Subunit Level Top-down ECD Characterization of Biotherapeutic Proteins: Improving Mass Spectral Clarity with Fragment Ion Level Ion Mobility

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Introduction

- Electron Capture Dissociation (ECD) generates complementary data to CID by cleaving N–Cα bonds in protein/or peptide backbone and preserves labile side-chain modifications.
- ECD provides more detailed structural information, increased sequence coverage and greater confidence for characterization of proteins and their PTMs.
- Bovine Carbonic Anhydrase II (BCA) and NIST mAb subunits are used to optimize system operation conditions for top-down ECD study on a SELECT SERIES™ Cyclic IMS (cIMS) system.
- Increased sequence coverage (when compared ECD to CID) demonstrates the potential benefit of using ECD on a cIMS system.

Experimental

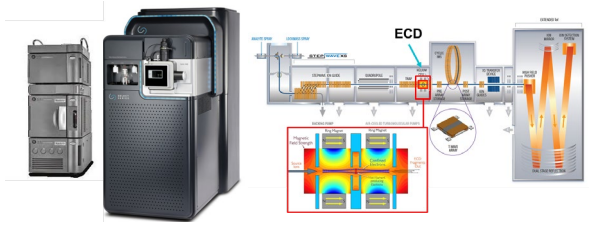

Figure 1, Premier ACQUITY UPLC™ H-Class system coupled to a SELECT SERIES™ Cyclic™ IMS mass spectrometer with standard electrospray source (left). Schemetic diagram of cIMS system with ECD unit highlighted (right).

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BCA (10 µM in 50:50 methanol: ddH20) was infused at 10 µL/min for ECD system qualification and optimization of the ECD cell lens voltages. LC/MS analysis of NISTmAb subunits (scFc, LC and Fd) was run on an ACQUITY™ Premier System with Quaternary Solvent Management with a BioResolve™ RP mAb Polyphenyl Column (p/n: 186008944). The ECD cell (e-MSion, Corvallis, Oregon) was installed in a pre-ion mobility (pre-IM) configuration on the Cyclic IMS mass spectrometer (Figure 1). The resulting data were processed in MassLynx™ 4.2 and/or waters_connect™ for BayesSpray deconvolution followed by mass spectral fragment annotation in ProSite Lite2.

Results and Discussions

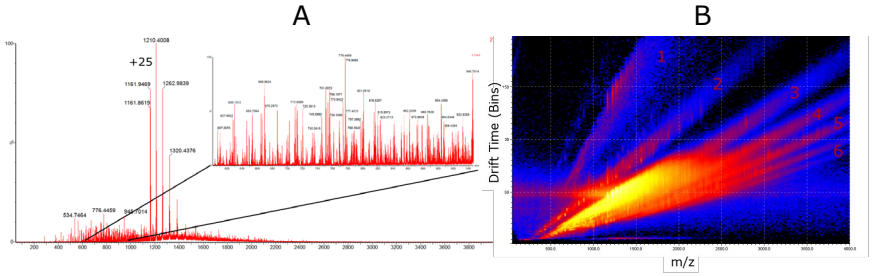

Figure 2. Panel (A) Combined ECD raw spectrum of Carbonic Anhydrase II (BCA) with precursor ion of m/z=1162 (25^+ charge state). The expanded view region demonstrates the high complexity of the ECD fragment ion spectrum prior to ion mobility separation. Panel (B) The ECD-IMS experiment enables the extraction of additional fragment ion mass spectral clarity from a highly complex mass spectrum.

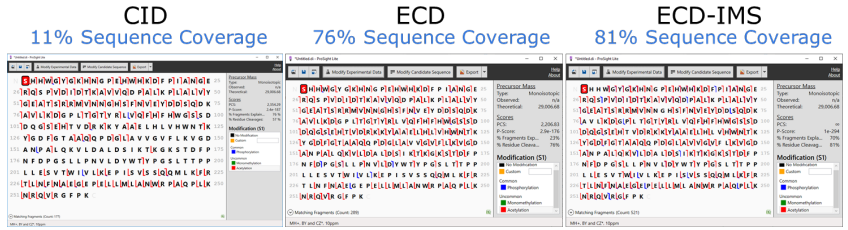

Figure 3. Comparison of sequence coverage of BCA when experiments performed in CID (11%), ECD (76%) and ECD-IMS (81%) modes. This comparison clearly demonstrates the benefit of increased sequence coverage of protein analysis when ECD and ECD-IMS were used. Note that 154 additional fragment ions are identified with the ECD-IMS experiment.

Results and Discussions (continued)

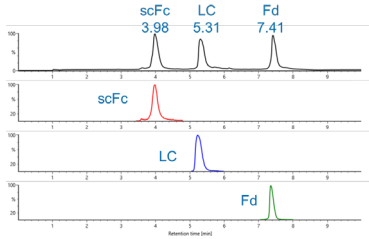

Figure 4. TICs of NISTmAb IdeS digested/reduced subunits. scFc, LC and Fd were eluted at 3.98, 5.31 and 7.41 min respectively with a total run time of 10 min for the method. Targeted ECD experiments acquired separately to test the sequence coverage of the three subunits as shown in the individual traces.

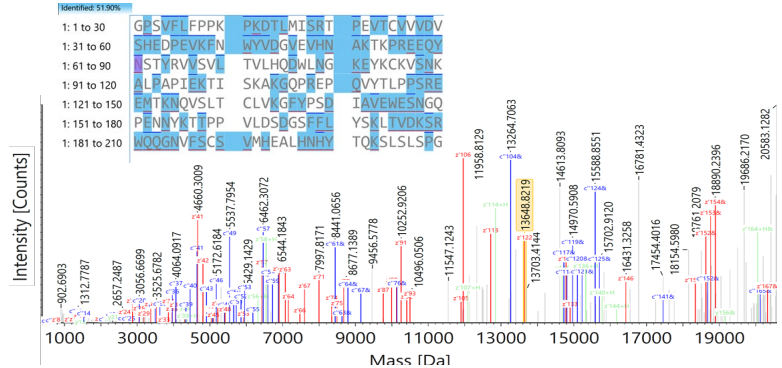

Figure 5. LC/MS ECD BayesSpray deconvoluted spectrum (zoomed in region, processed within waters_connect) for NISTmAb scFc subunit with the sequence coverage shown at 52%. This demonstrates that ECD top-down approach works well at the analytical scale LC/MS level for mAb's subunits.

Conclusion

- ECD top-down analysis of proteins and NIST mAb subunits on the Cyclic IMS system is very efficient with high sequence coverages.
- IMS enables improved fragment ion mass spectral clarity and sequence coverage.
- ECD top-down approach not only works well with nanoscale infusion experiments, but also with analytical scale LC/MS approach.

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