Microfluidic Capillary Electrophoresis – Mass Spectrometry for Glycomics and Glycoproteomics

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Overview

As mass spectrometry instrumentation becomes faster and more sensitive, overall method time, resolution and depth of analysis are now limited by the front-end separation methods. CE offers high peak capacity for glycoform resolution because separation is based on charge, size and shape. Traditional CE systems lacked convenient interfaces for mass spectrometers, thus affecting their popularity for applications in glycomics and glycoproteomics. With recent developments in technology, integrated microfluidic CE systems with integrated nano-electrospray ionization interfaces are now commercially available for easy coupling to mass spectrometers. The reduced scale of the CE device also improves sensitivity and allows analysis of small sample quantities. We demonstrate the application of an integrated CE-ESI-MS setup for glycomics and glycoproteomics.

Introduction

CE offers high peak capacity for glycoform resolution because the separation is based on charge, size and shape. In contrast to LC and nano-LC, CE systems have lacked convenient interfaces for mass spectrometers, and this situation has limited their utilization for applications in large-scale glycomics and glycoproteomics studies. Due to recent developments in technology, microfluidic CE systems with integrated nano-electrospray ionization interfaces are commercially available for easy coupling to mass spectrometers. The compact scale of these CE devices improves sensitivity and facilitates analysis of small sample quantities.

The CE-MS setup used in this work integrates a microfluidic separation system with a nanoESI source on a single piece of hardware that works as a removable interface for mass spectrometers. As shown in the schematic in Figure 1, sample injection, background electrolyte and ESI solution are controlled independently in a manner similar to the liquid-junction interface described above. The microfluidic setup minimizes gaps, dead volume, sample dilution, and band broadening, to maintain speed and sensitivity in analyses. The separation capillary surfaces are coated with an aminopropyl silane reagent to minimize EOF, as described previously (1).

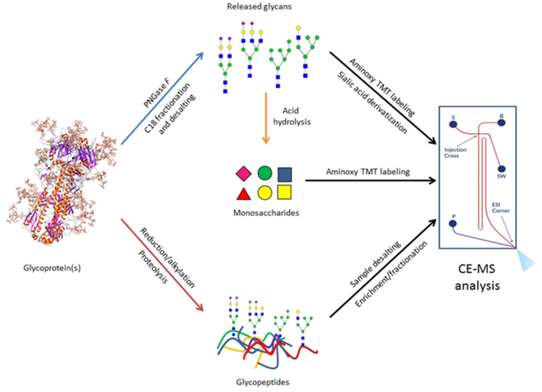

Figure 1: Figure 1: Overview of workflow for analysis of monosaccharides, oligosaccharides and glycopeptides using microfluidic CE-ESI-MS. Chip schematic on the right shows sample reservoir "S", background reservoir "B", ESI pump "P" and sample waste "SW".

We developed a CE-MS method for analysis of monosaccharides, oligosaccharides and glycopeptides to enable comprehensive glycoprotein analysis. The method uses the same electrophoresis solutions for all three compound classes, greatly simplifying the task of glycoprotein characterization. We used the aminoxy TMT regent (Figure S-1) to improve electrophoretic migration of neutral saccharides and to enable multiplexing in tandem-MS experiments. We also used a sialic acid derivatization method to prevent interaction of the negatively charged carboxyl groups with the positively charged capillary surface coating and discriminate sialic acid linkages. In summary, we present a CE-MS method using a convenient mass spectrometry interface for glycan, monosaccharide and glycopeptide analysis.

Methods

All experiments were performed on a ZipChip capillary electrophoresis-electrospray ionization interface (CE-ESI) (908 Devices, Boston, MA), using a high-resolution chip with capillary length 22 cm, coupled to a Q-Exactive Plus mass spectrometer (Thermo Scientific, San Jose, CA). A CE voltage of 20 kV was applied with a shield potential set at 500 V. ESI voltage varied between 2200-2700 V for the different samples and chips. A background electrolyte (BGE) and ESI solution containing 50% methanol, 48% water and 2% formic acid was used for all experiments. Pressure-assisted CE, a feature of the CE-MS interface, was used for oligosaccharide sample analysis to speed analysis times. Pressure-driven injection allows transient isotachophoresis for the analyte but dilutes the sample solution in cases where low sample volumes are loaded in the sample well. All MS data were acquired in the positive-ion mode. Released glycans and monosaccharides were derivatized with aminoxy-TMT prior to analysis. Sialic acid derivatization was performed using the method described by Reiding and coworkers**(2)**.

Results

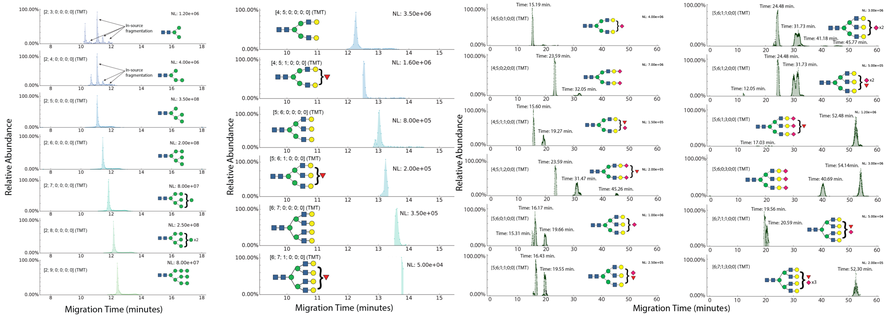

Figure 2: Neutral glycans – stacked EIEs showing pauci- and high-mannose N-glycans (left panel) and complex-type asialo N-glycans from a mixture of released glycans from bovine RNAseB human transferrin and human AGP. Glycan compositions are represented as [HexNAc; Hex; dHex; Total NeuAc; Esterified NeuAc; Lactonized NeuAc].

Figure 3: Sialylated glycans – EIEs showing sialylated glycan with underivatized sialic acids, from human transferrin and AGP. Glycan compositions are represented as [HexNAc; Hex; dHex; Total NeuAc; Esterified NeuAc; Lactonized NeuAc].

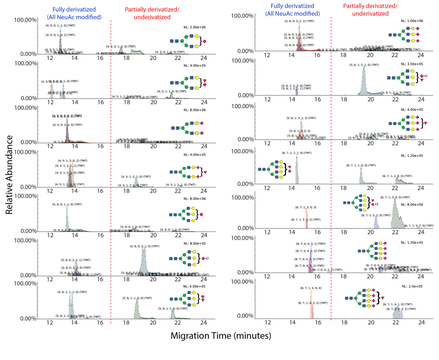

Figure 4: Derivatized sialylated glycans – EIEs showing sialylated glycan with derivatized sialic acids, from human transferrin and AGP. Glycan compositions are represented as [HexNAc; Hex; dHex; Total NeuAc; Esterified NeuAc; Lactonized NeuAc].

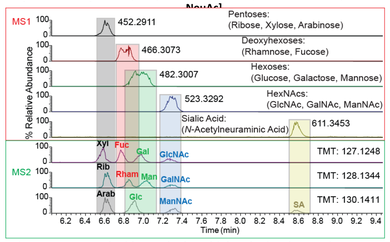

Figure 5: Monosaccharide standards multiplexed using three aminoxyTMT reagents to enable identification of isomers on the basis of CE migration and tandem MS.

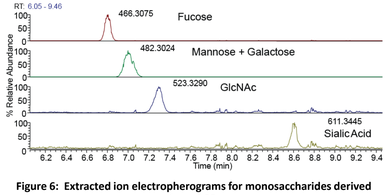

Figure 6: Extracted ion electropherograms for monosaccharides derived from AGP.

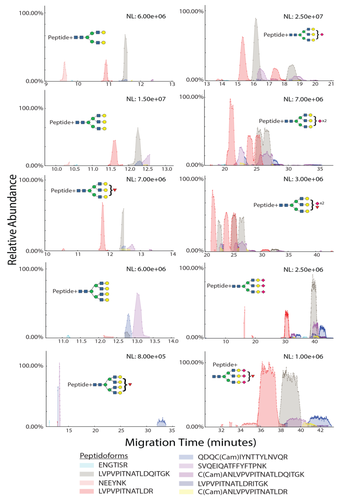

Figure 7: Glycopeptide migration by peptide backbone. Each electropherogram overlays the extracted ion trace for the same glycoform linked to a different peptide backbone. "Cam" stands for cysteine carbamidomethylation. Glycan topologies shown are speculative.

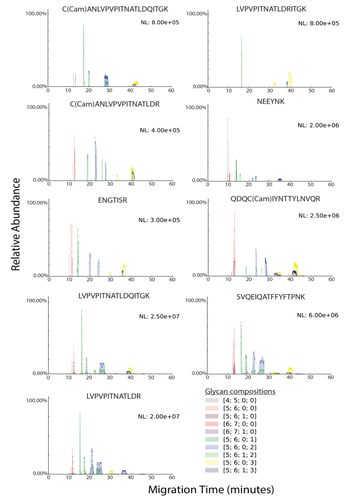

Figure 8: Glycopeptide migration by glycan composition. Each electropherogram overlays the extracted ion trace for a different glycan composition linked to the same peptide backbone. "Cam" stands for cysteine carbamidomethylation. Glycan compositions are represented as [HexNAc; Hex; dHex; NeuAc].

Discussion and conclusions

As shown in Figure 2, migration of neutral oligosaccharides was based on size and number of saccharide units. The addition of sialic acids to oligosaccharides appeared to retard migration, as shown in Figure 3. To eliminate the effects of sialic acid on migration due to possible interactions with the capillary surface, we performed sialic acid linkage specific derivatization that neutralized the carboxylic acid group and introduced linkage specific mass shifts. The migration of derivatized oligosaccharides is shown in Figure 4. The migration times of fully derivatized oligosaccharides decreased to under 16 minutes. Monosaccharide analysis was first performed using standards to estimate exact migration times, as shown in Figure 5. Monosaccharide isomers were pre-labeled with isobaric TMT-tags with different MS2 reporter ions and mixed together prior to analysis, which allowed discrimination of their migration profiles by tandem MS. Monosaccharides obtained from Human AGP were then analyzed using the migration times of standards, as shown in Figure 6. Glycopeptide migration was found to be a factor of both glycan size/monosaccharide units and the peptide length. Figures 7 shows effects of changing peptide backbone for a group of glycopeptides with the same glycan composition, on overlaid EIEs. Figure 8 shows the effects of changing glycan composition on glycopeptides with the same peptide backbone.

The ZipChip CE-ESI device allowed easy interfacing of the CE separation capabilities with mass spectral detection. The integrated sprayer obviated the need for complex ESI source setups on CE-MS systems and delivered stable nanoESI for all experiments. Our methods utilized the same mass spectrometer friendly buffer system for glycopeptides, released glycans and monosaccharides. We showed that the same CE conditions allow analysis of three different analyte classes, thus eliminating the need for switching among different CE methods and capillary chemistries. The CE device delivered rapid separation and sensitive detection of oligosaccharides, with the sialic acid derivatization procedure allowing identification of sialic acid linkages.

Acknowledgements

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References

1. Batz, N. G.; Mellors, J. S.; Alarie, J. P.; Ramsey, J. M. Anal. Chem. 2014, 86, 3493–3500.
2. de Haan, N.; Reiding, K. R.; Haberger, M.; Reusch, D.; Falck, D.; Wuhrer, M. Anal. Chem. 2015, 87, 8284–8291