GLYCOSYLATION ANALYSIS IN ACCORDANCE WITH ICH Q6B GUIDELINES

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ABSTRACT

Characterization of the carbohydrate structure of a biopharmaceutical is an essential regulatory requirement. The ICH guideline Q6B (1) states, "For glycoproteins, the carbohydrate content (neutral sugars, amino sugars and sialic acids) is determined. In addition, the structure of the carbohydrate chains, the oligosaccharide pattern (antennary profile) and the glycosylation site(s) of the polypeptide chain is analyzed, to the extent possible." Characterization is essential to assess if the glycosylation is compatible with human application of the drug.

INTRODUCTION

Glycosylation is a dynamic biosynthetic process and one of the most complex forms of post-translational modification. The biological activity of a glycoprotein can be significantly affected by the structure of the carbohydrate chains.

Two main types of protein glycosylation exist: (i) N-glycosylation of Asparagine residues and (ii) O-glycosylation of Serine and Threonine residues.

Glycan profiles can be quite different depending on the cell-lines in which a glycoprotein is produced. Different cell lines (e.g. CHO cells, insect cells, etc) can produce the same glycoprotein with differences in overall structure, linkage and composition of the glycans, affecting bioactivity.

Bioreactor conditions can also affect the glycosylation profile of a glycoprotein. Variation in nutrient levels, pH or O_2 content can all effect glycosylation and therefore potentially the efficacy of the final product.

For these reasons, characterization of the glycan structure in biopharmaceutical products is a key consideration even from early cell line selection. Ultimately, it is an essential regulatory requirement to assess consistency of the final product and ensure that glycosylation is compatible with human application of the drug.

This poster outlines the techniques that SGS M-Sca n employs to study a glycoprotein in accordance with the ICH Q6B guidelines.

METHODS

- N- and O-glycans are released, permethylated and analyzed by Matrix Assisted Lazer Desorption Ionization (MALDI) mass spectrometry. This technique produces limited fragmentation and provides data on the overall glycan population.
- Released, permethylated N- and O-glycans are analyzed by Electrospray Mass Spectrometry (ES-MS). This provides useful fragment ion information from which non-reducing terminal structures can be studied. These are often the regions of the glycan where most structural variance occurs hence it is necessary to examine them in detail. MS/MS based techniques can be used to select a specific glycan precursor ion for fragmentation and the data obtained can then be related to a specific component in the population.
- The linkages of the monosaccharides within a released glycan population are studied by Gas Chromatography Mass Spectrometry (GC-MS). When used in conjunction with specific chemical derivatization procedures this technique produces fragment ions from which the linkages of the variously detected monosaccharides can be determined. GC-MS can also be used with the trimethylsilyl derivatives to provide a profile of the monosaccharides present in a sample from which quantitation can be performed.
- Chromatographic procedures such as High pH Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) or LC/ES-MS (with or without fluorescence detection) of 2-aminobenzamide labeled glycans can be used to produce a profile which can serve as a comparator for future batches once characterization of the glycans has been performed.

RESULTS

- Initial studies involving the determination of intact mass of the product using the mass spectrometric technique of ES-MS shows the presence of mass differences consistent with variation in glycan structure.
- Comparison of the mass(es) obtained compared to that predicted by the amino acid sequence will give some information on the presence and basic structure of the relevant glycosylation, such as degree of heterogeneity (Figure 1).
- Monosaccharide analysis by Gas Chromatography – Mass Spectrometry (GC-MS) can also be used to provide initial data on protein glycosylation. This allows monosaccharides to be identified and quantitated. The presence of unusual or undesirable monosaccharides (e.g. Xylose in plant cell derived glycoproteins) can be monitored.
- Specific sialic acid analysis can also be performed by High pH Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD). This will allow the identification and quantitation of the sialic acid species N-Acetylneuraminic acid (NeuAc) and N-Glycolylneuraminic acid (NeuGc). The levels of sialic acid can affect residence time in the bloodstream and therefore efficacy of the therapeutic.

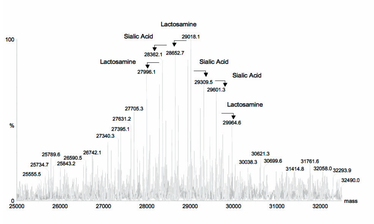

FIGURE 1: Electrospray mass spectrum (ES-MS) of a glycoprotein carrying multiple N-glycans. The high degree of heterogeneity can be readily seen by the mass differences observed between peaks

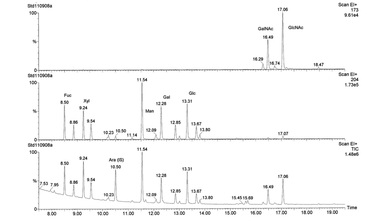

FIGURE 2: Chromatogram of monosaccharides present in a mixture following trimethylsilyl derivatization and analysis by Gas Chromatography – Mass Spectrometry (GC-MS)

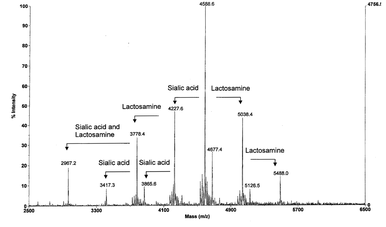

FIGURE 3: MALDI mass spectrum of permethylated N-glycans. Taken from the same glycoprotein as shown in Figure 1

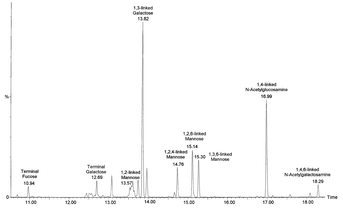

FIGURE 4: Gas chromatography mass spectrum of derivatized monosaccharides produced during linkage analysis

GLYCAN POPULATION ANALYSIS

- Glycan population analysis is performed to give a detailed picture of the N- and O-glycan structures present on a glycoprotein.
- The isolated glycans are analyzed by MALDI-MS. The data obtained, in conjunction with the known biosynthetic pathways of glycans, can be used to determine possible compositions (Figure 3).
- Information on antennal structures is required to assist in determining the arrangement of the monosaccharides in the glycan population, since some compositions can have different structural arrangements (an example is shown in Figure 5). This can be carried out by ES-MS and/or ES-MS/MS of the derivatized samples.
- Knowledge of the antennal structures are important since these are the main sites of glycan heterogeneity and the structures present can have an impact on the bioactivity of the molecule. Potential immunogenic epitopes such as Galα1-3Gal are also present on antennae.
- For the fullest characterization, linkage (methylation) analysis should also be performed. This technique uses chemical derivatization and GC-MS to determine the monosaccharide linkages in the sample. This can determine how, for example, sialic acid species are attached (α2-3 vs α2-6) and assess the presence of multiantennary or bisecting N-glycans (Figure 4).
- Glycan based receptor recognition will only occur if the monosaccharides are linked together in the correct way. Furthermore incorrect linkages and structures may result in the formation of epitopes with potential immunogenicity.

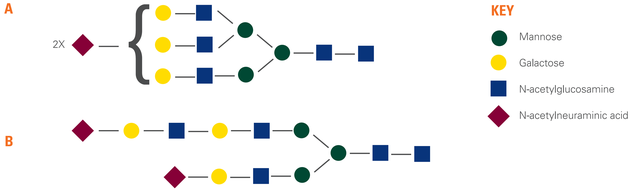

FIGURE 5: Structures A and B have the same mass as they have the same number and type of monosaccharides. The triantennary nature of structure A will give different fragment ions in ES-MS compared to structure B, which contains a lactosamine repeat. The two structures will also show a difference in linkage analysis by GC-MS

CONCLUSION

Detailed characterization of N- and O-glycans can be performed using various different ionization techniques and methodologies to address the requirements of the ICH Q6B guidelines and to assist from the early stage and throughout on-going development of a biotherapeutic.

USEFUL REFERENCES

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