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Negative Polarity Helium Charge Transfer Dissociation Tandem Mass Spectrometry: Radical-Initiated Fragmentation of Complex Polysulfated Anions

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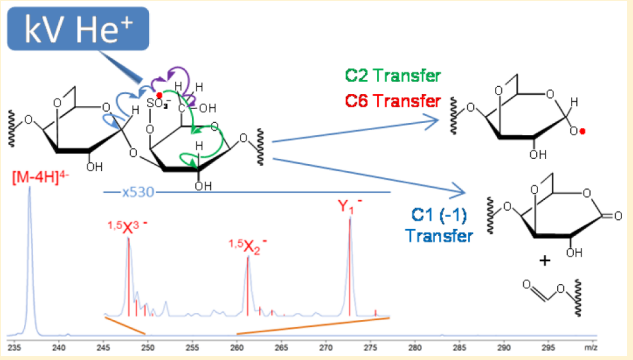
Supporting Information

ABSTRACT: This work provides the first use of helium charge transfer dissociation (He-CTD) tandem mass spectrometry (MS/MS) in negative polarity mode. Three sulfated oligosaccharides of natural origin were chosen as representative structures that are difficult to solve by conventional MS/MS approaches. Negative polarity He-CTD provided a full set of structurally informative fragments, which permitted the unambiguous determination of the complete structures of these molecules, including the characterization of labile sulfated functional groups. Despite close structural features, the three molecules led to distinct fragmentation patterns depending on the position of the sulfate group in the heterocycle. The observed fragments showed a consistent radical-initiated mechanism of dissociation, which shares similarities with fragment types produced in electron detachment dissociation (EDD), negative electron transfer dissociation (NETD), or extreme UV photodissociation (XUV-PD). Short times of data collection and the fact that the technique can be affordably implementable in any standard laboratory and with a classical ion trap mass spectrometer were other remarkable characteristics of negative polarity He-CTD.



Helium charge transfer dissociation (He-CTD) using a 6 keV helium cation beam was introduced recently and proved very beneficial for the structural characterization of positively charged biological ions.[1−3] He-CTD is one of several alternative activation or dissociation approaches in tandem mass spectrometry (MS/MS) that seeks to overcome some limitations of collision induced dissociation (CID).[4] In this work, we show the first example of negative polarity He-CTD. Sulfated oligosaccharides were selected as examples of the difficulties encountered when attempting to resolve the structure of oligosaccharides with conventional MS/MS approaches. The difficulties of CID include the lack of structurally informative fragments, such as cross-ring fragments, the presence of many uninformative neutral losses, the loss of labile modifications (e.g., sulfations), and the occurrence of consecutive fragmentations, which leads to ambiguous assignments.[4−6]

Sulfated polysaccharides are present in a wide variety of organisms and have major biological functions. In particular, they are the main structural components of the cell walls of red (carrageenans, porphyrans), green (ulvans), and brown (fucoïdans) algae. Glycosaminoglycans (GAGs), a heterogeneous family of sulfated polysaccharides, are found in bacteria and animals. Intact or after degradation into oligosaccharides, sulfated polysaccharides are of interest for several industrial applications, including food, cosmetic, pharmaceutical, and health/supplement industries. The biological properties and the end-uses of sulfated poly-/oligosaccharides are closely linked to their structures and, notably, to their sulfation patterns. In mass spectrometric analysis, these molecules are very difficult to ionize in positive mode due to the strongly acidic sulfate groups. Fragmentation using CID usually fails to reach complete structural characterization because of the lack of cross-ring fragments and because of the dominant losses of the very labile sulfate groups.

EXPERIMENTAL SECTION

Oligosaccharides. Oligosaccharides were produced by the laboratory CNRS-UPMC UMR 8227, Station Biologique, Roscoff, France. Iota carrageenans from *Eucheuma denticulatum* (Danisco), Kappa carrageenans from *Euchema Cottonii* (CP-Kelco), and porphyrans extracted from *Porphyra umbilicalis* (collected on-site in the bay of Morlaix, France) were degraded enzymatically into oligosaccharides using a κ-carrageenase, a ι-carrageenase, and a β-porphyranase A, respectively. For all

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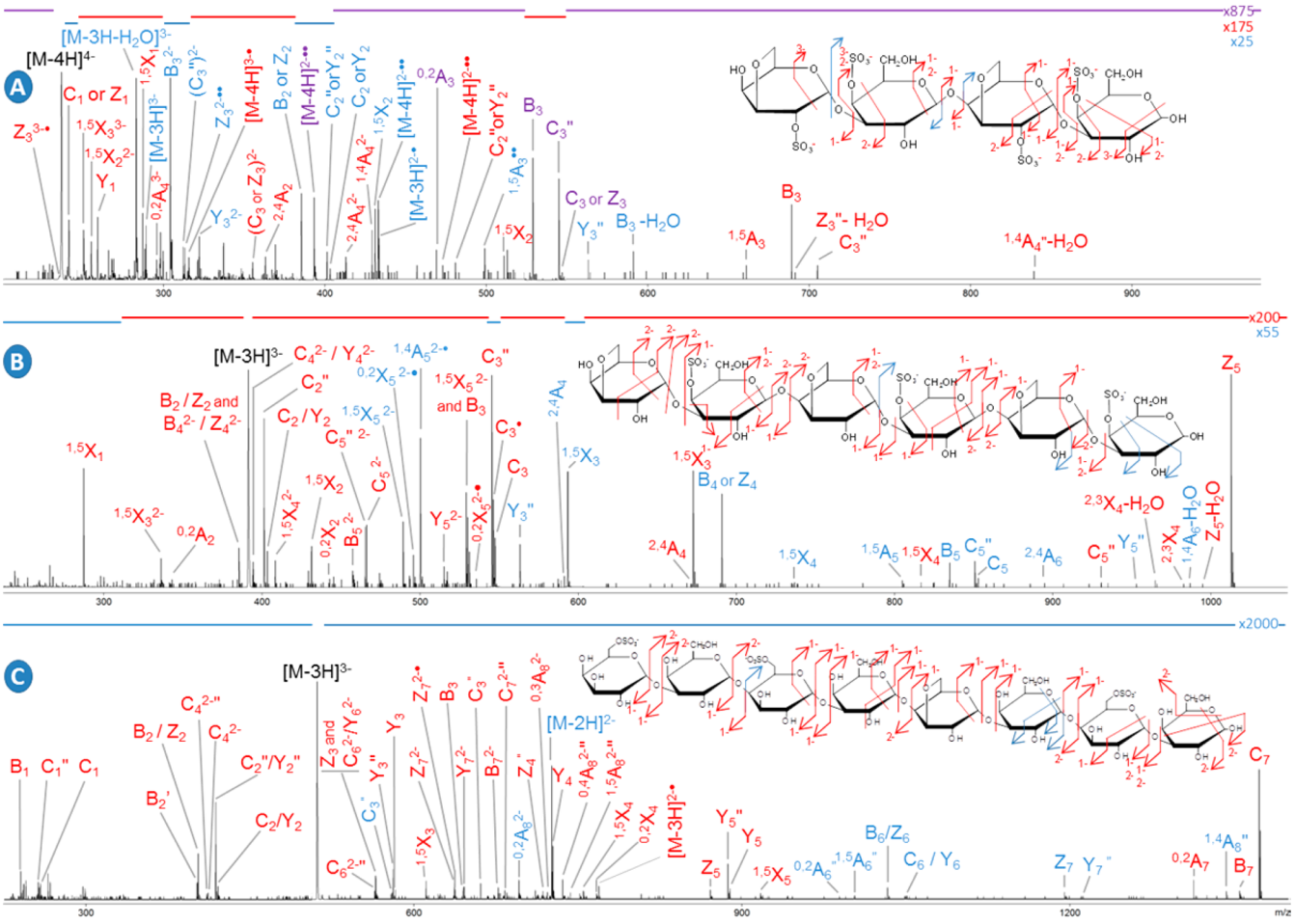

Figure 1. Negative polarity He-CTD MS/MS spectra of (A) oligo-ι carrageenan DP4 ($(DA2S\text{-}G4S)_2$, precursor ion isolated as $[M - 4H]^{4-}$ at m/z 236.72); (B) oligo-κ carrageenan DP6 ($(DA\text{-}G4S)_3$, precursor ion isolated as $[M - 3H]^{3-}$ at m/z 391.21); (C) hybrid oligo-porphyran DP8 ($(L6S\text{-}G)_2$-(LA-G)-(L6S-G), precursor ion isolated as $[M - 3H]^{3-}$ at m/z 511.49). Red: fully sulfated fragments. Blue: fragments with one sulfate loss. Purple: two sulfate losses. Distinct m/z ranges were amplified with the indicated factor and color code. Signal was accumulated over 1 min. Unless specified in the label, fragments are singly charged. Fragmentation efficiencies: (A) 12%, (B) 6.6%, and (C) 2%.

samples, purification was carried out by preparative size exclusion chromatography. An additional purification for the hybrid oligo-porphyran DP8 was performed by ion pairing reverse phase chromatography.[6]

He-CTD Tandem MS. He-CTD tandem MS experiments were performed using a modified ion trap mass spectrometer operated in negative polarity. A saddle field fast ion source (VSW/Atomtech, Macclesfield, UK) was interfaced with an AmaZon 3D ion trap (Bruker Daltonics) via a custom vacuum chamber cover.[1,2] The helium gas flow was controlled via a variable leak valve to the saddle field source and measured by the ion trap gauge (readout approximately 1.2×10^{-5} mbar). Precursor ions were isolated with a window width of 4 m/z and accumulated for 30 ms in the ion trap. They were exposed to the 6 keV helium cation beam for 100 ms. The total scan time, including isolation/accumulation, activation, cooling, and scanning, was approximately 200 ms. The fragmentation spectra displayed in the manuscript correspond to the signal averaged for 1 min. Oligosaccharides were diluted to 20 μg/mL in a 1:1 water/methanol mixture (vol/vol) and infused at a flow rate of 5 μL/min. Fragmentation was performed on the most abundant species detected in the MS spectrum, which corresponded, for each sample, to the fully deprotonated species.

RESULTS AND DISCUSSION

Three structures produced from sulfated algal polysaccharides, differing in their sulfation degree and in the positioning of the sulfate groups along the backbone, were used to highlight the potential of negative polarity He-CTD. Their chemical structures are shown in Figure 1. The first structure is an ι-carrageenan with a polymerization degree of 4 (DP_4, named (DA2S-G4S), MW = 950.0 Da). This structure is composed of anhydro-D-galactose (DA) and D-galactose (G) units. ι-Carrageenans are highly sulfated, and all subunits bear a sulfate group at position 2 and 4 of the ring for DA and G units, respectively (Figure 1A). The second structure is larger but less sulfated: this is a κ-carrageenan of DP6 ($(DA\text{-}G4S)_3$, MW = 1176.2 Da) (Figure 1B). This structure is built of anhydro-D-galactose and sulfated D-galactose at C4. The third structure is larger still and represents a hybrid agar-porphyran of DP8 ($(L6S\text{-}G)_2$-(LA-G)-(L6S-G), MW = 1536.3 Da), composed of D-galactose (G), sulfated L-galactose at C6 (L6S), and anhydro-L-galactoses (LA) (Figure 1C).

Figure 1 shows the fragmentation spectra recorded under He-CTD activation for the ι-carrageenan DP4, the κ-carrageenan DP6, and the hybrid agar-porphyran DP8. Polyanions ($[M - xH]^{x-}$, $x = 3$ or $x = 4$) were isolated as precursor ions. Identities of all fragments for the three structures were confirmed by ^{18}O labeling of the reducing ends (data not shown). In all cases,

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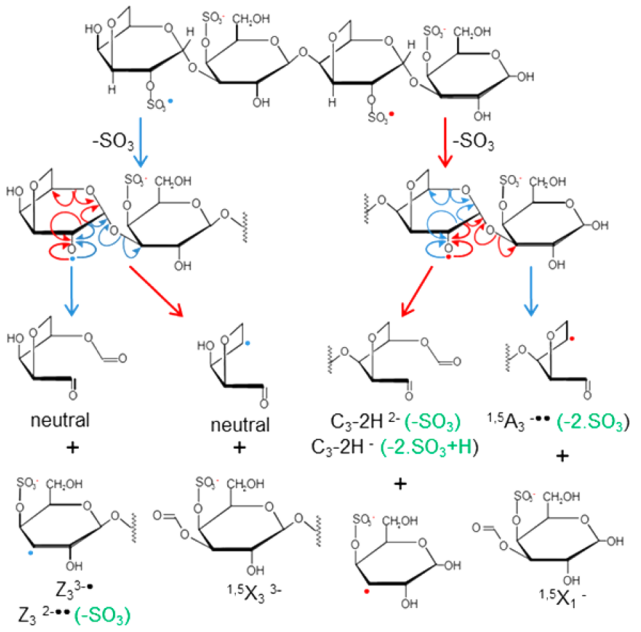
extensive fragmentation was observed, with numerous fragments observed from both ends (reducing and nonreducing ends) of the structures. Among the various fragments, we observed numerous structurally informative cross-ring fragments (mostly $^{1,5}X_n$ and $^{0,2}X_n$ for reducing-end containing fragments and $^{0,2}A_n$, $^{1,4}A_n$, $^{2,4}A_n$, and $^{1,5}A_n$ for the nonreducing end containing fragments). In the presence of an anhydro-bridge in the ring, only $^{1,5}X_n$ and $^{0,2}A_n$ fragments were produced, whereas $^{1,4}A_n$, $^{2,4}A_n$, and $^{1,5}A_n$ were absent. All those fragments, together with the between-ring fragments (B, C, Y, and Z types) permitted unambiguous structural identification for the three oligosaccharides. More importantly, many fragments retained the sulfate group and permitted the unambiguous localization of the sulfates on the backbone. Sulfate groups were even maintained on large fragments, as illustrated in the case of the agar-porphyran DP8 with the fragments C_7, B_7, and $^{0,2}A_7$ and with the doubly charged fragments $^{0,2}A_8^{2-\prime\prime}$, $^{1,5}A_8^{2-\prime\prime}$, and $^{1,4}A_8^{2-}$. One characteristic of He-CTD fragmentation, which was previously emphasized for positive ions,[2] is the absence of consecutive fragmentations. This capability is in contrast to CID, which often provides consecutive cleavages and internal fragments (CID spectra of the three oligosaccharides are presented in Figure S1). Like He-CTD, photodissociation in the extreme UV-range also minimizes consecutive or internal fragmentation.[7] Consecutive or double fragmentations contribute to false interpretation of glycan structures, and their absence is therefore a remarkable property of He-CTD and XUV-PD fragmentation spectra. The absence of consecutive fragmentation was also confirmed for negative polarity He-CTD, especially through the fragmentation of the agar-porphyran DP8, for which asymmetry of the molecule allowed the annotation of fragments with no ambiguity. None of the observed fragments arose from a consecutive fragmentation of the molecule, as illustrated in further detail in Figure S2.

Fragmentation efficiency (Σ(fragments intensities)/(precursor ion intensity + Σ(fragments intensities))) ranged from 2% for the agar-porphyran up to 12% for the ι-carrageenan. Although this efficiency is lower than that usually achieved with CID activation, this efficiency is in the same range as that commonly achieved with other negative-mode activation methods, such as XUV-PD (e.g., 8% for a 3 s activation and a signal accumulation of 2 min),[8] electron detachment dissociation (EDD; 6−13% with an activation time between 1 and 2 s and a signal accumulation of 1 min),[9−12] or negative electron transfer dissociation (NETD; a weak intensity of the fragments was mentioned by the authors due to a low efficiency in generating radical cations, with reaction times of up to 1 s and acquisition times of up to 10 min).[13,14] However, the background is extremely low, which resulted in a high signal-to-noise ratio without background subtraction for all fragments (Figure S3). The complete structural information could thus be retrieved through accumulation of the signal for 1 min of spectral averaging, representing approximately 300 individual acquisition cycles of 200 ms. He-CTD was also effective on lower charge state anions, as long as the sulfate groups were present in fully deprotonated states (e.g., doubly sulfated oligo-κ carrageenan DP4 in 2^- charged state, Figure S4).

The three structures represented in Figure 1 all encompass anhydro-D-galactoses and sulfated D- or L-galactoses. However, the positions of the anhydro-galactosyl residues in the sequence and the sulfate groups in the sulfated rings are different. Sulfation is at position C2 and C4 for the ι-carrageenan, C4 for the κ-carrageenan, and C6 for the agar-porphyran. Despite closely related structures, striking differences were observed in the fragments produced for the three molecules (Figure 1). Fragments in He-CTD arise from a radical-initiated mechanism following the detachment of electrons, or charge transfer, from the parent ion. In the structures herein explored, the fragments' formation was likely induced from radical sites located at the SO_3^- groups.

For the ι-carrageenan DP4, the observation of charge-reduced molecular ions ($[M-4H]^{3-\bullet}$ and $[M-4H]^{2-\bullet\bullet}$) indicated that the two sites were able to keep the radical without dissociating. Given the structure of this molecule, these two sites were hypothesized to be at C2 of anhydro-galactosyl residues (Scheme 1). In fact, the neighboring sulfates at C2 and the presence of an

Scheme 1. Proposed Mechanisms for the Formation of Fragments Produced by Electron Detachment from the Sulfate Groups Positioned at C2 of ι-Carrageenan DP4



anhydro-bridge are disadvantages of any potential hydrogen rearrangement for the delocalization of the radical that may lead to dissociation. The favored pathway thus goes through the concomitant delocalization of the radical at C2 and the loss of SO_3 (Scheme 1). The resulting species ($-SO_3$) can undergo decomposition by radical migration along the cyclic backbone. For simplification, the $[M-4H]^{2-\bullet\bullet}$ structure was depicted in this scheme. However, the fragments represented do account for the two charged-reduced molecular species $[M-4H]^{3-\bullet}$ and $[M-4H]^{2-\bullet\bullet}$. The odd electron fragments $Z_3^{3-\bullet}$ and even electron fragments $^{1,5}X_3^{3-}$ and $C_3\text{-}2H^{2-}$, i.e., $C''_3{}^{2-}$, were formed from $[M-4H]^{3-\bullet}$. Doubly radical species $^{1,5}A_3^{-\bullet\bullet}\text{-}2SO_3$ or $Z_3^{2-\bullet\bullet}\text{-}2SO_3$ were formed from the $[M-4H]^{2-\bullet\bullet}$ species, while $^{1,5}X_1^-$ and $C_3{-}SO_3{-}2H^{2-}$ could be formed from both oxidized species. Notably, all charged fragments represented were actually observed in the He-CTD fragmentation spectrum of the ι-carrageenan (Figure 1A).

Concerning the κ-carrageenan, no charge-reduced molecular ions were found (Figure 1B). Contrary to ι-carrageenan, the electron detachment from sulfate groups at C4 induces a rapid dissociation by intracyclic hydrogen rearrangement (Schemes 2 and S1). Among the fragments, two features were remarkable: the high intensity of the Z_5 ion and a triplet C_3'', $C_3^\bullet$, and C_3. As shown in Scheme S1, when the radical is formed on a sulfate at

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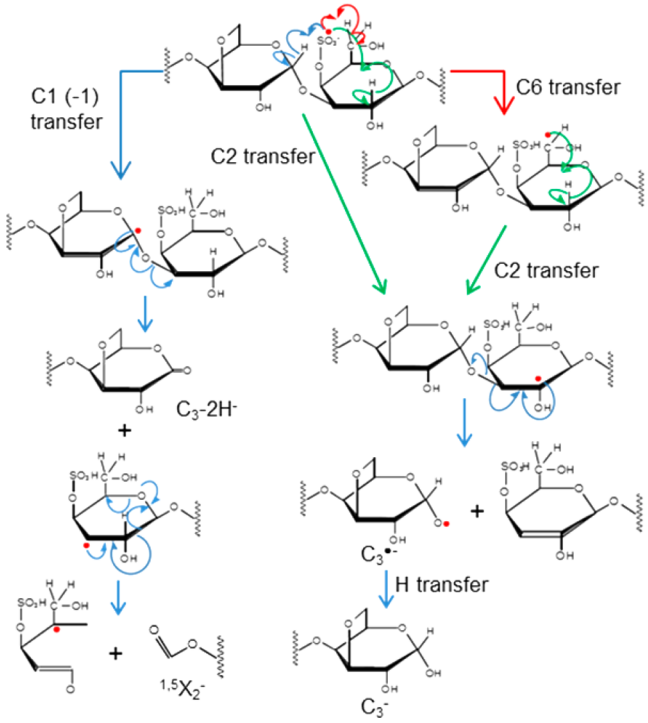
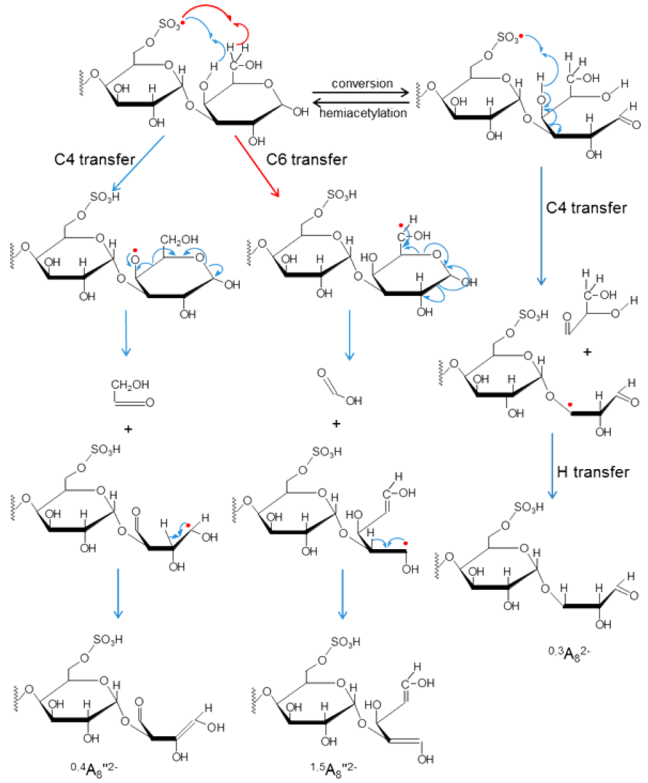
Scheme 2. Proposed Mechanisms for the Formation of Fragments Produced by Electron Detachment from the Sulfate Group Positioned at C4 on the Middle (LA-G) Dimer of the κ-Carrageenan DP6



Scheme 3. Proposed Mechanisms for the Formation of Fragments Produced by Electron Detachment from the Sulfate Group Positioned at C6 on the Reducing-End (L6S-G) Dimer of the Agar-Porphyran DP8



the nonreducing end (LA-G) dimer, two pathways can be proposed, both leading to the formation of the Z_5 fragment. When, instead, the radical is formed on the sulfate at the middle (LA-G) dimer (Scheme 2), intracyclic H atom rearrangement can be generated from the C6 and the C2. Both pathways produce $C_3^{-\bullet}$, which can be stabilized by H-transfer into a C_3^- species. Due to their vicinity, an intercyclic H atom rearrangement can also occur with the C1 of the adjacent anhydrogalactose. The latter pathway induces the formation of a C_3-2H (i.e., C_3'') ion. The Z_5 ion and C_3'', $C_3^{\bullet}$, and C_3 ions thus assign the position of the galactose bearing the sulfate group within the κ-carrageenan structure.

Concerning agar-porphyrans, the first relevant observation is that numerous large between-ring fragments were present as doubly charged ions in the spectrum; e.g., Z_7^{2-}, $Z_7^{2-\bullet}$, Y_7^{2-}, B_7^{2-}, and $C_7''^{2-}$, Figure 1C. Their formation is favored by the transfer of an H atom from all carbons of the sulfated heterocycle, which can occur at both the reducing-end (Scheme S2) and nonreducing end (Scheme S3). The second observation is the presence of doubly charged cross-ring fragments as oxidized ($^{0,4}A_8''^{2-}$ and $^{1,5}A_8''^{2-}$) and nonoxidized ($^{0,3}A_8^{2-}$) forms. Oxidized species can be explained by an intercyclic H atom rearrangement with the neighboring C4, which leads to the $^{0,4}A_8''^{2-}$ after hydrogen elimination. Alternatively, reaction with the neighboring C6 leads to the $^{1,5}A_8''^{2-}$ by the same pathway (Scheme 3). The nonoxidized $^{0,3}A_8^{2-}$ species, however, can only originate from the reducing-end heterocycle, after naturally occurring conversion into a hemiacetal. An intercyclic H atom rearrangement with the C4 and a H-transfer process then lead to the formation of the $^{0,3}A_8^{2-}$ fragment.

Finally, in all of the above examples, no fragments keeping the charge state of the isolated precursor ion were observed. This suggests that alternative pathways to electron detachment, such as those described as "direct fragmentation" for EDD,[8] were absent in negative polarity He-CTD.

In summary, negative polarity He-CTD provides remarkable structural information from highly acidic compounds. It allows the localization of labile modifications such as sulfate-esters by maintaining these modifications on many fragments. Notably, He-CTD produces specific patterns of fragmentation depending on the position of the sulfate groups, and those patterns could be rationalized as detailed above. No evidence of processes other than radical-initiated mechanisms of fragmentation was observed in our experiments. He-CTD led to the successful fragmentation of triply- and quadruply deprotonated species, as illustrated in Figure 1, but also of doubly charged anions (Figure S4). This suggests that the mechanism exhibits a weak dependency on precursor charge state.

Compared to EDD[9−12] and NETD,[13,14] shorter times of signal acquisition were achieved with He-CTD, i.e., in the 1 min scale or less, with the capability of obtaining enriched structural information on the target molecules. Comparable performance has been achieved with XUV-PD,[15] yet, until now, XUV-PD has been restricted to the use of synchrotron radiation. Given the short acquisition times achieved with He-CTD and the affordable implementation of this technique in a conventional laboratory, direct LC-MS/MS approaches through He-CTD activation ought to be possible, especially given that LC provides a means to efficiently concentrate the compounds and enhance the sensitivity of both MS and MS/MS measurements. Given the previous publications on positive mode He-CTD[1−3] and the rapid polarity switching that is possible between positive and negative polarities afforded by recent ion trap instruments, He-CTD may thus provide a powerful approach to obtain a

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comprehensive view of the structures present in a complex biological sample from a single LC-MSMS experiment in both ionization modes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.7b00473.

Materials and methods, CID MS/MS spectra, He-CTD fragmentation spectra, proposed mechanisms for the formation of fragments produced by electron detachment from the sulfate group, and example of negative polarity He-CTD (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Hoffmann, W. D.; Jackson, G. P. *J. Am. Soc. Mass Spectrom.* **2014**, *25* (11), 1939−1943.

(2) Ropartz, D.; Li, P.; Fanuel, M.; Giuliani, A.; Rogniaux, H.; Jackson, G. P. *J. Am. Soc. Mass Spectrom.* **2016**, *27* (10), 1614−1619.

(3) Li, P.; Jackson, G. P. *J. Am. Soc. Mass Spectrom.* **2017**, DOI: 10.1007/s13361-016-1574-y.

(4) McLuckey, S. A.; Mentinova, M. *J. Am. Soc. Mass Spectrom.* **2011**, *22* (1), 3−12.

(5) Brodbelt, J. S. *Anal. Chem.* **2016**, *88* (1), 30−51.

(6) Qi, Y. L.; Volmer, D. A. *Analyst* **2016**, *141* (3), 794−806.

(7) Ropartz, D.; Lemoine, J.; Giuliani, A.; Bittebiére, Y.; Enjalbert, Q.; Antoine, R.; Dugourd, P.; Ralet, M.-C.; Rogniaux, H. *Anal. Chim. Acta* **2014**, *807*, 84−95.

(8) Ropartz, D.; Giuliani, A.; Hervé, C.; Geairon, A.; Jam, M.; Czjzek, M.; Rogniaux, H. *Anal. Chem.* **2015**, *87* (2), 1042−1049.

(9) Wolff, J. J.; Amster, I. J.; Chi, L. L.; Linhardt, R. J. *J. Am. Soc. Mass Spectrom.* **2007**, *18* (2), 234−244.

(10) Yang, J.; Hakansson, K. *Int. J. Mass Spectrom.* **2008**, *276* (2−3), 144−148.

(11) Zhou, W.; Hakansson, K. *Electrophoresis* **2011**, *32* (24), 3526−3535.

(12) Wolff, J. J.; Laremore, T. N.; Busch, A. M.; Linhardt, R. J.; Amster, I. J. *J. Am. Soc. Mass Spectrom.* **2008**, *19* (2), 294−304.

(13) Wolff, J. J.; Leach, F. E.; Laremore, T. N.; Kaplan, D. A.; Easterling, M. L.; Linhardt, R. J.; Amster, I. J. *Anal. Chem.* **2010**, *82* (9), 3460−3466.

(14) Leach, F. E.; Wolff, J. J.; Xiao, Z. P.; Ly, M.; Laremore, T. N.; Arungundram, S.; Al-Mafraji, K.; Venot, A.; Boons, G. J.; Linhardt, R. J.; Amster, I. J. *Eur. Mass Spectrom.* **2011**, *17* (2), 167−176.

(15) Ropartz, D.; Giuliani, A.; Fanuel, M.; Hervé, C.; Czjzek, M.; Rogniaux, H. *Anal. Chim. Acta* **2016**, *933*, 1−9.

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