

AP-MALDI Ion Trap MS Analysis of Selective Phosphopeptides Using Different Immobilized Metal Affinity Chromatography Materials

N. I. Taranenko¹, V. M. Doroshenko¹, A. K. Shukla², M. M. Shukla²

¹MassTech, Inc., Columbia, MD, US, ²Glygen Corp., Columbia, MD, US

Introduction

The identification of post-translation modification (PTM) in proteins remains a challenge in current proteomic research and analysis. Several methods have been developed to analyze the different phosphorylation sites on peptides and proteins. There is, however, no universal method for the purification and analysis of modified peptides and proteins. Currently, different affinity chromatographic and other chromatographic materials such as immobilized metal affinity chromatographic (IMAC) - material, titanium dioxide (titania), graphite carbon, anion exchanger, and antibody affinity purification are used for the purification of phosphopeptides. Atmospheric pressure matrix-assisted laser desorption/ionization (AP-MALDI) mass spectrometry is particularly beneficial for phosphopeptides analysis because of minimal fragmentation of analyte ions, large tolerance to the laser energy and the ability to produce primarily singly-charged ions.

Experimental

Molecular Biology Grade Water from Biowittaker (Walkersville, MD, USA) was used for the matrix and sample preparation solutions. Bovine β -Casein Ovalbumin was obtained from Sigma (St. Louis, MO, USA). The matrix material, α -cyano-4-hydroxycinnamic acid (4-HCCA) was obtained from Fluka (Buch, Switzerland). Trypsin beads (Poroszyme Bulk Immobilized Trypsin, PerSeptive Biosystems) was obtained from Applied Biosystems (Foster City, CA, USA). All materials were used as received, without any further purification or modification. ZipTipMC pipette tips were obtained from Millipore (Bedford, MA, USA). Different affinity materials were obtained by different manufacturers and embedded in the micropipette tips by using Glygen (Columbia, MD, USA) Nutip Technology.

Experiments were carried out on a Thermo Finnigan (San Jose, CA, USA) LCQ Deca XP ion trap MS integrated with an AP/MALDI ion source (MassTech Inc., Columbia, MD, USA) [1-2]. A Thermo Laser Science Inc. (Franklin, MA, USA) Model 337 Si nitrogen UV laser was used. Its wavelength was 337 nm, laser pulse duration was about 4 ns, and the laser beam was focused to approximately 500 μ m size spot.

Seven different IMAC-materials were charged with iron and gallium and used for selective enrichment of the phosphopeptides from tryptic digest of bovine β -Casein and chicken ovalbumin. Micro adsorptive pipette tips are used for the IMAC purification. Trypsin digestion has been carried out at +37° C, overnight shaking. The NuTip were prepared with different IMAC materials and charged by gallium metal for enrichment of phosphopeptides obtained from tryptic digests. Phosphopeptides isolated from peptides mixture were eluted from immobilized gallium by 1.5 mM α -cyano-4-hydroxycinnamic acid (4-HCCA) matrix solution for analysis.

Results

The five phosphorylation β -Casin sites are denoted with pS. The sequence purified from cow milk might also have a signal sequence. We conducted a detailed analysis on the β -Casein product and found some contaminating phosphopeptides from alpha-casein, kappa-caseins. Ovalbumin contains two phosphorylated serines at positions 68 & 344. Trypsin cleavage of ovalbumin releases two phosphopeptides, LPGFGDpSIEAQCGTTSVNVHSSLR & EVVGpSAEAGVDAAS VSEEEFR with expected masses of 2,354.11 and 2,088.91 Da, respectively. After partial purification and enrichment with different commercially available micro pipette tips that contain IMAC materials, phosphopeptides are analyzed using AP-MALDI ion trap mass spectrometer.

Discussion

This study shows that various materials provide different results and result in the identification of different phosphopeptides from bovine β -casein tryptic digest and ovalbumin tryptic digest. We did not find any correlation between tridentate, tetradenate and pentadenate chelating agents. Furthermore, there is a difference in the results depending on whether the same chelating agent is immobilized on silica or polymer based chromatographic material.

The results (MSⁿ) showed that in positive ion mode or negative ionization mode without any labeling or modification AP-MALDI exhibited a neutral phosphate group loss of 98 Da (H₃PO₄) from peptides containing phosphoserine. Such phosphate loss was also observed on the MS/MS spectra of all other detected phosphopeptide peaks (data are not shown).

Conclusion

By using AP-MALDI-MS in combination with various micro affinity pipette tips (that contain a wide variety IMAC materials), a larger coverage of sequence of proteins as well as phosphorylation information about the post-translation modification has been obtained.

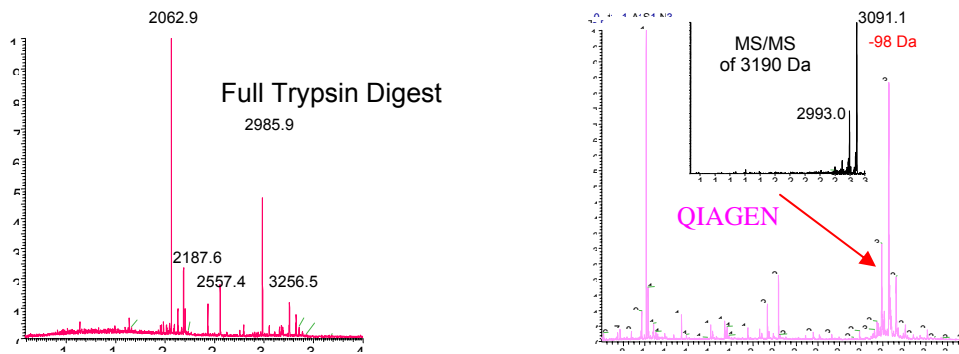


Figure 1. AP-MALDI spectra of tryptic digest β -Casein.

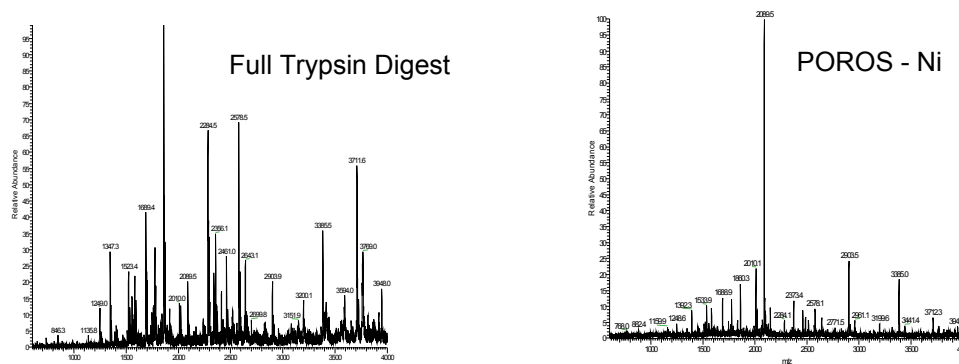


Figure 2. AP-MALDI spectra of tryptic digest Ovalbumin.

References

1. Moyer SC; Cotter RJ; Woods AS. Fragmentation of Phosphopeptides by Atmospheric Pressure MALDI and ESI/Ion Trap Mass Spectrometry. J. Am. Soc. Mass Spectrom. 2002, 13, 274-283.
2. Doroshenko VM; Laiko VV; Taranenko NI; Berkout VD; Lee HS. Recent Development in Atmospheric Pressure MALDI MS. J. Mass Spectrom. 2002, 221, 39-58.