Wavelength-Tunable Ultraviolet Photodissociation (UVPD) of Heparin-Derived Disaccharides in a Linear Ion Trap

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A set of three heparin-derived disaccharide deprotonated ions was isolated in a linear ion trap and subjected to UV laser irradiation in the 220–290 nm wavelength range. The dissociation yields of the deprotonated molecular ions were recorded as a function of laser wavelength. They revealed maximum absorption at 220 nm for the nonsulfated disaccharide, but centered at 240 nm for the sulfated species. The comparison of the fragmentation patterns between ultraviolet photodissociation (UVPD) at 240 nm and CID modes showed roughly the same distribution of fragment ions resulting from glycosidic bond cleavages. Interestingly, UVPD favored additional cross ring cleavages of A and X type ion series enabling easier sulfate group location. It also reduced small neutral losses (H₂O). (J Am Soc Mass Spectrom 2009, 20, 1645–1651) © 2009 Published by Elsevier Inc. on behalf of American Society for Mass Spectrometry

The major advances achieved in mass spectrometry have revolutionized the structural analysis of proteins and led to the emergence of proteomic strategies. Although software-assisted de novo sequencing of peptides resulting from unknown proteins has become a routine task, the structural assignment of complex N- and O-glycans is much less trivial. Unlike proteins accurately translated from a unique template that follows a universal code, a similar set of glycosyl transferases may, across different species, build oligosaccharides of different structure according to an *a priori* unpredictable chronological catalysis activity.

Compared with N- and O-linked glycans, the issue of structural complexity is continuing to increase in the world of glycosaminoglycans. Glycosaminoglycans are linear polymerized disaccharidic repeating units containing hexuronic acids and hexosamines. Their essential roles in governing numerous biologic functions [1] have led many groups to focus their efforts on defining generic strategies based on mass spectrometry, to obtain the structural definition of these acidic oligosaccharides. In the case of oligosaccharides derived from heparin or heparane sulfate, both glucuronic and iduronic acids and glucosamine may be sulfated at their OH and NH₂ groups. The fragmentation behavior of heparin-derived oligosaccharides has proven to be difficult to predict. Sulfate groups often impede access to

exhaustive structural information by collision induced dissociation (CID)-MS/MS. Indeed, when the charge state of the selected molecular ion is low, neutral SO₃ losses most often predominate in comparison with glycosidic backbone cleavages [2]. This situation can be improved by combining MS/MS data resulting from different charge states.

Very recently, Amster and coworkers published outstanding results showing how promising the electron detachment dissociation (EDD) of deprotonated sulfated oligosaccharides can be in providing informative fragment ions [3]. In particular, they documented the discrimination between the C5 epimeric glucuronic and iduronic acids based on specific fragment ions [4]. As in CID mode, they also observed that SO₃ loss can be dramatically reduced when EDD is performed on the charge states where the carboxylate group is deprotonated and subsequently subjected to electron removal [5]. A similar feature is obtained by replacing protons with sodium cations. UV and IR gas-phase photodissociation (i.e., ultraviolet photodissociation (UVPD) and infrared multiphoton dissociation (IRMPD) of positive and negative precursor ions also appears to be a very promising activation method for providing new or complementary structural information in CID. These techniques have been used for the analysis of biomolecules, such as proteins [6–11], peptides [12–16], and nucleic acids [17–19]. In the case of permethylated and fixed-charge derivatized oligosaccharides, the 157 nm UVPD spectra yield intense cross ring fragment ions corresponding to a high-energy dissociation pathway,

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Table 1. Name, abbreviation and structure of each heparin-derived disaccharide studied in this work

Name	Abbrev.	Structure
Disaccharide II-H	Δ UA-GlcN6S	
Disaccharide III-H	Δ UA2S-GlcN	
Disaccharide IV-H	Δ UA-GlcN	

useful in the assignment of branching and glycosidic linkages [20]. An alternative to UVPD is the reductive amination of oligosaccharides with a fluorophore. Hence, Wilson and Brodbelt noticed that in contrast to CID, the 355 nm photodissociation of fluorophore-labeled glycans produced additional nonreducing end fragment ions for improved comprehensive structural assignment [21].

With the perspective of access to new fragmentation pathways, the present work explores for the first time the merits of near UV photodissociation of polyanionic precursor ions of acidic disaccharide models in a linear quadrupole ion trap.

Materials and Methods

Materials

Disaccharides (Table 1) were obtained from Dextra Laboratories and dissolved at a concentration of 100 μ M

Figure 2. Photofragmentation yields measured as a function of the laser wavelength for deprotonated Δ UA-GlcN6S, Δ UA2S-GlcN, and Δ UA-GlcN.

in H₂O/MeOH (50:50, vol/vol). Additional experiments were also performed on saccharose. Saccharose was dissolved at a concentration of 150 μ M in H₂O/MeOH (50:50, vol/vol). Samples were introduced by electrospray ionization in negative mode. They were infused at a flow rate of 5 μ L/min using a syringe pump.

Mass Spectrometry and UV Photodissociation

The experimental setup (Fig. 1) consists of a mass spectrometer coupled to a visible/UV tunable OPO laser. The mass spectrometer is a quadrupole linear ion trap (LTQ; Thermo Fisher Scientific, San Jose, CA, USA). A quartz window was fitted on the rear of the LTQ chamber to allow the introduction of the laser beam. The OPO laser (Panther OPO laser pumped by a 355-nm Nd:YAG Surelite; Continuum, Santa Clara, CA, USA) can be scanned from 215 nm up to 2 μ m. Its main

Figure 1. Schematic of the experimental set up used for UVPD in a linear quadrupole ion trap.

characteristics are a repetition rate of 10 Hz and a pulse width of 7 ns. The laser beam passes through two diaphragms, lenses (3:1 telescope), and a mechanical shutter electronically synchronized with the mass spectrometer, after which it is injected into the axis of the linear trap through a 1 mm-diameter pinhole. The laser energy after the pinhole is between 0.1 and 1 mJ/pulse. The mechanical shutter is used to synchronize laser irradiation with ion trapping. To perform laser irradiation for a given number of laser pulses, we add an MS^n step with activation amplitude of 0% in the ion trap RF sequence, during which the shutter located on the laser beam is opened. The laser power is monitored in front of the mass spectrometer by a power meter (Ophir, Logan, UT, USA).

In the UVPD experiments, precursor ions were first isolated in the trap. After isolation, they were irradiated for n laser shots, and the resulting mass spectrum was recorded. Optical spectra (action spectra) were obtained by systematically recording UVPD spectra as a function of the laser wavelength. The photodissociation yield is given by $\ln((\text{precursor} + \text{fragments})/\text{precursor})/(\lambda \times$

$P_\lambda)$, where λ is the laser wavelength, precursor the intensity of the precursor ion, fragments the sum intensity of the identified fragment ions and P_λ the laser power measured at the entrance of the mass spectrometer. CID experiments were performed with the same apparatus. This was accomplished using helium gas at a normalized collision energy of 13% to 15% for 30 ms. The activation q value was set to 0.200 for CID and for UVPD. The isolation width for selecting the ion precursors (both for UVPD and CID) was 3 Da. A total of 50 microscans were averaged to produce a spectrum.

Results and Discussion

Whereas high photon energy [20, 22] enables the photofragmentation of native oligosaccharides, derivatization with a fluorophore is used for visible and near UV photofragmentation of oligosaccharides [21, 23]. To delineate the wavelength ranges that could be used in the near UV range to fragment acidic oligosaccharides, we recorded the first gas-phase optical spectra as a

Figure 3. (a) UVPD ($\lambda = 240$ nm) product ion mass spectrum of $\Delta\text{UA-GlcN6S}$ $[\text{M} - \text{H}]^-$. Insert, $\Delta\text{UA-GlcN6S}$ structure with major cleavages indicated. (b) CID product ion mass spectrum of $\Delta\text{UA-GlcN6S}$ $[\text{M} - \text{H}]^-$. Insert, $\Delta\text{UA-GlcN6S}$ structure with major cleavages indicated.

function of laser wavelength for three heparin-derived disaccharides.

Heparins are acidic molecules that readily form negative ions. In the full MS spectrum of Δ UA-GlcN6S and Δ UA2S-GlcN molecules, the most abundant molecular ions correspond to the deprotonated species $[M - H]^-$. Less intense $[M - 2H]^{2-}$ ions as well as sodium adduct ions $[M - 2H + Na]^-$ were also observed. In the case of mono acidic Δ UA-GlcN heparin-derived disaccharide, only the singly charged $[M - H]^-$ ion was observed. Hence, all the subsequent experiments for studying optical properties and for comparing the respective UVPD and CID dissociation pathways were performed on the singly $[M - H]^-$ deprotonated species.

The measurement of the fragmentation yield for the nonsulfated uronic acid containing Δ UA-GlcN disaccharide, which reflects the action spectrum, displays a broad band with a maximum at $\lambda \sim 228$ nm (Fig. 2). For the two sulfated Δ UA-GlcN6S, Δ UA2S-GlcN isomeric

disaccharides, the overall dissociation yield was higher and the band was centered at $\lambda = 240$ nm.

To our knowledge, these are the first gas-phase UV-optical spectra reported for oligosaccharides, whereas Polfer et al. [24] have published the possible differentiation of isomeric glucose-containing disaccharide by wavelength-tunable infrared multiple photon dissociation. More generally, these UV-optical spectra can be used as a reference for the optical absorption of carboxylate and sulfate groups in the gas phase. By contrast, the three solution spectra display an onset of absorption at $\lambda \sim 260$ nm after which the absorption increases regularly as the photon energy increases, with no identified band above 230 nm (aqueous solution, 100 μ M at pH = 5, spectra not shown). According to time-dependant density functional theory (TDDFT) calculations performed for small molecular mimics with sulfate and carboxylate groups (results to be published), the absorption in this region is mainly due to the

Figure 4. (a) UVPD ($\lambda = 240$ nm) product ion mass spectrum of Δ UA2S-GlcN $[M - H]^-$. Insert, Δ UA2S-GlcN structure with major cleavages indicated. (b) CID product ion mass spectrum of Δ UA2S-GlcN $[M - H]^-$. Insert, Δ UA2S-GlcN structure with major cleavages indicated.

Figure 5. (a) UVPD ($\lambda = 240$ nm) product ion mass spectrum of Δ UA-GlcN $[M - H]^-$. Insert, Δ UA-GlcN structure with major cleavages indicated. (b) CID product ion mass spectrum of Δ UA-GlcN $[M - H]^-$. Insert, Δ UA-GlcN structure with major cleavages indicated.

excitation of the excess electron localized on the acidic groups that results in partial charge-transfer from the negative site to the bonds in the vicinity of this acidic function. DUA-GlcN6S and Δ UA2S-GlcN are expected to be deprotonated on the sulfate group whereas Δ UA-GlcN is deprotonated on the carboxylate group. So, the transitions leading to the maximum of UV light absorption at $\lambda = 240$ nm for the two sulfated disaccharides likely involves electrons localized on the sulfate groups.

Figures 3a, 4a, and 5a show the UVPD spectra at $\lambda = 240$ nm recorded after three laser pulses for isolated $[M - H]^-$ ions with respectively Δ UA-GlcN6S, Δ UA2S-GlcN, and Δ UA-GlcN structures. They are similar to those previously reported by Saad and Leary [25]. Figures 3b, 4b, and 5b provide their corresponding CID spectra. In the case of a single shot irradiation (data not shown), identical photofragments with similar relative abundances but with lower signal intensities were observed. Moreover, the total yield of fragmentation increases linearly with laser power, whatever the disac-

charide structure, which is in agreement with a parent ion fragmentation driven by a one photon process, as observed in the case of peptides [26]. Most of the fragment ions resulting from CID were also observed in UVPD mode (Table 2). This is an indication that part of the UVPD fragmentation occurs after internal vibrational energy redistribution (IVR) and then global heating of the precursor ions. As expected from our gas-phase optical spectroscopic study, the UVPD depletion rate at 240 nm of $[M - H]^-$ for Δ UA-GlcN heparin derived disaccharide is lower than its two sulfated counterparts.

The pattern of fragment ions stemming from dissociation at the glycosidic bond shows close similarities between UVPD (Figure 3a, 4a, and 5a) and CID (Figure 3b, 4b, 5b) activation spectra. Thus Y_1 (m/z 258.0) and Z_1 (m/z 240.0) ions, B_1 -SO₃ (m/z 157.0) ion, C_1 (m/z 175.0), and B_1 (m/z 157.0) ions are observed, respectively, for Δ UA-GlcN6S, Δ UA2S-GlcN, and DUA-GlcN disaccharides, whatever the activation method.

Table 2. List of fragment ions resulting from UVPD at 240 nm and CID of [M–H][–] ions for ΔUA GlcN6S, ΔUA2S–GlcN, and ΔUA–GlcN disaccharides

Ion	Calc. <i>m/z</i>	UVPD Obs. <i>m/z</i>	CID Obs. <i>m/z</i>
ΔUA–GlcN6S			
[M–H–H ₂ O] [–]	398.04		398.1
^{0,2} A ₂	357.01	357.1	357.2
^{0,2} X ₁	300.04	300.1	300.2
Y ₁	258.03	258.1	258.2
^{0,2} X ₁ / ^{0,2} A ₂	241.00	241.1	
Z ₁	240.02	240.1	240.1
Z ₁ –H ₂ O	222.01		222.1
Y ₁ / ^{0,2} A ₂	199.00	199.1	199.1
^{0,4} X ₀	138.97	139.1	
HCO ₄ [–]	96.96		97.0
ΔUA2S–GlcN			
[M–H–H ₂ O] [–]	398.04		398.0
^{0,2} A ₂	357.01	357.1	357.2
^{0,2} X ₁	300.04	300.1	300.2
^{0,2} X ₁ / ^{0,2} A ₂	241.00	241.1	
B ₁	236.97	237.0	237.1
^{1,5} A ₁	208.98	209.0	
B ₁ –SO ₃	157.01	157.1	157.1
^{1,3} A ₁	138.97	139.0	
HCO ₄ [–]	96.96		97.0
ΔUA–GlcN			
[M–H–H ₂ O] [–]	318.08		318.0
^{0,2} A ₂	277.06		277.0
^{0,2} A ₂ –H ₂ O	259.05		259.1
^{2,4} A ₂	217.03	217.1	
C ₁	175.02	175.1	175.1
B ₁	157.01	157.1	157.1
^{0,2} A ₁	115.00	115.1	

In contrast, UVPD and CID activation spectra differ markedly in the fragment ion patterns originating from cross-ring cleavage and neutral loss. UVPD spectra are characterized by more intense cross-ring fragmentations of A and X series than CID, which in turn favors H₂O neutral loss. This increase in cross-ring cleavages in UVPD spectra in comparison with CID spectra was also observed with higher photon energy by Reilly and coworkers [27]. In the case of ΔUA–GlcN6S disaccharide, UVPD specifically initiates the formation of ^{0,4}X₀ and ^{0,2}X₁/^{0,2}A₂ fragment ions next to the ^{0,2}A₂ and ^{0,2}X₁ species common to CID. For the ΔUA2S–GlcN compound the ^{1,3}A₁ fragment appears specific to UVPD, whereas for the ΔUA–GlcN structure two cross ring cleavages are restricted to this mode of activation, i.e., ^{2,4}A₂ and ^{0,2}A₁. In all cases, UVPD leads to more informative cross-ring fragmentations than the CID mode, enabling unambiguous delineation of the sulfate group. All these fragments correspond to cross-ring cleavages that retain the carbon atom to which the sulfate is attached. This specific fragmentation is localized near the initial electronic excitation and may then occur before internal conversion or total IVR. This may be due to the nature of the UV excitation that leads to a partial charge-transfer to and destabilization of the bonds in the vicinity of the sulfate group. Most importantly, sulfate excitation does not result in unspecific SO₃ losses.

Conclusion

The present work reports for the first time on the optical spectra and photodissociation patterns of gas-phase deprotonated anions of three heparin-derived disaccharides. Whereas disaccharide with a carboxylic function displays maximum optical absorption at λ = 228 nm, the latter is observed at 240 nm for sulfated disaccharides. UVPD spectra appear more informative in all cases than CID spectra due to additional cross-ring cleavages that provide key fragment ions informative of the sulfate group location. For sulfated disaccharides, absorption of UV photons at λ = 240 nm mainly leads to the electronic excitation of the sulfate group, which results in specific cross-ring cleavages close to the sulfate group without breaking the SO₃ labile bond. This feature is currently being evaluated among larger oligosaccharides derived from acidic proteoglycans. The advantage of combining UVPD or EPD with CID in MSⁿ experiments for extensive structural analysis of such oligosaccharides, which has already proven to be successful for peptides [12–15], DNA [17, 18], and glycans with higher energy photons [20–22, 27], will also have to be evaluated.

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