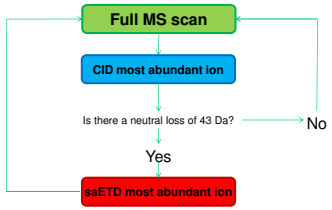
Neutral Loss-Triggered Electron Transfer Dissociation Mass Spectrometry for the Identification of Citrullination of Arginine.

Andrew J. Creese[1], Melissa M. Grant[1], Iain L C. Chapple[1], Helen J. Cooper[2]

1. School of Dentistry, University of Birmingham, St Chads Queensway, Birmingham, B4 6NN, UK.
2. School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK.

Overview

- Labile post-translational modifications are often lost from precursor ions during collision induced dissociation (CID). Data-dependent neutral loss tandem mass spectrometry targets this loss. If one of the dominant peaks in the CID spectrum represents a neutral loss the ion is re-analysed using supplemental activation electron transfer dissociation (saETD).



- Here we ask: Can data-dependent liquid chromatography NL-saETD be used to confidently identify sites of citrullination of proteins in bottom-up proteomics?

Introduction

- Citrullination of arginine is a post-translational modification which increases the mass of arginine by 0.9804 Da. It is a biomarker for rheumatoid arthritis, multiple sclerosis and Alzheimer's disease.
- We show online data-dependent liquid chromatography (LC) NL-saETD analysis of a six protein mix and a saliva digest spiked with three synthetic citrullinated peptides performed on a Thermo Fisher Scientific LTQ Orbitrap-Velos-ETD mass spectrometer.
- The results demonstrate that data-dependent NL-saETD is a highly useful tool in the targeted identification of citrullination.

Method

- A 100 µL saliva sample was reduced and alkylated prior to digestion with trypsin and diluted to ~40 ng/µL in 0.1% formic acid.
- Three synthetic peptides (ILN$_c$RTSFAK, VVE$_c$RHQSACK, LYNLHGD$_c$RSYVLSK. $_c$R is citrullinated arginine) were resuspended in water to a final concentration of 1 pmol/µL.
- Either 50 fmols of six protein mix spiked with 25 fmols of the three peptides or 5 µL of saliva trypsin digest with 25 fmols of the three peptides was loaded onto a 75 µm C18 reversed phase analytical column (LC Packings). Peptides were separated over a 30 minute gradient from 3.2 to 44% acetonitrile.
- Samples were infused by use of an Advion Triversa Nanomate nanospray ionization source into a Thermo Fisher Scientific LTQ Orbitrap-Velos-ETD hybrid mass spectrometer.
- ETD was performed with fluoranthene anions with activation for 130 ms. Supplemental activation was used (normalized collision energy of 25%). saETD spectra were recorded in the Orbitrap.
- Data were analysed with Xcalibur 2.1 and Proteome Discoverer sp 1.0 software (Thermo Fisher Scientific). All spectra are from the saliva analysis.

Results

Peptide	Saliva		Sixmix	
	CID	saETD	CID	saETD
$[ILN_cRTSFAK]^{2+}$	1.7	1.7	2.06	1.64
$[VVE_cRHQSACK]^{3+}$	-	2.82	0.67	2.89
$[LYNLHGD_cRYVLSK]^{2+}$	1.54	2.8	1.6	-
$[LYNLHGD_cRYVLSK]^{3+}$	1.1	4.56	0.91	4.57

Table 1: XCorr scores for CID and saETD the three citrullinated peptides from the SEQUEST searches.

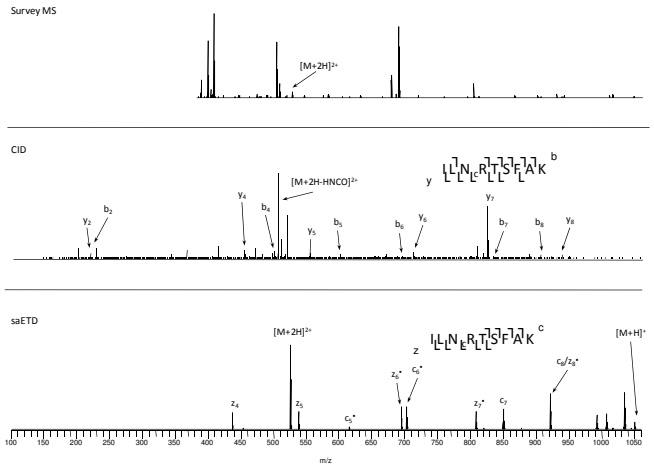


Figure 1: The neutral loss process for the citrullinated peptide $[ILN_cRTSFAK]^{2+}$ ions. In both the CID (middle) and saETD (bottom) spectra all N-C$_o$ (CID) or N-C$_\alpha$ (saETD) bonds are cleaved. Both were identified in a SEQUEST search.

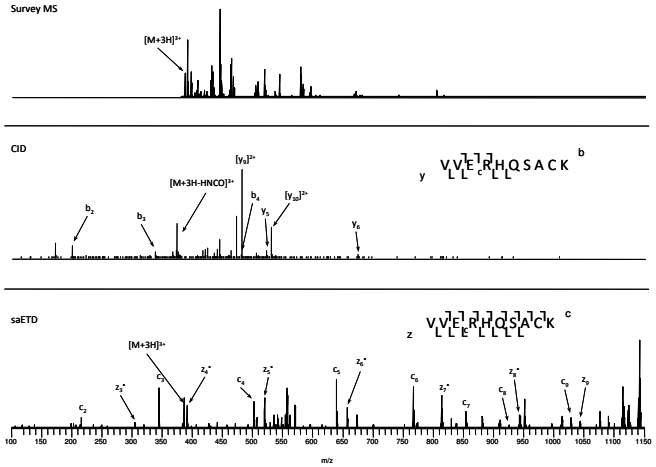


Figure 2: The neutral loss process for the citrullinated peptide $[VVE_cRHQSACK]^{3+}$ ions. All N-C$_\alpha$ bonds are cleaved in the saETD spectrum (bottom). Only 5 N-C$_o$ bonds were cleaved in the CID spectrum (middle). The CID spectrum did not result in a positive SEQUEST identification.

	Saliva				
	MS/MS Events	Positive peptide ID	Positive ID of synthetic peptides	False ID of citrullinated peptides	False positive rate (%)
CID	1280	324	8	17	5.2
saETD	64	21	18	0	0.0

	Sixmix				
	MS/MS Events	Positive peptide ID	Positive ID of synthetic peptides	False ID of citrullinated peptides	False positive rate (%)
CID	721	186	8	4	2.2
saETD	56	11	8	0	0

Table 2: Identification rates for CID and saETD from the SEQUEST searches of saliva and sixmix datasets.

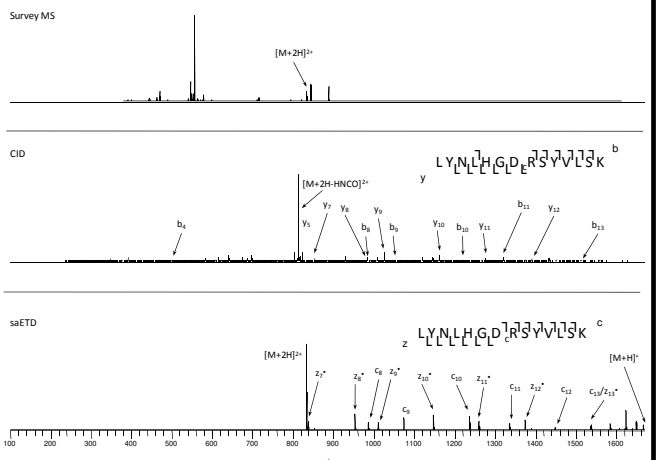


Figure 3: The NL process for the citrullinated peptide $[LYNLHGD_cRSYVLSK]^{2+}$ ions. In the saETD spectrum (bottom), all 13 N-C$_\alpha$ bonds are cleaved. In the CID spectrum (middle), twelve N-C$_o$ bonds are cleaved. Both the CID and saETD spectra resulted in positive SEQUEST identifications.

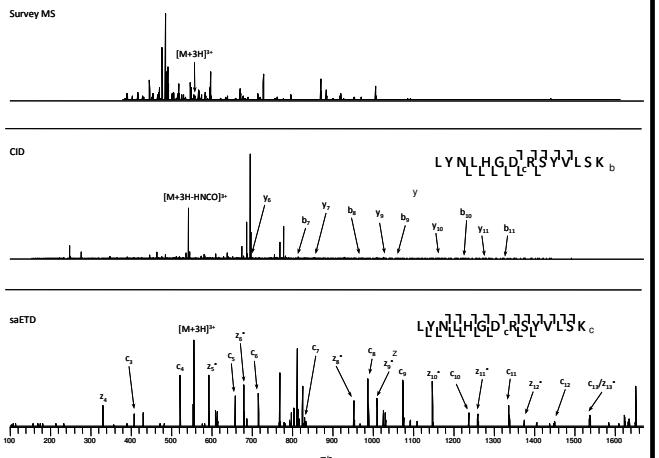


Figure 4: The neutral loss process for the citrullinated peptide $[LYNLHGD_cRSYVLSK]^{3+}$ ions. In the saETD spectrum (bottom), all N-C$_\alpha$ bonds are cleaved however in the CID spectrum, nine N-C$_o$ bonds are cleaved. Both spectra resulted in positive identifications by SEQUEST.

Conclusion and Future Work

- We have demonstrated data-dependent NL-saETD of tryptic proteins is suitable for the targeted identification of citrullinated peptides.
- Analysis of the CID mass spectra alone resulted in several false positive citrullinated peptides. None of these triggered saETD and on manual inspection were observed as either incorrect assignments or deamidated peptides.
- This method combines the high scan speed of CID with the high assignment confidence of saETD.
- Further work in this area will include analysis of diseased samples to identify novel citrullinated proteins.

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Andrew Creese, School of Dentistry, University of Birmingham, St Chads Queensway, Birmingham, B4 6NN, UK. E-mail:a.creese.2@bham.ac.uk