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Deciphering the structure of isomeric oligosaccharides in a complex mixture by tandem mass spectrometry: Photon activation with vacuum ultra-violet brings unique information and enables definitive structure assignment

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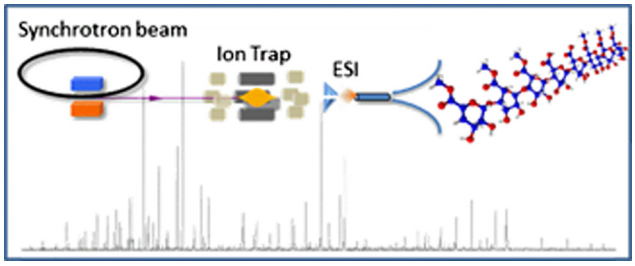
HIGHLIGHTS

- A complex mixture of methylated oligogalacturonans was fractionated by IP-RP-UHPLC.
- Synchrotron-radiation in VUV range was used as an activation process for tandem MS.
- VUV activation brought rich structural information compared to LE-CAD.
- Resolution of more than 35 structures, including isomers, was successfully completed.

GRAPHICAL ABSTRACT



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ABSTRACT

Carbohydrates have a wide variety of structures whose complexity and heterogeneity challenge the field of analytical chemistry. Tandem mass spectrometry, with its remarkable sensitivity and high information content, provides key advantages to addressing the structural elucidation of polysaccharides. Yet, classical fragmentation by collision-activated dissociation (CAD) in many cases fails to reach a comprehensive structural determination, especially when isomers have to be differentiated. In this work, for the first time, vacuum ultra-violet (VUV) synchrotron radiation is used as the activation process in tandem mass spectrometry of large oligosaccharides. Compared to low energy CAD (LE-CAD), photon activated dissociation brought more straightforward and valuable structural information. The outstanding feature

Abbreviations: VUV, vacuum ultra-violet; CAD, collision activated dissociation; LE, low-energy; HE, Hhigh-energy; DM, degree of methylation; MS, mass spectrometry; EID, electron induced dissociation; ETD, electron transfer dissociation; ECD, electron capture dissociation; EDD, electron detachment dissociation; UHPLC, ultra-high performance liquid chromatography; IP, ion pairing; RP, reverse-phase; PD, photo-dissociation; DPI, dissociative photoionization; HPAEC, high-performance anionic exchange chromatography; SEC, size exclusion chromatography; HILIC, hydrophilic interaction liquid chromatography; PGC, porous graphitized carbon; MALDI, matrix assisted laser desorption ionization; ESI, electrospray ionization; TOF, time of flight; IC, internal conversion; IVR, internal vibrational redistribution.

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Vacuum ultra-violet synchrotron photon activation
Tandem mass spectrometry
Structure

was that complete series of informative ions were produced, with only minor neutral losses. Moreover, systematic fragmentation rules could be drawn thus facilitating the definitive assignments of fragment identities. As a result, most of the structures present in a complex mixture of oligogalacturonans could be comprehensively resolved, including many isomers differing in the position of methyl groups along the galacturonic acid backbone.

1. Introduction

Biological carbohydrates play a crucial role in living organisms as structure or storage-related components, but also in signaling and recognition processes such as those involved in protein targeting and extracellular interactions [1,2]. These molecules also represent the most abundant biopolymers on earth and in the oceans, and have a wide variety of structures and properties. As such, they provide a vast reservoir of molecules of putative interest for several industries (e.g. anticoagulation and antitumor effects in the pharmaceutical industry, source of energy, dietary fibers, texturing and gelling agents in the cosmetics and food industries). Biological functions and end-uses of carbohydrates are strongly linked to their chemical structures, although, for many of them, these structures remain unknown or unexplored. If the glycosidic composition plays a major role, minor changes in linkage, branching as well as modifications of glycan residues might have a huge influence on the glycan properties.

Our work herein focused on pectins. These biopolymers account for about 30% of the composition of primary cell walls in terrestrial plants [3]. The degree of methylation (DM) and the distribution of the methyl groups along the galacturonan backbone contribute to distinct mechanical properties of the cell walls (e.g. cell expansion, intercellular adhesion, and porosity) and chemical properties (e.g. control of the cell wall ionic status) [4]. This backbone essentially consist of a linear chain of α-(1→4)-linked D-galacturonic acid (GalA) units, called homogalacturonan, that can be methyl esterified on their acidic functions. These features impact strongly on the pectin functionalities, such as the gelling properties or the ability to stabilize proteins, both of which are closely connected to the ionic binding capacities of pectins [5]. To better understand the structure/property relationships and tailor molecules with enhanced functionalities, a fine structural elucidation of pectins is needed, including the exact positioning of the methyl groups along the galacturonan backbone.

A variety of analytical methods have been applied for the structural characterization of polysaccharides. Among them, tandem mass spectrometry (MS/MS) remains at the forefront owing to its outstanding sensitivity and high information content. The most popular method for fragmentation is low energy collision activated dissociation (LE-CAD), a slow thermal excitation of ions produced by collision with gaseous molecules or rare gases. A series of fragment ions arising from cleavage of the glycosidic bonds is usually predominant in this process, such as Y and Z fragments, containing the reducing end, and B and C fragments, containing the non-reducing end (according to the nomenclature of Domon and Costello [6]). However, knowledge of B/C and Y/Z fragments is not always sufficient to comprehensively determine oligosaccharide structures, for instance, to pinpoint subtle modifications or provide information about branching and linkages. Additional product ions might be observed by multiple stages of MS/MS (so-called MSn), albeit that the sensitivity drops at each step and might limit the practical use of this strategy. An alternative is offered by high energy CAD (HE-CAD, ca. 1000 eV). This method was described using MALDI instrumentation, either combining sector fields and orthogonal time-of-flight (TOF) [7,8] or TOF/TOF analyzers [9,10] More cross-ring cleavages are observed, bringing valuable structural information on oligosaccharides. However, both in LE- and HE-CAD, occurrence of rearrangement ions, neutral losses, and internal fragments originating from multiple possible pathways (e.g. B/Y internal cleavages) complicate the interpretation of mass spectra, thereby preventing this method, in some cases, from achieving unambiguous structure assignment. In spite of these limitations, LE-CAD was successfully employed to characterize methyl-esterified galacturonans, both from positive and negative ions [11,12]. Nevertheless, it is worth noting that, in these studies, the dissociation pathways were found to vary greatly with the number and distribution of methyl-esterified galacturonic acid residues and no clear rules could be drawn for spectra interpretation. The authors concluded that definitive structural determination was possible only in cases of low sample heterogeneity.

Ion/electron interactions can be used as alternative dissociation methods to CAD. These include EID (electron induced dissociation [13]), ETD (electron transfer dissociation [14]), and ECD (electron capture dissociation [15]). Use of ECD and ETD for glycans is exemplified in [16,17]. In contrast to the dissociation of even electron molecular ions, significantly different fragmentation behavior is observed under ECD and ETD conditions and leads to more informative spectra. They contain many cross-ring fragments (A and X fragments), and thus provide information on branching and linkages. However, these electron-based activation methods are limited to components able to form positively- and multiply-charged species, and therefore are not directly amenable to acidic compounds, like pectins. For negatively-charged or neutral compounds, electron detachment dissociation (EDD), EID and negative ETD have been applied to the analysis of chitooligosaccharides [18], glycosaminoglycans (GAGs) [19–22] and sialylated oligosaccharides [23]. Although these electron-based methods offer interesting alternatives to CAD, they tend to produce highly complex spectra that are that are difficult to interpret for unknown structures, with fragments arising from many pathways and undergoing numerous neutral losses (H_2O, CO_2, and methanol, etc.).

An alternative technique was introduced more recently and employs ion/photon interactions as a way to induce dissociation. It is particularly attractive because of the potential for obtaining wavelength-dependent information, thus adding a degree of specificity. In the infra-red (IR) range, multi-photon dissociation may use 10.6 μm photons resulting in a slow thermal excitation close to that undergone by ions in CAD [24,25]. However, the features of ultraviolet (UV) [26] are more promising and even more so is vacuum UV (VUV, λ < 200 nm) photoexcitation, which produces a fast and high energy deposition from a single-photon absorption [27–30]. Photodissociation (PD) at 157 nm (7.9 eV) of positive ions was thoroughly investigated by Reilly and co-workers, in particular for glycans after Girard's T derivatization [27]. These studies ubiquitously observed that VUV PD yields unique fragment distribution and highly informative spectra, including extensive cross-ring fragments. Electron photodetachment may arise from photon activation of negative ions, leading to the formation of odd electron species. The latter exhibit specific reactivity and produce distinct fragmentation patterns complementary to those obtained from the activation of even electron species. Interestingly, Dugourd and co-workers introduced a near-UV PD (220–240 nm) of deprotonated ions which does not require any derivatization [31,32]: the activation is promoted by the presence of a C=C double bond at the non-reducing end of