International Journal of Mass Spectrometry 285 (2009) 70–77

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Structural characterization of negatively charged glycosaminoglycans using high-energy (50–150 keV) collisional activation

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ARTICLE INFO

Article history:
Received 25 March 2009
Received in revised form 24 April 2009
Accepted 27 April 2009
Available online 3 May 2009

Keywords:
Glycosaminoglycan
Multiply charged anions
Sulphated sugars
Collision-induced dissociation
High-energy mass spectrometry

ABSTRACT

Anionic glycosaminoglycan mono- and disaccharides (IVA, IH and IS) were subjected to very high-energy collisions (50–150 keV ion kinetic energy prior to collision) with Neon gas in an accelerator mass spectrometer, to explore the possibility of using this method to structurally characterize anionic sugars. Experiments were also conducted for the $Na^+ \cdot IH^{2-}$ and $Na^+ \cdot IS^{3-}$ sodiated complexes. This high-energy ion collision technique is applied here to sugars for the first time. Low-energy collision-induced dissociation (CID) measurements obtained using resonance excitation in a quadrupole ion-trap are presented for comparison. The high-energy measurements produce a rich variety of fragment ions, illustrating the general utility of the technique for providing detailed information for structurally characterizing sugar ions. We discuss the observed fragmentation patterns with reference to the known fragmentation behavior of small gas-phase monoanions, multiply charged anions and cation-dianion complexes.

1. Introduction

Over recent years, the study of gas-phase biological molecules has developed into a rapidly expanding field of research, with considerable experimental and theoretical effort being expended to provide detailed insights into the geometric and electronic properties of isolated biomolecules [1–11].

Progress in this area has been made possible by the application of generic chemical physics techniques to biomolecular systems, in particular through the application of high-resolution laser spectroscopy [1,4–10]. While laser spectroscopy is particularly powerful at providing detailed geometric structures, there is also scope for applying other molecular physics techniques in this area [11–14]. In this work, we describe the application of a high-energy ion-collision technique to the structural characterization of gas-phase sugar molecules.

Glycosaminoglycans (GAGs) are linear, sulfated polysaccharides found on the cell surfaces of a wide range of organisms. GAGs have been implicated in numerous biological roles including cell signaling, cancer progression, and in the regulation of the inflammatory immune response [15]. Although GAGs are unbranched and composed of repeating chains of acidic and basic sugar subunits, their structure is complex due to the varying levels of sulphation and N-acetylation. Heparin and Heparan Sulphate are the most intensively studied GAGs and are composed of a repeating disaccharide subunit made up of hexuronic acid followed by glucosamine. The structural complexity of heparins arises through several factors, including whether the hexuronic acid is either a glucuronic or an iduronic acid, and the positions of sulphation and acetylation [15].

In this study, we explore the possibility of structurally characterizing anionic GAGs by performing electron detachment *via* very high-energy collisions (50–150 keV ion kinetic energy prior to collision) with a nobel gas. This high-energy collision technique has previously been applied to studying the structures and potential energy surfaces of a number of gas-phase chemical systems, [16–21] including multiply charged oligonucleotide anions, [20] but is applied here to sugars for the first time. We also present low-energy collision-induced dissociation (CID) measurements obtained using resonance excitation in a quadrupole ion-trap for comparison, since low-energy CID is widely used to characterize the structures of oligosaccharides. Fig. 1 illustrates the acidic sugars that have been investigated in this initial study, including three prototypical GAG disaccharides (IVA, IH and IS), and three monomer sugar units (which we label A, B and C). These sugars are deprotonated in solution, so that negative ion electrospray ionization produces deprotonated gas-phase anions. The selected sugars are more densely charged than the related systems that have been studied previously using lower energy activated dissociation [22–24].

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doi:10.1016/j.ijms.2009.04.009

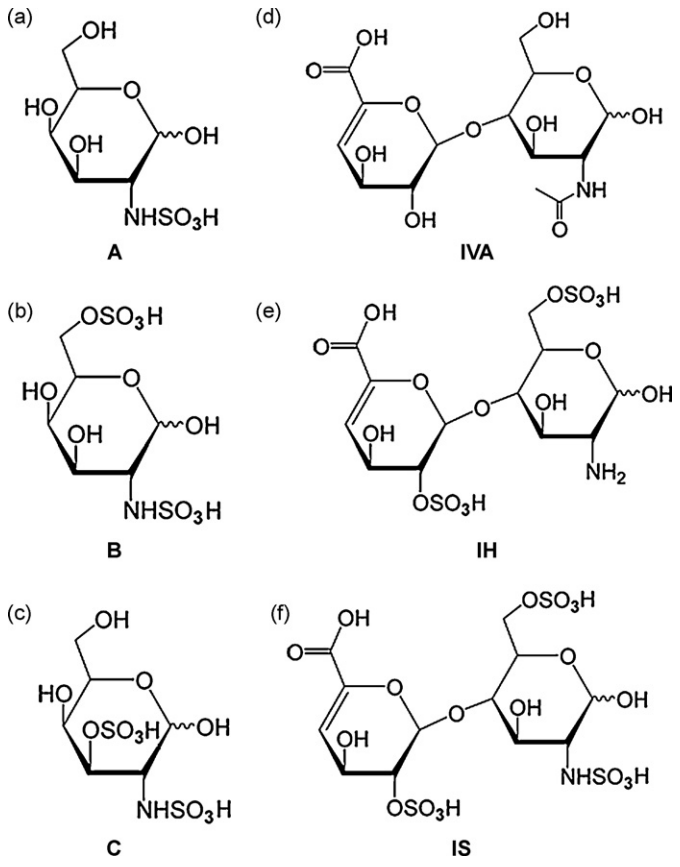

Fig. 1. The chemical structures of the six sulphated sugars studied in this work. (a) The A monosaccharide, GlcNS, (b) the B monosaccharide, GlcNS-6S, (c) the C monosaccharide, GlcNS-3S, (d) the IVA disaccharide, α-ΔUA-[1 → 4]-GlcNAc, (e) the IH trianionic disaccharide, α-ΔUA-2S-[1 → 4]-GlcN-6S, and (f) the IS disaccharide, α-ΔUA-2S-[1 → 4]-GlcNS-6S. (The sugar names have been abbreviated as follows: ΔUA = 4-deoxy-L-threo-hex-4-enopyranosyluronic acid; GlcN = D-glucosamine; Ac = Acetyl; NS, 2S, 3S and 6S = N-sulfo, 2-sulfate, 3-sulfate and 6-sulfate, respectively.)

Determining the pattern of sulphate and acetylate substitution displayed on Heparin and Heparan Sulphate is a matter of particular biological importance as it is thought to be the key to their biochemical activity. The availability of samples is limited to naturally occurring species since GAGs cannot yet be routinely synthesized. For this reason, structural characterization by NMR is severely limited by the purity and volume of material available [25]. Enzymatic digestion and Capillary Electrophoresis/MS^n based characterization techniques suffer from high sample consumption, low turnaround times, and low accuracy relative to mass spectrometry techniques, hence limiting the structural information that can be obtained from these methods [26]. A full complement of cross-ring and glycosidic cleavage fragments is required to determine the sequence of the sugar polymer and the sulphation sites within the sugar units. The suitability of mass-spectral techniques for obtaining structural information on GAG systems is therefore strongly dependent on the method's ability to retain the fragile sulphate groups following molecular fragmentation.

Recent advances in the structural characterization of anionic saccharides have included the application of Electron Detachment Dissociation (EDD) to effect the fragmentation of GAG tetrasaccharides [22,23]. This method involves the irradiation of a multiply charged anionic GAG with electrons of 15–20 eV, initiating electron detachment and subsequent fragmentation. Analysis of the resulting products has revealed the presence of fragments not seen by either low-energy CID or infrared multi-photon dissociation (IRMPD) techniques, and has shown an abundance of cross-ring and glycosidic cleavages. In addition, EDD was shown to be capable of distinguishing between the epimers iduronic and glucuronic acid based on the fragments produced [23]. To date however, experiments have been limited to model tetrasaccharides containing a lower level of sulfation than has been seen previously in biologically active GAG chains. We anticipate that high-energy ion collisions may also lead to EDD-type fragmentation of sugars and may therefore prove highly useful for performing structural characterization of GAGs.

2. Experimental

2.1. Mass-analyzed ion kinetic energy (MIKE) spectrometry

The experimental set-up of the SEP 1 accelerator mass spectrometer has been described in detail previously [17]. Monosaccharides (Dextra Laboratories) and disaccharides (Sigma–Aldrich) were purchased as the sodium salt complexes. All compounds were used without further purification. The ions studied were prepared *via* ESI (negative ion mode) from 1:1 water–methanol solutions. The sugars studied were mass selected in the following charge states, A^-, B^{2-}, C^{2-}, IVA^-, IH^{2-} and IS^{3-}, where we denote A^- to be equivalent to the more commonly used $[A-H]^-$, etc., to simplify the labeling of the fragmentation mass spectra.

Accelerated ions of 50 keV (monoanions), 100 keV (dianions) and 150 keV (trianions) kinetic energy were mass selected by a magnet, and subjected to single-collision activation in a 3 cm collision cell containing neon gas at a pressure of approximately 0.2 mTorr. Mass analyzed ion kinetic energy scans of the anions exiting the collision cell were recorded using a 180° hemi-spherical electrostatic analyzer coupled to a channeltron detector. The flight time from the collision cell to the detector is a few microseconds, setting a limit on the time available for rearrangement of the parent or fragment ions following collision. We note that due to the design of the detector on the SEP1 instrument, a peak is present below the parent ion. For dianionic species, this means that artificial peaks can mask the presence of (dianionic) fragment ions corresponding to loss of a neutral water unit from the parent ion.

2.2. Low-energy collision-induced dissociation

A Finnigan LCQ electrospray quadrupole ion-trap mass spectrometer in negative ion mode was used to perform the low-energy CID experiments as described previously [27]. Sample solution concentrations were 10^{-4} M in methanol, prior to electrospraying in an 80:20 methanol/water solution. Signal optimization was performed using the LCQ automated tuning feature with the cone voltage set at 4.2 kV and a capillary temperature of 150 °C. The sample flow rate was set to 5 μL/min.

Low-energy CID (or resonance excitation) was performed by applying an AC excitation voltage to the end caps of the ion trap. The LCQ uses a Mathieu q_z value of 0.25 and an excitation time of 30 ms. This induces multiple low-energy collisions with the Helium background gas (1×10^{-4} Torr) at a rate of $\sim 10^4\ s^{-1}$. When the high-energy tail of the ion energy envelope reaches the activation energy for the lowest energy decomposition pathway, fragment ions are observed [28]. The excitation voltage applied is varied between 0 and 2.5 V to optimize the number of product fragment ions for each sample, with CID energies being quoted as a percentage of the 2.5 V excitation voltage in line with standard practice [29,30].