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International Journal of Mass Spectrometry 272 (2008) 204–207

International Journal of Mass Spectrometry

www.elsevier.com/locate/ijms

Short communication

A carbonyl oxygen migration of *N*-phosphoryl dipeptide methyl esters containing β-alanine or γ-amino butyric acid in electrospray ionization mass spectrometry

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Received 6 December 2007; received in revised form 29 January 2008; accepted 29 January 2008
Available online 9 February 2008

Abstract

The electrospray ionization tandem mass spectra (ESI-MS^n) of the sodium adducts of *N*-diisopropyloxyphosphoryl (DIPP) dipeptide methyl esters containing unnatural amino acids, such as DIPP-β-Ala-Phe-OMe and DIPP-γ-amino butyric acid-Phe-OMe, were studied. An unusual rearrangement reaction with a carbonyl oxygen migration through a six- or seven-membered ring tetracoordinate phosphorus transition state was observed. In addition, ESI-MS^n of DIPP-L/D-α-Ala-Phe-OMe was also investigated. The transition state of their rearrangement was a five-membered ring pentacoordinate phosphorus transition state. It was the spatial configuration of phosphorus atom and the strong affinity between phosphorus and oxygen atoms that induced the carbonyl oxygen migration through different cyclic transition state. The results imply that the carbonyl oxygen migration in the ESI-MS/MS of dipeptide methyl esters was not dependent on the chain length of the amino acid at the N-terminus.

Keywords: ESI-MS/MS; *N*-Phosphoryl dipeptide methyl ester; Rearrangement reaction; Tetracoordinate phosphorus species; Pentacoordinate phosphorus species

1. Introduction

It is worth noting that phosphorus plays an important role in the chemistry of life [1]. Increasing work has been devoted to the study of the intrinsic chemical relationships between phosphorus and many kinds of life actions. Although most studies were carried out in the liquid phase, the study under gas phase condition is a more directive and effective method. Mass spectrometry offers a suitable tool for such investigations. Since the introduction by Yamashita and Fenn in 1984 [2], electrospray ionization mass spectrometry (ESI-MS) has been widely used in chemical and biological research, such as the sequencing of peptides [3] and proteomics [4], the investigation of reaction mechanism [5], the detection of non-covalent complexes [6], chiral recognition [7], etc. The reaction of phosphoryl derivatives in the gas phase could provide some basic information about the nature of phosphorylation. In our previous work, phosphorylated amino acids and peptides have been analyzed by several kinds of mass spectrometry [8–10]. A rearrangement reaction with a carbonyl oxygen migration was observed in the electrospray ionization tandem mass spectra of *N*-diisopropyloxyphosphoryl (DIPP) dipeptides and analogues, but not in the mass spectra of β-alanyl dipeptides [11]. In this paper, an unusual rearrangement reaction of the sodium adducts of *N*-diisopropyloxyphosphoryl dipeptide methyl esters containing unnatural amino acids, such as DIPP-β-Ala-Phe-OMe and DIPP-γ-amino butyric acid (γ-Aba)-Phe-OMe, was reported. In addition, the ESI-MS/MS of DIPP-D-α-Ala-Phe-OMe was also investigated.

2. Experimental

All phosphoryl dipeptide methyl esters were synthesized by literature methods [12]. Chemicals for syntheses were purchased from Sigma or Baitai Biochemical Co. (Shanghai, China). All their structures were confirmed by ^{31}P NMR, ^{1}H NMR and ESI-MS.

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doi:10.1016/j.ijms.2008.01.008

Table 1
ESI-MS/MS of $[M+Na]^+$ ions of different dipeptide methyl esters [*m/z* (relative abundance, %), $m = M+23$]

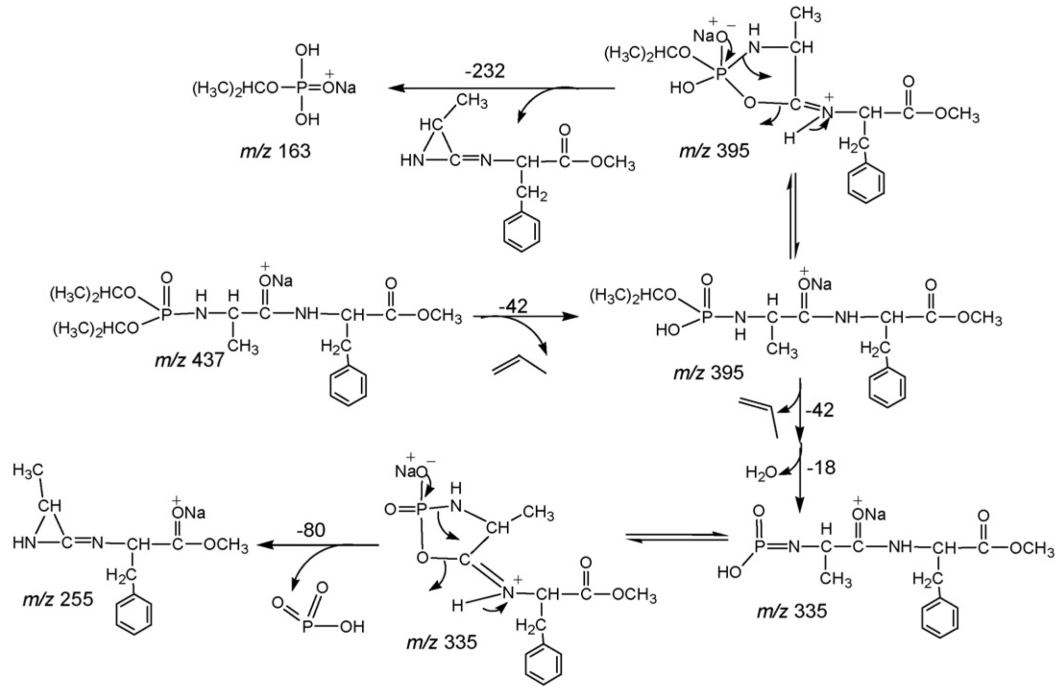
No.	Compound	Precursor ions	$[m-42]^+$	$[m-84]^+$	$[m-102]^+$	$[m-164]^+$	Rearrangement product
1	DIPP-L-α-Ala-Phe-OMe	437(MS^2)	395(43)	353(6)	335(25)	273(100)	163(11)
		395(MS^3)		353(11)	335(35)	273(100)	163(13)
		335(MS^3)					255(10)
2	DIPP-D-α-Ala-Phe-OMe	437(MS^2)	395(56)	353(4)	335(41)	273(100)	163(14)
		395(MS^3)		353(6)	335(49)	273(100)	163(10)
		335(MS^3)					255(9)
3	DIPP-β-Ala-Phe-OMe (in CH_3OD)	437(MS^2)	395(77)	353(8)	335(20)	273(100)	255(17)
		439(MS^2)	397(40)	355(6)	336(16)	275(100)	256(16)
		395(MS^3)		353(13)	335(29)	273(100)	255(16)
		353(MS^3)			335(53)	273(100)	255(37)
		335(MS^3)					255(100)
4	DIPP-γ-Aba-Phe-OMe (in CH_3OD)	451(MS^2)	409(23)	367(2)	349(2)	287(41)	269(100)
		453(MS^2)	411(46)	369(6)	350(2)	289(74)	270(100)
		409(MS^3)		367(2)	349(5)	287(23)	269(100)
		349(MS^3)					269(100)

High resolution mass spectra were obtained with an ESI-Q-TOF-MS spectrometer (Micromass, England). Other mass spectra were acquired using an Esquire 3000 ESI-MS with an ion trap mass spectrometer (Bruker Daltonik Gmbh, Germany). The MS^n spectra were obtained by collision-induced dissociation (CID) with helium after isolation of the appropriate precursor ions. Ionization was carried out using the following setting of the ESI: nebulizer gas flow 7 psi, dry gas 4.5 L/min, dry temperature 300 °C, spray voltage 4000 V. The samples dissolved in methanol were continuously pumped into the ESI source at a flow-rate of 4 μL/min with a Cole-Parmer 74900 syringe pump. Scan range was 15–500 *m/z* and scan resolution was normal (13,000 *m/z*/s).

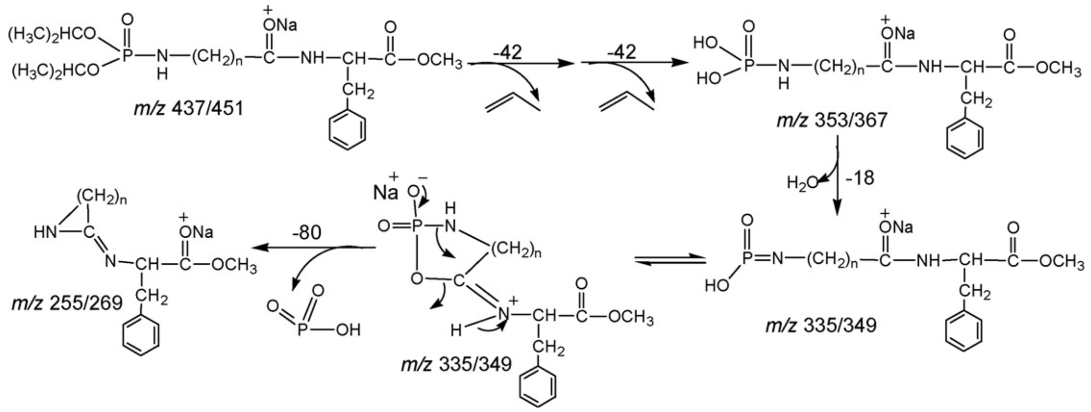
3. Results and discussion

The main ESI-MS/MS of $[M+Na]^+$ of DIPP-D-α-Ala-Phe-OMe are the same as those of DIPP-L-α-Ala-Phe-OMe (Table 1) and consistent with the literature results [11]. They both have a characteristic ion at *m/z* 163, which was generated through a five-membered ring pentacoordinate phosphorus transition state formed by a carbonyl oxygen migration (Scheme 1).

An unusual fragment at *m/z* 255 appeared in the spectra of $[M+Na]^+$ of DIPP-β-Ala-Phe-OMe (Table 1). Interestingly, another relevant fragment at *m/z* 269 was also observed in the spectra of $[M+Na]^+$ of DIPP-γ-Aba-Phe-OMe, and it was even the base peak of the spectra (Table 1). In the ESI-MS^3



Scheme 1. Possible rearrangement mechanism for DIPP-L/D-α-Ala-Phe-OMe.



Scheme 2. Possible rearrangement mechanism for DIPP-β-Ala-Phe-OMe and DIPP-γ-Aba-Phe-OMe. $n = 2$ for DIPP-β-Ala-Phe-OMe; $n = 3$ for DIPP-γ-Aba-Phe-OMe.

of $[M+Na]^+$ of DIPP-L/D-α-Ala-Phe-OMe, there was also a weak peak at *m*/*z* 255 (Table 1). Based on the ESI-MS^3 study of $[M+Na]^+$ of DIPP-β-Ala-Phe-OMe and DIPP-γ-Aba-Phe-OMe, it is presumed that the ions at *m*/*z* 255 and 269 were possibly derived from the fragments at *m*/*z* 335 and 349, respectively, through a six- or seven-membered ring tetracoordinate phosphorus transition state (Scheme 2).

To identify the composition of the ions at *m*/*z* 255 and 269, and to therefore infer their structure, DIPP-β-Ala-Phe-OMe and DIPP-γ-Aba-Phe-OMe were analyzed by high resolution ESI-MS/MS. The experimental data was 255.1083 and 269.1275, and was very close to the exact data by calculation, 255.1109 and 269.1266, respectively. In order to testify the possible rearrangement mechanism for DIPP-β-Ala-Phe-OMe and DIPP-γ-Aba-Phe-OMe in Scheme 2, DIPP-β-Ala-Phe-OMe and DIPP-γ-Aba-Phe-OMe were deuterium labeled through exchange with CH_3OD, and their sodium adducts were subjected to ESI-MS^n. The data is shown in Table 1. The formation of the ions at *m*/*z* 256 and 270 in ESI-MS/MS (Fig. 1) shows that one deuterium atom was included in rearrangement products. All the data are coincident with the proposed structures of the ions at *m*/*z* 255 and 269 in Scheme 2. Because the molecular weight of the fragments at *m*/*z* 255 and 269 is even after subtracting the weight of Na, the fragments at *m*/*z* 255 and 269 both have two nitrogen atoms according to the general N rule in the element analysis. Therefore, there is a nitrogen atom without active hydrogen. It means that there was an active hydrogen migrated from one of the two nitrogen atoms (Scheme 2), that is, the proton migrated from imine to the oxygen atom. At the same time, the six- or seven-membered ring might break down by the elimination of a neutral molecule containing an active hydrogen to yield the fragments at *m*/*z* 255 and 269 with a four- or five-membered ring.

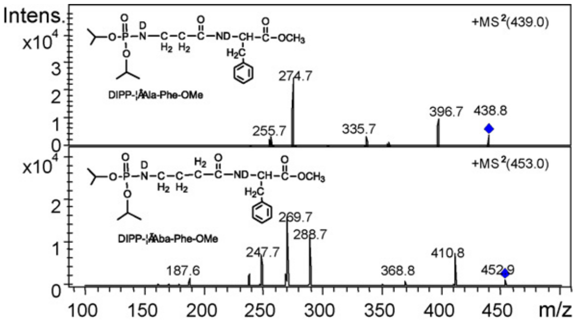


Fig. 1. ESI-MS^2 of the sodium adduct of deuterium-labeled DIPP-β-Ala-Phe-OMe and DIPP-γ-Aba-Phe-OMe.

In the rearrangement reaction with a carbonyl oxygen migration, the dominant pathway of DIPP-L/D-α-Ala-Phe-OMe was a five-membered ring pentacoordinate phosphorus transition state (Scheme 1), while that of DIPP-β-Ala-Phe-OMe and DIPP-γ-Aba-Phe-OMe was a six- or seven-membered ring tetracoordinate phosphorus transition state (Scheme 2). All the base peaks for the spectra of DIPP-γ-Aba-Phe-OMe are the peak at *m*/*z* 269 (Table 1), which shows that the seven-membered ring tetracoordinate phosphorus transition state was formed more easily. Schlosser and Lehmann have proposed that five-membered ring interaction represents the most effective structural principle governing low energy CID of peptides [13]. Cyclization rates are strongly dependent on ring size, with five-membered rings closing most rapidly, because larger ring sizes are less favorable for entropic reasons, and smaller ring sizes exhibit a larger enthalpy of activation [14,15]. As a result, five-membered ring structures in intermediates and products in low-energy CID of peptide ions appear to be favored over other ring sizes. Therefore, the results possibly relate to the spatial configuration of pentacoordinate and tetracoordinate phosphorus species. Pentacoordinate phosphorus species show a trigonal bipyramid configuration and they are less stable, but the five-membered ring could most stabilize them [16], while, tetracoordinate phosphorus species containing P=O show a tetrahedron configuration and they are more stable. Because the five-membered ring pentacoordinate phosphorus transition state could not appear in their rearrangement pathway, the rearrangement of DIPP-β-Ala-Phe-OMe and DIPP-γ-Aba-Phe-OMe proceeded through a six- or seven-membered ring tetracoordinate phosphorus transition state. Therefore, it was the spatial configuration of phosphorus atom that led the carbonyl oxygen migration through different cyclic transition state, that is, the five-, six- or even seven-membered ring transition state. Here, the strong affinity between phosphorus and oxygen atoms, which was proposed in a previous paper [10], might play an important role in this specific rearrangement. This implies that the carbonyl oxygen migration in the ESI-MS/MS of dipeptide methyl esters was not dependent on the length of the amino acid backbone at the N-terminus at least within these limits.

4. Conclusions

In conclusion, in the ESI-MS/MS of $[M+Na]^+$, DIPP-D-α-Ala-Phe-OMe showed the same rearrangement reaction with a carbonyl oxygen migration as DIPP-L-α-Ala-Phe-OMe. The transition state of their rearrangement was a five-membered ring pentacoordinate phosphorus transition state. However, in the ESI-MS/MS of $[M+Na]^+$ of DIPP-β-Ala-Phe-OMe and DIPP-γ-Aba-Phe-OMe, the carbonyl oxygen migration involved a six- or even seven-membered ring tetracoordinate phosphorus transition state. It was the spatial configuration of phosphorus atom that led the carbonyl oxygen migration through different cyclic transition state. This revealed the strong affinity between phosphorus and oxygen atoms in the gas phase, which might provide some useful clues for the study of reactions in liquid phase.

Acknowledgments

The authors would like to thank the National Natural Science Foundation of China (Nos. 20572061 and 20672104), the Chinese Ministry of Education and Zhengzhou University for financial support.

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