Glycoproteomics-compatible MS/MS-based quantification of glycopeptide isomers

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Glycosylation is an essential protein modification occurring on the majority of extracellular human proteins, mass spectrometry (MS) being an indispensable tool for its analysis. Not only can MS determine glycan compositions, but also position the glycan at specific sites via glycoproteomics. However, glycans are complex branching structures with monosaccharides interconnected in a variety of biologically relevant linkages such as α2,3- and α2,6-linked sialylation (1,2) - isomeric properties which are invisible when the readout is mass alone. We developed an LC-MS/MS-based workflow for determining glycopeptide isomer ratios. Making use of isomerically-defined glyco(peptide) standards, facilitated by methods of chemoenzymatic synthesis (3), we observed marked differences in fragmentation behavior between isomer pairs when subjected to collision energy gradients, specifically in terms of galactosylation/sialylation branching and linkage (figure 1). These behaviors were developed into component variables that allowed relative quantification of isomerism within mixtures. Importantly, at least for small peptides, the isomer quantification appeared largely independent from the peptide portion of the conjugate, allowing broad application of the method. If this broad application is successful, MS-based differentiation between structural glycan isomers would not only revolutionize the field of glycoproteomics, but also, for example, open up new insights into the mechanisms behind diseases and enable better therapeutical antibody design and quality control.

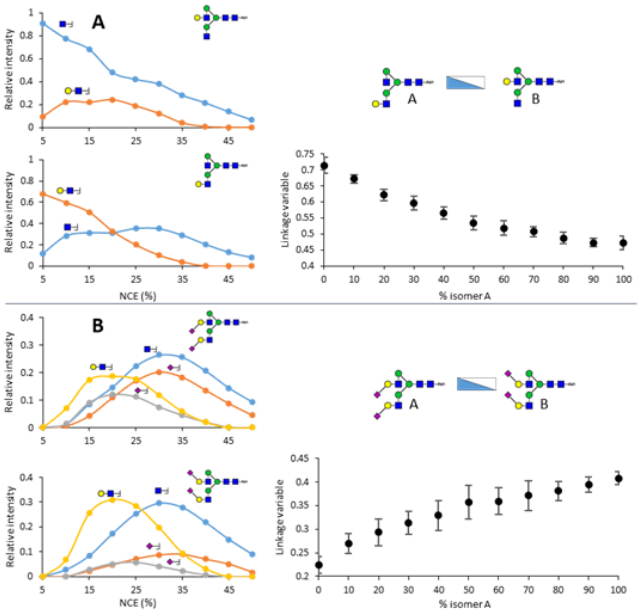

Figure 1: HCD-MS/MS patterns and average linkage variables with standard deviations for galactose branching (A) and sialic acid linkage (B) isomers.

Bibliographic references:

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