Characterization of Glycoproteins by Top Down UVPD Analysis

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ABSTRACT

Purpose: To demonstrate the potential of UVPD MSMS allows for the structural characterization of glycoproteins.

Methods: Static nano-electrospray, UVPD and orbitrap mass spectrometry

Results: UVPD-MSMS enables for the characterization of glycan diversity and glycosite occupancy in glycoproteins.

INTRODUCTION

Ultraviolet photodissociation (UVPD) is a powerful tool for top-down proteomics due to the high efficiency nature of its fragmentation and large number of fragment ion types produced. While UVPD has been demonstrated for glycopeptide [1-3] and glycan [4-6] analysis, it has not yet been tested on intact glycoproteins. Mass spectrometry analysis of glycoproteins typically consists of either protein digestion followed by glycopeptide separation and identification by LC-MS/MS, or removal of the glycans and separate LC-MS/MS analysis of the glycans and/or peptides. Key disadvantages of these methods are that glycopeptides can go undetected due to poor ionization efficiency, and that removal of glycans from glycopeptides before mass spectrometry eliminates glycosylation site information.

Here we show the first steps in characterizing top-down UVPD for glycoprotein analysis. Glycoprotein standards ribonuclease B and κ-casein (models for N- and O-glycosylation, respectively) are used to show the diversity of glycan and glycosite information that can be obtained with top-down UVPD.

MATERIALS AND METHODS

Disulfide intact and reduced and alkylated glycoprotein ions were produced by nanoelectrospray ionization via either static nanospray or nanoLC/MS on a Dionex Ultimate 3000 RSLC. Ions were analyzed on a Thermo Orbitrap Fusion Lumos Tribrid MS (below), and top-down fragmentation was performed on isolated precursors with ultraviolet photodissociation (UVPD) at 213 nm using a solid-state Nd/YAG. Spectra were analyzed by hand. Bottom-up analysis was performed on CTLA4 using a Thermo Q Exactive Plus and high-energy collision-induced dissociation (HCD). Spectra were analyzed using Byonic.

Ribonuclease B, and κ-casein were purchased from Sigma Aldrich, both as lyophilized powders..

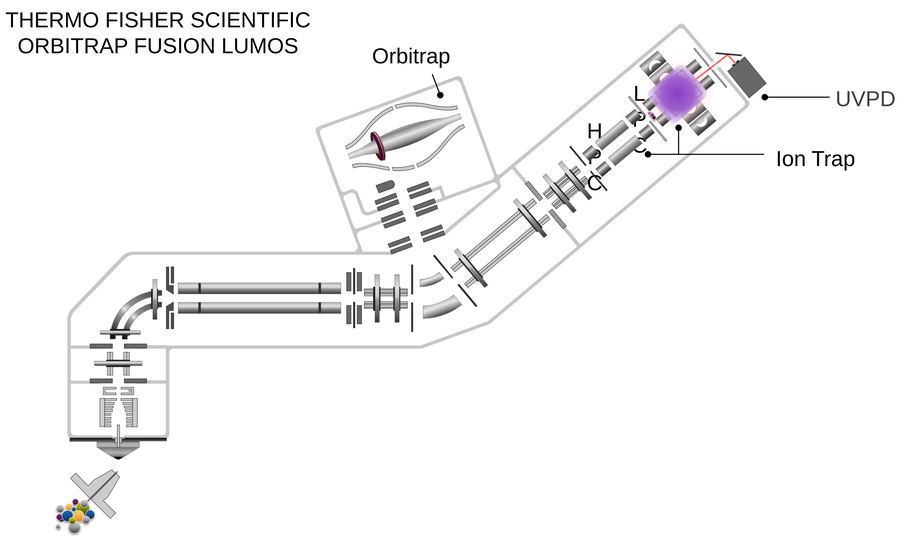

Figure 1. Schematic of a Thermo Fisher Scientific Orbitrap Fusion Lumos with a UVPD source

RESULTS

Ribonuclease B (RNase B): model for N-glycosylation

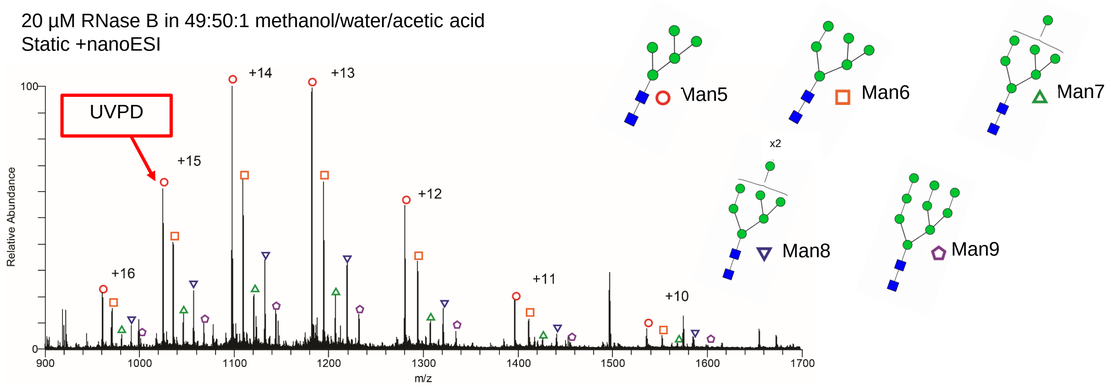

Figure 2. Full MS Rnase B showing one N-glycosite with five different high mannose glycoforms. Spectrum was acquired with static nanoESI which allows signal averaging over a long period of time and requires a minimal amount of sample compared to LC/MS or direct infusion

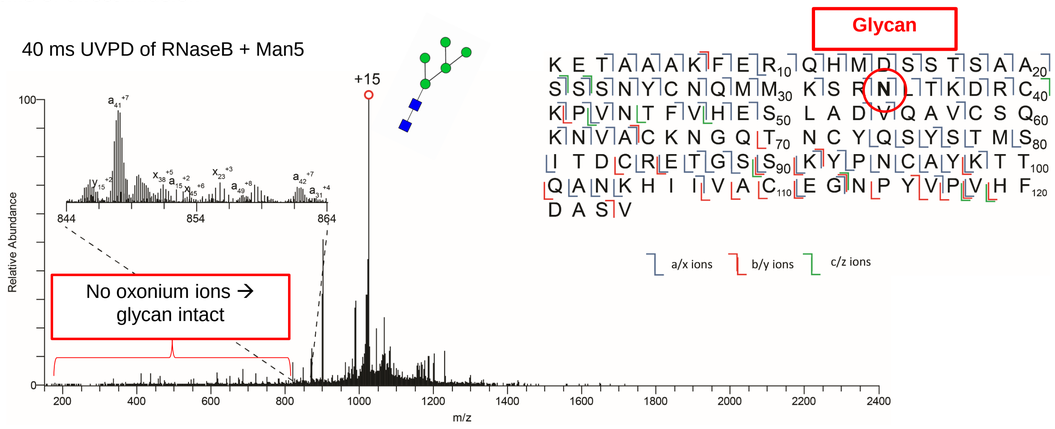

Figure 3. Top-down UVPD MSMS spectra of Man5 15+ species that resulted in fragmentation at 88/123 sites (72%). Predominantly a/x, often a+1 fragment ions were formed, consistent with previous UVPD experiments [7] No oxonium ions or fragments corresponding to glycan fragmentation indicating that glycans remain intact, which is consistent with the high gas phase stability of high mannose glycans [8]

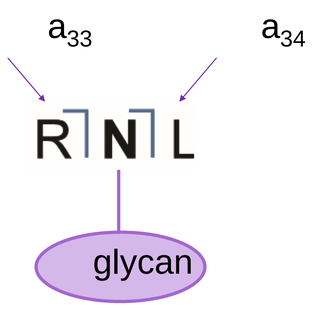

Figure 4. Single amino acid resolution obtained on the glycosite at N34 allows definitive localization of the glycan

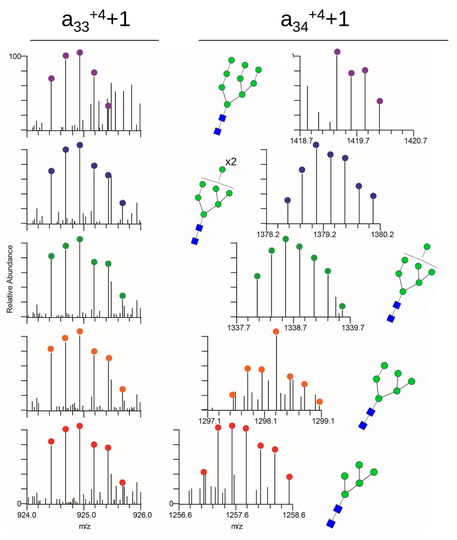

Figure 5 Monitoring bracketing fragment ions from each protein isoform indicating the existence of different glycan structures at a single site

RESULTS CONT'

K-CASEIN: MODEL FOR O-GLYCOSYLATION

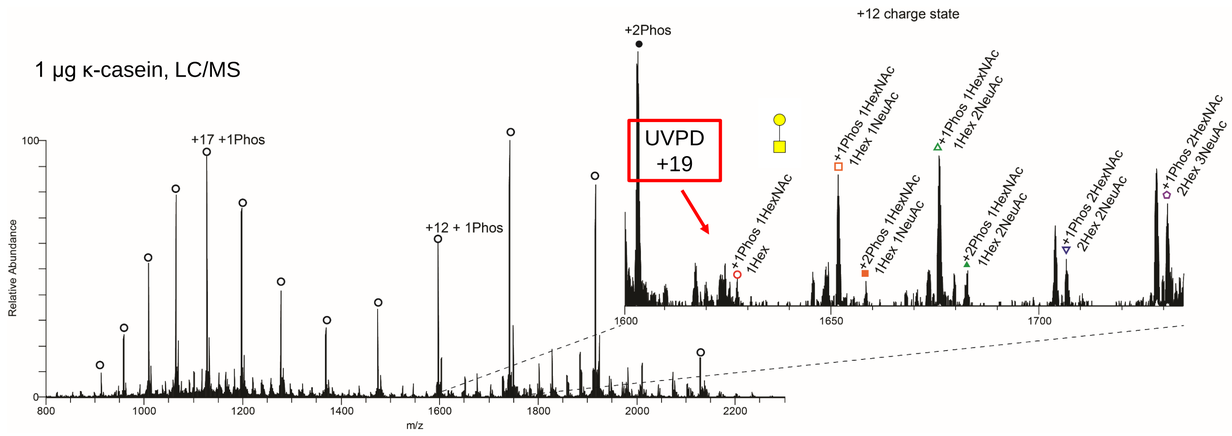

Figure 6. Full scan showing multiple O-glycosylated and phosphorylated proteoforms. K-Casein has 27 serine & threonine residues that are all possible O-glycosylation sites. However most glycosites are modified by simple sugar structures not exceeding 1HexNAc 1Hex 2NeuAc

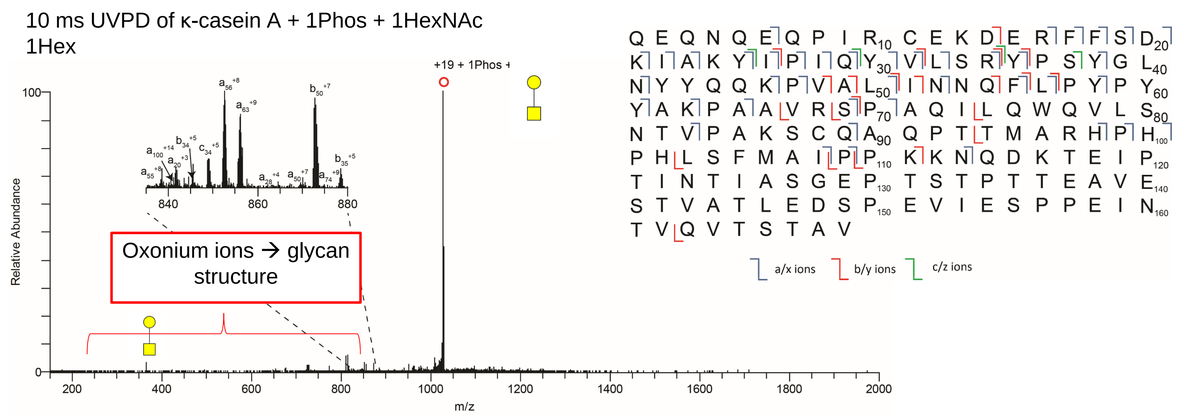

Figure 7 Top-down UVPD spectrum of 1Phos 1HexNAc 1Hex 19+ precursor (left) resulted in fragmentation at 54/168 sites (32%). Limited sequence coverage at C-terminus due to possible UVPnoD as there are very few basic residues in the C-terminal region. Predominantly a, b, and y ions with glycan intact were identified. Top-down UVPD of the 19+ ions of the other glycoforms of the monophosphorylated κ-casein A yielded nearly equivalent sequence coverage Oxonium ions present inform on glycan presence and structure.

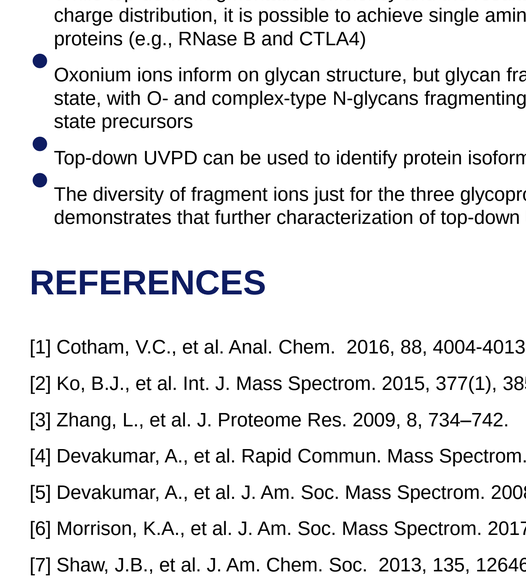

Figure 8. MSMS spectra showing site localization for one phosphosite and all glycosites to a 49 residue region, but no resolution of phosphosite and glycosites possible. Monitoring y fragment ions bracketing this region implies different glycan structures in region

CONCLUSIONS

- While top-down fragmentation efficiency and the resultant degree of localization of glycosites is highly dependent on precursor charge distribution, it is possible to achieve single amino acid resolution on glycosites and resolve different glycosites for some proteins (e.g., RNase B and CTLA4)
- Oxonium ions inform on glycan structure, but glycan fragmentation in UVPD is dependent on glycan type and precursor ion charge state, with O- and complex-type N-glycans fragmenting most easily and more glycan fragmentation occurring for higher charge-state precursors
- Top-down UVPD can be used to identify protein isoforms (e.g., CTLA4) – this is unique to top-down method!
- The diversity of fragment ions just for the three glycoproteins in this work and the potential for high resolution on glycosites demonstrates that further characterization of top-down UVPD of glycoproteins is warranted

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TRADEMARKS/LICENSING

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