Sequencing of oligosaccharides by collision-induced dissociation matrix-assisted laser desorption/ionization mass spectrometry

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A study of the collision-induced dissociation post-source decay (PSD) spectra of free oligosaccharides is presented. These spectra, when obtained with helium as collision gas, show $1.5X$ fragments containing the reducing end sugar. The presence of these fragments permits $Y$ ions and, consequently, $B$ and $C$ peaks to be identified. This is a common behaviour from which it has been possible to delineate a general method for the easy assignment of the peaks in PSD spectra of underivatized neutral sugars, allowing the sequence of a real unknown to be obtained. Copyright © 2000 John Wiley & Sons, Ltd.

KEYWORDS: oligosaccharides; matrix-assisted laser desorption/ionization; post-source decay; sugars; sequencing

INTRODUCTION

Analysis of carbohydrates is an important problem in biochemical analysis but the structural elucidation of a complex carbohydrate molecule is analytically difficult because it requires the knowledge of several parameters such as sugar sequence, reducing end, linkage type between the monosaccharides and the anomeric configuration.

The use of mass spectrometry for the structural analysis of carbohydrates has increased considerably in the last few years and it has been shown that fast atom bombardment (FAB) and electrospray ionization mass spectrometry (ESIMS) are powerful techniques for the determination of the sequences of permethylated oligosaccharides, and negative ionization FABMS or ESIMS can be used to discriminate linkage positions in underivatized oligosaccharides. Because of its large mass range and high sensitivity, matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry is now widely used in the molecular mass determination of underivatized oligo- and polysaccharides and since the introduction of the post-source decay (PSD) technique, providing fragmentation spectra, some papers dealing with the PSD of oligosaccharides obtained using TOF, magnetic sector or ion cyclotron resonance analysers have been published. A good review has recently been published by Harvey et al.

Concerning PSD experiments using MALDI-TOF, the most commonly used technique, it has been shown that although a lot of information can be obtained, it is not possible to sequence an unknown oligosaccharide from the PSD spectrum alone because of the presence of internal fragments and the difficulty of distinguishing $B$ and $C$ ions from $Z$ and $Y$ fragments. In order to overcome these problems, derivatization procedures or a combination of enzymatic degradation with MALDI-MS have been proposed.

In this paper, we show that PSD spectra of oligosaccharides obtained using collision-induced dissociation (CID) are different from those obtained without a collision gas and recently reported. The most important difference is the presence of $1.5X$ fragments which are highly diagnostic and provide the sequence of the oligosaccharides being analysed. An explanation of this behaviour, that is due to the presence of helium as the collision gas, is also presented.

EXPERIMENTAL

CID PSD spectra

CID PSD spectra were measured using a Voyager-DE STR MALDI-TOF instrument (Perseptive Biosystems, Framingham, MA, USA) equipped with a pulsed nitrogen laser (337 nm) and with a collision cell, with helium being used as the collision gas. For PSD spectra, the precursor ion was selected by the time ion selector, and spectra were acquired at a 20 kV accelerating voltage, while the reflector voltage was decreased step by step, allowing the
detection of certain fragments at a time. In order to shorten the analysis time, without losses of peaks, the reflector voltage was decreased by 5% in the first step, by 10% in the second step and by 20% in subsequent steps. Generally, 6–7 segments were acquired at 128 scans for each segment. The PSD spectrum was the combination of all acquired segments generated by the standard software. For CID experiments, the collision cell was filled with helium until the pressure in the source chamber reached \( \sim 1 \times 10^{-6} \text{ Torr} \) (1 Torr = 133.3 Pa).

2,5-Dihydroxybenzoic acid (DHB) was used as the matrix. A 1 \( \mu \text{l} \) volume containing about 10 pmol of the samples was premixed with 1 \( \mu \text{l} \) of the matrix (50 g l\(^{-1}\) water–acetonitrile), deposited on the sample target and allowed to dry. Recrystallization from ethanol or methanol was performed according to Harvey et al.’s procedure. We found that recrystallization leads to a stronger signal.

**Results and Discussion**

In Fig. 1(a) the CID PSD spectrum of the [M + Na]\(^{+}\) ion of lacto-N-fucopentaose I (LNFP I) is reported. In this spectrum peaks from one glycosidic linkage cleavage (Y, B and C fragments, Scheme 1) or from two or more glycosidic linkage cleavages (‘internal fragments’) are present in addition to \( ^{1.5}X \) peaks coming from sugar ring fragmentation. The \( ^{1.5}X \) peaks at \( m/z \) 231, 393, 596 and 759 always contain the reducing end and were missing in the PSD spectra obtained without a collision gas.\(^{15}\)

It has been reported previously\(^{11,12,15–19}\) that in the PSD spectra of free oligosaccharides it is not possible to assign \textit{a priori} a peak as Y or C or to an internal fragment and therefore the interpretation of the spectrum of a real unknown may be not simple. In this spectrum, because the \( ^{1.5}X \) peaks always contain the reducing end, we can use the \( ^{1.5}X \) peaks to attribute the Y fragments and from these assignments it is easy to attribute the corresponding B (\( M – Y + Na + 23 \)) and C (\( B + 18 \)) ions, thus obtaining the sequence of the oligosaccharide. Hence the Y ions \( (^{1.5}X – 28) \) are at \( m/z \) 203, 365, 568 and 731, the B and C ions are at \( m/z \) 697, 534, 331 and 169 (B ions) and \( m/z \) 715, 552, 349 and 187 (C ions). The remaining peaks are fragments produced with two or more cleavages (internal fragments) or very weak signals from other sugar ring fragmentation.

Although in the case of a linear structure an internal ion can give three peaks, we always observe only two mass signals, which differ by 18 u. The peaks originating from a combination of C and B or Z and Y fragmentation are, in fact, not observed. A similar behaviour will be present also in the analysis of branched structures.

Peaks at \( m/z \) 203 and 231 indicate that the reducing unit is a hexose. Peaks at \( m/z \) 365 and 393 contain 162 u more and indicate the presence of another hexose unit linked to the reducing unit. Peaks at \( m/z \) 568 and 596 differ by 203 u so they represent the sequence GlcNAc–Hex–Hex. The last couple of \( ^{1.5}X \) and Y ions at \( m/z \) 759 and 731 show that another hexose is linked to the GlcNAc. Finally,  

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**Materials**

Lacto-N-fucopentaoses were purchased from Dextra Laboratories (Reading, UK), samples of GPs from Dionex (Sunnyvale, CA, USA) and DHB from Aldrich (Milwaukee, WI, USA).

**Reproducibility**

We checked the reproducibility of the PSD spectra both by replicating data acquisition in our laboratory several times, and by sending samples of lacto-N-fucopentaoses to Perseptive Biosystems (Framingham, MA, USA) for analysis. The sets of data matched very well.

**Nomenclature**

We used Domon and Costello’s nomenclature\(^{21}\) to indicate the fragment ions observed as shown in Scheme 1. It is known\(^{1,12,15,16,19}\) that all ions (molecular and fragment ions) from MALDI of oligosaccharides contain sodium and this is implicit in the abbreviations used.

![Scheme 1. Oligosaccharide fragmentation nomenclature for peaks produced by a single fragmentation (from Ref. 21). Subscripts indicate the positions relative to the termini analogous to the system used in peptides, and superscripts indicate cleavages within carbohydrate rings.](image-url)

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the Y₄ peak at m/z 731 corresponding to a loss of 146 u from the parent ion and the presence of C₁ and B₁ peaks at m/z 187 and 169 indicates that the fucose is located at the terminal end.

This procedure can be applied here because ¹,5X peaks always contain the reducing end. If this behaviour is confirmed for other oligosaccarides, it delineates a general rule for sequencing free oligosaccharides by MALDI-MS.

The CID PSD spectra of LNFP II and LNFP III are shown in Fig. 1(b) and (c). ¹,5X fragments are present at m/z 231, 393, 596, 743 and 759 and from these peaks it is possible to assign the corresponding Y, B and C ions, except for the peak at m/z 596 because, although containing the reducing end, it corresponds to an internal fragment, involving a ¹,5X cleavage of the non-reducing terminal galactose or the fucose together with a Y cleavage on the other residue. In fact, the calculated C ion corresponding to the ¹,5X peak at m/z 596 is at m/z 350 and it is missing in the spectra. These assignments allow the sequencing of the oligosaccharides.

Figure 1. CID PSD spectra of [M + Na]⁺ (m/z 877) ions of LNFP I (a), LNFP II (b), LNFP III (c) and LNFP IV (d). The masses given on the structure are average values and they correspond to B, C, Y and ¹,5X ions, according to Domon and Costello’s nomenclature. Internal fragments are marked with (i) and abbreviations used are: N = N-acetylhexosamine, H = hexose and D = deoxyhexose.
Peaks at \( m/z \) 231 and 393 indicate the presence of the sequence Hex–Hex. The next \( 1.5X \) fragments at \( m/z \) 743 and 759 indicate the presence of a branching point with a hexose and a deoxyhexose linked to a GlcNAc. Good information is also obtained from the internal fragments; in this case the peak at \( m/z \) 550 suggests that a sequence Hex–Hex–HexNAc is present in these oligosaccharides, while peaks at \( m/z \) 372 and 354 in Fig. 1(b) allow us to discriminate between the two oligosaccharides as already reported.\(^{15}\)

Figure 1(d) shows the PSD spectrum of LNFP V. In this oligosaccharide, the reducing unit is composed of one glucose and one fucose unit; as expected the \( 1.5X \) fragment at \( m/z \) 231 is missing and a new \( 1.5X \) peak at \( m/z \) 377 corresponding to a fucose linked to an hexose unit is now present. The next \( 1.5X \) fragment is at \( m/z \) 539, 162 \( \mu \) higher, indicating that another hexose is linked to the reducing hexose. The next \( 1.5X \) fragments at \( m/z \) 743 indicate that a GlcNAc is linked to this unit and that a hexose is one terminal end. The last \( 1.5X \) peak at \( m/z \) 759 suggests that the fucose is other terminal monosaccharide.

The spectra of the lacto-N-fucopentaoses shown here confirm that by using the presence of the \( 1.5X \) peaks we have a good procedure for the assignment of peaks in CID.
PSD spectra of real unknowns and we further checked the method with the more complex N-linked oligosaccharides shown in Figs 2–4. The CID spectra shown in Figs 2–4 indicate that also for these oligosaccharides the $^{1,5}X$ peaks containing the reducing end are present, confirming that we can apply our rule to sequence oligosaccharides.

The CID PSD spectrum of GP-18 is shown in Fig. 2. In the low-mass range, peaks corresponding to mannose ($m/z$ 203) and GlcNAc ($m/z$ 244) are present; the presence of the peak at $m/z$ 272 ($244 + 28$) indicates that the GlcNAc is the reducing end. The next couple of $^{1,5}X$ and Y peaks are at $m/z$ 447 and 475. They differ by 203 u.
from the preceding peaks and indicate the presence of the sequence GlcNAc–GlcNAc. Peaks at m/z 771 (Y_2a) and 799 (1.5X_2a), containing two hexose units more than the peaks at m/z 447 and 475, indicate that the oligosaccharide has a branched hexose bearing another hexose linked to GlcNAc–GlcNAc. The presence of C_2 and B_2 fragments at m/z 527 and 509, containing three hexose units, and the M_r value permit us, finally, to deduce the complete sequence.

The CID PSD spectra of GP 14 and GP 12 are shown in Figs 3 and 4. In these spectra 1.5X peaks are also present, allowing the identification of the Y, B and C fragments. In the spectrum in Fig. 3, Y fragments at m/z 244, 447, 975, 1178 and 1340 indicate that two branching points are present in this oligosaccharide. In particular, peaks at m/z 975 and 1178 indicate that a GlcNAc–Man is one of the two antenna while the other antenna is (GlcNAc)_2–Man. Furthermore, the presence of the 1.5X peak ion at m/z 1368 and the absence of the 1.5X peak at m/z 1165 indicate that the two GlcNAc are linked to the mannose. Similar considerations can be made for the spectrum in Fig. 4.

CONCLUSIONS

We have shown that CID spectra of underivatized oligosaccharides, unlike other compounds such as peptides, are different from those obtained without CID. The difference is the presence abundant 1.5X ions containing the reducing end.

The 1.5X fragments are easily recognized, because they differ by 28 u from the corresponding Y fragments, so that if two peaks with a difference of 28 u are present, they are a 1.5X and a Y ion and, if we have a linear oligosaccharide, the sequence is assigned. In the case of more complex oligosaccharides, we must also find the B (M – Y + 46) and C (B + 18) ions in order to detect internal fragmentation. When all the B, C and Y peaks besides the internal fragments are assigned we should be able to draw the sequence.

The presence of helium as the collision gas is indispensable for the formation of 1.5X ions in the MALDI PSD spectra, because they are not present in the PSD spectra obtained without CID or using argon as the collision gas. We confirmed this by measuring PSD spectra of the same compounds as presented here without a collision gas or using argon as the collision gas, and no 1.5X fragments were observed at all. Furthermore, in the PSD spectrum of maltoheptaose obtained by using helium as collision gas, abundant 1.5X fragments at m/z 231, 393, 555, 717, 879, 1041 were present, as shown in Fig. 5, whereas they were missing in the spectrum obtained with argon reported recently.

These findings are not surprising because even if, in general, a heavier target gas is more effective in causing dissociation, it has been demonstrated that the energy transferred in helium collisions can be greater because of its high ionization energy and low scattering losses.

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Figure 5. CID PSD spectrum of [M + Na]+ (m/z 1176.0) ion of maltoheptaose. The masses given on the structure are average values and they correspond to B, C, Y and 1.5X ions, according to Domon and Costello’s nomenclature.

REFERENCES