STRUCTURAL IDENTIFICATION OF N-GLYCANS AND N-GLYCOPEPTIDES USING LOGICALLY DERIVED SEQUENCE TANDEM MASS SPECTROMETRY

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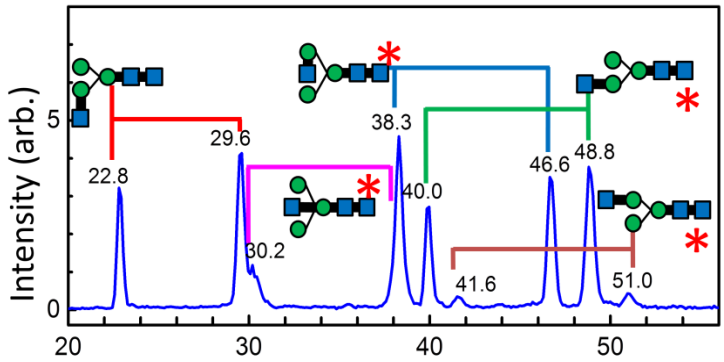
The *N*-linked glycosylation is an important post-translational modification of proteins. Current knowledge of multicellular eukaryote *N*-glycan biosynthetic pathways suggests N-glycans are produced in endoplasmic reticulum and Golgi apparatus through conserved biosynthesis. According to these biosynthetic pathways, there is only isomer of *N*-glycan with the composition of GlcNAc($Man_3GlcNAc_2$) and two isomers of *N*-glycan with composition of $GlcNAc_2(Man_3GlcNAc_2)$. In this study, we applied our newly developed mass spectrometry method, i.e., logically derived sequence tandem mass spectrometry (LODES/MS^n), and enzyme digestion to re-examine the structures of *N*-glycan extracted from various samples, including human milk, human saliva, bovine milk, HEK 293, HeLa cell, hen egg, duck egg, squid, and *Drosophila melanogaster*. Many isomers not predicted by the biosynthesis were identified, and many samples show these unusual isomers are the dominant isomers, indicating additional biosynthetic pathways are involved in these *N*-glycan generation. The complex *N*-glycans extracted from fused lobes, MGATII, MGATIVa, or MGATIVb knock out of *Drosophila melanogaster* confirm some additional biosynthetic pathways. We also applied LODES/MS^n to *N*-glycopeptide analysis. We show that LODES/MS^n provides detailed information on glycosylation sites as well as resolving isomeric N-glycan structures. Using this approach, we have successfully characterized N-glycopeptides from a variety of samples.



References:
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