Intact Mass Analysis of an 8-arm Branched PEG-protein Conjugate using Native Liquid Chromatography and Ultra-high Mass Range Orbitrap MS

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ABSTRACT

Purpose: Demonstration of a new platform for assessing protein-conjugation level on a macromolecular therapeutic PEG-protein conjugate drug.

Methods: Several workflows were explored through the combined use of native MS, proton sponge ESI dopants, liquid chromatography separation, and ultra-high mass range Orbitrap MS intact mass analysis.

Results: We found that there are several viable routes to determining protein conjugation level.

INTRODUCTION

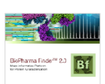
Monoclonal antibody Fab domains can be utilized therapeutically to bind to and sequester specific antigens to decrease activity of disease targets in the cells of patients. Large multi-pronged polyethylene glycol (PEG)-based structures can be utilized to "bundle" together multiple Fab domains and yield a drug with highly concentrated therapeutic activity and increased bioavailability. PEG-Fab conjugates pose enormous potential for sample heterogeneity, and thus present a significant analytical challenge in upstream characterization. Native MS and other charge reduction strategies yield increased spectral separation to allow the accurate intact mass analysis of such highly complex spectra. We present a semi-automated workflow for analyzing high mass PEG-protein conjugates utilizing native ion exchange coupled directly to an ultra-high mass range Orbitrap MS system.

MATERIALS AND METHODS

PEG-Fab conjugate was analyzed intact in native MS conditions using nanospray infusion or ion exchange chromatography (IEC). PEG-Fab conjugate was loaded onto a Thermo Scientific™ ProPac™ WCX ion exchange column and eluted using a gradient of pH 6.6 to 10.2 in a background of 50 mM ammonium acetate at a flow rate of 200 uL/min. LC-MS was performed using a Thermo Scientific™ Vanquish™ H-Class UHPLC with single wavelength UV detection coupled directly to an Orbitrap MS instrument capable of ultra-high mass range detection up to 80,000 *m/z*.

Vanquish Horizon UHPLC system

Thermo Scientific™ Q Exactive™ UHMR Orbitrap MS



Thermo Scientific™ BioPharma Finder™ 3.0 Data Analysis Software

RESULTS

Designing an Improved Drug Delivery System for Monoclonal Antibody Drug

A major aspect of characterizing PEG-Fab conjugates is determining the weighted average of Fab domains which are attached to an individual PEG core structure. In order to assess the degree of Fab-conjugation, PEG-Fab conjugate was analyzed by static nano-spray infusion using an ultra-high mass range Orbitrap MS system operated with nitrogen or xenon as the HCD trapping gas for cooling ions during transmission. PEG-Fab conjugate in native conditions resulted in a complex spectrum which did not yield sufficient definition in order to confidently determine accurate masses to measure the average ratio of Fab-to-PEG. Addition of the charge reducing reagent TMGN produced a more simplified spectrum spanning a range of 20,000 to 50,000 *m/z* which enabled deconvolution for accurate mass determination. These results showed an average load of approximately 7 Fab domains, which is consistent with a low resolution result attained by MALDI-TOF MS data. We coupled IEC directly to our Orbitrap MS system to allow charge-based separation and on-line native intact mass analysis. We found PEG-Fab conjugate isoforms elute as individual peaks in increasing order of Fab load. IEC-MS spectra became further simplified compared to direct nano-spray infusion. Isoform identity and abundance could be further confirmed and relatively compared by leveraging both chromatographic and mass spectral data for intact mass analysis.

Therapeutic antibodies such as Ranibizumab (trade name, Lucentis™), have been approved as an anti-angiogenic therapy for Age-related Macular Degeneration (AMD).

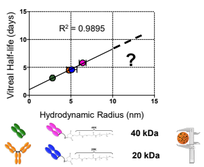
Adapted from https://en.wikipedia.org/wiki/Macular_degeneration

Adapted from http://thevisioncarecenter.com/we-treat/macular-degeneration/avastin/

PEGylation technology has been used for the modification of proteins, peptides, and antibody fragments for use as drugs.

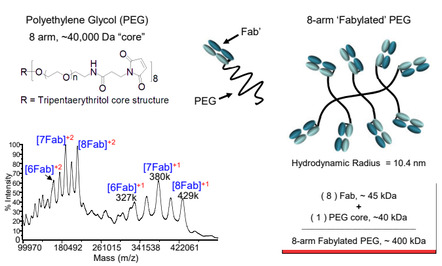
Advantages of PEGylation in Drug Design

- Water Soluble
- Increased bioavailability
- Increased blood circulation of the drug
- Optimized pharmacokinetics
- Decreased immunogenicity
- Decreased frequency of administration



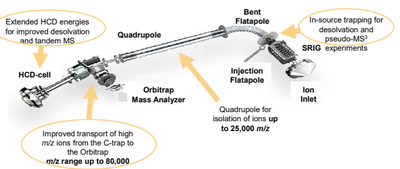
Adapted from Whitney Shatz

PEG-Protein Dendrimer Assemblies are Large, Labile Complex Mixtures

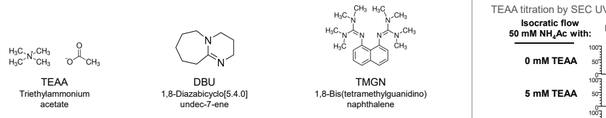


"Proton Sponge" Reagents Allow Further Simplification of Native MS Spectra Simplification at Ultra High *m/z*

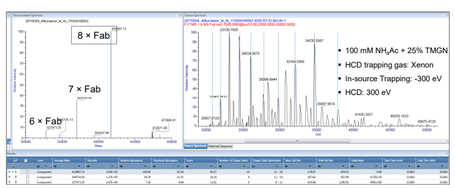
Q Exactive UHMR



Superbasic "proton sponges" reduce charge states of proteins by positive mode ESI-MS.

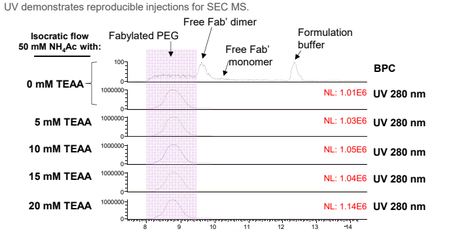


Nano-infusion MS

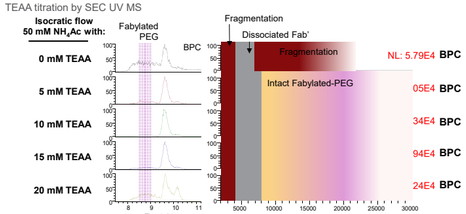


Size Exclusion LC-MS

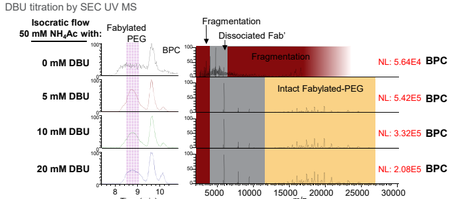
UV demonstrates reproducible injections for SEC MS.



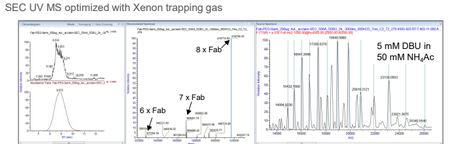
TEAA titration by SEC UV MS



DBU titration by SEC UV MS

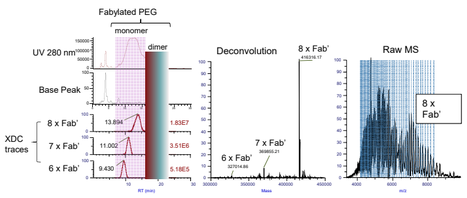


SEC UV MS optimized with Xenon trapping gas



Ion Exchange LC-MS

Weak cation exchange UV MS



CONCLUSIONS

Intact mass analysis of a complex PEG-protein conjugate using native ion exchange chromatography ultra-high mass range Orbitrap MS

TRADEMARKS/LICENSING

PO65314-EN 0518S

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