Glycomics & Lipidomics

Extended Abstract

Analytical glycomics: Biopharmaceutical applications

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Abstract

Full characterization of the N-glycosylation moieties of biopharmaceuticals is of high importance, especially when glycovariants may impact the biological effect. Well over half of the new generation protein therapeutics are monoclonal antibodies, in which the attached oligosaccharides not only affect their physicochemical properties and stability, but also their receptor binding activity, circulating half-life and last but not least, their immunogenicity. Therefore, high performance glycoanalytical techniques are of great demand for N-glycosylation analysis of therapeutic antibodies, especially during clone selection, process development and lot release. Analysis of complex carbohydrates is a very challenging task due to the lack of their chromophore/fluorophore activity and in many instances, easily ionizable groups, necessitating derivatization before electric field mediated analysis. Full N-glycosylation characterization may also require sequencing with consecutive exoglycosidase digestion steps, followed by capillary electrophoresis analysis. In this presentation, the state of the art of liquid phase separation methods will be conferred for comprehensive structural elucidation of protein N-glycosylation, mostly using capillary electrophoresis and its combination with mass spectrometry (CESI-MS). Assisted by the emerging field of glycoinformatics, assignment of the identity of the separated glycan structures will be demonstrated by using the recently introduced GUcal software.

This review covers advances in analytical technologies for high-throughput (HTP) glycomics. Our focus is on structural studies of glycoprotein glycosylation to support biopharmaceutical realization and the discovery of glycan biomarkers for human disease. For biopharmaceuticals, there is increasing use of glycomics in Quality by Design studies to help optimize glycan profiles of drugs with a view to improving their clinical performance. Glycomics is also used in comparability studies to ensure consistency of glycosylation both throughout product development and between biosimilars and innovator drugs. In clinical studies there is as well an expanding interest in the use of glycomics—for example in Genome Wide Association Studies—to follow changes in glycosylation patterns of biological tissues and fluids with the progress of certain diseases. These include cancers, neurodegenerative disorders and inflammatory conditions. Despite rising activity in this field, there are significant challenges in performing large scale glycomics studies. The requirement is accurate identification and quantitation of individual glycan structures. However, glycoconjugate samples are often very complex and heterogeneous and contain many diverse branched glycan structures. In this article we cover HTP sample preparation and derivatization methods, sample purification, robotization, optimized glycan profiling by UHPLC, MS and multiplexed CE, as well as hyphenated techniques and automated data analysis tools. Throughout, we summarize the advantages and challenges with each of these technologies. The issues considered include reliability of the methods for glycan identification and quantitation, sample throughput, labor intensity, and affordability for large sample numbers.

Glycans play key roles in important biological processes such as protein folding, host-pathogen interaction and signal transduction. They are present both in free form, or bound to proteins or lipids, and can modify the biological activities of the conjugate. The glycomics technologies covered in this review are mainly for analysis of protein glycosylation, the major types of which are N-linked glycans attached at Asn-X-Ser/Thr motifs on the peptide backbone and O-linked glycans attached to Ser/Thr. Protein glycosylation is a co-translational and post-translational modification where glycans are attached to proteins during translation, assisting in the folding and quality control of the protein. Attached glycans are subsequently modified in the endoplasmic reticulum and Golgi to varying levels of complexity, causing glycosylation to be one of the most complex and diverse types of protein modification.

Glycomics is of interest to biopharma companies because glycans can greatly modify safety and efficacy profiles of the therapeutic protein to which they are attached. For example, IgG effector functions such as antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity of monoclonal antibodies are modulated by *N*-glycosylation of its fragment crystallizable (Fc) portio. *N*-glycosylation modifications are also involved in determining the plasma half-life of glycoproteins by modulating the interaction with various receptors. Even small changes in glycosylation can lead to serious issues such as anaphylaxis in patients and the destruction of therapeutic activity. These effects have been found in drugs such as Cetuximab (a monoclonal antibody (mAb) for treatment of metastatic colorectal cancer), Herceptin (a mAb for treatment of breast cancer) and erythropoietin (EPO—a drug for increasing red blood cells in patients with renal failure and cancer patients undergoing chemotherapy). Given this, there are now increasing pressures from regulatory authorities for drug manufacturers to measure,

optimize and control their drug's glycosylation. This task is very challenging given the structural complexity of biopharmaceutical glycosylation and achieving it has required adoption of a new approach to drug design—namely Quality by Design (QbD). This methodology has been actively promoted by the FDA and other drug regulators and is being adopted by leading biopharma companies as the system of best practice for the design, development, and manufacture of biologic drugs. QbD has the potential to deal with the complexities of drug glycans and could help simplify difficult tasks such as demonstrating comparability of biopharmaceutical glycosylation. However, the glycoprofiling effort required for QbD is considerable. Studies of the QbD approach by the CMC Biotech Working Group (a consortium of experts from leading biopharma organizations including Genentech, Amgen, Eli Lilly, and Pfizer), indicate that definition of the QbD Design Space may require analysis of tens of thousands of drug samples. Successful adoption of QbD for development of glycoprotein therapeutics requires glycoprofiling systems that can cope with this very high level of sample throughput—in effect it will need to be built on a glycomics framework.

In addition to its use in biopharmaceutical realization, there is also increasing interest in glycomics for development of new clinical diagnostics based on glycosylation markers of diseases. This follows from the key roles that glycans play in major biological processes from the fertilization of eggs by sperm to cell death and their involvement in a multitude of disease etiologies and progressions. The potential of glycans as sensitive disease biomarkers is particularly high, since they are synthesized by the concerted action of multiple proteins, and influenced by various disease-associated factors such as genetic variations, epigenetic signatures and metabolic distortions. Glycan patterns of secreted proteins often confer information on the pathophysiological status of the secreting cells, making glycosylation analysis of relevant glycoproteins a suitable diagnostic tool. Changes in glycosylation are associated with various diseases like cancer, neurodegenerative and inflammatory diseases. These types of associated changes have resulted in an increased interest in studying the alterations in glycosylation patterns of biological fluids as disease biomarkers as well as for patient stratification and personalized medicine

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