Chinese Journal of Chemistry, **2004**, *22*, 477—481

Article

Novel Rearrangement of Small Peptides in Electrospray Ionization Tandem Mass Spectrometry

CHEN, Yi[a,b](陈益) WANG, Jin[c](王津) LIU, Xiao-Hong[b](刘晓红) SUN, Ming[b](孙命)
CHEN, Jing[a](陈晶) JIANG, Yang[b](江洋) ZHAO, Yu-Fen*[,a,b](赵玉芬)

[a] *Key Laboratory of Chemical Biology, Department of Chemistry, Zhengzhou University, Zhengzhou, Henan 450052, China*

[b] *Key Laboratory for Bioorganic Phosphorus Chemistry, Ministry of Education, Department of Chemistry, School of Life Sciences and Engineering, Tsinghua University, Beijing 100084, China*

[c] *Department of Chemistry, Peking University, Beijing 100871, China*

Electrospray ionization mass spectrometry (ESI-MS) is a powerful method for sequencing peptides. A novel fragmentation pattern with the loss of a neutral fragment of 45 Da was observed with the dipeptides, tripeptides, tetrapeptides and pentapeptides containing phenylalanine or histidine residues. A novel rearrangement reaction with the extrusion of a formamide piece was studied and the rearrangement mechanism was proposed and confirmed by deuterium labeling experiments with ESI-MS^n and high-resolution mass spectrometry. These findings are potentially helpful in identifying the specific sequence pattern in the peptide sequencing.

Keywords rearrangement, small peptides, electrospray ionization tandem mass spectrometry

Introduction

Electrospray ionization mass spectrometry (ESI-MS) has been widely used in sequencing peptides. Various methods, including change of activation methods,[1,2] chemical derivatization[3,4] and isotopic labeling,[5] have been used to simplify the spectra and facilitate the interpretation. Although most of the fragment ions can be well interpreted and named according to the system of Roepstorff and Fohlman,[6] the discovery of new fragmentation pattern will be greatly helpful for the identification of complicated sequences.

In our studies on the small peptides with ESI tandem mass spectrometry (ESI-MS^n), a novel fragmentation pattern was observed with the loss of a neutral 45 Da molecule. Herein this novel fragmentation is reported and the mechanism is proposed and studied with deuterium labeling experiments, and confirmed by high-resolution mass spectrometry and *ab initio* theoretical calculations.

Materials and methods

All small peptides consist of *L*-amino acids and purchased from Sigma (St. Louis, MO, USA) and used without any further purification. Mass spectra were acquired using a Bruker ESQUIRE-LC^{TM} ion trap spectrometer (Bruker Daltonics Inc. Billerica, MA, USA) equipped with a gas nebulizer probe, capable of analyzing ions up to *m/z* 6000. Nitrogen was used as drying gas with a flow rate of 4 L/min and the nebulizer pressure is 7 psi. Capillary was typically held at 4 kV. The samples dissolved in methanol were ionized by electrospray ionization and continuously infused into the ESI chamber at a flow rate of 4 μL/min by a Cole-Parmer 74900 syringe pump (Cole-Parmer Instrument Company, Vernon Hills, IL, USA). The $[M+H]^+$ ions were analyzed by multistage ESI-MS^n through collisions with helium and a high-resolution mass spectrum of Phe-Phe was obtained using a Bruker APEX II FT-ICRMS instrument equipped with an ESI source.

Results and discussion

As a representative of the novel fragmentation pattern, the ESI tandem mass spectra of Ser-Phe are shown in Figure 1A, 1B and 1C. A characteristic loss of 45 Da was observed for the fragment ion at *m/z* 207 to give the ion at *m/z* 162. The odd number of 45 Da indicated that the lost piece contains one nitrogen atom and that it has a molecular formula of CH_3NO with the confirmation of high-resolution mass. This fragmentation pattern was also observed for 8 out of 17 peptides we studied (Table 1). A common feature for those peptides with the characteristic loss of 45 Da is the existence of a Phe or His residue next to the N-terminal residue. For example, peptides Phe-Phe-Phe and Ala-His showed the loss of

* E-mail: yinyw@mail.tsinghua.edu.cn; Fax: 86-10-62781695
Received July 7, 2003; revised and accepted January 1, 2004.
Project supported by the Chinese National Natural Science Foundation (No. 20175026), the Ministry of Science and Technology, the visiting scholar fund of the Chinese Education Ministry, and Tsinghua University.

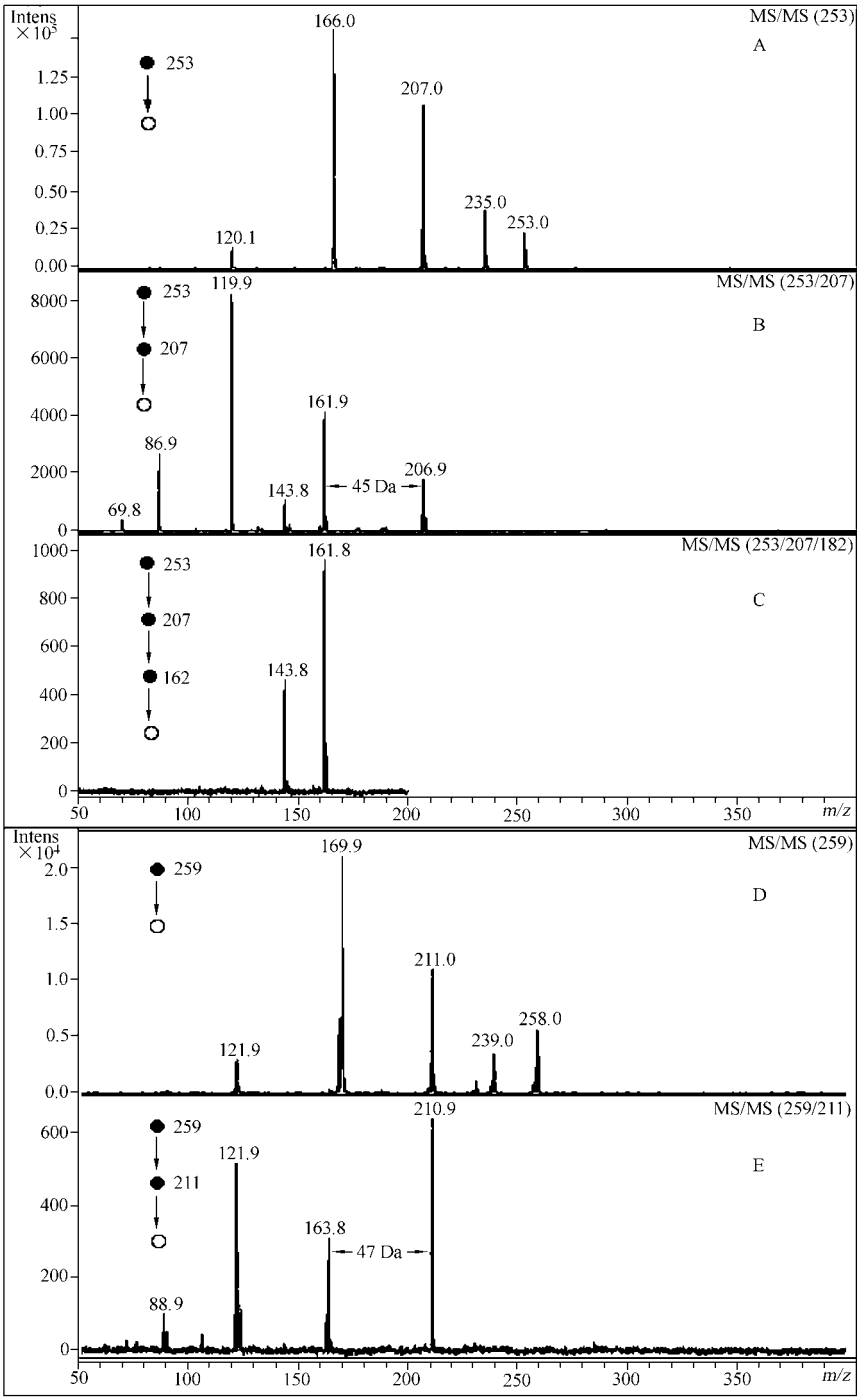


Figure 1 Fragmentation of seryl-phenylalanine in ESI-MS^n. A, B, C, native Ser-Phe, D, E, deuterium labeled Ser-Phe.

45 Da in their ESI-MS^n, respectively (Figure 2). Instead, those peptides without Phe or His residue next to the N-terminal did not provide the characteristic fragmentation pattern. Exceptions are observed for the dipeptides Gly-Phe and Leu-Phe; they both have a Phe residue but did not give the 45 Da loss in their ESI-MS^n.

Based on the structure of peptides, we proposed a novel rearrangement reaction responsible for the loss of the 45 Da (Scheme 1). As illustrated with Ser-Phe, the loss of a formic acid molecule from the C-terminus provided the fragment ion **I** at *m/z* 207, in which the activated phenyl ethyl group was attacked by the N-terminal amino group[8,9] through a five-membered ring intermediate **II** and with a migration of one of the two hydrogen atoms of the N-terminal amino group to give the rearranged ion **III**. Subsequently, a hydrogen atom is migrated from the N-terminal amino group to the carbonyl group, via a four-membered ring transition state, leading to the elimination of a 45 Da amide moiety (CH_3NO) and the fragment ion **IV** at *m/z* 162. An elimination of H_2O from the rearrangement product at *m/z* 162 supports the existence of a hydroxyl group in the fragment at *m/z* 162.

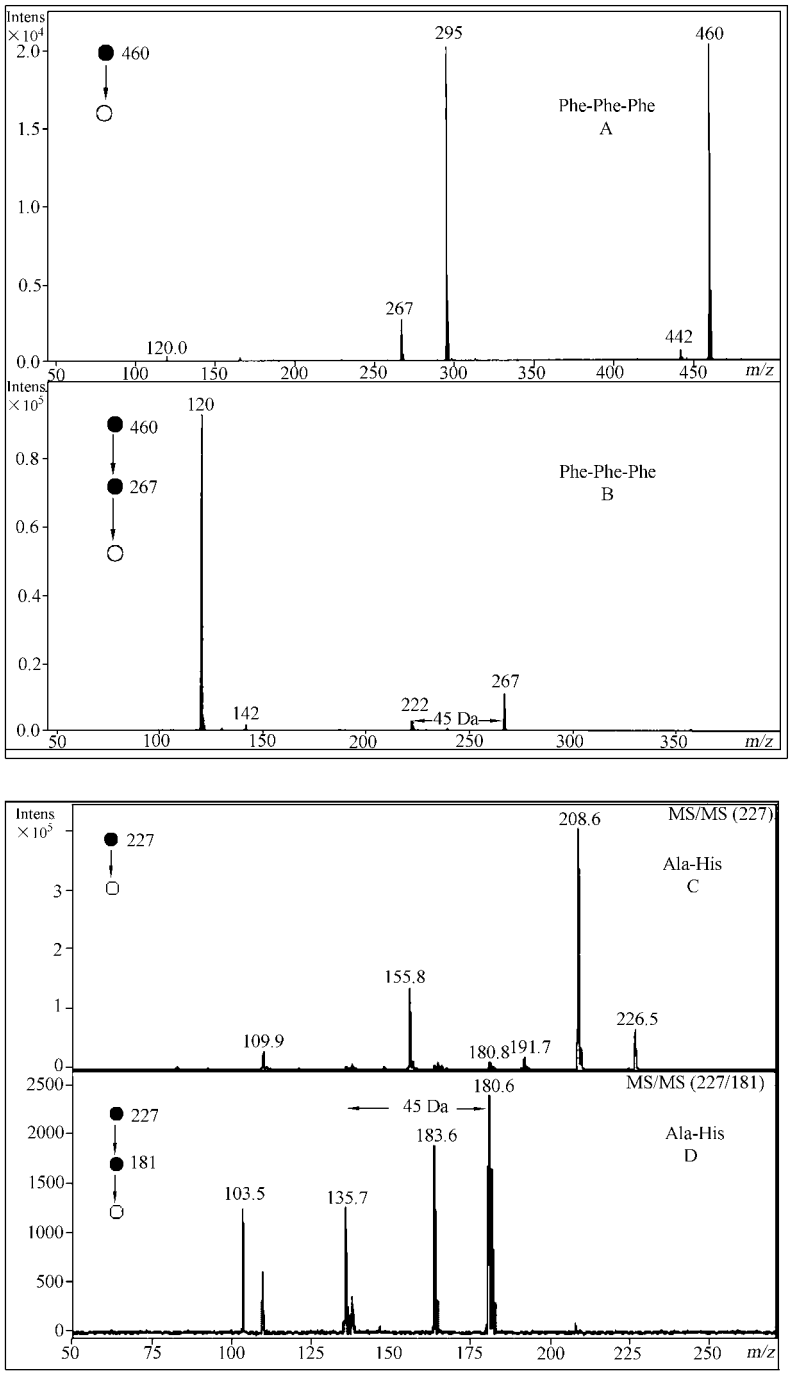


Figure 2 Fragmentation of Phe-Phe-Phe (A, B) and Ala-His (C, D) in ESI-MS^n.

To identify the elemental composition of these fragment ions, Phe-Phe high-resolution mass spectrometry (FTICR) was used. The exact mass of [Phe-Phe − HCOOH]$^+$ was 267.1491793 Da, corresponding to $C_{17}H_9N_2O_1^+$, and the exact mass of the fragment ion [Phe-Phe − HCOOH − 45]$^+$ 222.1277182 Da, corresponding to $C_{16}H_{16}N^+$. By difference, it was concluded that a neutral fragment with atomic composition (CH_3NO) was expelled.

In order to elucidate which hydrogen atoms are involved in the rearrangements, all active hydrogen atoms of Ser-Phe were exchanged with deuterium and the labeled Ser-Phe was studied by ESI-MS^n (Figures 1D, 1E). An extrusion of a neutral molecular of 47 Da was observed from the fragment ion at *m/z* 259, indicating that only two active hydrogen atoms were included in the lost molecule. Since one active hydrogen atom comes from the amide group during the first rearrangement while another hydrogen atom is from the amide bond, the last hydrogen should mainly come from the serine residue side-chain, instead of from the amide group.

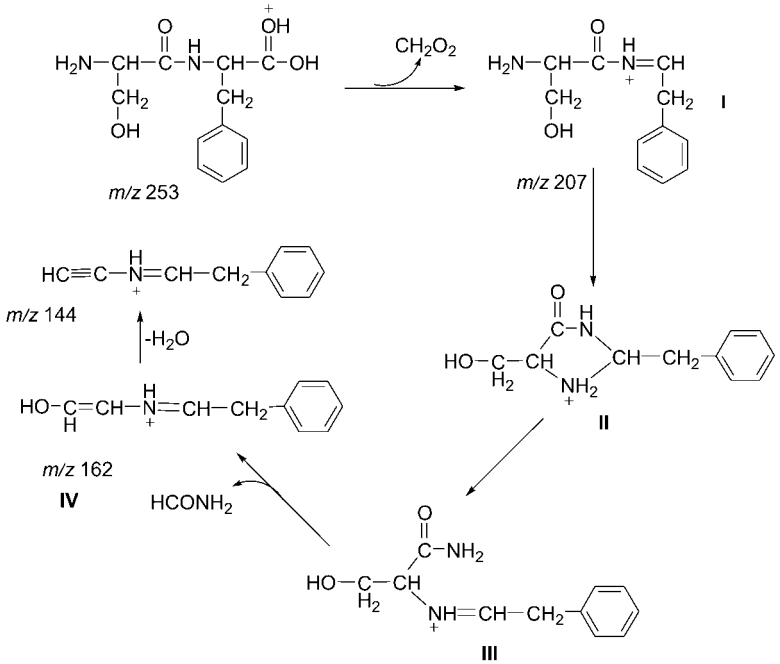
The extrusion of a 45 Da neutral molecular observed for the first eight peptides in Table 1 is consistent with

Table 1 Fragmentation of dipeptides, tripeptides and tetrapeptide in ESI-MS$^{n\,a,b}$

Peptide Examined	$[M+H]^+$	$[M-HCOOH+H]^+$ or $[M-residue-HCOOH+H]^+$	$[M-HCOOH-45+H]^+$ or $[M-residue-HCOOH-45+H]^+$	Phe or His
Ser-Phe	MS^2 253 (24)	MS^2 207 (69); MS^3 (22)	MS^3 162 (50)	Phe
Ala-His	MS^2 227 (16)	MS^2 181 (3); MS^3 181 (100)	MS^3 136 (53)	His
Ser-His	MS 243 (72)	MS 197 (21)	MS^2 152 (24)	His
Phe-Phe	MS^2 313 (48)	MS^2 267 (11); MS^3 (67)	MS^3 222 (3)	Phe
Phe-Phe-Phe	MS^2 460 (100)	MS^2 267 (14) MS^3 267 (13)	MS^3 222 (3)	Phe
Phe-Phe-Phe-Phe	MS^2 607 (44)	MS^2 267 (12) MS^3 267 (15)	MS^3 222 (6)	Phe
Phe-Phe-Gly-Leu-Met	MS^2 613(0)	MS^2 267 (7) MS^3 267 (5)	MS^3 222 (2)	Phe
Phe-Tyr-Gly-Pro-Val	MS^2 581(0)	MS^2 283 (6) MS^3 283 (18)	MS^3 238 (12)	Tyr
*Gly-Phe	MS^2 223 (25)	MS^2 177 (100)	—	Phe
*Leu-Phe	MS^2 279 (28)	MS^2 233 (15)	—	Phe
Ser-Ala	MS^2 177 (92)	MS^2 131 (14)	—	no
Leu-Val	MS^2 231 (46)	MS^2 185 (37)	—	no
Ala-Ala	MS^2 161 (100)	MS^2 115 (11)	—	no
Ala-Ala-Ala	MS^2 232 (73)	MS^2 115 (7)	—	no
Glu-Cys-Gly	MS^2 308 (100)	MS^2 205 (17)	—	no
Leu-Ala-Phe	MS^2 350 (44)	MS^2 157 (18)	—	no
Arg-Val-Tyr-Ile-His-Pro-Ile	MS^2 898 (45)	MS^2 283 (0)	—	no

[a] Abbreviations used: MS^2: MS/MS; MS^3: MS/MS/MS; [b] Numbers in parentheses indicate the relative intensity; * The fragment ion $[M-HCOOH-45+H]^+$was not observed .

Scheme 1 Proposed rearrangement mechanism for Ser-Phe to form $[F-45]^+$ in ESI-MS^n



our proposed mechanism. However, the requirement for a Phe or His residue at the second position to the N-terminal indicated a specific role of these residues /or side chains (*e.g.* phenyl group for Ser-Phe*, Phe-Phe*-Phe and Phe-Phe*-Phe-Phe, Phe-Phe*-Gly-Leu-Met, Phe-Tyr*-Gly-Pro-Val; imidazole group for Ala-His*) on this kind of rearrangement, which might serve to stabilize the intermediate. It is surprising that the fragment ion $[M-HCOOH-45+H]^+$ was not observed for dipeptides Leu-Phe and Gly-Phe although they both have a Phe residue at the right position. This can be explained that Glycine has no side chain to provide the addition hydrogen for the fragmentation, and the steric hindrance of the isopropyl group in Leucine might make the rearrangement difficult to proceed.

Theoretical calculation at the B3LYP/6-31G* level[10] provided us further insight into the rearrangement mechanism. It is found that the peptide fragment ion $[M-HCOOH]^+$ with phenyl group or imidazole group at C-terminus (*e.g.* Ser-Phe or Ala-His) is energetically favorable than those without phenyl group or imidazole group (*e.g.* Ala-Ala or Ser-Ala) (Table 2). The phenyl or imidazole group could possible provide additional stability to the positive ion and make it more likely undergo rearrangement, which partially explains why they are essential for this type of rearrangement.

Table 2 Calculation results of fragment ion $[M-HCOOH+H]^+$ generated by some dipeptides by Gaussian98 B3LYP/6-31G

Dipeptide used	ΔE/a.u.	Relative stability
Ser-Phe	−4.79845	Ser-Phe > Ser-Ala
Ser-Ala	−2.82194	
Ala-His	−3.99377	Ala-His > Ala-Ala
Ala-Ala	−2.66617	

In conclusion, we have observed a characteristic fragmentation pattern with the extrusion of a formamide molecule for peptides with a Phe or His residue next to the N-terminal residue. Such a fragmentation was proposed to go through a novel sequential rearrangement and was supported by our studies with deuterium labeling experiments and high-resolution mass spectrometry and theoretical calculation. This result is very helpful in identifying peptides with a Phe or His residue at the second position to the N-terminus; the fragment ion $[M-HCOOH-45]^+$ or $[M-residue-HCOOH-45]^+$ can be indicative of such Phe or His residues and therefore is of potential use in peptide sequencing.

References

1 Johnson, R. S.; Martin, A.; Biemann, K. *Int. J. Mass Spectrom. Ion Processes* **1998**, *86*,137.

2 Reiber, D. C.; Grover, T. A.; Brown, R. S. *Anal. Chem.* **1998**, *70*, 673.

3 James, E. V.; Biemann, K. *Int. J. Mass Spectrom. Ion Processes* **1990**, *100*, 287.

4 Lindh, I.; Sjövall, J.; Bergman, T.; Griffiths, W. J. *J. Mass Spectrom.* **1998**, *33*, 988.

5 Chen, X. H.; Anderson, V. E.; Chen, Y. H. *J. Am. Soc. Mass Spectrom.* **1999**, *5*, 448.

6 Roepstorff, P.; Fohlman, J. *Biomed. Mass Spectrom.* **1984**, *11*, 601.

7 Chen, J.; Jiang, Y.; Fu, H.; Chen, Y.; Cheng, C. M.; Zhao, Y. F. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 1489.

8 Dua, S.; Chia, C. S. B.; Bowie, J. H. *Int. J. Mass Spectrom.* **1996**, *61*, 179/180.

9 Chia, C. S. B.; Taylor, M. S.; Dua, S.; Blanksby, S. J.; Bowie, J. H. *J. Chem. Soc., Perkin Trans.* **1998**, *2*, 1435.

10 Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M.A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, Jr., V. G.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J.V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, H.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, Revision A.9, Gaussian, Inc., Pittsburgh PA, **1998**.

(E0307077 ZHAO, X. J.)