Overview

Purpose: To demonstrate the use of SimGlycan software for automated structural isomer differentiation of permethylated glycan samples. Methods: Step-by-step MSn spectra of permethylated chicken ovalbumin glycans were obtained and analyzed using SimGlycan software. Results: The combination of permethylation and SimGlycan software enabled automated differentiation of the various structural isomers of chicken ovalbumin glycans.

Introduction

Mass spectrometry has emerged as a powerful tool for the structural elucidation of glycans. The use of permethylation in combination with multistage fragmentation mass spectrometry (MSn) is a critical step in glycan structural characterization. Because it enables the characterization of complex glycan structures, MSn is often the method of choice for the identification of branching patterns, linkages and the resolution of isobaric structures. Here we present the use of SimGlycan software, a bioinformatics tool, for the structural elucidation of complex permethylated glycan structures.

Methods

Sample Preparation

Ovalbumin (10 mg) was reduced, alkylated and digested overnight with trypsin (1:20 w/w) in 0.1% formic acid. The sample was incubated at 37°C for 16 hours, then digested with trypsin (1:20 w/w) in 0.1% formic acid. The sample was washed three times using a Sep-Pak C18 cartridge (Waters). The Sep-Pak C18 cartridge was washed with 10 mL of acetonitrile, followed by 10 mL of 0.1% formic acid. The sample was then washed with 10 mL of ultrapure water, followed by 10 mL of methanol. Finally, the sample was washed with 10 mL of acetonitrile, and the glycan sample was dried under nitrogen gas.

Results

A temperature of 80°C with a ramp of 1°C per minute was used to vaporize the permethylated glycans. The MALDI-TOF mass spectrometer was used to acquire the mass spectra. The spectra were then analyzed using SimGlycan software. MS2 spectra were acquired for the major glycan peaks. The MS2 spectra were then analyzed using SimGlycan software. MSn spectra were acquired for the major glycan peaks. The MSn spectra were then analyzed using SimGlycan software.

Conclusion

The combination of permethylation and SimGlycan software enabled automated structural isomer differentiation of the various structural isomers of chicken ovalbumin glycans. The overall analysis time was reduced to a matter of minutes. SimGlycan software is a powerful tool for the structural elucidation of complex permethylated glycan structures.

References

4. SimGlycan is a trademark of PREMIER Biosoft International. Sep-Pak is a registered trademark of Waters Corporation.

Automated Glycan Structural Isomer Differentiation Using A Bioinformatics Tool

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Table 1. Mass Spectrometer Settings

<table>
<thead>
<tr>
<th>Source</th>
<th>Capillary Temperature</th>
<th>Rep Pen</th>
<th>Source Voltage [V]</th>
<th>Full Mass Range</th>
<th>Scan Rate</th>
<th>Mass Spectrometer Type</th>
<th>Target Value Full MS</th>
<th>Target Value MS/MS</th>
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<tbody>
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<td>220°C</td>
<td>2</td>
<td>1000</td>
<td>1000-2000</td>
<td>1000</td>
<td>LTQ</td>
<td>3e4</td>
<td>1e4</td>
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Table 2. SimGlycan Software 2D Chromatography

<table>
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<tr>
<th>Ice Make</th>
<th>Pressure/ anxiety</th>
<th>Temperature</th>
<th>Cutoff</th>
<th>Glucose</th>
<th>Man</th>
<th>Gal</th>
<th>1D Chromatography</th>
<th>2D Chromatography</th>
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<td>1.0</td>
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<td>1.0</td>
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</table>

Table 3. Structures of chicken ovalbumin-derived glycans identified in this study (structures drawn using GlycoWorkbench).

<table>
<thead>
<tr>
<th>Structure</th>
<th>Data Source</th>
<th>SimGlycan</th>
<th>Figure 5b</th>
<th>Figure 5d</th>
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<tr>
<td>(Gal)(Man)4(GlcNAc)4</td>
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<td>19</td>
<td>(a)</td>
<td>(c)</td>
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<tr>
<td>(Gal)(Man)4(GlcNAc)3</td>
<td>MS3</td>
<td>5</td>
<td>(b)</td>
<td>(d)</td>
</tr>
<tr>
<td>(Gal)(Man)4(GlcNAc)2</td>
<td>MS4</td>
<td>9</td>
<td>(a)</td>
<td>(d)</td>
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</table>

Figure 5. (a) 2D top full mass range spectra of permethylated chicken ovalbumin glycans. (b) MS2 spectrum of the major glycan at m/z 1054.68 (+2) with the predicted structures shown. (c) MS3 spectrum of the major glycan at m/z 1054.68 (+2) with the predicted structures shown. (d) MS4 spectrum of the major glycan at m/z 1054.68 (+2) with the predicted structures shown.

For example, we selected the most significant glycan structures from the list and performed MS2 and MSn fragmentations to confirm the proposed structures. In fact, when the MS2 data is submitted to SimGlycan, the software correctly identifies the correct glycan structure. In this case, the MS2 data is submitted to SimGlycan and the software correctly identifies the correct glycan structure.

Conclusion

The combination of permethylation and SimGlycan software enabled automated structural isomer differentiation of the various structural isomers of chicken ovalbumin glycans. The overall analysis time was reduced to a matter of minutes. SimGlycan software is a powerful tool for the structural elucidation of complex permethylated glycan structures.