Introduction

Glycosylation is a cotranslational and a posttranslational modification found in proteins. Monoclonal antibody (mAb) is a class of immunoglobulin. The antibody coated oligosaccharides vary in composition and branching within different classes of immunoglobulins. Glycosylation is vital for bioactivity and pharmacokinetics of therapeutic 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 deglycosylated and deglycosylated IgG revealed that the sugar moiety has mass of approximately 1468 Daltons corresponding to the asparagine (N-linked) linked oligosaccharide with core fucose. Signature ion scan for mass 204 m/z in the peptide map of IgG revealed 3 distinct glycolipidic 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 GO glycosylated IgG was also obtained and the composition of the oligosaccharides confirmed by daughter ion assignments.

Experimental

MS Conditions
- Source: ESI(+)
- Capillary (kV) = 3.3
- Cone (V) = 30
- Temperature (C) = 150
- Desolvation = -425
- Gas Flow (L/Hr) = 80
- Collision Energy = 10

HPLC Conditions
- SPE-MS (Figure 2;IIA,B,C) Poster # P-33-W
- SEC-MS (Figure 2;IIA,B,C) Poster # P-33-W
- Affinity-MS (Figure 2;IIA,B,C) Poster # P-33-W

Peptide mapping (Figure 3;III A,B,C,D)Poster # P-34-Th

IgG deglycosylation

Deglycosylation 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: MS analysis of deglycosylated IgG, partially deglycosylated IgG and glycosylated IgG1 is shown in Figure 2. The charge envelope of deglycosylated IgG is shown in II A. The spectrum reveals a peak at 1444 Daltons corresponding to the monomeric weight of IgG. A minor peak also is seen which corresponds to an IgG2 (G2) peak. The deconvoluted spectrum of the charge envelope in II A shows a major peak at 1412 Daltons corresponding to the monomeric weight of IgG1. A minor peak also is seen which corresponds to an IgG2 (G2) peak. The deconvoluted spectrum of the charge envelope in II B shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II C shows a major peak at 147018 Daltons corresponding to the monomeric weight of IgG1. A minor peak is seen which corresponds to an IgG2 (G2) peak. The deconvoluted spectrum of the charge envelope in II D shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II E shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II F shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II G shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II H shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II I shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II J shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II K shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II L shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II M shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II N shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II O shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II P shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II Q shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II R shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II S shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II T shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II U shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II V shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II W shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II X shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II Y shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in II Z shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III I shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III J shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III K shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III L shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III M shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III N shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III O shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III P shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III Q shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III R shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III S shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III T shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III U shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III V shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III W shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III X shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III Y shows charge isoforms with charge state of +41 and +40. the deconvoluted spectrum of the charge envelope in III Z shows charge isoforms with charge state of +41 and +40.

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 biochemical structure.
- MS/MS analysis of GO glycosylated IgG confirms the sequence of the sugar moiety.
- Intact protein analysis and MS/MS analysis are useful techniques for the characterization of carbohydrate structure.

References