Isomeric Complexity of Glycosylation in MCF-7 and MDA-MB-231 Cell Lines Revealed by Detailed Analysis with Ion Trap Mass Spectrometry

David J. Ashline1,2, Hailong Zhang2, Vernon N. Reinhold2
1Glycan Connections, LLC Lee, NH; 2University of New Hampshire, Durham, NH;

Introduction
Glycosylation is a ubiquitous post-translational modification of proteins and lipids. Glycans function as important ligands in intercellular and intracellular interaction. The template-free biosynthesis of oligosaccharides frequently results in multiple isomeric glycoforms. Examination of two well studied cell lines shows the ubiquity and complexity of this problem. While localizing fucose to a core GlcNAc or antenna is important, determining the specific antennal structure, i.e. Lewis X or H2, is also an important detail.

Methods
Cultured MCF-7 and MDA-MB-231 cell lines were processed to yield pools of glycoproteins, N-linked glycoprotein glycans, and O-linked glycoprotein glycans. Glycospingolipids were isolated by solvent extraction and purified by silica phase extraction, followed by permethylation using sodium hydroxide and iodomethane. N-linked glycans were released enzymatically, purified by graphitized carbon solid phase extraction, reduced with borane/ammonia, and permethylated. N-linked glycans were released by reduction, base-elimination, purified by carbon exchange and C18 solid phase extraction, and permethylated. Permethylation of oligosaccharides were directly infused into an ion-trap mass spectrometer (LTQ, Thermo Scientific, San Jose, CA) via nano-electrospray (Triversa Nanomate, Advion, Ithaca, NY). MSn peak selection was performed manually.

Results
Many biological samples can contain glycan isomers, particularly with larger structures. These isomers can contain important structural features such as disaccharide core structures. H antigens, Lewis structures, etc. Methods relying solely on intact mass or composition data may miss these additional structures. Further, MS/MS analysis may not be sufficient to empirically distinguish isomeric fragments such as Lewis a and Lewis b.

Conclusions
This composition contains multiple isomers, with variations in fucose position and number of antennae. Metabolic samples were isolated from MCF-7 cultured cells and analyzed. The biantennary, digalactosylated, monofucosylated composition found in MDA-MB-231 cultured cells, particularly important when important ligands such as Lewis structures can be found. Most biological samples can contain glycan isomers, particularly with larger structures. These isomers can contain important structural features such as disaccharide core structures.

References
1. Isomeric Complexity of Glycosylation in MCF-7 and MDA-MB-231 Cell Lines Revealed by Detailed Analysis with Ion Trap Mass Spectrometry
2. Glycosylation is a ubiquitous post-translational modification of proteins and lipids.