Automated Glycan Structural Isomer Differentiation Using Bioinformatics Tool

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Overview

Purpose: To demonstrate the use of SimGlycan® software for automated structural elucidation of glycan MS^n spectra.

Methods: Sequential MS^n spectra were acquired for permethylated chicken ovalbumin glycans. Structural elucidation was performed on SimGlycan software 2.92.

Results: The combination of permethylation, MS^n and SimGlycan software enabled successful identification and differentiation of various structural isomers of chicken ovalbumin released glycans.

Introduction

The use of permethylation in combination with multistage fragmentation (MS^n) is a critical aspect for glycan structural characterization. Only MS^n truly characterizes a glycan structure as it allows identification of branching, linkages and resolution of isobaric structures that are otherwise indistinguishable in MS^2 spectra. However, MS^n analysis is complicated by the large number of spectra generated for a single structure. It is very common that one must acquire MS^6 or MS^7 level of fragmentation to differentiate potential glycan structural isomers. Here we present the use of a bioinformatics tool (SimGlycan software) for glycan structural isomer differentiation from MS^n data.

Methods

Sample Preparation

Ovalbumin (1 mg, Sigma) was reduced, alkylated and digested overnight with trypsin in 25 mM ammonium bicarbonate buffer (pH ~8) at 37 ºC. PNGase F solution (3 µL, Roche) was added to 200 µL of digested sample and incubated for another 16 hours at 37 ºC. Released glycans were separated from peptides using a C_{18} cartridge. The cartridge was conditioned by washing with acetonitrile, followed by water. PNGaseF digested sample was loaded onto the cartridge and released glycans were eluted with 1% ethanol while peptides remain bound to the cartridge. Released oligosaccharides were first purified using PGC (PhyNexus) and then permethylated as described previously.[1]

Mass Spectrometry

All MS^n experiments were carried out on a Thermo Scientific Velos Pro dual-pressure linear ion trap mass spectrometer using a nanoESI source via direct infusion. Mass spectrometric settings and SimGlycan software (version 2.92) search parameters are listed in Tables 1 and 2.

TABLE 1. Mass spectrometer settings

Source	nano-ESI
Capillary Temperature	200 ºC
S-lens RF Level	50%
Source Voltage	1.3 kV
Full MS Range (*m/z*)	150-2000
Scan Rate	Enhanced
Maximum Injection Time	MS 50 ms
	MS^n 50 ms
Isolation Width	3
Collision Energy	30
Activation Time	10 ms
pAGC Enabled	Yes
Full MS µscans	5
Target Value MS	3e4
Target Value MS^n	3e4

TABLE 2. SimGlycan software version 2.92 search parameters

Ion Mode	Positive
Adducts	Sodium
Precursor *m/z* Tolerance	0.8 Da
Spectrum *m/z* Tolerance	0.8 Da
Chemical Derivatization	Permethylated
Reducing Terminal	Reduce
Class	Glycoprotein
SubClass	N-Glycan
Biological Source	Chicken
Pathway	Unknown
Search Structure	All
Glycan Type	All

Results

Automated structural interpretations of MS^n glycan spectra were tested on glycans released from chicken ovalbumin (Figure 1). This was an ideal system to test the capability of SimGlycan software because the glycan content of ovalbumin has been characterized in depth.[2] In parallel, we manually interpreted the MS^n spectra and compared it with previously presented data, thus providing a perfect control.[3] Figure 2a shows the MS profile of permethylated glycans derived from ovalbumin on a Velos Pro™ mass spectrometer. This was an ideal instrument for these experiments because the dual-pressure ion trap and S-Lens ion optics provide increased ion transmission and better trapping and fragmentation efficiency, which are critical for performing MS^n experiments. Table 3 shows all glycans identified in this study. Figure 2b shows the MS/MS spectrum for a peak at *m/z* 1054.68 (+2), which was selected for software evaluation as it was interrogated before.[3] To fully characterize the glycan structure, sequential MS^n fragmentation was utilized for this precursor. The Velos Pro mass spectrometer was operated in “Enhanced Scan” profile mode for all MS experiments. The enhanced scan mode allows charge stage determination of precursors and fragment ions.

FIGURE 1. Workflow for automated structural interpretation of MS^n glycan spectra

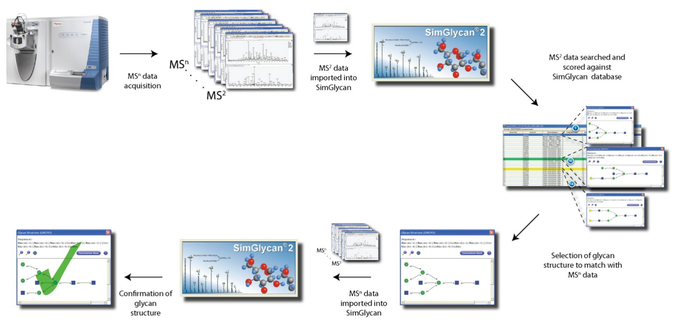
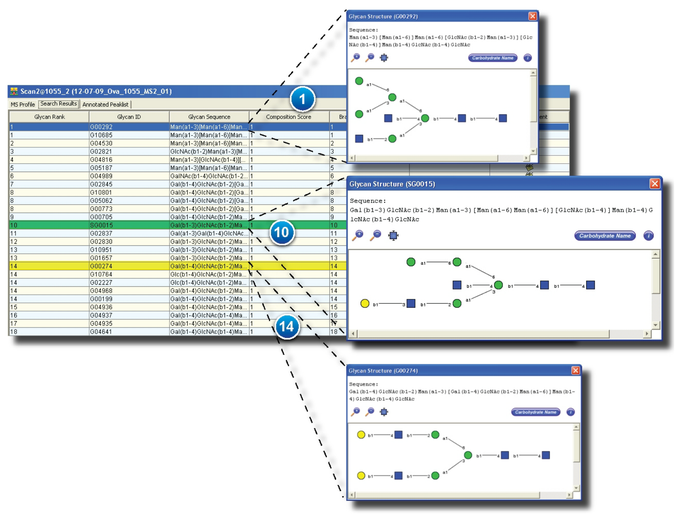


FIGURE 2. (a) ion trap Full MS of permethylated ovalbumin released glycans (labeled peaks correspond to Table 3). (b) ion trap MS/MS of peak at *m/z* 1054.68. (c) Set of sequential MS^n spectra acquired for peak at *m/z* 1054.68 (+2).

Figure 2c shows sets of MS^n spectra acquired for this precursor that can be imported into SimGlycan software for analysis. The workflow undertaken for MS^n data interpretation is as follows: The user submits the MS^2 spectrum for automatic compositional identification. Based on the criteria selected, SimGlycan software searches its database to match the MS^2 data. If the user strictly relies on MS^2 data, then the fragmentation pattern for *m/z* 1054.68 (+2) can be interpreted as a hybrid glycan with a bisecting GlcNAc from the top ranked glycan from the SimGlycan database search results (Figure 3). Examination of the glycan list reported by SimGlycan software for the submitted MS^2 spectrum shows additional glycan compositions having the same mass but ranked much lower. These glycans, though reported to have much lower probability of matching the submitted MS^2 spectrum, could represent additional isomers that might be present since not every major fragment in the spectra was assigned (Figure 2b). To determine if these glycans are additional isomers, SimGlycan software was used to examine the lower-ranked glycan structures to see if they matched the MS^n fragmentation pathway. From the list generated by SimGlycan software, specific structures can be selected to compare with the MS^n fragmentation pathway. Each successive level of fragmentation can be brought in to match with the specific precursor selected for fragmentation in the previous level of MS^n spectrum.

FIGURE 3. SimGlycan software search results for ion trap MS/MS spectrum of precursor ion at *m/z* 1054.68 (+2). Symbolic representation of search results for top ranked and two lower ranked glycans is shown.



For example, from our list we selected the asialyl digalactosyl biantennary glycan, which is ranked lower based on MS^2 data but has the same precursor mass as the top match, to confirm or deny as a potential isomer (Figure 3, ranked 14 on the list). In the MS^2 spectrum (Figure 2b) of *m/z* 1054.68 (+2), we selected the fragment ion at *m/z* 1623.25 (+1) for further fragmentation. The detection of this ion indicates the loss of Gal-GlcNAc from the non-reducing end of the glycan. Figure 2c shows the MS^3 spectra for this ion. Of particular interest in the MS^3 spectrum is the fragment ion at *m/z* 1159.93 (+1), which corresponds to additional loss of Gal-GlcNAc structure. This is only possible from our selected asialyl digalactosyl biantennary glycan structure, as additional loss is possible from the non-reducing end. Figure 4a shows the overall sequential fragmentation pathway for the proposed structure, which is only compatible with the selected structure and the set of sequential MS^n spectra acquired in Figure 2c (1054.68→662.50). Figure 4b shows the sequential fragmentation pathway (1054.68→925.42, as in Figure 2c) for the hybrid glycan with the bisecting GlcNAc. This further confirms that this structure is also present in the precursor at *m/z* 1054.68 (+2). An additional hybrid glycan is also identified for this precursor in Figure 4c (1054.68→621.59, as in Figure 2c). As illustrated in Figures 4a-c, SimGlycan software was able to resolve isobaric oligosaccharides and perform detailed characterization of selected structures.

FIGURE 4. Representation of Y-type glycosidic fragments. MS^n fragmentation pathways for (a) $(Gal)_2(Man)_3(GlcNAc)_4$ (b) $(Man)_5(GlcNAc)_4$ and (c) $(Gal)(Man)_4(GlcNAc)_4$.

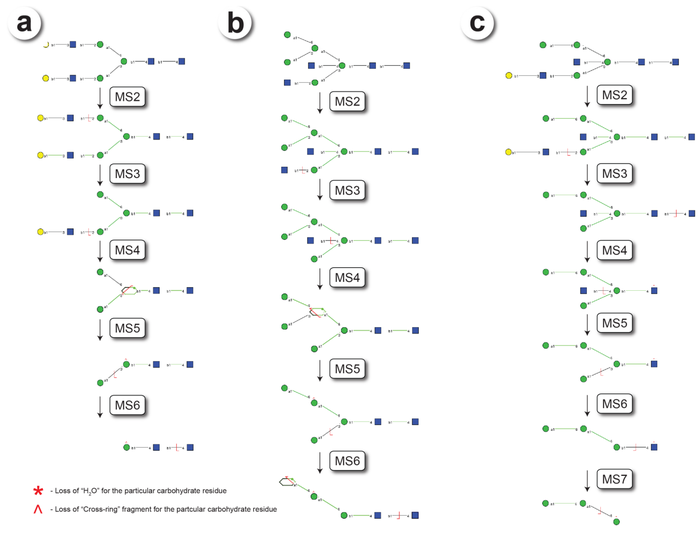


TABLE 3. Structures of chicken ovalbumin *N*-linked released glycans identified in this study (structures drawn using the GlycoWorkbench tool[4]).

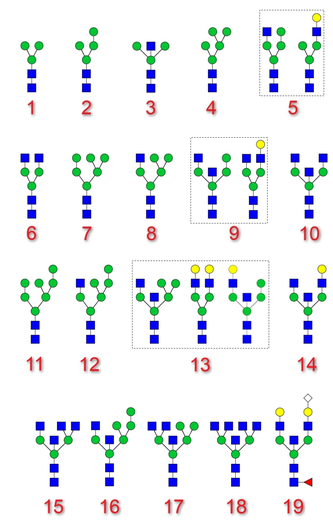
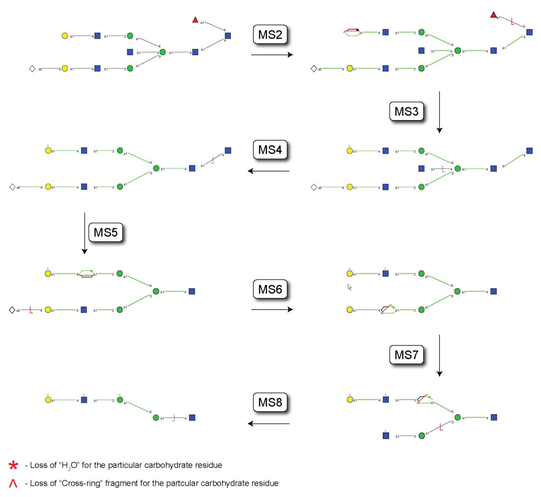


Table 3 shows two other glycan structural isomers (labeled as 5 and 9) identified by SimGlycan software using the approach described above. In addition to differentiating structural isomers, MS^n can be used to confidently elucidate correct glycan structure when insufficient fragmentation is generated at the MS^2 level. For example, the peak at *m/z* 1422.92(+2) represents a single glycan structure. However, the MS^2 spectrum does not provide enough information to clearly elucidate the correct structure. Submission of MS^2 data to SimGlycan software results in an incorrect structure being ranked first due to the absence of key fragment ions. The correct structure of this precursor is shown in Table 3 (labeled as 19). Figure 5 highlights the MS^n sequential fragmentation pathway required for this glycan identification and the use of SimGlycan software to interpret the MS^n spectra.

FIGURE 5. Ion trap MS^n fragmentation pathway for precursor at *m/z* 1422.92 (+2).



Conclusion

- The combination of permethylation, MS^n and SimGlycan software enabled successful identification and differentiation of various structural isomers of chicken ovalbumin released glycans.
- The overall analysis time was reduced to a matter of minutes, thus enabling truly automated, high-throughput data analysis.
- SimGlycan software simplifies data analysis by providing comprehensive support for performing MS^n experiments on Thermo Scientific ion traps and ion trap-Orbitrap™ hybrid mass spectrometers.

References

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3. Ashline, D. J. *et al.,* Software-assisted Peak Annotation and Isomer Detection for Oligosaccharide Mass Spectra: A Case Study. ASMS 2010 poster, ThP13.
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