Detailed Glycan Structure Characterization by Electron Activated Dissociation (ExD)-Based Tandem Mass Spectrometry

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Abstract

Challenges in glycan structure determination arise from their structural complexity, lack of glycan amplification methods, limited glycan database information, and their presence as complex mixtures. Thus, there is a need for developing a *de novo* approach for glycan structure characterization, using analytical tools with high sensitivity, and compatible with chromatographic separation.

Detailed glycan structure characterization is traditionally achieved by multiple stages of tandem mass spectrometry, often employing collisionally activated dissociation (CAD)-based methods in ion trap mass spectrometers. However, such approach suffers from its low sensitivity, low throughput, low mass measurement accuracy, and difficulty in automation. Here we present an MS^2-based, *de novo* approach employing ExD methods in high-resolution mass spectrometers for high-throughput structural determination of glycans at biological concentrations.

Electron capture dissociation (ECD) has shown great potential in glycan structural analysis, but is only applicable towards multiply-charged precursor ions. Additionally, fragmentation remote from the initial electron capture (metal-binding) site is limited, and the ECD efficiency is severely reduced in the presence of a radical trap, which is often the case in derivatized glycans commonly used in LC-MS/MS analyses. Electronic excitation dissociation (EED) can overcome these difficulties, as it is initiated by electronic excitation rather than electron capture, and does not rely on radical cascade for remote fragmentations. As such, EED is amenable to singly-charged as well as negatively charged ions, capable of producing extensive fragmentation, even for derivatized glycans. Finally, we show that EED can generate rich structural information with consumption of small amount of sample on a chromatographic timescale.

Recent development of bioinformatics tools for interpretation of complex, high-resolution glycan tandem mass spectral data will also be discussed.

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