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Electrospray ionization mass spectral characteristics and fragmentation mechanisms of Angiotensin II and its analogues

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Abstract

The characteristic fragmentation pathways of Angiotensin II and eight analogues were investigated by electrospray ionization tandem mass spectrometry. The main fragmentations involve the cleavages of the C─CO and CO─NH bonds with the loss of water, ammonia or carbon monoxide and rearrangements involving hydrogen atoms, and the MS/MS spectra give significant sequence information of these octapeptides. In addition, the two members of the analogues with the same mass and different elemental composition can be distinguished by the MS/MS spectra of $[M+H]^+$ and fragment ions. These results show that ESI tandem mass spectrometry is an excellent tool for the structural identification of Angiotensin II and its analogues.

Keywords: Angiotensin II; Fragmentation mechanism; Electrospray ionization; Tandem mass spectrometry; Collision-induced dissociation

1. Introduction

The renin–angiotensin system is very important to regulate of blood pressure in human. Angiotensin II is a potent bioactive peptide containing eight amino acids (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), which can cause hypertension and plays an important role in this renin–angiotensin system. The block action of Angiotensin II is a strategy of medication, and recently, some analogues of Angiotensin II were synthesized by the replacement of Asp, Ile and Phe in position 1, 5 and 8 of this peptide with different amino acids for the investigation of the antagonistic properties. The analysis of the structures and the fragmentation of Angiotensin II and its analogues is very useful in research the efficiency of Angiotensin II receptor antagonists [1–3].

Electrospray ionization-mass spectrometry (ESI-MS) is a powerful tool for the analysis of peptides [4–11], which provide abundant structural information. In this paper, ESI mass spectral characteristics and fragmentation mechanisms of Angiotensin II and its eight analogues were investigated (Scheme 1). The results show that the sequence information can be obtained from the MS/MS of the peptide, and the fragmentation pathways involve kinds of rearrangements releasing small neutrals such as water, carbon monoxide or ammonia [12–21]. In addition, two analogues with the same mass were able to be distinguished by electrospray ionization tandem mass spectrometry.

2. Experimental

2.1. Materials

Angiotensin II and its analogues are from ICN Biomedicals without further purification. All of the other chemicals were of analytical grade.

2.2. Mass spectrometry

ESI-MS spectra were obtained by using a Finnigan LCQ Deca XP Plus ion trap mass spectrometer (San Jose, CA), and all experiments were carried out in the positive-ion mode. Samples were dissolved in 20:80 (v/v) methanol–water containing 100 mmol L^{-1} ammonium acetate (pH = 6.5) to a concentration of 20 μmol L^{-1}. Inclusion of methanol was necessary to obtain good electrospray behavior [22]. The peptide solution was infused directly into the mass spectrometer at a flow

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X–Arg-Val–Tyr-Y-His–Pro-Z

Angiotensin Ⅱ (**P1**):	X=Asp, Y=Ile, Z=Phe
[Val5]-Angiotensin Ⅱ (**P2**):	X=Asp, Y=Val, Z=Phe
[Asn1, Val5]-Angiotensin Ⅱ (**P3**):	X=Asn, Y=Val, Z=Phe
[Sar1]-Angiotensin Ⅱ (**P4**):	X=Sar, Y=Ile, Z=Phe
[Sar1, Ala8]-Angiotensin Ⅱ (**P5**):	X=Sar, Y=Ile, Z=Ala
[Sar1, Gly8]-Angiotensin Ⅱ (**P6**):	X=Sar, Y=Ile, Z=Gly
[Sar1, Ile8]-Angiotensin Ⅱ (**P7**):	X=Sar, Y=Ile, Z=Ile
[Sar1, Thr8]-Angiotensin Ⅱ (**P8**):	X=Sar, Y=Ile, Z=Thr
[Sar1, Val5, Ala8]-Angiotensin Ⅱ (**P9**):	X=Sar, Y=Val, Z=Ala

Scheme 1. The structures of Angiotensin II and its analogues.

rate of 2.0 μL min^{-1}. The electrospray source conditions were optimized to favor the detection of peptides (spray voltage 4.0 kV, capillary temperature 180 °C). The MS/MS spectra were obtained by collision-induced dissociation (CID) after isolation of the appropriate precursor ions. The collision conditions were maintained at normalized collision energy of 0–120%, and activation time of 400 ms [10]. In all experiments, the scanned mass range was set at 50–2000 u. Data were collected and analyzed by using the Xcalibur software developed by ThermoFinnigan, and 10 scans were averaged for each spectrum.

3. Results and discussion

3.1. Characteristics of ESI mass spectra of Angiotensin II and its analogues

Angiotensin II and eight analogues were analyzed by electrospray ionization-mass spectrometry. The ESI mass spectra of these peptides showed two main peaks corresponding to protonated molecular ions with single and double charge. For example, the ESI mass spectrum of Angiotensin II (**P1**) in Fig. 1 shows two ions at *m/z* 1046.55 ($[\mathbf{P1}+H]^+$) and 524.05 ($[\mathbf{P1}+2H]^{2+}$),

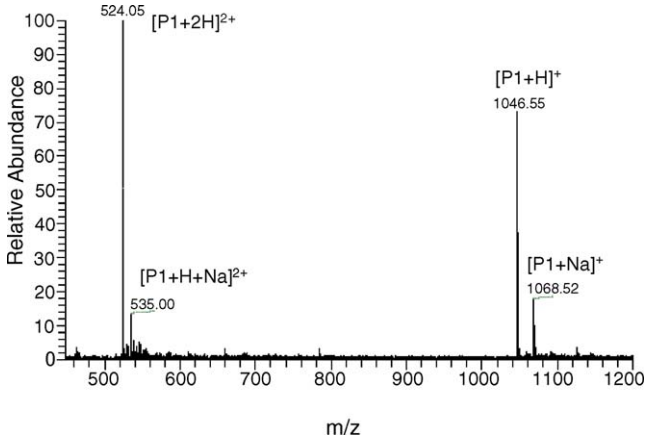


Fig. 1. ESI mass spectrum of Angiotensin II (**P1**).

respectively. The ions with low intensities at *m/z* 535.00 and 1068.52 correspond to Na adducts, $[\mathbf{P1}+H+Na]^{2+}$ and $[\mathbf{P1}-H+Na]^+$, respectively.

3.2. MS/MS spectral characteristics and fragmentations of Angiotensin II and analogues

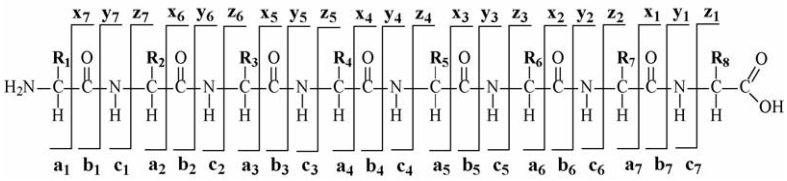
The MS/MS spectral data of the nine octapeptides are summarized in Table 1. The main fragmentation pathways of these peptides can be rationalized by the a_n, b_n and y_n ($n=1, 2, 3, \ldots 7$) cleavages in Scheme 2, which involve the cleavage of the C–CO bond (a path) and CO–NH bond (b and y paths).

It was observed that ESI mass spectral characteristics of **P1** and **P2** differ entirely from those of **P4**–**P9**. In the case of **P1** and **P2**, the ions at *m/z* 931.4 and 917.4 by the y_7 cleavage were observed in the MS/MS spectra of $[\mathbf{P1}+H]^+$ and $[\mathbf{P2}+H]^+$ with a relative abundance of 100%, respectively. In contrast, the ions corresponding to the y_7 cleavage of **P4**–**P9** were barely observable. When the Asp was changed to Asn in the case of **P3**, the y_7 ion at *m/z* 917.4 was shown with lower abundance of 12%. These results suggest that the dissociation of the amide bond between Asn and Arg is easier than that of Sar-Arg, but more difficult than that of Asp-Arg. The MS/MS spectrum of $[\mathbf{P1}+H]^+$ with the collision energy of 100% is shown as an example in Fig. 2.

Table 1
MS/MS mass spectral data of $[\mathbf{P}n+H]^+$ of Angiotensin II and its analogues

Peptide **P*n***	Parent ion (*m/z*)	Main fragment ions [*m/z* (%)]							
		Base peak	$[\mathbf{P}\text{-}NH_3+H]^+$	$[\mathbf{P}\text{-}H_2O+H]^+$	b_7+18	b_6	b_5	b_4	$b_3\text{-}NH_3$
P1	1046.6	931.4 (y_7)	1029.3 (18)	1028.4 (25)	899.3 (16)	784.3 (21)	647.2 (7)	N.D.[a]	N.D.[a]
P2	1032.7	917.4 (y_7)	1015.3 (18)	1014.5 (23)	885.4 (13)	770.2 (21)	633.2 (7)	N.D.[a]	N.D.[a]
P3	1031.7	752.3 ($b_6\text{-}NH_3$)	1014.4 (14)	N.D.[a]	884.4 (67)	769.3 (79)	632.3 (33)	533.2 (13)	353.1 (9)
P4	1002.5	740.2 (b_6)	985.3 (37)	984.4 (16)	855.3 (93)	Base peak	603.2 (27)	490.2 (16)	310.0 (8)
P5	926.6	740.3 (b_6)	909.3 (41)	908.3 (13)	855.4 (77)	Base peak	630.2 (28)	490.1 (14)	310.0 (10)
P6	912.5	723.2 ($b_6\text{-}NH_3$)	895.2 (59)	894.3 (29)	855.1 (9)	740.3 (72)	603.2 (28)	490.1 (14)	310.1 (8)
P7	968.5	855.3 (b_7+18)	951.2 (28)	N.D.[a]	Base peak	740.3 (58)	603.2 (14)	490.2 (6)	N.D.[a]
P8	956.7	894.4 ($-C_2H_6O_2$)	939.3 (17)	938.4 (45)	855.4 (35)	740.3 (59)	603.2 (20)	490.2 (8)	N.D.[a]
P9	912.5	709.3 ($b_6\text{-}NH_3$)	895.3 (40)	894.4 (21)	841.3 (77)	726.3 (91)	589.2 (26)	490.1 (8)	310.0 (7)

[a] Not detectable.



Scheme 2. Main fragmentation modes of octapeptides.

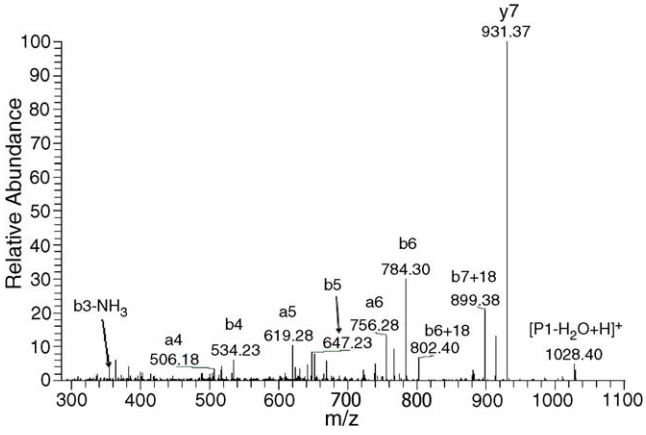
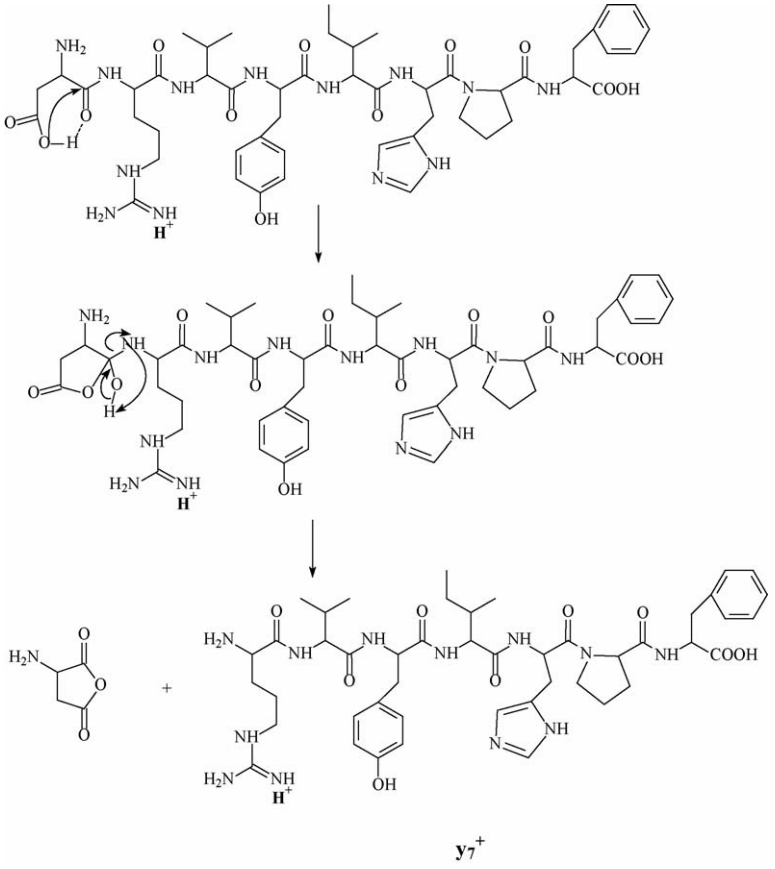


Fig. 2. MS/MS spectrum of $[\mathbf{P1}+H]^+$.

In this peptide, the most basic site is $-NH_2$ group at the side chain of arginine, which tightly binds a proton, and the acidic H of aspartic acid is the 'reactive' proton that initiates cleavage as shown in Scheme 3. In this case, charge-remote fragmentation pathways usually dominate in the low-energy CID process and the backbone cleavage between Asp and Arg residues at C-terminal side of acidic residues can take place, and yield y_7 ion [7,23–26].

As for **P1** and **P2**, the b_7+18 ion is generated through the C-terminal rearrangement process from $[\mathbf{P}n+H]^+$ with lower abundance, and **P6** was especially difficult to generate this ion (relative abundance is only 9%). In contrast, the significant ions of b_7+18 at *m/z* 884.4, 855.3, 855.4, 855.3, 855.4 and 841.3 were observed in the MS/MS spectra of $[\mathbf{P}n+H]^+$ of **P3**–**P5**, **P7**–**P9**, respectively. Especially, the b_7+18 ion of **P7** has a highest abundance (*m/z* 855.3, 100%). The b_7+18 ion in Fig. 3 is generated through the C-terminal rearrangement process from



Scheme 3. Main fragmentation pathway for the MS/MS spectrum of $[\mathbf{P1}+H]^+$.

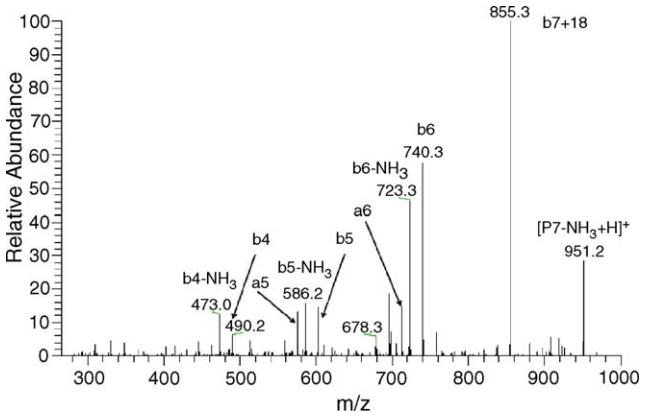
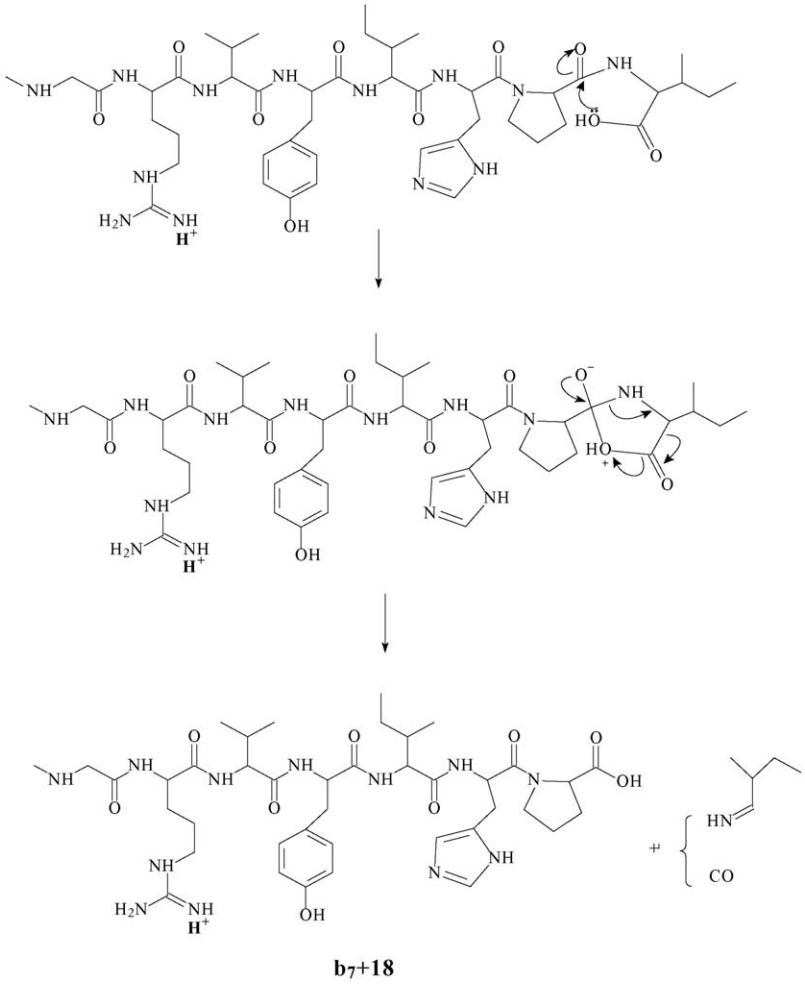


Fig. 3. The MS/MS spectrum of $[\mathbf{P7}+H]^+$.

$[\mathbf{P7}+H]^+$ (Scheme 4). This process begins with the oxygen atom of carboxylic acid at C-terminal attacking the carbonyl carbon of Pro. The cyclic structure of Pro on the backbone of the peptide increases the rigidity of the C-terminal backbone, which makes the carbon atom more susceptible to nucleophilic attack [3,27–31]. After a rearrangement of cyclic structure, the b_7+18 ion is generated with the transfer of –OH group to the Pro carbonyl carbon [32–41].

The fragmentation with the loss of water and ammonia were observed in the MS/MS spectra. The loss of water is usually from side chains of Asp, Glu, Ser and Thr residues as well as the carboxyl group of C-terminal [10]. The loss of ammonia is usually from side chains of Lys, Asn, Gln and Arg residues as well as the amine of N-terminal from these peptides. In the case of **P1** and **P2**, the fragment ions at *m/z* 1029.3 and 1015.3 were generated by the loss of NH_3 from the side chain of arginine and the N-terminal amine, respectively. For **P4**–**P9**, the loss of NH_3 is from the side chain of Arg. In the case of **P3**, $[\mathbf{P3}\text{-}H_2O+H]^+$ could not be detectable in the MS/MS spectrum, while the relative abundance of $[\mathbf{P3}\text{-}NH_3+H]^+$ is 14% (Table 1). In the MS/MS spectrum of **P8** (Fig. 4), the significant product ion at *m/z* 894.4 with the highest intensity was probably produced from a process combined with the loss of H_2O and acetaldehyde from the C-terminal carboxylic acid (Scheme 5), which should be proved further by ion at *m/z* 938.4 ($[\mathbf{P8}\text{-}H_2O+H]^+$).

In the case of **P3**–**P6** and **P9**, the proline cycle in the backbone goes against the stabilization of the amide bond of Pro, so b_6 ions had remarkable intensity. Especially for **P4** and **P5**, the b_6



Scheme 4. Main fragmentation pathway of $[\mathbf{P7}+H]^+$.

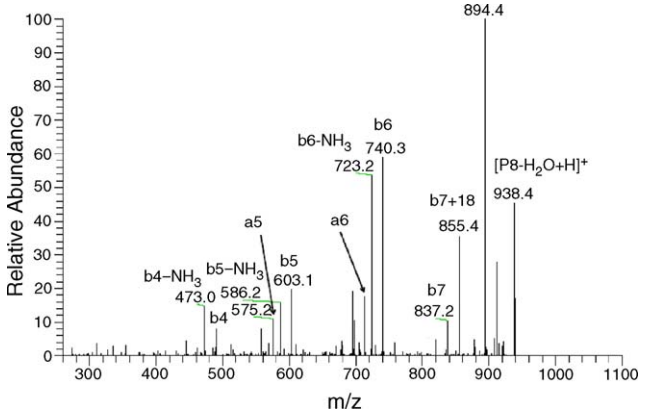


Fig. 4. The MS/MS spectrum of $[\mathbf{P8}+H]^+$.

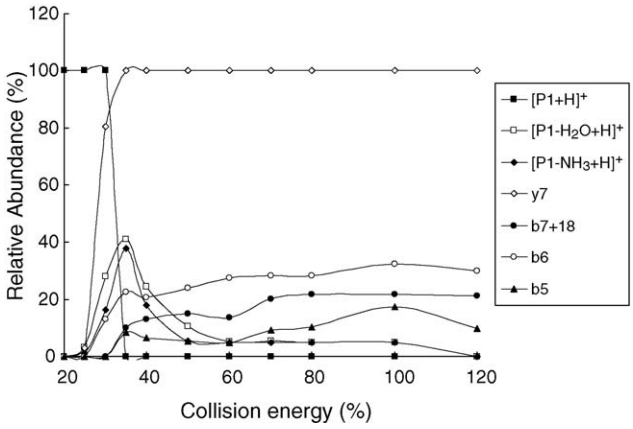
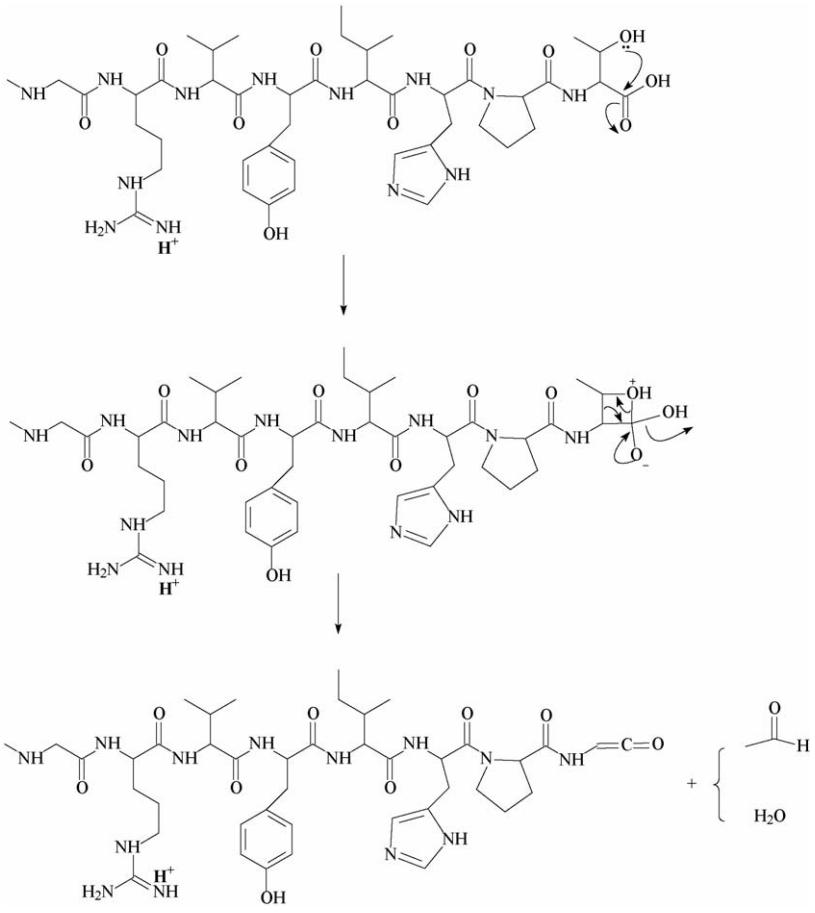


Fig. 5. The effect of collision energy on the relative intensities of fragment ions.

ions at *m/z* 740.2 and 740.3 had the highest intensity (100%), respectively. Besides, the b_6-NH_3 ions at *m/z* 752.3, 723.2 and 709.3 had the highest abundance (100%) in MS/MS spectra of **P3**, **P6** and **P9**, respectively, which might be produced by the cleavage of His-Pro amide bond and the loss of NH_3 from the side chain of arginine simultaneously.

3.3. The effect of the collision energy on fragment ions

In the MS/MS analysis, first fragment ion of $[\mathbf{P1}+H]^+$ was observed at *m/z* 931.4 by an y_7 cleavage using 25% collision energy. Other fragment ions of $[\mathbf{P1}+H]^+$ appeared by different styles of cleavage when the collision energy was increased. It was found that the main fragmentations produce y_7 ion, $[\mathbf{P1}\text{-}H_2O+H]^+$, $[\mathbf{P1}\text{-}NH_3+H]^+$, b_6 ion, b_7+18 ion and b_5 ion at *m/z* 931.4, 1028.4, 1029.4, 784.3, 899.2 and 647.2, respectively. However, y_7 fragment ion always has the highest intensity in the MS/MS spectrum at different collision energy due to Arg residue has high basicity in gas-phase. Fig. 5 demonstrates the effect of collision energy on the relative intensities of fragment ions of **P1**. When the energy was increased from 0 to 30%, the relative inten-



Scheme 5. Main fragmentation pathway of $[\mathbf{P8}+H]^+$.

Table 2
Sequence information from ions of **P4**

m/z	Precursor ion	Amino acid (residue mass)
1002.2	$[\mathbf{P4}+H]^+$	-Phe + H_2O (147)
855.3	b_7+18	-Pro, H_2O (115)
740.3	b_6	-His (137)
603.2	b_5	-Ile (113)
490.1	b_4	-Tyr, NH_3 (180)
310.0	b_3-NH_3	

Table 3
Sequence information of fragment ions of **P6**

m/z	Precursor ion	Amino acid (residue mass)
912.5	$[\mathbf{P6}+H]^+$	-Gly + H_2O (57)
855.3	b_7+18	-Pro, H_2O (115)
740.3	b_6	-His (137)
603.2	b_5	-Ile (113)
490.1	b_4	-Tyr, NH_3 (180)
310.1	b_3-NH_3	

Table 4
Sequence information of fragment ions of **P9**

m/z	Precursor ion	Amino acid (residue mass)
912.5	$[\mathbf{P9}+H]^+$	-Ala + H_2O (71)
841.3	b_7+18	-Pro, H_2O (115)
726.2	b_6	-His (137)
589.2	b_5	-Val (99)
490.1	b_4	-Tyr, NH_3 (180)
310.0	b_3-NH_3	

sity of $[\mathbf{P1}+H]^+$ significantly decreased from 100% to 0. The relative intensity of y_7 significantly increased and then maintained to 100% even when the energy was increased to 120%. However, the relative intensity of other fragment ions gradually increased to about 20%, such as b_7+18 and b_6. The relative intensities of $[\mathbf{P1}\text{-}H_2O+H]^+$ and $[\mathbf{P1}\text{-}NH_3+H]^+$ increased to about 40% with the energy of 35%, then these intensities decreased to quite low along with the collision energy, and which were almost constant at above 60% energy.

The low collision energy is favorable for the loss of water and ammonia from parent ion, but the high collision energy leads to the cleavage of CO–NH bonds. According to the order of intensities of fragment ions from the cleavage of CO–NH bonds (Asp-Arg > His-Pro > Ile-His), we could conclude that some side chains of residues catalyze fragmentations easier than others since the amide bond strength at different sites is probably quite similar. For other analogues, the influence of collision energy on fragment ions is similar with that of **P1**.

*3.4. Sequence information and distinguishing **P6** and **P9** from the MS/MS spectra*

The sequence information of Angiotensin **II** and its analogues could be obtained from the MS/MS and MS^3 spectra of $[\mathbf{P}n+H]^+$. For example, in the case of **P4**, a series of b_n ions (*m/z* 740.2, 603.2 and 490.1), b_7+18 ion (*m/z* 855.3) and b_3-NH_3 ion (*m/z* 310.0) were yielded in the MS/MS spectrum. Table 2 shows these sequence information of **P4**. The mass difference between b_n and b_{n-1} ions equals the residue mass of an amino acid, respectively. Starting from b_3-NH_3, the whole peptide can be reconstructed by these sequence information (b_n ions, b_7+18 ion, b_3-NH_3 ion ...). The remaining sequence information of the three amino acids near C-terminus could be obtained by the MS^3 spectrum of b_3-NH_3 ion. The sequences of other analogues could be determined also by this method.

P6 and **P9** ($[\mathbf{P}n+H]^+$, *m/z* 912.5, $n=6$, 9) are two octapeptides with the same mass, but with different structure. In the MS/MS spectra with the same collision energy of 40%, there is the significant b_7+18 ion (77%) at *m/z* 841.3 for **P9**, while the b_7+18 ion has rather low intensity of 9% for **P6**. As mentioned above, the b_7+18 ion is generated through the C-terminal rearrangement process, which begins with the oxygen atom of carboxylic acid at C-terminal attacking the carbonyl carbon of Pro. Other than **P9**, **P6** is especially difficult to generate this kind of ion. The Gly residue without the side chain at C-terminal has an effect on the rigidity of the C-terminal backbone, which is not favorable for the nucleophilic attack to carbon atom. In addition, the *m/z* of b_n ($n=5$–7) ions are different and the value of b_n–b_{n-1} equals that of the residue mass of the amino acid, respectively. The two peptides could be distinguished based on the sequence information obtained from the MS/MS spectra of $[\mathbf{P}n+H]^+$ in Tables 3 and 4.

4. Conclusions

The MS/MS spectra of Angiotensin II and its analogues showed characteristic fragmentation pathways by the cleavage of C–CO and CO–NH bonds with the loss of water, ammonia or carbon monoxide and H rearrangement(s). Sequence information of Angiotensin II and its analogues could be obtained from the low-energy CID mass spectra. In addition, the two analogues with the same mass and different elemental composition were distinguished by the MS/MS spectra. These results show that ESI tandem mass spectrometry is an excellent tool for the structural identification of Angiotensin II and its analogues.

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