Fragmentation of oligosaccarides using MALDI LTQ Orbitrap for multi-stage MS

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INTRODUCTION. Glycomics, the methodological approach aiming at the mapping of the entire set of glycans of individual organisms, exploits the recent advances in analytical and computational tools developed for proteomics. In this context mass spectrometry, thanks to its high sensitivity and selectivity, is a very powerful tool, allowing characterization of glycopeptides as well as glycans released from them.
MALDI-MS is a rather sensitive technique, presenting a high tolerance to salts contaminants frequently co-eluting with glycans during purification procedures, allowing rapid analyses without much effort. Structural information on glycans requires fragmentation procedures using tandem mass spectrometry techniques. Glycans fragmentation presents some main cleavage sites, termed glycosidic cleavages, between adjacent sugar rings. They provide much information on sequence and branching but little on linkage. Other cleavages, named cross-ring cleavages and involving two bonds cleavages are useful in the linkage determination [1]. Fig.1 provides a clear representation of the fragmentation of oligosaccharides.
Further fragmentation events can be achieved in multi-stage mass spectrometry, allowing re-fragmentation of product ions obtained during MS^2 experiments. MS^n experiments can be carried out only on ion traps, FT-ICR and Orbitrap instruments. Here we report on the structural characterization of glycans by MS^n analyses of $[M+Na]^+$ ions using the versatility of the MALDI ionization source coupled with the high mass accuracy and mass resolution provided by the Orbitrap analyzer. The goal of a confident structural discrimination of the oligosaccharidic moieties is achieved without enzymatic digestions or more complex structural analyses, like NMR. During CID fragmentation Q-value remains constant and it is not possible to trap fragments at m/z lower than the 30% of the precursor. Attempts to overcome the problem of low mass cut-off were faced using PQD and HCD by Karas and coworkers [2]. Thus, the absence of significant signals at low masses and the need for this information in structural elucidation of oligosaccharides sequences convinced us to explore the potential of multi-stage fragmentation.

MATERIALS AND METHODS. N-glycans were extracted from 500 µg of fetuin or chicken ovalbumin(Sigma Aldrich) that were treated with 3 units of PNGase F (Sigma Aldtrich) at 37°C for 12-16 h. Glycans were separated from proteins upon ethanol precipitation. MALDI-LTQ-Orbitrap (Thermo Fisher scientific) analyses were carried out using DHB as matrix (30 mg/ml in 70% ethanol, 0.1% TFA. All the spectra were recorded in positive ion mode. Full scan mass spectra were carried out using a the Orbitrap mass analyzer, using resolving power of 60,000 or 100000 and recording 3 microscans for one position. A system for the automatic recognition of crystals, the survey crystal positioning system(survey CPS) allowed the random choice of shot. Laser intensity was generally set at 35 µJ. For MS^n scans the filling status of the linear ion trap was regulated by automatic gain control (AGC). CID fragmentation spectra were performed using the ion trap as mass analyzer and collision energy (CE) was optimized to 45%-50%; the activation Q value was set to 0.250 and the activation time was 30 ms. Spectra were manually interpreted and checked against GlycoWorkbench software.

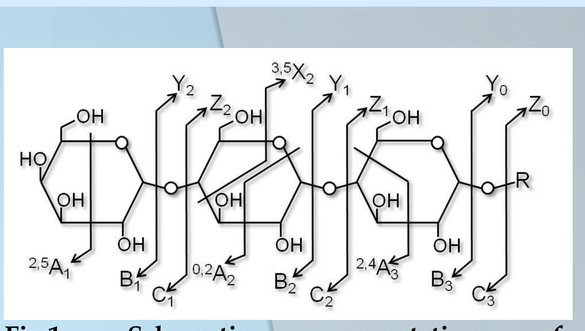
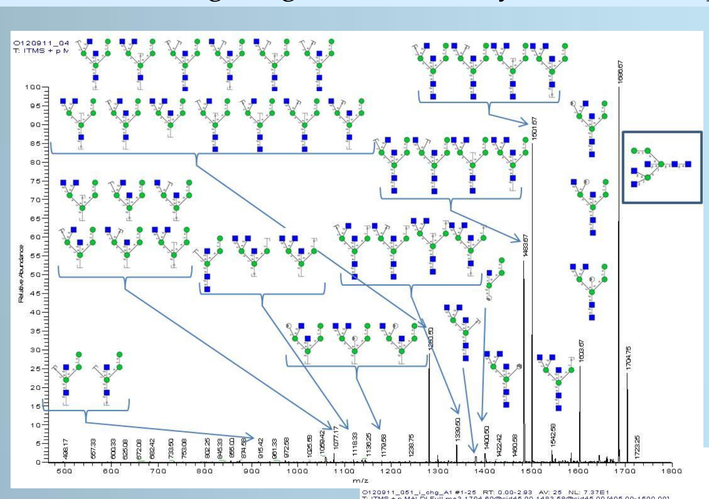


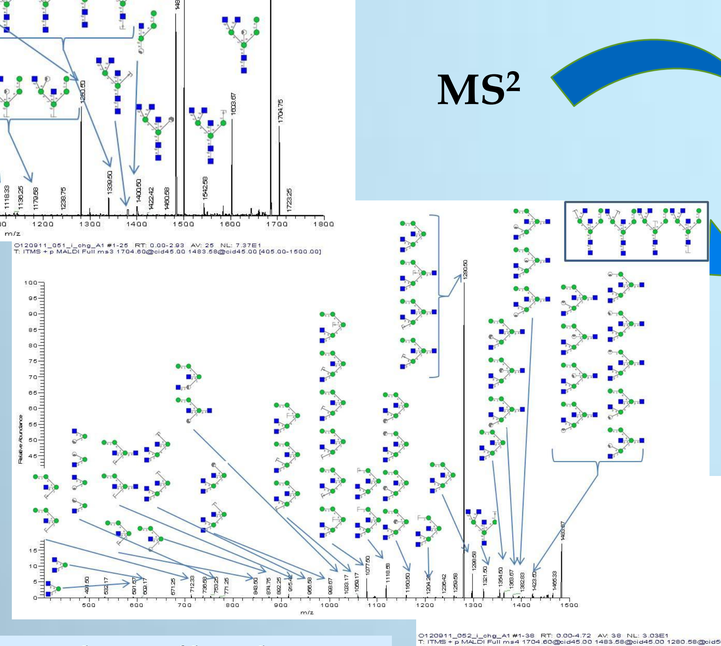
Fig.1: Schematic representation of nomenclature for the fragmentation of oligosaccharide ions

RESULTS. N-glycans extracted from standard proteins were analyzed using DHB as matrix. All the spectra were recorded in positive ion mode. Full scan mass spectra were carried out revealing a high mass accuracy due to orbitrap analysis. This offered the advantage to perform a reliable glycan profiling based on full scan spectra.



CID fragmentation spectra were performed using the ion trap as mass analyzer. As an example we show the ovalbumin specie at m/z 1704.61, that was chosen to set up collision energy and laser intensity. This ion can have different attribution on the base of the knowledge of the N-glycans biosynthetic pathway.

Yet, a database searching of the exact mass of the specie revealed that the most probable structure in chicken egg is the hybrid bisecting one in Fig.2. Multi-stage mass spectrometry experiments were set up on MALDI-LTQ Orbitrap to achieve a structural elucidation of N-linked oligosaccharides.



Multi-stage MS

MS^2

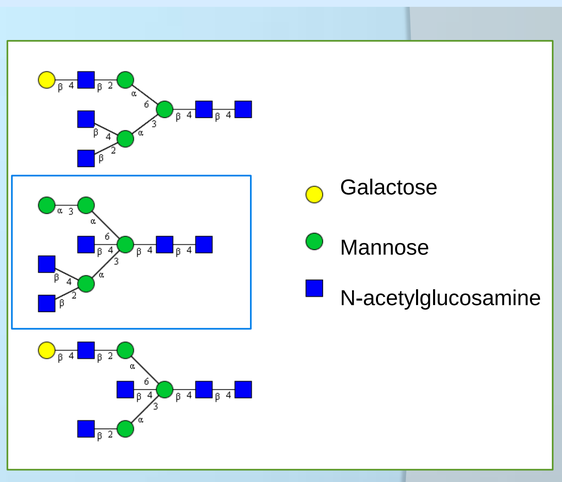
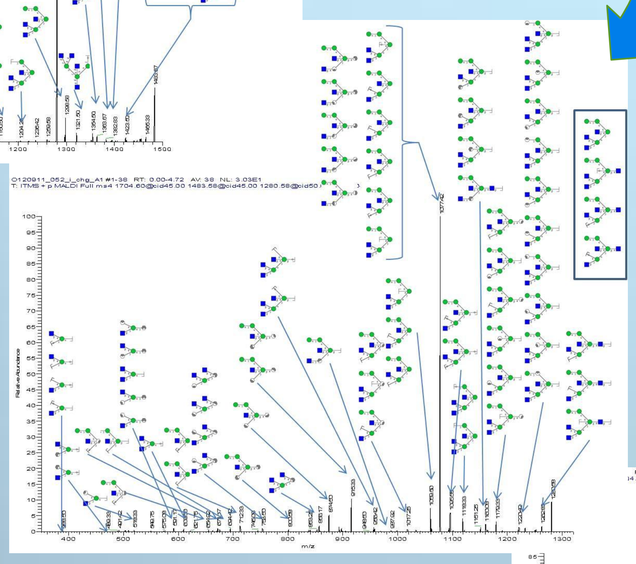
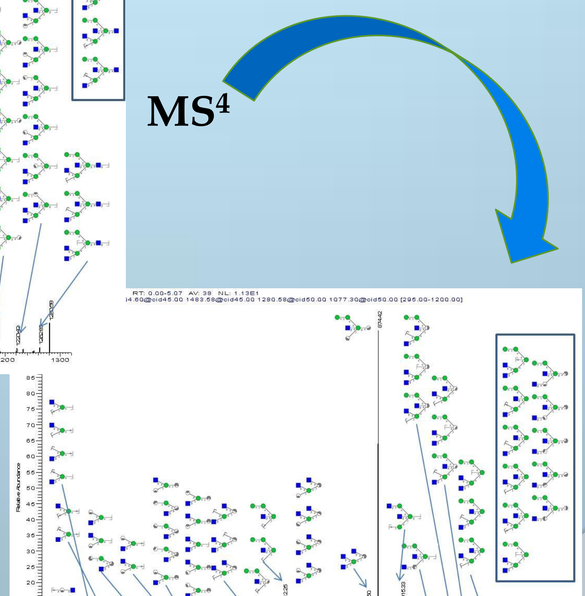


Fig.2: Most probable interpretation for the ion at m/z 1704.6 on the basis of the exact mass determined in full MS.

MS^3

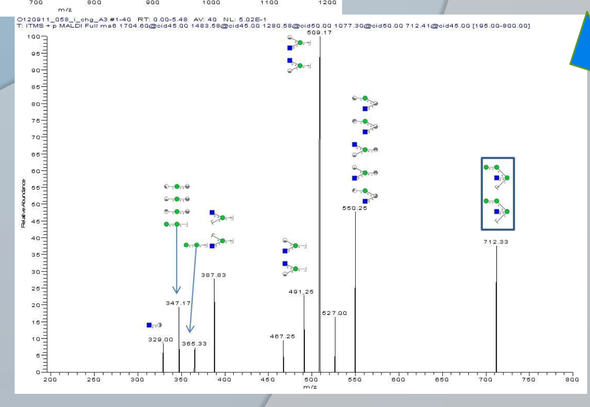


MS^4



MS^5

MS^6



The MS^2 spectra revealed the prevalence of b and y-type ions, covering a narrow mass range, due to the one third rule of orbitrap analyzers. The ion at m/z 1483.58 was chosen for the further fragmentation event in MS^3. A wider sequence coverage was achieved performing an further fragmentation events until MS^6. The multi-stage mass spectrometry experiments allowed a better sequence coverage, also thanks to the presence of cross-ring cleavages and a wider mass range.

CONCLUSIONS. In conclusion the coupling of the high sensitivity and throughput of MALDI-MS with the extremely high accuracy of the Orbitrap analyzer opened the way for a better structural elucidation of oligosaccharides as post-translational modifications.
The methodological approach opens up the way for the use of this instrumentation in the characterization of glycosylation in different systems.

[1] Domon B, Costello CE. A Systematic Nomenclature for Carbohydrate Fragmentations in FAB-MS/MS of Glycoconjugates., Glycoconjugate J. 1988, 5, 397-409.
[2] Rohmer M, Baeumlisberger D, Stahl B, Bahr U, Karas M. Fragmentation of neutral oligosaccharides using the MALDI LTQ Orbitrap. Int J Mass Spectrom 305, (2-3), 15 August 2011:199-208