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Recent advances in ion mobility–mass spectrometry for improved structural characterization of glycans and glycoconjugates

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Glycans and glycoconjugates are involved in regulating a vast array of cellular and molecular processes. Despite the importance of glycans in biology and disease, characterization of glycans remains difficult due to their structural complexity and diversity. Mass spectrometry (MS)-based techniques have emerged as the premier analytical tools for characterizing glycans. However, traditional MS-based strategies struggle to distinguish the large number of coexisting isomeric glycans that are indistinguishable by mass alone. Because of this, ion mobility spectrometry coupled to MS (IM–MS) has received considerable attention as an analytical tool for improving glycan characterization due to the capability of IM to resolve isomeric glycans before MS measurements. In this review, we present recent improvements in IM–MS instrumentation and methods for the structural characterization of isomeric glycans. In addition, we highlight recent applications of IM–MS that illustrate the enormous potential of this technology in a variety of research areas, including glycomics, glycoproteomics, and glycobiology.

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Introduction

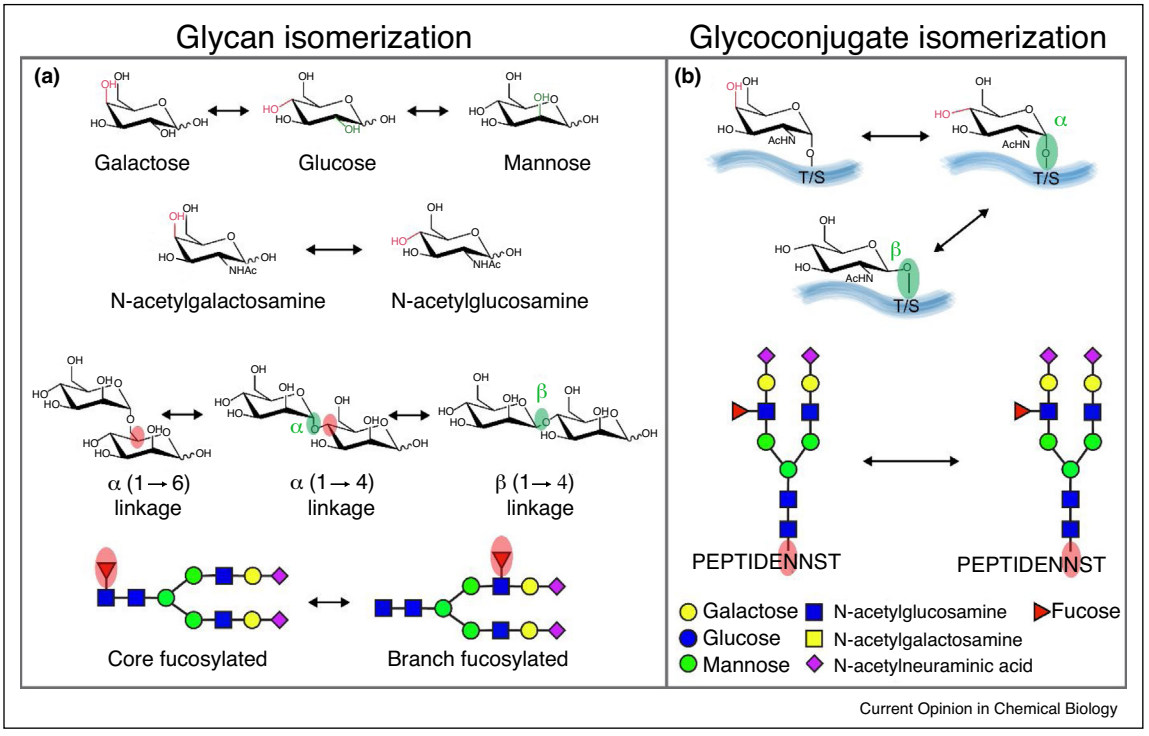
As one of the most abundant and complex protein post-translational modifications (PTMs), glycosylation is associated with many key biological processes including cell adhesion, molecular trafficking, receptor activation, and signal transduction [1]. The analysis of glycans and glycoconjugates is challenging due to the large diversity of structures resulting from the non-template driven biosynthesis [2,3]. In addition, many of the monosaccharides that compose larger glycans are structural isomers, and they can be connected via either α-stereochemistry or β-stereochemistry at multiple linkage positions, resulting in many glycan isomers (Figure 1a). Isomeric glycans can also have a variety of connectivities to numerous sites on other classes of molecules such as proteins, contributing to their structural complexity (Figure 1b).

Separation and detailed structural characterization of glycan or glycoconjugate isomers is crucial for understanding their roles in various biological processes. Benefiting from speed and sensitivity of analysis, liquid chromatography (LC)–MS and capillary electrophoresis (CE)–MS have emerged as powerful techniques for glycan characterization [4–6]. Despite recent improvements to nearly all aspects of MS-based analytical workflows for glycan and glycoconjugate characterization, it remains challenging to achieve complete structural elucidation due to the complexity of glycans and lack of standard reference databases. Therefore, new techniques and methods that enhance the differentiation of glycan and glycoconjugate isomers would be highly desirable.

Although it has been two decades since IM–MS was originally used to separate glycan isomers, recent technological advancements have sparked increased interest in IM–MS for glycan and glycoconjugate analysis [7]. Unlike other commonly used separation techniques such as LC and CE, IM–MS is a post-ionization gas-phase technique that separates ions based on differences in shape and charge as they travel through a buffer gas under the influence of an electric field [8,9]. The time it takes for an analyte ion to travel through the IM cell can be used to calculate rotationally averaged collision cross section (CCS) which provides an additional parameter that can be used to identify compounds as well as information about molecular conformation [8–10].

Initial applications of IM to carbohydrate analysis focused on distinguishing small isomeric carbohydrate standards [11,12]. Due to the advancement and commercialization of IM–MS instrumentation, a growing number of labs continue to demonstrate that IM–MS is a fast, sensitive, and effective method for resolving carbohydrate isomers. For example, IM–MS has been used to separate a variety of isomeric species, including connectivity and configurational isomers [13]. Furthermore, studies have been

Figure 1



The isomerization of glycan and glycoconjugates. **(a)** The building blocks (monosaccharides) that compose larger glycans are structural isomers (hexose: galactose, glucose, mannose, N-acetylhexosamine: N-acetylgalactosamine, N-acetylglucosamine); monosaccharides can be connected either α-stereochemistry or β-stereochemistry at multiple potential linkage position; fucose could be either attached to N-glycan core or branches. **(b)** Epimeric glycoconjugates results from alternative configurations (α- or β-) at the anomeric linkages or the presence of epimeric glycan monomers (galactose or glucose), scheme modified from Ref. [43]; two isomeric N-glycopeptides differ in the site of N-glycan attachment.

extended to more complex systems such as N-glycans and O-glycans and intact glycopeptides [14,15•,16••,17–20]. Here, we discuss the latest developments in IM–MS methods and technology that have allowed for enhanced separation and structural characterization of glycans and glycoconjugates and discuss advances necessary for IM to become more widely used in glycomics and glycoproteomics workflows.

Improving IM-based isomer separations

Although many proof-of-principle experiments have shown the potential of IM to separate glycan isomers, baseline separation of isomeric glycans is difficult as they often have minor differences in CCS. This is especially problematic as studies are expanded to larger glycans and glycoconjugates because minor changes in the glycan composition often result in subtle differences in the overall structure. Furthermore, improved glycan separation will be crucial for extending the applications of IM–MS technology to large-scale studies of glycans and glycoproteins in complex mixtures from biological systems (i.e., glycomics and glycoproteomics). Thus, various analytical workflows that include IM separation have been developed to enhance the separation of isomeric glycans.

One of major factors that has limited the utility of IM–MS for many applications is that many instrument platforms lack the mobility resolution necessary to resolve isomeric species that have minor differences in CCS. The development of high resolution IM instrumentation using structures for lossless ion manipulations (SLIM) technology has shown great potential to enable separations of a variety of isomeric species [21]. Recently, a novel instrument was developed capable of ultralong pathlength travelling wave ion mobility (TWIM) separations on a serpentine-shaped SLIM device that has a 30-fold increase in IM resolution compared to traditional drift tube IM and TWIM instruments [22••]. In addition to providing baseline separation of isomers lacto-N-hexaose and lacto-N-neohexaose, high resolution SLIM IM–MS revealed a new conformation of lacto-N-neohexaose. This suggests that SLIM-based IM separations will provide a level of conformational information about glycans that was previously inaccessible.

An alternative approach to increase isomer separation by improving IM–MS instrumentation is to optimize the