CrossMark

RESEARCH ARTICLE

Charge Transfer Dissociation of Complex Oligosaccharides: Comparison with Collision-Induced Dissociation and Extreme Ultraviolet Dissociative Photoionization

David Ropartz,[1] Pengfei Li,[2] Mathieu Fanuel,[1] Alexandre Giuliani,[3,4] Hélène Rogniaux,[1] Glen P. Jackson[2,5]

[1]INRA, UR1268 Biopolymers Interactions Assemblies, 44316, Nantes, France
[2]C. Eugene Bennett Department of Chemistry, West Virginia University, Morgantown, WV 26506, USA
[3]Synchrotron SOLEIL, L'Orme des Merisiers, 91190, Gif-sur-Yvette, France
[4]UAR 1008 CEPIA, INRA, 44316, Nantes, France
[5]Department of Forensic and Investigative Science, West Virginia University, Morgantown, WV 26506-6121, USA
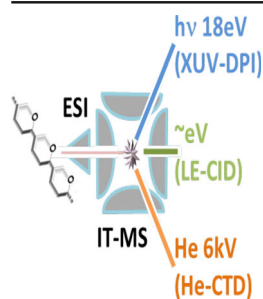
Abstract. The structural characterization of oligosaccharides still challenges the field of analytical chemistry. Tandem mass spectrometry offers many advantages toward this aim, although the generic fragmentation method (low-energy collision-induced dissociation) shows clear limitations and is often insufficient to retrieve some essential structural information on these molecules. In this work, we present the first application of helium charge transfer dissociation (He-CTD) to characterize the structure of complex oligosaccharides. We compare this method with low-energy collision-induced dissociation and extreme-ultraviolet dissociative photoionization (XUV-DPI), which was shown previously to ensure the successful characterization of complex glycans. Similarly to what could be obtained by XUV-DPI, He-CTD provides a complete description of the investigated structures by producing many informative cross-ring fragments and no ambiguous fragmentation. Unlike XUV-DPI, which is performed at a synchrotron source, He-CTD has the undeniable advantage of being implementable in a conventional benchtop ion trap in a conventional laboratory setting.

Keywords: Tandem mass spectrometry, Fragmentation method, Extreme Ultra-violet photon activation, Helium-charge transfer dissociation, Characterization of complex oligosaccharide structures

Introduction

Low-energy collision-induced dissociation (LE-CID) is by far the most popular approach in tandem mass spectrometry (MS) and is based on a slow thermal excitation by colliding ions with gaseous molecules and conversion of kinetic energy to internal energy [1, 2]. Nevertheless, in LE-CID, the predominance of glycosidic bond cleavages (i.e., Y and Z fragments and/or B and C fragments) and the lack of cross-ring cleavages often leads to the unsuccessful structural determination of complex oligosaccharides [3, 4]. In addition, LE-CID sometimes suffers from rearrangement ions, neutral losses, and internal fragments originating from multiple pathways, which together complicate the interpretation of tandem mass spectra and, as exemplified in [3], may even prevent the unambiguous assignment of numerous fragments. LE-CID has also been used in combination with infrared multiphoton dissociation to provide superior results [5].

Other ion activation methods that exhibit selective advantages over LE-CID have been explored in recent years. Some methods involve ion–electron interactions as a means to activate precursor ions, such as electron capture dissociation [6],

Correspondence to: Hélène Rogniaux; *e-mail:* helene.rogniaux@nantes.inra.fr

electronic excitation dissociation [7–10], hot electron capture dissociation [11], electron-induced dissociation [12, 13], and electron detachment dissociation [14]. These electron-based approaches are most frequently and most successfully performed with expensive Fourier transform ion cyclotron resonance MS instruments. When combined, these electron-based approaches can sometimes lead to stereospecific forms of chondroitin sulfate [15]. Another activation method based on ion–ion interaction, electron transfer dissociation [16], has become very popular for proteins and peptides and, in contrast to free electron methods, is now available on many commercial instruments. Unfortunately, electron transfer dissociation is limited to the fragmentation of multiply charged precursor ions. This limits its use for the structural analysis of oligosaccharides because they predominantly form singly charged species (e.g., sodium cationized species) in positive ionization mode. Only a few examples of negative electron transfer dissociation of oligosaccharides exist [15]. Tandem MS of anionic oligosaccharides has shown promise for acidic oligosaccharides such as chondroitin sulfate, but the labile sulfate group is often missing in the product ion spectra [17, 18]. Although cross-ring cleavages have been observed in negative ion tandem MS studies of functionalized oligosaccharides [15, 19], full characterization of glycan modifications is typically limited to relatively small oligosaccharides by means of a combination of chemical modification and tandem MS approaches [20–24].

Two studies recently highlighted the potential of extreme-UV (XUV) photons as a means to activate ions and resolve the structures of complex oligosaccharides by tandem MS [3, 25]. Ion–photon activation with UV photons leads to the absorption of a single photon with sufficient energy to induce the dissociation of precursor ions. These approaches include UV (400 nm $> \lambda > 200$ nm, 3 eV $< E < 6.2$ eV) [26] vacuum UV (200 nm $> \lambda > 100$ nm, 6.2 eV $< E < 12.4$ eV) [27–29], and XUV (100 nm $> \lambda > 10$ nm, 12.4 eV $< E < 124$ eV) [30]. Compared with photons of other wavelengths, XUV photons far exceed the ionization threshold of trapped molecular ions and do not require the presence of any specific chromophore for absorption. Without exclusion of other relaxation processes, the fragmentation then predominantly occurs following a dissociative photoionization (DPI) mechanism [30]. As shown in [3], the structural information provided by XUV-DPI on complex oligosaccharides is strikingly better than that obtained by LE-CID of the same compounds, and allows a complete sequencing of the oligosaccharides, including the positioning of lateral branching and labile modifications. Unfortunately, the scope and practicality of XUV-DPI is limited at the moment to the use of synchrotron radiation sources.

Very recently, charge transfer dissociation (CTD), another ion activation method, was described as a promising alternative to the aforementioned methods [31]. Chingin et al. [32] described the use of an ion gun fed with air for the dissociation of multiply charged polypeptides precursor ions, and the concept was extended by Hoffmann and Jackson [31] for the fragmentation of singly charged peptide ions by use of a beam of pure helium cations. Helium CTD (He-CTD) is very attractive because, compared with the reagent air cations such as $O_2^{+\bullet}$ and $N_2^{+\bullet}$, the electron affinity of helium cations (24.6 eV) is the largest of any singly charged cation. Notably, this energy is in the energy range used for activation by XUV photons, which suggests that He-CTD can be extremely promising as a method complementary to XUV-DPI. This experimental setup is not currently available commercially, yet a benchtop ion trap mass spectrometer modified with a saddle field source can be implemented in a conventional laboratory [31, 32].

In this work, we compared the fragmentation results obtained by LE-CID, XUV-DPI, and He-CTD for two classes of oligosaccharides. Both sugars are challenging to characterize because of the possibility of isomeric forms and the presence of labile modifications. The fragmentation pattern obtained by He-CTD shows remarkable similarity to that obtained by XUV-DPI. The two methods produce fragments resulting from a variety of glycosidic bond cleavages and cross-ring cleavages, allowing the unambiguous determination of the complex structure of the glycans. Promisingly, He-CTD thus opens the possibility of achieving high-energy fragmentation with an instrumental setting that, in principle, is more practical and affordable than other high-energy tandem MS methods.

Experimental

Oligosaccharides

A pure oligogalacturonan with a degree of polymerization of 5 and a degree of methylation of 3 (DP5DM3) was produced from the homogalacturonan portion of highly methylated pectins [33] and fractionated by reversed-phase liquid chromatography as in [3]. A hybrid agar–porphyran with a degree of polymerization of 6 (DP6) was produced as described in [34], except that no pretreatment by a β-agarase was applied.

^{18}O-Labeling of the Reducing Ends

Two micrograms of the dried oligosaccharides was solubilized in 50 µL of $H_2^{18}O$ and incubated for 1 week at room temperature in the dark.

Tandem MS

Oligosaccharides were analyzed with the use of modified ion trap mass spectrometers. Samples were diluted to a concentration of 10 µg/mL and infused at a flow rate of 5 µL/min. Measurements were performed in positive ion mode on the singly charged sodium adducts. The nomenclature used for annotations is according to that in [35]. Raw data were treated with mMass 5.3.0 [36] (subtraction of the background). All the tandem MS spectra presented were obtained by our summing the signal for 1 min for each tandem MS technique.

XUV-DPI

The experimental setup was developed at the SOLEIL synchrotron radiation facility at the endstation of the DISCO
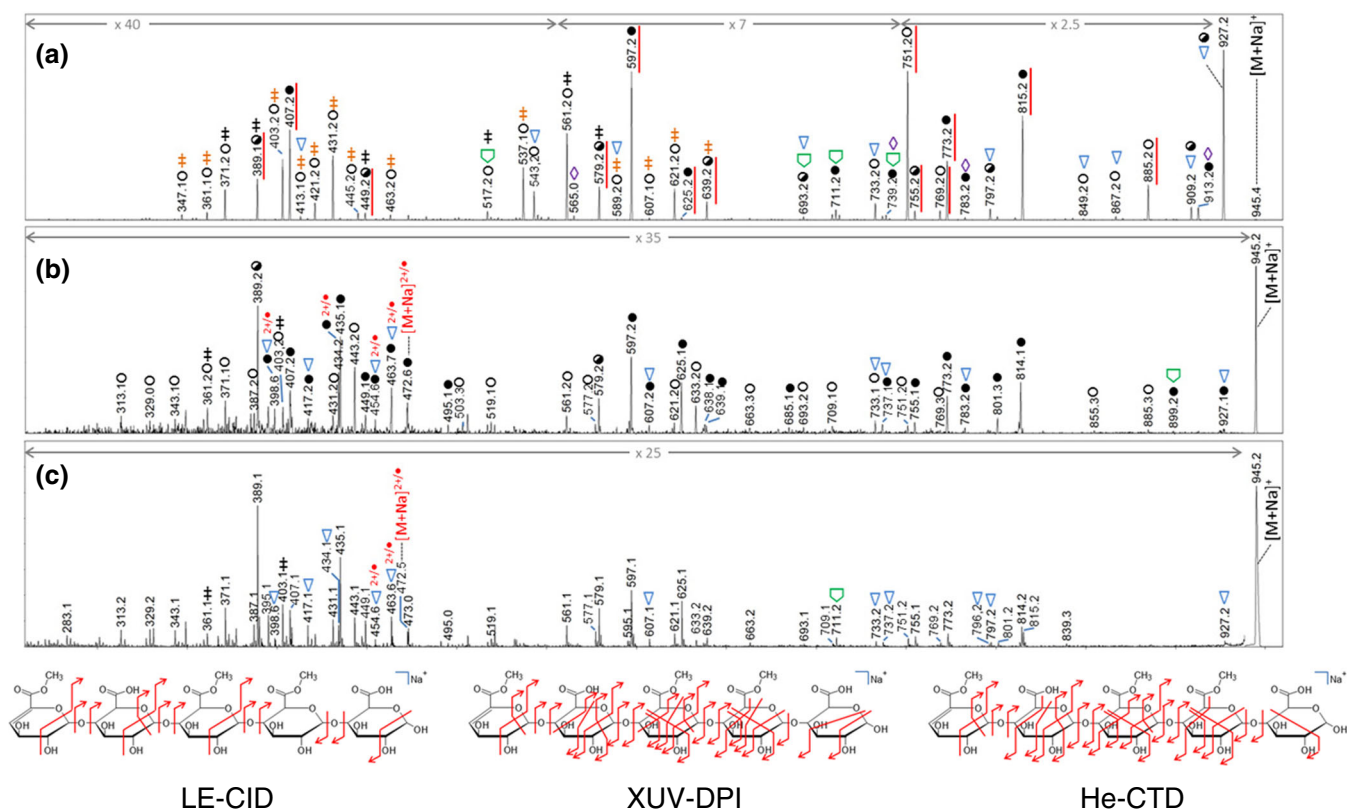
Figure 1. Fragmentation spectra of the oligogalacturonan with a degree of polymerization of 5 and a degree of methylation of 3, isolated as an [M + Na]$^+$ species, obtained by low-energy collision-induced dissociation (*LE-CID*; A), extreme-UV dissociative photoionization (*XUV-DPI*, B), and helium charge transfer dissociation (*He-CTD*; C). Spectra correspond to a 1-mn registration. Validated fragments in the LE-CID experiment are underlined in *red*. Schematic annotation of ions: *filled circle* reducing-end-containing fragments (evidenced by the ^{18}O labeling), *empty circle* nonlabeled fragments, *half-filled circle* ions encompassing both ^{18}O-labeled and nonlabeled fragments, *triangle* H_2O losses, *pentagon* CO_2 losses, *diamond* CH_3OH losses, *double dagger* ions arising from a double fragmentation. Doubly charged fragments are annotated with the superscript label $2+/\bullet$. Unambiguous fragments for each tandem mass spectrometry approach are reported on the corresponding structures below. Fragments are further detailed in Table 1

beamline [37]. A bending magnet-based synchrotron beamline was coupled to a linear ion trap (LTQ XL, Thermo Fisher Scientific). An automatic shutter was used to synchronize the photon beam (tuned to 18 eV, 68.9 nm) with the trapped precursor ions. Precursor ions were isolated with a window of 2 Da and exposed to XUV photons for 1000 ms.

LE-CID

Experiments were performed on the modified linear ion trap used for XUV photon activation tandem MS. Precursor ions were isolated with a window of 2 Da. The collision energy was set at 10 (arbitrary units) for the DP5DM3 oligogalacturonan and at 37 (arbitrary units) for the DP6 oligoporphyran, with an activation time of 30 ms.

He-CTD

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. The

instrument modification and working principle are highly analogous to those in previous work on a linear ion trap [31]. Precursor ions were isolated with a window of 4 Da and exposed to the 6-kV helium cation beam for 30 ms. 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).

Results and Discussion

Figure 1 and Table 1 show the results obtained following LE-CID, XUV-DPI, and He-CTD activation of an oligogalacturonan consisting of five glycosidic units bearing three methyl groups (DP5DM3), isolated as [M + Na]$^+$ species at *m/z* 945.2. Labeling of the reducing end of the glycan with ^{18}O was performed to help annotate isobaric fragments. Fragments arising from the reducing end are expected to display a shift of 2 Da for the ^{18}O-labeled species (spectra of ^{18}O-labeled species are not presented).

Table 1. Fragments observed for the oligogalacturonan with a degree of polymerization of 5 and a degree of methylation of 3 by low-energy collision-induced dissociation (*LE-CID*), extreme-UV dissociative photoionization (*XUV-DPI*), and helium charge transfer dissociation (*He-CTD*). Unambiguous fragments are displayed in *red*. The fragments presenting possible ambiguity are indicated in *black and italic*. Neutral losses and double fragmentations (details are provided in Table S1) are indicated in *green and purple* respectively

m/z	^{18}O	CID assignation	^{18}O	XUV-DPI assignation	He-CTD assignation
283.1					$^{3,5}A_2$
313.1			o	$^{2,5}A_2$	$^{2,5}A_2$
329.0			o	$^{0,2}A_2$	$^{0,2}A_2$
343.1			o	$^{1,5}A_2$	$^{1,5}A_2$
347.1	o	DF			
361.2	o	DF	o	DF	DF
371.1	o	B_2 ; DF	o	B_2	B_2
387.2			o	C_2''	C_2''
389.2	⊘	$Z_2+(C_2; DF)$	⊘	$Z_2 + C_2$	$Z_2 + C_2$
398.6			●	$^{0,2}X_4^{2+/•}$-H_2O	$^{0,2}X_4^{2+/•}$-H_2O
403.2	o	DF	o	DF	DF
407.2	●	Y_2	●	Y_2	Y_2
413.1	o	DF - H_2O			
417.2			●	$^{1,5}X_2$-H_2O	$^{1,5}X_2$-H_2O
421.1	o	DF			
431.2	o	DF	o	$^{2,4}A_3$	$^{2,4}A_3$
434.2			o	$^{0,2}A_5^{2+/•}$-H_2O	$^{0,2}A_5^{2+/•}$-H_2O
435.1			●	$^{1,5}X_2$	$^{1,5}X_2$
443.2			o	$^{1,4}A_3$-H_2O	$^{1,4}A_3$-H_2O
445.2	o	DF			
449.1	⊘	$^{0,2}X_2 + DF$	●	$^{0,2}X_2$	$^{0,2}X_2$
454.6			●	$-2.H_2O^{2+/•}$	$-2.H_2O^{2+/•}$
463.2	o	DF			
463.7			●	$-H_2O^{2+/•}$	$-H_2O^{2+/•}$
472.6			●	$[M+Na]^{2+/•}$	$[M+Na]^{2+/•}$
473.0					$^{3,5}A_3$
495.1			●	$^{3,5}X_2$	$^{3,5}X_2$
503.3			o	$^{2,5}A_3$	
517.2	o	$(B_3; DF)$-CO_2			
519.1			o	$^{0,2}A_3$	$^{0,2}A_3$
537.1	o	DF			
543.2	o	B_3-H_2O			
561.2	o	B_3 ; DF	o	B_3	B_3
565.0		Y_3-MeOH			
577.2			o	C_3''	C_3''
579.2	⊘	$Z_3+(C_3; DF)$	⊘	$Z_3 + C_3$	$Z_3 + C_3$
589.2	o	DF - H_2O			
595.1					Y_3''
597.2	●	Y_3	●	Y_3	Y_3
607.2	o	DF	●	$^{1,5}X_3$-H_2O	$^{1,5}X_3$-H_2O
621.2	o	DF	o	$^{2,4}A_4$	$^{2,4}A_4$
625.1	●		●	$^{1,5}X_3$	$^{1,5}X_3$
633.2			o	$^{1,4}A_4$-H_2O	$^{1,4}A_4$-H_2O
638.2			●	$^{0,2}X_3^{•}$	
639.1	⊘	$^{0,2}X_3 + DF$	●	$^{0,2}X_3$	$^{0,2}X_3$
663.3			o	$^{3,5}A_4$	$^{3,5}A_4$
685.1			●	$^{3,5}X_3$	
693.2	●	DN	o	$^{2,5}A_4$	$^{2,5}A_4$
709.1			o	$^{0,2}A_4$	$^{0,2}A_4$
711.2	●	Z_4-CO_2			Z_4-CO_2
733.1	o	B_4-H_2O	o	B_4-H_2O	B_4-H_2O
737.1			●	Z_4-H_2O	Z_4-H_2O
739.2	●	DN			
751.2	o	B_4	o	B_4	B_4
755.1	⊘	Z_4	●	Z_4	Z_4
769.3	o	C_4	o	C_4	C_4
773.2	●	Y_4	●	Y_4	Y_4
783.2	●	$^{1,5}X_4$-H_2O	●	$^{1,5}X_4$-H_2O	
796.2					$^{0,2}X_4^{•}$-H_2O
797.2	⊘	$^{0,2}X_4$-H_2O			$^{0,2}X_4$-H_2O
801.3			●	$^{1,5}X_4$	$^{1,5}X_4$
814.1			●	$^{0,2}X_4^{•}$	$^{0,2}X_4^{•}$
815.2	●	$^{0,2}X_4$			$^{0,2}X_4$
839.3					$^{3,5}A_5$
849.2	o	$^{0,2}A_5$-$2.H_2O$			
855.3			o	$^{0,3}A_5$	
867.2	o	$^{0,2}A_5$-H_2O			
885.3	o	$^{0,2}A_5$	o	$^{0,2}A_5$	
899.3			●	$-CO_2$	
909.2	⊘	$-2.H_2O$			
913.1		-MeOH			
927.1	⊘	$-H_2O$			$-H_2O$
945.2	●	$[M+Na]^{+}$	●	$[M+Na]^{+}$	$[M+Na]^{+}$

DF double fragmentation, *DN* double neutral loss, *empty circle* nonlabeled fragments, *filled circle* reducing-end-containing fragments, *half-filled circle* ions encompassing both ^{18}O-labeled and nonlabeled fragments

In the LE-CID experiment, among the 41 detected fragments, only 13 could be unambiguously assigned to the structure of the DP5DM3 oligogalacturonan (underlined in red in Fig. 1, spectrum A) and only five cross-ring fragments were detected. Sixteen peaks correspond to neutral losses (H_2O, CH_3OH, and CO_2) of other fragments and do not provide any valuable structural information. Among the 13 assigned fragments, 5 led to a doublet with ^{18}O labeling, which indicates that they have different origins. However, with LE-CID, the absence of ^{18}O labeling is not absolute proof that the fragment contains the nonreducing end of the oligosaccharide. This is because double fragmentation may occur, as exemplified by the 19 fragments marked by a double cross in Fig. 1, spectrum A (noted as "DF" in Table 1). Among those fragments, the actual occurrence of double fragmentation is evidenced for 13 fragments (marked by an orange double cross in Fig. 1, spectrum A). One example is given by the ion at *m/z* 639.1. This fragment shows a doublet under ^{18}O labeling conditions and thus can be interpreted only as a mixture of a reducing-end-containing fragment ($^{0,2}X_3$) and a $^{0,2}X_4/C_4$ double fragmentation (Table S1). The occurrence of double fragmentations complicates the interpretation of the spectrum and may even lead to erroneous assignments.

The picture is strikingly different after XUV-DPI analysis of the same glycan (Fig. 1, spectrum B). The number of fragment ions observed is drastically increased. A systematic series of fragments are produced from both ends of the oligosaccharide, including numerous cross-ring fragments ($^{0,2}X_n$, $^{1,5}X_n$, $^{0,2}A_n$, $^{2,5}A_n$). The number of neutral losses is significantly reduced, and very few double fragmentations are observed. This is exemplified by the intense peak observed at *m/z* 537.1 in LE-CID, which can originate only from a double fragmentation ($^{0,2}A_4/Y_5$ or $^{0,2}A_5/Y_4$), and is entirely absent in the DPI tandem MS spectrum. Together, these features simplify the annotation: 37 fragments were thus unambiguously identified (in red in Table 1). The glycosidic bond cleavages allow the retrieval of the osidic sequence, whereas the cross-ring fragments permit the differentiation of most of the hydroxyl functions and thus provide information on the branching pattern, the localization of the chemical modifications, and therefore the characterization of the exact isomeric form.

The same oligosaccharide was exposed to the 6-kV He^{+} beam and subjected to CTD for 30 ms (Fig. 1, spectrum C). The first observation is that He-CTD produces a variety of between-ring and cross-ring cleavages, which shows remarkable similarity to the product ion spectrum observed under XUV-DPI activation conditions. As in DPI, double fragmentations are

Table 2. Fragments observed for the oligoporphyran with a degree of polymerization of 6 by LE-CID, XUV-DPI, and He-CTD. Unambiguous fragments are displayed in *red*. The fragments presenting a possible ambiguity are indicated in *black and italic*. Neutral losses, sulfates losses and double fragmentations are indicated in *green*, *orange*, and *purple* respectively (details of double fragmentations and sulfate losses are provided in Table S2)

m/z	^{18}O	CID assignation	^{18}O	XUV-DPI assignation	He-CTD assignation
347.3	◐	SL ; DF	◐	SL	SL
365.3			●	SL	SL
375.3			●	SL	SL
377.1	●	$^{1,4}X_1$			
393.3			●	SL	SL
448.3			○	B_2'	
449.3	○	B_2 ; DF	◐	$Z_2 + B_2$	$Z_2 + B_2$
465.2			●	Y_2''	Y_2''
467.3	◐	$Y_2 +(C_2$; DF)	●	Y_2	Y_2
479.3			◐	$(^{0,2}X_5 + ^{0,2}A_3)^{2+\bullet}$	$(^{0,2}X_5 + ^{0,2}A_3)^{2+\bullet}$
491.3	○	SL ; DF	●	$^{0,2}X_2$-H_2O	$^{0,2}X_2$-H_2O
495.3			●	$^{1,5}X_2$	$^{1,5}X_2$
509.3	●	$^{0,2}X_2$; SL	◐	$^{0,2}X_2 + SL$	$^{0,2}X_2 + SL$
529.3			●	$SL^{2+\bullet}$	
539.3	●	$^{1,4}X_2$			
550.8			○	$^{0,2}A_6''^{\,2+\bullet}$-2.$H_2O$	
551.4			○	$^{0,2}A_6'^{\,2+\bullet}$-2.H_2O	$^{0,2}A_6'^{\,2+\bullet}$-2.H_2O
559.3			○	$^{0,2}A_6''^{\,2+\bullet}$-$H_2O$	$^{0,2}A_6'^{\,2+\bullet}$-H_2O
560.4			○	$^{0,2}A_6^{\,2+\bullet}$-H_2O	$^{0,2}A_6'^{\,2+\bullet}$-H_2O
569.2			◐	$^{2,4}X_2 + ^{0,2}A_6'^{\,2+\bullet}$	$^{2,4}X_2 + ^{0,2}A_6^{\,2+\bullet}$
581.6			◐	-2.$H_2O^{2+\bullet}$	
590.3			◐	-$H_2O^{2+\bullet}$	
593.3	○	B_3 ; SL	◐	B_3	B_3
599.3			●	$[M+3.Na-2H]^{2+\bullet}$	
609.4					C_3''
611.3	○	C_3 ; SL	○	C_3	C_3
627.3			●	Y_3''	
629.3	●	Y_3	●	Y_3	Y_3
639.3			●	$^{1,5}X_3$-H_2O	$^{1,5}X_3$-H_2O
653.4			◐	SL	SL
657.3			●	$^{1,5}X_3$	$^{1,5}X_3$
671.4			●	$^{0,2}X_3$	$^{0,2}X_3$
699.4			◐	SL	SL
713.2	○			$^{0,2}A_4$	
737.4			◐	(Z_4+B_4)-H_2O	
754.3			○	B_4'	
755.3	○	B_4 ; DF	◐	$Z_4 + B_4$	$Z_4 + B_4$
771.3			○	C_4''	C_4''
773.4	●	Y_4	●	Y_4	Y_4
783.3			●	$^{1,5}X_4$-H_2O	
801.3			●	$^{1,5}X_4$	$^{1,5}X_4$
815.5			●	$^{0,2}X_4$	$^{0,2}X_4$
827.3			○	$^{1,4}A_5$-H_2O	
875.3	○	SL	●	$^{2,4}X_4$	$^{2,4}X_4$
899.5	○	SL			
917.4	○	SL	◐	$Z_5 + SL$	$Z_5 + SL$
933.4			●	Y_5''	Y_5''
935.4	◐	$Y_5 + SL$	◐	$Y_5 + SL$	$Y_5 + SL$
945.3			●	$^{1,5}X_5$-H_2O	
947.3			○	$^{0,3}A_5$	
963.3			●	$^{1,5}X_5$	$^{1,5}X_5$
977.5			◐	$^{0,2}X_5+ ^{0,2}A_5$	$^{0,2}X_5+ ^{0,2}A_5$
1015.1			○	$(C_5$-$Na+H)$	$(C_5$-$Na+H)$
1019.3	○	B_5 ; SL	○	B_5	
1037.4	◐	$C_5 + SL$	○	C_5	C_5
1055.3			●	$^{4,5}X_5$	$^{4,5}X_5$
1079.4	●	SL			SL
1081.3			●	$^{1,4}X_5$-H_2O	$^{1,4}X_5$-H_2O
1097.6					SL
1109.3	●	$^{1,4}X_5$			
1137.5			○	$^{0,2}A_6''$	
1139.4					$^{0,2}A_6$
1163.3	○	-2.H_2O			
1181.3			●	-H_2O	
1199.4		$[M+3.Na-2H]^+$		$[M+3.Na-2H]^+$	$[M+3.Na-2H]^+$

DF double fragmentation, *SL* sulfate loss, *empty circle* nonlabeled fragments, *filled circle* reducing-end-containing fragments, *half-filled circle* ions encompassing both ^{18}O-labeled and nonlabeled fragments

also drastically reduced, as exemplified here again by the absence of the fragment at *m/z* 537.1.

The doubly charged radical species ($[M + Na]^{2+\bullet}$) at *m/z* 472.6, along with other radical ions (e.g., $^{0,2}A_5^{2+\bullet} - H_2O$ at *m/z* 434.2; $^{0,2}X_4^{2+\bullet} - H_2O$ at *m/z* 398.6), indicates that the two activation methods go through an electron detachment process and the production of a charge-increased radical intermediate. The lack of data acquired over a larger range of species prevents us from making generalizations about mechanistic differences between the two methods. However, the similarity between the CTD and the 18-eV DPI spectra gives us some idea of the activation energies available through He-CTD.

The second glycan studied is a hybrid oligoporphyran, a class of sulfated oligosaccharides. Sulfation is one of the most labile modifications of polysaccharides and has a crucial impact on their biological and end-use properties [38, 39]. Sulfation modifications are often lost on LE-CID. Table 2 describes the fragments observed for the $[M + 3Na - 2H]^+$ ion of the DP6 oligoporphyran for the three activation methods (tandem MS spectra and structures are presented in Fig. S1).

On LE-CID, a predominant fragment is observed at *m/z* 1079.4, corresponding to the loss of H_2SO_4Na. Similarly to the previous example, the LE-CID spectrum contains few diagnostic fragments, among which only seven could be unambiguously assigned to the structure of the oligoporphyran. In contrast, the DPI and CTD spectra contain a variety of informative fragments with few sulfate losses. In both cases, most of the hydroxyl functions can be differentiated from the cross-ring fragments, thereby making possible an accurate localization of

the sulfate groups. Some of the observed fragments indicate a slight difference in the activation mechanism between DPI and CTD. In DPI, a desulfated species was detected at *m/z* 529.3, corresponding to a doubly charged radical cation $[M + 2Na - 2H - NaHSO_4 - H_2O]^{2+\bullet}$. This species is totally absent in the CTD spectrum, but two ions corresponding to a loss of SO_3Na (*m/z* 1097.6) and HSO_4Na (*m/z* 1079.5) are produced in CTD. Although they require further confirmation, these observations may indicate that the electron detachment does not occur at the same position along the oligosaccharide in DPI as in CTD.

Conclusion

These two examples illustrate that He-CTD shares the same distinctive characteristics as XUV-DPI in the fragmentation of oligosaccharides. Both activation methods are based on the formation of a radical cation from photoionization and charge transfer respectively. The intermediate radical cation fragments readily and leads to a variety of diagnostic ions. As illustrated for DPI [3, 25], CTD provides rich and informative spectra which allow the sequencing of oligosaccharides as well as the characterization of the branching pattern between subunits and the positioning of chemical modifications, including labile ones such as sulfation. All these features, obtained previously with use of a synchrotron beam to activate ions, are now reachable with He-CTD on modified benchtop mass spectrometers, which may considerably increase the use of MS in glycomics.

Acknowledgments

References

1. Kailemia, M.J., Li, L., Ly, M., Linhardt, R.J., Amster, I.J.: Complete mass spectral characterization of a synthetic ultralow-molecular-weight heparin using collision-induced dissociation. Anal. Chem. **84**, 5475–5478 (2012)
2. Kailemia, M.J., Ruhaak, L.R., Lebrilla, C.B., Amster, I.J.: Oligosaccharide analysis by mass spectrometry: a review of recent developments. Anal. Chem. **86**, 196–212 (2014)
3. Ropartz, D., Lemoine, J., Giuliani, A., Bittebiere, Y., Enjalbert, Q., Antoine, R., Dugourd, P., Ralet, M.C., Rogniaux, H.: 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. Anal. Chim. Acta **807**, 84–95 (2014)
4. Ko, B.J., Brodbelt, J.S.: 193 nm Ultraviolet photodissociation of deprotonated sialylated oligosaccharides. Anal. Chem. **83**, 8192–8200 (2011)
5. An, H.J., Lebrilla, C.B.: Structure elucidation of native N- and O-linked glycans by tandem mass spectrometry (tutorial). Mass Spectrom. Rev. **30**, 560–578 (2011)
6. Zubarev, R.A., Kelleher, N.L., McLafferty, F.W.: Electron capture dissociation of multiply charged protein cations. a nonergodic process. J. Am. Chem. Soc. **120**, 3265–3266 (1998)
7. Nielsen, M.L., Budnik, B.A., Haselmann, K.F., Olsen, J.V., Zubarev, R.A.: Intramolecular hydrogen atom transfer in hydrogen-deficient polypeptide radical cations. Chem. Phys. Lett. **330**, 558–562 (2000)
8. Leach 3rd, F.E., Arungundram, S., Al-Mafraji, K., Venot, A., Boons, G.J., Amster, I.J.: Electron detachment dissociation of synthetic heparan sulfate glycosaminoglycan tetrasaccharides varying in degree of sulfation and hexuronic acid stereochemistry. Int. J. Mass Spectrom. **330–332**, 152–159 (2012)
9. Wolff, J.J., Chi, L., Linhardt, R.J., Amster, I.J.: Distinguishing glucuronic from iduronic acid in glycosaminoglycan tetrasaccharides by using electron detachment dissociation. Anal. Chem. **79**, 2015–2022 (2007)
10. Wolff, J.J., Amster, I.J., Chi, L., Linhardt, R.J.: Electron detachment dissociation of glycosaminoglycan tetrasaccharides. J. Am. Soc. Mass Spectrom. **18**, 234–244 (2007)
11. Kjeldsen, F., Haselmann, K.F., Budnik, B.A., Jensen, F., Zubarev, R.A.: Dissociative capture of hot (3–13 eV) electrons by polypeptide polycations: an efficient process accompanied by secondary fragmentation. Chem. Phys. Lett. **356**, 201–206 (2002)
12. Budnik, B.A., Haselmann, K.F., Elkin, Y.N., Gorbach, V.I., Zubarev, R.A.: Applications of electron-ion dissociation reactions for analysis of polycationic chitooligosaccharides in Fourier transform mass spectrometry. Anal. Chem. **75**, 5994–6001 (2003)
13. Wolff, J.J., Laremore, T.N., Aslam, H., Linhardt, R.J., Amster, I.J.: Electron-induced dissociation of glycosaminoglycan tetrasaccharides. J. Am. Soc. Mass Spectrom. **19**, 1449–1458 (2008)
14. Zubarev, R.A.: Reactions of polypeptide ions with electrons in the gas phase. Mass Spectrom. Rev. **22**, 57–77 (2003)
15. Leach 3rd, F.E., Ly, M., Laremore, T.N., Wolff, J.J., Perlow, J., Linhardt, R.J., Amster, I.J.: Hexuronic acid stereochemistry determination in chondroitin sulfate glycosaminoglycan oligosaccharides by electron detachment dissociation. J. Am. Soc. Mass Spectrom. **23**, 1488–1497 (2012)
16. Syka, J.E., Coon, J.J., Schroeder, M.J., Shabanowitz, J., Hunt, D.F.: Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. Proc. Natl. Acad. Sci. U. S. A. **101**, 9528–9533 (2004)
17. Zaia, J., Miller, M.J.C., Seymour, J.L., Costello, C.E.: The role of mobile protons in negative ion CID of oligosaccharides. J. Am. Soc. Mass Spectrom. **18**, 952–960 (2007)
18. Naggar, E.F., Costello, C.E., Zaia, J.: Competing fragmentation processes in tandem mass spectra of heparin-like Glycosaminoglycans. J. Am. Soc. Mass Spectrom. **15**, 1534–1544 (2004)
19. Seymour, J.L., Costello, C.E., Zaia, J.: The influence of sialylation on glycan negative ion dissociation and energetics. J. Am. Soc. Mass Spectrom. **17**, 844–854 (2006)
20. Kailemia, M.J., Park, M., Kaplan, D.A., Venot, A., Boons, G.J., Li, L., Linhardt, R.J., Amster, I.J.: High-field asymmetric-waveform ion mobility spectrometry and electron detachment dissociation of isobaric mixtures of glycosaminoglycans. J. Am. Soc. Mass Spectrom. **25**, 258–268 (2014)
21. Zaia, J., McClellan, J.E., Costello, C.E.: Tandem mass spectrometric determination of the 4S/6S sulfation sequence in chondroitin sulfate oligosaccharides. Anal. Chem. **73**, 6030–6039 (2001)
22. McClellan, J.E., Costello, C.E., O'Connor, P.B., Zaia, J.: Influence of charge state on product ion mass spectra and the determination of 4S/6S sulfation sequence of chondroitin sulfate oligosaccharides. Anal. Chem. **74**, 3760–3771 (2002)
23. Zaia, J., Li, X.Q., Chan, S.Y., Costello, C.E.: Tandem mass spectrometric strategies for determination of sulfation positions and uronic acid epimerization in chondroitin sulfate oligosaccharides. J. Am. Soc. Mass Spectrom. **14**, 1270–1281 (2003)
24. Zaia, J., Costello, C.E.: Tandem mass Spectrometry of sulfated heparin-like glycosaminoglycan oligosaccharides. Anal. Chem. **75**, 2445–2455 (2003)
25. Ropartz, D., Giuliani, A., Herve, C., Geairon, A., Jam, M., Czjzek, M., Rogniaux, H.: High-energy photon activation tandem mass spectrometry provides unprecedented insights into the structure of highly sulfated oligosaccharides extracted from macroalgal cell walls. Anal. Chem. **87**, 1042–1049 (2015)
26. Antoine, R., Dugourd, P.: Visible and ultraviolet spectroscopy of gas phase protein ions. Phys. Chem. Chem. Phys. **13**, 16494–16509 (2011)
27. Reilly, J.P.: Ultraviolet photofragmentation of biomolecular ions. Mass Spectrom. Rev. **28**, 425–447 (2009)
28. Brodbelt, J.S.: Photodissociation mass spectrometry: new tools for characterization of biological molecules. Chem. Soc. Rev. **43**, 2757–2783 (2014)
29. Devakumar, A., Thompson, M.S., Reilly, J.P.: Fragmentation of oligosaccharide ions with 157 nm vacuum ultraviolet light. Rapid Commun. Mass Spectrom. **19**, 2313–2320 (2005)
30. Giuliani, A., Milosavljevic, A.R., Canon, F., Nahon, L.: Contribution of synchrotron radiation to photoactivation studies of biomolecular ions in the gas phase. Mass Spectrom. Rev. **33**, 424–441 (2014)
31. Hoffmann, W.D., Jackson, G.P.: Charge transfer dissociation (CTD) mass spectrometry of peptide cations using kiloelectronvolt helium cations. J. Am. Soc. Mass Spectrom. **25**, 1939–1943 (2014)
32. Chingin, K., Makarov, A., Denisov, E., Rebrov, O., Zubarev, R.A.: Fragmentation of positively-charged biological ions activated with a beam of high-energy cations. Anal. Chem. **86**, 372–379 (2014)
33. Ralet, M.C., Williams, M.A., Tanhatan-Nasseri, A., Ropartz, D., Quemener, B., Bonnin, E.: Innovative enzymatic approach to resolve homogalacturonans based on their methylesterification pattern. Biomacromolecules **13**, 1615–1624 (2012)
34. Correc, G., Hehemann, J.-H., Czjzek, M., Helbert, W.: Structural analysis of the degradation products of porphyran digested by Zobellia galactanivorans β-porphyranase A. Carbohydr. Polym. **83**, 277–283 (2011)
35. Domon, B., Costello, C.E.: A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra of glycoconjugates. Glycoconj. J. **5**, 397–409 (1988)
36. Niedermeyer, T.H., Strohalm, M.: mMass as a software tool for the annotation of cyclic peptide tandem mass spectra. PLoS One **7**, e44913 (2012)
37. Giuliani, A., Jamme, F., Rouam, V., Wien, F., Giorgetta, J.L., Lagarde, B., Chubar, O., Bac, S., Yao, I., Rey, S., Herbeaux, C., Marlats, J.L., Zerbib, D., Polack, F., Refregiers, M.: DISCO: a low-energy multipurpose beamline at synchrotron SOLEIL. J. Synchrotron Radiat. **16**, 835–841 (2009)
38. Capila, I., Linhardt, R.J.: Heparin-protein interactions. Angew. Chem. Int. Ed. **41**, 391–412 (2002)
39. Pomin, V.H., Mourao, P.A.: Structure, biology, evolution, and medical importance of sulfated fucans and galactans. Glycobiology **18**, 1016–1027 (2008)