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Top-down characterization of native monoclonal antibodies obtained with electron capture dissociation on Q-TOF instruments

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Introduction

Monoclonal antibodies (mAbs) are an important class of therapeutics that are used for the treatment of a variety of illnesses from cancer to autoimmune diseases. Their production is often accompanied by unwanted post-translation modifications and structure changes that should be reliably monitored. Bottom-up and middle-down approaches are currently the most common mass spectrometric (MS) methods for mAbs analysis. Both these methods presume long and complex sample preparation procedures and complete or partial loss of information on protein's proteoforms. Top-down MS is free of these drawbacks; however, the top-down approach usually suffers from low ion abundance. In this study, we show that electron capture dissociation (ECD) with top-down approach can be used for analysis of native intact mAbs with very high efficiency. Characterization of the complementarity determining regions (CDRs) in the light (LC) and heavy (HC) , particularly the CDR-3 regions, was the focus of these efforts.

Experimental

Sample Preparation

NIST mAb was purchased from NIST (Gaithersburg, MD). SigmaMAb and Infliximab (European Pharmacopoeia Reference Standard) were purchased from Sigma-Aldrich (St. Louis, MO). Intact mAbs were buffer exchanged into 100 mM ammonium acetate using Amicon Ultra 0.5 mL 10 kDa centrifugal molecular weight cutoff filters (Sigma-Aldrich, St. Louis, MO, USA). Working solutions of intact mAb were prepared at 1 mg/mL in 100 mM ammonium acetate before to introduce into mass spectrometer.

Data Analysis

Data processing was performed with Agilent MassHunter Qualitative Analysis and ExDViewer (e-MSion part of Agilent, USA) softwares. ExDViewer allowed determination of protein sequence coverage and evaluation of the ECD efficiency based on ion intensities for all isotopic peaks. ExDViewer was also used in real-time streaming experiments for tuning instruments equipped with the ExD cells in both MS1 and MS2 modes.

Experimental

Instrumental Analysis

Electron Capture Dissociation (ECD) experiments were performed using both 6545 and 6545XT AdvanceBio LC/Q-TOF (Agilent, USA) mass spectrometers modified to enable ECD by installation of a second generation ExD cell (e-MSion part of Agilent, USA). The ExD cell (Figure 1) consists of seven electrostatic lens elements, two ring magnets, and an electron emitting filament. All voltages are supplied by a separate power supply regulated by ExD Control software (e-MSion - Part of Agilent, USA). The new generation ExD cell allowed dramatic increase of the ECD efficiency for both peptide and proteins. Static nanospray was used to introduce the antibodies (1 mg/ml, 100 mM ammonium acetate). The mass scale was calibrated with Agilent tune mix. Peptide Substance P and native carbonic anhydrase were used for tuning ExD cell. ECD spectra of antibodies were recorded in MS1 mode using a low-mass cut-off to eliminate ions below approximately *m/z* 4,000. On-line streaming with ExDViewer was applied to determine the optimal collisional energy to assist in ECD fragmentation. For these intact antibodies, supplemental energies of 100 V or higher were found to be beneficial.

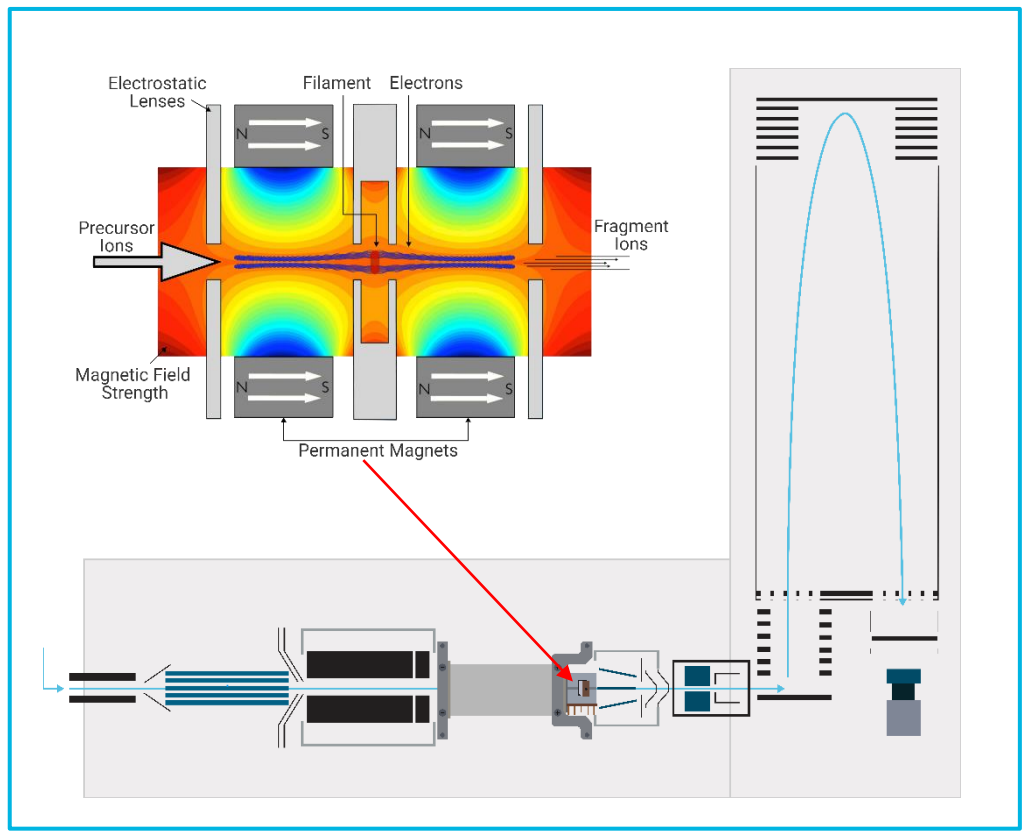

Figure 1. Schematic of Agilent 6545XT AdvanceBio LC/Q-TOF mass spectrometer with e-MSon- a part of Agilent ExD cell (top left; its position in the instrument is indicated by the red arrow) implemented between the shortened collision cell and IBC.

Results and Discussion

In the present work, intact monoclonal antibodies NIST mAb, SigmaMAb, and Infliximab were fragmented using ECD with and without complementary collisional activation. Collisional energies from 0 to 200 V were applied in experiments to find the optimal supplementary vibrational activation prior to ECD to break noncovalent intramolecular bonds and thus partially unfold antibodies (Figure 2). For these experiments, the intensities of ECD peaks were higher in comparison to CID peaks over all range of collision energies used (Figure 3). ECD fragmentation resulted in 45-50% for HCs (Figure 4) and in 75-90% sequence coverage for the LCs (Figure 5) of intact monoclonal antibodies.

Only the complementarity determining region CDR-3 was determined with a high confidence for the HCs (Figure 4). For the LCs, sequence-specific ECD fragments were identified with high confidence for all three CDRs (Figures 5 and 6). With a slightly different tune profile, ECD was able to selectively fragment disulfide bonds of antibodies, with generation of prominent peaks of the intact LC in 9+ to 12+ charge states, which, in turn, are ECD-fragments from intact mAbs, (Figure 2, bottom panel). With this tune, a stronger competition between ECD and CID processes was observed. ECD peaks were more abundant at lower CID energies, whereas CID-fragment peaks became more intense at higher collision energies (Figure 7).

ECD analysis of NIST mAb

Two ECD spectra of NIST mAb both recorded with supplementary activation of 100 V are depicted in Figure 2. The top spectrum was recorded with the instrument tuned for efficient ECD. The bottom spectrum was recorded with a tune file made for selective cleavage of disulfide bonds to enhance formation of intact LC peaks from NIST mAb.

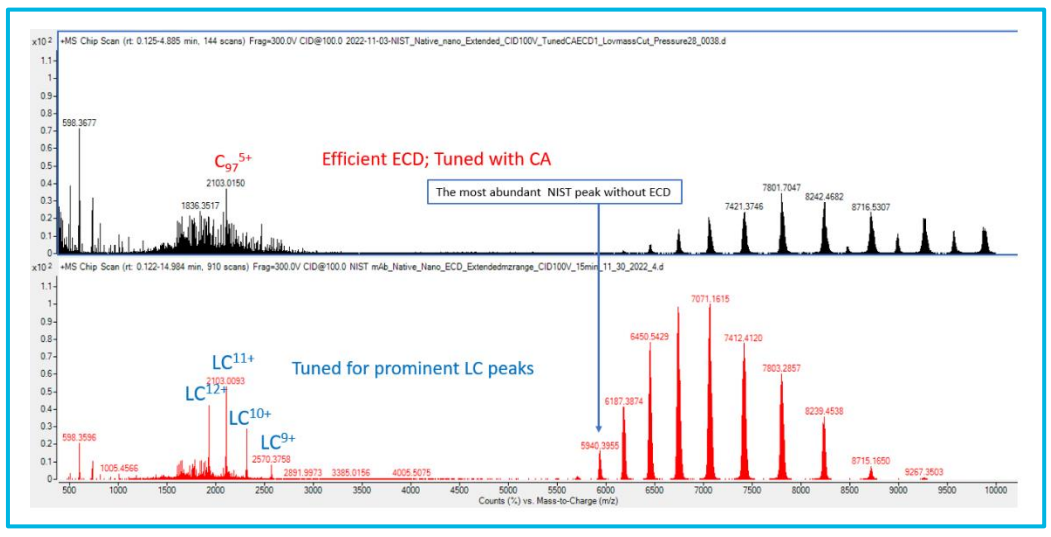

Figure 2. ECD product ion spectrum of NIST mAb performed in MS1 mode with low-mass cut-off and collisional energy of 100 V. Peaks below *m/z* 4,000 are fragmentation products. The top is spectrum produced when the ExD cell was tuned for efficient ECD. Bottom spectrum was recorded when ExD cell was tuned for generation of prominent LC peaks as ECD-fragments from intact NIST mAb.

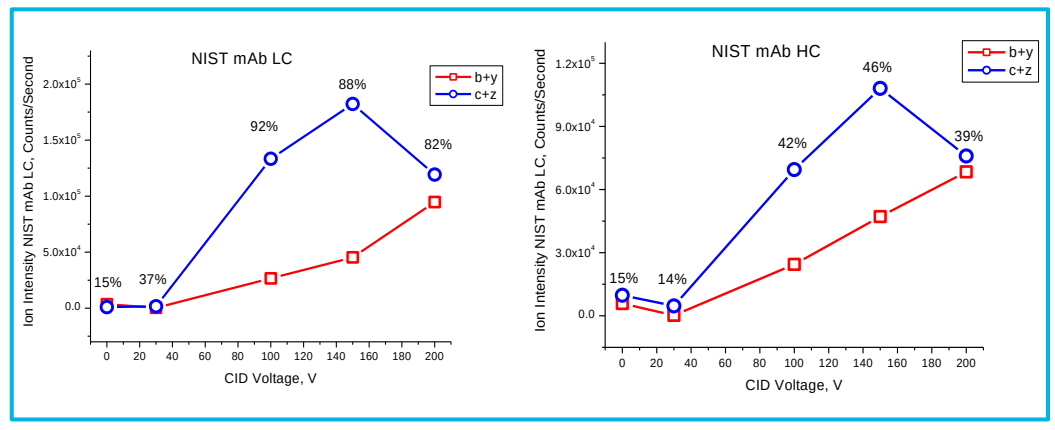

Figure 3. Ion intensities for the sum of the ECD-fragments (c- and z-type) and CID fragments (b- and y-type) as a function of collisional energy for NIST mAb light chain (left) and heavy chain (right) determined with ExD cell tuned for higher efficient ECD.

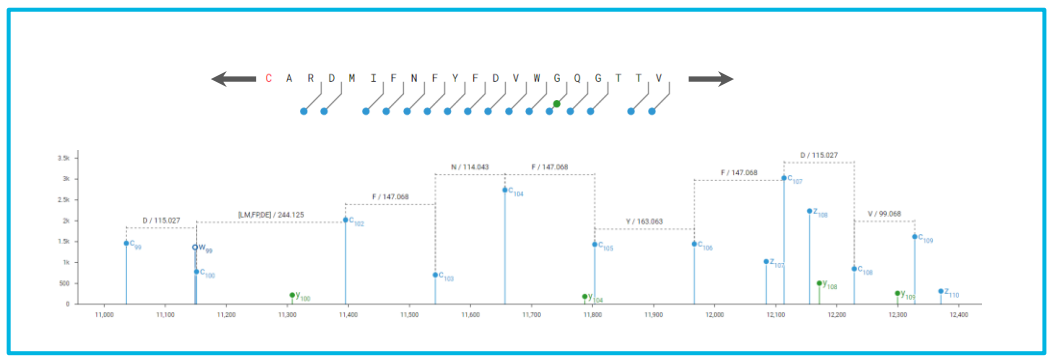

Figure 4. The decharged NIST mAb Heavy Chain CDR-3 region in ExDViewer, showing the feasibility of *de novo* sequencing. Sequence coverage for the HC was 48% in this experiment.

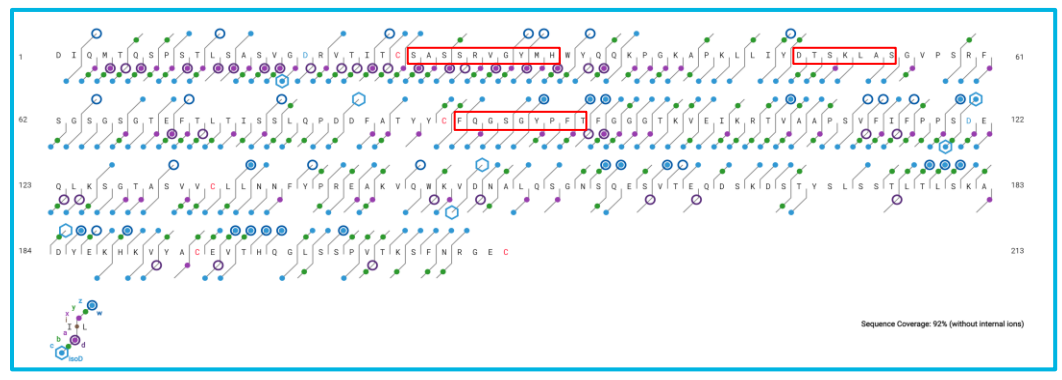

Figure 5. Sequence map showing greater than 90% coverage of NIST mAB LC (including internal fragments resulted in 100% coverage). The supplemental collision energy was 100 V and the ExD cell tuned for higher ECD efficiency. Localization of the CDR regions is indicated by red-line boxes.

ECD analysis of Infliximab

Intact Infliximab was also analyzed with ECD and complementary collisional energies at two different ECD tune-files. Figure 6 shows analysis of ECD spectrum made with ExDViewer.

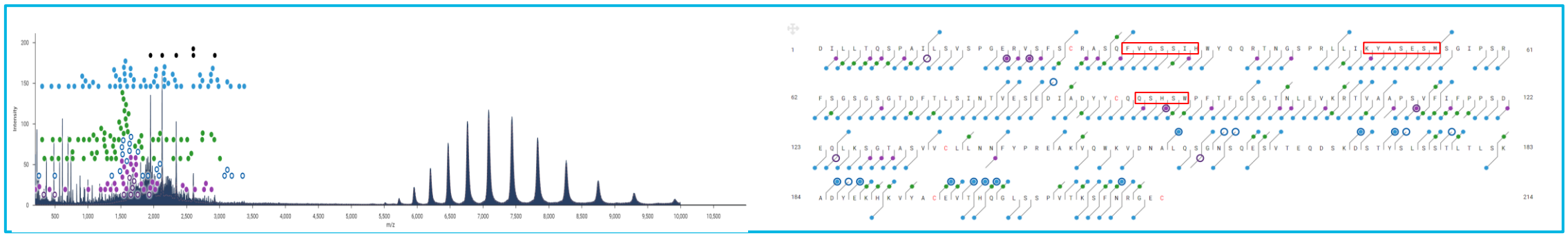

Figure 6. ECD spectrum (left) and sequence map (right) with 79% coverage for Infliximab LC with ECD and supplementary activation of 100 V and ExD cell tuned for prominent LC peaks. Localization of the CDR regions is indicated by red-line boxes (right).

ECD analysis of SigmaMAb

Based on the ECD profile tuned to produce prominent LC peaks, SigmaMAb was the only antibody studied that showed more abundant CID fragments compared with ECD fragment' intensities with increasing collisional energy (Figure 7).

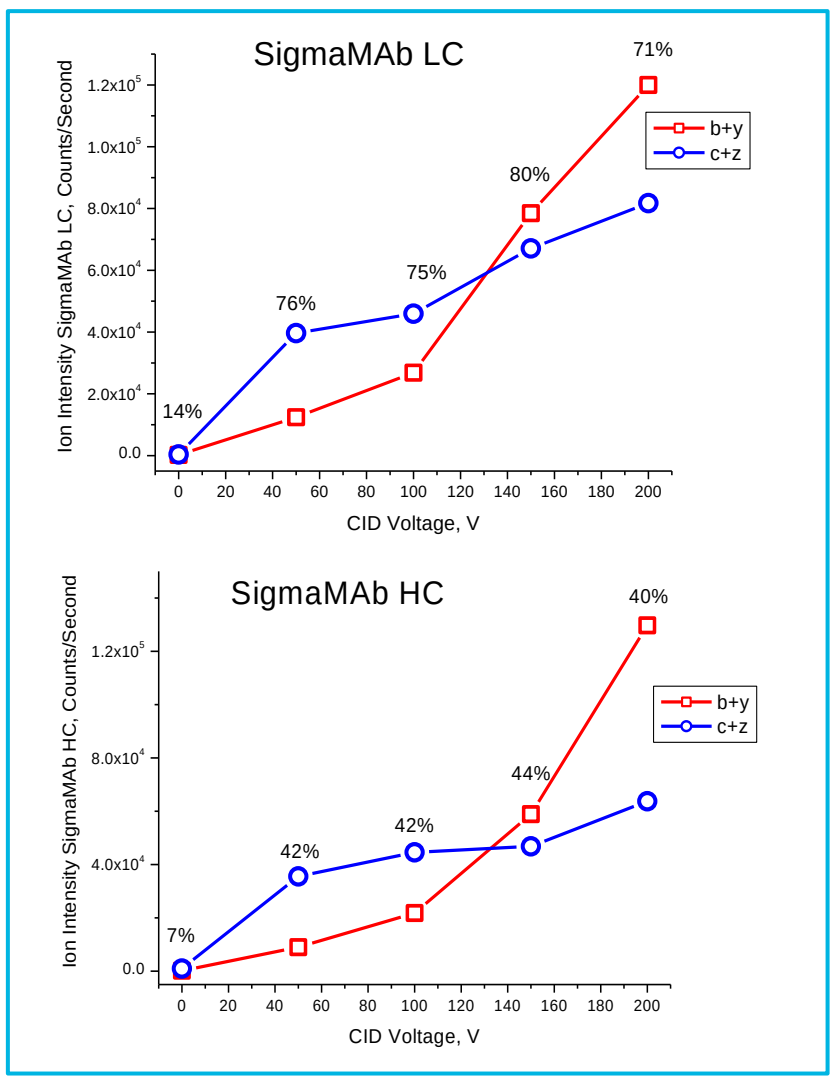

Figure 7. Ion intensities for the sum of the ECD fragments and CID fragments as functions of collisional energy for SigmaMAb light chain (top) and heavy chain (bottom) determined with the ExD cell tuned for prominent LC peaks.

Conclusions

- ECD spectra of intact monoclonal antibodies were studied with supplementary collisional activation applied prior to ECD to break internal fragments and partially unfold mAbs. Collisional energy of 100 V was found to be the most universal supplementary energy sufficient for protein partial unfolding and for better ECD.
- Two types of the ExD cell tuning profiles were used, one for efficient ECD-type fragmentation, and one to selectively fragment disulfide bonds for production of prominent peaks of the antibody's intact light chains.
- Applying the efficient ECD profile always resulted in dominant ECD peaks even at very high collisional energies. Using different tuning for prominent LC peaks in the case of SigmaMAb resulted in higher CID peaks at high CID energies showing competition between ECD and CID-type fragmentations.
- ECD fragment ions covering all three CDR regions were identified for the antibody light chains, whereas only the CDR-3 region was determined with a high confidence for the heavy chains.
- Including internal fragments in the sequence map analysis resulted in localization of the type and positions for the glycans and their combinations in the heavy chains.

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