Profiling and Heightened Characterization of O-linked Glycans from Therapeutic Glycoproteins

Elaine Sun[1], Mario DiPaola[1], Marshall Bern[2], Andrew Hanneman[1]

[1]Charles River Laboratories, Woburn, MA; [2]Protein Metrics Incorporated, San Carlos, CA

charles river

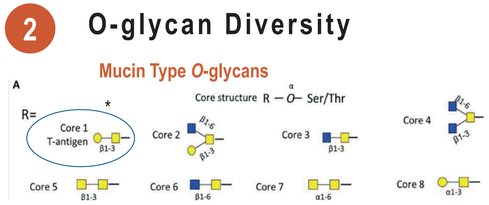
PROTEIN METRICS
Powerful software for protein characterization

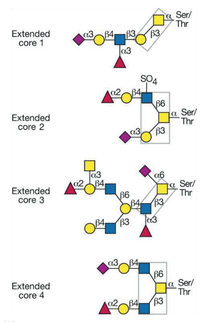
1 Key Points

- Biopharma requires robust methods for routine profiling and detailed structural characterization of O-linked glycans
- Monitoring the degradation reactions associated with maintaining an intact reducing end may dictate the most suitable approaches
- Advantageous if: the same method used to profile oligosaccharides includes monosaccharide O-glycans in the same chromatogram
- The exact methods for pursuing detailed structural analysis (accurate mass, MS/MS, and MS^n) is an important consideration, especially for complex glycoproteins such as blood derived protein products

2 O-glycan Diversity

Mucin Type *O*-glycans





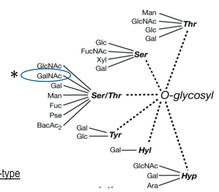
Front. Genet., 19 March 2015 | Louise E. Tailford†, Emmanuelle H. Crost†, Devon Kavanaugh† and Nathalie Juge*

* Common in CHO-expressed proteins

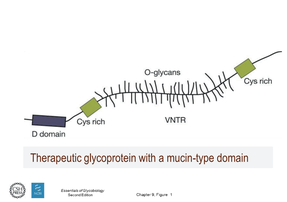
Essentials of Glycobiology Second Edition — Chapter 9, Figure 2

Profiling and Characterization of *O*-glycans from Diverse/Complex O-glycoproteins

1) A wide variety of *O*-glycans exist in nature



* Mucin-type



Therapeutic glycoprotein with a mucin-type domain

Essentials of Glycobiology Second Edition — Chapter 9, Figure 1

Non-CHO Glycoproteins

1. Emerging Expression Systems:
***O*-glycosylation in *Spodoptera frugiperda* (Sf9) and *Trichoplusia ni* (Hi-5) insect cell lines** is complex and include abundant hexuronic acid (Sf9 and Hi-5) and *O*-linked phosphocholine (Sf9)

Glycobiology vol. 22 no. 11 pp. 1593 Abstract #217

Stefan Gaunitz, Chunsheng Jin, Anki Nilsson, Jining Liu, Niclas G. Karlsson and Jan Holgersson

2. Glycoengineering: e.g. GlycoSwitch®

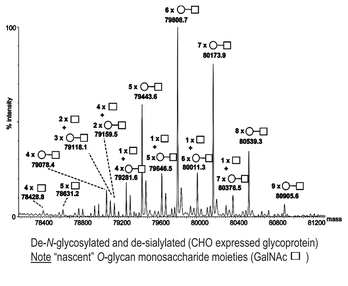
Jacobs PP, Geysens S, Vervecken W, Contreras R, Callewaert N. Engineering complex-type N-glycosylation in *Pichia pastoris* using GlycoSwitch technology. Nat Protoc. 2009; 4:58–70.

Hamilton SR, Cook WJ, Gomathinayagam S, Burnina I, Bukowski J, Hopkins D, Schwartz S, Du M, Sharkey NJ, Bobrowicz P, Wildt S, Li H, Stadheim TA, Nett JH. **Production of sialylated O-linked glycans in *Pichia pastoris*.** Glycobiology. 2013;23:1192–1203

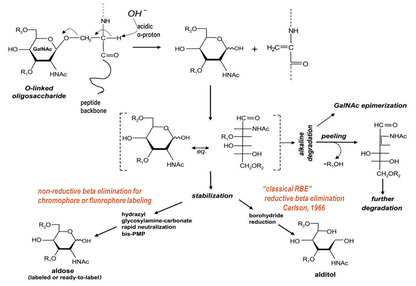
3. Various therapeutic glycoproteins are derived from human blood

3 MS-based Methods used to Profile and Characterize O-linked Glycans

1. **Analyze O-glycans while they still reside on the protein or peptide backbone (may need to de-N-glycosylate, de-sialylate, etc.)**
2. **Release by non-reductive β-elimination for subsequent chromophore or fluorophore labeling (important to monitor degradation)**

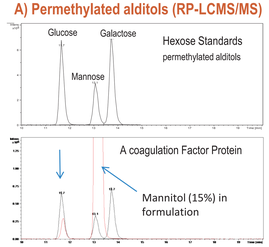


De-*N*-glycosylated and de-sialylated (CHO expressed glycoprotein)
Note "nascent" *O*-glycan monosaccharide moieties (GalNAc □)

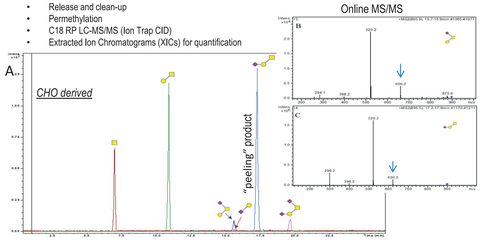


3. Release using "classical" reductive β-elimination (minimal degradation "peeling" product)

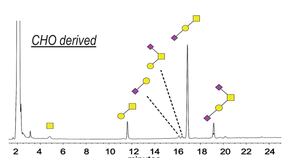
A) Permethylated alditols (RP-LCMS/MS)



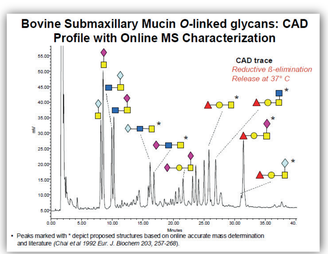
- Release and clean-up
- Permethylation
- C18 RP LC-MS/MS (Ion Trap CID)
- Extracted Ion Chromatograms (XICs) for quantification



B) Native Alditols (PGC-LC-CAD-MS/MS)

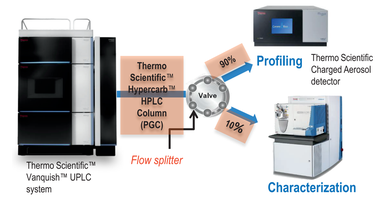


Dual detection data streams (CAD, and MS/MS) analyzed in collaboration with Protein Metrics

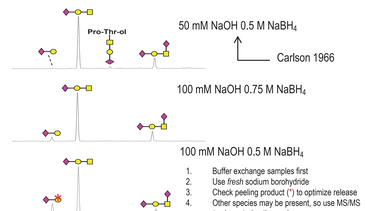


4 LC-MS with Charged Aerosol Detection (CAD)

1. Configuration



2. Release optimization

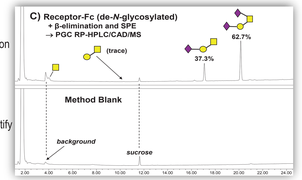


3. Applications

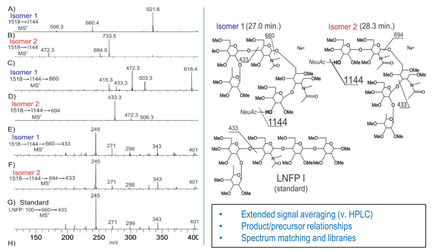
A) Biopharma samples by PGC-CAD-MS (CHO derived)

Buffer exchange to remove formulation components.

Buffer blank Include to help identify unknowns.



B) Blood derived *O*-glycosylated products (MS^n characterization of permethylated O-glycans)



- Extended signal averaging (v. HPLC)
- Product/precursor relationships
- Spectrum matching and libraries

5 Conclusions

1. Currently, we prefer traditional reductive beta elimination due to the diversity of structures potentially presented in a contract research organization (CRO) environment (potential degradation reactions cannot be tolerated)
2. We closely monitor peeling reactions during method development
3. Place monosaccharides within the same chromatogram as oligosaccharides
4. CAD detection may be promising for routine batch-to-batch comparability
5. Prefer MS/MS for structural confirmation (vs. accurate mass)
6. Permethylation and MS^n are highly useful for detailed or *de novo* structure analysis

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