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Matrix-assisted ionization vacuum for protein detection, fragmentation and PTM analysis on a high resolution linear ion trap-orbitrap platform

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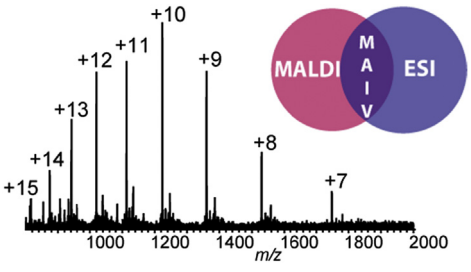
HIGHLIGHTS

- MAIV can generate multiply charged ions without laser ablation or high voltage.
- Detects proteins up to 18.7 kDa with 18 charges in a HRAM mass spectrometer.
- Soft ionization technique suitable for labile PTM analysis.
- Improved fragmentation efficiency compared to MALDI-MS/MS of singly charged ions.
- Capable of complex tissue sample analysis both in liquid extracts and *in situ*.

GRAPHICAL ABSTRACT



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ABSTRACT

Matrix-assisted ionization vacuum (MAIV) is a novel ionization technique that generates multiply charged ions in vacuum without the use of laser ablation or high voltage. MAIV can be achieved in intermediate-vacuum and high-vacuum matrix-assisted laser desorption/ionization (MALDI) sources and electrospray ionization (ESI) sources without instrument modification. Herein, we adapt MAIV onto the MALDI-LTQ-Orbitrap XL platform for biomolecule analysis. As an attractive alternative to MALDI for in solution and *in situ* analysis of biomolecules, MAIV coupling to high resolution and accurate mass (HRAM) MS instrument has successfully expanded the mass detection range and improved the fragmentation efficiency due to the generation of multiply charged ions. Additionally, the softness of MAIV enables potential application in labile post-translational modification (PTM) analysis. In this study, proteins as large as 18.7 kDa were detected with up to 18 charges; intact peptides with labile PTM were well preserved during the ionization process and characterized MS/MS; peptides and proteins in complex tissue samples were detected and identified both in liquid extracts and *in situ*. Moreover, we demonstrated that this method facilitates MS/MS analysis with improved fragmentation efficiency compared to MALDI-MS/MS.

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1. Introduction

In the late 1980s, the development of MALDI [1] and ESI [2] revolutionized the field of mass spectrometry (MS) application in the analysis of large biomolecules [3]. MALDI utilizes laser desorption to produce mostly singly charged ions from a solid matrix, while ESI employs high voltage to produce multiply charged ions from solution. The capability of ionizing analytes directly from solid surface makes MALDI an ideal tool for *in situ* tissue analysis. However, as MALDI predominantly produces singly charged ions, a few challenges remain for MALDI analysis, especially for HRAM protein analysis. With the commonly used ion activation methods such as collisional induced dissociation (CID) and high energy collisional induced dissociation (HCD) techniques, the singly charged ions usually have lower fragmentation efficiencies compared with that of multiply charged ions, making the identification of analyte molecules difficult. Additionally, most current commercially available high-performance Orbitrap instruments cannot detect ions larger than m/z 10,000. Moreover, in-source or post-source fragmentations of molecules are usually observed in MALDI process [4,5]. On the other hand, although ESI overcomes most of these problems mentioned above, it is impossible to preserve the spatial information on tissue sections using conventional ESI analysis since the analytes must be dissolved in a volatile solution.

In recent years, many approaches have been taken to address these challenges. “Electron-free” MALDI was observed to produce higher percentage of multiply charged ions, when the number of electrons was limited in the plume [6]. Desorption electrospray ionization (DESI) ionizes analytes from surface by electrospray generated charged droplets and solvent ions [7,8]. Matrix-assisted laser desorption electrospray ionization (MALDESI) generates ESI-like multiply charged ions with the assistance of matrix [9,10]. Electrospray-assisted laser desorption ionization (ELDI) is capable of ionizing peptides and proteins in solid materials by laser desorption and post-ionization electrospray without matrix [11–15].

Laserspray ionization (LSI) and MAIV are relatively new ionization techniques that produce ESI-like multiply charged ions while being able to preserve spatial information on tissue sections. Unlike DESI, MALDESI or ELDI, LSI and MAIV can be readily achieved using commercially available MALDI sources without any instrumentation modification. These two ionization techniques were first introduced by the Trimpin lab and have been demonstrated on several MS platforms [16–19]. LSI utilizes volatile small molecule matrices with laser ablation to ionize analytes under atmosphere pressure (1.01 bar) [20], intermediate-vacuum (10^{-3} to 33 mbar) [18,21] and high-vacuum (10^{-6} to 10^{-3} mbar) MALDI sources [22]. However, the involvement of laser in LSI could induce in-source or post-source fragmentations. In contrast, MAIV is a softer ionization method that generates multiply charged ions in a triboluminescence process, which does not involve either laser or high voltage application during the ionization process [23]. With the assistance of small volatile matrices, MAIV can generate highly charged ions from a wide variety of compounds [24]. The original MAIV study was performed on both LTQ and quadrupole time-of-flight instruments with low to medium mass resolution. The use of this ionization method was also demonstrated on high resolution Fourier transform instruments for full MS analysis [17,19]. However, to our knowledge, no study has been performed on a MALDI-Orbitrap platform, and MAIV-MS/MS data, especially for post-translational modification (PTM) analysis, is also quite limited in previous studies.

Many biological processes are regulated by PTM of peptides and proteins [25]. Understanding PTMs is essential for understanding the biological functions of various proteins and studying cell regulations. However, some labile PTMs, such as glycosylation, can be easily detached during ionization process, making them difficult to be analyzed by MS. Due to its softness, we hypothesize that MAIV can reduce in-source and post-source fragmentation of biomolecules, especially peptides and proteins with labile PTMs.

The introduction of hybrid MALDI mass spectrometers has expanded the capability of MALDI MS analysis. The MALDI-LTQ-Orbitrap XL system (Thermo Fisher Scientific, Bremen, Germany) incorporates linear ion trap and orbitrap mass analyzers [26], making HRAM analysis as well as MS^n by collisional induced dissociation (CID) and high-energy collision dissociation (HCD) possible on one platform. However, this instrument has a limited m/z range of 50–4000. With singly charged ions generated in the MALDI source, this instrument cannot analyze large molecules such as proteins, polysaccharides and polynucleotides.

The development of LSI [27,28] and MAIV [29] prompted us to investigate the possibility of employing the MALDI-LTQ-Orbitrap XL as a HRAM platform for protein characterization. LSI has been adapted to MALDI-LTQ-Orbitrap XL hybrid system and the application has been expanded to *in situ* MS/MS analysis and *de novo* sequencing [18]. In this study, we adapted the recently developed MAIV technique to our hybrid MALDI-LTQ-Orbitrap XL system for peptide and protein analysis. Multiply charged ions were detected with this HRAM MS platform in the analysis of protein and peptide standards, tissue extracts and *in situ* tissue sections. Furthermore, MAIV-MS/MS analysis has been achieved in both standards and tissue protein extraction analysis. The fragmentation efficiency of multiply charged species has been greatly improved in comparison to that of the singly charged ions. We also demonstrated that MAIV-MS could be used for the analysis of labile PTMs on peptides because of the soft ionization nature of MAIV.

2. Materials and methods

2.1. Materials

Methanol (MeOH), ethanol (EtOH), acetonitrile (ACN), acetic acid (AA) and formic acid (FA) were purchased from Fisher Scientific (Pittsburgh, PA). 3-nitrobenzonitrile (3-NBN), α-cyano-4-hydroxycinnamic acid (CHCA), insulin (bovine), cytochrome C (bovine heart), lysozyme (chicken egg white) and myoglobin (equine heart) were purchased from Sigma Aldrich Inc. (St. Louis, MO). Peptide standard bradykinin was purchased from American Peptide Company (Sunnyvale, CA). Kinase domain of insulin receptor was purchased from AnaSpec (Fremont, CA) and glycosylated erythropoietin (EPO) 117–131 was purchased from Protea Biosciences Group, Inc. (Morgantown, WV). All standards and reagents were used without additional purification.

2.2. Sample preparation

2.2.1. Peptide and protein standard preparations

Peptide and protein stock solutions with concentration of 10 mg mL^{-1} were prepared by dissolving standards in water (0.1% FA). Standards with concentrations of 1 mg mL^{-1}, 100 μg mL^{-1}, 10 μg mL^{-1} and 1 μg mL^{-1} were prepared by serial dilutions in water (0.1% FA). Samples were stored at −20 °C until analysis.

2.2.2. Tissue analysis

Animal experiments were conducted following institutional guidelines (UW-Madison IACUC). Female Sprague–Dawley rats were anesthetized, perfused with chilled phosphate buffered saline, decapitated and removed brains. The brain tissues were either stored in Eppendorf tubes for protein extraction or embedded in