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Short communication

Sequencing of Lys-containing peptides through phosphonylation modification and electrospray ionization mass spectrometry

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Abstract

Lys-containing peptides were phosphonylated at their N-termini by reacting with ethoxyphenylphosphinate (EPP-H) in the presence of tetrachloromethane and triethylamine, and picomolar amount of the modified phosphonamidate peptides were determined by electrospray ionization mass spectrometry, together with tandem mass spectral technique, the intensive b_n-type ions relative to other type ions were observed, which clearly provided sequences of the original peptides.

Keywords: Peptide sequencing; Electrospray ionization mass spectrometry; Tandem mass spectrometry; Phosphonylation; Phosphonamidate peptide

1. Introduction

The rapid and convenient peptide sequencing is highly desirable in proteomics and related bioscience, and tandem mass spectrometry (MS/MS) provides a means for rapid identification and characterization of peptides/proteins [1–5]. Unfortunately, a protonated peptide in mass spectrometry is typically dissociated into a wide variety of fragment ions including the a_n, b_n, and c_n ions that correspond to the N-terminal fragments and the x_n, y_n, and z_n ions that represent the C-terminal fragments [6,7]. In general, it is difficult to predict which type of fragment ions will be formed for a given peptide, and the MS/MS spectra are often so complicated that de novo sequence determination is impossible. Many efforts have been made to simplify the mass spectra by modifying peptides with either positively or negatively charged groups. For positive derivatization, a positively charged group such as trimethylammoniumacetyl or tris[(2,4,6-trimethoxypheny)phosphonium]acetyl is introduced to the N-terminus of a peptide [8]. In all these derivatization methods, less investigation involved the original peptides containing lysine residue [9]. In our previous research, some peptides were phosphonylated and analyzed, and the phosphonamidate peptides simplified multistage mass spectra of the protonated molecules relative to unmodified peptides that allow ready sequence determination of the original peptides [10]. As the continuum of this program, we would like to report ESI-MS/MS of the protonated phosphonamidate peptides containing lysine residue.

2. Experimental

2.1. Chemistry

About 0.5 mg of peptide-bound resin (∼1000 beads) was added to a 1.5 ml microcentrifuge tube, 50 μl of trifluoroacetic acid (TFA) was added, and the peptide was cleaved from the Rink or Wang resin within 30 min. The TFA solution was dried with a stream of nitrogen leaving the crude peptide residue. Five microliters of triethylamine, 10 μl of tetrachloromethane, 480 μl of methanol and 5 μl of ethoxyphenylphosphinate (EPP-H) were added to the microcentrifuge tube containing the cleaved peptide at 0 °C. The solution was stirred for 30 min at this temperature, and the corresponding phosphonamidate peptide was formed (Scheme 1) [10]. The tube was centrifuged, and 10 μl of the

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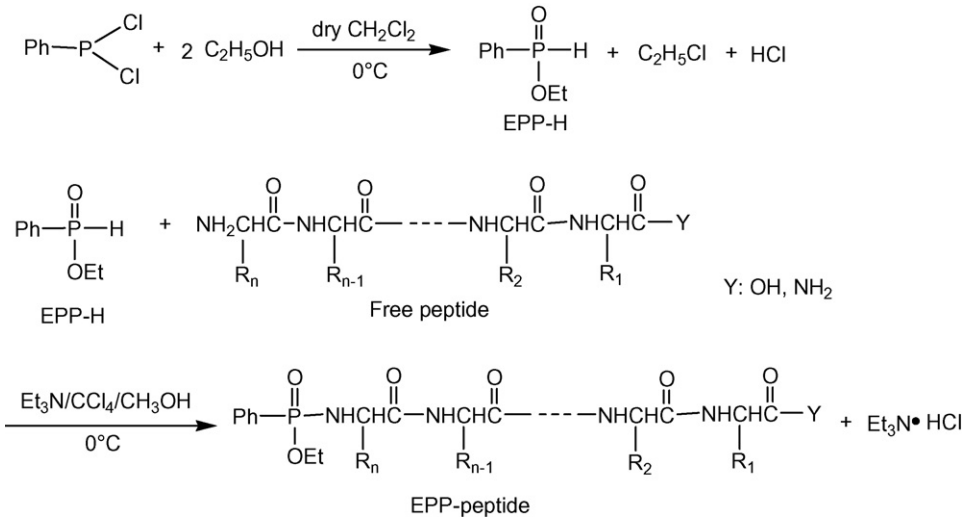

Scheme 1. Synthetic route of phosphonamidate peptides.

clear solution was drawn and diluted with 990 μl of methanol containing 0.05% TFA, and 40 μl of the resulting solution (picomol level of the phosphonamidate peptide) was used to analyze by ESI mass spectrometry without sample cleanup.

2.2. 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 methanol and ionized by electrospray ionization. The scan range was from *m/z* 100 to 1000 in positive-ion mode. The selected protonated molecules [EPP-$M+H]^+$ and $[M+H]^+$ (M represents molecular weight of a free peptide) were analyzed by multistage tandem mass spectrometry through collision with helium.

3. Results and discussion

ESI-MS spectrum (Fig. 1) of the phosphonamidate peptide EPP-AKDWV-NH_2 shows the protonated molecule [EPP-$M+H]^+$ at *m/z* 785, and the diphosphonylated product ion [EPP_2-$M+H]^+$ at *m/z* 953 was also observed because of the existence of Lys residue. The protonated molecule [EPP-$M+H]^+$ at *m/z* 785 was chosen to perform tandem mass spectrometry (Fig. 2), the b_n-type ions (*m/z* 669, 483, 368, 240) were observed, so sequence of the phosphonamidate peptide was easily identified.

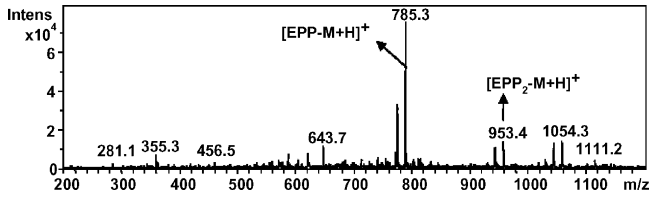

Fig. 1. ESI-MS spectrum of EPP-AKDWV-NH_2.

Interestingly, the intensive fragment ions in Fig. 2 all contained phosphonyl group, and the phosphonyl group was at their N-terminus not on ε-amino group of lysine residue, the results showed that monophosphonylation selectively occurred on the N-terminal amino group not on ε-amino group of lysine residue of the original peptide under the phosphonylating conditions above. The examples of selective derivatization at N-terminus of peptides have been found using *S*-pentafluorophenyl [tris(2,4,6-trimethoxyphenyl)phosphonium]acetate bromide [8] and 5-bromonicotinic acid *N*-hydroxysuccinimide ester [9] as the modifying reagents described by Watson and Tsunasawa's groups, respectively.

We also sequenced the phosphonamidate peptide EPP-VYKDH-OH using similar procedure. Reaction of 0.5 equivalent of ethoxyphenylphosphinate (EPP-H) with VYKDH-OH for a short time (about 0.5 h) produced EPP-VYKDH-OH (corresponding to the protonated molecule *m/z* 829) with unmodified peptide (corresponding to the protonated molecule *m/z* 661) being remained so that the mass spectral fragmentation pathways of the modified and original peptides could be compared (Fig. 3).

ESI-MS spectrum (Fig. 4A) shows the protonated molecule [EPP-$M+H]^+$ at *m/z* 829 with the unmodified peptide ion at *m/z* 661 appearing. ESI-MS/MS spectrum of the protonated molecule [EPP-$M+H]^+$ at *m/z* 829 produced the b_n-type ions at *m/z* 674 (b_4), 559 (b_3), 431 (b_2) and 268 (b_1), so sequence of the phosphonamidate peptide could be easily assigned to EPP-VYKDH-OH.

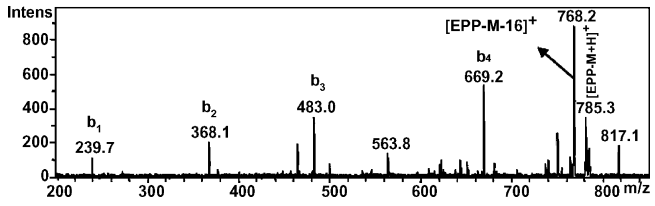

Fig. 2. ESI-MS/MS spectrum of the protonated molecule [EPP-$M+H]^+$ at *m/z* 785 of EPP-AKDWV-NH_2 in Fig. 1.

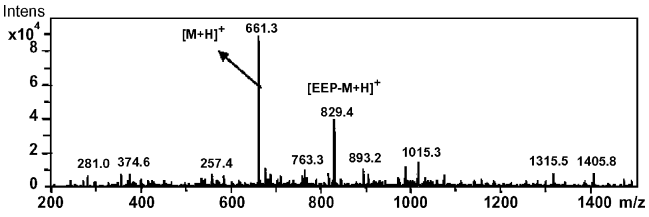

Fig. 3. ESI-MS spectrum of EPP-VYKDH-OH.

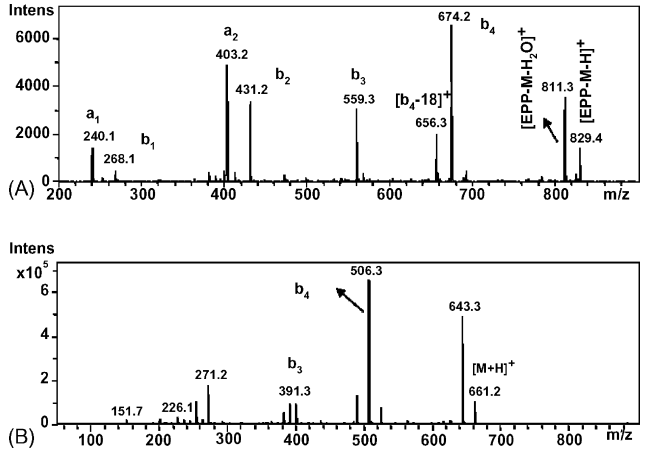

Fig. 4. ESI-MS/MS spectra of the protonated molecule [EPP-M + H]$^+$ at m/z 829 (EPP-VYKDH-OH) (A) and [M + H]$^+$ at m/z 661 (VYKDH-OH) (B) in Fig. 3.

We compared ESI-MS/MS spectra of unmodified and phosphoramidate peptides. Fig. 4B show ESI-MS/MS spectrum of the unmodified peptide VYKDH-OH. Compared with Fig. 4A and B exhibits complex spectrum, and only characteristic fragment ions b_4 and b_3 were observed. The N-terminal phosphonylating method could selectively increase the intensities of b_n-type ions relative to other ion types, and the resulting simplified mass spectra clearly showed the sequential loss of amino acid residues from the C-termini of peptides.

4. Conclusions

Lys-containing peptides were phosphonylated at their N-termini by reacting with ethoxyphenylphosphinate (EPP-H) in the presence of tetrachloromethane and triethylamine, and their monophosphonylation reaction mainly occurred at N-termini not on ε-amino group of lysine residue. Picomolar amount of the modified phosphonamidate peptides were determined by electrospray ionization mass spectrometry, together with tandem mass spectral technique, the intensive b_n-type ions relative to other type ions were observed, and sequences of peptides could be rapidly identified. Therefore, the method can be applied for sequencing of Lys-containing peptides.

Acknowledgments

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