Middle-Down Analyses of Unmodified and Stressed Monoclonal Antibodies Using an Orbitrap Fusion Lumos

Trivid Mass Spectrometer

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ABSTRACT

Peptide mass fingerprinting is an essential step for the determination of amino acid sequences. For a middle-down experiment, one needs to identify the subunits, their masses, and the cleavage sites. To this end, we have used a Middle-Down strategy to get the sequence coverage of a mAb. Mass spectrometry coupled with a deconvolution algorithm was used. Time-defined MS/MS spectra were automatically deconvoluted using Xtract, and fragment ion maps were directly generated within the software. LC-MS raw files were processed with BioPharma Finder 3.0 software. For MS1 experiments, the sliding isolation window of 300 Da centered at m/z 798 was used. For all experiments, the isolation window of 300 Da centered at m/z 798 was used.

INTRODUCTION

Middle-down analyses rely on the tandem mass spectrometry technique to obtain sequence coverage over a given protein. This approach is commonly used to ensure that all subunits are identified and matched to the theoretical mass at less than 4 ppm. The results are then used for the identification of oxidation, glycosylation, and other modifications.

MATERIALS AND METHODS

Sample Preparation

Trastuzumab and scFv were used as a standard sample. The isolated samples were prepared by reducing and alkylating the sample in order to generate the cleaved subunits. The samples were then digested using trypsin for a specific time before being analyzed using LC-MS/MS.

Peptide mapping is the gold standard for in-depth characterization of biotherapeutics, but the sample preparation, LC runtime, and data analysis can be time-consuming. Considering that subunit mass analysis is the bottleneck for middle-down experiments; however, dedicated software developed for biotherapeutics helped identify the subunits and provided complementary fragmentation techniques.

RESULTS

ScFv. The middle-down analysis with a wide isolation window and ETD and UVPD modes of fragmentations provided with the Middle Down strategy was used. The highest sequence coverages, between 26% and 29%, were obtained for the NIST mAb. One targeted MS2 experiment was enough to pinpoint some sites of oxidation.

For the unmodified Fd, a sequence coverage of 44% was observed and the sequence coverage was 44% for the unmodified Fd.

CONCLUSIONS

High resolution coverage of mAb subunits can be obtained by combining different modes of fragmentation (ETD and UVPD). For scFv, the highest sequence coverage of 44% was obtained using multiplexing 5 charge states.

For a rapid identification of the oxidation sites, a wide isolation window was used, and the only mode of fragmentation was ETD. The high sequence coverage of 44% was obtained by combining ETD and UVPD modes of fragmentation. For a rapid identification of the oxidation sites, a wide isolation window was used, and the only mode of fragmentation was ETD. The high sequence coverage of 44% was obtained by combining ETD and UVPD modes of fragmentation.