Structural Characterization of Glycans by Electron-Transfer/Higher-Energy Collision Dissociation

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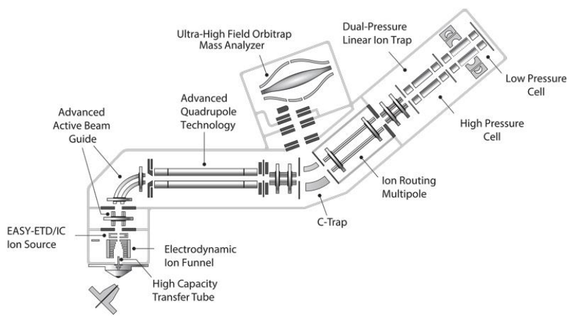
INTRODUCTION

- Structural characterization of glycans poses a significant analytical challenge because of their structural complexity, diversity and the frequent occurrence of isomers in biological samples.
- Electronic excitation dissociation (EED) tandem mass spectrometry (MS/MS) can provide detailed glycan structural information that is often unobtainable by collision-induced dissociation (CID) MS/MS. However, EED MS/MS has yet to be broadly applied to glycomics studies because of the limited access to FTICR MS instruments by the glycoscience community.
- We have previously shown that electron transfer dissociation (ETD) with supplemental activation can provide glycan structural details. Here, we studied the electron-transfer/higher-energy collision (EThcD) fragmentation behavior of permethylated and metal-adducted glycans, and explored its potential as an alternative to EED for detailed glycan structural characterization and isomer differentiation.

METHODS

- LNFP II and III (20 μg) were reduced by sodium borodeuteride (0.25 M) in 200 μL of NH_4OH (0.1 M) for 2 h at room temperature before addition of 10% acetic acid to stop the reaction.
- Reduced glycans were dried and re-suspended in 120 μL of DMSO and 5 μL of water, and permethylated with addition of 100 μL of methyl iodide on a DMSO-conditioned NaOH spin column. Reduced and permethylated glycans (10 pmol/μL) were dissolved in 50:50 water:methanol solution containing cobaltous chloride (200 μM) and directly infused into the mass spectrometer.
- ETD, HCD, and EThcD analyses were performed on an Orbitrap Fusion Lumos Tribrid mass spectrometer. EED analysis was performed on a 12-T solariX FTICR mass spectrometer.



Schematic diagram of an Orbitrap Fusion Lumos Tribrid mass spectrometer

RESULTS

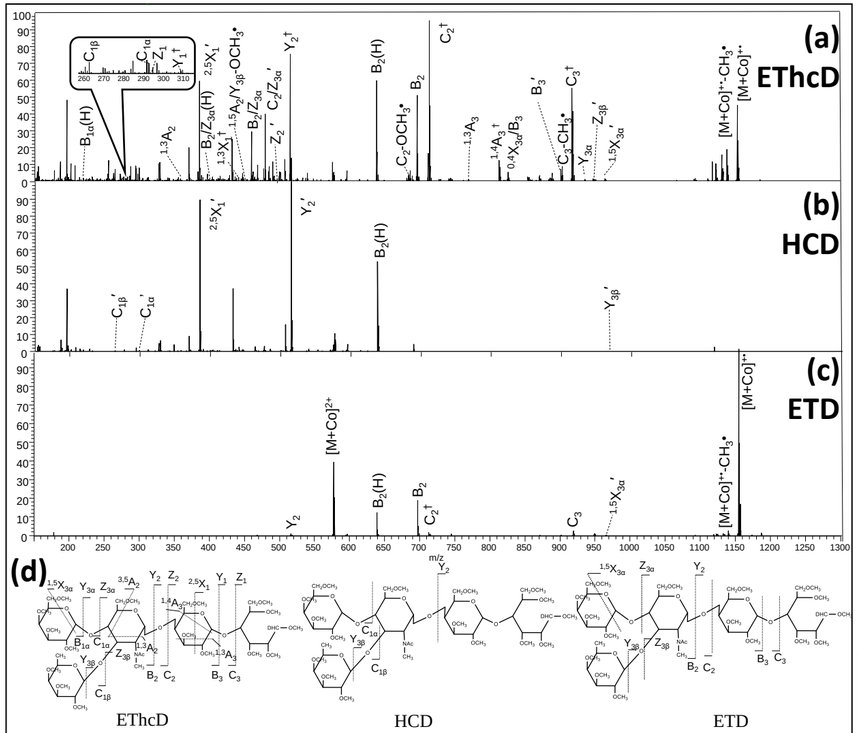


Figure 1. Comparison of (a) EThcD (reaction time: 100 ms; supplemental activation: 35%), (b) HCD (collision energy: 17%), and (c) ETD (reaction time: 100 ms) MS/MS spectra of permethylated LNFP III ($[M+Co]^{2+}$); and (d) cleavage maps. All fragments were cobalt adducts unless labeled otherwise. Symbols ′/† denote the gain/ loss of a single H atom, respectively.

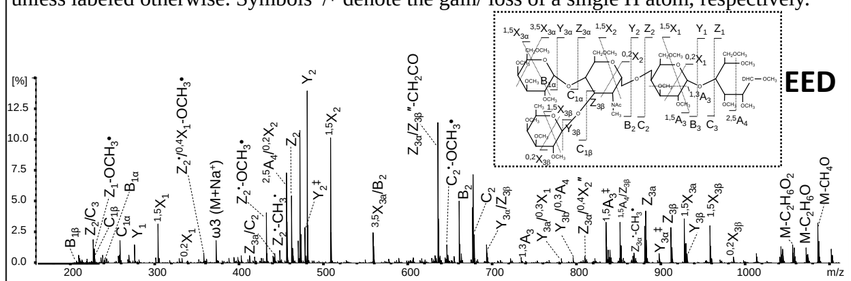


Figure 2. EED MS/MS spectrum of deutero-reduced and permethylated LNFP III ($[M+Na]^+$) (Energy: 18 eV). The inset shows the EED cleavage map.

- Neither HCD nor ETD produced complete glycosidic cleavage coverage.
- Both EThcD and EED produced a complete series of glycosidic cleavages, many with complementary fragments.
- EThcD and EED also produced many linkage-diagnostic ions.
- The EThcD and EED fragmentation patterns were noticeably different. For example, the high-abundance $^{1,5}X$ ions typically found in the EED spectra were often absent in the EThcD spectra.

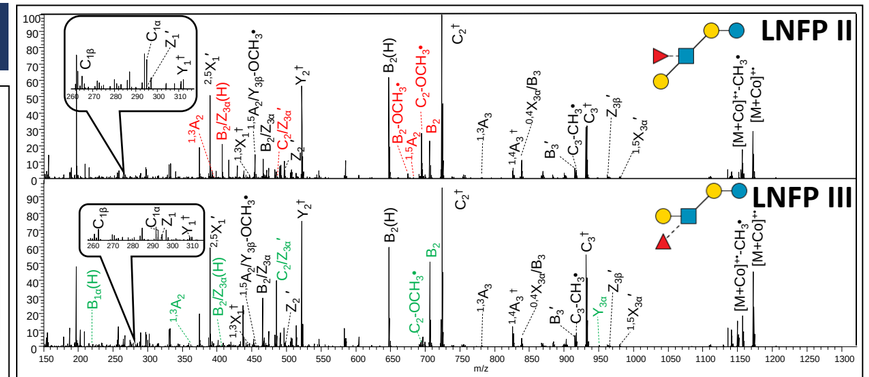


Figure 3. Comparison of the EThcD MS/MS spectra of deutero-reduced and permethylated LNFP II and III ($[M+Co]^{2+}$). Linkage-diagnostic fragments are labeled in color.

- The EThcD MS/MS spectra of LNFP II and III were very similar. However, a number of fragment ions were either unique to one isomer or displayed significant differences in their abundances between two isomers, and may be used to infer the linkage positions.
- Unique fragments: $^{1,5}A_2$ and B_2-OCH_3• were only observed in the LNFP II spectrum; $B_{1\alpha}(H)$ and $Y_{3\alpha}$ were only present in the LNFP III spectrum.
- Fragments displaying significant differences in their relative abundances: $C_2/Z_{3\alpha}'$, $B_2/Z_{3\alpha}(H)$, C_2-OCH_3•, and B_2.

SUMMARY

- EThcD can produce an abundance of structurally informative fragments for elucidation of the glycan topology and linkage configuration, and for isomer differentiation.
- EThcD can serve as a more accessible alternative to EED for detailed glycan structural elucidation.
- EThcD and EED MS/MS spectra are very different due to differences in their underlying fragmentation mechanisms. Further studies of the EThcD process are needed to guide adaptation of existing bioinformatics software or development of new strategies for spectral interpretation.

REFERENCES AND ACKNOWLEDGEMENTS

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