FACILITATED DE NOVO SEQUENCING OF PEPTIDES BY CHARGE DERIVATIZATION AND C-TERMINAL DIGESTION

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OVERVIEW

- Improved peptide *de novo* sequencing by MALDI Q-Tof MS
- The method coupled a charge derivatization reaction with C–terminal digestion to yield peptides with a fixed charge group attached to the N termini and the removal of C–terminal basic amino acid residue (Arginine and Lysine)
- Fragmentation of the modified peptides by MALDI Q-Tof MS produced a simplified ion series allowing *de novo* sequencing be readily accomplished

INTRODUCTION

Peptide *de novo* sequencing infers 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 algorithm require 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 combine TMPP-Ac-OSu derivatization reaction with a C-terminal digestion to improve peptide *de novo* sequencing in MALDI mass spectrometry. The results demonstrate that TMPP derivatized peptides generated simplified fragmentation pattern in a MALDI Q-Tof mass spectrometer with predominate a or b ions in the MS/MS spectra. In addition, the removal of C-terminal basic residues such as Arginine and Lysine greatly improve the fragmentation efficiency of the derivatized peptides, thus making the method applicable to relative complex samples when a large scale of *de novo* sequencing experiment are undertaken.

METHODS

Peptide Derivatization

1. TMPP-Ac-OSu solutions were prepared in anhydrous acetonitrile
2. Peptides/protein digests were placed in 0.4M 4-methylmorpholine (pH 9.0, 70% CH_3CN). A 30-fold molar excess of TMPP-Ac-OSu solution was added and incubated at room temperature for 30 minutes

METHODS

SCX Clean-up

An Alliance™ Bioseparations HPLC system (Waters) was used with a PolySulfoethyl A column (4.6 mm × 50mm, 5 µm, 300 Å) to remove excess amount of derivatizing reagent. After 20-minute wash, peptides were eluted with a linear gradient of 10—500 mM ammonium formate over 20 minutes at a flow rate of 0.2 mL/min, with fractions collected at 5-min intervals. The fraction were lyophilized using a Speedvac.

Carboxypeptidase B Digestion of Derivatized Peptides

The lyophilized fractions were reconstituted in 20% acetonitrile/50 mM ammonium biocarbonate, and digested with Carboxypeptidase B (a molar ratio of 1:100) at 37 °C for one hour.

LC-MALDI Spotting

The derivatized and carboxypeptidase B digested peptides were separated by reverse phase chromatography on a Waters nanoACQUITY UPLC™ System

Column: Waters BEH 75 µm × 100 mm column
Mobile Phases: A: 0.01% TFA H_2O, B: 0.01% TFA ACN

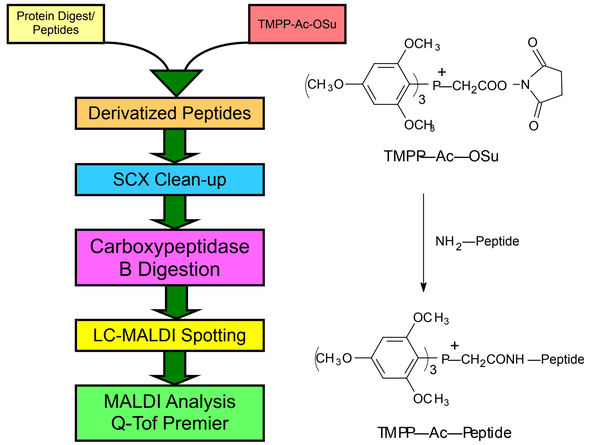
Gradient: 10—50% B over 30 mins,
Flow Rate: 300 nL/min, Injection Volume: 2µL

LC-MALDI Spotting

The eluent from the nanoACQUITY BEH column was directly collected onto a 96 well MALDI target using a micro fraction collector (CTC MALDI Spotter):
Collection Time: 40 sec/spot
Matrix: α cyano-4-hydroxycinnamic acid (2 mg/ml), added post-column at 1.7 µL/min via a syringe pump

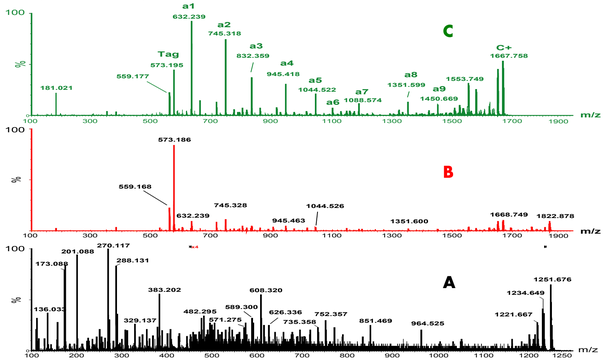
Mass Spectromtry

MS analysis was performed on a Waters MALDI Q-Tof Premier™ mass spectromter operated in the positive ion mode.

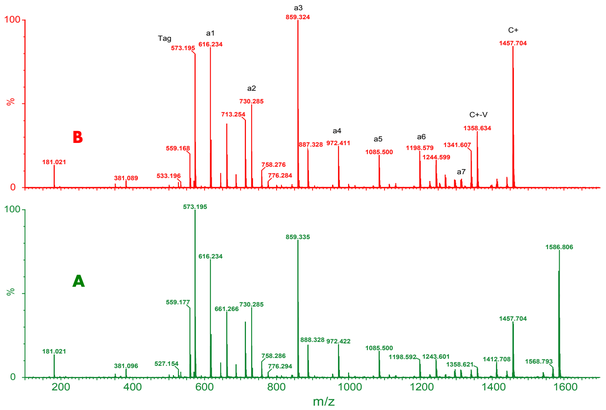


RESULTS

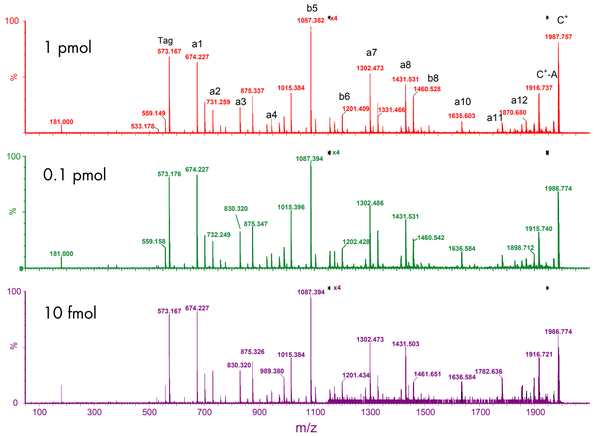
1. Comparison of the MSMS spectra of a Arg-containing peptide (SISIVGSYVGNR) from ADH under different treatments. (A). MSMS spectrum of the native peptide; (B) MSMS spectrum of the TMPP derivatized peptide; (C) MSMS spectrum of the TMPP derivatized peptide after treated with carboxypeptidase B. The derivatization changed fragmentation pattern and the removal of Arginine from the derivatized peptide greatly improved the fragmentation efficiency.



2. Comparison of the MSMS spectra of a Lys-containing peptide (ANELLINVK) from ADH under different treatments. (A). MSMS spectrum of the TMPP derivatized peptide; (B) MSMS spectrum of the TMPP derivatized peptide after treated with carboxypeptidase B. Removal of Lysine from the derivatized peptide has less effects with Lys-containing peptides compared to Arg-containing peptides on the fragmentation efficiency.



3. MS-MS spectra of Q Tof Premier of TMPP derivatized [Glu]-Fibrinopeptide B with the removal of C-terminal Arginine, 1 pmol (upper); 100 fmol (middle); 10 fmol(lower). All spectra were obtained at a collision energy of 80 v.



CONCLUSIONS

- TMPP-Ac 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 N-terminal fragments (such as a- or b- 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.

References

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2. W. Chen, P.J. Lee, D.B. Wall, Y.Q. Yu, J. C. Gebler, ASMS 2004 Poster http://www.waters.com/WATERSDIVISION/pdfs/WA40494.pdf

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