Disulfide Mapping Using Accurate-Mass ETD and MS^n Techniques

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Overview

Purpose: Disulfide mapping with minimal sample preparation and data interpretation.

Methods: Following limited proteolysis, beta-lactoglobulin peptides were analyzed using an electron-transfer dissociation (ETD) triggered MS^3 experimental paradigm, and the disulfide bonds were identified using multiple software platforms.

Results: We demonstrated a workflow for the discovery of disulfide bonds using ETD-triggered MS^n techniques.

Introduction

The biological function of proteins is a result of a number of factors including conformation, alternative splicing and a plethora of known and unknown modifications. Among the important contributors to tertiary and quaternary structure are disulfide linkages. Disulfide linkages influence not only the structural integrity of proteins, but also their biological functions. Characterization of these linkages is important in many biological assay protocols. Protein structural characterization continues to present major challenges for biological mass spectrometry research. Traditional collision-induced dissociation (CID) is inefficient at cleaving disulfide bonds and produces difficult-to-interpret heterogeneous MS/MS ion populations. We present a complimentary approach leveraging multiple software platforms along with a novel ETD-triggered MS^3 experimental paradigm.

Methods

Sample and Preparation

Beta-lactoglobulin was used as a model protein to study disulfide linkages. The native protein was partially digested using trypsin for limited proteolysis. This method obviates the need for reduction/alkylation and extensive sample manipulation.

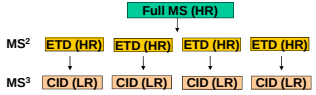
Uniprot ID P02754:

Disulfide bond	82 ↔ 176	Ref.1, Ref.2
Disulfide bond	122 ↔ 137	Alternate Ref.1
Disulfide bond	122 ↔ 135	Ref.1, Ref.2

Data Acquisition

An aliquot of the digest was loaded onto a microcapillary analytical column. Peptides were gradient-eluted and analyzed using a Thermo Scientific LTQ Orbitrap Velos mass spectrometer for the ETD-triggered CID MS^3 experiment. In this method, the mass spectrometer was utilized to acquire high-resolution precursor ion spectra with the top four high-resolution tandem mass spectra (MS/MS). Also, each MS/MS spectrum was followed by a CID MS^3 spectrum of the most intense ion from the corresponding MS/MS scan. Figure 1 illustrates the ETD-triggered MS^3 experimental paradigm.

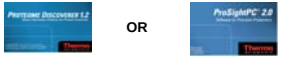
Figure 1. Schematic view of a ETD-triggered MS^3 experimental paradigm. HR represents high-resolution data detected by the LTQ Orbitrap Velos system; LR stands for nominal resolution data acquired by the LTQ Orbitrap Velos system.



Data Analysis

For disulfide mapping, LC-MS data can be analyzed using either Thermo Scientific Proteome Discoverer or ProSightPC software as shown in Figure 2.

Figure 2. Accurate mass ETD and MS^3 spectra can be analyzed using either Proteome Discoverer or ProSightPC software.



Results

Unlike CID, ETD favors fragmentation of disulfide linkages[Ref.3]. As shown in Figures 3, ETD fragmentation dissociated the two peptides linked by a disulfide bond, producing intense fragments corresponding to the unlinked peptides which could then be isolated and fragmented by CID for identification. As such, we established a method in which the most abundant fragment in each ETD MS^2 scan was then selected for ion trap CID MS^3 scans. Figure 4 illustrates the detection of disulfide linked peptides LSFNPTQLEEQCHI and WENGECAQKK in high-resolution full MS scan with 0.7ppm mass accuracy, which further confirms the structural identification.

Figure 3. ETD MS^2 spectrum of disulfide linked peptides. ETD dissociated the two peptides linked by a disulfide bond, producing intense fragments corresponding to the unlinked peptides. Ions corresponding to the dissociated chains were among the most abundant species since ETD favors fragmenting disulfide bonds.

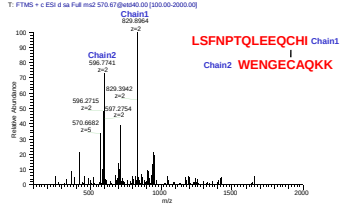
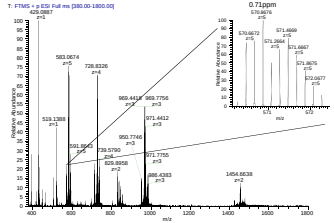


Figure 4. Detection of disulfide linked peptides LSFNPTQLEEQCHI and WENGECAQKK in full MS scan.



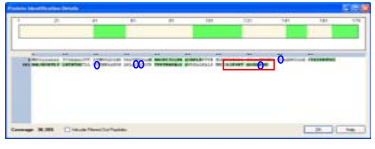
Disulfide Mapping using Proteome Discoverer Software

As shown in Figure 5, no cysteine-containing peptide is identified by ETD with strict tolerances due to disulfide linkage.

Figure 5. Protein coverage by ETD MS^2 spectra. Green highlights represent peptides identified by ETD MS^2 .

As shown in Figure 6, a cysteine-containing peptide was identified by CID MS^3 as a fragment from ETD ms^2 scan. It is a peptide that dissociates from the other chain in a pair of disulfide linked peptides.

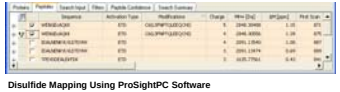
Figure 6. Protein coverage by CID MS^3 spectra. Green highlights represent peptides identified by CID MS^3 .



Using the mass of LSFNPTQLEEQCHI with the loss of two hydrogen [M-2H] as a dynamic chemical modification to cysteines, ETD MS^2 spectra were re-searched using SEQUEST® (Figure 7). The other chain of the disulfide linked peptides was identified as WENGECAQKK (Figure 8).

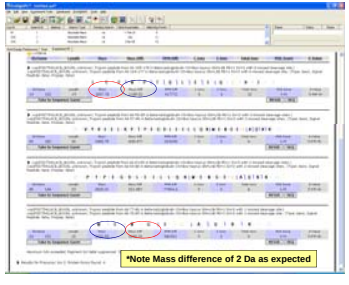
Figure 7. From CID MS^3, identification of the cysteine-containing peptide LSFNPTQLEEQCHI using the SEQUEST® search algorithm within Proteome Discoverer software. Upper panel shows a list of peptides identified by CID MS^3 spectra including LSFNPTQLEEQCHI; bottom panel demonstrates a CID MS^3 spectrum for LSFNPTQLEEQCHI with fragment ions annotated.

Figure 8. From ETD MS², WENGECAQKK was identified as the other chain of the disulfide linked peptides using LSFNPTQLEEQCHI with loss of two hydrogen [M-2H], a dynamic chemical modification.



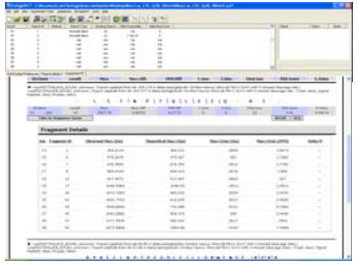
Disulfide Mapping Using ProSightPC Software

Figure 9. Search results from ProSightPC software, Delta-M mode.



Delta-M mode in the ProSightPC™ software was used to map disulfide bonds. ProSightPC software does not have a "variable" modification search mode for unknown post translational modification identification. Instead, it can search for any single modification of any mass using the Delta-M mode. In Delta-M mode, another set of fragments are matched to the sequence: b + "Delta m" and y + "Delta m". The delta m is calculated as the mass difference between the measured precursor and the matched sequence. In this case, to each chain of the disulfide linked peptides, the mass of the other chain with the loss of two hydrogen will be the delta mass. Correlation of the delta masses to the peptide masses leads to identification of disulfide linked peptides (Figures 9 and 10).

Figure 10. Using ProSightPC software, the disulfide linkage between LSFNPTQLEEQCHI and WENGECAQKK was confirmed by searching using WENGECAQKK as a chemical modification to the other chain.



Conclusion

We present a comprehensive approach for disulfide mapping which leverages multiple software platforms along with a novel ETD-triggered MS^3 experimental paradigm.

- ETD fragmentation dissociates the two peptide chains linked by a disulfide bond, producing intense fragments corresponding to the unlinked peptide, which can then be isolated and fragmented by CID for identification.
- Without a priori knowledge of the disulfide linkages, we were able to detect inter-peptide disulfide linkages using this method. In this case, a disulfide linkage between Cys82 and Cys176 was identified unambiguously.
- Analysis of the data using Proteome Discoverer™ software allowed the identification of the unlinked peptide in the CID MS^3 spectra and then to be used as a modification to identify the linked peptide in the ETD MS^2 spectrum.
- Analysis of the data using Delta-M mode feature in ProSightPC software also allowed identification of the inter-peptide disulfide linkage.

References

1. Biochemistry. 1972 Nov 21;11(24):4539-47.Location of sulfhydryl and disulfide groups in bovine-lactoglobulins and effects of urea. McKenzie HA, Ralston GB, Shaw DC.
2. Protein Sci. 1999 Nov;8(11):2541-5.Solution structure and dynamics of bovine beta-lactoglobulin A.Kuwata K, Hoshino M, Forge V, Era S, Batt CA, Goto Y.
3. Biochim Biophys Acta. 2006 Dec;1764(12):1811-22. The utility of ETD mass spectrometry in proteomic analysis. Mikesh LM, Ueberheide B, Chi A, Coon JJ, Syka JE, Shabanowitz J, Hunt DF.