

Fragmentation of Neutral Carbohydrates in MALDI TOF/TOF™ Mass Spectrometers

Tools for data interpretation in Data Explorer® Software

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Carbohydrate Analysis by Mass Spectrometry

The analysis of carbohydrates is of high importance in modern biochemistry. Structure determination requires the knowledge of the sugar sequence, the assignment of the reducing end, the linkage type between the monosaccharides and the anomeric configuration. Because of its large mass range, the high sensitivity and the soft desorption and ionization MALDI time-of-flight (MALDI-TOF) mass spectrometry is today widely used in the molecular weight determination of underivatized oligo- and polysaccharides [1, 2].

Since the introduction of the post-source decay (PSD) technique for MALDI TOF mass spectrometers, which provides fragment ion spectra, some publications were dealing with the PSDTOF analysis of oligosaccharides [3, 4]. It has been shown that much information can be obtained by MALDI-PSD-TOF experiments. PSD (metastable or unimolecular decay) spectra of sodiated ions from neutral carbohydrates are dominated by glycosidic and internal cleavages, providing information related to sequence and branching; however, a lack of abundant cross-ring cleavages limits the linkage information to be deduced from such experiments. Cross-ring fragmentation providing information on glycosidic linkages can be induced by high energy collision-induced dissociation (CID) on MALDI-TOF/TOF™ instruments [5, 6].

quantification package that enables many analytes and samples to be processed all at the same time with additional support for protein / peptide quantitative workflows.



Figure 1. The AB SCIEX TOF/TOF™ 5800 System: With its patented TOF/TOF™ optics and collision cell the 5800 System is capable low-energy and high-energy MALDI CID MS/MS generating many more useful fragments for carbohydrate analysis than other mass spectrometry techniques.

Key Features

- MALDI TOF/TOF™ analyzer spectra of neutral oligosaccharides can be interpreted using a Data Explorer® macro, assigning 1,5X, Y, Z, and C ions
- Cross-ring fragments do not appear in metastable fragmentation or PSD-type fragmentation (attributed to high energy requirements)
- Ring fragments (eg r,sA) can be used to determine branching patterns of glycans
- MALDI TOF/TOF™ spectra show greater number of fragments than electrospray CID MS/MS spectra or MALDI PSD spectra

Carbohydrate Fragmentation Nomenclature

The fragmentation nomenclature for oligosaccharide in mass spectrometry is shown in Figure 2. The fragment ions that contain a non-reducing terminus are labeled with uppercase letters A, B, C and the ions that contain the reducing end of the oligosaccharide are labeled with letters X, Y, Z. The subscripts indicate the sugar residue numbered from the nonreducing end. B ions are oxonium ions, Y ions are protonated species that include a H-transfer. C as protonated molecular ions and Z ions result from cleavage of glycosidic (O-C bond), Z fragments are rarely observed in MALDI mass spectrometry (see Figure 3).

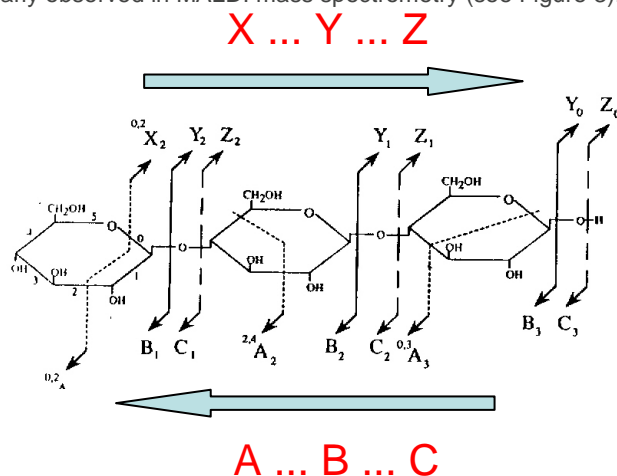


Figure 2. Structure of celotriose Glc(β 1 \rightarrow 4)Glc(β 1-4)Glc (MW 504 Da) illustrating the fragment ion nomenclature [7, modified from 8]

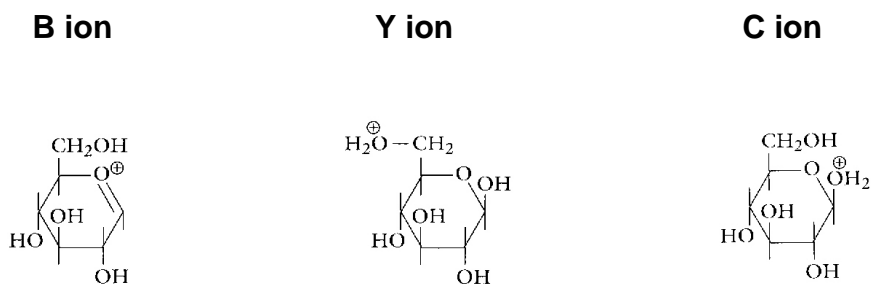


Figure 3. Structure of B Y and C ions

Ring Fragmentation

The A and X ions are produced by cleavage across the glycosidic ring, and are labeled by assigning a number to the cleaved bond counting clockwise. Figure 4 shows an example. r,sA , r,sX are formed by ring cleavages through the r , s bonds. Both ions retain the charge of the molecular species. Mechanisms of two-bond ring cleavages have been described in references [9, 10, 11].

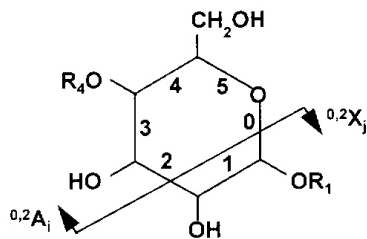


Figure 4. Example of ring fragmentation: r,sA , r,sX are formed by ring cleavages through the r , s bonds. Both ions retain the charge of the molecular species.

MALDI TOF/TOF™ Mass Spectrometry

Either the 4700 Proteomics Analyzer, 4800 *Plus* MALDI TOF/TOF™ Analyzer or the AB Sciex TOF/TOF™ 5800 (AB Sciex, Framingham, MA, USA) can be used for CID fragmentation of carbohydrates. In case of the 4700 and 4800 the instrument is equipped with an Nd:YAG laser with $\lambda = 355$ -nm wavelength of <500 ps pulse and 200 Hz repetition rate in both MS and MS/MS modes. In case of the 5800 the instrument is equipped with an Nd:YLF Laser with $\lambda = 345$ -nm wavelength of <500 ps pulse length and up to 1000 Hz repetition rate.

All measurements were made in automatic mode. In MS mode, up to 1200 shots were accumulated and in MS/MS mode up to 4800 laser shots were accumulated. All spectra shown were obtained using DHB as the matrix and argon as the collision gas, although no large differences were observed when argon was replaced by air. The potential difference between the source acceleration voltage and the collision cell was set as 1 kV.

Sample Preparation

Both alpha-cyano-4-hydroxycinnamic acid (a-cyano) and 2,5-dihydroxybenzoic acid (DHB) were used at a concentration of 10 mg/mL, DHB in pure water, and a-cyano in a 1:1 mixture (v/v) of acetonitrile and 0.1% trifluoroacetic acid/water. Preparation method for neutral carbohydrates: DHB is recrystallized using 1 μ l of ethanol to create an amorphous preparation in order to promote sodium adducts in MALDI spectra.

Automated Sequence Ion Matching

Because of the large number of peaks present in the MALDI CID TOF/TOF™ spectra of sugars a macro was developed (available as a Data Explorer® macro [6]) able to attribute 1,5X, Y, Z, B and C ions in these spectra. The macro finds all the pairs of peaks with a difference of 27.99 Da (CO) corresponding to possible 1,5X,Y ion pairs.

1. The macro is able to attribute 1,5X, Y, Z, and C ions
2. The macro finds pairs in the spectra eg. CO (27.99 Da) for 1,5X and Y ion pairs, similar to a,b pairs with 28 Da for peptides
3. All ions 1,5X, Y, B, C and Z have to be present in the spectrum
4. $(B + Y) = (C + Z) = (M + 2Na - H)$

A copy of the macro routines is available upon request via <http://www.ictmp.ct.cnr.it/ictmp/mass6.htm> or via http://www.absciex.com/mk/get/CONTACT_US Figure 5 to 7 show the Data Explorer® macro, the user interface and some examples of the results format.

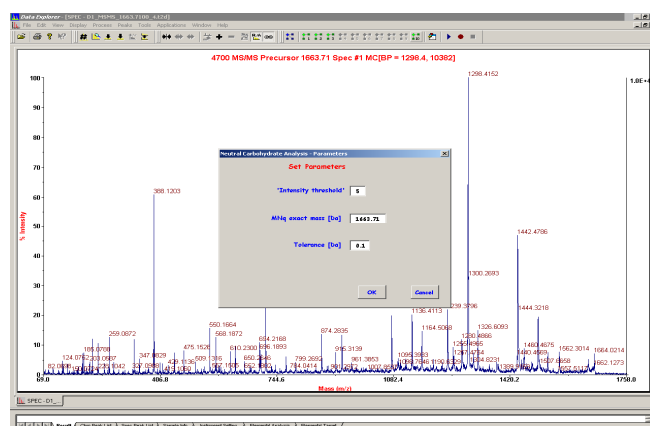


Figure 5. Data Explorer® Macro for evaluation of CID MSMS spectra of neutral carbohydrates

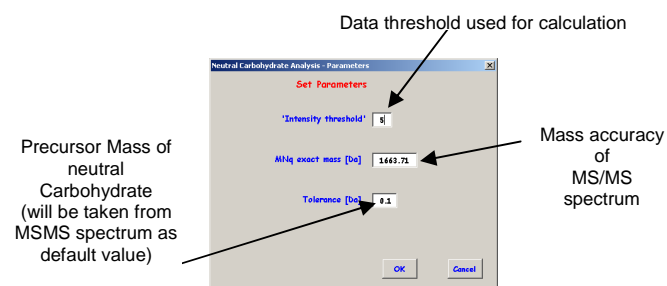


Figure 6. Data Explorer® Macro for evaluation of CID MSMS spectra of neutral carbohydrates: user interface and required settings

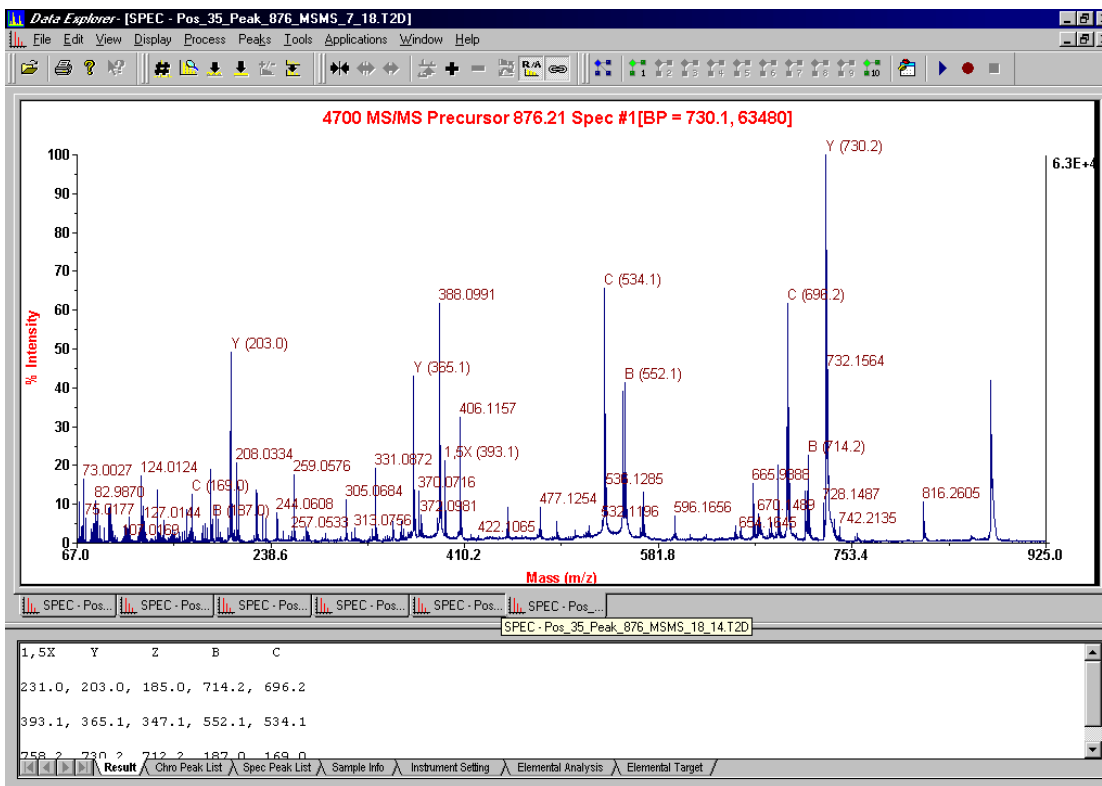


Figure 7. Data Explorer® Macro: The macro calculates the 1,5X, Y, Z, B and C and copies the result to the output window of Data Explorer® Software.

Conclusion

MALDI CID TOF/TOF™ spectra allow differentiation of isomeric structures, and the determination of the sequence of the oligosaccharides and the linkage position of the reducing residue. In the MALDI CID MS/MS spectra a considerably greater number of fragments are found in comparison to other mass spectrometry fragmentation techniques, and, although this may complicate their interpretation, it permits acquisition of more information on linkage position and points of branching. A macro can be used to automate the interpretation of the peaks present in a MALDI CID TOF/TOF™ spectra.

References

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