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Structural characterization by both positive and negative electrospray ion trap mass spectrometry of oligogalacturonates purified by high-performance anion-exchange chromatography

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Abstract

The off-line coupling of high-performance anion-exchange chromatography to electrospray ion trap mass spectrometry (ESI-IT-MS) is described. Two sets of isocratic conditions were optimised for the semi-preparative purification of oligogalacturonates of degree of polymerisation from 4 to 6 by monitoring eluates with either pulsed amperometric detection or evaporative light scattering detection in the presence of an online Dionex Carbohydrate Membrane Desalter (CMD). In these conditions, purified oligogalacturonate solutions were suitable, without further desalting steps, for infusion ESI-IT-MS experiments. This paper provides some interesting features of positive and negative ESI-IT-multiple MS (MS^n) of these acidic oligosaccharides. The spectra acquired in both ion modes show characteristic fragments resulting from glycosidic bond and cross-ring cleavages. Under negative ionization conditions, the fragmentation of the singly-charged $[M-H]^-$ ions, as well as the C_i^- and Z_i^- fragment ions through sequential MS^n experiments, was always dominated by product ions from C- and Z-type glycosidic cleavages. All spectra also displayed 0.2 A-type cross-ring cleavage ions which carry linkage information. Collision-induced dissociation (CID) spectra of sodium-cationized species obtained under positive ionization conditions were more complex. Successive MS^n experiments also led to the 0.2 A-type cross-ring cleavage ions observed together with B- and Y-type ions. The presence of the 0.2 A ion series was related to M_r 60 ($C_2H_4O_2$) losses. Combined with the absence of the M_r 30 (CH_2O) and the M_r 90 ($C_3H_6O_3$) ions, these ions were indicative of 1–4 type glycosidic linkage.

Keywords: Mass spectrometry; Detection, LC; Oligogalacturonates; Carbohydrates

1. Introduction

The structural analysis of cell wall polysaccharides requires fractionation steps and enzymic or chemical degradation of these compounds in oligosaccharides more amenable to structural characterization. High-performance anion-exchange chromatography (HPAEC) has become one of the most powerful techniques for the separation of oligosaccharides but

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the elution behavior in the case of oligogalacturonates, which may be partially methylated or acetylated when originated from pectins, is rather unpredictable because the presence of free carboxyl groups and hydrophobic substituents has opposite effects on the elution time. Moreover, standard oligomers are not often commercially available, so techniques such as NMR or mass spectrometry may be very useful for the identification and the structural characterization of separated oligosaccharides. The main drawback of NMR techniques is the relatively large amounts of material required. The new ionization techniques used in mass spectrometry are more sensitive. In this way, fast atom bombardment (FAB) and electrospray ionization mass spectrometry (ESI-MS) of derivatized oligosaccharides have provided molecular mass and sequence information [1–8]. FAB and ESI in combination with tandem mass spectrometry of lithium-cationized di- and oligosaccharides allowed differentiation of linkage position [9,10]. In the same manner, FAB-MS or ESI-MS in negative ion mode were successful in discriminating linkage positions in underivatized oligosaccharides [11,12]. Recently, the sequencing of underivatized neutral oligosaccharides was obtained by collision-induced dissociation matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) [13]. However, high salt concentrations are prejudicial to most MS interfaces. HPAEC with pulse amperometric detection (PAD) of acidic oligosaccharides necessitates alkaline conditions in the presence of quite high concentrations of sodium acetate (typically 100–400 m*M* of NaOAc). As a consequence, various on-line anion membrane suppressors were previously used. Anion micromembrane suppressors (AMMS-I and AMMS-II) and anion self-regenerating suppressors (ASRS) from Dionex were successfully used before thermospray MS analysis [14–17]. HPAEC, using a 2-mm i.d. column and a flow-rate of 0.4 ml/min, was also coupled to ESI-MS via an AMMS interface [18]. As these anion membrane suppressors only remove the sodium ions by exchange with hydronium ions, a cation self-regenerating suppressor (CSRS) was recently connected in series with an ASRS unit in order to also exchange the acetate ions for hydroxide ions and removing by the way the acetic acid from the ASRS eluate [19]. In fact, this acetic acid hinders the crystallization of the samples for further MALDI-TOF-MS analysis. Recently the new Carbohydrate Membrane Desalter (CMD) from Dionex was used for the off-line coupling of HPAEC to MALDI-MS analysis system [20]. The focus of this work was (i) to assess the use of evaporative light scattering detection (ELSD) for monitoring oligosaccharides separated by HPAEC and (ii) to develop efficient conditions, using this new membrane as an on-line desalting system, for the coupling of HPAEC with ESI-ion trap (IT) MS for the further structural characterization of oligogalacturonates (1,4-linked-α-D-galacturonic acid oligomers).

2. Experimental

2.1. Materials

Carbohydrate standards (mono, di- and trigalacturonic acids) and polygalacturonic acid from orange (purity, 89% dry matter basis) were purchased from Sigma (St. Louis, MO, USA). $H_2^{18}O$ (normalized 95 atom ^{18}O) used for labelling purified oligomers was purchased from Sigma–Aldrich. Cation-exchange resin AG 50W-X8 (200–400 mesh) was obtained under hydrogen form from Bio-Rad (Richmond, CA, USA).

2.2. Autohydrolysis of polygalacturonic acid

Ultrapure water (50 ml) was added to polygalacturonic acid (500 mg) and autohydrolysis was carried out under magnetic stirring during 48 h at 100 °C. After centrifugation, the supernatant containing oligogalacturonates was lyophilized. The lyophilized sample was then solubilized in water (10 ml) under magnetic stirring and pure ethanol (80–90 ml) was added to partially precipitate the highest oligomers (degree of polymerisation higher than 7–8) at 4 °C for 1 h. After centrifugation, the supernatant was evaporated under vacuum and redissolved in water (2.5 ml). This solution was filtered using a 0.45-μm Millipore filter before HPAEC injection.

2.3. HPAEC purification of oligogalacturonates

HPAEC was performed using either a Dionex

4500i LC gradient pump equipped with a pulsed electrochemical detector (working in pulsed amperometric mode for sugar detection and in conductivity mode for salts determination) and with an AS 1000 autosampler (Thermo Separation Products, CA, USA) or with a Waters (Milford, MA, USA) 626 pump equipped with a Waters 600S controller and an autosampler Waters 717 plus. Oligomers were monitored in this case using either a pulse amperometric detector (EC 2000, Thermo Separation Products) or an evaporative light scattering detector (PL-ELS 1000, Polymer Labs, Amherst, MA, USA). Borwin (JMBS, France) chromatography software was used for controlling both chromatographs and for collecting and processing the data. The mobile phases were all degassed with helium in order to prevent absorption of carbon dioxide and transformation to carbonate. Columns used were thermostated to 20 °C.

The oligogalacturonates produced from polygalacturonic acid autohydrolysis were applied in fractions of 50 μl to a Dionex (Sunnyvale, CA, USA) semi-preparative Carbopac PA-100 anion-exchange column (9×250 mm) with a Carbopac PA-100 guard column (4×50 mm). Oligogalacturonates were eluted isocratically at 1 ml/min either in 370 m*M* sodium acetate–$5 \cdot 10^{-3}$ *M* NaOH (94:6, v/v) using pulsed amperometric detection (PAD) or 450 m*M* ammonium acetate replacing PAD by ELSD. The CMD was placed either just after the PAD instrument (and before collection) or just before the ELSD instrument. Diluted sulphuric acid (75 m*M*) was used as the regenerant and was supplied through the CMD at a flow rate of ~7.5 ml/min by adjusting the pressure regulator of the regenerant bottle to 20–25 p.s.i. (1 p.s.i.=6894.76 Pa). In order to enhance the counter-current cation-exchange mechanism of Na^+ (present in eluate) by H^+, the current level of the Self Regenerating Controller (SRC-1), which supplies power to the CMD for electrolytic generation of hydronium ions, was set at the highest setting (level 4). The galacturonic acid content of purified oligogalacturonates was determined colormetrically by the *m*-hydroxybiphenyl method [21]. The efficiency of the CMD in eliminating sodium and ammonium cations from eluents, respectively, used with PAD and ELSD was measured by applying eluate aliquots to a Dionex Ionpac CS12A (4×250 mm) cation-exchange column equipped with an Ionpac CG12A guard column (4×50 mm). Quantitation of sodium and ammonium in the eluate of the cation-exchange column was performed by conductivity detection using a CSRS (4-mm) Cation Self-Regenerating Suppressor and water as the regenerant supplied at ~15 p.s.i. In order to enhance the counter-current anion-exchange mechanism, the current level of the SRC-1 was set at level 2 (100-mA current).

2.4. Resin treatment

Cation-exchange resin was converted from the hydrogen to the ammonium form by washing a column filled with 10 g of resin with 5% ammonia solution (100 ml) followed by 3 *M* ammonium acetate solution (500 ml). Ultrapure water was then passed through the resin up to a eluate conductivity of 1–2 $\mu S/cm^2$. Aliquots of ~5 ml were poured into small plastic columns for further cation-exchange of 1–2 ml of purified hexamer of galacturonic acid.

2.5. Labelling with ^{18}O

The reducing end of purified tetramer of galacturonic acid was labelled with ^{18}O [9,10,22] by adding 60 μl of $H_2^{18}O$ and 0.2 μl of formic acid to 60 μg of lyophilized tetramer. The sample was incubated with $H_2^{18}O$ for at least 72 h at ambient temperature in a dessicator.

2.6. Electrospray mass spectrometry

ESI-IT-MS experiments were carried out on a Finnigan LCQ ion trap mass spectrometer using negative and positive electrospray as the ionization processes. Sample solutions of purified galacturonates were diluted in methanol in order to obtain methanol–water mixtures (20:80 to 40:60, v/v) before their infusion at a flow rate of 3 μl/min into the ESI source. All analyses were carried out under automatic gain control conditions, using a typical needle voltage of 4.2 kV and a heated capillary temperature of 200 °C. For multiple MS (MS^n) experiments, the various parameters (collision energy, activation q_z parameter, activation time) were adjusted for each sample in order to optimize the signal and obtain maximal structural information

from the ion of interest. For MS^n analysis of lithium cationized oligomer, 140 μl of pentamer of galacturonic acid under NH_4^+ form were mixed with 85 μl of methanol and with 25 μl of 0.002 *M* LiCl just before infusion into the ESI source.

3. Results and discussion

3.1. HPAEC of non-esterified oligogalacturonates

In order to investigate in detail the influence of cationic forms (namely sodium but also ammonium) in the negative and positive ESI-IT-MS analysis of purified non-esterified oligogalacturonates and to assess evaporative light scattering detection for their semi-preparative purification, two sets of isocratic eluents were optimized. Selected conditions, namely 370 m*M* sodium acetate–$5{\cdot}10^{-3}$ *M* NaOH (94:6, v/v) using PAD and 450 m*M* ammonium acetate replacing PAD with ELSD, were compatible with the effective desalting capacity of the CMD and allowed rather fast separation of oligogalacturonates of polymerisation degree from 4 to 6. It is known that eluents containing ammonium are not compatible with pulse amperometric detection but, because they are volatile, they are suitable for ELSD. HPAE–PAD and HPAE–ELSD chromatograms resulting from a 50-μl injection of polygalacturonic acid hydrolysate after partial purification in 90% ethanol are shown in Fig. 1A and B, respectively. The baseline drift was less accentuated in the HPAE–ELSD than in the HPAE–PAD chromatogram and this feature combined with a faster elution of oligomers facilitated peak collection in this case. Respective responses with both detection modes were not very different. The low concentration of NaOH used for the detection in PAD was chosen in order to decrease the PAD signal and to allow the injection of quite high concentrated samples. The tetra-, penta- and hexamer galacturonic acid concentrations were, respectively, estimated to approximately 5.8, 5.4 and 5.2 mg/ml in 80% ethanol samples, using MHDP colorimetric determination [21]. As their concentrations in 90% ethanol sample were lower (5, 4.4 and 3.4 mg/ml, respectively) and as oligomers of superior DP were always present (Fig. 1B), 80% ethanol sample was used for collecting the tetra-, penta- and hexa-mer of galacturonic acid. PAD is more sensitive than ELSD when typical 100 m*M* NaOH is used. However, for semi-preparative purposes, these alkaline conditions would cause complete saturation of the PAD signal and a drastic increase in the highest oligomer elution time. Indeed, the retention time of oligosaccharides on an anion-exchange column depends on their global pK_a value in the eluent used. The lower the pK_a, the longer the retention time. This means that anionic oligosaccharides as oligogalacturonic acids are more retained than neutral ones. The pK_a value of galacturonic acid is 3.42 and the hydroxyl at the reducing end has more acidic pK_a than the other hydroxyl groups in

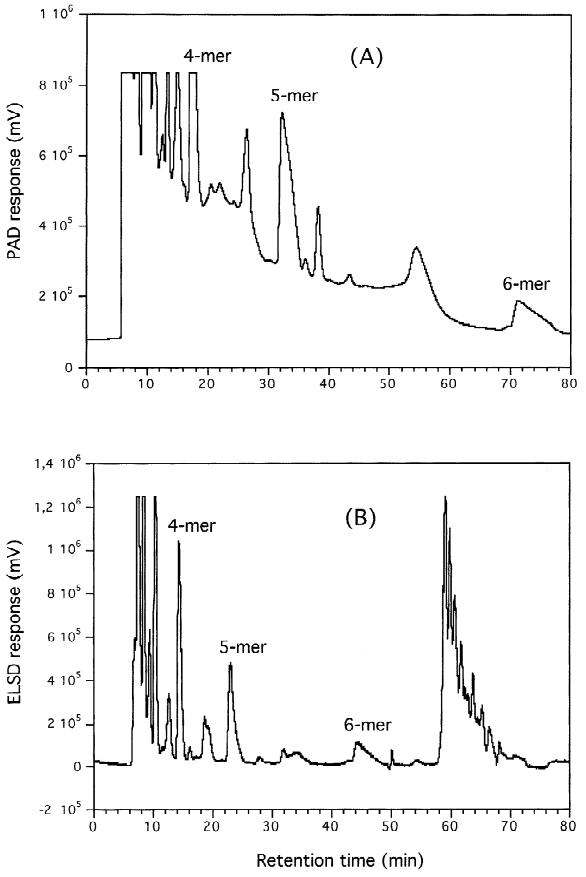

Fig. 1. HPAEC traces of oligogalacturonates released by autohydrolysis from polygalacturonic acid: (A) PAD chromatogram and (B) ELSD chromatogram. 4-mer, 5-mer and 6-mer corresponded to tetra-, penta- and hexa-mer of galacturonic acid, respectively; injected amounts were 250 μg (4-mer), 220 μg (5-mer) and 170 μg (6-mer).

neutral oligosaccharides (2-OH>6-OH>3-OH>4-OH) [23]. In order to optimize the recovery yield, the delay between the PAD system and collection, due to the presence of the CMD, was measured and estimated to 10.5 s. Taking into account this delay, the chromatographic resolution allowed a purity greater than 95% for all collected oligomers to be obtained. We have to underline the use of ELSD as an appropriate means for monitoring oligosaccharides in ammonium acetate eluent when high sensitivity is not required. Under the experimental conditions used, the concentrations of ammonium and sodium ions in both eluates were lower than 10 μg/ml and were compatible, without additional desalting steps, with further infusion ESI-IT-MS experiments of the various purified oligogalacturonates solutions (5–50 μg/ml). It is important to note that the pH of the collected fractions was ~2.5 as expected and this value is due to the formation of acetic acid from the acetate salts by the action of CMD. These results, obtained with an eluent flow of 1 ml/min, correspond to a desalting efficiency greater than 99.9%. This high on-line desalting capacity of CMD is due to the combination of water electrolysis (under an applied 500-mA current) and pneumatically supplied H_2SO_4 used as regenerant at a flow rate of 7–7.5 ml/min. After 1 month of constant use with these conditions the efficiency of membrane was had not changed much and was compatible with further ESI-IT-MS analysis. Assays performed with an eluent flow rate of 2 ml/min, as allowed with the used semi-preparative column, failed as the dynamic capacity of CMD was exceeded.

3.2. Negative ESI-IT-MS analysis of purified oligogalacturonates

Purified non-esterified oligogalacturonates were diluted with methanol (20–40%) before analysis by electrospray mass spectrometry, in order to obtain enhanced spray stability. However the proportions of methanol are limited by the relatively low solubility of acidic oligomers in the organic solvent. Mass spectra of oligogalacturonates acquired in the negative ion mode revealed that purified fractions corresponded to the tetra-, penta- and hexa-mers of galacturonic acid (M_r of 722, 898 and 1074, respectively). All spectra were similar, irrespective of the cationic form. They were generally dominated by the singly-charged $[M-H]^-$ ion although doubly-charged $[M-2H]^{2-}$ minor ions were sometimes observed. Two cluster ions series were also observed: $[M-nH+(n-1)Na]^-$ and $[M-nH+(n-1)Na+m98]^-$ ions with m and $n \geq 1$. The 98 additional mass was attributed to the presence of sulphuric acid in the purified samples as confirmed by an MS–MS experiment carried out on the $[M+98-H]^-$ ion which showed the $[M-H]^-$ species as the only daughter ion. This can be explained considering a little permeability of the CMD membrane to the sulphuric acid used as the regenerant. Although they allow confirmation of the molecular mass of the oligogalacturonates, the presence of these adducts can be considered as a drawback since it is associated with a lower intensity of the singly-charged $[M-H]^-$ ion used as the parent species for MS^n experiments.

In order to establish the fragmentation pathway through MS^n experiments of analyzed galacturonates, the purified tetramer was ^{18}O-labelled at the reducing end. This ^{18}O-labelling allows differentiation of isobaric ions, especially B_i from Z_j ions and C_i from Y_j ions, according to the nomenclature established by Domon and Costello [24] which is depicted in Fig. 2 for the ^{18}O-labelled tetramer of galacturonic acid. Ions from Y_j and Z_j series contained the labelled ^{18}O from the reducing end hydroxyl and their respective m/z is 2 mass units higher than respective C_i and B_i ions (with $i=j$). The MS–MS spectrum of the ^{18}O-labelled $[M^*-H]^-$ ion at m/z 723 (Fig. 3A), showed C_i and Z_j fragment ions which were predominant but not exclusive as minor ions from the B and Y series were also observed. The fragmentation pattern of the parent ion and of the C_i ions, showing major daughter ions from the C- and Z-series, indicated that under negative ion conditions the glycosidic cleavage occurred preferentially on the reducing side of the glycosidic oxygen, as previously reported for neutral disaccharides [11] and for partially methyl-esterified oligogalacturonic acids [22] by tandem mass spectrometry. When trapped for MS^3 fragmentation, the ^{18}O-labelled Z_3^- ion at m/z 529 (Fig. 3B) also produced ions from the C- and Z-series and minor Y_1 ions at m/z 195. Note that the C_i' ions (i.e. fragment ions of the C-series for which the counting from the

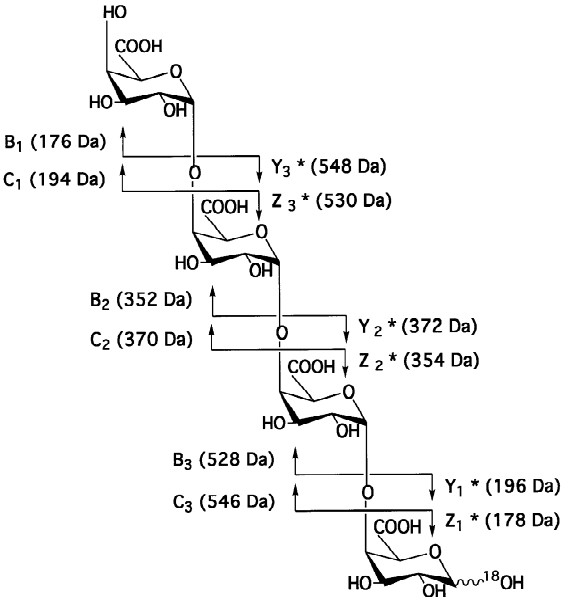

Fig. 2. Fragmentation nomenclature according to Domon and Costello for the ^{18}O-labelled tetramer of galacturonic acid. Designation " * " is used to indicate the labelling of Y_j and Z_j fragments.

non-reducing end has changed and depends on the trapped Z_j ion) have a mass 18 u lower than C_i ions produced from the trapping of either a $[M–H]^-$ or a C_i species. Both spectra also showed cross-ring cleavage fragment ions such as the 0.2 A ion series at m/z 661 and 485 from the $[M^*–H]^-$ and the C_3 ions, respectively (Fig. 3A) and at m/z 467.0 and 291.0 from the Z_3^* and the Z_2^* ion, respectively (Fig. 3B). In order to differentiate 0.2 A ion fragments derived from C or Z parent ions, we have used the symbol " / " followed by Z_j ion designation. As expected, these ions correspond to the loss of $C_2H_4O^{18}O$ (62 u) at the ^{18}O-labelled reducing end [11]. The 0.2 A ion fragments are formed, in two steps, involving the ring opening of the reducing sugar followed by a retro-aldol rearrangement [25]. These cross-ring cleavage ions from the 0.2 A ion series were always observed on the MS^n spectra of all the purified oligogalacturonates analysed when ions from the C series were selected, as illustrated by the MS^6 experiments performed on the purified pentamer of galacturonic acid (Fig. 4). The presence of the 0.2 A ion series, combined with the absence of the M_r 30 (CH_2O) and the M_r 90 ($C_3H_6O_3$), were indicative of 1–4 type glycosidic linkage [11]. However, the loss of neutral $C_2H_4O_2$ from the non-labelled C_i fragment ions is more informative because it is related to the glycosidic bond present at the "new" reducing end produced. In other terms, each C_i ion can provide information about the $i-1$ glycosidic linkage. When Z_j^* fragment ions are concerned, the loss of $C_2H_4O^{18}O$ is only related to the first glycosidic bond counted from the ^{18}O-labelled reducing end. Sequential MS^6 (MS^7 on the hexamer) experiments were possible because the loss of signal through subsequent MS stages into the trap was not too important, ~30–50 from MS to MS^5 stage). The CID spectrum of the galacturonic acid residue obtained in MS^6 of Z_1 ion showed fragments corresponding to decarboxylation and to loss of water. One important feature of the ion trap possibilities is illustrated in Fig. 3. The feasibility of subsequent trapping and collision induced dissociation of a particular ion allows for example to enhance the relative abundance of ion C_2^- at m/z 369.0 which, from minor ion (Fig. 3A), becomes the major ion $C_2'^-$ at m/z 351 (Fig. 3B). Of course, it was interesting to take advantage of this important feature in order to increase the relative abundance of the 0.2 A ion series through all MS^n experiments.

3.3. Positive ESI-IT-MS analysis of purified oligogalacturonates

Positive ESI spectra of purified oligomers under Na^+ form were characterized by the predominant $[M+Na]^+$ ion sometimes with the presence of minor $[M–(n-1)H+nNa]^+$ ions which are due to a high degree of sodium exchange [26]. In the case of the galacturonic acid tetramer, the ^{18}O-labelled $[M^*+Na]^+$ ion at m/z 747.3 was also accompanied by the unlabelled $[M+Na]^+$ ion at m/z 745.4. All MS spectra also contained $[M+NH_4]^+$ ions.

The ^{18}O-labelling of the tetramer at the anomeric position allowed identification of fragment ions as B_i and Y_j through CID performed either on the $[M^*+Na]^+$ ions or on the predominant Y_j ions (Fig. 5). Thus, in the positive ion mode, as previously reported for neutral disaccharides [9,10], the glycosidic cleavage occurs preferentially on the non-reducing side of the glycosidic oxygen with charge retention

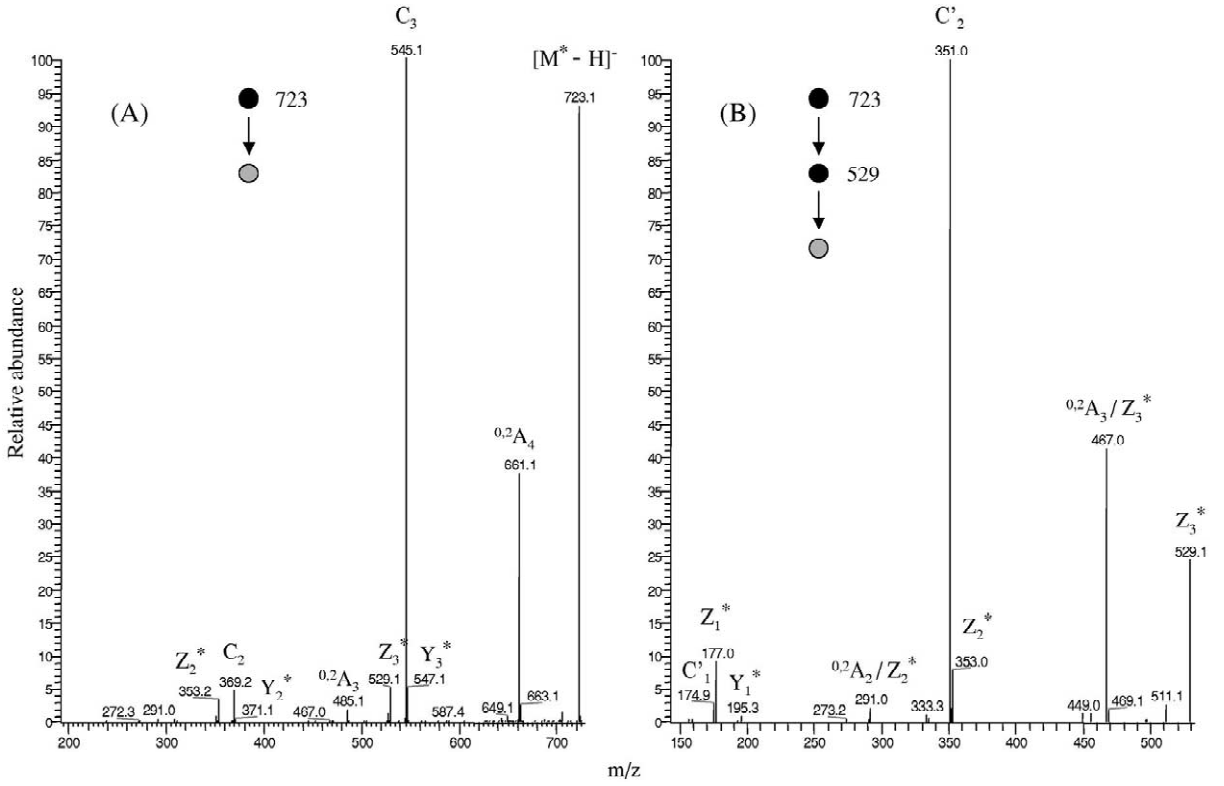

Fig. 3. CID mass spectra obtained from the singly deprotonated ^{18}O-labelled purified tetramer of galacturonic acid: (A) MS–MS spectrum of the $[M^*–H]^-$ ion (m/z 723) and (B) MS^3 spectrum of the Z_3^* ion (m/z 529). Designation " * " is used to indicate the labelled fragments.

on either side of this cleavage. However, there were also minor ions of the C-type, such as C_1 at m/z 217 (Fig. 5C). MS–MS experiments performed on $[M+Na]^+$ and $[M+Li]^+$ cationized forms of the pentamer showed that the dissociation pathway of both metal cationized species were very similar (data not shown). Isolation and subsequent fragmentation of ions from Y series produced 0.2 A ions due to the loss of 60 u (62 for ^{18}O-labelled tetramer), corresponding to the elimination of $C_2H_4O_2$ (Fig. 5). These 0.2 A ions correspond, as previously observed under negative ionization conditions, to the reducing ring cleavage and are also specific of 1–4 glycosidic linkage type, when associated with no other neutral losses than water [9,10]. On the other hand, the subsequent isolation and fragmentation of NH_4^+ cationized species in MS^2 only yielded the protonated B_i and Y_j species and ions at 18 mass units lower due to successive water losses. The instability of NH_4^+ cationized ions under the trapping and resonant excitation parameters used may explained why subsequent MS^n experiments were impossible on the NH_4^+ cationized species beyond the MS^2 stage. Moreover, diagnostic fragment ions corresponding to the $C_2H_4O_2$ loss (60 u) were absent in the MS^2 spectrum. Indeed, it is known that protonated molecular ions exhibit only glycosidic but no ring cleavages using FAB ionization and low-energy CID [9]. In other terms the fragmentation pathway of $[M+NH_4]^+$ is similar to that of $[M+H]^+$ ions and gives rise to only B- and Y-type fragments. In fact, it is less informative than the metal-cationized ion fragmentation which produces both glycosidic and A-type cross-ring cleavage ions. Treatment of the samples under Na^+ form with cation-exchange resin (ammonium form) increased $[M+NH_4]^+$ ions species to the detriment of $[M+Na]^+$ ion and gave MS and MS–MS spectra similar to the MS spectra of samples collected using ammonium acetate as eluent.

Finally, the loss of signal through the subsequent

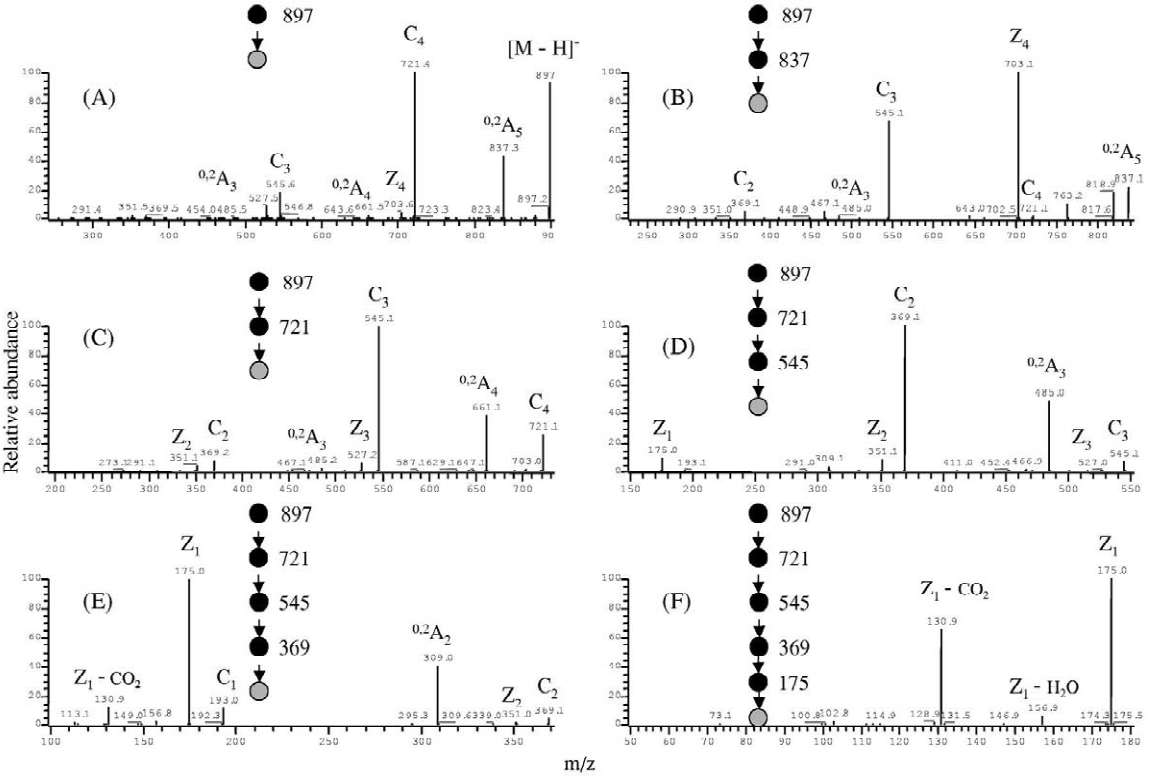

Fig. 4. CID mass spectra of the singly deprotonated purified pentamer of galacturonic acid: (A) MS–MS spectrum of the $[M–H]^-$ ion (m/z 897), (B) MS^3 spectrum of the 0.2 A_5 ion (m/z 837), (C) MS^3 spectrum of the C_4 ion (m/z 721), (D) MS^4 spectrum of the C_3 ion (m/z 545), (E) MS^5 spectrum of the C_2 ion (m/z 369), and (F) MS^6 spectrum of the Z_1 ion (m/z 175).

MS stages of Na-cationized ions was more marked in the positive mode (~300 from MS to MS^5) than in the negative mode. Only MS^5 experiments were possible on the pentamer and hexamer (Na^+ form) when they were infused at 30 and 13 μg/ml, respectively (results not shown). However, the signal loss from MS to MS^5 experiments was only ~30 with the Li^+ cationized pentamer infused at twice the concentration (58 μg/ml). This result is in agreement with previous results reported on neutral oligosaccharides [10].

4. Conclusion

HPAEC performed in light alkaline conditions or in ammonium acetate using either PAD or ELSD provides, when coupled to an online CMD suppressor, a convenient method for the separation and purification of non-esterified oligogalacturonates suitable for further structural characterization by mass spectrometry. The CMD revealed to be very efficient to eliminate sodium or ammonium at a high enough extent to avoid the additional desalting steps generally necessary for ESI-MS analyses. However, some sulphates originating from the regenerant passed through the membrane and were responsible for the presence of corresponding adduct ions in MS spectra. Nevertheless, the developed HPAEC system using an online CMD suppressor may be suitable for direct coupling with ESI-IT-MS or other ESI-MS analysers. Online coupling is of greater interest than off-line coupling, but this coupling operation could not be performed in our laboratory because the HPAE system and the MS spectrometers were not available in the same geographic location. The

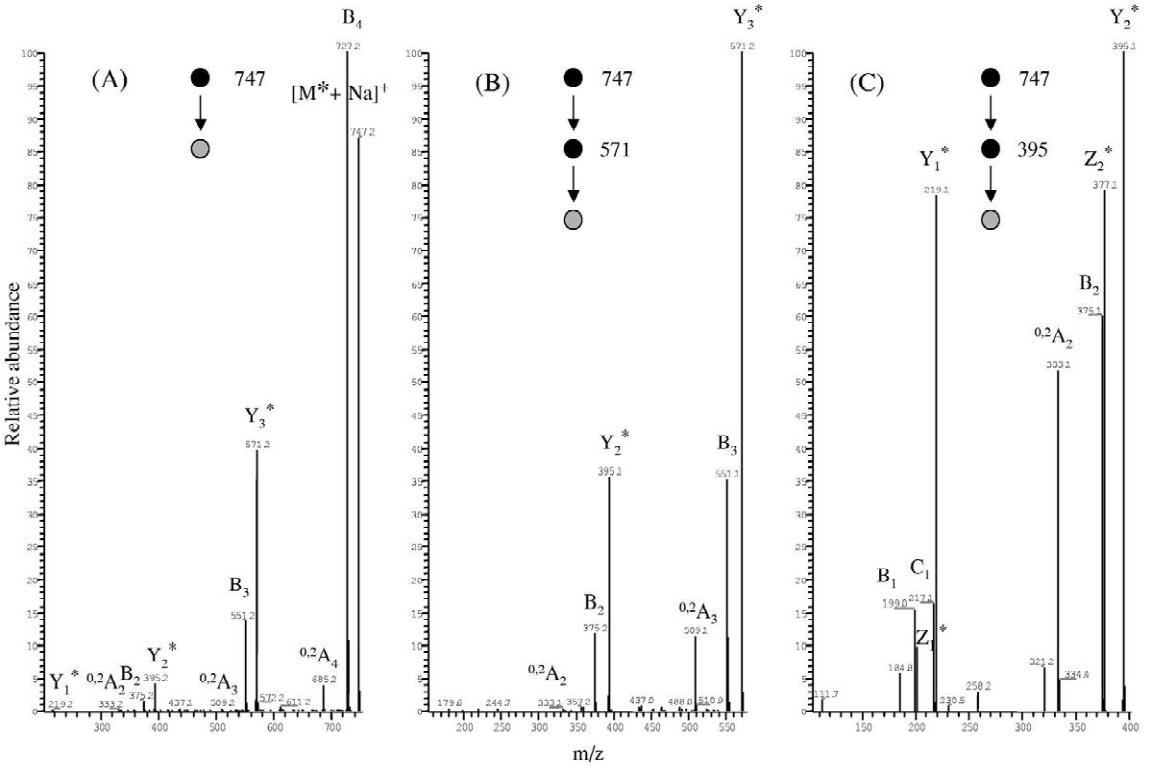

Fig. 5. Positive ion mode MS^n spectra of sodiated ^{18}O-labelled purified tetramer of galacturonic acid; $[M+Na]^+$ m/z 747: (A) MS–MS spectrum of the $[M^*+Na]^+$ ion (m/z 747), (B) MS^3 spectrum of the Y_3^* ion (m/z 571), and (C) MS^3 spectrum of the Y_2^* ion (m/z 395). Designation " * " is used to indicate the labelled fragments.

negative ion mode provided the highest sensitivity and the simplest fragmentation spectra of the oligosaccharides. Fragmentation of the singly-charged $[M–H]^-$ ions, as well as of C_i^- ions through successive MS^n experiments, was dominated by products of C- and Z-type glycosidic cleavages and all spectra also contained, to a lower extent, 0.2 A-type cross-ring cleavage ions which carry linkage information. CID spectra in positive ion mode of sodium-cationized species were more complex but as structurally informative as lithium-cationized species spectra. They always contained, besides B- and Y-type ions, the 0.2 A-type cross-ring cleavage ions.

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