Strategies for the Identification of Posttranslational Modifications in Biopharmaceuticals by Mass spectrometry

K. Vivekanandan

Analytical and Formulation Division, Research and Development
Biocon Limited, Bangalore, India. 560100

Protein based therapeutics are emerging as the largest class of new chemical entities being developed in drug industry. Presently there are approximately 140 therapeutic proteins approved and additional 500 are in clinical trials and people who use these drugs worldwide are over 250million. Recombinant technologies used for large scale production leads to modifications of proteins depending on expression systems. Although there are number of posttranslational modifications known, the most common form of protein posttranslational modification is glycosylation. During glycosylation, monosaccharides are attached to the protein side chain and form a glycoprotein. Glycosylation can either be an N-linked or O-linked depending on the expression system. The glycosylation pattern of a protein also depends on number of factors such as expression system, media, pH, cell density and age, which makes the structural diversity of glycoproteins vast and impossible to predict.

In biological processes, glycosylation plays an important role in immune defense, fertilization, viral replication, parasitic infection, cell growth, and inflammation and cell-cell adhesion. In case of pharmaceutical glycoproteins, glycosylation affects stability of protein conformation, immunogenicity, clearance rate, and protection from proteolysis and improves protein solubility.

Since different glycoforms can exhibit different biological properties, it is essential to control glycosylation to maintain the quality of biopharmaceutical molecule. Removal of glycoproteins not only eliminates undesirable changes it also improves the process yields. For analyzing glycoprotein, purification and different chemical and instrumentation techniques are utilized. The most commonly employed ionization techniques in mass spectrometry are electrospray and matrix assisted laser desorption ionization (MALDI). The advantage of electrospray is that it can be coupled with HPLC for separating mixtures for online peptide mapping. Glycoproteins are first isolated by reverse phase HPLC to find the intact mass by ESI-MS and MALDI-MS. Chemical methods such as reduction and alkylation, peptide digest were employed to find out the site of glycosylation and type of glycan in the aminoacid sequence. The nature of carbohydrate can be predicted by employing different glycosidases. A full characterization of a glycoprotein helps in optimizing the level of glycosylation in biopharmaceuticals by glyco-engineering.

References

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Dr. K. Vivekanandan
Analytical and Formulation Division, Research and Development
Biocon Limited, Bangalore, India. 560100

Ph.D Chemistry, IIT Kanpur 1997
Two years postdoctoral work (USA)
Eight years experience in industrial (petroleum, pharma and biotech) Mass spectrometry.
Nine publications and a patent in anticancer drug
Life member of ISMAS since 2000