Glycan Structural Elucidation On A Novel Quadrupole Dual Cell Linear Ion Trap Orbitrap Hybrid Mass Spectrometer

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Overview

Purpose: To demonstrate the use of HCD MS³ for glycan structural elucidation on a novel hybrid mass spectrometer, based on mass resolving quadrupole, Thermo Scientific™ Orbitrap™ analyzer, collision cell, linear ion trap architecture.

Methods: HCD MS² and MS³ spectra of permethylated chicken ovalbumin glycans were acquired on a Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer. Structural elucidation was performed using SimGlycan® software.

Results: The combination of permethylation, HCD MS³ and SimGlycan software enabled successful identification and differentiation of structural isomers of chicken-ovalbumin-released glycans.

Introduction

Glycans in glycoproteins are involved in a wide-range of biological and physiological processes including recognition and regulatory functions, cellular communication, gene expression, cellular immunity, growth and development. Mass spectrometry (MS) has emerged as a powerful tool for glycan structural elucidation. The use of permethylation in combination with multistage fragmentation (MSⁿ) is critical to the success of this approach. Only analysis by MSⁿ truly characterizes glycans as it allows identification of heterogeneity, branching, linkages and resolution of isobaric structures which are otherwise indistinguishable in MS² spectra. Traditionally, MSⁿ has been restricted to low-energy, collisional-induced dissociation (CID) in linear ion trap mass spectrometers, thereby, requiring multiple stages of fragmentation (MS³, MS⁴….) for structural elucidation. Here we demonstrate for the first time the use of higher-energy collisional dissociation (HCD) MSⁿ for glycan structural elucidation on a novel instrument, the Orbitrap Fusion™ Tribrid™ mass spectrometer based on a mass resolving quadrupole, Orbitrap analyzer, collision cell, linear ion trap (Q-OT-qIT) architecture (Figure 1). The primary advantage of HCD fragmentation is the production of glycosidic, cross-ring, and internal double cleavage ions at the MS² level, where branching, linkage and resolution of isobaric structures are derived from the latter two types of ions. The availability of HCD MSⁿ enables comprehensive glycan structural elucidation at much lower MS³ stages.

Methods

Sample Preparation

Ovalbumin (1 mg, Sigma) was reduced, alkylated and digested overnight with trypsin in of 25 mM ammonium bicarbonate buffer (pH=8) at 37 °C. PNGase F solution (3 µL, Roche) was added to 200 µL of digested sample and the mixture was incubated for another 16 hours at 37 °C. The released glycans were separated from the peptides using a Sep-Pak® C18 cartridge (Waters). The Sep-Pak C18 was conditioned by washing with acetonitrile, followed by water. PNGaseF digested sample was loaded onto the cartridge and the released glycans were eluted with 1% ethanol while the peptides remained bound to the Sep-Pak C18. The released ovalbumin oligosaccharides were first purified using a porous graphite carbon column (PhyNexus) and then permethylated using an in-house protocol.

Mass Spectrometry

All MS experiments were performed using an Orbitrap Fusion Tridend mass spectrometer via direct infusion into the nano-electrospray source. The mass spectrometer settings and SimGlycan software [1,2] version 4.50 (PREMIER Biosoft International) search parameters are listed in Tables 1 and 2.

Table 1. Mass Spectrometer Settings

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Introduction

The combination of permethylation, HCD MS3 and SimGlycan software enabled successful identification and differentiation of structural isomers of chicken ovalbumin released glycans.

Results

HCD fragmentation provides much more informative spectra for glycan analysis compared to low-energy CID fragmentation available on linear ion trap mass spectrometers. (Figure 2). Targeting glycans with HCD fragmentation results in the production of cross-ring fragment ions and internal double-cleavage ions which, even with permethylation, can be lacking with low energy CID fragmentation. The ability to generate these types of ions at the MS2 level provides sufficient information in a lot of cases to successfully elucidate glycan structures.

Though HCD can produce much more informative fragmentation at the MS2 level, differentiating structural isomers can be an issue as fragments from mixed spectrum can complicate spectral assignment. MS3 would still be required for differentiation of these isomers. Currently the acquisition of HCD fragmentation is still limited to MS2 on commercial mass spectrometers. However, we have recently built a new quadrupole, dual cell linear ion trap, Orbitrap hybrid mass spectrometer that has a novel architecture and ion transfer path that enables HCD MSn analysis.

Structural elucidation using HCD MSn was initially tested on glycans released from chicken ovalbumin. Since the glycan content of ovalbumin has been characterized in depth,[3] it was an ideal system to examine the capabilities of the novel Orbitrap Fusion Tribrid mass spectrometer. Figure 3 shows the MS profile of permethylated glycans derived from ovalbumin acquired on this instrument. Data-dependent MS2 spectra were acquired on all precursors with a charge state greater than two (Z ≥ 2) and for each MS2 spectra subsequent top 20 MS3 spectra were acquired.

The MSn data interpretation workflow is as follows. The acquired MS2 data is brought into SimGlycan software for automatic compositional identification. Based on the criteria selected (Table 2), SimGlycan software searches its database for glycan matches. For example the MS2 spectrum (Figure 4) for the glycan at m/z 1046.511 (Figure 2) is brought into SimGlycan for identification. Based on the MS2 fragmentation pattern SimGlycan interprets the spectrum as most likely being a hybrid glycan with a bisecting GlcNAc, as this is the glycan ranked as the top structure in SimGlycan search result (Figure 5).

Table 2. SimGlycan Software 4.50 Settings

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<td>Reducing Terminal</td>
<td>Free</td>
<td>Glycan Type</td>
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</table>

FIGURE 1. Schematic representation of the novel Orbitrap Fusion Tribrid mass spectrometer architecture.

FIGURE 2. Comparison of CID MS2 vs HCD MS2 spectrum of glycan at m/z 1046.511. An additional hybrid glycan with the bisecting GlcNAc (ranked 3) was also identified. We indeed the hybrid glycan with a bisecting GlcNAc is present at rank 1 and not from other structures (Figure 6).

FIGURE 3. FT full scan mass spectrum of permethylated ovalbumin released glycan at m/z 1046.511. An additional hybrid glycan with the bisecting GlcNAc (ranked 3) was also identified. We indeed the hybrid glycan with a bisecting GlcNAc is present at rank 1 and not from other structures (Figure 6).

FIGURE 4. HCD MS2 spectrum of the peak at m/z 1046.511. A pattern SimGlycan interprets the spectrum as most likely being a hybrid glycan with a bisecting GlcNAc is present at rank 1 and not from other structures (Figure 6).

FIGURE 5. SimGlycan software search results for the HCD MS2 spectrum of the peak at m/z 1046.511. An additional hybrid glycan with the bisecting GlcNAc (ranked 3) was also identified. We indeed the hybrid glycan with a bisecting GlcNAc is present at rank 1 and not from other structures (Figure 6).
Glycan Structural Elucidation On A Novel Quadrupole Dual Cell Linear Ion Trap Orbitrap Hybrid Mass Spectrometer

**Introduction**

Purpose:

Overview

**Methods:**

Collisional dissociation (HCD) MSn for glycan structural elucidation on a commercial mass spectrometers. However, we have recently built a new quadrupole, and then permethylated using an in-house protocol.

Peptides remained bound to the Sep-Pak C18. The released ovalbumin another 16 hours at 37 ºC. The released glycans were separated from the peptides and then permethylated using an in-house protocol.

**Results:**

Ovalbumin-released glycans enabled successful identification and differentiation of structural isomers of chicken ovalbumin. Since the glycan content of ovalbumin has been characterized in depth,[3] it was an ideal system to examine the capabilities of the novel Orbitrap Pro™ mass spectrometer using CID MSn.[1] In order to differentiate the structural isomers, cellular communication, gene expression, cellular immunity, growth and development. Mass spectrometry (MS) has emerged as a powerful tool for glycan structural elucidation. The use of permethylation to generate these types of ions at the MS2 level provides sufficient information in a lot of cases to successfully elucidate glycan structures.

Though HCD can produce much more informative fragmentation at the MS2 level, HCD fragmentation provides much more informative spectra for glycan analysis. Of glycosidic, cross-ring, and internal double cleavage ions at the MS2 level, where architecture (Figure 1). The primary advantage of HCD fragmentation is the production of cross-ring fragment ions and internal double-cleavage ions which, even though HCD can produce much more informative fragmentation at the MS2 level, even though HCD can produce much more informative fragmentation at the MS2 level, even though HCD can produce much more informative fragmentation at the MS2 level, even though HCD can produce much more informative fragmentation at the MS2 level, even though HCD can produce much more informative fragmentation at the MS2 level, even though HCD can produce much more informative fragmentation at the MS2 level, even though HCD can produce much more informative fragmentation at the MS2 level, even though HCD can produce much more informative fragmentation at the MS2 level, even though HCD can produce much more informative fragmentation at the MS2 level, even though HCD can produce much more informative fragmentation at the MS2 level, even though HCD can produce much more informative 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Glycans in glycoproteins are involved in a wide range of biological and physiological expression, cellular immunity, growth, and development. Mass spectrometry (MS) has emerged as a powerful tool for glycan structural elucidation. The use of permethylation enabled successful identification and differentiation of structural isomers of chicken-egg ovalbumin acquired on a Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer, thereby requiring multiple stages of fragmentation (MS6, MS7) for glycan structural elucidation on a novel hybrid mass spectrometer, based on mass resolving quadrupole, Orbitrap analyzer, collision cell, linear ion trap (Q-OT-qIT) architecture.

Methods

In combination with multistage fragmentation (MSn) is critical to the success of this approach. Only analysis by MSn truly characterizes glycans as it allows identification of heterogeneity, branching, linkages and resolution of isobaric structures. Though HCD can produce much more informative fragmentation at the MS2 level, the acquisition of HCD fragmentation is still limited to MS2 on international search parameters are listed in Tables 1 and 2. HCD fragmentation provides much more informative spectra for glycan analysis compared to collisional dissociation (CID) MSn for glycan structural elucidation on a Thermo Scientific™ Orbitrap™ analyzer, collision cell, linear ion trap architecture. SimGlycan is a trademark of PREMIER Biosoft International. Sep-Pak is a trademark of Waters Corporation. All mass spectrometric data using SimGlycan are a result of searching the Intelligent Mass Spectrometry (IMS) database using the software.

Methods:

Structural elucidation was performed using SimGlycan® software. The availability of HCD MSn enables comprehensive glycan structural elucidation at much lower MSn stages. Further examination of the glycan result reported by SimGlycan software for the MS2 spectrum shows additional glycan compositions that possess the same mass but are ranked much lower. In order to ensure that we characterized all possible structural isomers, we incorporated MS3 spectra in our data analysis. Figure 6 shows that indeed the hybrid glycan with a bisecting GlcNAc is present at m/z 1046.511, as the combination of MS2 and MS3 spectra show fragmentation specific to the hybrid glycan with a bisecting GlcNAc (rank 1) and not from other structures (Figure 6).
It should be pointed out that the HCD MS² and MS³ spectra generated are information rich (Figure 6), containing informative glycosidic and cross-ring fragment ions. An additional advantage of HCD fragmentation is that the ions are measured within the Orbitrap mass analyzer with high-resolution, accurate-mass (HR/AM). This allows for differentiation of near mass fragment ions, which we observed to be very useful for correctly assigning branching and linkage ions.

Though we were able to confidentially identify the hybrid glycan with a bisecting GlcNAc (rank 1), there were additional structural isomers present. Closer examination of the MS³ spectrum for the ion at m/z 1046.511 reveals numerous unidentified peaks that we could not attribute to the top ranked glycan. The acquisition of MS³ spectra on these peaks enabled identification of additional isomers. For example, the fragment ion observed at m/z 1606.789 in the MS² spectrum (Figure 4) indicates the loss of Gal-GlcNAc, which is most likely to occur from the non-reducing end of either asialyl digalactosyl biantennary glycan (ranked 4) or the other hybrid glycan with bisecting GlcNAc (ranked 3). The acquisition of MS³ spectrum for the ion at m/z 1606.789 provided additional fragment ions unique to the asialyl digalactosyl biantennary glycan that confirmed the presence of this structure at m/z 1046.511.

Overall, we were able to identify three structural isomers at m/z 1046.511. An additional hybrid glycan with the bisecting GlcNAc (ranked 3) was also identified. We had previously characterized these structural isomers on a Thermo Scientific ™ Velos Pro™ mass spectrometer using CID MSn. [1] In order to differentiate the structural isomers, it required MS⁷, MS⁸ stages of fragmentation with CID MS⁰.

**Conclusion**

- The novel Orbitrap Fusion Tribrid mass spectrometer based on mass resolving quadrupole, Orbitrap, collision cell, linear ion trap (Q-OT-qIT) architecture enables HCD MS⁰.
- Additional glycan fragmentation pathways are accessible with this mass spectrometer as it has the ability to use any fragmentation mode, at any stage of MS⁰ analysis.
- The combination of permethylation, HCD MS³ and SimGlycan software enabled successful identification and differentiation of structural isomers of chicken ovalbumin released glycans.

**References**
