Oxonium ions-dependent mass spectrometry method to measure the glycosylation features of glycoproteins

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Introduction: Protein glycosylation is a Post-Translational Modification (PTM) that involves the covalent attachment of mono, oligo and polysaccharides or glycans to selected residues of proteins such as asparagine (N-linked) or serine/threonine (O-linked). It plays an important role in the stability and function of proteins and consequently in the control of biological processes. Therapeutic antibodies and biosimilars have glycans attached to their light and/or heavy chains, which play an important role in their pharmacokinetics, efficacy and safety. Several LC-MS/MS methods and computational tools have been developed to evaluate the glycosylation pattern of these molecules. We are proposing the development of a rapid, sensitive and accurate method to screen the glycosylation features of glycoproteins, which can be used in the quality control of therapeutic antibodies and other glycoproteins.

Material and Methods: Three standard glycoproteins were used for method standardization: RNAseB, Fetuin and Human IgG. LC-MS/MS analyses were performed on an EasyII nLC coupled to LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific). The tested methods are 35 min long, with a 10min gradient, and consisted of several full scans MS1 windows immediately followed by MS2 of all ions from the previous MS1, using HCD with 35% collision energy and resolution 7500. A computational algorithm was developed to extract oxonium ions, quantify them throughout the chromatograms and compare their intensity between different LC-MS/MS runs. To test the method accuracy, the standard glycoproteins were treated with PNGase F, for N-linked glycans removal, and then the glycans specific oxonion ions intensity were compared between samples.

Results: Initially, four LC-MS/MS methods were compared, in which the MS1 and MS2 m/z ranges, as well as the number of MS1 windows, were modified to obtain the best intensity of glycans oxonium ions. Two methods showed the best results and then standard proteins were analyzed in triplicate: **Method I** - MS1 600-2000 m/z; MS2 100-900 m/z - 4 MS1 windows; **Method II** - MS1 600-2000 m/z; MS2 100-1600 m/z - 4 MS1 windows. The analysis of standard glycoproteins showed that it is possible to relatively quantify glycosylation features using oxonium ions specific for general glycans (mz138.05; mz186.07; mz204.08) and sialic acid - NeuAc (mz292.10; mz274.09) and NeuGc (mz308.09; mz290.08). Comparison between control and PNGaseF-treated samples showed a significant decrease of glycan-specific oxonium ions intensities.

Conclusion: The method presented in this work is fast, accurate and can be used to evaluate the glycosylation profile of glycoproteins and other biomolecules.

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