

Glycosylation Profiling of Immunoglobulin G (IgG) Subclasses

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Introduction

Human plasma IgG glycosylation is dependent on various parameters, including age, pregnancy, and health status. Various infectious diseases and autoimmune diseases including rheumatoid arthritis, Myasthenia gravis, Lyme disease, and tuberculosis, are associated with a reduction in galactosylation of the single biantennary N-glycan found at the conserved N-glycosylation site of each heavy chain of the four IgG subclasses (Fig. 1). Moreover, IgG with sialylated N-glycan chains seems to have anti-inflammatory effects (Fig. 1). Here we present a method for sub-class specific glycosylation profiling of plasma IgG. Mass spectrometry is performed on a three-dimensional ion trap allowing the detailed structural analysis of IgG-derived glycopeptides.

Methods

Using a 96-well plate sample preparation method, IgG1, IgG2, and IgG4 were captured from plasma by ProtA beads. From the flow-through, IgG3 was captured using ProtG. ProtA-eluate and ProtG-eluate were separately trypsinized and analyzed by nano-LC-MS/MS on a Bruker HCTultra ion trap-MS. Observed glycopeptide species were quantified by integrating all the observed charge states using QuantAnalysis. Samples were analyzed in fourfold.

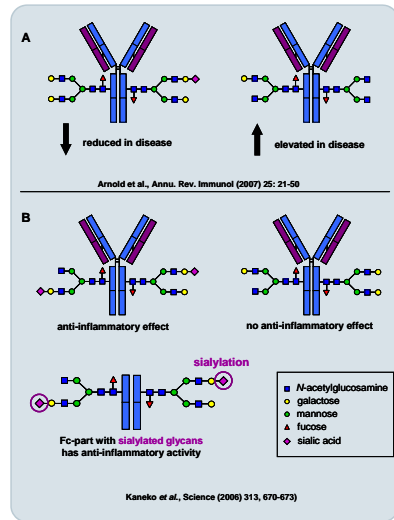


Fig. 1. (A) Glycosylation changes associated with various infectious and autoimmune diseases. (B) Anti-inflammatory properties of IgG has been shown to be mediated by Fc-parts carrying sialylated N-glycans.

Results

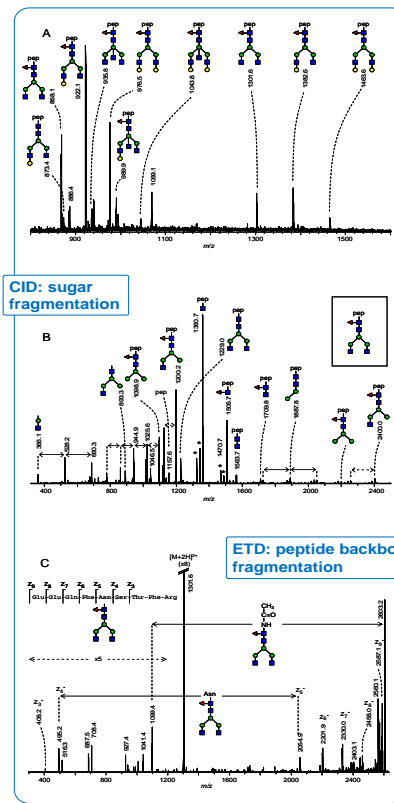


Fig. 2. (A) MS profile of the registered neutral glycoforms of IgG3. (B) Analysis of the glycopeptides at m/z 1301.6 by CID and (C) by ETD with Smart-decomposition™.

Glycosylation of IgG1, IgG2, and IgG4 (captured by ProtA) as well as IgG3 (captured subsequently by ProtG) was analyzed at the glycopeptide level by LC-MS/MS. Tryptic glycopeptides were characterized by MS/MS using collision-induced fragmentation (CID) and electron-transfer dissociation (ETD) in the alternating mode, as shown for an IgG3 glycopeptide (Fig. 2). These experiments resulted in the unambiguous assignment of the observed glycopeptides to specific IgG subclasses. The method allows the registration of IgG glycosylation features like core-fucosylation, bisecting GlcNAc, galactosylation and sialylation for all four IgG subclasses. The method was applied to plasma samples of rheumatoid arthritis patients obtained before, during, and after pregnancy. The time course obtained for one patient is shown in Fig. 3: An increased

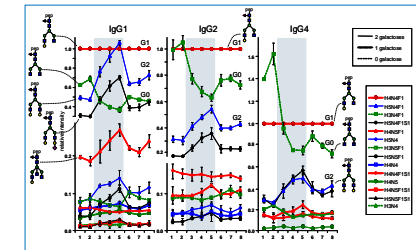


Fig. 3. Subclass-specific IgG glycosylation profiling of a rheumatoid arthritis patient before, during and after pregnancy. 1 and 2, two timepoints before pregnancy, with 1 year in between; week 10 (3), week 20 (4), and week 30 (5) of the pregnancy; 6 weeks (6), 12 weeks (7), and 24 weeks (8) post partum. For each time point IgG1, IgG2, and IgG4 were isolated from 2 µl of serum using Prot A, trypsinized, and analyzed in fourfold by nano-LC-MS. Signals were normalized to the H4N4F1 signal (G1; circles and dotted line), and averages as well as standard deviations are given.

IgG galactosylation during pregnancy was observed for all the analyzed IgG subclasses, which is in accordance with literature data (Rook *et al.* (1991) J. Autoimmun. 4: 779-94). The method is currently applied to various patient cohorts of infectious and autoimmune diseases. Currently the approach is adapted to look at glycosylation profiles of IgGs directed against specific antigens in various diseases. Preliminary results show that these profiles may differ enormously from total plasma IgG glycosylation profiles. This indicates that subsets of human B-cells produce IgGs with highly distinct glycosylation profiles. These distinct profiles are expected to have immunological relevance, as IgG Fc glycosylation is known to modulate the affinity of IgGs to various Fc receptors present on many immune cells.

Wuhrer *et al.* (2007) Proteomics, *in press*

Conclusions

- Glycosylation profiles of the Fc-glycosylation of IgG subclasses can be analyzed on an ion trap MS, providing information on core-fucosylation, bisecting GlcNAc, galactosylation, and sialylation.
- The HCTultra ion trap MS allows the detailed characterization of glycopeptides by CID and ETD fragmentation.
- The sensitivity of the ion trap MS analysis allows both: the acquisition of total plasma IgG glycosylation profiles and the acquisition of glycosylation profiles of antigen-specific IgG.

Ion Trap / ETD