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Quantitation and Improved *de Novo* Sequencing of Proteins by Isotopic N-Terminal Labeling of Peptides with a Multifunctional Charged Tag

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Overview

- A charge-derivatization reaction was optimized to effectively add a pre-charged tag to the N-terminus of peptides to enhance ionization efficiency.

- The fragmentation behaviors of the derivatized peptides were investigated using MALDI Q-TOF, showing easy-interpretive CID spectra.

Introduction

Ionization of peptides and protein digests via MALDI preferentially yields singly charged analyte ions, and the fragmentations of these precursor ions often generate higher background in MS/MS spectra and undergo preferential cleavages. Selective fragmentation reactions limit the amount of *de Novo* peptide sequence information that can be obtained in these experiments. In this presentation, we report our investigation to overcome this obstacle by modifying peptides using a fixed-charge derivatization reagent, tris(2, 4, 6-trimethoxyphenyl) phosphonium acetic acid N-hydroxysuccinimide esters (TMPP-Ac-OSu). Peptides, after derivatization, show enhanced ionization efficiency. Collision-induced dissociation (CID) of derivatized peptides and protein digests on the new generation of MALDI Q-TOF instrument significantly enhances the amount of protein/peptide sequence information obtained, thus greatly facilitating *de Novo* sequencing of peptides.

Experimental

1. Synthesis of TMPP-Ac-OSu

TMPP-Ac-OSu reagent was synthesized in-house using the method published previously.[1] The reagent was characterized by ^{1}H-, ^{13}C-, ^{31}P-NMR, ESI-MS and MALDI MS.

2. Derivatization and Sample Preparation

- TMPP-Ac-OSu solutions were prepared in anhydrous acetonitrile at a stock solution of 120 nmol/μL.
- Peptides/protein digests were dissolved in 20 mM 4-methylmorpholine (pH 9.0, with 20% CH$_3$CN)
- Add 20 times molar excess of TMPP-Ac-OSu solution to peptide solution
- Vortex the solution, then incubate at room temperature for 30 minutes
- Add TFA to acidify the reaction mixture
- Mix the reaction mixture with matrix solution (HCCA, 5 mg/ml)
- Spot 1 μL on a stainless steel target for MALDI-TOF MS analysis (Micromass M@LDI-LR) or MALDI Q-TOF analysis (Micromass Q-TOF Ultima MALDI)

Results

1. The Derivatization Reaction and Ionization Efficiency Comparison of Native and Derivatized Peptides
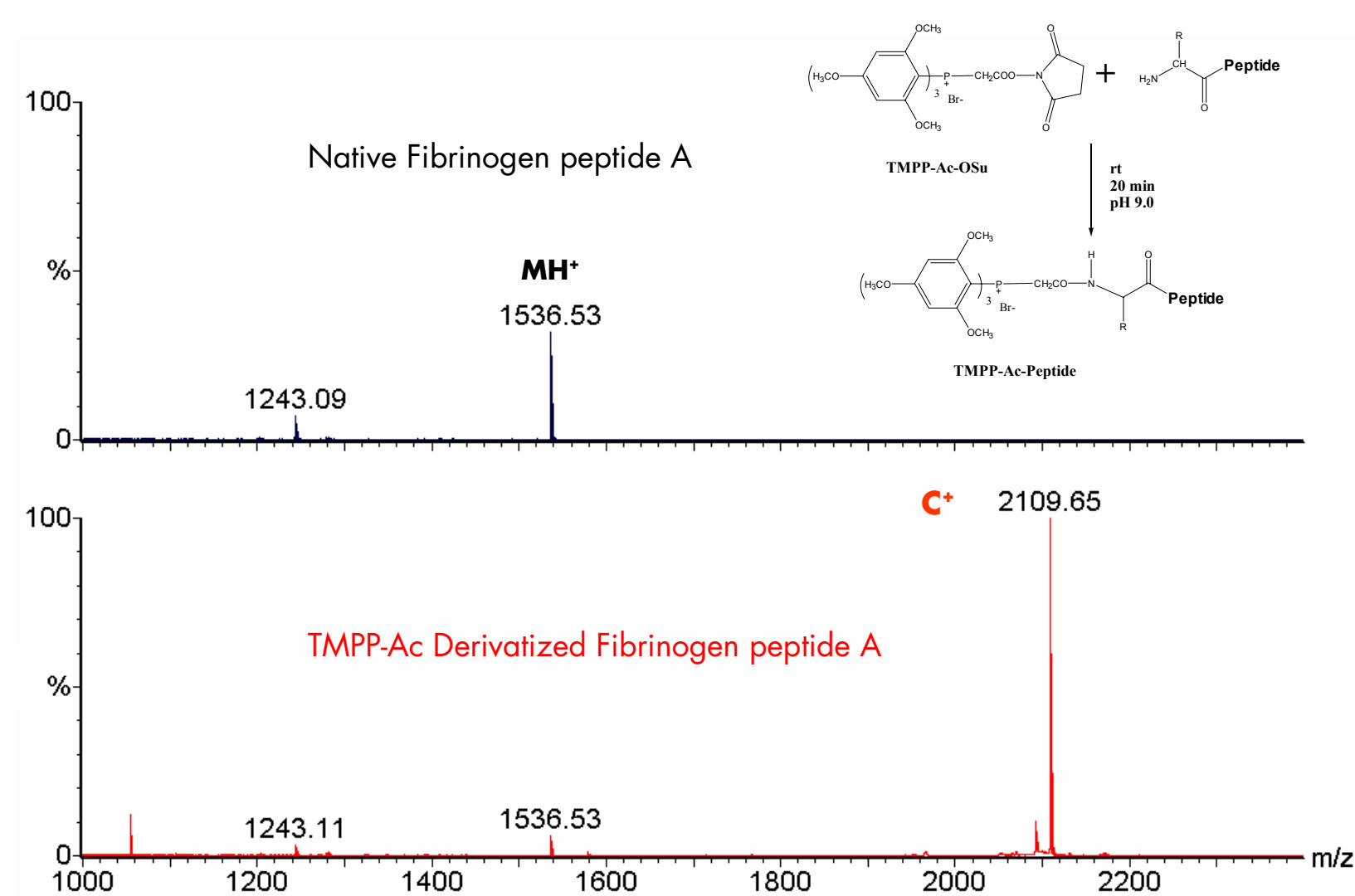

Figure 1. Normalized MALDI spectra of 200 fmol of native (Top) and TMPP derivatized (Bottom) Fibrinogen peptide A (ADSGEGDFLAEGGGVR).

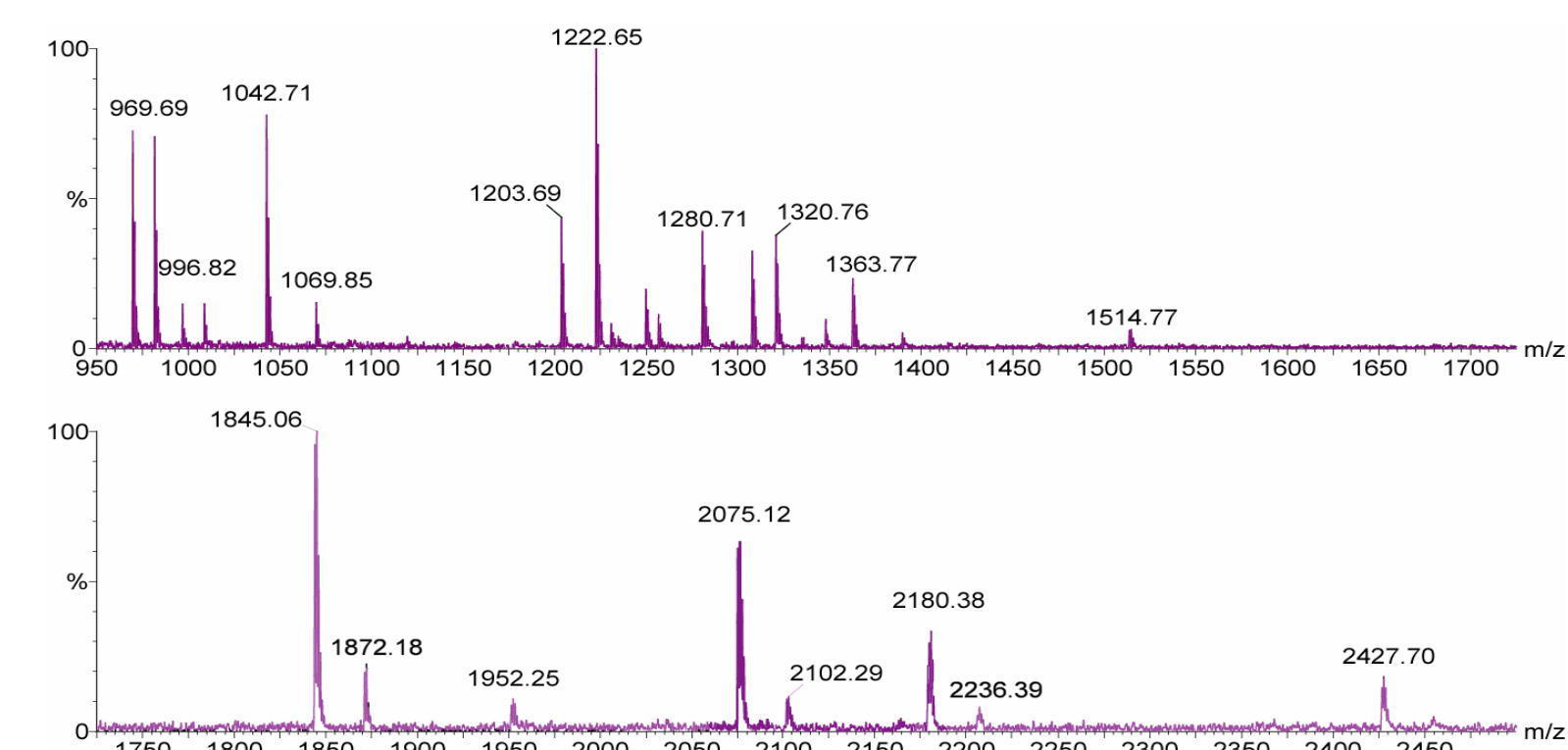### 2. Relative Quantifications Using Isotopically Labeled Reagents

Figure 2. MALDI-TOF spectrum of apomyoglobin tryptic digests labeled by either light or heavy TMPP-Ac-OSu reagent. The mixture ratio of TMPP-Ac tagged digests was 5:1 (light : heavy). The spectrum was generated with 50 fmol of digest.

3. Fragmentation of TMPP-Ac Derivatized Lys-containing Peptide— VQGEESNDK
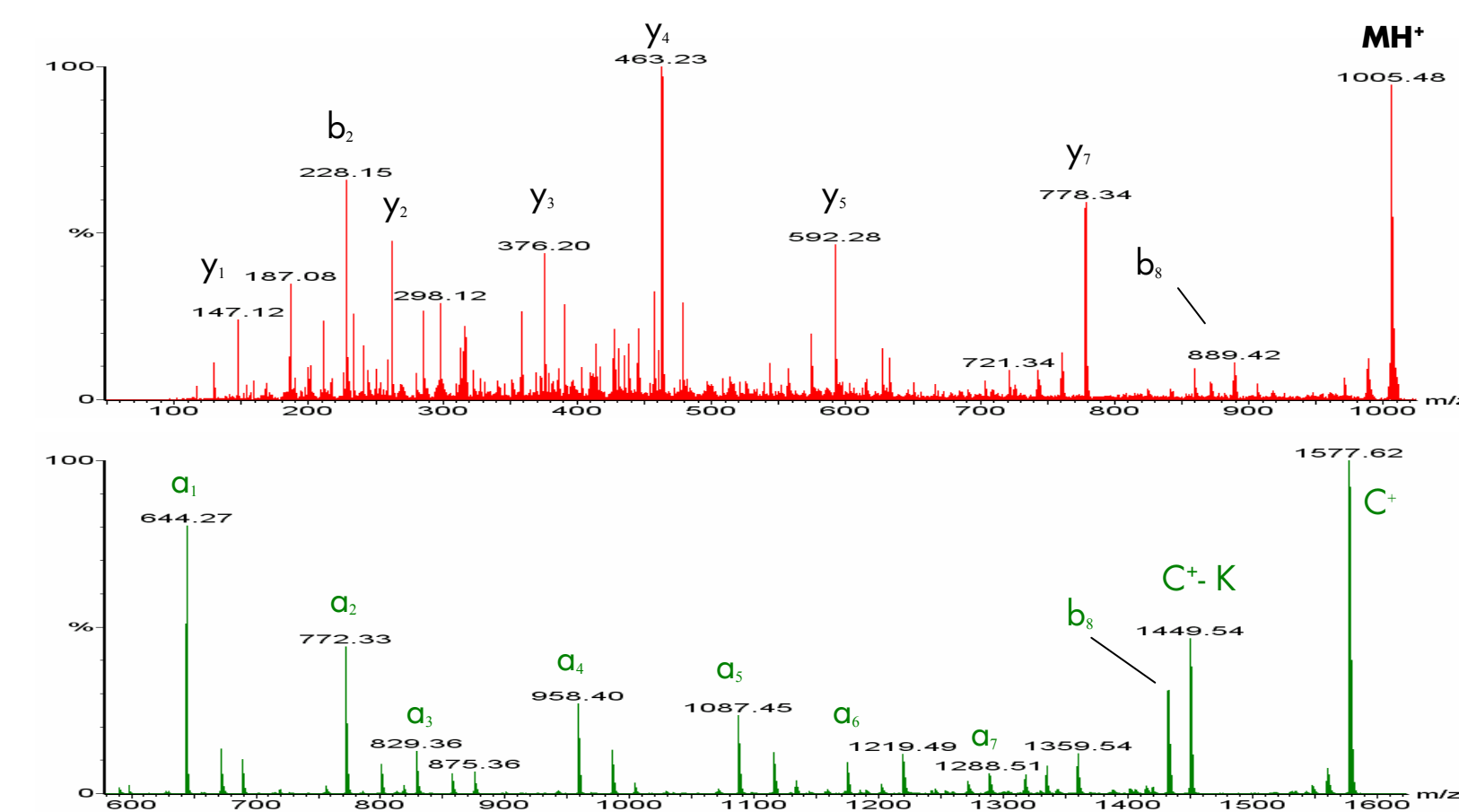

Figure 3. MALDI Q-TOF MS/MS spectra of native (top) and TMPP-Ac derivatized (bottom) peptide VQGEESNDK.

4. Fragmentation of TMPP-Ac Derivatized Arg-Containing Peptide— ASHLGLAR
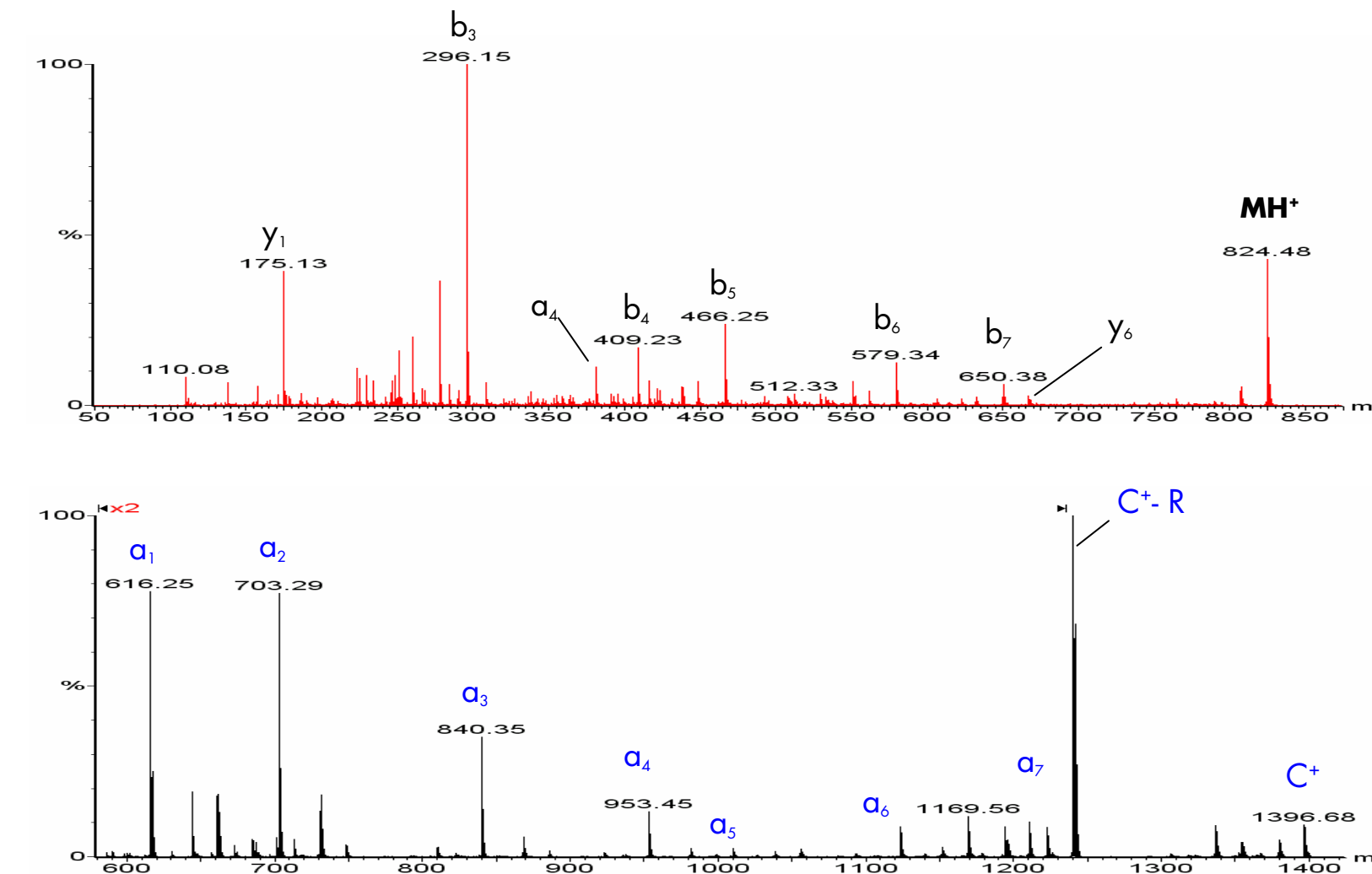

Figure 4. MALDI Q-TOF MS/MS spectra of native (top) and TMPP-Ac derivatized (bottom) peptide ASHLGLAR.

5. *De Novo* Sequencing of TMPP-Ac Derivatized Peptides

Figure 5. MALDI Q-TOF spectrum of a derivatized peptide from Apomyglobin tryptic digest. The MS/MS spectrum was submitted to BioLynx for *de novo* sequencing. The predicted sequences from the program are shown in the sequence pane (up left corner of the figure). The only return from MS/MS data generated using the derivatized peptide matched exactly the sequence of the myoglobin peptide.

Conclusions

- It is demonstrated that fragmentations of charged TMPP-Ac derivatives follow different pathways under low energy CID performed in a 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.

- The charge derivatization improved signal intensity for peptides in MALDI MS analysis.

Reference

1. Z. H. Huang, T. Shen, J. Wu, D.A. Gage and J. T. Watson Anal. Biochem. **268**, 305-317