Protein disulfide bond characterization with DMS and ECD

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ABSTRACT

Evaluating a collision cell with dual CID and ECD capability to characterize disulfide bond location in digested proteins.

INTRODUCTION

Identification and characterization of disulfide linkage yields critical information pertaining to biologic compound integrity throughout the entire development. Many of the approaches proposed have been relying on differential MS mapping of reduced and non-reduced digested sample. This is mainly due to the complexity of the MSMS fragmentation by CID, and the lack of automated tools for interpretation. It has been shown that ECD and ETD could yield simplified fragmentation that would improve the confidence in the identification and linkage of peptides [1-3]. When combined with differential mobility (DMS), additional selectivity could be obtained over LC separation [3] for sequence analogues. Here we explored the roles that both of these techniques (ECD and DMS) could play in the characterization of biologic compounds.

MATERIALS AND METHODS

Sample Preparation:

Bovine serum albumin (BSA) was digested with trypsin after alkylation but without the reduction step. BSA was used a simple model to generate mixture of intra- and cross-linked peptides. Table 1 give a list of the cross-linked peptides observed and detected with BioPharmaview Software(Sciex).

HPLC Conditions:

A Shimadzu Prominence-XR LC system with an Aries XB C18, 100x2.1mm, 2.6μm column (Phenomenex) at 45ºC with a gradient of eluent A water/acetonitrile (98/2) + 0.1% formic acid and eluent B water/acetonitrile (5/95) + 0.1% formic acid was used at a flow rate of 300μL/min. Peptide elution was performed with a gradient to 45% of eluent B in 24 minute.

MS/MS Conditions:

An Sciex Turbo V™ source and Electrospray Ionization (ESI) probe was used. The mass spectrometer was a research grade hybrid quadrupole time-of-flight system that share many of the ion optic element typically used on a TripleTOF® 5600 LC/MS system. The CID/ECD cell operational details were reported previously [4]. The ECD-enabled quadrupole time-of-flight (Q-ToF) mass spectrometer that can operated in 2 modes: 1) flow thought for CID and ECD and 2) simultaneous trapping of precursor ions with continuous beam of electron. This is achieved with a unique RF ion guide (Figure 1), whose branched structure enables the inter-crossing of an adjustable energy electron beam with the conventional analytical ion beam of the mass spectrometer.

Figure 1. Details of the Chimera-ECD device in terms of location on the ion optics of the QqTOF system
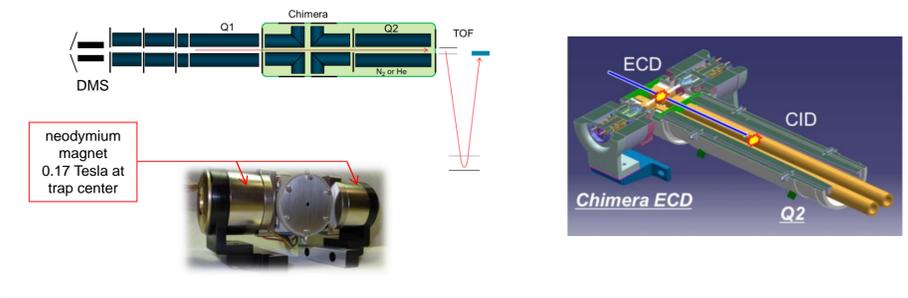
RESULTS

When digested under non-reducing conditions, disulfide linked peptide yield larger charge state peptides are easily separated from others ones with DMS. Using BSA as a model protein, most of the disulfide linked peptides generated have charge state ranging from +3 to +7 (Table 1). Though all peptide originate from a single protein chain, a wide range of intra- and inter- linked disulfide peptides are generated and represent a good pool of peptides to evaluate both ECD and DMS as tools for characterization of proteins. Our attention focused on a particular case where it was not possible to distinguish between 2 possible sequence combination; neither high mass accuracy or CID fragmentation. The isobaric nature of the cases requires alternative tool to distinguish which combination is present in the sample and detected (Figure 1). Since ECD fragmentation yields dominant fragment ions that originate from the cleavage of the S-S bond, complementary sequence information can be used to identify the proper peptide.
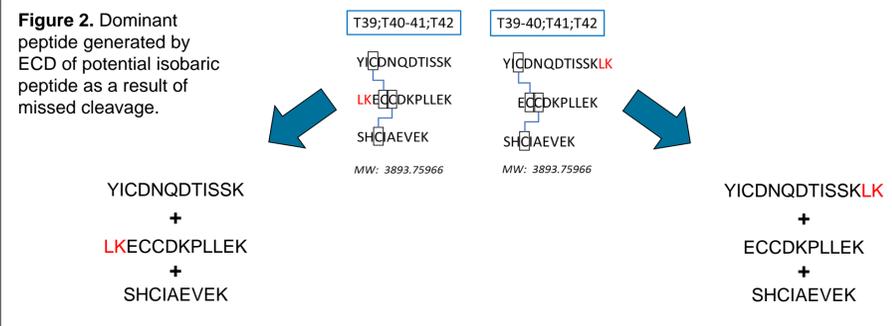
Figure 2. Dominant peptide generated by ECD of potential isobaric peptide as a result of missed cleavage.

Tabel 1. List of detected peptides containing disulfide links originating from BSA when digestion is performed with alkylation and without reduction (allowed single missed cleavage). A mix of intra- and inter-sequence peptide are generated to evaluate CID and ECD fragmentation.
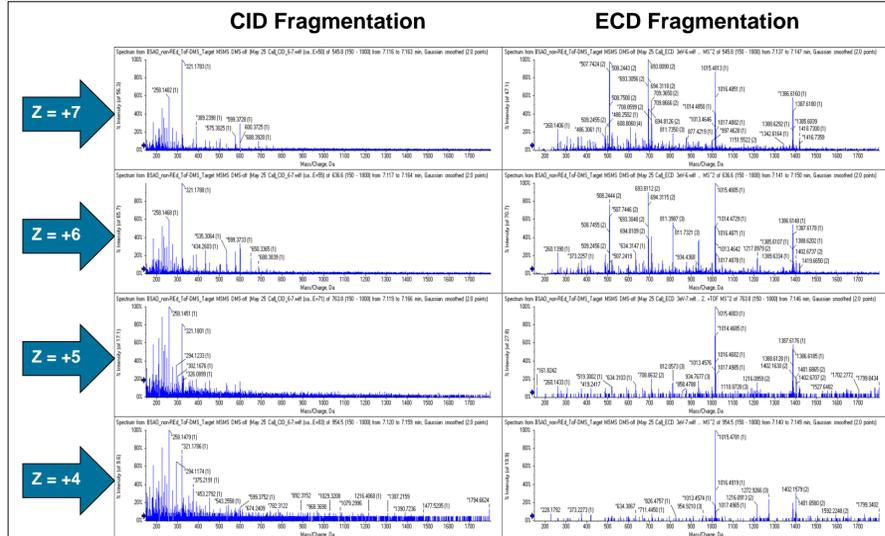

Figure 3. CID and ECD spectra for peptide depicted in Figure 1. The precursor charge state ranged from z=+4 to +7. ECD conditions were kept constant; 3eV with 10ms fill and 30ms reaction time.
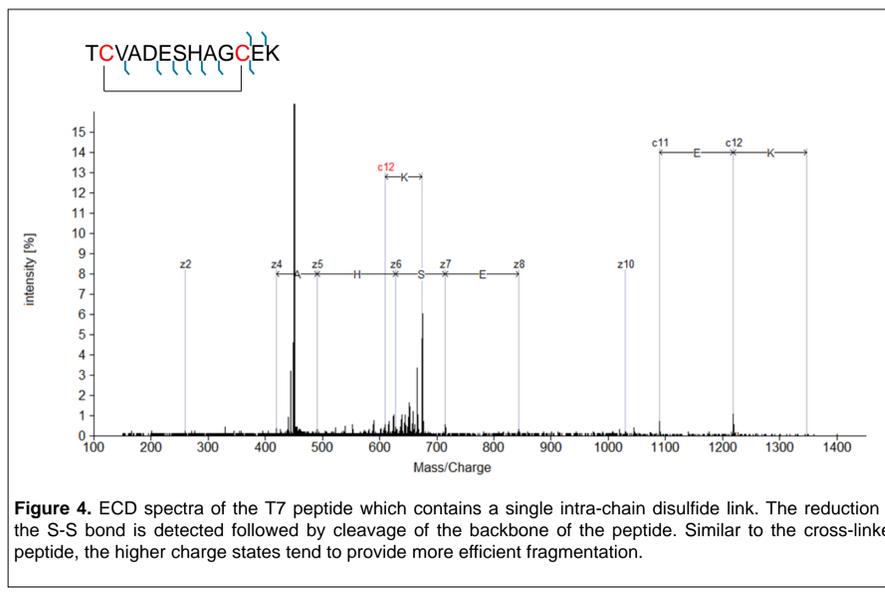

Figure 4. ECD spectra of the T7 peptide which contains a single intra-chain disulfide link. The reduction of the S-S bond is detected followed by cleavage of the backbone of the peptide. Similar to the cross-linked peptide, the higher charge states tend to provide more efficient fragmentation.

Figure 2 shows that ECD fragmentation yields fragmentation information that confirm detection of the T39-40 and T41 peptide in both the M(SH) and M(S) form as doubly and singly charged species. As show in Table 2, the higher charge states (Z=7+ and 6+) yielded fragment ions that confirmed the proper peptide. Additional fragmentation allowed confirmation that the proper peptide detected. From the data, it is possible to extract additional fragmentation information that confirmed the proper linkage S-S linkage (depicted in Figure 2)

	SH for [M+H]+	SH for [M+2H]2+	S for [M+2H]2+ ·	2xS for [M]2+	Observed for Z
YICDNQDTISSKLK	1627.79959	814.40343	813.89952	n.a.	7 & 6
ECCDKPLLEK	1177.55915	589.28321	589.28321	588.27539	7 & 6
SHCIAEVEK	1015.4877	508.24749	507.74357	n.a.	7 & 6
YICDNQDTISSK	1386.62057	693.81392	693.31001	n.a.	n.d.
LKECCDKPLLEK	1418.73818	709.87273	709.36881	708.8649	n.d.
SHCIAEVEK	1015.4877	508.24749	507.74357	n.a.	7 & 6

Table 2. Expect list of masses predicted and observed to confirm which of the peptide is present (green highlight). Of note, the higher charge states tend to generates the diagnostic fragment ions that confirm the T39-40;T41;T42.

CONCLUSIONS

ECD fragmentation offers additional complimentary information over CID in the case of disulfide containing peptides. When the link is intra-peptide, ring opening is observed and minimal fragmentation information is obtained. When the disulfide link is across peptide originating from different section of the sequence, cleavage of the bond generates individual chains which can yield information about the individual chains as well as some sequence information for each. This provides improved confidence in the location of the disulfide bonds.

Future efforts will concentration of development of comprehensive IDA logic to ensure data collection in automated way to optimize sequence information from the ECD and CID perspective. This future work would also include integration of DMS into the work flow. DMS has demonstrated that multiply charged species can be segregated ahead of mass selection, thus further simplifying the data reduction.

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