P-01

Structural Analysis of Human Glycoproteins by Tandem Mass Spectrometry

ANDRÁS ÁCS[1,2]; DÁNIEL SZABÓ[1]; KÁROLY VÉKEY[1]; GABRIELLA SÁRMAY[3]; LÁSZLÓ DRAHOS[1]; LILLA TURIÁK[1]

[1] *MS Proteomics Research Group, Research Centre for Natural Sciences, 2 Magyar tudósok körútja, H-1117, Budapest, Hungary*
[2] *Semmelweis University, 26 Üllői út, H-1085, Budapest, Hungary*
[3] *Department of Immunology, Eötvös Loránd University, 1/C Pázmány Péter sétány, H-1117, Budapest, Hungary*

Correspondence: dr.andras.acs@gmail.com

Keywords: *Glycoproteomics, mass spectrometry, glycopeptides, energy-dependent fragmentation, immunoglobulins*

1. Introduction

The process of glycosylation is a highly conserved mechanism among species. The enzymatic addition of oligosaccharide chain to the protein significantly changes the physicochemical properties and protein-protein interactions. Therefore glycosylation considered as a CQA (Critical Quality Attribute) for biopharmaceutical products. At this point there is no routinely used, widespread analytical method to determine glycosylation. This may derive from the fact that the term glycosylation summarizes various features. The most often examined feature is the relative abundance of glycoforms. The position of different sugar residues within the oligosaccharide chain has been less frequently analyzed. In this study we have developed a method which, in addition to the commonly investigated features, determines the structural position of the fucose residue. We have examined glycosylation of three proteins: AGP (alpha-1-acid glycoprotein), PSA (Prostate Specific Antigen) and IgG (Immunoglobulin G).

2. Materials and methods

AGP standard and IgG isolated from human serum were enzymatically digested in-solution sequentially by Lys-C/trypsin and trypsin. PSA standard was digested using Arg-C enzyme. The resulting peptide mixture was separated by a reversed-phase nanoLC coupled to a Bruker Maxis II Q-TOF. Relative abundance of glycoforms were determined by EIC (Extracted Ion Chromatogram). Selected glycopeptides were subjected to MS/MS measurements by CID (Collision-induced dissociation). Collision energy-dependence of the fragmentation was examined. Raw data were evaluated using Compass DataAnalysis and GlycoPattern.

3. Results

Various glycoforms were identified on each glycoprotein. AGP contains five *N*-glycosylation sites. In addition to the abundant, biantennary structures, tri- and tetraantennary glycoforms also occur. PSA has one *N*-glycosylation site, which is occupied by dominantly biantennary structures. IgG contains one *N*-glycosylation site at the constant region. The ten most abundant glycoforms of IgG are listed in Table 1. Overall, 90% of the glycoforms are fucosylated and the sialic acid content is less abundant, around 12%.

Table 1 *Relative abundance of the ten most abundant IgG glycoforms. N: N-acetylglucosamine, H: Hexose, S: Sialic acid, F: Fucose*

Canonical name	Sugar composition	RA (%)
G1F	N4H4F1	32.77%
G0F	N4H3F1	15.96%
G2F	N4H5F1	13.52%
G2SF	N4H5S1F1	8.59%
G0FB	N5H3F1	3.47%
G1FB	N5H4F1	7.40%
G1SF	N4H4S1F1	2.50%
G1	N4H4	4.31%
G2	N4H5	2.78%
G2FB	N5H5F1	1.66%
G0	N4H3	1.52%
G1B	N5H4	1.13%
G2S	N4H5S1	0.99%
G2S2F	N4H5S2F1	0.17%

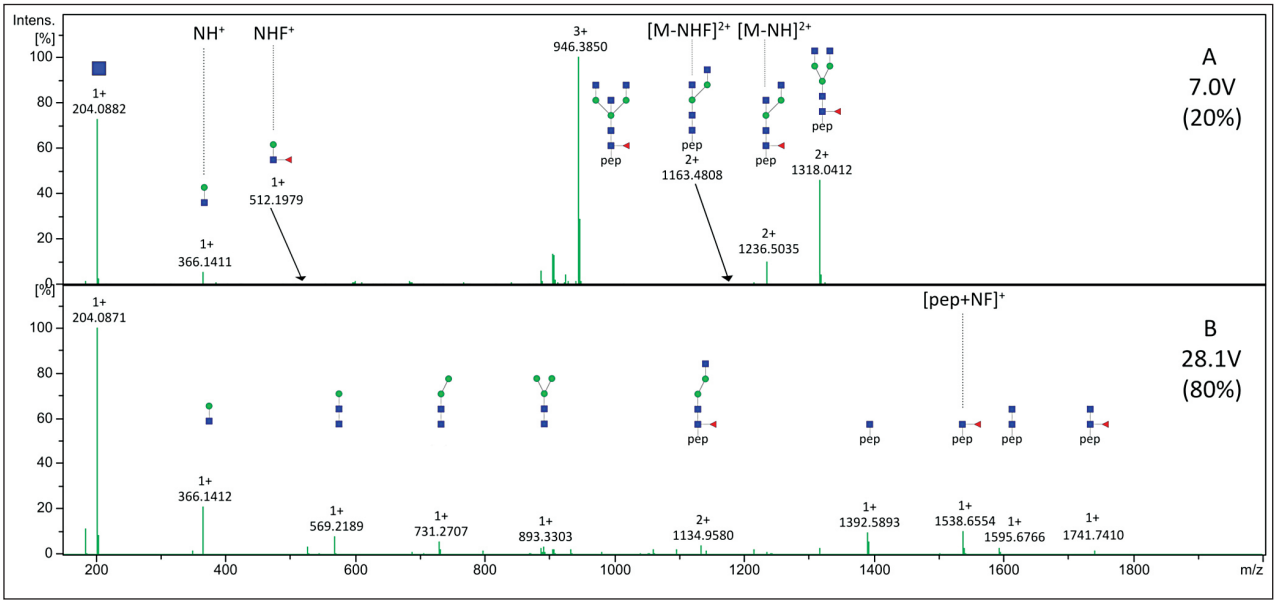

Figure 1 *MS/MS spectra of IgG derived EEQYNSTYR-N5H3F1 glycopeptide (m/z 946.3803) at 7.0 V (A) (20% of the "standard" collision energy), 28.1 V (B) (80% of the "standard" collision energy)*

In addition to glycoform identification and quantitation we have developed a method which can distinguish core and antenna fucosylated glycopeptides. The technique is based on low-energy tandem mass spectra. By identifying the fragment ions in the spectra, position of the sugar residues can be determined. We have identified diagnostic fragment ions, which characterize core and antenna fucosylation. Antenna fucosylation can be confirmed by the presence of $NH(S)F^{+}$ ion and it's complementary $[M\text{-}NH(S)F]^{2+}$ ion. These ions are abundant at low collision energy. The absence of these ions are indirect sign of core fucosylation. Such a case is illustrated in ***Figure 1/A***. At higher collision energies the location of the fucose residue can also be identified using the $[pep+NF]^{+}$ and $[pep+N]^{+}$ ions (***Figure 1/B***). We have combined the above mentioned diagnostic fragment ion's relative abundance into different mathematical formulas to characterize the degree of antenna fucosylation. These equations are appropriate for quantifying the amount of antenna fucosylation.

4. Conclusions

We have developed a straightforward analytical method which is suitable to identify and determine the relative abundance of glycoforms and provide structural information about the position of the fucose residue. In-depth characterization of glycan structures contributes to improve quality control over antibody production and promotes refined biomarker research.

5. Acknowledgements

The authors are grateful for support from the National Research Development and Innovation Office (OTKA 119459).

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