposed to initiate a rearrangement reaction of hydroxyl group leading to sequential loss of the C-terminal amino acid during multiple stages of MS/MS process [34–36]. Complementary fragment information generated by  $[M+H]^+$  and  $[M+Na]^+$  as well as C-terminal cleavages had been used for determination of peptide sequences. These previous reports lead us to hypothesize that the product ions formed via sodium ion promoted selective cleavage of the peptide backbone adjacent to Asp residue might allow distinction of Asp isomers in peptides.

To test this hypothesis, three sets (eight pairs) of heptapeptides (Table 1) were designed and the low energy

CID reactions of their  $[M+Na]^+$  were studied. In each set, each pair of the peptides was composed of same amino acid sequence, with two distinct  $\alpha$ - or  $\beta$ -Asps in second and sixth amino acid positions, respectively; the normal N- and C-terminals of the same peptides were acylated and amidated, respectively, to generate additional two pairs of the peptides. In different sets the sequence of the peptides was changed. Using these peptides, the fragment patterns of  $[M+Na]^+$  were studied, with the aim of discovering diagnostic product ions that would allow exact identification of  $\alpha$ - and  $\beta$ -Asp isomers in the different positions of the heptapeptides. Also, the effects of neighboring amino acids and of the terminal groups of the peptides on the formation of product ions of the heptapeptides were examined.

## Experimental

### Sample Preparation

The peptides were synthesized by Changchun BCHT Biotechnology Co., Ltd. (Changchun, China) and used without further purification. Methanol (chromatographic grade), formic acid (analytical grade), sodium acetate (analytical grade), were purchased from Beijing Chemical Plant. Each sample was dissolved in Milli-Q water (Millipore, MA, USA) to make a stock solution of approximate 2 mM concentration, and then was diluted to a final concentration of 30  $\mu$ M in a solution containing 8 mol/L of sodium acetate and methanol/water/formic acid at a ratio 49.5:49.5:1, (vol/vol/vol) for direct ESI-MS analysis.

### Mass Spectrometry

Mass spectrometric analysis was performed in ESI positive ion mode of an Agilent G6300 Trap mass spectrometer (Agilent Technologies, CA, USA). Experiments were repeated three times on three different days. The samples were infused at a rate of 5  $\mu$ L/min. The instrument conditions: spray voltage, 4000 V; capillary exit voltage, 305 V; skimmer voltage 25 V; dry gas, He, 300 °C (5.00 L/min); nebulizer gas, N<sub>2</sub> (15 psi). For all CID MS/MS experiments: isolation selection, standard; fragmentation cutoff, default; isolation width, 4.0  $m/z$ ; fragmentation amplitude, 0.80 V. The data were analyzed with Agilent 6300 Ion Trap LCMS software.

**Table 1.** Studied Peptides and their Sequences

| Peptide        | Sequence                                               | Peptide       | Sequence                                             |
|----------------|--------------------------------------------------------|---------------|------------------------------------------------------|
| 1- $\alpha$    | HSD $_{\alpha}$ AAVD $_{\alpha}$ TOH                   | 1- $\beta$    | HSD $_{\beta}$ AAVD $_{\beta}$ TOH                   |
| 1-Ac- $\alpha$ | CH <sub>3</sub> CO-SD $_{\alpha}$ AAVD $_{\alpha}$ TOH | 1-Ac- $\beta$ | CH <sub>3</sub> CO-SD $_{\beta}$ AAVD $_{\beta}$ TOH |
| 1-Am- $\alpha$ | HSD $_{\alpha}$ AAVD $_{\alpha}$ T-NH <sub>2</sub>     | 1-Am- $\beta$ | HSD $_{\beta}$ AAVD $_{\beta}$ T-NH <sub>2</sub>     |
| 2- $\alpha$    | HSD $_{\alpha}$ AASD $_{\alpha}$ AOH                   | 2- $\beta$    | HSD $_{\beta}$ AASD $_{\beta}$ AOH                   |
| 2-Ac- $\alpha$ | CH <sub>3</sub> CO-SD $_{\alpha}$ AASD $_{\alpha}$ AOH | 2-Ac- $\beta$ | CH <sub>3</sub> CO-SD $_{\beta}$ AASD $_{\beta}$ AOH |
| 2-Am- $\alpha$ | HSD $_{\alpha}$ AASD $_{\alpha}$ A-NH <sub>2</sub>     | 2-Am- $\beta$ | HSD $_{\beta}$ AASD $_{\beta}$ A-NH <sub>2</sub>     |
| 3- $\alpha$    | HVD $_{\alpha}$ TAVD $_{\alpha}$ TOH                   | 3- $\beta$    | HVD $_{\beta}$ TAVD $_{\beta}$ TOH                   |
| 3-Am- $\alpha$ | HVD $_{\alpha}$ TAVD $_{\alpha}$ T-NH <sub>2</sub>     | 3-Am- $\beta$ | HVD $_{\beta}$ TAVD $_{\beta}$ T-NH <sub>2</sub>     |

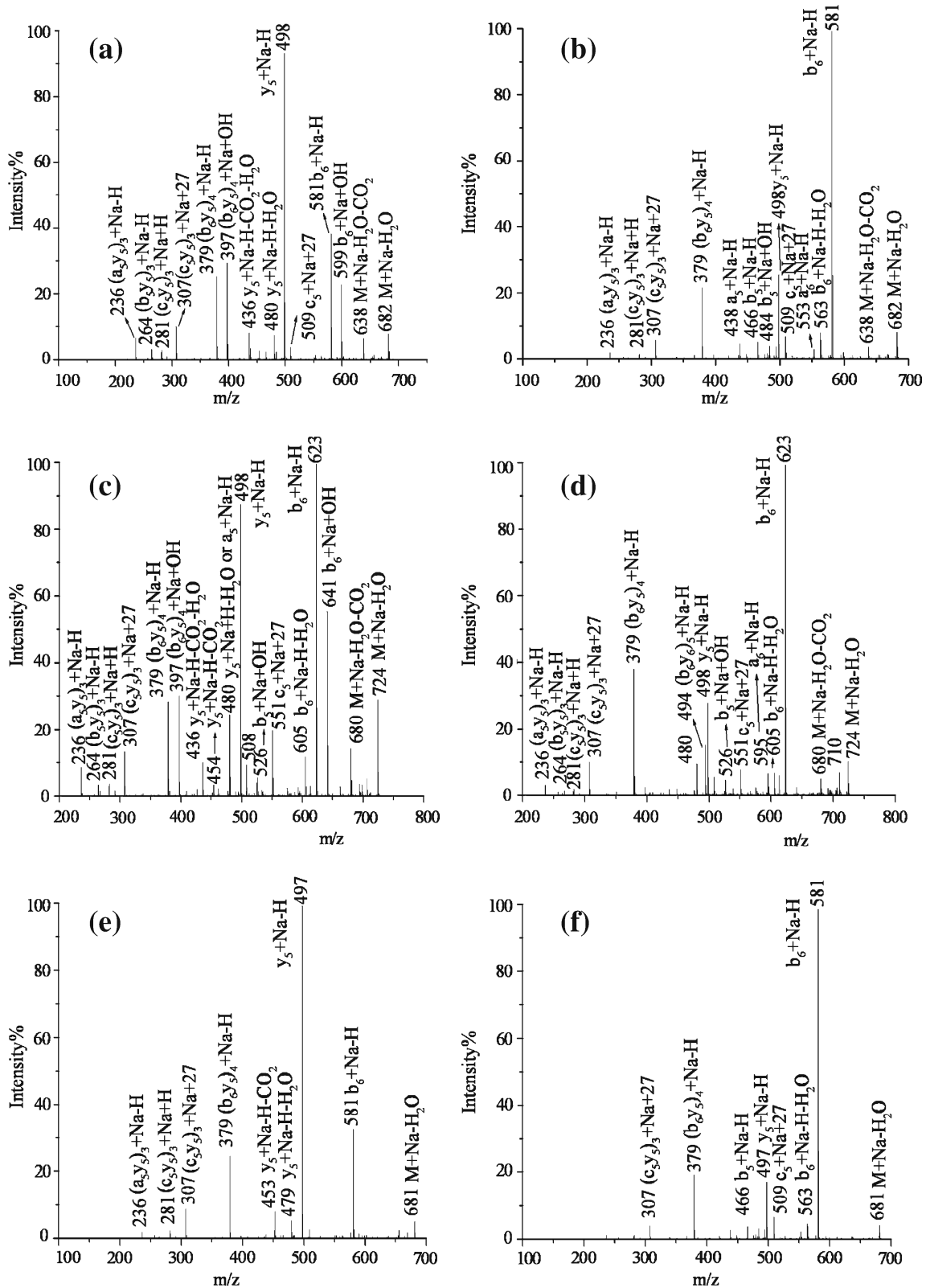


Figure 1. Tandem mass spectra of  $[M+Na]^+$  of the heptapeptides (a)1- $\alpha$ ; (b)1- $\beta$ ; (c)1-Ac- $\alpha$ ; (d)1-Ac- $\beta$ ; (e)1-Am- $\alpha$ ; and (f)1-Am- $\beta$

## Results and Discussion

### Fragmentation Patterns of $[M+Na]^+$

The tandem mass spectra of the  $[M+Na]^+$  of the heptapeptides from Set 1 are shown in Figure 1. Several series of ions were detected. C-terminal ions including  $[y_5 + Na - H]^+$ ,  $[y_5 + Na - H - H_2O]^+$ , and  $[y_5 + Na - H - CO_2 - H_2O]^+$  were generated by selective cleavage of the peptide amide bond C-terminal from Asp2; N-terminal ions such as  $[b_6 + Na + OH]^+$ ,  $[b_6 + Na - H]^+$ ,  $[c_5 + Na + 27]^+$ ,  $[b_5 + Na - H]^+$ , and  $[a_5 + Na - H]^+$  were formed by selective cleavage of the peptide backbones adjacent to the Asp6 residue. Moreover, the internal ions  $[(b_6y_5)_4 + Na + OH]^+$ ,  $[(b_6y_5)_4 + Na - H]^+$ ,  $[(a_5y_5)_3 + Na - H]^+$ ,  $[(b_5y_5)_3 + Na - H]^+$ , and  $[(c_5y_5)_3 + Na + 27]^+$  were produced by simultaneous cleavage from the peptide backbones of the C-terminal from Asp2 and attachment to Asp6, respectively. The predominant product ions from the  $[M+Na]^+$  precursors shown in the mass spectra are ions generated by selective cleavage of the amide bonds adjacent to the Asp residues.

### Terminal Group Effect on Fragmentation

Comparing the mass spectra of peptide 1- $\alpha$  with 1-Ac- $\alpha$  and 1-Am- $\alpha$  (Figure 1a, c, and e), the terminus of the two latter peptides were either acylated or amidated; 1- $\alpha$  and 1-Ac- $\alpha$  displayed similar fragment patterns, indicating that the N-terminal groups of the peptide had less effect on the fragmentation patterns of  $[M+Na]^+$ . In contrast, peptide 1-Am- $\alpha$  formed less fragment ions than peptide 1- $\alpha$ . Abundant  $[b_6+Na+OH]^+$  and several N-terminal ions appeared in the mass spectrum of the 1- $\alpha$  missing in the mass spectrum of 1-Am- $\alpha$ , suggesting that the reactivity of the C-terminal carboxylic group was blocked by amidation reaction, therefore restricting the formation of  $[b_6 + Na + OH]$  and some N-terminal ions.

### Distinction of $\alpha$ - or $\beta$ -Asp Isomers

Comparing the fragmentation patterns of  $[M+Na]^+$  formed by each  $\alpha$ - and  $\beta$ -Asp pair-containing peptides, two typical peaks ( $[y_5 + Na - H]^+$  and  $[b_6 + Na + OH]^+$ ) were generated by the amide bond cleavage C-terminal from Asp2 and Asp6, respectively. The relative abundance of the two ions were more intense for the  $\alpha$ -Asp peptides than the  $\beta$ -Asp peptides, with the exception of the C-terminal amidated peptides for  $[b_6 + Na + OH]^+$  (Figure 1 a, c, and e). Thus, the  $\alpha$ - or  $\beta$ -isomers of Asp2 and Asp6 could be distinguished by two diagnostic peaks, respectively.

To examine the formation and specificity of  $[y_5 + Na - H]^+$  and  $[b_6 + Na + OH]^+$ , the ratios  $[y_5 + Na - H]^+ / [(b_6y_5)_4 + Na - H]^+$  and  $[b_6 + Na + OH]^+ / [b_6 + Na - H]^+$  of the three measurements for each pair of  $\alpha$ - and  $\beta$ -Asp-containing peptides were calculated and listed in Table 2. Ions  $[(b_6y_5)_4 + Na - H]^+$  and  $[b_6 + Na - H]^+$  were compared because both were stable and thus visible in

the mass spectra of both  $\alpha$ - and  $\beta$ -Asp peptides. Table 2 shows that the ratio  $[y_5 + Na - H]^+ / [(b_6y_5)_4 + Na - H]^+$  of the  $\alpha$ -containing peptides was ten fold higher than that of the  $\beta$ -Asp-containing peptides. In fact,  $[y_5 + Na - H]^+$  was the base peak in most mass spectra of the  $\alpha$ -Asp containing peptides (Figure 1). Similarly, the ratio  $[b_6 + Na + OH]^+ / [b_6 + Na - H]^+$  for  $\alpha$ -containing peptides without C-terminal amidation were also much higher than that of the  $\beta$ -Asp peptides. The  $[b_6 + Na + OH]^+$  ion of the C-terminal amidated peptides were less abundant regardless of Asp isomer presence. These results support the use of the two diagnostic peaks for the differentiation of the Asp isomers in different positions. Table 2 also shows that the difference of peptide sequence in this study has no effect on the trends of diagnostic peak abundance.

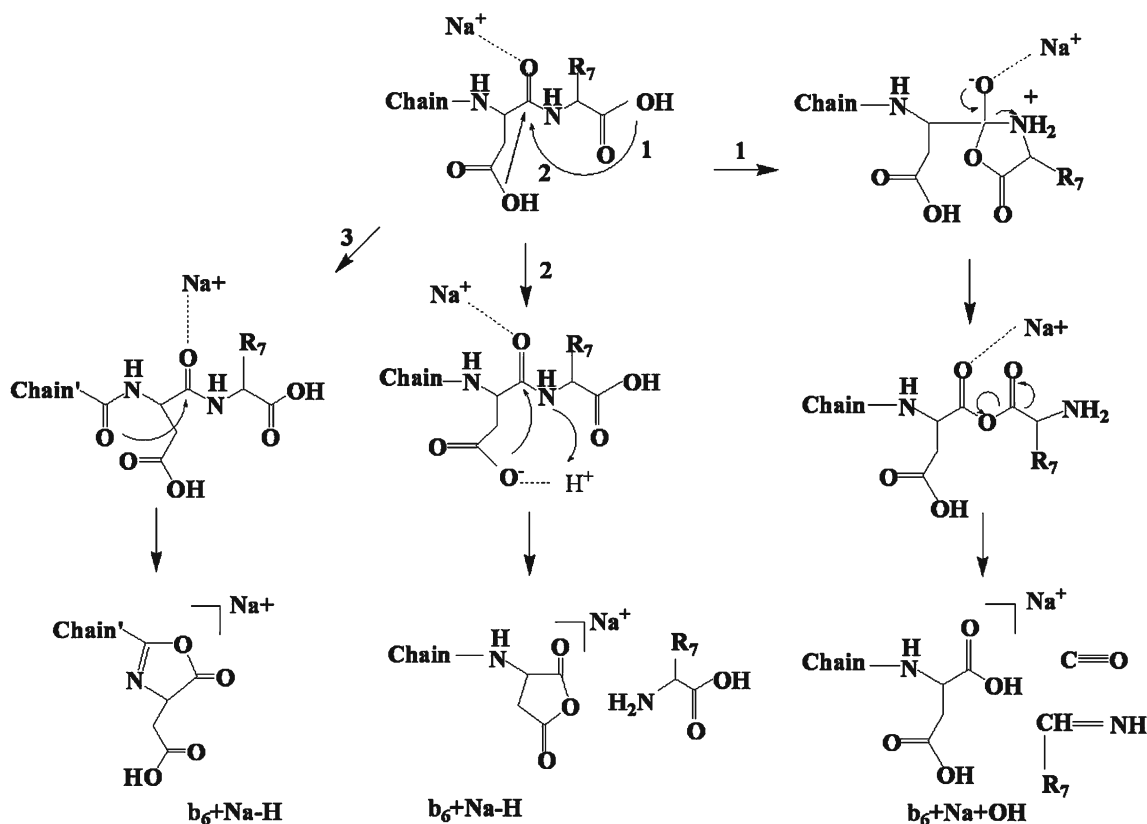
### Formation of $[b_6 + Na + OH]^+$ and $[b_6 + Na - H]^+$

The selective cleavages that occurred suggest that the acidic carboxylic group on the side chain of the Asp residue is involved in the dissociation reactions. All product ions of  $[M+Na]^+$  in the mass spectra are associated with the sodium ion, indicating the key role of sodium ions in selective cleavage. In fact, Lee et al. reported the highly selective cleavage of sodium-associated peptides from Asp residues; the sequential loss of amino acids from the C-terminus under low-energy CID were also observed when the peptide lacked acidic residues [32]. Rollgen et al. first reported that the sodium ion interacts selectively with the polar functional groups of the peptides, initiating the backbone cleavage through a “charge remote” mechanism resulting in significant differences of the product ions from those formed by protonated peptides [38]. Various fragmentation mechanisms of the sodium-associated peptides have been proposed

**Table 2.** Ratios of  $[b_6 + Na + OH]^+ / [b_6 + Na - H]^+$  and  $[y_5 + Na - H]^+ / [(b_6y_5)_4 + Na - H]^+$  Formed in the Low Energy CID of the Heptapeptides  $[M+Na]^+$

| Sample         | $[b_6 + Na + OH]^+ / [b_6 + Na - H]^+$ | $[y_5 + Na - H]^+ / [(b_6y_5)_4 + Na - H]^+$ |
|----------------|----------------------------------------|----------------------------------------------|
| 1- $\alpha$    | 0.61±0.01                              | 3.64±0.12                                    |
| 1- $\beta$     | 0.02±0.01                              | 1.13±0.14                                    |
| 1-Ac- $\alpha$ | 0.56±0.02                              | 2.86±0.15                                    |
| 1-Ac- $\beta$  | 0.02±0.00                              | 0.73±0.03                                    |
| 1-Am- $\alpha$ | nd                                     | 3.88±0.41                                    |
| 1-Am- $\beta$  | nd                                     | 0.84±0.04                                    |
| 2- $\alpha$    | 1.24±0.01                              | 5.29±0.22                                    |
| 2- $\beta$     | 0.03±0.00                              | 1.02±0.13                                    |
| 2-Ac- $\alpha$ | 1.06±0.04                              | 4.28±0.22                                    |
| 2-Ac- $\beta$  | 0.03±0.00                              | 0.80±0.01                                    |
| 2-Am- $\alpha$ | nd                                     | 3.69±0.09                                    |
| 2-Am- $\beta$  | nd                                     | 0.73±0.01                                    |
| 3- $\alpha$    | 0.46±0.02                              | 4.34±0.26                                    |
| 3- $\beta$     | 0.02±0.00                              | 1.37±0.03                                    |
| 3-Am- $\alpha$ | nd                                     | 4.65±0.20                                    |
| 3-Am- $\beta$  | nd                                     | 1.12±0.08                                    |

nd=indicates not detected

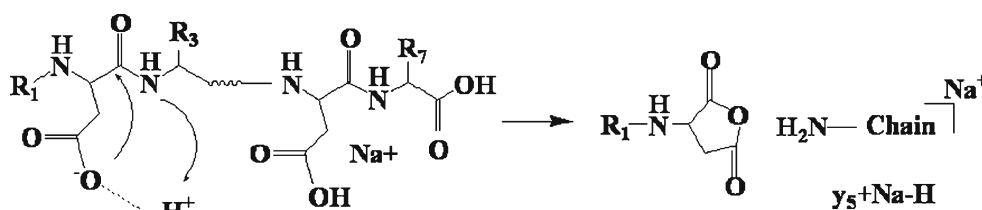


Scheme 2. Formation pathways of  $[b_6 + Na - H]$  and  $[b_6 + Na + OH]$

[24, 26–37], a recent study on C-terminal fragmentations of sodium-associated peptides indicate that sodium was attached to the carbonyl oxygen; the hydroxyl oxygen nucleophilically attacked the carbon center of the amide bond to form a oxazolidin-5-intermediate that underwent a rearrangement into an anhydride intermediate leading to the formation of the ion  $[b_{n-1} + Na + OH]^+$  [34]. In this study, two adjacent carboxylic groups presented in the heptapeptides; the carboxyl oxygen on the side chain of Asp 6 and on the C-terminus competitively attacked the carbon center of the sodiated amide bond, resulting in the formation of the ions  $[b_6 + Na + OH]^+$  and  $[b_6 + Na - H]^+$ , respectively (Scheme 2). Lin et al. studied more than 100 peptides having 2–10 amino acid length; most of them generated the dominant peak of  $[b_{n-1} + Na + OH]^+$  by sequential loss of the C-terminal amino acid and the less abundant  $[b_{n-1} + Na - H]^+$  ion [35]. The ion  $[b_{n-1} + Na - H]^+$  could be formed by nucleophilic attack of the Asp side

chain or an adjacent amide oxygen in the peptide backbone [27, 30, 32, 33]. Clearly, the formation process of  $[b_{n-1} + Na + OH]^+$  from the C-terminal group and of  $[b_{n-1} + Na - H]^+$  from the adjacent Asp side chain is competitive, but the formation of  $[b_{n-1} + Na - H]^+$  from the carbonyl amide oxygen was energetically and kinetically unfavored.

The less abundant  $[b_6 + Na + OH]^+$  ion was detected in the mass spectra of the  $\beta$ -Asp-containing peptides, where an additional methylene group was introduced into the peptide backbone adjacent to the carbon center. This methylene group might decrease the nucleophilicity of the carboxylic oxygen to the carbon center compared to tertiary carbon of the  $\alpha$ -Asp. The less abundant  $[b_6 + Na + OH]^+$  ion was detected for C-terminal amidated peptides. These observations suggest that not only amidated C-terminus but also the presence of an adjacent  $\beta$ -Asp residue restricts the reactivities of the C-terminal carboxylic group.



Scheme 3. Formation pathway of  $[y_5 + Na - H]$

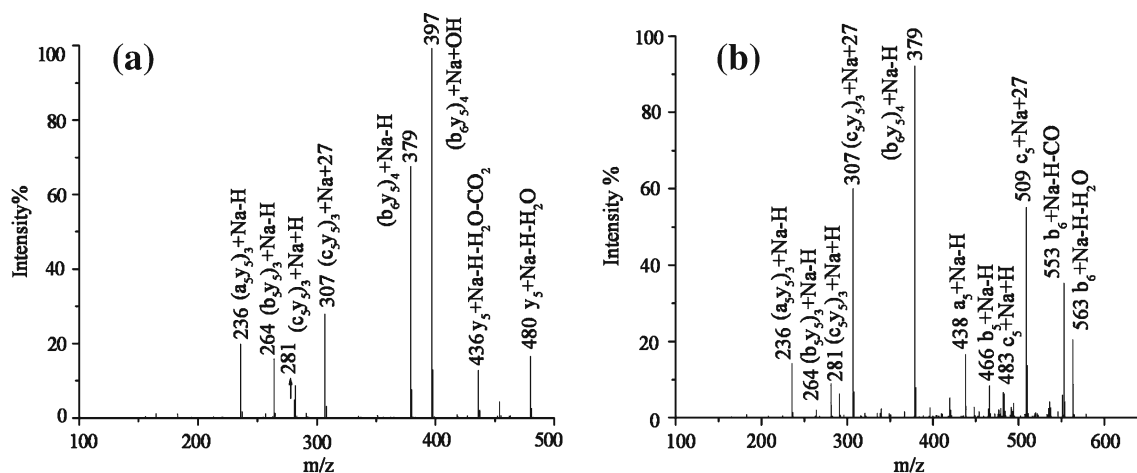


Figure 2. Low energy CID MS<sup>3</sup> spectra of (a) and (b) of the heptapeptide 1- $\alpha$

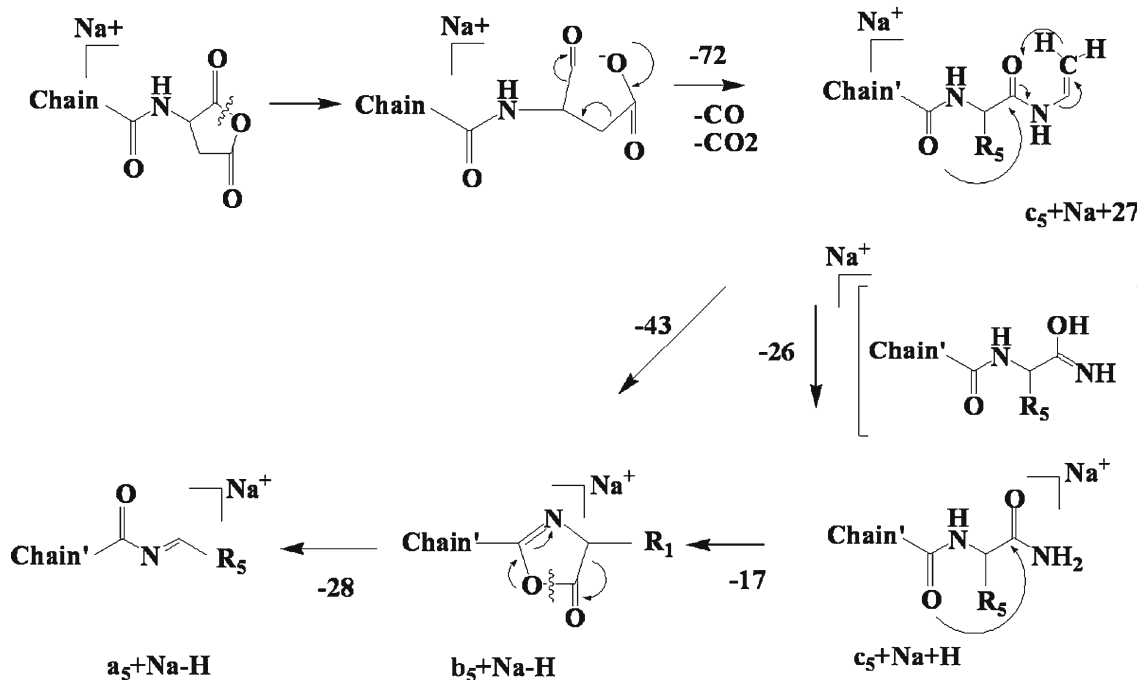
### Formation of $[y_5 + Na - H]^+$

The ion  $[y_5 + Na - H]^+$  was formed by selective cleavage of the peptide at Asp2 with charge remaining on the C-terminal ion; no corresponding  $b_2$  ion was detected. In the protonated peptides, the mobile proton rapidly moves along the peptide backbone, finds the basic sites, and induces charge-directed cleavage upon activation [27, 32]. The sodium ion remained at the chain with more carboxylic groups; the sodium ion likely associated with acidic carboxylic groups and induced a charge-remote cleavage. The abundance of the  $[y_5 + Na - H]^+$  ion formed by the  $\alpha$ -Asp-containing peptides is more abundant than those by the  $\beta$ -Asp peptides. The formation mechanism of the ion is shown in Scheme 3. The same principle for interpreting the

relative abundance of  $[b_6 + Na + OH]^+$  applies: the secondary carbon from the  $\beta$ -Asp peptide adjacent to the carbon center makes the carbon center with less positive charges compared to the  $\alpha$ -Asp tertiary carbon, so that the carboxylic attack of  $\beta$ -Asp is less favored.

### Formation of Internal Ions

The same internal ions including  $[(a_5y_5)_3 + Na - H]^+$ ,  $[(b_5y_5)_3 + Na - H]^+$ ,  $[(c_5y_5)_3 + Na - H]^+$ ,  $[(c_5y_5)_3 + Na + 27]^+$ , as well as  $[(b_6y_5)_4 + Na - H]^+$  were observed in both of the tandem mass spectra of  $[y_5 + Na - H]^+$  and  $[b_6 + Na - H]^+$  (Figure 2). Ion  $[y_5 + Na - H]^+$ , a C-terminal ion formed by selective cleavage at Asp2 was



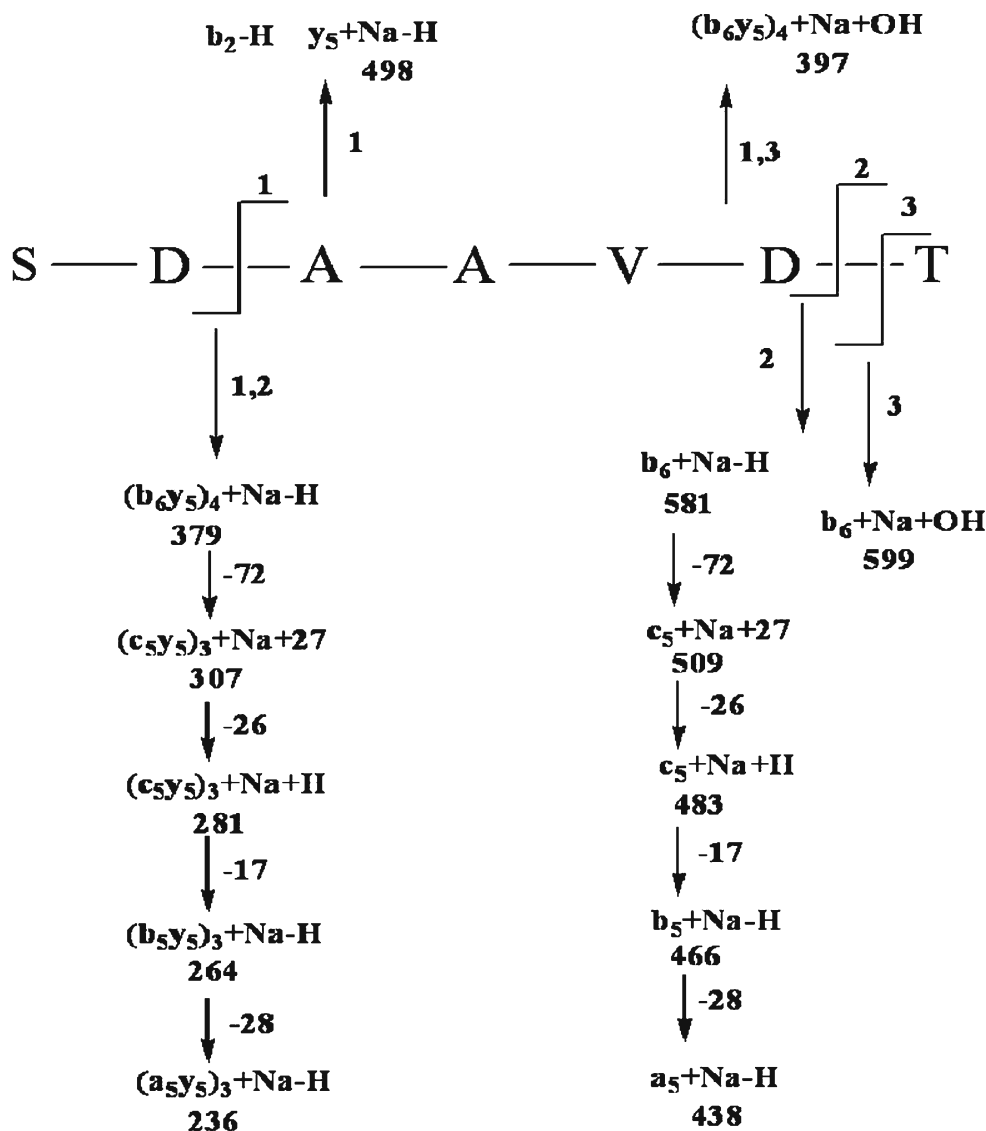
Scheme 4. Formation pathways of  $[a_5 + Na - H]$ ,  $[b_5 + Na - H]$  and  $[c_5 + Na - H]$



further broken at Asp6 to form  $[(b_6y_5)_4 + Na - H]^+$ . Similarly,  $[b_6 + Na - H]^+$  was broken at the N-terminal Asp2, forming  $[(b_6y_5)_4 + Na - H]^+$  and then broken at the C-terminus to form a series of ions including  $[b_6 + Na - H - O]^+$ ,  $[b_6 + Na - H - H_2O]^+$ ,  $[c_5 + Na + 27]^+$ ,  $[c_5 + Na - H]^+$ ,  $[b_5 + Na - H]^+$ , and  $[a_5 + Na - H]^+$ . The ion formation mechanisms are shown in Scheme 4.

### Fragmentation Pathways

The fragmentation pathways of the heptapeptides 1- $\alpha$  and 1- $\beta$  are shown in Scheme 5; this is also applicable for the other heptapeptides in this work. Selective cleavages of the peptide backbone occur at Asp residues first; further cleavages generate series a, b, c, and the internal ions.



Scheme 5. Fragmentation pathways of the heptapeptides 1- $\alpha$  and 1- $\beta$

### Conclusions

In this work, the fragmentation patterns of  $[M+Na]^+$  of heptapeptides with Asp2 and Asp6 isomers were studied by ESI ion trap mass spectrometer. Mass spectra showed that selective cleavages of the peptide backbone occurred at Asp residues forming series  $y_5$ ,  $b_6$ , and the internal ions. Two diagnostic ions ( $[y_5 + Na - H]^+$  and  $[b_6 + Na + OH]^+$ ) are identified with higher abundance for  $\alpha$ -Asp containing peptides than that the peptides with  $\beta$ -Asp. Since they are formed by backbone cleavage at the peptide bond C-terminal to Asp2 and Asp6, respectively, this can be used for the distinction of Asp isomers in different positions. The  $y_5$  series of ions are formed by Asp cleavage near the N-terminus, whereas the  $b_6$  series of ions are generated by Asp cleavage close to the C-terminus; no corresponding  $b_2$  and  $y_1$  ions are detected. The number of acidic carboxylic



groups is likely to decide the charge of the remaining sodiated fragments. The presence of  $\beta$ -Asp adjacent to the C-terminus restricts the formation of the  $[b_6 + Na + OH]^+$  ion; the formation of  $[b_6 + Na - H]^+$  may be limited by the presence of an adjacent C-terminus due to competitive reaction. Otherwise, the b ion formed at the Asp cleavage could also be used for identifying the Asp isomer. Further work is needed to test these assumptions.

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