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ScienceDirect
Carbohydrate Polymers 68 (2007) 797–807
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Review

Introduction to ion trap mass spectrometry: Application to the structural characterization of plant oligosaccharides

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Received 20 April 2006; received in revised form 21 July 2006; accepted 31 July 2006
Available online 18 September 2006

Abstract

This review will focus on ion trap mass spectrometry (ITMS) and the application of this technique to the structural analysis of carbohydrates. The basic principles of operation of the electrostatic ion traps are briefly described and the applicability of the technique to the structural characterization of carbohydrates is illustrated with the analysis of arabinoxylan oligosaccharides by ion trap mass spectrometry.

Keywords: Ion trap mass spectrometry; Carbohydrate structural characterization; Plant oligosaccharides

1. Introduction

The fact that carbohydrates are non-linear polymers creates a high structural diversity that prevents the development of a single analytical method for their characterization (Laine, 1994). Structural characterization of carbohydrates is achieved usually using a combination of different techniques which include (but not limited to) NMR (Duus, Gotfredsen, & Bock, 2000), GC-MS–EI (gas chromatography with electron impact ionization), specific chemical reactions (Pazur, 1986) or specific glycosidases (Sutton, O'Neill, & Cottrell, 1994). Until the beginning of the 90s the analysis of carbohydrates by mass spectrometry was limited to the use of electron impact, chemical (Reinhold, 1987) and fast atom bombardment ionization. The development of desorption/ionization techniques like matrix assisted laser desorption (MALDI) and electrospray (ESI) as well as the instrumental developments in mass spectrometry in the past 10 years allowed the application of multiple mass spectrometry techniques to the structural characterization of carbohydrates (Reinhold, Reinhold, & Chan, 1994, 1996; Reinhold, Reinhold, & Costello, 1995).

Ion trap mass spectrometry (ITMS) coupled with either MALDI or ESI is a very useful technique for the structural characterization of carbohydrates. This utility comes from the ion trap's capacity to perform multiples stages of mass spectrometry (MS^n). Despite this fact, previous reviews in ITMS have focused more on general principles (McLuckey, Van Berkel, Goeringer, & Glish, 1994), the application to protein and peptide analysis (Jonscher & Yates, 1997) or the analysis of organic compounds (March, 1997, 1998) rather than on the application of this particular technique to carbohydrate structural characterization. Therefore, this review will focus on the basic aspect of ITMS and the application of the technique to the analysis of carbohydrates by ITMS. A very interesting review covering historical aspects has been published (Todd & March, 1999) and readers are oriented to this paper for further details on the different stages of ITMS development. The physical principles of another mass spectrometry technique with intrinsic MS^n capacity, Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) will not be covered here

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doi:10.1016/j.carbpol.2006.07.033

but readers are directed to a recent review on the application of this technique in carbohydrate structural characterization (Park & Lebrilla, 2005).

Ion traps (three-dimensional, 3D) were invented by Wolfgang Paul in 1953 (Paul & Steinwedel, 1953, 1956, 1960), who received the 1989 Nobel Prize of Physic for this work. Despite its early invention, the development of ion traps as mass analyzers took some time. In the early stages of their development of ion trap instruments, in-house built ion traps were primarily employed to investigate the properties of isolated ions (Dehmelt, 1967, 1969), storage of microparticles (Wuerker, Shelton, & Langmuir, 1959), measurement of ion/molecule reaction rates (Bonner, Lawson, Todd, & March, 1976) and residual gas analysis (Rettinghaus, 1967). At that time ion traps were operated in the *mass-selective stability* mode which was not practical for many analytical purposes due to the limited mass range, low mass resolution and low sensitivity obtained.

A turning point occurs in 1983 when George Stafford (Stafford, Kelley, Syka, Reynolds, & Todd, 1984) and coworkers developed the *mass-selective instability* mode and discovered that helium used as dampening gas at a pressure of ~1 mtorr within the ion trap greatly improved the mass resolution and the trapping capacity for externally injected ions. These two facts led to the development of the first commercial ion trap instrument by Finnigan MAT. Subsequent innovations like the extension of the mass range, development of MS^n capabilities and high mass resolution measurement further improved the performance of the ion trap and lead to its use in the analysis of biological macromolecules. Today commercially available ion trap mass spectrometers are characterized by their small size, a relatively low cost, high sensitivity and MS^n capability.

2. Principles of operation

2.1. Ion trapping

Three-dimensional (3D) quadrupole ion traps consist of an arrangement of three electrodes, a hyperbolically shaped ring electrode and two hyperbolically shaped end caps electrodes (Fig. 1). Both end cap electrodes have orifices for ion injection and for ion extraction. Trapping or storage of externally produced ions is achieve by applying a high voltage oscillating potential called the fundamental radio frequency (RF) to the ring electrode while the end caps are kept grounded. The confinement of the ions within the trap is illustrated by the quadrupole potential that is represented in Fig. 2. As can be seen from this figure, ions in the central part of the trap are confined in the axial z-direction; however, in the radial r-direction ions are accelerated towards the end caps and are not confined. Simultaneous confinement (trapping) of the ion in both directions can be obtained by changing the direction of the field every time the ion is approaching the electrodes. The magnitude of the trapping potential is described by

$$D_z = z/mV_2/(4z_0^2\Omega^2)$$

Where D_z is the depth of the quadrupolar potential, z/m is the inverse of the mass to charge ratio of the ion, V is the amplitude of the RF voltage, z_0 is the distance from the center to an end cap, and Ω is the frequency of the RF voltage. Depending on the value of the fundamental RF voltage, ions of different m/z are trapped inside the ion trap.

Ions produced externally (by ESI or MALDI) and then injected into the ion trap will have kinetic energy values that will prevent their effective trapping. The ion trap is therefore filled with helium gas at a pressure of ~1 mtorr to reduce the kinetic energy content of injected ions through elastic collisions with the helium atoms making ion trajectories collapse toward the center of the ion trap after several milliseconds (Stafford et al., 1984). Because non-linear resonance caused by distortions of the quadrupolar field is minimal in the center of the ion trap, ion confinement in the center of the trap reduces ion losses thus enhancing sensitivity. A positive effect is also obtained on the mass resolution because displacements of the trapped ions remain small throughout the mass analysis allowing ejection of ions of the same m/z value in compact ion packets. There is an upper limit for the number of ions that can be trapped in the ion trap owing to the coulombic repulsion between the trapped ions, when this limit is exceeded a decrease in the mass accuracy and the resolution occurs. The main concerns in the operation of the ion trap are the conditions that affect the ion trajectory within the quadrupolar field, and will determine whether an ion can be trapped or not under a set of particular conditions. The motion of ions in a quadrupolar field can be described mathematically by the Mathieu differential equation

$$a_r = -8eU/m(r_0^2 + 2z_0^2)\Omega^2 \quad a_r = -4eV/m(r_0^2 + 2z_0^2)\Omega^2$$

and

$$a_z = -16eU/m(r_0^2 + 2z_0^2)\Omega^2 \quad q_z = 8eV/m(r_0^2 + 2z_0^2)\Omega^2$$

The equation and the relevant mathematics has been already discussed in details (Dawson, 1976; March & Londry, 1995; McLachlan, 1947). Solutions to these equations in the (radial) r- and (axial) z-directions are of two kinds (i) solutions, which represent stables trajectories and (ii) solutions, which represent unstable trajectories. The set of solutions can be readily represented in the form of *stability diagram* (Fig. 3). In physical terms the stability diagram allows the operation of the quadrupole ion trap to be reduced from a six-dimensional problem (involving e, Ω, r_0, m, U and V) to a two-dimensional problem involving only the parameters a_z and q_z. Additionally, the range of masses that can be trapped simultaneously under a set of well-defined operative conditions can be readily appreciated from this diagram.

2.2. Mass separation with the ion trap

The use of the ion trap as a mass analyzer requires that the trapped ions are ejected from the ion trap in a mode

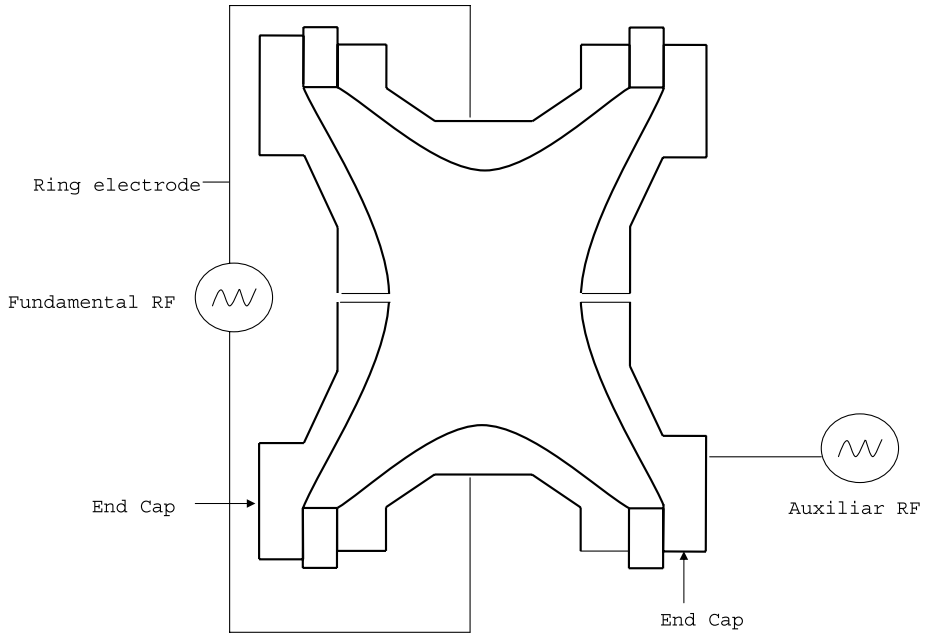

Fig. 1. A diagram of a quadrupole ion trap cut in half along the axis of cylindrical symmetry. The ion trap is composed of three electrodes with the ring electrode located between the two end cap electrodes. The ring electrode is connected to a RF voltage while the end caps are grounded.

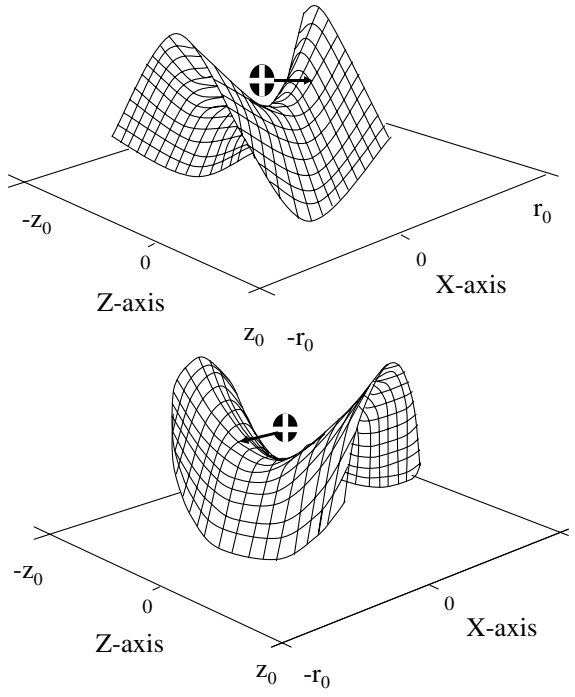

Fig. 2. The quadrupole potential is depicted for a plane that intersects the trap's symmetry axis. For an ideal quadrupole ion trap ($r^2 = 2z^2$) the potential will be purely quadrupolar.

dependent of their m/z value. There are currently two operation modes, which are used today: The *mass-selective instability* mode and the *mass-resonant ejection* mode.

The *mass-selective instability mode* (Stafford et al., 1984) is described in Fig. 3a. In this figure, the position of different ions is depicted in the stability diagram for different RF amplitudes. In *mass-selective instability* the fundamental RF voltage is linearly increased to move the ions toward the boundary of the stability region ($q_z = 0.908$, $a_z = 0$). When ions of increasingly m/z reach the $q_z = 0.908$ point they become unstable in the axial direction and are ejected from the trap as a function of the applied RF voltage. To obtain the mass spectra the voltage scale is converted into a mass scale by rearranging the expression for q_z

$$m/e = 4V/r_0^2\Omega^2 q_z$$

From this equation it can be readily seen that the mass range in which ions can be analyzed is limited by the frequency (Ω) and the amplitude (V) of the fundamental RF voltage. The simplest way to extend the mass range is to caused the ion to become unstable at a value of q_z lower than 0.908. This is achieve by applying an auxiliary RF voltage to the end cap with a frequency (auxiliary frequency) matching the oscillation frequency of an ion of particular m/z in the axial direction (q_z) (Kaiser, Cooks, Stafford, Syka, & Hemberger, 1991) while ramping the fundamental RF. This ejection method is known as *mass-resonant ejection* and conceptually can be described as creating a line of resonant ejection in the stability diagram at a q_z value corresponding to the applied auxiliary frequency (Fig. 3b).

Similar to quadrupoles mass analyzers, resolution depends on the number of cycles that the ion spends interacting with the trapping field (March & Hughes, 1989), this make resolution in the ion trap a function of the scan speed. If the rate at which the fundamental RF is ramped (scanned) is too fast, ions will fail to respond to the instability condition completely before ions of the next value begin to be ejected. This will result in a loss of resolution.

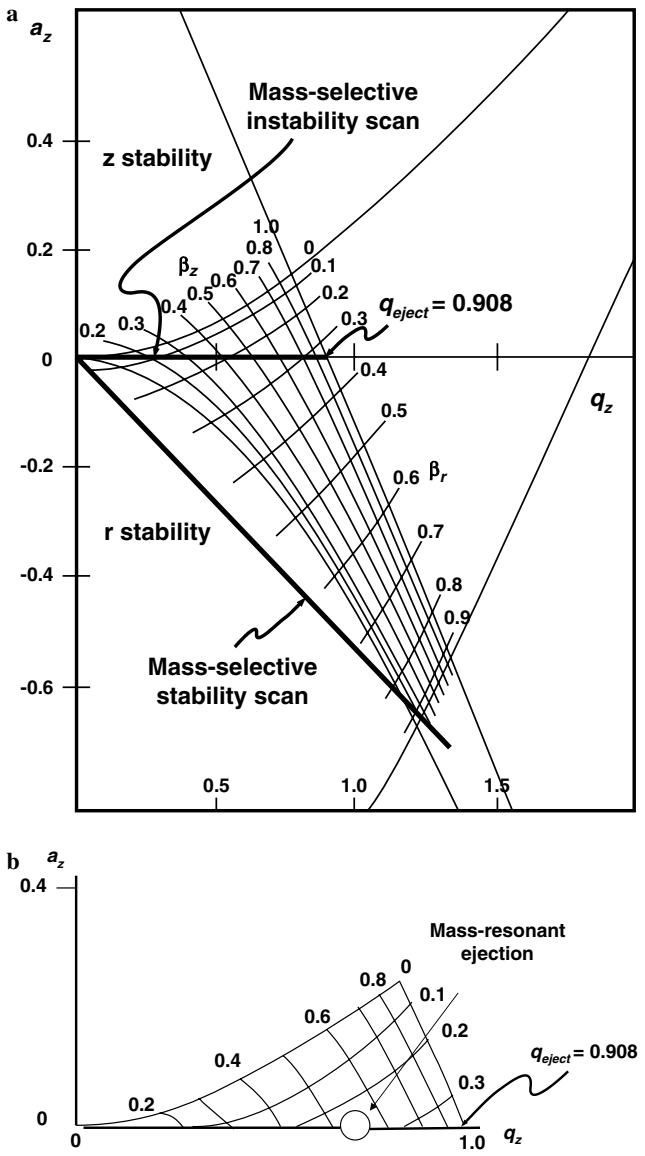

Fig. 3. (a) Stability diagram for an ion trap for the regions of simultaneous stability in both the *r*- and *z*-directions. The operative lines for *mass-selective stability* and *mass-selective instability* scans are shown. (b) Expanded region of the stability diagram. The mass-resonant ejection is shown.

Conversely, slowing the rate at which the fundamental RF is increased will improve resolution (Schwartz, Syka, & Jardine, 1991).

2.3. Tandem mass spectrometry in the ion trap

Fragmentation of ions of a particular m/z value can be achieved by collision with helium atoms within the ion trap. Before this fragmentation step is carried out, a mass isolation step is performed to eject all the ions except for the ion of interest (precursor ion) thus, upon fragmentation; only product or fragment ions related to the precursor ion are present. The isolation step is carried out using *mass-resonant ejection* of all ions except for the ions that are to be isolated. This *mass-resonant ejection* can be achieved by scanning the amplitude of the fundamental RF voltage in a reverse- then forward manner while applying a resonant signal (Kaiser, Cooks, Syka, & Stafford, 1990; Louris et al., 1987), using the stored waveform inverse Fourier transform (SWIFT) method (Julian & Cooks, 1993; Soni & Cooks, 1994) or filtered noise fields (McLuckey, Goeringer, & Glish, 1991).

The isolation step is followed by *resonant excitation* of the isolated ions to induce fragmentation. In this *resonant excitation* technique a low amplitude (1–3 kV) resonance frequency is applied to the end cap electrode making the resonantly excited ions gain kinetic energy from the resonance field. This amplitude (1–3 kV) resonance frequency causes collision induced dissociation (CID) of the ion with helium atoms rather than its ejection of the ion trap (Charles, McLuckey, & Glish, 1994; Louris et al., 1987). The efficiency of the CID process in the ion trap is dependent on parameters like the nature and the pressure of the bath gas, collision time and the q_z value of the ion to be fragmented (Charles et al., 1994; Louris et al., 1987). A key feature of the *resonant excitation* method is that it is mass selective; meaning that since fragment ions have different oscillation frequencies only the precursor ion is activated by the applied resonant frequency. Thus, fragment ions are stable and do not further fragment in the ion trap. The produced fragment ions are then sequentially ejected from the ion trap to obtain a mass spectrum or specific fragment ions can be selected to perform additional steps of isolation and fragmentation. This process of isolation, fragmentation and mass analysis can be repeated several times and is known as MS^n capability. The number of MS^n steps that can be performed in the ion trap have been show to be dependent on chemical (the ion activation method used, e.g. photodissociation, electron capture dissociation), the efficiency of the CID process and diverse instrumental parameters (McLuckey, Glish, & Van Berkel, 1991). Typically MS^3 and MS^4 are used although MS^{12} have been reported (Louris et al., 1990). An inconvenience of the CID process in the ion trap is the existence of a low mass cut-off value owing to the fact that fragment ions with m/z values 1/3 of the m/z value of the parent ion will not be detected. This problem can be overcome by using fragment ions with m/z values lower than the m/z value of the precursor ion to perform additional steps of isolation and fragmentation.

2.4. Scan function

The events previously described comprise the basic steps of the ion trap operation and can be described by the *scan function* (Fig. 4a and b). The *scan function* sets the amplitude of the fundamental and supplementary potentials and the temporal variation of the potentials applied to the ion trap electrodes. Either using the *mass-selective instability* mode or the *mass-resonant ejection* mode, operation of the ion trap as a mass analyzer means creating *scan*

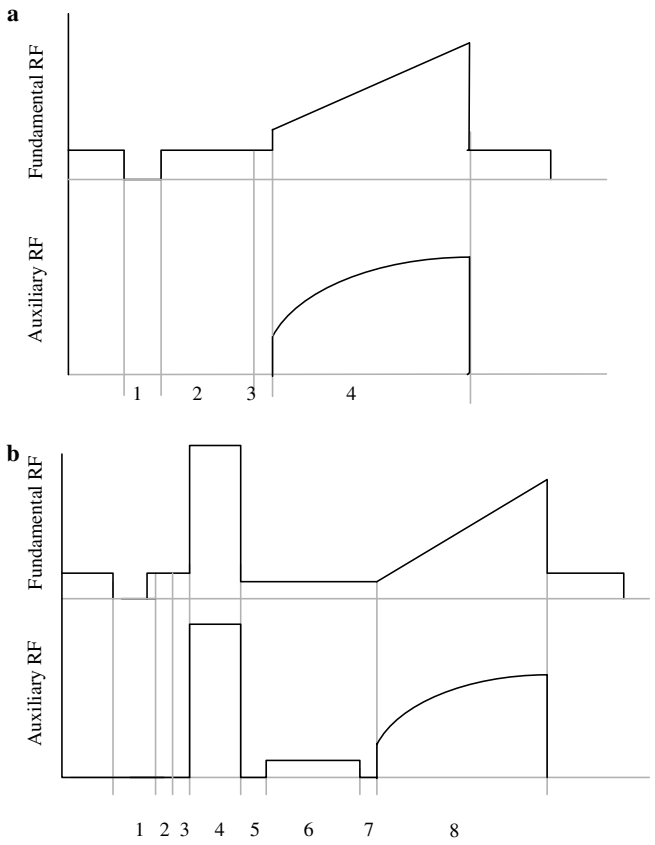

Fig. 4. (a) The auxiliary and fundamental potentials applied to end cap electrodes to obtain the scan function for an MS cycle in the ion trap, 1. trap clearing, 2. accumulation time, 3. scan delay, 4. mass analysis. (b) The auxiliary and fundamental potentials applied to end cap electrodes for obtain the scan function for an MS/MS cycle in the ion trap 1. clear trap, 2. accumulation time, 3. isolation delay, 4. precursor ion isolation, 5. fragmentation delay, 6. fragmentation, 7. scan delay, 8. mass analysis.

functions that will move ions through the different points of the stability diagram until they are ejected from the ion trap.

3. Application

A common problem with the analysis of carbohydrates by MS/MS is that fragment ions in the spectra sometimes cannot be assigned unambiguously, this means that more than one carbohydrate structure can be usually assigned to a particular spectrum, preventing structural identification base only on the mass spectra. This limitation becomes more evident when the analysis of isomeric carbohydrates structures is the main goal. Owing to the structural diversity of carbohydrates the analysis of mixtures of isomeric compounds is a common scenario in the field of carbohydrate analysis. Therefore, the capacity to analyze mixtures is a highly desirable feature in any analytical method employed in this field. Despite the fact that separation of isomers is not possible by mass spectrometry, identification of oligosaccharide isomeric structures based on their fragmentation pattern has been reported using MALDI-TOF-PSD (Garozzo, Nasello, Spina, & Sturiale, 1997; Yamagaki, Mitsuishi, & Nakanishi, 1998), ESI-MS (Reinhold et al., 1994), and ESI-ITMS (Konig & Leary, 1998; Minamisawa & Hirabayashi, 2005; Reis et al., 2004; Viseux, de Hoffmann, & Domon, 1998).

The application of a combination of MALDI-MS and ESI-ITMS to analyze mixture of oligosaccharides is illustrated in this section. In this example arabinoxylans (AX) polysaccharides were digested with an endoxylanase (that specifically cleaves the β-(1 → 4) linkages of the xylose backbone) in order to obtain oligosaccharides of smaller size amenable of analysis by mass spectrometry. The enzymatic digestion was monitored by MALDI-MS (Fig. 5) revealing the presence of a complex mixture of oligosaccharides.

From this data (m/z values) only the degree of polymerization (DP) and partial monosaccharide composition of the oligosaccharides can be inferred as they are in good agreement with the expected m/z values of pentose oligosaccharides. Because the two component monosaccharides in arabinoxylans (arabinose and xylose) are isobaric, direct analysis of the native oligosaccharides by mass spectrometry does not allow obtaining information on the presence of branching arabinose residues, linkage position and the localization on a specific xylose residue.

Sample permethylation combined with analysis by ESI-ITMS with CID is a well-established methodology to obtain branching and linkage position information (Reinhold, Reinhold, & Chan, 1996). Introduction of methyl groups labels free hydroxyl groups with the result that carbohydrate residues with branching will be methylated at fewer positions than those in linear structures. Therefore, upon fragmentation, branched residues are identified from their non-branched counter part by a mass decrement of 14 Da per branching point. An additional advantage of the permethylation procedure is an increase in sensitivity of the analysis due to enhanced ionization properties for carbohydrates and elimination of ionic contaminants.

Analysis by ITMS of the signal at m/z 1029.7 corresponding to a permethylated oligosaccharide with a DP = 6 showed an MS^2 spectrum were the main signals are Y and B fragment ions (Fig. 6a) indicating the presence of a mixture of at least four structures with the same molecular mass (m/z 1029.7) that differed in the degree of branching and in the position of the branched xylopyranose (Xyl*p*) residue relative to the position of enzyme's cleavage.

The different structures are designated with Greek capital letters: A (alpha), B (beta), Γ (gamma) and Δ (delta), and are represented on top of the spectrum. In this spectrum two situations are presented (i) fragment ions with different m/z values have the same label, (ii) some fragment ions have more than one label. Therefore, the nomenclature of the fragment ions has been slightly modified to aid in the interpretation, and to each fragment ion a Greek letter has been added as a superscript on the left side to

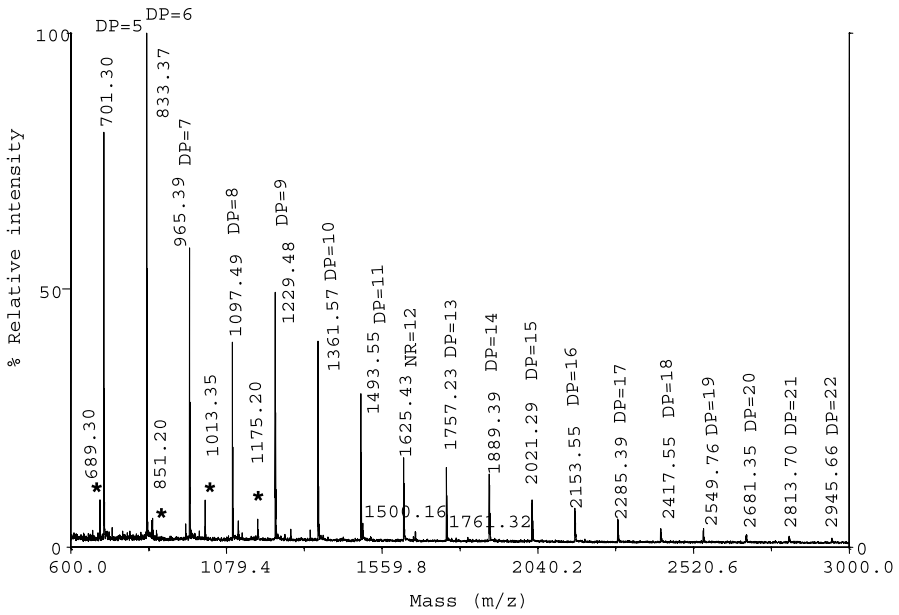

Fig. 5. MALDI-TOF reflector mode spectrum of the permethylated arabinoxylan sample AX 70–80%. Signals labeled with an asterisk represent hexose polymers. DP, degree of polymerization (taken from characterization of plant oligosaccharides by matrix-assisted laser desorption/ionization and electrospray mass spectrometry, *J. Mass Spectrom.* 38: 427–437, Matamoros et al., ©2003 John Wiley & Sons, Ltd. Reproduced with permission).

correlate the fragment ion with a particular oligosaccharide structure.

Owing to the above mentioned complexity, the interpretation of the MS^2 spectrum of the signal at *m/z* 1029.7 is not conclusive as some ambiguities need to be resolved e.g. the ion at *m/z* 535.3 can be produced from two different precursor ions, either by a mass loss of 146 from the ion at *m/z* 681.4 or a mass loss of 160 from the ion at *m/z* 695.4. The reduced intensity of some fragment ions in the low mass region of the spectra is also an element that complicates the spectra interpretation. This reduced intensity of some ions in the low mass region of the spectra is a consequence of the reduced trapping efficiency as a consequence of the low mass cut-off phenomena. Using fragment ions with lower *m/z* than the precursor ion to perform further sequential isolation/fragmentation steps (MS^n) allows extension of the accessible mass range toward ions of lower mass by decreasing the 30% cut-off value.

The fragment ion at *m/z* 855.6 was selected for MS^3. The resulting spectrum is shown in Fig. 6b and the structures derived from its interpretation are on the top of the spectrum. Its interpretation supports the previously proposed structures from the MS^2 spectrum with the same ambiguities for corroboration of the presence of the structures A and Γ. In a third step, the fragment ions at *m/z* 681.3 and 695.3 derived from the fragmentation of the ion at *m/z* 855.6 were selected as precursor ions for MS^4 analyses. The MS^4 spectrum for the ion at *m/z* 681.0 is shown in Fig. 6c. The presence of the signal at *m/z* 535.2 corroborated the presence of the structure A. The presence of the structure (Δ) (delta) is also confirmed with this spectrum. The MS^4 spectrum of the ion at *m/z* 695.4 (Fig. 6d) showed the presence of the signal at *m/z* 535.2 corroborating the presence of structure Γ (gamma).

Despite these four MS/MS steps some ambiguities in the interpretation still remained due to the complexity of the mixture for the oligosaccharide with DP = 6. Thus, the presence of a linear polymer cannot be unambiguously excluded. It should be noted also that the position of the arabinofuranose (*Araf*) substitutions on the *Xylp* ring could not be established from these results, as the dissociation pathways did not generate fragment ions carrying this information. Information on the position of *Araf* substitution can be obtained by analysis of the mixture by methylation analysis as reported recently (Matamoros Fernandez, Obel, Scheller, & Roepstorff, 2004; Matamoros Fernandez, Sorensen, Pedersen, Meyer, & Roepstorff, 2006).

The capacity of analyzing mixtures of isomeric oligosaccharides by ion trap mass spectrometry is limited. Therefore, when analyzing very complex mixtures a chromatographic separation step is mandatory. For instance, in this particular case owing to the complexity of the arabinoxylans oligosaccharide mixture, oligosaccharides with DP > 6 could not be analyzed, as the fragmentation spectra obtained are too complex to allow unambiguous structural identification. Despite this limitation of ITMS there are several advantages on this approach, which should be highlighted: even when the analytical method of choice is NMR and a purification process is required, the capacity of analyzing complex mixture of oligosaccharides by ITMS can be used at very early steps to evaluate the sample complexity and to monitor the purity of the fractions obtained from the isolation method with

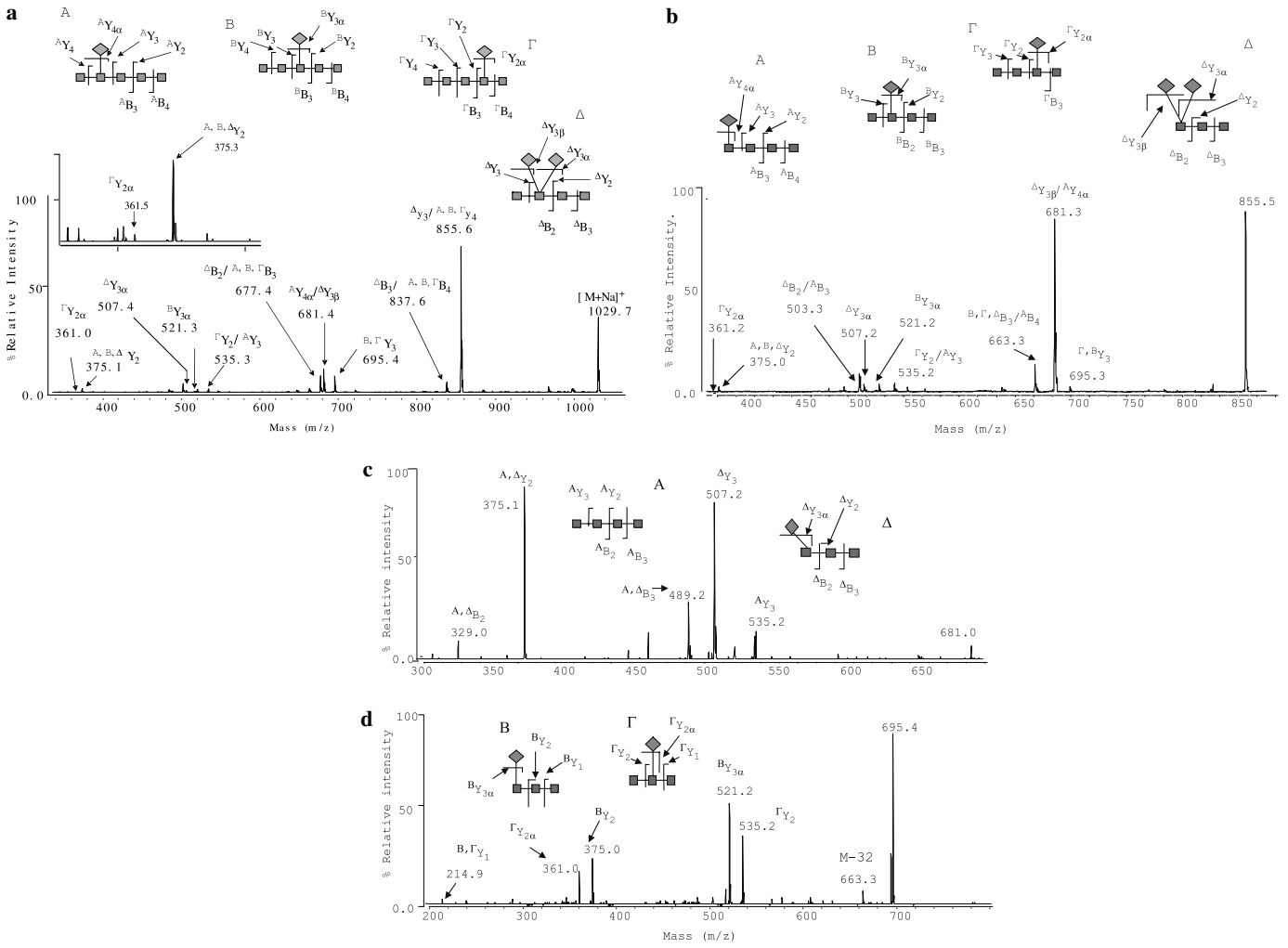

Fig. 6. (a) ESI-IT MS^2 spectrum of the signal with *m/z* 1029.7 (polymer with DP = 6) from the permethylated AX 70–80% sample. Inset (top left): zoom of the low mass region. Structures have been labeled with Greek capital letters, alpha (A), beta (B), gamma (Γ) and delta (Δ), and the nomenclature has been modified accordingly, see text for details. (b) ESI-IT MS^3 spectrum of the ion with *m/z* 855.6 from a. (c) ESI-IT MS^4 spectrum of the ion with *m/z* 681.4 from b. Note that *m/z* 507 is produced by a loss of methylated branching *arabinofuranose* residue (−174). *m/z* 535 is produced by a loss of *xylopyranose* residue. Mass difference is 28.0 (2× -CH_3). (d) ESI-IT MS^4 spectrum of the signal at *m/z* 695.4 from b. Key: □ xylopyranose, △ arabinofuranose (modified from *J. Mass Spectrom.* 38: 427–437, Matamoros et al., ©2003 John Wiley & Sons, Ltd. Reproduced with permission).

minimal sample consumption, allowing to adjust the procedure accordingly, ITMS analysis of the mixture can be performed rapidly (2–3 h including sample permethylation and data acquisition) and the procedure is compatible with well-established analytical methods like methylation analysis. Additionally, the fragmentation pathways of oligosaccharides in mass spectrometry are well characterized a fact that allows obtaining structural information on sequence, linkage and branching (Cancilla, Wong, Voss, & Lebrilla, 1999; Harvey, 2005a, 2005b, 2005c; Harvey, Bateman, & Green, 1997; Hofmeister, Zhou, & Leary, 1991; Lemoine et al., 1993; Morelle et al., 2004; Ngoka, Gal, & Lebrilla, 1994; Zhou, Ogden, & Leary, 1990). As an alternative to the use of NMR, mass spectrometry with CID has been used for the characterization of pectines (An et al., 2005; Bonnin et al., 2002; Brull et al., 1998; Guillaumie et al., 2006; Korner, Limberg, Christensen, Mikkelsen, & Roepstorff, 1999, 1998; Limberg et al., 2000; Mutenda, Korner, Christensen, Mikkelsen, & Roepstorff, 2002; Quemener, Desire, Lahaye, Debrauwer, & Negroni, 2003; Schols et al., 1994; van Alebeek, Schols, & Voragen, 2001; van Alebeek, Christensen, Schols, Mikkelsen, & Voragen, 2002, 2000), xylans (Deery, Stimson, & Chappell, 2001; Fukuyama et al., 2005; Jacobs & Dahlman, 2001; Jacobs, Larsson, & Dahlman, 2001; Quemener, Ordaz-Ortiz, & Saulnier, 2006; Reis et al., 2004), and amylopectins (Richardson, Nilsson, Bergquist, Gorton, & Mischnick, 2000, 2001; van der Burgt et al., 2000a, 2000b, 2000c).

4. Conclusions and perspectives

The advantages of mass spectrometry for the analysis of carbohydrates are sensitivity, high throughput and capacity to analyze mixtures. Information on structural features of carbohydrates like (i) identification of the monosaccharide units; (ii) determination of the anomericity and ring

size of each monosaccharide; (iii) determination of the monosaccharide sequence; (iv) determination of linkage; and (v) identification of modifying groups can be obtained by mass spectrometry. In addition, the analysis of oligosaccharides mixtures is possible. Analysis of isomeric mixtures is also feasible as long as the isomeric carbohydrate structures produce different fragmentation spectra.

Metal coordinates oligosaccharides have been analyzed by ion trap mass spectrometry for structural characterization of carbohydrates. The principle of using different metal coordinates oligosaccharides for MS^n analysis takes advantage of the fact that carbohydrate fragmentation is influenced among other factors by the type of precursor ion formed e.g. $[M + Na]^+$, $[M + Li]^+$. In this way, lithium cationization have been used to elucidate linkage position of disaccharides (Hofmeister et al., 1991; Zhou et al., 1990) and of four stereoisomers (Glc, Gal, Man and Tal) (Gaucher & Leary, 1998). The same kind of structural information was obtained with the analysis of branched oligomers complexed to alkaline earth and transition metals (Fura & Leary, 1993). Ion–molecule reactions with metal complexes were employed to quantify diastereomeric hexosamines (Desaire & Leary, 1999) and diastereomeric *N*-acetylhexosamine monosaccharides (Desaire & Leary, 2000).

Until relatively recently IT instruments were coupled only to ES ionization, but the introduction of IT instruments with MALDI ionization sources (Harvey, Martin, Jackson, & Sutton, 2004; Jonscher & Yates, 1998; Laiko, Moyer, & Cotter, 2000; Lennon & Glish, 1997) should be a major technical improvement for the analysis of carbohydrates by mass spectrometry. As oligosaccharides do not produce multiple charged ions they do not benefit from the use of ES ionization; in addition MALDI is more tolerant to ionic contaminants, offers higher sensitivity and lower sample consumption compare to ES ionization.

A new development in the field is the introduction of new ion trap analyzers coupled to conventional mass analyzers to obtain hybrid instruments. Thus, a linear ion trap (2D) has been developed (Schwartz, Senko, & Syka, 2002) and coupled to a quadrupole to produced a hybrid mass spectrometer (quadrupole/linear ion trap) known as QTRAP (Hager & Le Blanc, 2003). Linear ion traps are capable of operating in a manner similar to conventional 3D ion traps but they show an increased ion trapping capacity and therefore sensitivity, as well as an increase in the amount of structural information obtained from the spectra due to a triple quadrupole-like fragmentation and the absence of a low mass cut-off (Sandra, Devreese, Van Beeumen, Stals, & Claeyssens, 2004). Orbitrap is the most recently introduced ion trap mass analyzer. In Orbitraps, ions are electrostatically trapped in an orbit around a central, spindle-shaped electrode, this oscillation generates an image current in detector plates which is recorded. The frequencies of these image currents depend on the mass to charge ratios of the ions trapped. Mass spectra are then obtained by Fourier transformation of the recorded image currents. The Orbitrap has been coupled to a linear ion trap to create a new hybrid analyzer, the LTQ-Orbitrap that has been recently described (Hu et al., 2005; Makarov, Denisov, Lange, & Horning, 2006); features of the LTQ-Orbitrap are: high mass resolution, large space charge capacity, high mass accuracy (2–5 ppm), and a mass/charge range of at least 6000. The benefits of these instruments for the field of carbohydrate characterization remains to be evaluate.

Until recently, collision induced dissociation (CID) was the only ion activation method available in commercial ion trap instruments, but alternative ion activation methods have been used to circumvent some disadvantages of CID, see (Sleno & Volmer, 2004) for a detailed discussion. In this way, the use of electron capture dissociation (ECD) (Silivra, Kjeldsen, Ivonin, & Zubarev, 2005) and fast atom bombardment (FAB) (Misharin, Silivra, Kjeldsen, & Zubarev, 2005) have been recently reported on ion trap instruments. As ECD does not work very effectively with the radio frequency fields used in ion traps a new technique called electron transfer dissociation (ETD) was developed by Donald Hunt and colleagues in 2004 (Coon, Shabanowitz, Hunt, & Syka, 2005; Syka, Coon, Schroeder, Shabanowitz, & Hunt, 2004). In this technique, fluoranthene anions transfer an electron to a multiply charged peptide cation, leading to cleavage of the peptide backbone at random N–C alpha bonds. ETD can be combined with CID in a single analysis, and it is offer currently on commercially available instruments.

On the other hand, commercial FTICR instruments offer greater flexibility in terms of ion activation techniques for MS^n experiments and ECD have been use to localize protein glycosylation sites (Czeszak, Morelle, Ricart, Tetaert, & Lemoine, 2004; Mormann, Paulsen, & Peter-Katalinic, 2005; Peter-Katalinic, 2005) and for the characterization of gangliosides (McFarland et al., 2005). Infrared multiphoton dissociation (IRMPD) have been reported for characterization of O-linked mucin type oligosaccharides (Xie & Lebrilla, 2003; Zhang, Schubothe, Li, Russell, & Lebrilla, 2005) and oligosaccharides from bacterial lipopolysaccharides (Kondakova, Vinogradov, Knirel, & Lindner, 2005).

Ion trap mass spectrometry is already a well-established analytical tool in the field of carbohydrate characterization. Continuous developments in the field of mass spectrometry and in particular ion ITMS will undoubtedly expand the application of this technique; in particular new ion traps mass analyzers with increased trapping capacity and thus higher sensitivity, new hybrid instruments and the introduction of new ion activation methods in commercial instruments, should have the greatest impact in carbohydrate structural characterization.

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