Comprehensive Profiling of *O*-linked Glycans in Bovine Submaxillary Mucin

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INTRODUCTION

O-glycosylation is an important protein post-translational modification that plays significant roles in cellular signaling and cell-cell interactions. Comprehensive structural elucidation of glycan or glycoconjugate mixtures is challenging because of their structural complexity and the universal presence of isomeric structures. Here, *O*-glycans were released via reductive alkaline β-elimination to avoid peeling and eliminate anomerism-induced chromatographic peak splitting. Released *O*-glycans were permethylated to increase their detection sensitivity and prevent gas-phase structural rearrangement. Isomers were separated by PGC-LC, and characterized by electronic excitation dissociation (EED) MS/MS which allowed accurate determination of the glycan topology and linkages. Offline RPLC fractionation and enrichment were performed to accomplish the analysis of low-abundance species. Detailed structures of 58 *O*-glycans from bovine submaxillary mucin were determined, including 11 novel structures.

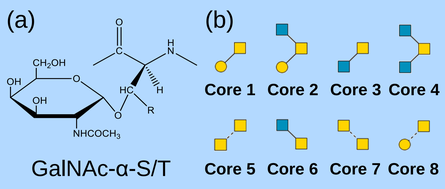

Figure 1. *O*-glycosylation: (a) linkage to the Ser/Thr residue; (b) core structures.

METHODS AND MATERIALS

O-linked glycans were released from bovine submaxillary mucin via reductive alkaline β-elimination. Released *O*-glycans were purified using C18 Sep-Pak cartridges and recovered by charcoal solid-phase extraction columns. Reduced glycans were permethylated before LC-MS/MS analysis. Liquid chromatographic separation was carried out on a Waters nanoACQUITY UPLC system, with an in-house packed nanoPGC column held at 60 °C. Online EED MS/MS analyses were performed on a Bruker 12-T solariX Fourier transform ion cyclotron resonance (FTICR) mass spectrometer, with the cathode bias set at 18 V. In order to facilitate detailed structural determinations of low-abundance glycoforms, offline fractionation by reversed-phase liquid chromatography was performed using a C18 RPLC column, prior to online PGC-EED MS/MS analysis. Multiple fractions were combined to achieve enrichment. EED spectra of all glycans were interpreted manually.

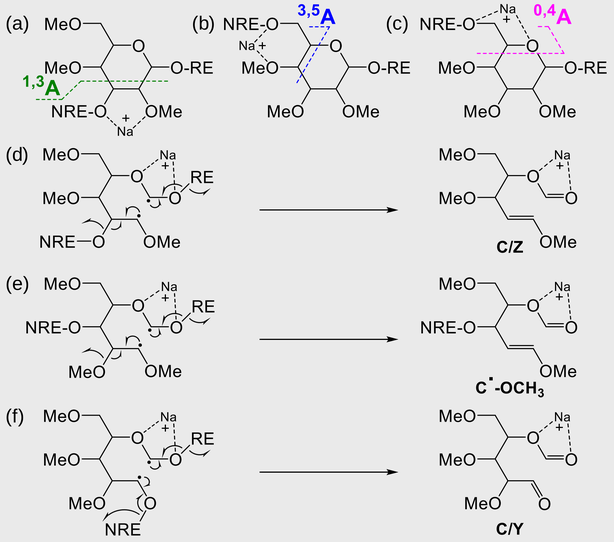

Figure 2. Formation of linkage-diagnostic fragments by EED. RE = reducing end; NRE = non-reducing end.

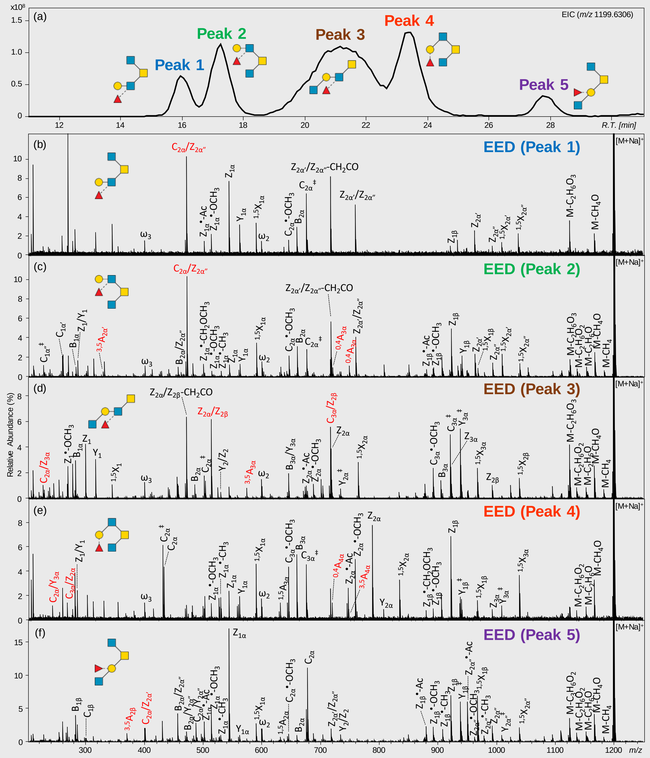

Figure 3. On-line nanoPGC-LC–EED-MS/MS analysis of enriched deutero-reduced and permethylated *O*-glycans. (a) EIC of the HexNAc(3)Hex(1)Fuc(1) glycans from bovine submaxillary mucin. (b-f) EED spectra of five isomers acquired at elution peaks marked in panel a. The stereochemical configurations were assumed based on biosynthetic knowledge. Key fragments are labeled in red.

RESULTS AND DISCUSSION

The superb separation performance of PGC-LC and the exceptional fragmentation efficiency of EED allowed detailed structural analysis of complex mixtures of *O*-linked glycans.

Compared with other LC methods, PGC-LC offers better glycan isomer resolution and its performance may be further improved when operated at an elevated temperature.

EED produced an abundance of glycosidic, as well as linkage-specific, cross-ring and secondary fragments for confident determination of the glycan topologies and linkage configurations.

Analyses of low-abundance species were facilitated by off-line fractionation and enrichment, which proved to be an essential step for analyzing complex mixtures with components whose concentrations span a wide dynamic range.

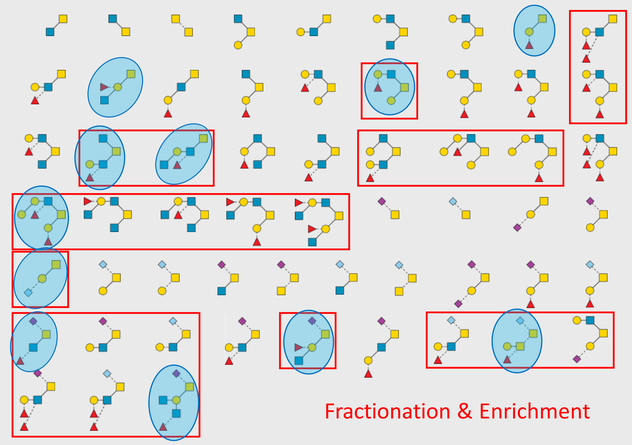

Figure 4. Structures of *O*-linked glycans identified in bovine submaxillary mucin by LC-EED-MS/MS. Red rectangles enclose low abundance glycoforms characterized after fractionation. Blue ovals highlight novel structures.

CONCLUSIONS

1. The potential of PGC-LC–EED-MS/MS for structural analysis of *O*-glycans was explored and demonstrated.
2. Fractionation and enrichment is a vital step for analysis of low-abundance glycoforms.
3. Detailed structures of 58 *O*-linked glycans were characterized by nanoPGC-LC–EED-MS/MS analysis, including 11 novel structures.

REFERENCES

1. Yu, X.; Jiang, Y.; Chen, Y.; Huang, Y.; Costello, C.E.; Lin, C. *Anal. Chem.*, **2012**, *84,* 7487-7494.
2. Tang, Y.; Wei, J.; Costello, C.E.; Lin, C., *J. Am. Soc. Mass Spectrom.*, **2018**, *29*, 1295-1307.
3. Wei, J.; Tang, Y.; Bai, Y.; Zaia, J.; Costello, C.E.; Hong, P.; Lin, C., *Anal. Chem.*, **2020**, *92*, 782-791.

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