Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Glycan analysis by ion mobility-mass spectrometry and gas-phase spectroscopy

Christian Manz[1,2] and Kevin Pagel[1,2]

Due to the existence of numerous isomers, the in-depth analysis of glycans represents a major challenge. Currently, the majority of glycans are analysed using mass spectrometry (MS)-based techniques, which can provide information on regioisomers but usually fail to differentiate stereoisomers. A promising approach to overcome this limitation is to implement ion mobility spectrometry (IMS) as an additional gas-phase separation dimension. This review highlights recent developments in which IM-MS was used as a tool for comprehensive glycan analysis or as rapid screening method for glycan feature analysis. Furthermore, we summarize a series of very recent investigations in which gas-phase spectroscopy is applied to study glycans and discuss the potential of the hyphenation between IM-MS and infrared (IR) spectroscopy as a future tool for glycomics and glycoproteomics.

Addresses
[1] Institute of Chemistry and Biochemistry, Freie Universität Berlin, Takustr. 3, 14195 Berlin, Germany
[2] Fritz Haber Institute of the Max Planck Society, Faradayweg 4-6, 14195 Berlin, Germany

Corresponding author: Pagel, Kevin (kevin.pagel@fu-berlin.de)

Current Opinion in Chemical Biology 2018, **42C**:16–24

This review comes from a themed issue on **Omics**

Edited by **Erin Baker** and **Perdita Barran**

http://dx.doi.org/10.1016/j.cbpa.2017.10.021

Introduction

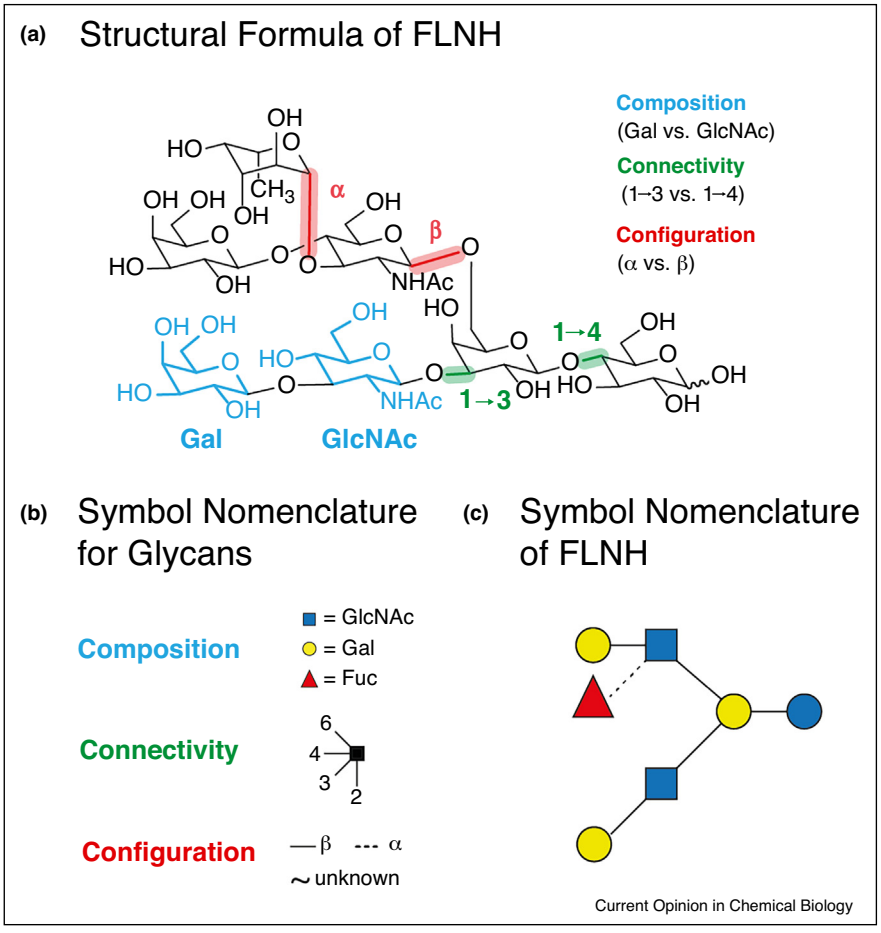
Glycans have long been known for their major role in the metabolism and structural properties of biological systems, especially for sensing in inter-molecular and intra-molecular interactions such as innate and adaptive immune responses [1]. Their underlying structure dictates their function and therefore comprehensive structural characterization of glycans is prerequisite to the elucidation of *in vivo* operation. However, in contrast to DNA and proteins, glycans often exhibit non-linear, branched, and stereochemically complex structures [2]. As a result, they possess a unique complexity, which is based on the type of building blocks (composition), the regiochemistry and branching (connectivity), and the stereochemistry (configuration) (Figure 1). Furthermore, glycans may exist in multiple conformations and, in case of a free reducing end, form an equilibrium between different configurations (*i.e.* α and β) and ring structures (*i.e.* pyranose and furanose) [1].

The combination of these structural categories frequently results in closely related structural isomers, which are often difficult to distinguish using established analytical tools. Today, a variety of hyphenated techniques are applied to elucidate glycan structures, with the majority deriving directly from technologies developed in genomics and proteomics. The most prominent example is the combination of liquid chromatography (LC) and mass spectrometry (MS). This combination has proved to be useful for the separation of released glycans and the characterization of glycan composition [4]. However, carbohydrates are inherently very polar, which makes them difficult to separate using traditional reversed-phase chromatography. As a result, other stationary phases such as porous graphitized carbon (PGC) [5] or techniques such as hydrophilic interaction chromatography (HILIC) [6] have to be used. However, both techniques often fail in the separation of glycoconjugates with amphiphilic character (*i.e.* mixed polarity) such as glycolipids. In addition, oligosaccharides do not contain a natural chromophore or fluorophore, which complicates their detection [7]. Established glycan LC–MS workflows therefore often involve a derivatization step to label the glycans with a fluorescent tag. This improves sensitivity and enables quantification, but is also time consuming and expensive. MS-based approaches, on the other hand, provide a larger degree of structural information but, due to their identical atomic composition and mass, often struggle to distinguish regio-isomers and stereo-isomers [8–12]. Sophisticated MS^n experiments can help to overcome this limitation, but they are elaborate and therefore of limited use in routine analysis [13]. Recently, other gas-phase analysis techniques such as ion mobility spectrometry (IMS) and gas-phase spectroscopy have emerged as promising alternatives. Here we summarize recent developments on the gas-phase structural analysis of oligosaccharides and glycopeptides.

Ion mobility spectrometry

IMS is a gas-phase separation technique in which ions are guided through a gas-filled drift cell by an electric field. On their way, they undergo collisions with a buffer gas,

Figure 1



Visual representation of glycans. **(a)** The most accurate depiction of glycans is the structural formula as shown for the milk oligosaccharide fucosyllacto-N-hexaose (FLNH). However, structural features such as the composition (blue), connectivity (green) and configuration (red) are often difficult to identify, due to the complex nature of oligosaccharides. **(b)** The symbol nomenclature for glycans (SNFG) was introduced to allow a better visualisation of complex structures. Here, monosaccharides are illustrated using specific symbols, while the regio-chemistry and stereo-chemistry of the glycosidic bond is represented by the angle and the type of the connecting lines, respectively [3]. **(c)** Depicting the milk oligosaccharide FLNH using the SNFG simplifies its visualisation and allows an easy identification of minute structural differences.

which separates them based on their charge, size and shape [14]. Coupling IMS with MS allows for the detection of the mobility-separated ions and the collection of mass-to-charge (m/z) and drift time information within one measurement. The obtained instrument-dependent drift time can furthermore be converted into a rotationally-averaged collision cross-section (CCS), which is correlated to the shape of an ion and can be used as additional identification parameter.

Group separation *via* IM-MS

One of the main advantages of ion mobility mass spectrometry (IM-MS) is its universal separation power. Unlike LC separations, which are very sensitive to the stationary phase, IM-MS depends on the much more universal interaction principles of the investigated ions with the drift gas. Due to their distinct atomic composition, different molecular classes can exhibit a different mobility behaviour, especially when a polarizable gas such as nitrogen is used. This property can be used to significantly reduce the spectral complexity of the acquired data [15]. It was recently demonstrated that whole molecular classes can be easily distinguished based on their trend lines in a plot of CCSs against m/z. For example, carbohydrates exhibit on average shorter drift times compared to lipids and peptides of the same m/z, allowing a rapid identification of each class [16]. This group separation can also be observed for different charge states of molecules (Figure 2) [17].

Multiply charged ions generally exhibit a higher mobility than singly charged ions of the same m/z, resulting in individual signal groups (Figure 2b). Selectively extracted data from complex spectra enables