

Characterization of Oligosaccharides Released from Glycoproteins by Mass Spectrometry

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Recently, mass spectrometry has been extensively applied to the proteomics study of various organisms. A keypart of proteomics research is examining the more than 100 different types of post-translational modifications (PTMs) that can occur to proteins, of which two of the most important are phosphorylation and glycosylation. The oligosaccharides attached to glycoproteins serve to modulate protein function and may influence folding, biological lifetime, and recognition of binding partners. Mass spectrometry is an important tool for the structural analysis of carbohydrates, and offers precise results, analytical versatility, and very high sensitivity. In my presentation, I want to introduce the characterization of oligosaccharides released from glycoproteins using various mass spectrometric techniques. The fragmentation characteristics of native and 3-aminophthalic hydrazide (3-APH)-derivatized oligosaccharides using a matrix-assisted laser desorption/ionization (MALDI) time-of-flight/time-of-flight tandem mass spectrometer will be also described. 3-APH-derivatized maltoheptaose showed a 50-fold improvement in sensitivity over the underivatized one and could be detected at a level of 10 fmol. The extensive fragmentation including glycosidic cleavages and cross-ring cleavages is shown to facilitate the detailed structural characterization of N-linked oligosaccharides released from standard glycoproteins. Furthermore, it was demonstrated that the technique could be used to the characterization of glycan moieties extracted by in-gel PNGase F treatment of glycoproteins on one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).