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Bottom-up and Top-down Disulfide Bond Mapping of Beta-lactoglobulin on a Q-TOF with the Capability to Perform both CID and ECD

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Introduction

Characterization of disulfide bonds is of high importance for the structural elucidation of proteins. Using conventional bottom-up approaches, disulfide bonds are reduced and alkylated prior to enzymatic digestion with loss of disulfide bond connectivity. Digestion under non-reducing conditions generates disulfide-linked peptides for analysis via liquid chromatography-tandem mass spectrometry (LC-MS/MS). With top-down MS, the presence of post-translation modifications can be determined by the intact protein mass spectrum and modification sites can be further determined by MS/MS. Collision-induced dissociation (CID) has reduced fragmentation of the protein backbone inside a disulfide loop, while electron capture dissociation (ECD) preferentially cleaves disulfide bonds. Here we present a bottom-up and top-down disulfide bond analysis of beta-lactoglobulin with a quadrupole time-of-flight (Q-TOF) instrument capable of both CID and ECD.

Experimental

Sample Preparation

Beta-lactoglobulin (Sigma-Aldrich, St. Louis, MO) was either alkylated with iodoacetamide and subjected to tryptic digestion or prepared in water and desalted with Amicon Ultra Centrifugal Filters (Sigma-Aldrich, St. Louis, MO). The digested sample was reconstituted in 99:1:0.1 water (H_2O)/acetonitrile (ACN)/formic acid (FA) at a concentration of 1 µg/µL, while the intact sample was diluted with 50:50:0.1 H_2O/ACN/FA to a final concentration of 10 µM.

Instrumental Analysis

Peptide mapping was performed with a 1290 Infinity II LC system coupled to the 6545XT AdvanceBio LC/Q-TOF (Agilent Technologies, Santa Clara, CA), while intact protein samples for top-down analysis were infused at a flow rate of 0.75 mL/hr. The Q-TOF incorporated an ExD cell (e-MSion Inc., Corvallis, OR) to perform both CID and ECD on the same instrument without any hardware modifications when switching between the two fragmentation techniques. Data was analyzed with Byos (Protein Metrics, Cupertino, CA) or ExD Viewer (e-MSion Inc., Corvallis, OR).

Figure 1. 6545XT AdvanceBio LC/Q-TOF retrofitted with an ExD cell

Experimental

Instrumental Analysis

Q-TOF conditions	Value
Source	Dual ESI
Gas Temp	250 °C
Gas Flow	11 L/min
Nebulizer	35 psig
Vcap	5000 V
Fragmentor	175 V
Skimmer	65 V
Reference Mass	922.0098 *m/z*
Acquisition Mode	Positive, Standard (3200 *m/z*) Mass Range, 2 GHz

Table 1. Q-TOF Instrument Parameters for the intact analysis of beta-lactoglobulin

LC and Q-TOF conditions	Value
Source	Dual Agilent Jet Stream
Gas Temp	300 °C
Gas Flow	13 L/min
Nebulizer	35 psig
Sheath Gas Temp	275 °C
Sheath Gas Flow	12 L/min
VCap	3500 V
Nozzle Voltage	0 V
Fragmentor	175 V
Skimmer	45 V
Column	AdvanceBio Peptide Plus, 2.1 X 150 mm, 100 Å, 2.7 µm
Thermostat	4 °C
Solvent A	0.1% Formic Acid in DI Water
Solvent B	0.1% Formic Acid in 100% Acetonitrile
Gradient (first three minutes to waste) (stoptime 107 minutes) (posttime 1 minute)	0 minutes 1% B 3 minutes 1% B 93 minutes 35% B 97 minutes 95% B 102 minutes 95% B 103 minutes 1% B
Column Temperature	60 °C
Flow rate	0.400 mL/min
Injection Volume	4 µg

Table 2. LC/Q-TOF Instrument Parameters for the non-reduced tryptic digest of beta-lactoglobulin

Top-down Analysis of beta-lactoglobulin

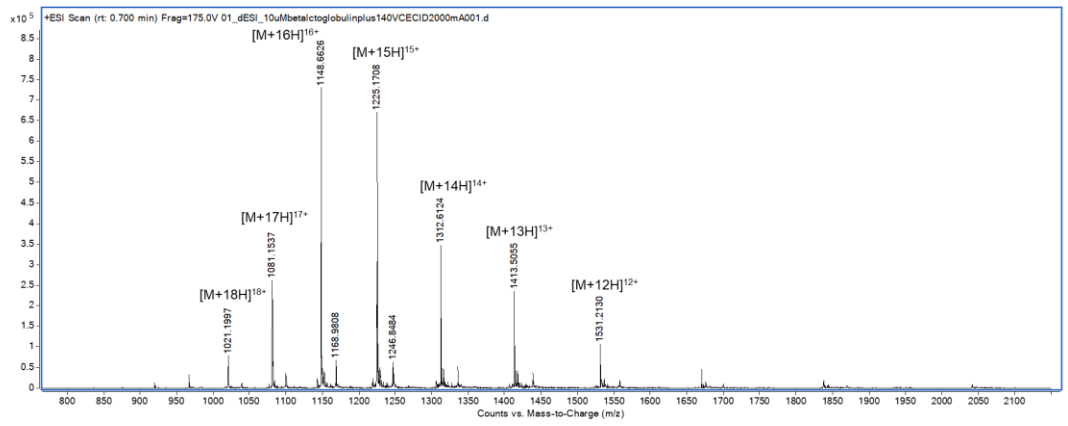

Figure 2. Mass spectrum of 10 µM beta-lactoglobulin

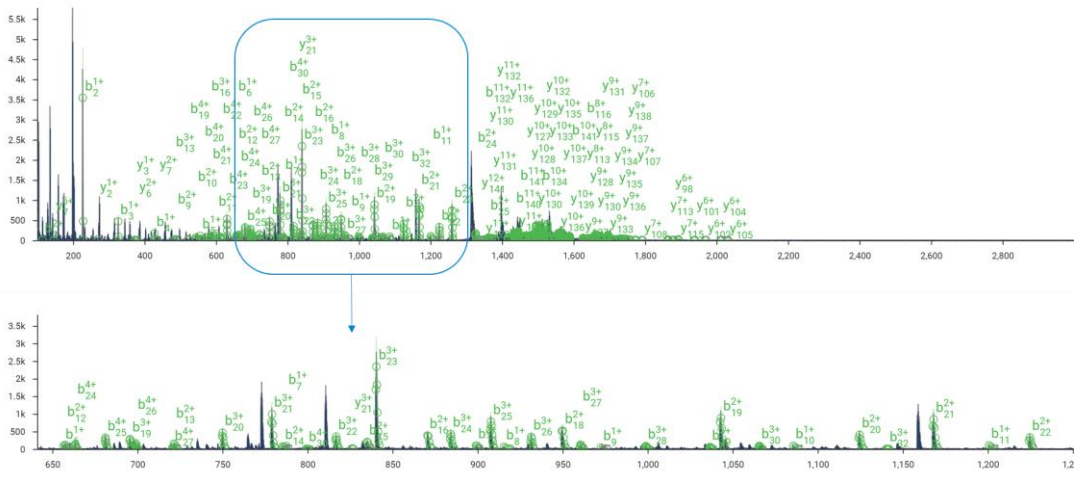

Figure 3. CID for the 14+ charge state of beta-lactoglobulin with a collision energy of 42 V applied

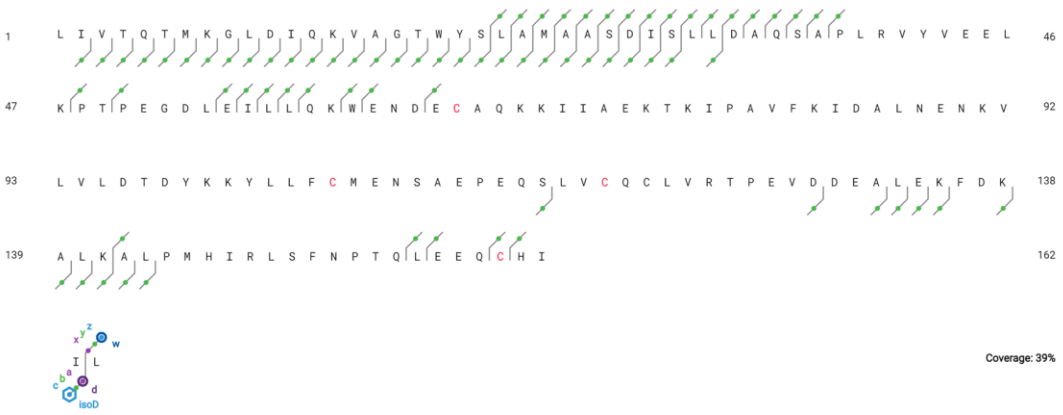

Figure 4. CID sequence coverage map for the 14+ charge state of beta-lactoglobulin

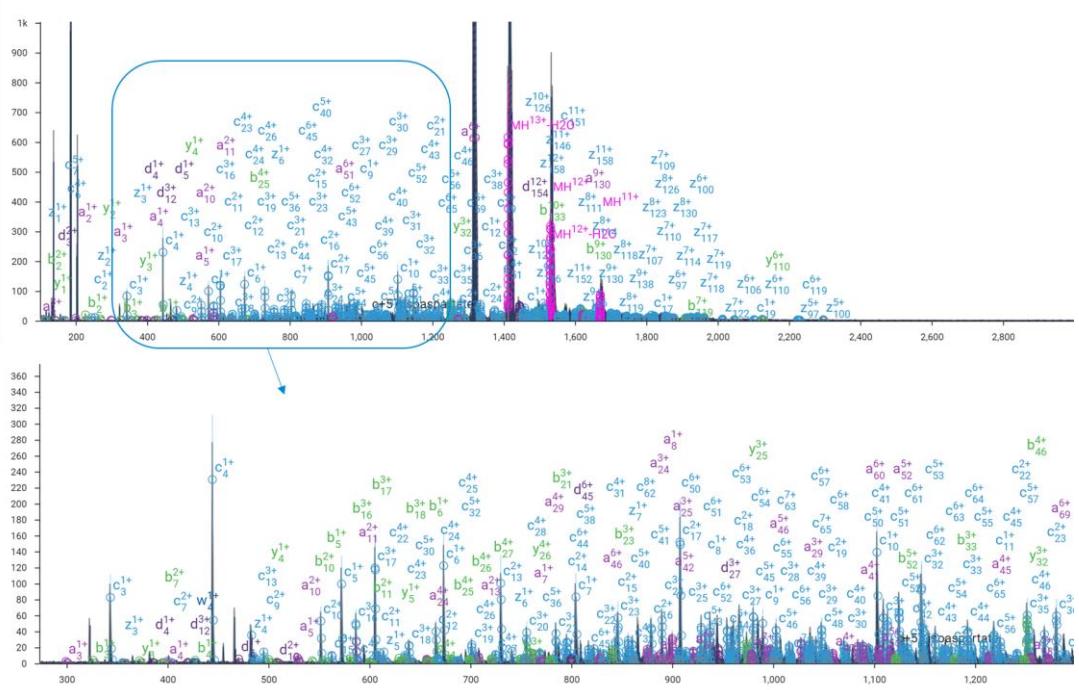

Figure 5. ECD for the 14+ charge state of beta-lactoglobulin

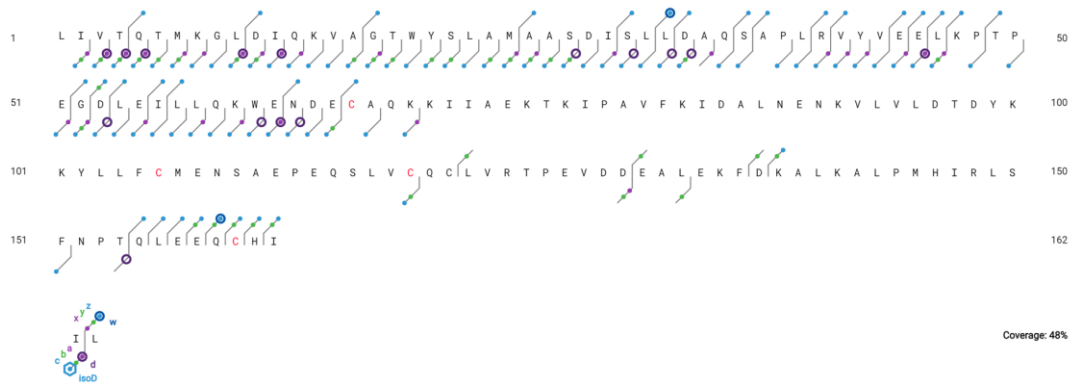

Figure 6. ECD sequence coverage map for the 14+ charge state of beta-lactoglobulin

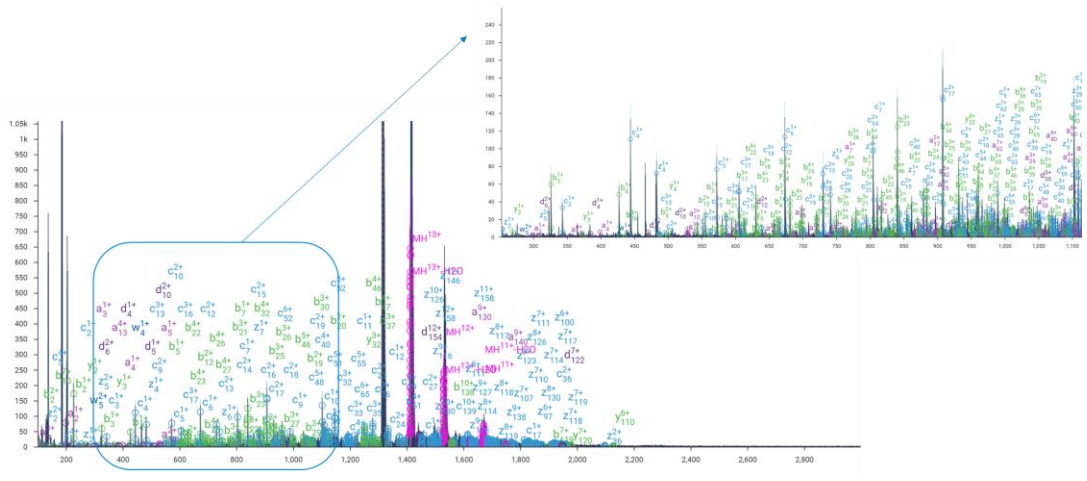

Figure 7. ECD for the 14+ charge state of beta-lactoglobulin with a CID collision energy of 10 V applied

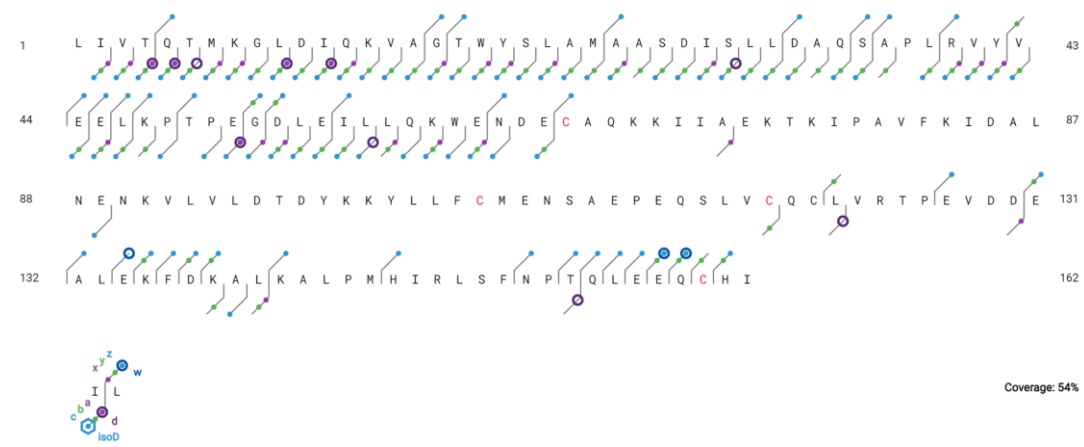

Figure 8. ECD sequence coverage map for the 14+ charge state of beta-lactoglobulin with a CID collision energy of 10 V applied

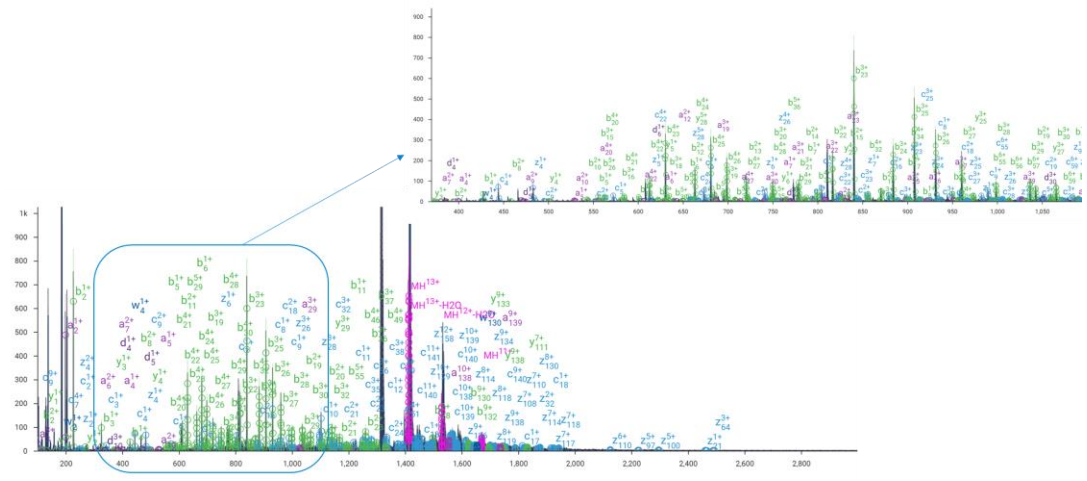

Figure 9. ECD for the 14+ charge state of beta-lactoglobulin with a CID collision energy of 20 V applied

Results and Discussion

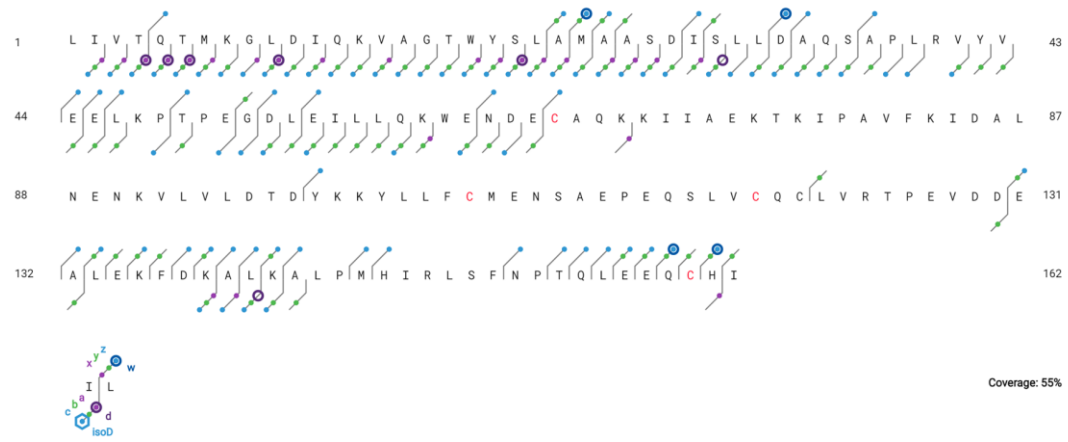

Figure 10. ECD sequence coverage map for the 14+ charge state of beta-lactoglobulin with a CID collision energy of 20 V applied

Bottom-up Analysis of beta-lactoglobulin

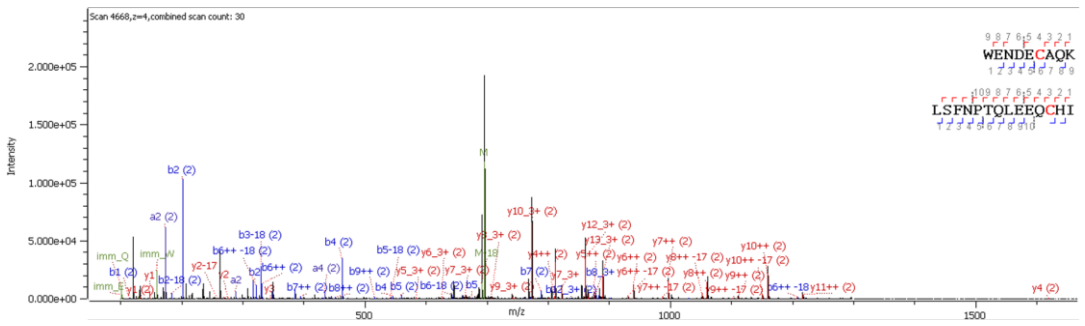

Figure 11. CID MS/MS spectrum for a peptide with an interpeptide disulfide bond of beta-lactoglobulin

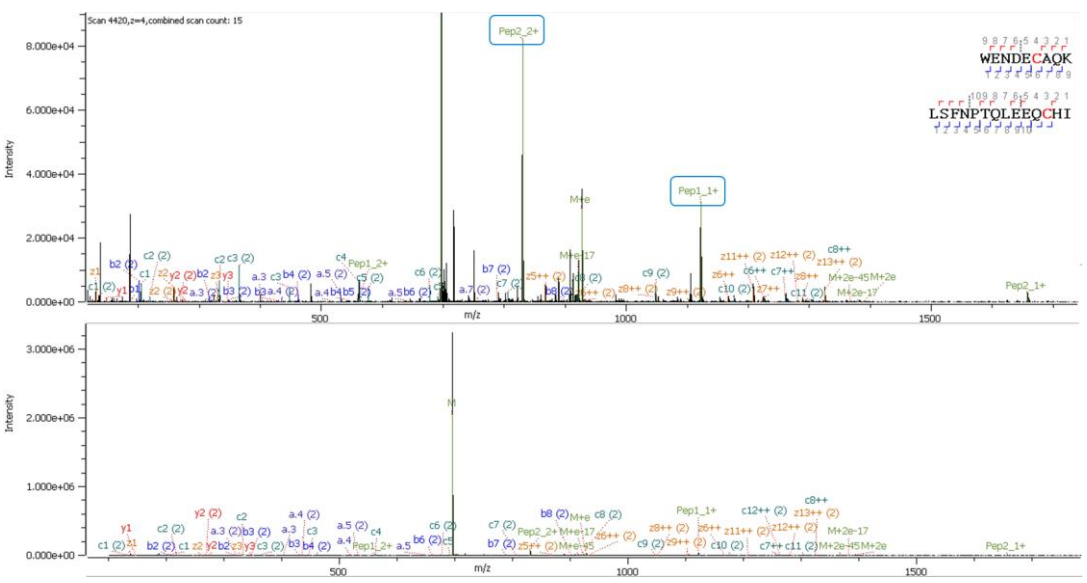

Figure 12. ECD MS/MS spectrum for a peptide with an interpeptide disulfide bond of beta-lactoglobulin. The inset in the top plot shows the mass spectrum of the individual peptides resulting from the cleavage of the disulfide bond

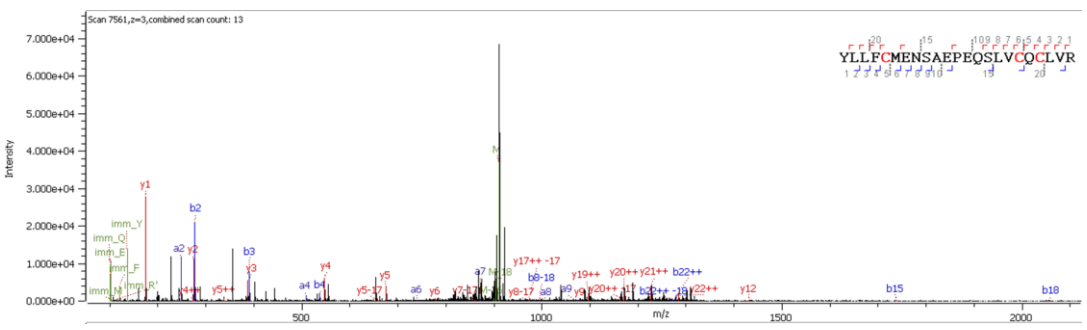

Figure 13. CID MS/MS spectrum for a peptide with an intrapeptide disulfide bond of beta-lactoglobulin between Cys_{106} and Cys_{119} (the fist two cysteines in the sequence above)

https://explore.agilent.com/asms

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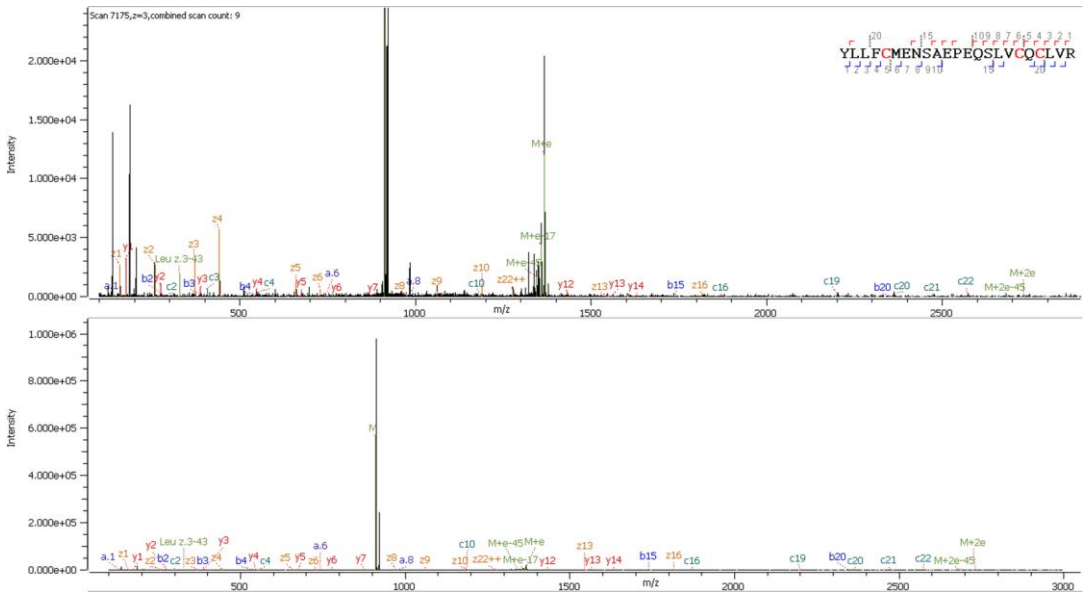

Figure 14. ECD MS/MS spectrum for a peptide with an intrapeptide disulfide bond of beta-lactoglobulin. The inset in the top plot displays the resulting fragmentation within the disulfide loop between Cys_{106} and Cys_{119} (the fist two cysteines in the sequence above)

Conclusions

Top-down Analysis of beta-lactoglobulin

- Top-down analysis was performed for the 14+ charge state in a targeted MS/MS method with the resulting sequence coverage obtained via CID and ECD resembling those obtained in other labs by the same or similar activation methods.
- With CID, the sequence coverage was 39 %, predominately around the N-terminus of the protein.
- Using ECD, the sequence coverage increased to 48 % with ECD with additional fragmentation around the C-terminus of the protein.
- Additional 10-20 V of CID collisional energy can be applied with the ECD fragmentation to increase the sequence coverage to 55 %. The combined CID and ECD fragmentation provides greater sequence information in a single scan than CID alone.

Bottom-up Analysis of beta-lactoglobulin

- CID and ECD provided a wealth of information regarding the expected disulfide bonds.
- Fragmentation via ECD yielded the individual masses of the peptides from the cleavage of the interpeptide disulfide bond in the resulting MS/MS spectrum that was not observed in the CID MS/MS spectrum, providing higher confidence in the assignments of the disulfide linkage.

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