These partially a-fucosylated antibodies can principally exhibit a heterogeneous a-fucosylation distribution within a pool of antibodies. For example, fucosylation rates can be different during fermentation. Also, the event of fucosylation could be cooperative, i.e. the second fucosylation on the homodimeric antibody may occur with an increased (positive cooperativity) or decreased (negative cooperativity) rate compared to the first one.

The FcγRIIIa/IgG complex has a 1:1 stoichiometry but IgG has two binding sites for FcγRIIIa. Consequently, in a single a-fucosylated antibody the receptor can bind with high affinity to the binding site formed by the IgG´s a-fucosylated glycan and protein core or with low affinity to the binding site consisting of the fucosylated carbohydrate and the protein core. It can therefore be concluded that a pool of antibodies with 50% a-fucosylation may consist of a homogeneous population of antibodies in which only one of the two N-glycans is fucosylated, or 50% of antibodies in which both N-glycans are fucosylated while in the other 50% none of the N-glycans are fucosylated. It is obvious that such a differential partition of a-fucosylation influences the overall affinity to FcγRIIIa and results in a different biological activity. It is therefore mandatory to analyze the biological activity of such an antibody preparation either directly by employing a biological test system (bioassay) or indirectly by biochemically measuring the rate and distribution of the a-fucosylation, which yields a more exact result.

The current state-of-the-art glycoanalytics uses N-glycosidase F (PNGase F) from *Flavobacterium meningosepticum* to cleave off the N-linked carbohydrates with a subsequent MALDI-MS (matrix-assisted laser desorption ionization mass spectrometry) analysis (according to Papac et al., Glycobiology (1998) 8, 445-454). By employing such a process, however, the linkage information is lost and the determination of fucosylation distribution within an antibody preparation is not possible.

On the other hand, analysis of the complete antibody using ESI-MS (electrospray ionization mass spectrometry) yields complex mass patterns that do not allow a quantitative interpretation due to the various modifications other than fucosylation – like galactosylation, C-terminal lysine heterogeneity, deamidation etc. – that may or may not occur in both subunits of the homodimeric IgG.

Therefore, there is a need for a new analytical method that eliminates the mentioned heterogeneity but maintains the linkage information.

Summary

In a first aspect, there is provided a method for detecting the presence or absence of fucose residues within a glycosylated antibody or a fragment thereof, comprising: a) enzymatic removal of heterogeneous saccharide residues from the protein b) enzymatic removal of other heterogeneous residues from the protein c) subsequent analysis of the protein, wherein the removal of step a) is performed by Endo S and Endo H, and wherein the analysis of step c) comprises LC-MS analysis, CE-SDS MW analysis or ESI-MS analysis.

In a second aspect, there is provided the use of the method as defined above for the determination of cooperative fucosylation in an antibody preparation during fermentation.

In a third aspect, there is provided the use of Endo S for cleavage of complex-type oligosaccharides from a glycoprotein in a method as defined above for detecting the presence or absence of fucose residues within a glycosylated antibody or a fragment thereof.

Description of the Invention