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Lasers and ion mobility: new additions to the glycosaminoglycanomics toolkit

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Glycosaminoglycans are biopolymers present in mammalian cells or in the extracellular matrix. To address their structure, the nature of the hexuronic acids and the position of sulfate groups must be determined. Tandem mass spectrometry using collision induced dissociation or electron-based fragmentation techniques, is a well-established approach for the identification of glycans but suffers from the frequent lack of diagnostic fragments in the case of glycosaminoglycans. This review presents alternative fragmentation techniques, namely photofragmentation in the IR and the UV ranges. Alternative approaches based on the direct analysis of the molecular structure, including ion mobility spectrometry and ion spectroscopies are reviewed. The potential of future multidimensional workflows for glycosaminoglycanomics is discussed.

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Introduction

Mass spectrometry (MS) has become a mainstream approach in glycoanalysis, yet glycosaminoglycanomics [1•] remains a particularly challenging area of glycomics. This is due to the structural specificities of glycosaminoglycans (GAGs), which feature a deceivingly simple linear backbone consisting of a disaccharide repeat (hexuronic acid — glucosamine or hexuronic acid — N-acetyl-galactosamine) with well-defined connectivity, as shown in Scheme 1. Two epimers of the hexuronic moiety and ubiquitous sulfate modifications however, generate an exponentially increasing set of combinations as the size of the backbone increases. It results in the frequent occurrence of isomers and multicharged ions, therefore it is essential to insure efficient separation of overlapping *m/z* ions before MS analysis. A variety of methods have been successfully applied to the separation of GAG fragments, including ion chromatography [2–7] and electrophoresis [8–11].

Yet, a major difficulty remains: indeed, information from mass determination alone does not disambiguate the isomeric structural features present in GAGs, that is, the epimers of hexuronic acid and the position of the sulfate groups. Tandem MS analysis (MS/MS), however provides additional information in the form of MS spectra of fragments resulting mainly from collision-induced dissociation (CID), which can be used in combination with a library of reference MS/MS data for structure identification. The two main limitations of the MS/MS approaches are (i) the reduced number of GAG standards available to populate MS/MS databases, and (ii) the low reproducibility of the MS/MS data, which are instruments-dependent and conditions-dependent. Besides, since the main dissociation reactions in MS/MS using standard CID are glycosidic cleavage and loss of sulfate, multi-stage tandem mass spectrometry (MSn) is often required to generate diagnostic ions [12].

Alternative fragmentation techniques have been extensively explored to improve the performance of MS/MS analysis. On one hand, high energy collisions can be used to increase the sequence coverage. On the other hand, electron-based fragmentation techniques tend to promote cross-ring fragmentation over the cleavage of glycosidic bond, which may provide valuable information on the sulfate position and epimers. Interesting alternative fragmentation patterns can also be obtained by modulating the charge state of the ion of interest. These strategies have been largely reviewed in the past decade by several authors [13–20].

To date, glycosaminoglycanomics relies on the combination of separation methods and the variety of fragmentation techniques available in commercial instruments. Nowadays, the identification of disaccharides is robust, and the sequencing of short oligosaccharides is becoming more and more reliable [21,22], even in mixtures [23]. Sequencing of a full chain however, remains a tremendous challenge [24,25••].

Glossary

Tandem MS analysis : also known as MS/MS or MS2; in Tandem MS, ions are selected according to their mass-to-charge ratio (*m/z*) in a first stage of MS, then dissociated. Product ions are analyzed in a second stage of MS.
Electron-based fragmentation techniques : these techniques are alternative to collisional-induced dissociation. They rely on the transfer of an electron to or from a selected multiply charged molecular ion, yielding a radical ion, which then undergoes fragmentation.
FT-ICR mass spectrometer : Fourier transform ion cyclotron resonance mass spectrometer. FT-ICR-MS is based on the circular movement of ions (cyclotron movement) trapped in a magnetic field. Their cyclotron frequency depends directly on the *m/z* ratio of the ions. The major advantage of FTICR-MS is ultrahigh resolution.
OPO laser : optical parametric oscillator LASER technology offering a wide wavelength tunability in the UV, IR or visible range.
Dp : degree of polymerization, that is, the number of constitutive monomer units in an oligomer or polymer.
Dp2 : oligosaccharide made of two monomer units, that is, a disaccharide.
Dp4 : oligosaccharide made of four monomer units, that is, a tetrasaccharide.
Synapt G2-Si HDMS : hybrid mass spectrometer integrating ion mobility into a tandem-in-space mass spectrometer. Ion mobility provides an additional dimension of separation based on molecular size and shape. Sample molecules are subjected to electrospray ionization (ESI) either in positive or in negative mode to investigate positive or negative ions.
TOFWERK IMS-TOF : atmospheric-pressure, drift-tube ion mobility spectrometer with high-speed TOF MS detection.
DFT B3LYP/6-311+G• : quantum chemistry method based on density functional theory for the accurate determination of structures, energies and physical/chemical properties of molecules.
Positive/negative ionization mode : ion source configuration leading to the formation of positively / negatively charged ions
Hyphenated approaches : methods based on the on-line coupling of two or more techniques, usually a separation technique (LC or IMS) and a detection by mass spectrometry (MS) such as LC–MS/MS, IMS–MS, or LC–IMS–MS
Cryogenic messenger-tagging : low temperature spectroscopy that generates high-resolution spectra able to distinguish subtle structural differences.
m/z **:** mass-to-charge ratio of an ion.

Here we present a review of works reporting the use of laser-induced fragmentation in the IR and UV domains for tandem MS analysis of GAGs. In the second section, we present a radically new direction in glycosaminoglycanomics aiming to the analysis of the molecular 'shape'. Indeed, several tools stemming from Physical Chemistry have recently shown great promise for direct analysis of the molecular structure of GAGs, in contrast with traditional tandem MS, which relies on the analysis of the fragmentation patterns. These approaches include ion mobility spectrometry (IMS) and ion spectroscopy. We describe the additional analytical dimensions offered by these tools, which can advantageously be implemented in almost any existing MS-based workflow. Finally, possible future integrations of these novel approaches in advanced hyphenated MS-based workflows are discussed.

Photofragmentation techniques

Photofragmentation of biomolecules, an alternative activation method to the widely spread CID and the more recent electron-based activation methods, has been largely applied to proteins, nucleic acids and glycans but comparatively much less to glycosaminoglycans. In such photodissociation MS experiments, photons are emitted at a fixed wavelength either in the UV energy range for ultra-violet photodissociation (UVPD) or in the IR energy range for infrared multiple photon dissociation (IRMPD). A review of studies reporting photofragmentation of GAGs in the UV and IR domains is presented below.

Photo-induced ion fragmentation in the IR domain

In IRMPD MS experiments, gas phase trapped ions are irradiated by an infrared beam, resulting in their fragmentation if the wavelength is resonant with molecular vibrations. Initially applied to the structural and mechanistic characterization of small molecules, IRMPD has shown great potential in the past decade for the analysis of biomolecules including peptides, oligonucleotides, and oligosaccharides, particularly N-glycans and O-glycans involved in protein glycosylation and comparatively few GAG-derived oligosaccharides [26].

IRMPD belongs like CID to low energy dissociation methods, inducing the rupture of the weakest bonds and labile modifications. Therefore, IRMPD and CID fragmentation patterns of GAG-derived oligosaccharides exhibit similar glycosidic bond cleavages and sulfate loss. Principal ion products observed upon IRMPD MS of chondroitin/dermatan sulfate, heparan sulfate (HS) and heparin ion precursors are thus Z-type, Y-type and B-type glycosidic fragments [27], while much less cross-ring fragments are observed, mainly A-type fragments [28–30]. For example, IRMPD activation of four synthetic (with saturated non-reducing end) disulfated HS tetrasaccharides yielded the dominant glycosidic fragment ions Y1-, Y32-, B2-, and B3-, and sulfate loss [31]. Glycosidic cleavage is often paired with sulfate loss as observed with IRMPD of HS disulfated tetrasaccharide ΔUA-GlcNSO3-IdoA-GlcNAc-6-SO4, which is detrimental to the determination of sulfate position [32]. Sulfate loss can be minimized by selecting the highest charged precursor in which all sulfate groups are charged and partially cationized by sodium, but at the expense of glycosidic and cross-ring fragmentation [33]. Highest degree of sulfation has been also examined by IRMPD as reported for the heparin hexasulfated tetrasaccharide ΔUA2S-GlcNS6S-IdoA2S-GlcNS6S [30]. In addition to glycosidic and cross-ring A_n fragment ions, informative X-type ions are observed in this case, such as $^{2,4}X_0$ which allows the location of the O-sulfo group on the terminal reducing N-sulfo-glucosamine [30,31]. A major advantage of IRMPD over CID is that IRMPD is much more efficient in fragmenting long oligosaccharides. Although multi-stage CID (MSn, $n > 2$) is required for gaining complete structural information, a single-stage IRMPD results in cleavage of most of the glycosidic bonds. It

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