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

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Introduction

Glycosylation is a ubiquitous post-translational modification of proteins and lipids. Glycans function as important ligands in intercellular and intermolecular 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, ie Lewis X or H2, is also an important detail. Comparison of sample spectra with known standards, along with *de novo* analysis, enables assignment of these differences, including common instances of isomeric mixtures.

Methods

Cultured MCF-7 and MDA-MB-231 cell lines were processed to yield pools of glycosphingolipids, N-linked glycoprotein glycans, and O-linked glycoprotein glycans. Intact glycosphingolipids were isolated by solvent extraction and purified by solid-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. O-linked glycans were released by reductive beta-elimination, purified by cation exchange and C18 solid phase extraction, and permethylated.

Permethylated oligosaccharides were directly infused into an ion-trap mass spectrometer (LTQ, Thermo Scientific, San Jose, CA) via nano-electrospray (Triversa Nanomate, Advion, Ithaca, NY). MS^n peak selection was performed manually. Standard materials were either obtained commercially or from the Consortium for Functional Glycomics. Materials were permethylated and disassembled via MS^n.

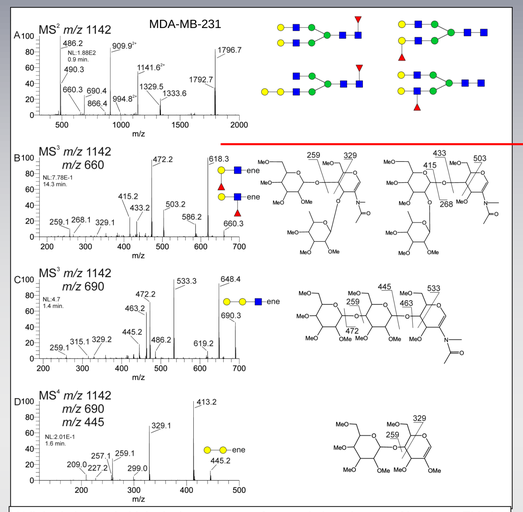
Relevant epitope fragment spectra were compared visually and computationally to generate similarity scores.

References

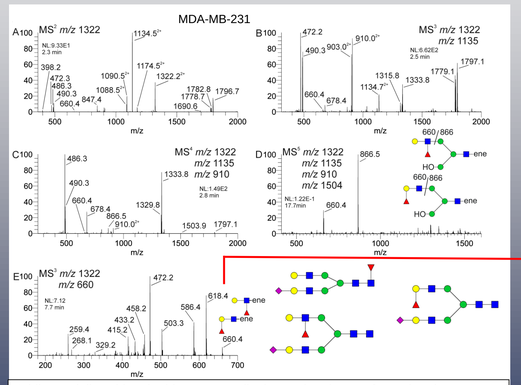
Ashline DJ[1], Zhang H[1], Reinhold VN[2]. **Isomeric complexity of glycosylation documented by MSn.** Anal Bioanal Chem. 2017 Jan;409(2):439-451.

Ashline DJ[1], Hanneman AJ, Zhang H, Reinhold VN. **Structural documentation of glycan epitopes: sequential mass spectrometry and spectral matching.** J Am Soc Mass Spectrom. 2014 Mar;25(3):444-53.

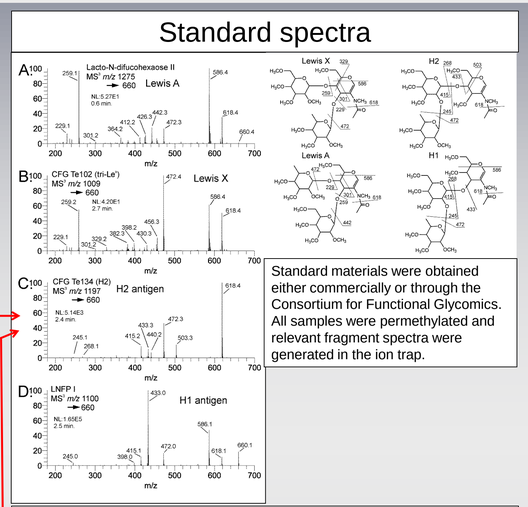
Results



N-linked glycans in biological systems can frequently be mixtures of isomers. This is particularly important when important ligands such as Lewis structures can be found. For the biantennary, digalactosylated, monofucosylated composition found in MDA-MB-231 cultured cells, this composition was found to contain four different isomeric structures, including LeX, H2, and Gal-Gal motifs. Spectral matching of the MS^3 *m/z* 660 spectrum with relevant standard spectra yields a similarity score of 0.997 with H2 and 0.633 with Lex. The low match for Lex is due to the small amount relative to H2; *de novo* assignment is required here.



Adding a sialic acid to the biantennary, digalactosylated, monofucosylated composition, also in MDA-MB-231 cultured cells, also yields a set of isomeric structures. As with the non-sialylated composition, LeX and H2 structures are present. Also of potential significance, no fragmentation consistent with sialylated Lewis X was detected. The similarity score for the MS^3 spectrum is 0.792 for H2 and 0.887 for Lex. The relatively low scores are because the spectrum is a mixture of both structures.



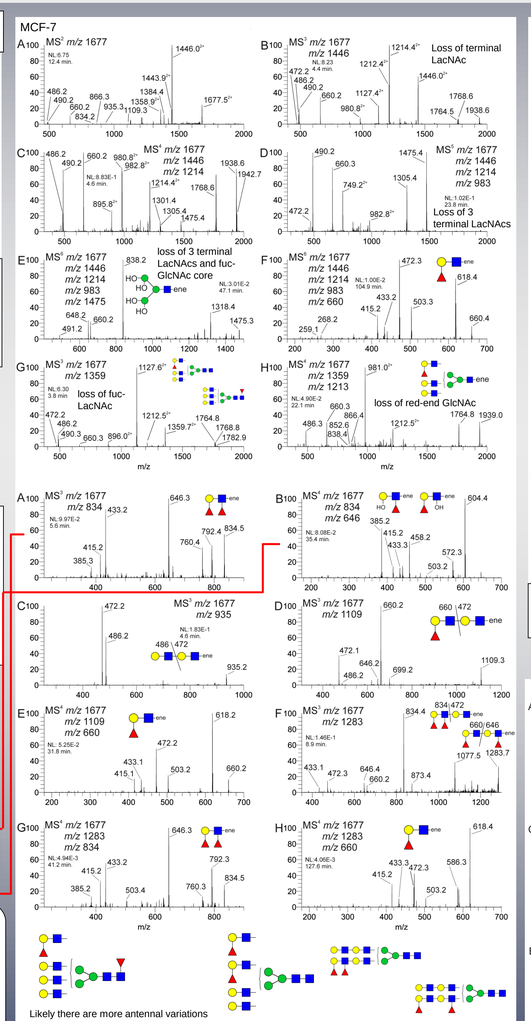
Standard materials were obtained either commercially or through the Consortium for Functional Glycomics. All samples were permethylated and relevant fragment spectra were generated in the ion trap.

Standard spectra were generated from authentic materials. Included, for monofucosylated lactosamines, are Lewis x, Lewis a, H1, and H2 b-type fragments of permethylated precursors. For difucosylated lactosamines, Lewis b (type 1 LacNAc) and Lewis y (type 2 LacNac) were used, again as b-type fragments of permethylated precursors. For monofucosylated LacNAcs, the single b-type *m/z* 660 trisaccharide fragment was compared for unknowns and standards. For the difucosylated LacNAcs, both the b-type m/z 834 tetrasaccharide fragment, and the b/y-type *m/z* 646 trisaccharide fragments were compared between standards and unknowns, where possible.

Conclusions

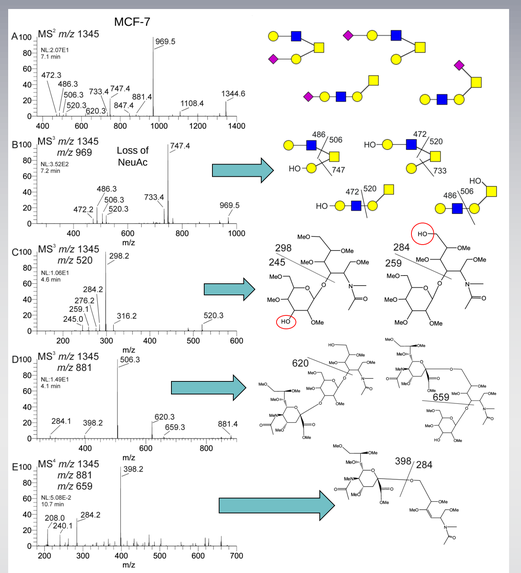
Many biological samples can contain glycan isomers, particularly with larger structures. These isomers can include potentially important structures such as H antigens, Lewis structures, etc. Methods relying solely on intact mass or composition data may miss these additional structures. Further, MS/MS analysis may not be sufficient to empirically distinguish isomeric fragments such as Lewis x/Lewis a/H2/H1 or Lewis y/Lewis b.

Comparison of fragment spectra with those of standard materials can be helpful for making structure assignments. However, in cases of mixture spectra, low computationally generated scores can confound analysis. In such cases, visual determinations must be made.,

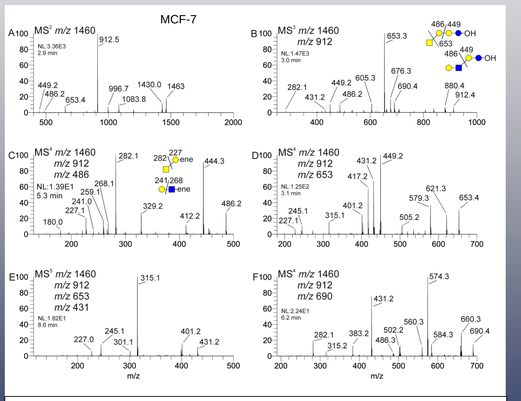


Likely there are more antennal variations

This composition contains multiple isomers, with variations in fucose position and antenna number. Singly fucosylated LacNAc antennae are consistent with H2 mixed with a small amount of Lex. The difucosylated LacNAc is consistent with Lewis y. Spectral similarity scores for the *m/z* 834 tetrasaccharide (0.961) and the *m/z* 646 trisaccharide (0.990) confirm this assignment.



O-linked glycans may also yield isomeric mixtures. This composition isolated from MCF-7 cultured cells shows at least four different isomers, both core 1 and core 2 with varying sialic acid position.



Glycosphingolipids can also contain isomeric mixtures. The tetraosylceramides in MCF-7 cells were found to be a mixture of globotetraosylceramide and neolactotetraosylceramide. Isolation of the terminal LacNAc (*m/z* 486) clearly shows both HexNAc-Hex and Hex-HexNAc motifs. Further, the Hex-HexNAc was 4-linked. The putative Gal-Gal motif (*m/z* 431) was also empirically determined to be 4-linked, distinguishing this structure from an isoglobo structure.