Overview - Introduction

- ISD results in the fragmentation of analytes in the MALDI source.
- ISD fragmentation of permethylated glycans leads to the formation of oxonium ions, resulting from the protonation of glycosydic bonds[1]

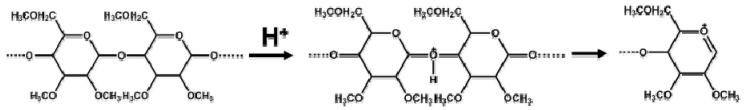


Figure 1 : Mechanism of fragmentation leading to the formation of glycan oxonium ions

- ISD is a « Janus type phenomenon »: it creates artefact peaks BUT allows « pseudo-MS[3] » analysis to be performed.

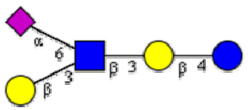
Our goal is to define optimal matrix conditions to induce ISD of permethylated glycans or, inversely, to minimize this phenomenon.

Methods

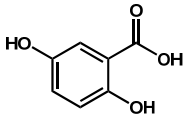
- LNDFH II and LS-tetrasaccharide B were used as model compounds.
- Permethylation was performed by resuspending lyophilized samples in 1 ml of DMSO/NaOH and addition of 0.5 ml of CH_3I. Samples were incubated 20 min at room temperature, extracted in chloroform, and then purified on Sep-Pack C18.
- DHB (2,5 dihydroxybenzoic acid), DHB + Pyridine ionic liquid matrix, DHB sodium salt, CHCA (α-Cyano-4-hydroxycinnamic), ATT (6-aza-2-thiothymine) and 9AA (9-aminoacridine) were tested as matrices.
- Spots were prepared using the « dried droplet » method by mixing equal volumes of matrix and analyte (model glycans or mouse IgG1 permethylated N-glycans).
- Spectra were recorded on a Bruker Ultraflex II TOF/TOF. MALDI-Imaging was used to test potential variations inside a same spot.

Results

Analyte = LS-Tetra

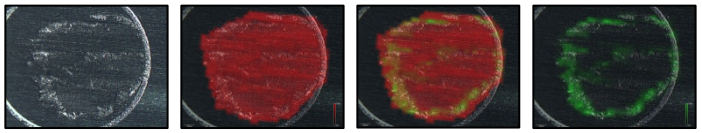


1) Matrix = DHB



With DHB, fragmentation is strongly favoured on the crystals, where the sodium concentration is lower[2,3]

Parent ion @ m/z = 1287.6 **Fragment @ m/z = 825.4**



Spectrum from the red region **Spectrum from the green region**

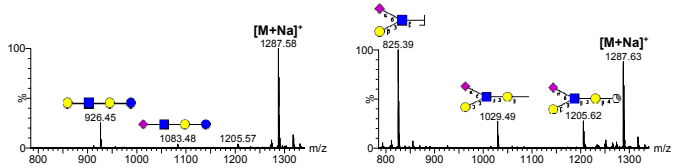
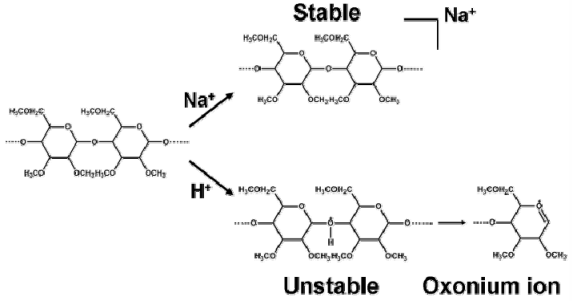


Figure 2 : In source decay of permethylated LS-tetrasaccharide B in DHB. Top: from left to right: photography of the spot, repartition of the sodiated parent ion, repartition of both sodiated parent ion and fragment at m/z = 825, repartition of the oxonium fragment. Bottom: left: spectrum recorded in a region where no ISD was observed. Right: spectrum recorded on a crystal displaying high level of ISD.

There is a competition between two ionization processes : cationization of the compounds leading to stable species, and protonation of glycosidic bonds favouring fragmentation



Neutralizing protons (ILM or basic matrix like 9AA) or including sodium into crystals (DHB salt) would therefore minimize ISD

2) Matrix = DHB + Py

3) Matrix = DHB + DHB Na salt

No ISD was observed in these conditions

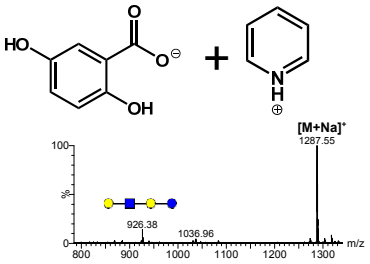
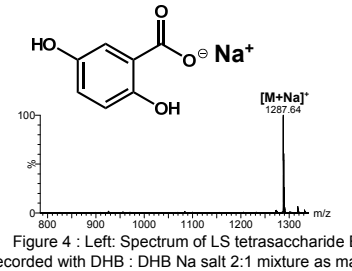


Figure 3 : Spectrum of LS tetrasaccharide B recorded with ILM DHB + Py. No ISD was observed



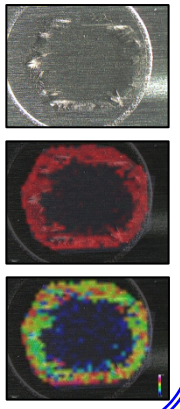


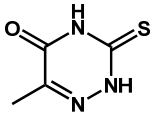
Figure 4 : Left: Spectrum of LS tetrasaccharide B recorded with DHB : DHB Na salt 2:1 mixture as matrix. No ISD was observed. Right: from top to bottom: photography of the spot, repartition of the sodiated parent ion, relative intensity of the sodiated parent ion.

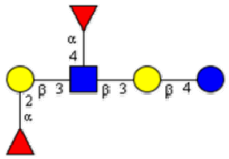
Other Matrices inducing ISD

Like DHB, HCCA and ATT were found to be able to promote ISD of permethylated glycans, in a location-dependant manner

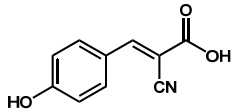
Analyte = LNDFH I

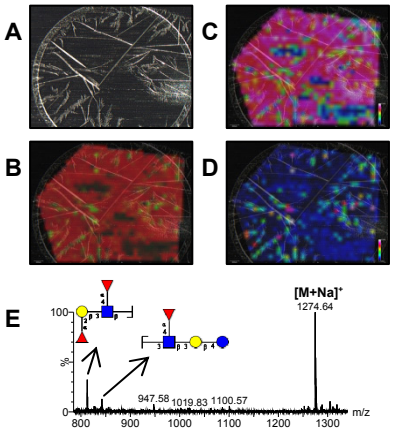
Matrix = ATT





Matrix = CHCA





In CHCA, fragmentation is favoured in the center of spots (even if the matrix layer seems to be homogenous), but is less important than in DHB.

In ATT, like in DHB, fragmentation is observed when laser shots is performed on crystals. However, not all crystals promote ISD.

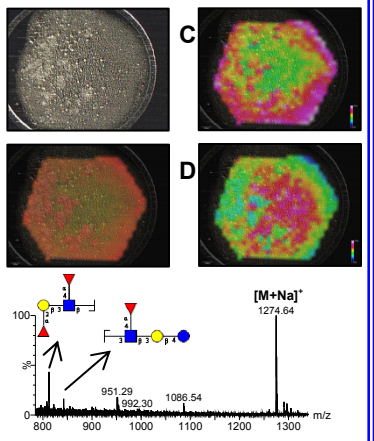
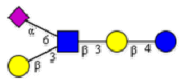


Figure 5 : A: photography of the spot (LNDFH I + ATT). B: repartition of both sodiated parent ion (red) and fragment (green). C and D: relative intensities of the sodiated parent ion and the fragment, respectively. E: spectrum recorded on a green zone of B

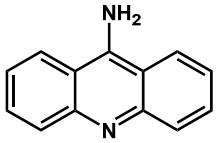
Figure 6 : A: photography of the spot (LNDFH I + CHCA). B: repartition of both sodiated parent ion (red) and fragment (green). C and D: relative intensities of the sodiated parent ion and the fragment, respectively. E: spectrum recorded on a green zone of B

9AA, which is generally used in negative ion mode, was found to avoid ISD of permethylated glycans

Analyte = LS-Tetra.



Matrix = 9AA



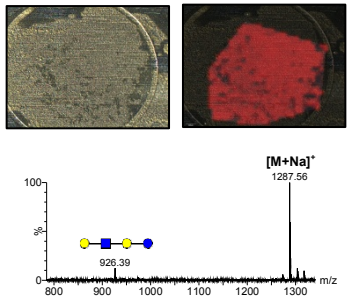


Figure 7 : Top: left: photography of the spot, repartition of the sodiated parent ion. Bottom spectrum of LS tetrasaccharide B recorded with 9AA.

No oxonium fragments were dectected with 9AA

Protons are probably bound to the matrix

Implication for biological samples

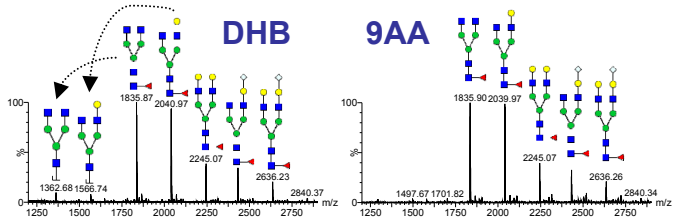


Figure 8 : Spectra of permethylated N-Glycans of mouse IgG1. PNGaseF was used to obtained N-glycans. The same sample was tested with DHB or 9AA as matrices. No oxonium ions were detected, indicating that 9AA completely avoids the in source fragmentation of permethylated glycans since.

N-glycans from mouse IgG1 : use of DHB leads to the detection of artefact ISD peaks, making the interpretation of spectra more ambiguous.

Conclusions

- **ISD** of permethylated glycans is **promoted** by the protonation of glycosidic bonds in **DHB, CHCA and ATT.**
- Due to differences in Na^+ repartition in spots, **ISD is enhanced in crystals of DHB** but avoided in amorphous zones.
- Trapping protons by **ILM** or use of **9AA** as well as including cations in DHB crystals allows **ISD** to be **avoided.**

It is therefore possible to effectively control the occurrence of ISD

Acknowledgements

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Influence of the matrix on the In Source Decay of permethylated glycans during MALDI-TOF analysis

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