Costello, C. E.; Bourgoin-Voillard, S.; Han, L.; Yu, X.; Huang, Y.; Lin, C. Optimization of ETD and ECD MS^n Approaches for Glycans, Glycopeptides and Glycoproteins. Japanese Human Proteomics Organization Annual Meeting, Niigata, Japan, July 2011.

Optimization of ETD and ECD MS^n Approaches for Glycans, Glycopeptides and Glycoproteins

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We are applying Electron Transfer Dissociation (ETD) and Electron Capture Dissociation (ECD), with and without prior or post-activation, to analyze oligosaccharides, glycopeptides and glycoproteins. We have investigated adduction with different metals, the use of alternative ETD reagent anions, and varied ECD parameters and are exploring the theoretical basis for the observed fragmentation.

We reported recently the generation of a variety of fragmentation types by ETD using fluoranthene as the chemical reagent [Han and Costello, *J Am Soc Mass Spectrom*, **2011**, 22, 997]. Cross-ring cleavages helped to clarify the different linkage types and branching patterns of the glycans. Stable isotopic labeling verified the fragment ion assignments. Now we report results for permethylated cation-adducted oligosaccharides, biantennary and disialylated biantennary *N*-linked glycans and native glycopeptides with ETD MS/MS on a AmaZon quadrupole ion trap (Bruker Daltonics, Billerica, MA) with different chemical reagents, adjusting parameters to obtain the best signals. We also report further glycan analyses and the top-down analyses of glycoproteins with a 12-T solariX hybrid QhFTICR MS (Bruker) equipped for CID, ECD, ETD and IRMPD.

ETD spectra with other chemical reagents now reveals complementary information. In ECD, the nature of the metal adduct and the electron energy strongly influences the ECD fragmentation of glycans; these results shed light on the dissociation mechanisms. In top-down experiments that used ETD or ECD, loss of any portion or the full glycan was minimal (<2%), protein sequence coverage was high and glycosylation sites could easily be determined. The methods are being applied to the analysis of milk sugars, glycans released from infectious microorganisms, and to intact glycoproteins, *e.g.*, Ribonuclease B, human serum amyloid P.

Acknowledgements: NIH grants P41 RR10888 and S10 RR025082. We are grateful to Bruker Daltonics for loan of the AmaZon instrument.