OVERVIEW

Peptide de novo sequencing by MALDI Q-ToF MS

The method coupled a derivatization reaction with C-Terminal digestion to yield peptides with a fixed charge group attached to the N terminus and the removal of C-terminal basic amino acids (Arginine and Lysine)

Fragmentation of the modified peptides by MALDI Q-ToF MS produced a simplified ion series allowing de novo sequencing to be readily accomplished

INTRODUCTION

Peptide de novo sequencing refers about a peptide sequence independently of any information extracted from an existing protein or DNA database. It represents an alternative viable approach to obtain peptide sequences, especially when such information is difficult to obtain via database searching methods. However, the main challenge imposed upon any de novo sequencing algorithms requires fragmentation spectra of high quality with a continuous ion series. Peptide fragmentation typically produces a variety of ion series, which often add ambiguity to sequence determination. This is a particular problem for peptides at low abundance in complex samples where only a small number of fragment peaks are observed above background. The difficulty is even more compounded when peptide sequencing is done through MALDI mass spectrometry, where higher energy is required for singly charged ions which introduces more internal fragmentations.

In this presentation, we report our strategy that combines TMPP-Ac-OSu derivatization reaction with a C-terminal digestion to improve peptide de novo sequencing experiment are undertaken.

METHODS

Peptide Derivatization

1. TMPP-Ac-OSu solutons were prepared in anhydrous acetonitrile
2. Peptides/protein digests were placed in 0.4M 4-methylmorpholine (pH 9.0, 100 μL) and incubated at room temperature for 30 minutes

SCX Clean-up

An Albufera® Reagents: LC columns (3.0 mm × 30mm, 5 μm) was used with a polyethylene A buffer column (3.0 mm × 15mm, 5 μm)

CARBOXYPEPTIDASE B DIGESTION OF DERIVATIZED PEPTIDES

MALDI Analysis Q-ToF Premier

RESULTS

1. Comparison of the MSMS spectra of a Lys-containing peptide (SISIVGSYVGNR) from ADH under different treatments (A). MSMS spectra of the TMPP derivatized peptide (B). MSMS spectra of the TMPP derivatized peptide after treated with carboxypeptidase B. Removal of Lysine from the derivatized peptide greatly improved the fragmentation efficiency.

2. Comparison of the MSMS spectra of a Arg-containing peptide (ANELLNVK) from ADH under different treatments (A). MSMS spectra of the TMPP derivatized peptide (B). MSMS spectra of the TMPP derivatized peptide after treated with carboxypeptidase B. Removal of Arginine from the derivatized peptide greatly improved the fragmentation efficiency.

CONCLUSIONS

• TMPPAc derivatives of peptides follow different fragmentation pathways under low energy CID performed on a MALDI Q-ToF mass spectrometer.
• CID spectra of derivatized peptides contain solely Nterminal fragments [such as α- or β- ion] and independent of the presence and position of acidic amino acids in the peptide chains.
• More complete sequence-specific fragments are generated, providing unambiguous sequence information of the peptides.
• Removal of C-terminal Arginine residues with Carboxypeptidase B helps improve the fragmentation efficiency greatly while the removal of C-terminal Lysine shows less effects.

MALDI Q-ToF Premier mass spectrometer is proven to be an ideal instrument to meet the stringent mass accuracy requirements in peptide de novo sequencing.

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