Characterization of IgG Glycosylation Using Intact Protein Analysis and Peptide Mapping.

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Introduction

Glycosylation is a cotranslational and a posttranslational modification found in proteins. N-linked glycosylation is found in the Fc region of immunoglobulins. The covalently bound oligosaccharides vary in composition and branching within different classes of immunoglobulins. Glycosylation is vital for bioactivity and pharmacokinetics of biotherapeutic proteins. We characterized glycosylation in monoclonal IgG by "top down" and "bottom up" approaches using intact protein mass analysis and peptide mapping. The intact protein analysis was carried out using SEC-MS and RP-MS. Global mass analysis of glycosylated, partially glycosylated and deglycosylated IgG revealed that the sugar moiety has mass of approximately 1468 Daltons corresponding to the asialo-biantennary N-linked oligosaccharide with core fucose. Signature ion scan for mass 204 in the peptide map of IgG revealed 3 distinct glycol-peptide peaks. Based on the mass of these resolved peaks we were able to confirm that they were composed of different glycoforms, confirming the heterogeneity observed at the intact protein analysis. The MS/MS analysis of the G0 glycopeptide was also obtained and the composition of the oligosaccharides confirmed by daughter ion assignments.

System Components

Waters® BioSuite™ Intact Protein System

- Waters® 2796 Separations module
- Waters® 2487 Dual Wavelength Absorbance Detector
- Waters® Q-Tof-2™

Waters® BioSuite™ peptide mapping System

- Waters® 2796 Separations module.
- Waters® 2487 Dual Wavelength Absorbance Detector
- Waters® Qtof micro ™

Columns:

- Waters® BioSuite™ PA-A C18 3µm (2.1X250 mm)
- Waters® Oasis® HLB 5µM (2.1 mm X 20 mm)
- Waters® BioSuite™ 250, 5 µm HR SEC (7.8 mm X 300mm)
- Protein A affinity column 4.6X50

Experimental

MS Conditions:

-Source = ESI(+)
-Capillary (kV) = 3.3
-Cone (V) = 30
-Temperature (°C)
  -Source = 150
  -Desolvation = 425
-Gas Flow (L/Hr)
  -Cone = 50
  -Desolvation = 500
-Scan Mode
-Collision Energy = 10

HPLC Conditions:

SPE-MS (Figure 2;IIIA,B&C) Poster # P-33-W
SEC-MS (Figure 2;IIA,B&C) Poster # P-28-W
Affinity-MS (Figure 2;1A,B&C) Poster # P-32-W
Peptide mapping (Figure 3) Poster # P-54-Th

IgG deglycosylation:

Deglycosytation was carried out with PNGase F obtained from Sigma St. Louis, Mo using protocol provided by the manufacturer. The incubation times were 2 hrs and 30 min for complete and partial deglycosylation respectively.

Figure 1:Biantennary sugar structure of IgG1

Figure 1 shows the N-linked biantennary carbohydrate structure commonly found in the Fc region of IgG1. A common source of heterogeneity in such a structure is the loss of one or

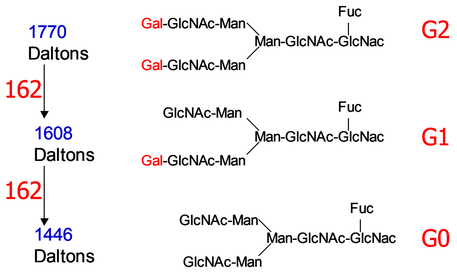
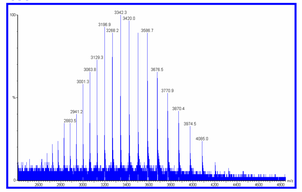


Figure 2: LC-MS analysis of IgG Glycosylation.

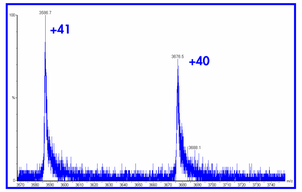
MS analysis of deglycosylated (I) partially deglycosylated (II) and glycosylated (III) IgG 1 is shown in Figure 2. The charge envelope of deglycosylated IgG1 is shown in I A while I B shows charge isoforms with charge state of + 41 and + 40. the deconvoluted spectrum of the charge envelope in 1C shows a major peak at 147018 Daltons corresponding to the molecular weight of IgG1. A minor peak can also be seen which corresponding in MW to IgG 1 with an additional lysine which is known to be present at the "C" terminus of the heavy chain. The spectrum for partially deglycosylated IgG is shown in IIA in. At least three distinct charge envelopes can be seen in the spectrum which is even more apparent in II B where two additional cluster of peaks can be observed in addition to the +40 and + 41 charge of deglycosylated IgG. The heterogeneity can also be seen in the deconvoluted spectrum shown in II C where peaks for deglycosylated IgG1 , partially deglycosylated IgG 1 (deglycosylation on only one heavy chain) and glycosylated IgG1 are observed. The charge envelope for glycosylated IgG1 is shown in III A although only single envelope is observed for glycosylated IgG 1 It is apparent from II B that each charge species is composed of several isoforms which corresponds to glycoform heterogeneity that is also represented in the deconvoluted spectrum in III C. This data was obtained with the Q-Tof-2™ instrument.

Deglycosylated IgG

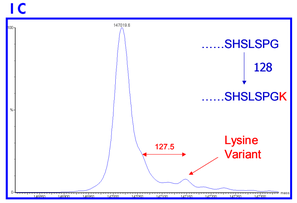
I A



I B

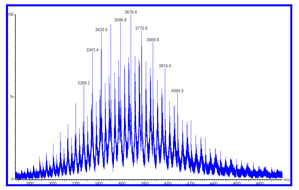


I C

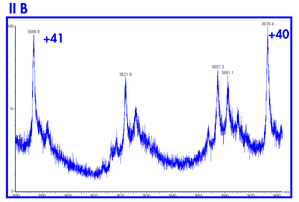


Partially Deglycosylated IgG

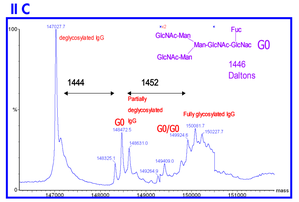
II A



II B

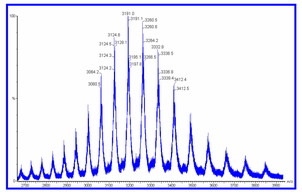


II C

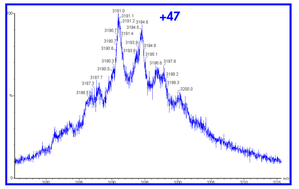


Glycosylated IgG

III A



III B



III C

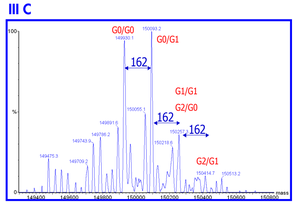
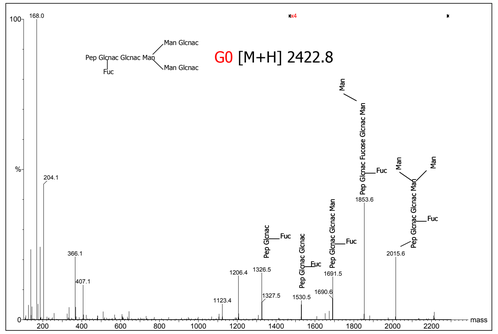


Figure 3: MS/MS analysis of G0 glycopeptide.

The MS/MS analysis of G0 glycopeptide is shown in Figure 3. The biantennary carbohydrate structure was confirmed by MS/MS analysis Several daughter ions corresponding to different sugar structures were observed. This data was obtained with the QTof micro™ instrument.



Conclusions

- Intact protein analysis of deglycosylated, partially deglycosylated and glycosylated IgG1 confirmed that the carbohydrate structures present in IgG1 have the same compositions the as previously published biantenary structure
- MS/MS analysis of G0 glycopeptide confirms the sequence of the sugar moiety.
- Intact protein analysis and MS/MS analysis are useful techniques for the characterization of carbohydrate structure.

References

Robert L. Shields , Jadine Lai , Rodney Keck, Lori Y. O'Connell , Kyu Hong, Y. Gloria Meng, Stefanie H. A. Weikert , and Leonard G. Presta J. Biol. Chem., Vol. 277, Issue 30, 26733-26740, July 26, 2002.