High Resolution Intact Glycoprotein Analysis by CESI-MS

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Introduction

The analysis of intact proteins by Electrospray Ionization - Mass Spectrometry (ESI-MS) provides the most direct route for the identification and characterization of these highly complex molecules. With the potential presence of multiple isoforms, it is also widely accepted that the presence of a liquid-phase separation technique upstream from the MS detection step would greatly contribute to a complete deciphering of the structure of these molecular entities. Capillary electrophoresis (CE), compatible with the preservation of delicate protein structure, post-translational and other modifications, while simplifying sample complexity through high resolution separation capabilities, appears as a very remarkable alternative prior to introduction into the mass spectrometer. To achieve the greatest advantage of coupling CE with ESI-MS for intact protein analysis, we are currently developing a novel technology called CESI that integrates CE with ESI into a single dynamic process. The CESI technique has been successfully implemented for the analysis of a model glycoprotein, Ribonuclease B (RNase B). The efficient coupling between these liquid and gas phase separation systems allowed the rapid, sensitive and high resolution detection of the five known Ribonuclease B glycoforms. Further deconvolution of the resulting Time of Flight (ToF) mass spectra by the Maximum Entropy algorithm identified differences in the attached high mannose structure as the source of the electrophoretic heterogeneity. Produced on multiple sheathless sprayers, the data confirm that these separations are highly repeatable and reproducible.

Materials and Methods

Instrumentation

All CE-MS separations were performed on a prototype CESI 8000 High Performance Separation - ESI Module. Separations were performed on OptiMS 100 cm long 150 µm o.d. x 30 µm i.d. Surface+ coated capillaries. Instrument temperature settings were 10°C for the sample storage module and the OptiMS capillary was maintained at 20°C with a rapidly circulating liquid coolant. MS detection was performed using a Waters Xevo® QToF set with a scanning range between 800 μm and 3000 Da. Electrospray ionization was achieved at 1.4 – 1.6 kV with the OptiMS 8000 sprayer needle filled with background electrolyte (BGE). 200 mM Ammonium Acetate pH 3.6 BGE Solution A A 200 mM acetic acid solution was produced by diluting 5.7 mL of 17.4 M glacial acetic acid (EMD P/N UN 2750) in 49.3 mL of 16-MΩ water using a 500 mL volumetric flask. The 200 mM acetic acid solution was titrated to a pH of 3.6 with a 200 mM ammonium acetate solution produced by diluting 5.7 mL of 15.9 M ammonium acetate (Sigma P/N A2706-1L) in 97.3 mL of 16-MΩ water using a 100 mL volumetric flask. 

Capillary Coating Procedure

The reagents used are as follows: Optima® LC/MS grade methanol (MeOH) (Fisher Scientific P/N 485-11), 0.1 M sodium hydroxide (NaOH) (Beckman Coulter P/N 391988), 0.1 M hydrochloric acid (HCl) (Beckman Coulter P/N 391946). Prior to introducing the Surface+ Coating Reagent (Beckman Coulter P/N B008746), the capillary was conditioned by performing a series of conditioning step washes. Following the conditioning step, a 20% mixture of Surface+ Coating Reagent in MeOH was introduced into the capillary at 50 psi for 65 min. The coating reagent was left to incubate in the capillary overnight to allow for the coating attachment between the capillary wall and the cationic polymer in the Surface+ coating reagent. After incubation, the system was purged of the Surface+ coating reagent using a series of purge steps.

Capillary Maintenance Procedures

Newly coated capillaries were cleaned, and used capillaries were prepared for storage following the completion of an analytical cycle by first purging the separation capillary and conductive solution capillary for 5 min. at 50 psi with MeOH, then electrokinetically cleaned at 20 kV in reverse polarity. After cleaning the capillaries, both capillaries were air-dried using pressure rinses with empty vials for 5 min. at 50 psi.

Protein Analysis Procedures

Prior to starting an analytical cycle, the OptiMS sprayer needle was filled with conductive by applying 50 psi on a vial in the buffer outlet tray containing BGE for 1 min. The cationic coating was conditioned by applying 50 psi on a vial in the buffer inlet for 10 min., then 20 kV in reverse polarity for another 10 min. Protein samples were hydrodynamically injected into the separation capillary using 5 psi for 5 sec, for a total plug volume of approximately 7 nL and separated using a 20 kV potential in reverse polarity. A separate BGE vial was used to rinse the capillary for 6 min. at 50 psi following the completion of the separation.

References

7. In development.

Table 1. Shows the results of 100 compositional analysis runs of RNase B glycoforms performed on 3 separate capillaries over 5 days. The results show that the analysis method is both highly repeatable and reproducible.

Conclusions

- Surface+ Coated OptiMS capillaries are capable of separating and detecting a broad range of proteins.
- Separations of RNase B produced five highly resolved peaks which were identified as high mannose glycoforms.
- Highly reproducible assessment of the percent composition were achieved with RSD ranging between 3.65% to 8.45%.