

A Novel Approach for Identification and Quantitation of Protein Glycosylation Pattern by Precursor Ion Scan and H-SRM

on Triple Quadrupole Mass Spectrometer

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Overview

Purpose: Develop a high throughput method to characterize and quantify the glycopeptide distribution patterns from different samples by using precursor ion scan, data dependent full MS/MS scan and H-SRM assay on a triple quadrupole mass spectrometer.

Methods: A recombinant glycoprotein from two different manufacturing processes was reduced, alkylated and enzymatically digested, respectively. The digest was then analyzed on a triple quadrupole mass spectrometer (Thermo Scientific TSQ Vantage) equipped with a nanoLC pump (nanoLC 1D plus, Eksigent) and a dynamic nanospray source (Thermo Scientific). The instrument was operated in the precursor ion scan mode (m/z 500-1500) first for detecting all possible glycosylated precursor ions by using the diagnostic sugar oxonium ions of m/z 204, 274, 292, and 366. Then a full data dependent MS/MS scan was used to trigger all detected possible glycopeptide precursor ions for confirming the identity of glycopeptides. For the identified glycopeptide precursor ions, the H-SRM method was further employed to quantitate these glycopeptide precursor ions by monitoring m/z 204, 274, 292, 366, and specific fragmented glycan ions from each particular peptide. Each sample was analyzed in triplicate using the same H-SRM assay to assess the reproducibility and %CV.

Results: The mixture of glycopeptides and nonglycosylated peptides of a recombinant glycoprotein from two different manufacturing processes (TRCM-I & TRCM-G) were analyzed. Without performing any enrichment for the glycopeptides, the mixture was analyzed directly and more than 30 major glycopeptides were identified and quantitated using above method. Over 60% glycopeptides gave RSD below 10% with the total RSD below 20%. Two major peptide glycosylation patterns were observed at two different chromatographic retention times, which represented glycosylated variants in two different peptides. The subtle difference of glycosylation, e.g. the sialylated and fucosylated glycopeptides, between these two manufacturing processes was revealed.

Introduction

The qualitative and quantitative characterization of glycopeptides remains one of the major challenges for mass spectrometry due to the carbohydrate's complex heterogeneity. Although the precursor ion scanning of the diagnostic sugar oxonium ions are capable to detect all glycopeptides, the resulted spectra are very complex with poor quantitative information (1, 2). In this study, we present a novel strategy to use combination of precursor ion scan and data dependent full MS/MS scan for detecting all possible glycopeptides first, then use high resolution selective reaction monitoring (H-SRM) assay for high throughput quantitation of detected glycopeptides on a triple quadrupole mass spectrometer. With this novel approach, the protein glycosylation patterns of a recombinant protein from two different manufacturing processes were established and compared. The subtle difference of glycosylation between the two processes was revealed.

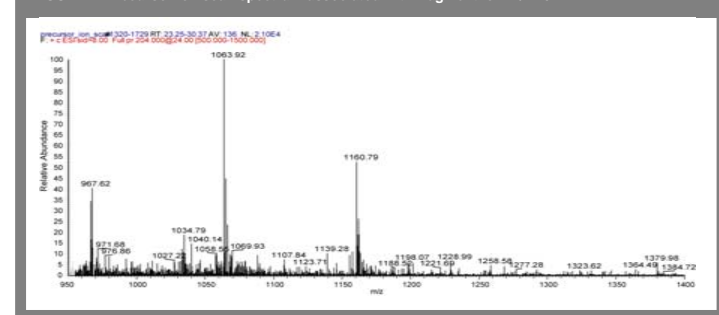
Methods

Sample Preparation: Glycoprotein solution (1 mg/mL) was reduced with 5 mM dithiothreitol (DTT) and alkylated with 20 mM of iodoacetamide (IAA). The reduced and alkylated protein was digested by trypsin (1:100 w/w) at room temperature for 8 hr and then added a second time (1:100 w/w) for 12 hr again at room temperature.

Nano – HPLC

Pump: nanoLC 1D plus, Eksigent
Column: C18 column (75 μ m x100 mm, New Objective); flow rate: 400 nL/min; Injection amount: 1 μ L
Buffer A: 0.1% FA; Buffer B: 0.1% FA/100% AcN; Gradient: 2% B to 40% B in 40 min.

FIGURE 1. Precursor ion scan spectrum associated with fragment ion m/z 204



MS

TSQ Vantage™ triple quadrupole equipped with a nanospray source
Capillary Temperature: 200 °C; Spray voltage: 2000 V

Precursor ion scans (fragment ions of m/z 204, 292 & 366)

Mass range, m/z 500 -1500, scan time, 1.00 s
Q1 – 0.7 FWHM Da; Q3 – 0.7 FWHM Da; Q2 – 1.5 mTorr; CE – 24 V

Data dependent MS/MS:

Scan event 1: Full MS (m/z 950 – 1400)
Scan event 2: DD precursor mass from scan event 1; signal threshold 50000 counts;
Q1 – 0.7 FWHM Da; Q3 – 0.7 FWHM Da; Q2 – 1.5 mTorr;
CE, 0.034 x precursor mass m/z ; scan time, 1.0 s; Dynamic exclusion, repeat 2,
Exclusion time, 2 min.

H-SRM

Q1 – 0.4 FWHM Da; Q3 – 0.7 FWHM Da; Q2 – 1.5 mTorr;
Time-based SRM (duty cycle time: 2.0 s); CE – 0.034 x precursor mass m/z

Results

Detecting all possible glycopeptides by using precursor ion and data dependent MS/MS scan

In order to detect all possible glycosylated precursor ions from the sample, a precursor ion scan mode (m/z 500-1500) to monitoring diagnostic sugar oxonium ions of m/z 204, 274, 292, and 366 was used. Figure 1 shows the averaged precursor ion spectrum of monitoring m/z 204. Except for abundant precursor ions of m/z 967.62, 1063.92 & 1160.79, many trace precursor ions, such as m/z 971.68, 1107.84, 1139.28 and 1379.98 were detected. All these detected precursor ions were output into the inclusion list of a data dependent MS/MS scan mode for characterizing possible glycopeptides. Figure 2 shows the extracted ion chromatograms of fragment ions of m/z 204, 274, 292 and 366. Two glycopeptide groups were identified clearly around retention time of 9.3 min and 21.8 min. The averaged full MS spectrum of each group was shown in the insert (a) and insert (b), respectively. The charge state of each precursor ion was further confirmed by using a high-resolution Thermo Scientific LTQ Orbitrap hybrid mass spectrometer with separate experiments (data not published). As shown from the Figure 2, although high-resolution and accurate-mass measurements are not necessary for quantitation, it is very useful for structure determination (results published separately).

The Group1 is more abundant and has more glycosylated variants compared to Group 2. Each variant's MS/MS spectrum from the same glycopeptide group gave specific fragment ions except for glycan ions of m/z 204, 274, 292 and 366 (Figure 3), showing that these glycosylated variants are related each other.

FIGURE 2. Selected fragment ion chromatograms from the data dependent MS/MS run. Insert (a) & (b): Averaged MS spectra of glycopeptide Group 1 & 2. High-resolution precursor charge state envelope insert for each glycopeptide group was acquired on an LTQ Orbitrap instrument.

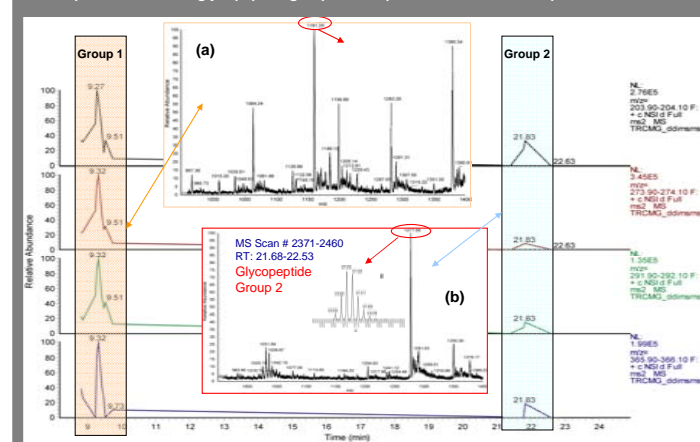
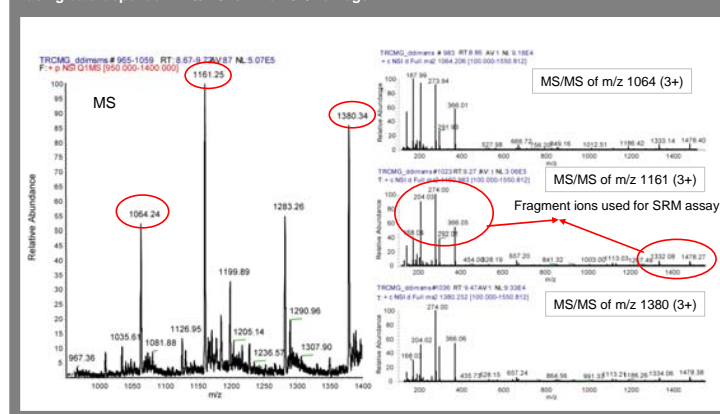


FIGURE 3. Average MS spectrum of glycopeptide group 1 and representative MS/MS spectra acquired using data-dependent MS/MS on the TSQ Vantage



Quantitative results using H-SRM assay

Forty glycopeptides were detected using a combination of precursor ion scan and data dependent MS/MS scan and targeted for quantitation using H-SRM assay. The glycan ions and specific fragment ions from each glycopeptide were used for the SRM assay design as shown in Figure 3.

Both samples from TRCM-I and TRCM-G were run in triplicate by using the same H-SRM assay. Table 1 summarize the analytical results. Over 60% glycopeptides gave %CV below 10% with the total % CV below 20%. The normalized glycopeptide distribution patterns for the two samples were shown in figure 3 and figure 4, respectively. Although both samples show similar patterns, the subtle differences of the glycopeptide distribution patterns between the two samples were identified for both groups, revealing that they do have exactly the same glycosylation. This information will help to improve the manufacture processes.

Table 1. The quantitative results of the glycoprotein from the two manufacturing processes

TRCM - I										TRCM - G													
precursor ion	RT	Area(n=1)	Area(n=2)	Area(n=3)	Mean Area	% CV	precursor ion	RT	Area(n=1)	Area(n=2)	Area(n=3)	Mean Area	% CV	precursor ion	RT	Area(n=1)	Area(n=2)	Area(n=3)	Mean Area	% CV			
glycopeptide group 1																							
1	967.62	8.76	88845	90953	91231	98233.0	4.0	1	967.62	9.04	88774	88852	92890	93800.7	6.3	2	1186.13	8.80	147405	139740	144508	150001.0	8.4
2	1186.13	8.80	150084	147373	137587	145138.0	4.4	2	1186.13	9.02	147405	139740	144508	150001.0	8.2	3	1034.84	10.02	171838	158391	190134	173404.3	9.2
3	1034.84	10.02	92239	97229	91242	93750.0	3.4	4	1064.14	9.2	502914	514229	505773	506206.3	4.3	5	1071.59	9.94	27968	32963	28027	27748.7	12.7
4	1064.14	9.2	501987	512500	493238	505971.7	9.7	6	1084.92	8.9	39544	29303	22992	32962.0	16.2	7	1131.82	9.7	32952	34844	34332	33776.0	4.6
5	1071.59	9.94	27968	32963	24071	28000.7	10.5	8	1148.42	8.89	42088	34858	30890	39804.7	15.9	9	1184.13	9.14	150564	147373	137587	137940.2	2.6
6	1084.92	8.9	39544	31128	28254	27778.7	10.4	10	1199.84	10.28	442328	466864	433168.0	446.8	11	1213.06	9.96	85408	81708	78092	80782.0	4.9	
7	1131.82	9.7	32952	34844	34332	33776.0	4.6	12	1259.78	9.1	2949	4464	9238	4500.7	14.3	13	1267.34	8.74	20789	23221	27896	23928.7	14.9
8	1148.42	8.89	42088	34858	30890	39804.7	15.9	14	1306.88	9.11	28868	33168	29248	32766.3	10.2	15	1362.59	9.11	28868	33168	29248	32766.3	10.2
9	1184.13	9.14	150564	147373	137587	144128.0	4.6	16	1427.98	9.35	355400	347892	305020	350730.3	11.2	17	1427.98	9.35	355400	347892	305020	350730.3	11.2
10	1199.84	10.28	442328	466864	433168.0	446.8	18	1501.41	9.49	14684	11120	14114	13228.3	13.9	19	1501.41	9.49	14684	11120	14114	13228.3	13.9	
11	1213.06	9.96	85408	81708	78092	80782.0	4.9	20	1526.88	9.86	254798	261277	269233	260666.0	2.1	21	1526.88	9.86	254798	261277	269233	260666.0	2.1
12	1259.78	9.1	2949	4464	9238	4500.7	14.3	22	1526.88	9.86	254798	261277	269233	260666.0	2.1	23	1526.88	9.86	254798	261277	269233	260666.0	2.1
13	1267.34	8.74	20789	23221	27896	23928.7	14.9	24	1526.88	9.86	254798	261277	269233	260666.0	2.1	25	1526.88	9.86	254798	261277	269233	260666.0	2.1
14	1306.88	9.11	28868	33168	29248	32766.3	10.2	26	1526.88	9.86	254798	261277	269233	260666.0	2.1	27	1526.88	9.86	254798	261277	269233	260666.0	2.1
15	1362.59	9.11	28868	33168	29248	32766.3	10.2	28	1526.88	9.86	254798	261277	269233	260666.0	2.1	29	1526.88	9.86	254798	261277	269233	260666.0	2.1
16	1427.98	9.35	355400	347892	305020	350730.3	11.2	30	1526.88	9.86	254798	261277	269233	260666.0	2.1	31	1526.88	9.86	254798	261277	269233	260666.0	2.1
17	1427.98	9.35	355400	347892	305020	350730.3	11.2	32	1526.88	9.86	254798	261277	269233	260666.0	2.1	33	1526.88	9.86	254798	261277	269233	260666.0	2.1
18	1501.41	9.49	14684	11120	14114	13228.3	13.9	34	1526.88	9.86	254798	261277	269233	260666.0	2.1	35	1526.88	9.86	254798	261277	269233	260666.0	2.1
19	1501.41	9.49	14684	11120	14114	13228.3	13.9	36	1526.88	9.86	254798	261277	269233	260666.0	2.1	37	1526.88	9.86	254798	261277	269233	260666.0	2.1
20	1526.88	9.86	254798	261277	269233	260666.0	2.1	38	1526.88	9.86	254798	261277	269233	260666.0	2.1	39	1526.88	9.86	254798	261277	269233	260666.0	2.1
21	1526.88	9.86	254798	261277	269233	260666.0	2.1	40	1526.88	9.86	254798	261277	269233	260666.0	2.1	41	1526.88	9.86	254798	261277	269233	260666.0	2.1
22	1526.88	9.86	254798	261277	269233	260666.0	2.1	42	1526.88	9.86	254798	261277	269233	260666.0	2.1	43	1526.88	9.86	254798	261277	269233	260666.0	2.1
23	1526.88	9.86	254798	261277	269233	260666.0	2.1	44	1526.88	9.86	254798	261277	269233	260666.0	2.1	45	1526.88	9.86	254798	261277	269233	260666.0	2.1
24	1526.88	9.86	254798	261277	269233	260666.0	2.1	46	1526.88	9.86	254798	261277	269233	260666.0	2.1	47	1526.88	9.86	254798	261277	269233	260666.0	2.1
25	1526.88	9.86	254798	261277	269233	260666.0	2.1	48	1526.88	9.86	254798	261277	269233	260666.0	2.1	49	1526.88	9.86	254798	261277	269233	260666.0	2.1
26	1526.88	9.86	254798	261277	269233	260666.0	2.1	50	1526.88	9.86	254798	261277	269233	260666.0	2.1	51	1526.88	9.86	254798	261277	269233	260666.0	2.1
27	1526.88	9.86	254798	261277	269233	260666.0	2.1	52	1526.88	9.86	254798	261277	269233	260666.0	2.1	53	1526.88	9.86	254798	261277	269233	260666.0	2.1
28	1526.88	9.86	254798	261277	269233	260666.0	2.1	54	1526.88	9.86	254798	261277	269233	260666.0	2.1	55	1526.88	9.86	254798	261277	269233	260666.0	2.1
29	1526.88	9.86	254798	261277	269233	260666.0	2.1	56	1526.88	9.86	254798	261277	269233	260666.0	2.1	57	1526.88	9.86	254798	261277	269233	260666.0	2.1
30	1526.88	9.86	254798	261277	269233	260666.0	2.1	58	1526.88	9.86	254798												