Characterization of Protein/PTM Changes by Chip Capillary Electrophoresis Mass Spectrometry

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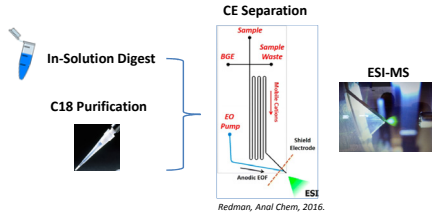
Overview

- Characterization of post-translational modifications (PTMs), specifically complex protein glycosylation, remains a challenge for high-throughput mass spectrometry applications.
- Standard C18 nano-LCMS approaches are sufficient for certain PTM compound classes but are unable to adequately resolve individual glycoforms.
- Capillary electrophoresis mass spectrometry (CE-MS) offers high-resolution separation capabilities which are unique and potentially offer advantages for glycoproteomics analyses.
- We successfully conducted a series of experiments in collaboration with 908 Devices (Boston, MA) using chip-based CE-MS and demonstrated the ability to obtain high-resolution separation on digests of proteins and glycoproteins.(1)
- Here we present results of this continued work and demonstrate improvements in the entire analytical pipeline including sample preparation, separation parameters, MS and MS/MS data acquisition and bioinformatics approaches for data interpretation.

Applications of CEMS



Redman, Anal Chem, 2016.

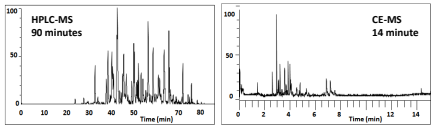
Approach

- CE was performed using both prototype and commercially available second generation ZipChip ™ CE interfaces obtained though collaboration with 908 Devices.
- CE-MS analyses were performed by interfacing the device with a QExactive MS or a QExactive HF MS (Thermo-Fisher).
- Samples included in-solution protein digests with emphasis on protein glycosylation.
- Peptides/proteins were prepared in up to 50% organic solvent (ACN, MeOH or isopropanol) prior to CEMS; picomole to low femtomole sample loads were analyzed.
- MS/MS was performed using DDA and AIF modes which were optimized for high-throughput bottom-up acquisition. A wide range of MS, MS/MS and AIF parameters were explored.
- Feature identification was enabled by analyzing the data using Proteome Discoverer (Thermo-Fisher), Mascot (Matrix Science), Scaffold (Proteome Software), PEAKS Studio (Bioinformatics Solutions) and Byonic (Protein Metrics).
- Detailed assignments of glycopeptides were achieved using GlycReSoft(2) and BUPID-PALM (Boston University Protein Identifier-Parallel Acquisition Library Matcher) software developed in our laboratory.
- Top down MS and MS/MS analysis was performed using BUPID software developed in our laboratory at BUSM.

Results

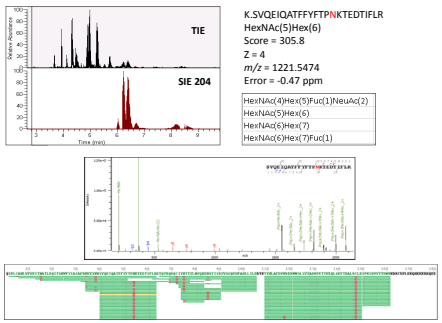
- While we have demonstrated that chip CE-MS allows for unique separation of glycopeptides (1), we have observed that we sometimes obtain broad peaks due to the presence of sialic acid termini on N- or O-linked glycans.
- We also observed that, in some cases, due to fast separation and narrow peaks, the quality of MS/MS data was relatively poor or precursors were missed.
- We further explored different electrolytes to improve CE separation, evaluated different MS and MS/MS data acquisition parameters to accommodate narrow peak widths, and expanded upon our initial set of CE and MS parameters.
- Chip-based CE-MS of proteolytic digests of A1AG1 yielded 7-minute to 15-minute run times, depending on conditions and obtained data comparable with >60-minute LC-MS experiments.

Comparison of CE-MS with LC-MS: A1AG1 Peptides



- Setting the *m/z* range 600-3000 resulted in an increase in S/N compared with *m/z* 370-1800 and did not result in the loss of glycopeptide MS/MS data.
- MS2 threshold was set at 2e5 to 5e5 and yielded more confident assignments of higher quality MS2 data.
- Reducing the duty cycle to Top 4 allowed for more cycle time, improved MS1 TIE (SIE) and accommodated narrow CE peaks.
- Glycopeptide MS/MS required 100msec fill/transient at 17,500 resolution. We observed that 2 uscans yielded improved glycopeptide MS/MS data.

CE-MS: A1AG1 Peptides/Asialo-Glycopeptides

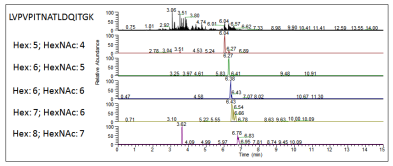


- Use of different enzymes allowed for improved digestion to peptides, and more control on peak capacity.
- Desialylation improved peak shapes and CE run times.
- Arg-C digests yielded larger glycopeptides which behaved well under the CE conditions of separation.

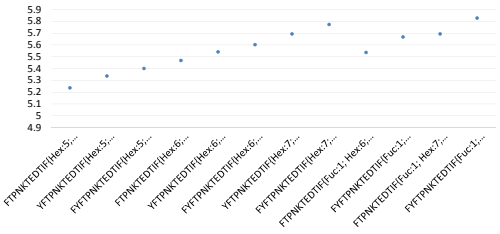
Results

- Glycopeptides were consistently well resolved from peptide peaks by CE-MS.
- The individual glycopeptides migrated in terms of increasing peptide mass and oligosaccharide composition.
- This improvement in resolution compared with LCMS would potentially allow for improved differential glycan structure quantification.

CE-MS of A1AG1 Glycopeptides: Increasing Migration Times

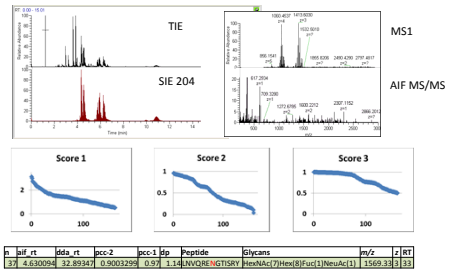


Migration Times: CE-MS Chymotrypsin Peptides FYFTPNKTEDTIF



- Optimal glycopeptide MS/MS data requires longer duty cycles in comparison to peptide MS/MS: 100 msec and 2 uscans. This resulted in loss of identifications for lower S/N glycopeptides.
- Use of targeted exclusion and inclusion lists via a 2-run strategy improved sequence coverage and assignment of glycopeptides.
- Implementation of wide precursor windows, AIF and alternating high/low collision energy also improved our ability to obtain high quality sequence information for glycopeptides.
- In order to interpret data obtained using large precursor window isolation, AIF and high/low fragmentation modes, we utilized a spectral library search approach.

A1AG1: CE-MS AIF MS/MS: Glycopeptide ID via BUPID-PALM

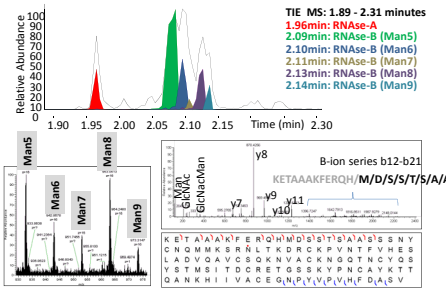


n	aif_rt	dda_rt	pcc-2	pcc-1	dp	Peptide	Glycans	m/z	z	RT
37	4.630094	32.89347	0.9003299	0.97	1.14	LNVQRNGTISRY	HexNAc(7)Hex(8)Fuc(1)NeuAc(1)	1569.33	3	33

Results

- Lastly we explored the use of Top Down MS and MS/MS for glycosylation characterization.
- Intact RNAse-B was used as a model glycoprotein due to its low molecular weight and single glycosylation site.
- Different CE voltages and electrolytes were used.
- MS and MS/MS parameters were varied in a similar means as the bottom up experiments.
- We also implemented targeted MS/MS windows and AIF modes for acquisition.
- We were able to separate RNAse-B from RNAse-A.
- Resolution of the different high-mannose forms of RNAse-B was achieved.
- Both targeted MS/MS and AIF modes yielded sufficient information to characterize RNAse-B and the individual glycoforms.

Top Down CE MS/MS of RNAse-B



Summary

- These strategies further improved the quality of the data and allowed for a greater number of confident glycopeptide assignments.
- In conclusion we demonstrate improved approaches for CE-MS for characterization of complex protein glycosylation.
- See poster MP 359 BUPID-PALM: Glycopeptide Identification by All-ion Fragmentation(AIF) Ion Mobility MS/MS; Christian Heckendorf; Joshua A. Klein; James A. Hill; Catherine E. Costello; Mark E. McComb; Boston University School of Medicine, Boston, MA

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- (1) Khatri K, et al. Anal. Chem. 2017 PMC5554952.
- (2) Maxwell, E., et al. PLoS ONE 2012 PMC3458864.