

Analysis of Polypeptide Ladders Produced by Microwave-induced Acid Hydrolysis of Proteins with Atmospheric Pressure Matrix-assisted Laser Desorption/ionization–Triple Quadrupole Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (AP-MALDI-QFT)

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INTRODUCTION

• Recently, a novel technique for protein sequencing has been demonstrated. Microwave-induced acid hydrolysis randomly hydrolyzes peptide bonds along the backbone, and quickly (<5 minutes) generates peptide ladders from both the N- and C-terminals, providing high sequence coverage for the examined proteins. (Zhong et al., 2004).

• We have implemented this protein sequencing method with AP-MALDI coupled to an FT-ICR mass spectrometer. The AP-MALDI source has been demonstrated to produce low-energy (M+H)⁺ ions without the fragmentation encountered in vacuum MALDI. Hence, information on low *m/z* peptide ions (<*m/z* 1000) is retained. Combining this benefit with the high resolution and high mass accuracy of FT-ICR results in additional information that is normally overwhelmed by fragment ions in vacuum MALDI.

METHODS

•Microwave-assisted Acid Hydrolysis

1-5 pmol of a protein (cytochrome C, eglin C, myoglobin, or lysozyme) was dissolved in 2μL of water, then placed in a siliconized tube with an equal volume of 6N HCl (High purity grade, Pierce). The sample was microwaved for 60-300s at 1kW. The sample was evaporated down, then reconstituted with 1μL of 50:50 ACN:H₂O + 0.2% TFA, then 1μL of 1mg/mL ACHA matrix. Finally, 1μL of this solution was spotted (dry drop) onto the AP-MALDI target plate.

Mass Spectrometry

AP-MALDI was performed on an IonSpec QFT-12 triple quadrupole-Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer, equipped with a 12 T magnet. Samples were ionized by AP-MALDI (MassTech). The sample was rastered in a spiral pattern while the laser fired and ions were accumulated in the Q3 region for 30-300s.

Data Analysis

The resulting AP-MALDI mass spectra were analyzed with software developed in-house for microwave hydrolysis. Peptide sequence tags were generated and searched against the SwissProt protein database.

RESULTS

Sensitivity

• AP-MALDI technique not as sensitive as analogous (vacuum) MALDI-ToF experiment (M@LDI-R, Micromass)

Resolution and Mass Accuracy

• Resolution achieved with AP-MALDI FT-ICR was between 100,000-500,000 in broadband mode (*m/z* 185 – 4000)

• Mass accuracy ranged from 3-5 ppm (internal calibration) to 5-10 ppm (external calibration)

• At lower *m/z*, the proton-bound dimer and trimer of ACHC were detected (used as internal calibrants).

Major Findings

• Protein IDs arising from the mass fingerprinting (using in-house software) were correctly assigned to the hydrolysed peptides.

• Unfortunately, the majority of low *m/z* hydrolysis products (<*m/z* 1000) result from cleavage at non-sequential locations. These hydrolysis products were uninformative.

• There was considerable variability in the efficiency of the protein hydrolysis (e.g., ions missing, variable intensity) between similarly prepared samples.

• For many of the hydrolyzed peptides, **dehydration (-H₂O, 18 Da) and deamination (-NH₂, 17 Da) products** were detected. These ions were *not a result of the AP-MALDI process*, as these degradation products were not formed when standard peptide mixtures were analyzed under identical conditions.

• **Adducts with ACHA** with larger ions (>*m/z* 2500) were observed (Kellersberger et al., 2002), even at diluted matrix concentrations

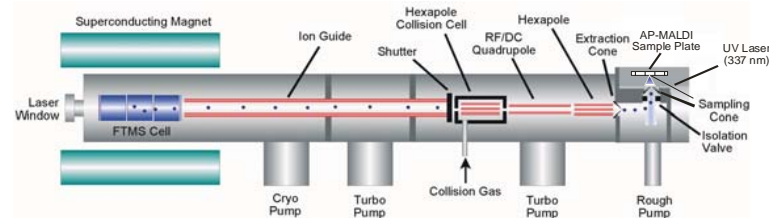
• For the conditions employed, ions >*m/z* 2000 were detected in greater abundance than ions <*m/z* 700.

CONCLUSIONS

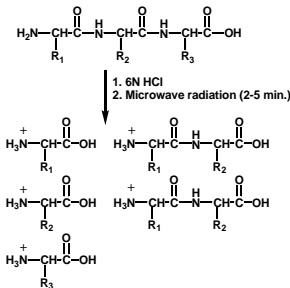
• While several small peptides (<*m/z* 700) were detected from non-sequential peptide bond hydrolyses, the majority of peptides detected were >*m/z* 2000.

• The microwave-induced acid hydrolysis method displayed considerable variability in the efficiency of protein hydrolysis.

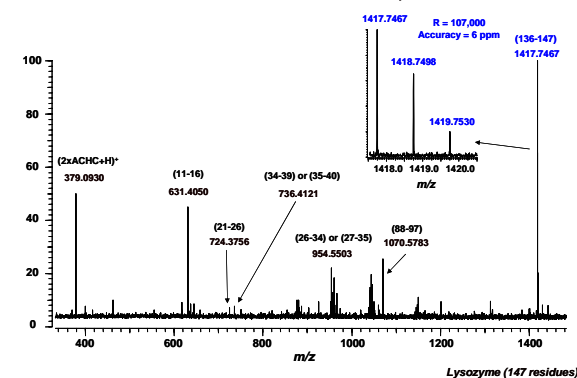
• Optimization of microwave-assisted acid hydrolysis technique, AP-MALDI sample preparation conditions, and QFT-12 instrument parameters is underway.



QFT-12 Schematic



Lysozyme microwave hydrolysis (90s)
Ion accumulation: 240s; laser@7.5; low *m/z* optimized



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Eglin C microwave hydrolysis (300s)
Ion accumulation: 300s; laser@7.5; high *m/z* optimized

