APPLICATION NOTE FOR THERMO SCIENTIFIC QE ORBITRAP™ MS

ECD spectra of NIST mAb with low and high mass resolution

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Introduction

Mass spectrometry is now widely used for sequencing monoclonal antibodies (mAbs). After digestion of antibodies into peptides, depending on the type of enzymes, either middle-down or bottom-up mass spectrometry (MS) approaches might be applied for the antibody sequencing. The digestion is, however, not only time consuming but also quite costly procedure since it requires availability of expensive enzymes and reducing agents. Another potential drawback of digestion is partial or complete loss of direct information on the proteoforms present in proteins[1,2]. Top-down MS technique is an alternative approach for protein sequencing where whole proteins are analyzed directly. Because of complexity of the data generated and various technical limitations, the top-down approach might have even more challenges than its bottom-up alternative. Applying traditional collisional activation (CID) during top-down analysis might result in unwanted loss of post-translation modifications reducing main advantage of the top-down technique in identification of proteoforms. Here we show how electron capture dissociation (ECD) with rather mild supplemental activation can be used in the MS top-down approach for sequencing antibodies using NIST mAb as a case study.

Experimental

Sample preparation

NIST mAb was bought from NIST and was cleaned via buffer exchange before using it in the experiments. The NIST mAb sample of 1 mg/mL concentration was dissolved in 100 mM ammonium acetate in both static nanospray injection and HPLC experiments.

Instrumentation

ECD spectra of the NIST mAb were obtained with an ExD cell (e-MSion, Inc.)[3,4] installed on a Thermo Scientific Q Exactive Orbitrap mass spectrometer (Figure 1). Agilent 1200 Series liquid chromatograph was used in liquid chromatography-MS experiments with the following characteristics: Agilent PLRP-S column at 8 min gradient, 1 µL injection volume, 0.2 mL/min flow rate. In the static nanospary experiments, custom-made tips of 1.5 micron ID were used. The autotune feature in the ExDControl software (e-MSion, Inc.) was used to optimize the ExD cell voltage profiles for transmission and for ECD fragmentation, both with the filament heating current set to 2.2 A. To tune for transmission, Thermo-Fisher calibrant solution was used, whereas tuning for ECD the peptide standard Substance P and carbonic anhydrase were used for efficient ECD, whereas for pronouncedly formed peaks of Light chain with less ECD-fragmentation in the ECD spectra, the NIST mAb itself was used for the ECD tuning. To analyze the EChcD spectra, Viewer software (e-MSion, Inc.) was used at 10 ppm tolerance for masses.

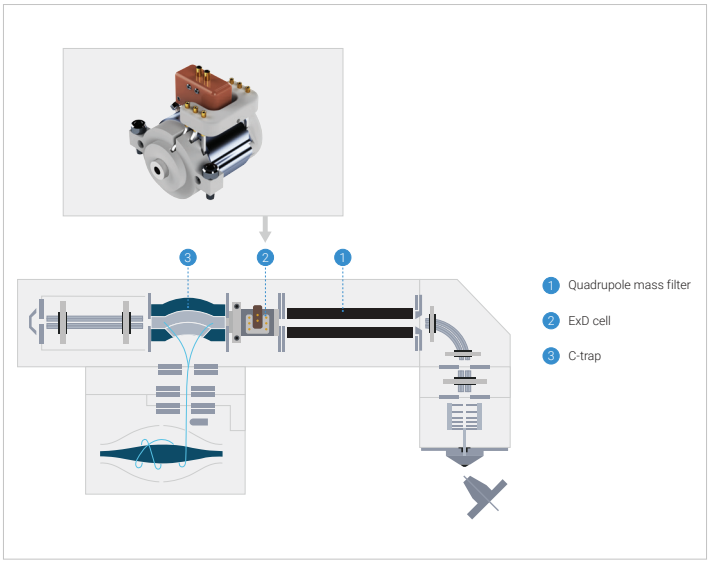

Figure 1. Schematic of the ExD cell in the Thermo Scientific Q Exactive Orbitrap mass spectrometer.

Results and Discussion

Figure 2 shows the mass spectrum of NIST mAb obtained in MS2 mode to involve the instrument HCD cell at higher gas pressure for better cooling and trapping the ion beam. Lowest default for the QE instrument HCD = 10 V energy was used to prevent fragmentation of the NIST mAb via CID processes. For better sample desolvation in-source CID (In-SCID) of 50 V was applied. The ExD cell was tuned for transmission. At these experimental conditions, fragmentation via ECD-CID was minimized and low-resolution mass spectrum consisted only of peaks of intact NIST mAb (Figure 2).

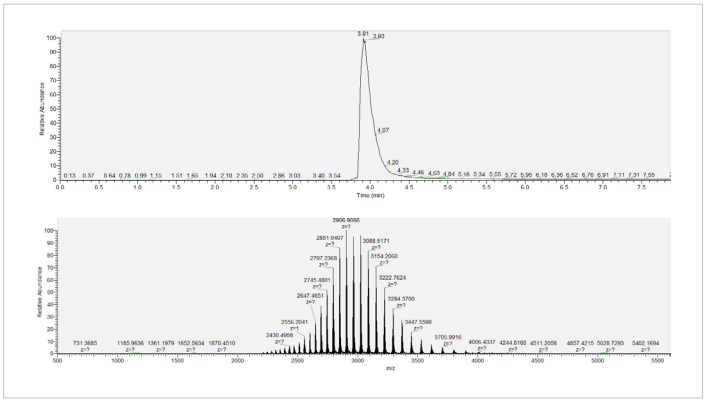

Figure 2. Low-resolution MS1 spectrum of NIST mAb recorded at higher HCD gas pressure (ca. 0.7 mPa), HCD = 10 V and In-SCID 50 V. The sample was introduced via PLRP-S column.

Increasing HCD energy to 30 V and In-SCID to 80 V with the ExD cell being tuned for pronounced formation of peaks of the light chain (LC), using the ExD tune profiles made for substance P and carbonic anhydrase as starting voltage set, resulted in the EChcD spectrum shown in Figure 3. Increasing the HCD energy to 40 V led to larger relative efficiency of the LC peaks with respect to the peaks of intact NIST mAb (spectrum is not shown here). Such top-down approach with detection of peaks of LC is fast and efficient approach for identification of antibodies, especially those modified with attachment of different drugs known as antibody-drug-conjugates (ADC).

Increasing mass resolution and decreasing the HCD gas pressure, allows for better identification of product ions formed via ECD. Figure 4 shows the ECD spectrum of NIST mAb recorded with the same ECD profile as was in the spectrum in Figure 3. Pronounced peaks of LC were still present in the spectrum. The dominant formation of *c*-ions over *z*-ions shows that ECD fragment ions were mainly

produced from LC as part of the intact NIST mAb. Using Viewer software helped to determine complementarity determining regions (CDR) in NIST mAb (Figure 5).

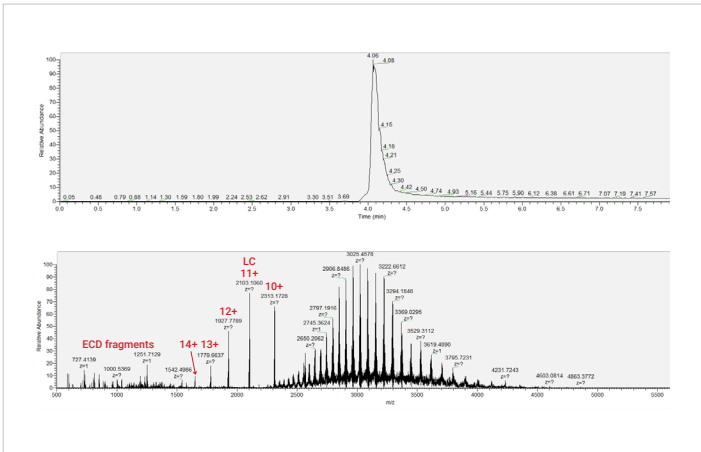

Figure 3. EChcD spectrum of NIST mAb recorded with low mass resolution and higher HCD gas pressure (ca. 0.7 MPa) at supplemental activation HCD = 30 V and In-SCID = 80 V. The sample was introduced via PLRP-S column. Isolation window was over *m/z* 2200-6000. Peaks of light chain (LC) of different charge state were formed via ECD fragmentation from intact NIST mAb.

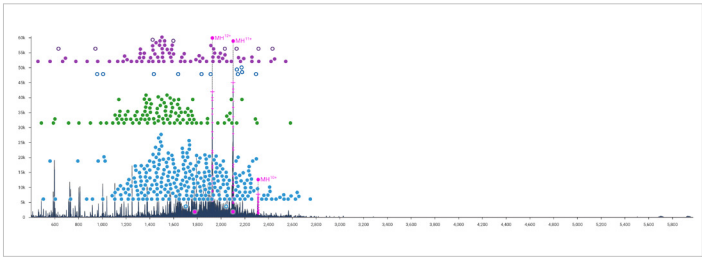

Figure 4. EChcD spectrum of NIST mAb recorded with High (140 K) resolution and HCD gas pressure ca. 0.5 MPa at supplememntal activation HCD = 15 V and In-SCID = 100V. Intact NIST mAb almost completely fragmented into intact light chain (charge states from 10+ to 13+ were observed) as well as ECD and CID-fragments of LC and heavy chain. Blue filled circles show ECD-type *c*- and *z*-fragments, green filled circles are CID-type *b*- and *y*-fragments, purple filled circles are *a*-ions, open circles are secondary *d*- and *w*-ions.

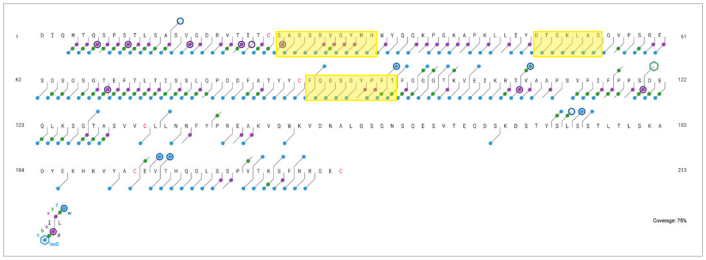

Figure 5. Sequence coverage determined by Viewer from EChcD spectrum of NIST mAb in Figure 4. Complementarity determining regions (CDR) are highlighted in yellow.

References

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