

Analysis of post-translationally modified peptides and proteins by APMALDI-*oa* TOFMS

Jun Tamura¹; Kenji Nagatomo¹; Tetsuichiro Morita¹; Yasunori Nishimura¹; Robert B. Cody², Phillip Tan³

¹JEOL Ltd., Akishima, Tokyo, Japan, ²JEOL USA, Inc., Peabody, MA, ³MassTech, Inc., Columbia, MD

Introduction

The combination of an atmospheric pressure matrix assisted laser desorption/ionization (APMALDI) ion source and an orthogonal acceleration time-of-flight mass spectrometer (*oa*TOFMS) is expected to have unique advantages over conventional vacuum MALDI-TOFMS or APMALDI-QITMS (quadropole ion trap mass spectrometer,) which has been popular after the introduction of the commercial APMALDI ion source for QITMS, for the following reasons: 1) Capability to detect molecular-related ions of very fragile species such as sulfopeptides and phosphopeptides 2) Very high mass accuracy (~ 5 ppm) regardless of sample morphology 3) Ability to realize very high throughput analyses using a high repetition rate (> 100 Hz) laser and robotics These characteristics are quite suitable for high throughput identification of proteins and peptides with post-translational modifications

Experimental Instruments

All the data have been acquired with a model 611 APMALDI ion source (MassTech, Inc., MD, U.S.A.) and a JMS-T100LC AccuTOF API-*oa*TOFMS (JEOL Ltd., Tokyo, Japan.) A prototype atmospheric pressure ionization interface employing a heated metal capillary has been developed for optimizing the transportation of MALDI generated ions from the atmospheric pressure into the high vacuum.

APMALDI ion source is represented schematically in Figure 1. Nitrogen laser with a wavelength of 337nm is send to the ion source through an optical fiber, reflects the laser which focused through the lens by the mirror, and irradiate a sample plate. The sample plate has spots in 12 by 8 arrangement, and each spot can be visually observed by the monitor connected to the CCD camera. The position of the spot to which the laser is irradiated can be controlled by the computer. The standard ESI needle potential of ~3kV was applied between the target plate and the metal capillary.

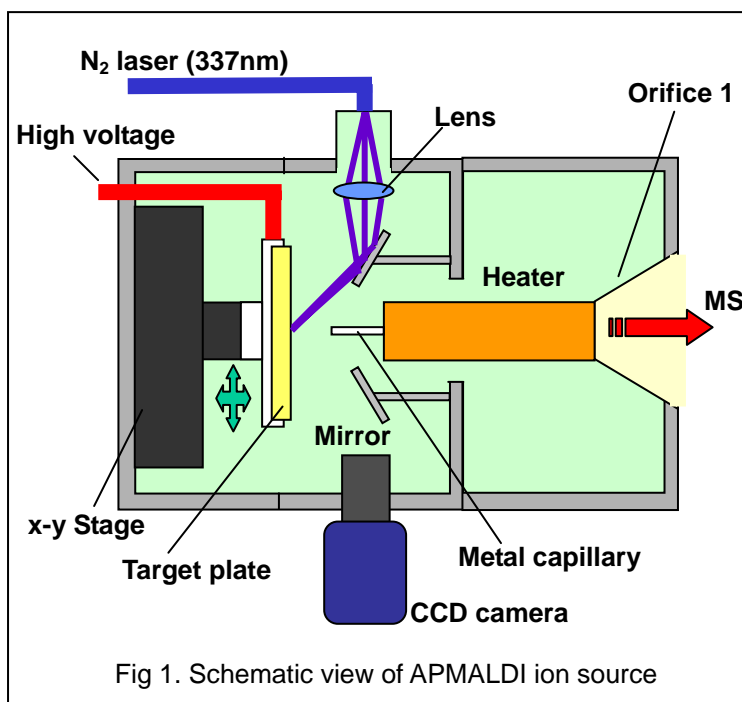


Fig 1. Schematic view of APMALDI ion source

Sample preparation

A standard MALDI sample preparation routine was used; each droplet of 1 μ L of matrix/analyte solution mixed on the target plate and allowed to dry. The matrix solution was α -cyano-4-hydroxycinnamic acid purchased (Agilent technologies).

Results

Basic performance characteristics of the instrument, such as sensitivity, mass resolution and mass accuracy were demonstrated with peptide standards. The protonated molecular ion (m/z : 1297) of

Angiotensin I (10 fmol) was observed with sufficient signal to noise ratio (>10). In the analysis of peptides mixture (100 fmol each), mass accuracy of each peptide, as compared with the theoretical value, was better than 4ppm with internal mass reference. The spectrum of Bovin Insulin (10 pmol) protonated molecular ion (m/z : 5734) was obtained with high resolution ~ 8000 (FWHM).

A tryptic peptide (1-25) from bovine beta-casein was analyzed by the APMALDI-*oa*TOFMS and the result was compared with that from a conventional vacuum MALDI-TOFMS in linear and reflectron mode [Fig. 2]. The peptide was expected to contain four phosphoserin residues. The protonated molecular ion of the peptide was hardly discernible in the spectrum from reflectron MALDI-TOFMS whereas it was very clearly observed in the spectra from APMALDI-*oa*TOFMS and linear MALDI-TOFMS, suggesting that APMALDI is indeed a very soft ionization method. The mass resolution ($> 6,000$, FWHM) and mass accuracy was much higher in the APMALDI-*oa*TOFMS spectrum than those in the linear MALDI-TOFMS spectrum.

Conclusions

A MassTech APMALDI ion source was successfully coupled with a JEOL AccuTOF TOFMS. Molecular related ion of the peptide with multiple phosphorylations was clearly detected. It was possible to detect molecular-related ions of very fragile species with high resolution by APMALDI-*oa*TOFMS.

References

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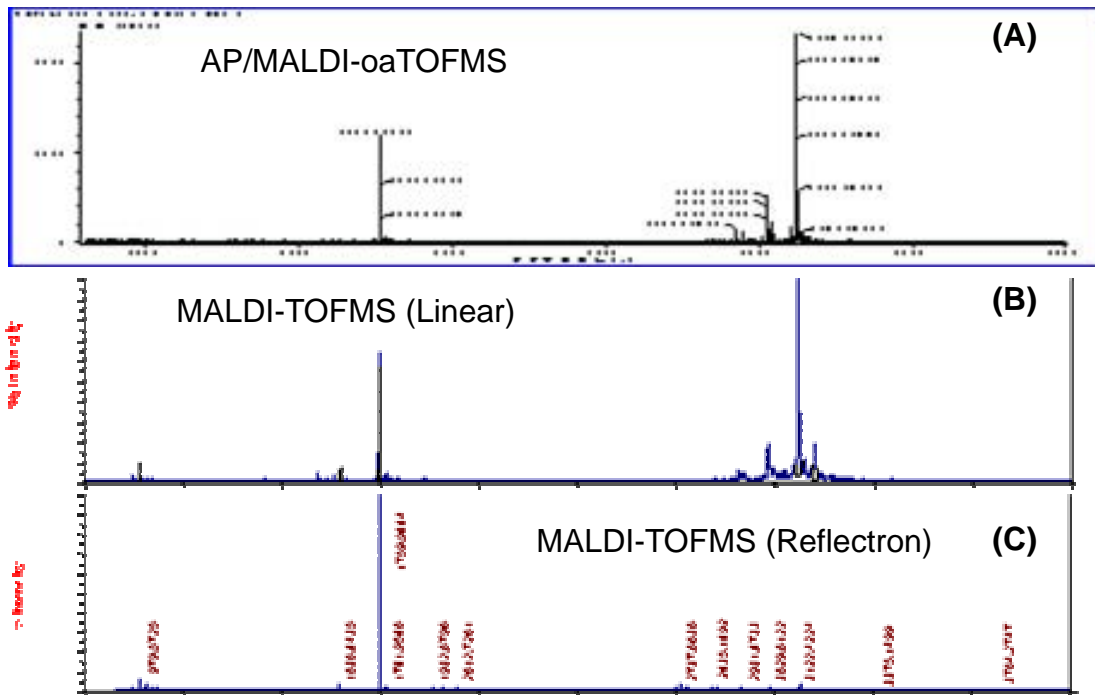


Fig 2. MALDI spectra of β -casein (bovine) fragment 1-25. (A) AP/MALDI-*oa*TOFMS: JMST100LC AccuTOF. (B) The conventional vacuum MALDI, Liner mode. (C) The conventional vacuum MALDI, Reflectron mode.