Disulfide Mapping Using Accurate-Mass ETD and MSⁿ 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 MS³ experimental paradigm, and the disulfde bonds were identified using multiple software platforms.

Results: We demonstrated a workflow for the discovery of disulfide bonds using ETDtriggered MSⁿ techniques.

Introduction

The biological function of proteins is a result of a number of factors including conformation, alternative splicing and a plethors of known and unknown modifications. Among the important contributors to tertiary and quaternary structure are disulfide linkages. Distuiled linkages influence of only the structural integrity of proteins, but also their biological langtonic. Profession structural characterization continues to prosent many biological assay protocole. Profession structural characterization continues to prosent many characterization (CD) is reflicions at characterization of these biological datasy processes that in integret heterogeneous MSMIS in on populations. We present a complementary approach leveraging multiple software platforms along with a novel ETD-riggered MS³ experimental paradigm.

Methods

Sample and Preparation

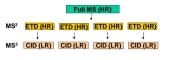
Beta-lactoglobulin was used as a model protein to study disulfide linkages. The native protein was partially digested using trypsin for limited proteolysis. This method obviates the need for reduction/alkylation and extensive sample manipulation. Uniornt ID P02764

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Disulfide bond	122 ++ 137	Alternate Ref.1	
Disulfide bond	122 ++ 135	Ref.1, Ref.2	

Data Acquisition

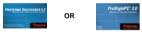
An alique of the digest was loaded onto a microcapillary analysical column. Peptides were gradient-tubed and analyzed using a Thermo Selerific LTO Orbitrap Volso mass spectrometer for the ETD-triggered CID MS² experiment. In this method, the mass spectrometer was using the solution acquired previous for a spectra with the top spectrometer was used to the solution of the most interest on from the corresponding MSMS son. Figure 11 lustrates the ETD-triggered MS² experiment paradigm. Figure 1. Schematic view of a ETD-triggered MS³ 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 MS³ spectra can be analyzed using either Proteome Discoverer or ProSightPC software.



Results

Unlike CID, ETD favors fragmentation of disulfiels linkages^{14,1} As shown in Figures 3, ETD fragmentation disocated the hew optical links day advalute boot, producing intense fragments corresponding to the unlinked peptides which could then be isolated and fragmented by CID for identification. As such, we stabilised an embod in which the most abundant fragment in each ETD MS² scans was then selected for ion trap CID MS² scans. Figure 4 illustrates the detection of disulfiels index optides LSFWPTOLEEDCH and VENBECAXKK in high-resolution full MS scans with 0.7pm mass accuracy, which further confirms tratitization.

Figure 3. ETD MS² 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.

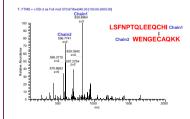
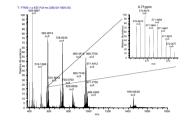


Figure 4. Detection of disulfide linked peptides LSFNPTQLEEQCHI and WENGECAQKK in full MS scan.



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 $\rm MS^2$ spectra. Green highlights represent peptides identified by ETD $\rm MS^2$.



As shown in Figure 6, a cysteine-containing peptide was identified by CID MS³ as a fragment from ETD ms² scan. It is a peptide that dissociates from the other chain in a pair of disulfide linked peptides.

Figure 6. Protein coverage by CID MS³ spectra. Green highlights represent peptides identified by CID MS³.



Using the mass of LSFNPTQLEEQCHI with the loss of two hydrogen [M-2H] as a dynamic chemical modification to cystelines, ETD MS² spectra were re-searched using SEOUEST® (Figure 7). The other chain of the disulfide linked peptides was identified as WENGECACKK (Figure 8).

Figure 7. From CID MS¹, identification of the cysteine-containing peptide LSFNPTQLEEQCH using the SEQUEST® search algorithm within Proteome Discoverer software. Upper panel shows a list of peptides identified by CID MS³ spectra including LSFNPTQLEEQCH; bottom panel demonstrates a CID MS³ spectrum for LSFNPTQLEEQCH with fragment ions annotated.

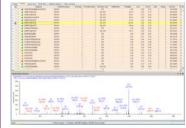


Figure 8. From ETD MS², WENGECAQKK was identified as the other chain of the disulfide linked peptides using LSFNPTQLEEQCHI with loss of two hydrogen [M-2H], a dynamic chemical modification.

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Disulfide Mapping Using ProSightPC Software

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

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

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Conclusion

We present a comprehensive approach for disulfide mapping which leverages multiple software platforms along with a novel ETD-triggered MS³ 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 MS³ spectra and then to be used as a modification to identify the linked peptide in the ETD MS³ spectrum.
- Analysis of the data using Delta-M mode feature in ProSightPC software also allowed identification of the inter-peptide disulfide linkage.

References

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