MALDI Coupled with Negative Ion Electron Capture Dissociation (niECD) for Characterization of Labile Acidic Biomolecules

UNIVERSITY OF MICHIGAN

Steven A. DeFiglia, and Kristina Håkansson*

Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1055, U.S.A.

Overview

- Negative ion electron capture dissociation (niECD) is compatible with matrix assisted laser desorption/ionization (MALDI)
- Matrix choice does not affect fragmentation
- MALDI appears to produce zwitterions
- niECD of MALDI-generated 1- phosphopeptide yields more fragments than for ESI-generated 2- anions
- Successful MALDI niECD allows for sulfation characterization in peptides with multiple sulfation sites
- MALDI niECD fragmenation of lipid A, RNA
- MALDI-generated 1- ubiquitin captures an electron but does not yield fragmentation

Introduction

Acidic chemical groups, e.g., phosphorylation/sulfation, are common constituents of biomolecules, including peptides, lipids, and glycans. To maximize precursor ion signal in tandem mass spectrometry (MS/MS), negative mode ionization is preferred; however, collision induced dissociation often shows poor structural information including preferential loss of such labile groups. Thus, alternative MS/MS activation approaches are needed. niECD [1] has been proposed to require gaseous zwitterionic structures and is most compatible with singly charged anions. The latter characteristic suggests that MALDI may be superior to electrospray ionization (ESI) for niECD implementation; however, the former characteristic suggests that MALDI may not be appropriate. Here, we show that niECD indeed proceeds for MALDI-generated gaseous anions for structural characterization of, e.g., isomeric sulfopeptides. Sulfation characterization is important because it impacts inflammation, fertility, viral entry, and protein-protein interactions.

Methods

Experiments were performed on a 7T Bruker SolariX FT-ICR with a MALDI source and an XR Paracell detector. The ECD cathode was set to 1.5 A, -6 V bias for up to 10 second irradiation time. Hirudin sulfopeptide was used to tune for niECD [2]. Matrices used include α-cyano-hydroxycinnamic acid (CHCA), 2,5-dihydroxybenzoic acid (DHB), 1,8-diaminonapthalene (DAN), sinapinic acid (SA), 9-aminoacridine (9AA), 4-chloro-cyanocinnamic acid (Cl-CCA), and 1,8-bis(diamino) napthalene (PS). Samples were spotted using the dried drop method at 0.5 µL. MALDI matrices were obtained from Sigma-Aldrich. Synthetic peptides were acquired from Genscript, oligonucleotide GCAUAC was obtained from Integrated DNA Technologies. Hirudin was purchased from Bachem, β-casein phosphopeptide was ordered from Anaspec. Matrices were dissolved in 75:25 MeOH:Isopropanol for matrix comparisons, and 30:70 acetonitrile:0.1% trifluoroacetic acid (TA30) for all other experiments.

Results

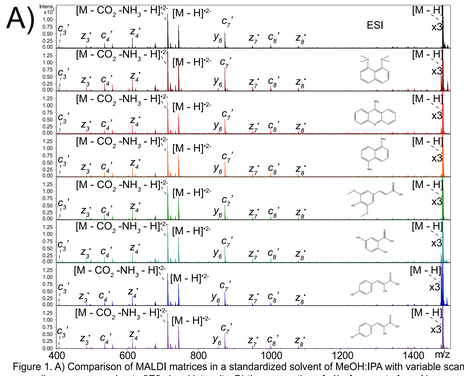
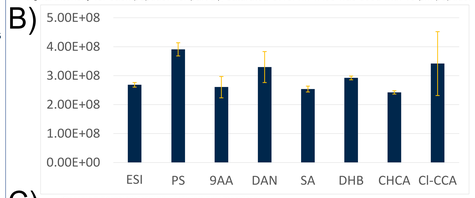
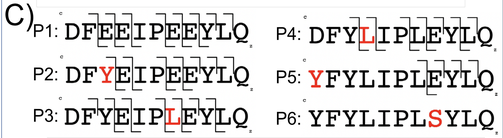
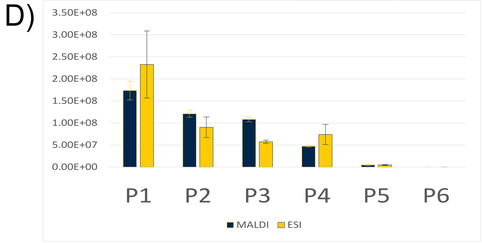


Figure 1. A) Comparison of MALDI matrices in a standardized solvent of MeOH:IPA with variable scans to normalize precursor anion to 3E8 signal intensity. B) the summation of $c'/z^{\bullet}$ fragments from A), arranged in order of decreasing matrix proton affinity. No significant differences occur based on proton affinity. C) Hirudin based synthetic peptide set with acidic residues modified to neutral residues to reduce zwitterion formation probablity. As the peptides become more neutral, fewer niECD fragments occur. D) The summation of $c'/z^{\bullet}$ fragments from the peptide set. A variable number of scans were averaged to reach 8E8 precursor signal intensity for each peptide. For part D), saturated CHCA in TA30 was used with 10 µM peptide.







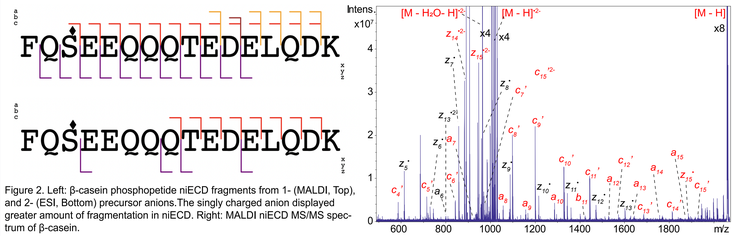


Figure 2. Left: β-casein phosphopetide niECD fragments from 1- (MALDI, Top), and 2- (ESI, Bottom) precursor anions.The singly charged anion displayed greater amount of fragmentation in niECD. Right: MALDI niECD MS/MS spectrum of β-casein.

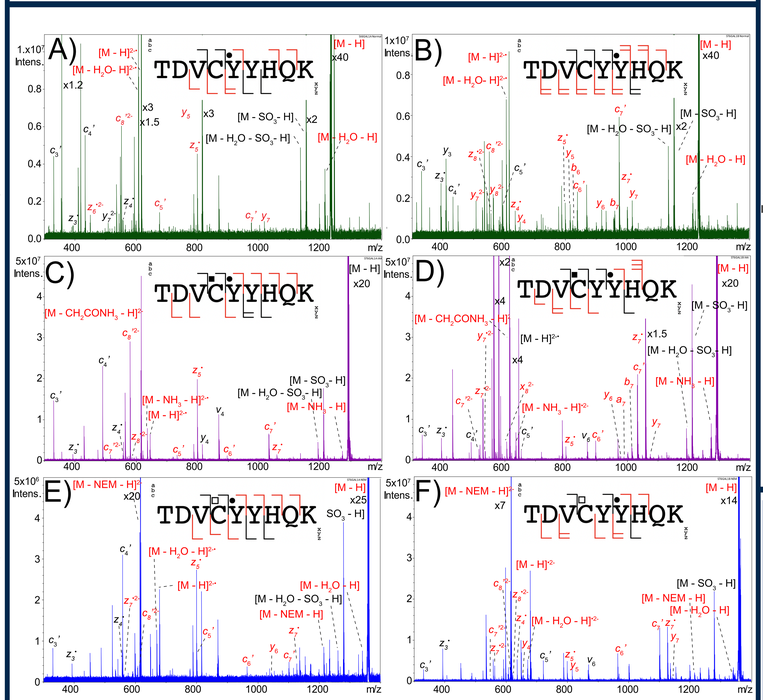


Figure 3. A) niECD spectrum of ST6GAL1A (TDVCY*YHQK). B) niECD spectrum of ST6GAL1B (TDVCYY*HQK). C) niECD spectrum of ST6GAL1A with iodoacetamide alkylated cysteine. D) niECD spectrum of ST6GAL1B with iodoacetamide alkylated cysteine. E) niECD spectrum of ST6GAL1A with N-ethylmaleimide alkylation. F) niECD spectrum of ST6GAL1B with N-ethylmaleimide alkylation. Sulfation location is characterized under every condition except for in D), in which the $z_4^{\bullet}$ fragment is obscured by an interfering ammonia loss from the charge-increased precursor ion. All spectra were collected with 256 scans averaged at 5 s irradiation at -6 V ECD bias and -5 V ECD lens. Red fragments contain sulfation.

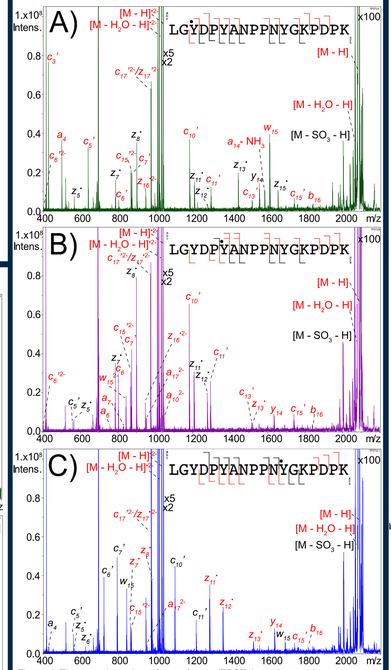


Figure 4. The tyrosylprotein sulfotransferase (TPST1) tryptic peptide LGYDPYANPPNYGKPDPK has 3 potential sulfation sites. niECD of A) LGY*DPYANPPNYGKPDPK, B) LGYDPY*ANPPNYGKPDPK, and C) LGYDPYANPPNY*GKPDPK. niECD spectra were collected with 32 scans averaged at 5 s irradiation at -6 eV ECD bias and -5 V ECD lens. Red fragments contain sulfation.

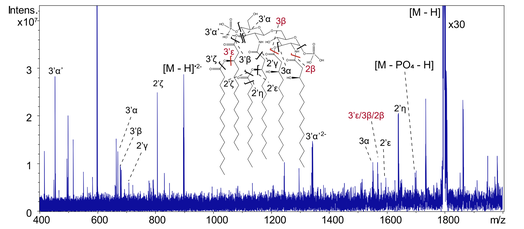


Figure 5. MALDI niECD of *E.coli* Lipid A with 10 s irradiation, -6 V ECD bias and -5 V ECD lens for 200 averaged scans. Typical acyl losses were observed but unique acyl chain fragment, 3'α˙, was also directly measured.

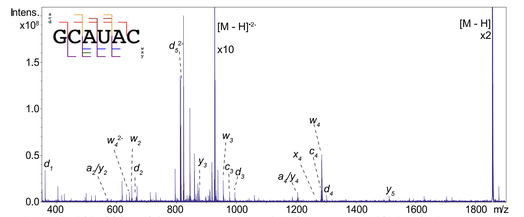


Figure 6. MALDI niECD of GCAUAC RNA with 5 s irradiation, -6 V ECD bias and -5 V ECD lens for 64 averaged scans.

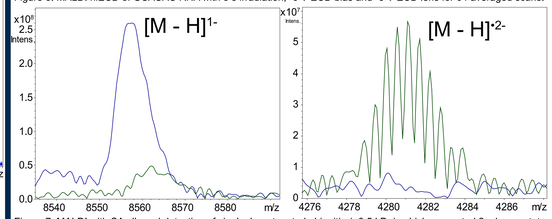


Figure 7. MALDI with SA allowed detection of singly deprotonated ubiquitin (~8.5 kDa), which generated 2- charge state upon 0.5 s irridiation with -6 V ECD bias and -5 V ECD lens over 200 averaged scans. These data represent the highest m/z anion undergoing electron capture to date. Left: Control, Right: After electron.

Conclusions

Matrix choice does not inhibit niECD and the decrease in $c'/z^{\bullet}$ ion abundance in a peptide set designed to decrease in zwitterionic potential suggests the presence of zwitterions in MALDI. niECD of MALDI generated ions yields greater fragmentation of acidic biomolecules than ESI 2- anions. MALDI niECD allows for sulfation localization of all ST6GAL1 isomers except for iodoacetamide alkylated ST6GAL1B. For the first time TPST1 is fully characterized without extensive derivatization [3]. MALDI niECD of lipid A, and GCAUAC yields fragmentation. Irradiation of MALDI generated ubiquitin 1- ions creates the largest anion charge increase species so far recorded.

References and Acknowledgements

[1] Yoo, H.J.; Wang, N.; Zhuang, S.; Song, H. Håkansson, K.; *J. Am. Chem. Soc. 2011, 133, 16790–16793.*
[2] Hersberger, K.E.; Håkansson, K. *Anal. Chem, 2012, 84, 6370–6377.*
[3] Jang, E., DeFiglia, A. S.; Håkansson, K. *Proc. 70th Annual ASMS Conference on Mass Spectrometry and Allied Topics; Minneapolis, 2022.*

NSF
NIH