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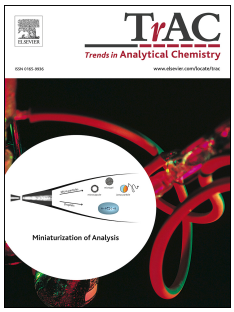

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The benefits of hybrid fragmentation methods for glycoproteomics

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ABSTRACT

Glycosylation is an important and variable protein modification that can have a profound effect on the physiological characteristics of the substrate, warranting careful examination in applications ranging from the development and quality control of biopharmaceuticals to clinical glycoproteomics.

Glycoproteomics describes the mass spectrometric analysis of protein glycosylation in a site-specific manner, typically of proteolytically digested glycoprotein samples. This may be achieved by interpreting the mass (over charge) values of (glyco)peptides across a run of liquid chromatography coupled to mass spectrometry (LC-MS), and acquiring the fragmentation patterns of selected precursors to sequence the peptide and characterize the composition/structure of the glycan. It has become apparent, however, that most fragmentation mechanisms do not equivalently affect the glycan and peptide portion of a glycopeptide. For example, collision-induced dissociation (CID) and higher-energy collisional dissociation (HCD) primarily yield abundant B- and Y-ions from the glycan portion of a glycopeptide conjugate, whereas electron-transfer dissociation methods such as electron-capture dissociation (ECD) and electron-transfer dissociation (ETD) mainly affect the peptide backbone to yield c- and z-ions.

Hybrid fragmentation, *i.e.*, the application of sequential or combinatorial fragmentation by orthogonal fragmentation strategies, has shown to greatly benefit the characterization of glycopeptides by combining the advantages of the individual methods. Examples of hybrid fragmentation methods include the sequential triggering of HCD and ETD on a closely situated precursor mass, using multiple steps of collision energy for the same precursor, and combining multiple methods on the same time/mass window. This is for instance the case with electron-transfer/collision-induced dissociation (ETciD) and electron transfer/higher-energy collisional dissociation (EThcD). Many modern-day mass spectrometers are capable of applying these fragmentation workflows, and the reported use of hybrid fragmentation for glycoproteomics is rapidly expanding.

This review will cover recent the developments and applications within the use of hybrid fragmentation for glycoproteomics. The work will be broadly centered on 1) energy-stepping in collisional activation, 2) sequential fragmentation, and 3) combinatorial fragmentation methods. We close by discussing remaining technical challenges, and outline possible future developments.

HIGHLIGHTS

- Mass spectrometry is a critical analytical method within the field of glycoproteomics.