Improved Mass Spectrometry Workflows for Glycopeptides Characterization

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Glycoproteins are proteins well represented as diseases biomarkers or as therapeutics. Glycosylation is a heterogenous post-translational modification (PTM) where a carbohydrate so called glycan, is covalently attached to a peptide. They are two main types of glycosylation, N-linked glycosylation which occurs when a glycan is linked to an asparagine (only when the special consensus N-X(≠P)-S/T occurs) and O-glycosylation which can occurs on a serine or a threonine. At one of this single glycosite, multiple glycoforms can be present.

The presence of two chemical groups, glycans and peptides, with large heterogeneity, makes their analysis challenging and liquid chromatography coupled to high resolution tandem mass spectrometry (LC-HRMS) using a bottom-up approach has become the method of choice for the characterization and quantification of glycopeptides. Most MS data dependent (DDA) and data independent (DIA) workflows apply multiple MS dissociation techniques including collision induced dissociation (CID) and electron induced dissociation (ExD) resulting in multiple LC-MS analysis and requires specific data analysis software tools. Glycan composition and position lead to the possible existence of glycopeptide isomers, either from isomeric glycan at a single glycosite or from identical glycan at a different glycosites calling for improved MS workflows. The present work describes the application of differential mobility spectrometry (DMS) as an additional separation dimension and integrates CID and electron activated dissociation (EAD) on a fast-acquiring MS platform for the characterisation of N and O glycopeptides. The combination of LC and DMS allows to resolve glycopeptide isomers improving the quality of CID and EAD spectra facilitating their identification. Also, with DMS glycopeptides cluster in a different space as non-glycosylated peptides enabling to resolve co-eluting species and allows to reduce the LC analysis time and improves sample-throughput.

References:

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