Background

- Protein drugs, such as Fc-fusion proteins are often modified by complex glycans with heterogeneity
- Glycosylation can impact safety, stability and efficacy of protein therapeutics, therefore an in-depth characterization of N- and O-glycans is critical
- Analysis of protein glycosylation poses major challenges
- No generic method for release of all glycans is available
- Poor ionization of neutral carbohydrates requires derivatization such as permethylation or labeling to enhance detectability of glycans during mass spectrometric analysis

Methodology

A. One-Pot N and O-glycan profiling of a model glycoprotein, Fetuin
B. Glycan profiling of Fc-Fusion Proteins Abatacept, Etanercept, and Affiberecept and LIFT-MS analysis for differentiation of N- and O-glycans by unique reducing ends and fragments
C. MALDI-TOF/MS

Figure 1. Workflow of the one-pot method for simultaneuously analysis of N-, and O-glycans of glycoproteins

A. Isolation of N-glycans and O-glycopeptides in tandem by FASP filter
B. Differentiation of permethylated N and O-glycans by unique reducing ends
C. Mass Spectrometry analysis of N- and O-glycans in one pot by MALDI-TOF/MS

Figure 2. One Pot Glycan analysis of Fetuin and Fc-Fusion Proteins
A. One-pot N and O-glycan profiling of a model glycoprotein, Fetuin
B. Glycan profiling of Fc-Fusion Proteins Abatacept, Etanercept, and Affiberecept and LIFT-MS analysis for differentiation of N- and O-glycans by unique reducing ends and fragments

Figure 3. Simultaneous N &O-glycan profiling of WT and KOC3 Cellular Glycoproteins
A. Isolation of N-glycans and O-glycopeptides in tandem by FASP filter
B. Differentiation of permethylated N and O-glycans by unique reducing ends
C. Mass Spectrometry analysis of N- and O-glycans in one pot by MALDI-TOF/MS

Figure 4. Summary and Reproducibility of One Pot Glycomics Method
A. Summary of unique glycan species and their relative abundances in Fc-Fusion proteins Abatacept, Etanercept, and Affiberecept (Data from 5 independent preparations)
B. Summary of one-Pot glycan analysis of two different lots of etanercept

Summary

- We developed a reproducible method for simultaneously profiling N- and O-glycans from purified proteins and cellular proteins from cell lysates in One-Pot.
- Relative abundances of unique N- and O-glycan species were determined consistently from three independent preparations of Fetuin and Fc-Fusion Proteins
- N- and O-glycans were differentiated by tandem mass spectra based on unique mass shifts (+16 Da) associated by reduced N-glycan core fragments.
- We evaluated our One-Pot method with O-glycoengineering CHO cell lines and identified unique Core 3 O-glycans in C3KO CHO Cells

References


Acknowledgments

This work is supported by the FDA Intramural Funding, also supported by an appointment to the Research Participation Program at the U.S. Food and Drug Administration administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration.