

Short communication

Peptide sequencing through N-terminal phosphonylation and multistage electrospray mass spectrometry of sodiated molecules

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Abstract

Peptides were phosphonylated at their N-termini through reaction of ethoxyphenylphosphinate (EPP) with peptides in the presence of triethylamine and tetrachloromethane under mild conditions. The phosphoramidate peptides were analyzed by tandem electrospray ionization mass spectrometry after addition of trace amounts of sodium chloride to the resulting solution above, and ESI-MS/MS of the sodiated molecules gave predominantly $[b_n + Na + OH]^+$ and $[b_n + Na - HCO]^+$ ions that clearly showed the sequential loss of amino acid residues from C-terminus of peptides, and the original peptides were readily assigned. The rapid, high sensitive and convenient method can widely be used for determination of peptide sequence.

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1. Introduction

The rapid and convenient peptide sequencing is highly desirable in proteomics and related bioscience. While the conventional method, Edman sequencing, does not fulfill this task, tandem mass spectrometry (MS/MS) provide a means for rapid identification and characterization of peptides/proteins [1–5]. MS/MS of a protonated peptides typically result in the formation of a variety of product ions, from both the C- and N-terminus which make mass spectra complex. In our previous research, some peptides were phosphonylated and analyzed, and the phosphoramidate peptides simplified multistage mass spectra of the protonated molecules relative to unmodified peptides that allow ready sequence determination of the original peptides [6]. As the continuous research of this program, we would like to report ESI-MS/MS of their sodiated phosphoramidate peptides, and the results unambiguously showed amino acid residue sequences of the original peptides.

2. Experimental

2.1. Materials

The peptides include Ala-Gly-Phe-Leu-ValOH (MW 505), Leu-Gly-Phe-Ala-ValOH (MW 505), Gly-Leu-Val-Ala-PheOH (MW 505), Phe-Phe-Phe-Phe-PheOH (MW 753), Phe-Ala-Ser-Asp-LeuOH (MW 551), Val-Ala-Ser-Phe-LeuOH (MW 535), Val-Glu-Gln-HisOH (MW 511) and Leu-Glu-His-GlnOH (MW 525). These peptides were commercially available from Shanghai Glsynthesis Company (Shanghai, China), and their purity is about 80%. The peptides were phosphonylated, and the phosphoramidate peptides were analyzed by ESI-MS/MS without further purification. Dichloro(phenyl)phosphine (DCPP) was purchased from Beijing Chemical Reagent Factory (Beijing, China). Ethanol was rendered anhydrous by refluxing with Mg.

2.2. Chemistry

³¹P NMR chemical shifts were reported in ppm downfield (+) or upfield (–) from external 85% H₃PO₄ as reference. Ethoxyphenylphosphinate and phosphoramidate peptides were

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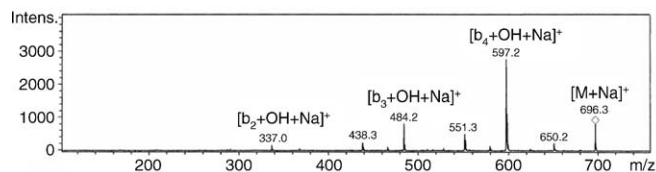


Fig. 1. ESI-MS² spectrum of the sodiated molecule $[M+Na]^+$ at m/z 696 of EPP-Ala-Gly-Phe-Leu-ValOH.

prepared according to the modified method in our previous report [6].

2.3. Mass spectrometric conditions

Mass spectra were obtained using a Bruker ESQUIRE–LC ion trap spectrometry equipped with a gas nebulizer probe. Nitrogen was used as drying gas at a flow rate of 4 L/min. The nebulizer gas fore-pressure was 7 psi. The electrospray capillary was typically held at 4 kV. Samples were dissolved in ethanol and ionized by electrospray ionization. The scan range was from m/z 100 to 1000 in positive-ion mode. The selected ions $[M+Na]^+$ were analyzed by multistage tandem mass spectrometry through collision with helium.

3. Results and discussion

Peptide (~ 0.1 mg) was dissolved in 10 μ L of triethylamine, 80 μ L of ethanol and 10 μ L of tetrachloromethane, and then 1.2–1.5 equiv. of ethoxyphenylphosphinate (EPP) (~ 2 μ L) was added to the solution at -5°C , the solution was stirred for 45 min, and the corresponding phosphoramidate peptide was obtained (Scheme 1) [6]. Trace amount of NaCl was added to the solution (molar ratio of peptide and NaCl 1:1), and the resulting solution was directly analyzed by ESI-MS/MS, without sample cleanup.

The multistage ESI-MS of $[M+Na]^+$ showed sequential loss of amino acid residues from C-termini of the modified peptides. For example, EPP-Ala-Gly-Phe-Leu-ValOH was determined by ESI-MS, the first stage MS showed the base peak $[M+Na]^+$ at m/z 696. ESI-MS² of the precursor ion (Fig. 1) produced b_n ions $[b_4+Na+OH]^+$ ($[M+Na-\text{Val}]^+$) at m/z 597, $[b_3+Na+OH]^+$ ($[M+Na-\text{LeuVal}]^+$) at m/z 484, $[b_2+Na+OH]^+$ ($[M+Na-\text{PheLeuVal}]^+$) at m/z 337 and ions $[b_4+Na-\text{HCO}]^+$ ($[M+Na-\text{Val}-\text{HCOOH}]^+$) at m/z 551,

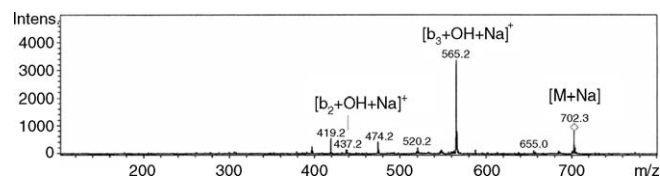
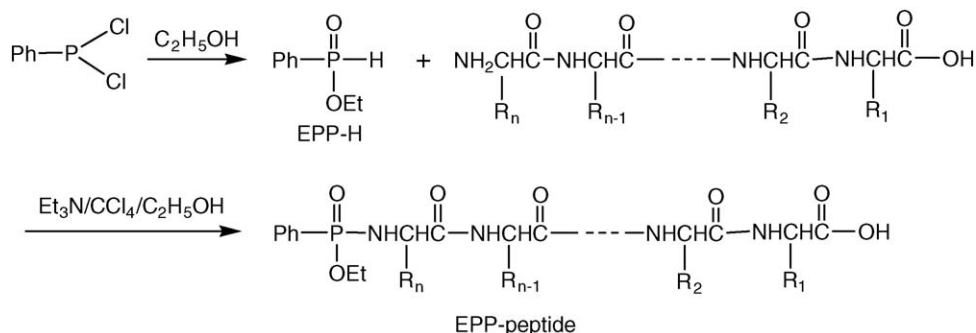


Fig. 2. ESI-MS² spectrum of the sodiated molecule $[M+Na]^+$ at m/z 702 of EPP-Val-Glu-Gln-HisOH.

$[b_3+Na-\text{HCO}]^+$ ($[M+Na-\text{LeuVal}-\text{HCOOH}]^+$) at m/z 438. Next, the ion $[b_3+Na-\text{HCO}]^+$ at m/z 484 was selected for further fragmentation to obtain the ESI-MS³ spectrum, which displayed ions $[b_2+Na+OH]^+$ ($[M+Na-\text{PheLeuVal}]^+$) at m/z 337 and $[b_1+Na+OH]^+$ ($[M+Na-\text{GlyPheLeuVal}]^+$) at m/z 280 (data not shown). Similarly, ESI-MS³ of the ion $[b_2+Na+OH]^+$ at m/z 337 yielded daughter ions $[M+Na-\text{GlyPheLeuVal}]^+$ at m/z 280 and $[b_0+Na+OH]^+$ ($[M+Na-\text{AlaGlyPheLeuVal}]^+$) at m/z 209 (data not shown). So the phosphoramidate peptide was sequenced by three-stage ESI-MS.

The phosphoramidate derivatives of other peptides including Ala-Gly-Phe-Leu-ValOH, Leu-Gly-Phe-Ala-ValOH, Gly-Leu-Val-Ala-PheOH, Phe-Phe-Phe-Phe-PheOH, Phe-Ala-Ser-Asp-LeuOH, Val-Ala-Ser-Phe-LeuOH, Val-Glu-Gln-HisOH and Leu-Glu-His-GlnOH were also analyzed using similar procedures, and their sequences were identified unambiguously. For example, Fig. 2 shows ESI-MS² of the sodiated molecule $[M+Na]^+$ at m/z 702 of EPP-Val-Glu-Gln-HisOH, and some fragment ions resulting from sequential loss of C-terminal amino acid residues were observed, i.e. $[b_3+Na+OH]^+$ ($[M+Na-\text{His}]^+$) at m/z 565 and $[b_2+Na+OH]^+$ ($[M+Na-\text{GlnHis}]^+$) at m/z 437. In addition, $[M+Na-\text{His}-\text{HCONH}_2]^+$ at m/z 520, $[M+Na-\text{His}-\text{HCONH}_2-\text{HCOOH}]^+$ at m/z 474 and $[M+Na-\text{GlnHis}-\text{H}_2\text{O}]^+$ at m/z 419 appeared, which showed the presence of Gln residue in the peptide chain. ESI-MS³ of the ion $[b_3+Na+OH]^+$ at m/z 565 (Fig. 3) yielded fragment ions $[M+Na-\text{His}-\text{NH}_3]^+$ at m/z 548, $[M+Na-\text{His}-\text{HCONH}_2]^+$ at m/z 520, $[b_2+Na+OH]^+$ ($[M+Na-\text{GlnHis}]^+$) at m/z 437, $[M+Na-\text{GlnHis}-\text{H}_2\text{O}]^+$ at m/z 419, $[b_2+Na-\text{HCO}]^+$ ($[M+Na-\text{GlnHis}-\text{HCOOH}]^+$) at m/z 391, $[b_1+Na+OH]^+$ ($[M+Na-\text{GluGlnHis}]^+$) at m/z 308, $[b_1+Na-\text{HCO}]^+$ ($[M+Na-\text{GluGlnHis}-\text{HCOOH}]^+$) at m/z 262 and $[b_0+Na+OH]^+$ ($[M+Na-\text{ValGluGlnHis}]^+$) at



Scheme 1. Synthetic route of phosphoramidate peptides.

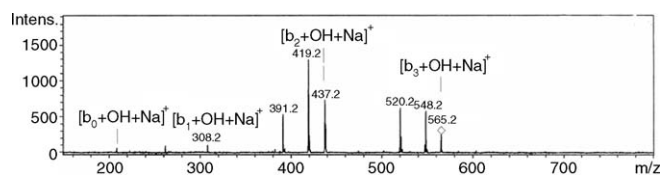


Fig. 3. ESI-MS³ spectrum of the ion at m/z 565 in Fig. 2.

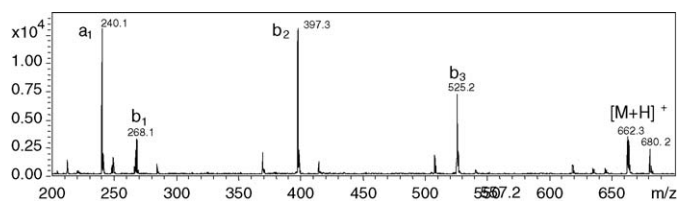


Fig. 4. ESI-MS² of the protonated molecule $[M+H]^+$ at m/z 680 of EPP-Val-Glu-Gln-HisOH.

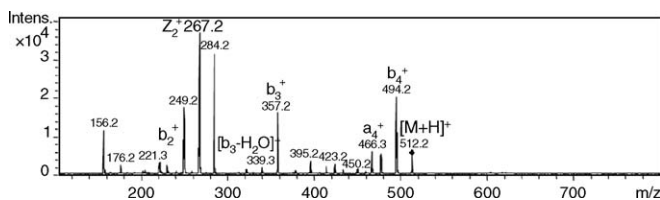


Fig. 5. ESI-MS² spectrum of the protonated molecule $[M+H]^+$ at m/z 512 of Val-Glu-Gln-HisOH.

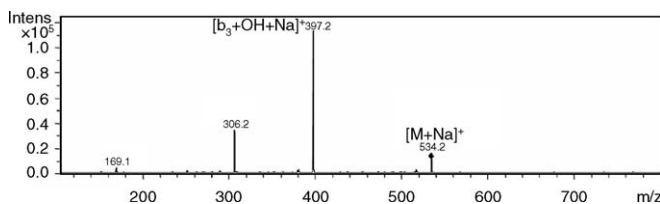


Fig. 6. ESI-MS² spectrum of the sodiated molecule $[M+Na]^+$ at m/z 534 of Val-Glu-Gln-HisOH.

m/z 209. So the modified peptide was clearly sequenced. We compared the ESI-MS/MS of the protonated molecule $[M+H]^+$ at m/z 680 (Fig. 4) and the sodiated molecule $[M+Na]^+$ at m/z 702 of EPP-Val-Glu-Gln-HisOH, the former predominantly produced the b_n -type ions, and the later one mainly yielded the ions $[b_n+Na+OH]^+$ and $[b_n+Na-HCO]^+$.

As shown in Fig. 5, ESI-MS² spectrum of the protonated molecule $[M+H]^+$ at m/z 512 of Val-Glu-Gln-HisOH shows many fragment ions though b-type ions were observed, which makes sequencing of the peptide difficult. Unfortunately, ESI-MS² spectrum of the sodiated peptide $[M+Na]^+$ at m/z 534

(Fig. 6) is too simple so that only one amino acid residue at C-termini could be identified.

We compared the response sensitivity of the phosphoramidate peptides and the corresponding original peptides in ESI-MS. The experiments showed that the addition of phosphonyl group at N-termini of peptides greatly enhanced response sensitivity of the phosphoramidate peptides relative to the free peptides in positive ion ESI-MS. For example, equimolar of the phosphoramidate peptide EPP-Ala-Gly-Phe-Leu-ValOH and the original peptide Ala-Gly-Phe-Leu-ValOH were mixed and dissolved in ethanol with trace amounts of NaCl, and then the mixture was analyzed by positive ion ESI-MS. The sodiated molecule $[M+Na]^+$ of the phosphoramidate peptide EPP-Ala-Gly-Phe-Leu-ValOH was observed as the base peak, however, we did not find the sodiated molecule $[M+Na]^+$ at m/z 528 and the protonated molecule $[M+H]^+$ at m/z 506 of the free peptide Ala-Gly-Phe-Leu-ValOH in the spectrum.

4. Conclusions

Multistage ESI-MS of the sodiated phosphoramidate peptides produced predominantly the ions $[b_n+Na+OH]^+$ and $[b_n+Na-HCO]^+$ that allow easy sequence determination of the original peptides by two-stage or three-stage ESI-MS after the free peptides were phosphonylated into the corresponding phosphoramidate peptides by EPP under mild conditions. The phosphonylating method combining with high sensitivity multistage ESI-MS may be generally used in sequencing of free peptides.

Acknowledgments

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References

- [1] P. Roepstorff, *Curr. Opin. Biotechnol.* 8 (1997) 6.
- [2] A. Shevchenko, M. Wilm, M. Mann, *J. Protein Chem.* 16 (1997) 481.
- [3] A.R. Dongré, J.K. Eng, J.R. Yates III, *Trends Biotechnol.* 15 (1997) 418.
- [4] J.R. Yates III, *J. Mass Spectrom.* 33 (1998) 1.
- [5] C. Gu, G. Tsapralis, L. Brecci, V.H. Wysocki, *Anal. Chem.* 72 (2000) 5804.
- [6] J.Y. Bao, H.W. Ai, H. Fu, Y.Y. Jiang, Y.F. Zhao, *J. Mass Spectrom.* 40 (2005) 772 (and references therein).