

Rapid Proteomics Approach for the Identification of Peptide Hydrazide Adducts by Atmospheric Pressure MALDI MS/MS

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INTRODUCTION

Carboxylic acid groups on proteins can be activated by carbodiimide mediated hydrazine reaction to create reactive hydrazide centers on them for many bio-conjugation purposes. Carrier proteins used in making bacterial polysaccharide-protein conjugate vaccines are often hydrazide activated prior to coupling with activated polysaccharides. Identifying hydrazide covalent modification centers on a carrier protein is crucial to better characterize the conjugated product. However, there are no qualitative tests available for identifying the location of involved amino acid residues in the hydrazide modification. In this study we present a rapid proteomics based AP MALDI MS/MS method of identification of peptide hydrazide adducts in bovine serum albumin (BSA).

METHODS AND MATERIAL

Reagents: α -cyano-4-hydroxycinnamic acid (4-CHCA) was from Fluka (Buch, Switzerland). Immobilized trypsin beads was from Applied Biosystems (Foster City, CA, USA). BSA, hydrazine, EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide) and 2,4,6-trinitrobenzenesulfonic acid (TNBS) and other chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA).

Hydrazide reaction: BSA was hydrazine activated using carbodiimide mediated hydrazine reaction using standard protocol. Formed hydrazide on BSA was estimated by TNBS assay.

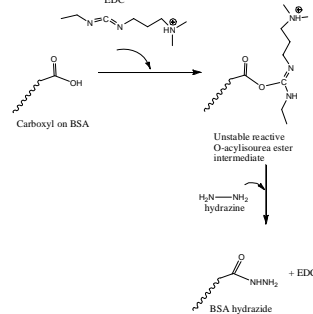
Mass Spectrometry:

All mass spectral experiments were carried out on a Thermo Finnigan (San Jose, CA, USA) LCQ Deca XP ion trap mass spectrometer integrated with an AP/MALDI ion source with pulsed dynamic focusing (MassTech Inc., Columbia, MD, USA) using positive ionization mode. C-18 coated MALDI target plates were prepared according to published procedure.

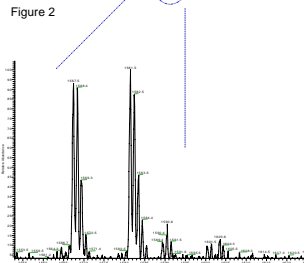
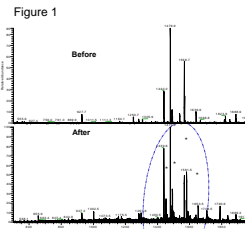
Sample processing protocol:

Control BSA and carbodiimide mediated hydrazine activated BSA were separately subjected to rapid tryptic digestion on C18 coated MALDI target plate at 50 °C. 1 μ l of sample was treated with 1 μ l of trypsin (immobilized on beads) and allowed to react until the sample dries out (about 1 minute). All sample processing was done on the MALDI target. Salts from reaction mix were then removed by washing with 3 μ l water. 1 μ l CHCA matrix solution was then added and AP-MALDI MS and MS/MS spectra were acquired on LCQ Deca XP ion trap MS fitted with an AP-MALDI ionization source. The MS/MS data was then used to search in the publicly available database using Mascot engine to confirm the identification of peptide sequences from BSA. In silico fragment analyses was done using PROWL.

EDC mediated hydrazine reaction on BSA



Results



Figures 1 and 2. AP-MALDI MS spectra of BSA before and after hydrazide activation. Region magnified to show m/z 1567.5 and 1581.5 to show the 14Da mass units increase as a result of hydrazide addition on the peptide.

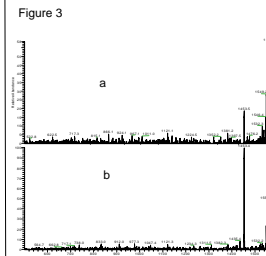


Figure 3. MS/MS patterns of peptide ions m/z 1567.5 from BSA before hydrazide modification (a) and peptide ion m/z 1581.5 after hydrazide modification (b).



Figure 4. PROWL peptide fragmentation of protonated peptide ion 1568 indicating the origin of this peptide from BSA

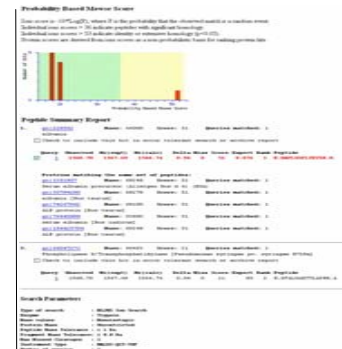


Fig 5. Mascot NCBI search result on MS/MS of m/z 1567.5

CONCLUSIONS

Using milder ionization method of AP-MALDI and rapid on probe tryptic digestion we have identified the hydrazide modified peptides. Four peptide ions from unmodified BSA (m/z of 1439.6, 1479.6, 1567.8 and 1639.9) show an increase of 14 Da mass units (m/z 1453.6, 1493.5, 1581.4 and 1653.9 respectively) in hydrazide activated BSA sample. Database search using Mascot confirmed the product ions origin.

PROWL analysis of MS/MS fragment spectra predicted the likely involvement of aspartic acid residue in hydrazide addition to peptide ion with m/z 1581.5. This approach using AP-MALDI (due to its mild ionization) can be applied to identify the hydrazide modified centers on any carrier protein whose sequence details are available.

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