IMS, HCD, and ETD for Improved Chemical Cross-linking Mass Spectrometry

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Overview

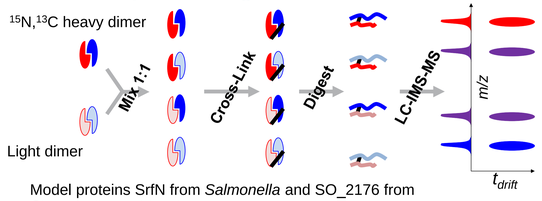
- Chemical cross-linking mass spectrometry can probe protein structure [1-2]
- Detection of rare cross-linked peptides is challenging
- Interpretation of MS/MS spectra is challenging for existing tools
- Ion mobility (IMS) plus mixed isotope labeling (MIX) provides a possible alternative approach to identify cross-linked peptides
- Alternative fragmentation mechanisms can provide fragments needed to identify cross-linking site

Introduction

- IMS increases dynamic range of peptide detection by LC-MS [3].
- MIX uses spectral multiplets to identify intermolecular cross-links [4]
- IMS drift time unchanged by heavy Isotopes [5]
- We combined MIX and LC-IMS-MS to analyze BS^3 cross-linking of homodimeric model proteins
- We tested whether MIX-LC-IMS-MS showed any improvement (versus MIX-LC-MS alone) in identifying cross-linked peptides [6]
- Heme-copper oxidases (HCOs) contain a natural His-Tyr cross-link [7-8] necessary for efficient enzymatic activity [9]
- A designed HCO built on a myoglobin scaffold (Cu_BMb) was treated with H_2O_2 to induce cross-link formation
- Using ETD, we found a novel His-His cross-link, rather than the expected His-Tyr link

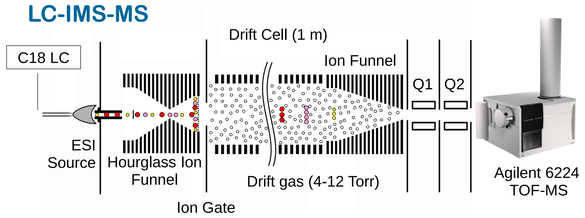
Methods: MIX-LC-IMS-MS

Mixed isotope labeling



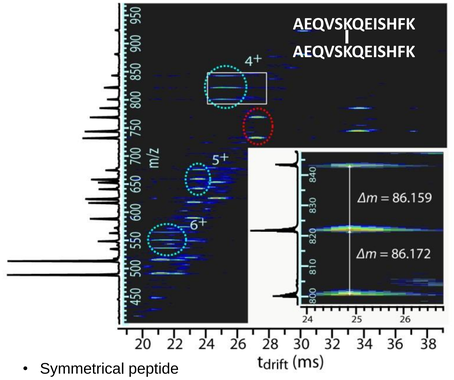
Model proteins SrfN from *Salmonella* and SO_2176 from *Shewanella oneidensis*

LC-IMS-MS



Results

Intermolecular cross-linked peptides



- Symmetrical peptide
- Triplet pattern
- 1:2:1 peak intensity ratio
- Coelution of multiple charge states
- ≤6 ppm mass measurement error
- Mass shift unique to this sequence

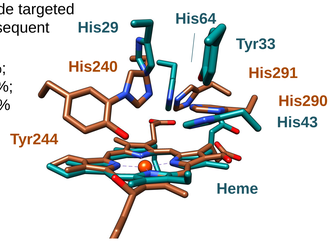
MIX-LC-IMS-MS compared to MIX-LC-MS

Model Protein:	SrfN		SO_2176	
IMS:	+	—	+	—
Forward hits	18	8	12	2
Peptides	9	4	8	1
Decoy Hits/100	0	0	0.44	0.42
Peptides/100	0	0	0.37	0.36
% FDR (features)	0	0	3.7	21
% FDR (peptides)	0	0	4.6	36

- Automated search compares theoretical cross-link masses and shifts to LC-IMS-MS features using Decon2LS [10] and LCMSFeatureFinder [11]
- Datasets searched against forward target sequence and 100 randomized target sequences
- Data searched with and without drift time information from IMS
- MIX-LC-IMS-MS increases the number of cross-linked peptides detected due to separation in drift time dimension
- MIX-LC-IMS-MS increases the confidence of peptide identifications

Methods: HCD, ETD of cross-linked F33Y- Cu_BMb

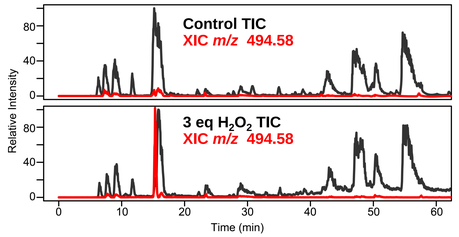
- Copper B site engineered into myglobin scaffold [12] and F33Y mutant constructed [13]
- Treated with 3 equivalents H_2O_2 to induce cross-linking
- Tryptic peptides analyzed by LC-MS/MS on a Thermo Scientific LTQ Orbitrap Velos mass spectrometer
- Shotgun analysis for first run; ions at expected *m/z* values for cross-linked peptide targeted for MS/MS in subsequent runs
- CID, NCE 35-45%; HCD, NCE 35-50%; ETD NCE 33-200%



Structural superposition of the active site of crystal structure of bovine cytochrome c oxidase (brown) and F33Y-Cu_BMb (cyan). After reference [13].

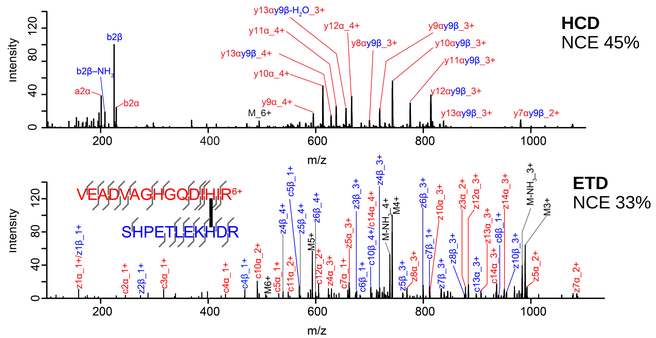
Results

H_2O_2 induces cross-linking



- Unique peak in H_2O_2-treated F33Y-Cu_BMb chromatogram
- Sequenced as VEADVAGHGQDIHIR/SHPETLEKHDR by CID
- XIC of 6+ charge state shown in red
- Average mass measurement error is 0.7 ppm
- Expected link is tyrosine to histidine (Y-H), but no tyrosine residue present

ETD allows determination of cross-linked site



- CID (not shown) and HCD (top) fail to specify exact linkage site
- Efficient fragmentation by ETD identifies novel His-His cross-link
- Sites identified by multiple fragment ions with mass measurement error < 5 ppm and most with good isotopic envelopes
- Consistent with Edman degradation results (not shown)

Conclusions

- MIX-LC-IMS-MS confidently identified cross-linked peptides
- MIX multiplet peak spacing is a powerful constraint on peptide sequence
- MIX-LC-IMS-MS identified more cross-linked peptides than MIX-LC-MS
- Separation of features in the drift time dimension is critical to identification
- Peroxide-treated F33Y-Cu_BMb contains an unexpected His-His cross-link
- Fragmentation by ETD was necessary to determine the linked residues
- For cross-links with unknown chemical specificity, efficient fragmentation and high-resolution data are essential

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References

1. Leitner A, et al. *Mol Cell Proteomics* 9:1634-1649 (2010).
2. Rappsilber J. *J Struct Biol* 173:530-540 (2011).
3. Baker ES, et al. *J Proteome Res* 9:997-1006 (2009).
4. Taverner T, et al. *J Biol Chem* 277:46487-46492 (2002).
5. Kindy JM, et al. *Anal Chem* 74:950-958 (2002).
6. Merkley ED, et al. *J Am Soc Mass Spectrom* 24:444-449 (2013).
7. Yoshikawa S, et al. *Science* 280:1723-1729 (1998).
8. Buse G, et al. *Protein Sci* 8:985-990 (1999).
9. Liu X, et al. *Angewandte Chemie International Edition* 51:4312-4316 (2012).
10. Jaitly N, et al. *BMC Bioinformatics* 10 (2009).
11. Crowell KL, et al. 59th ASMS Conference on Mass Spectrometry and Allied Topics, Denver, CO (2011).
12. Sigman JA, et al. *J Am Chem Soc* 122:8192-8196 (2000).
13. Miner KD, et al. *Angewandte Chemie International Edition* 51:5589-5592 (2012).

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