Prediction of the Tandem Mass Spectra of Cross-linked Peptides

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Introduction

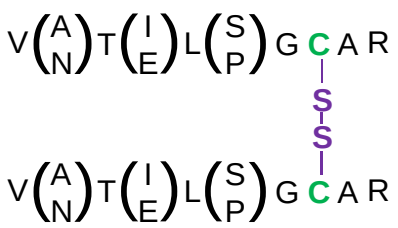
- Protein-protein interactions are involved in many biological processes, including both normal and abnormal growth.
- Mass spectrometry combined with covalent cross-likers has the potential to study many of these interactions as well as help to characterize protein structure.
- Both native (disulfide bond) and non-native (BS3, DEST, etc.) cross-linkers target specific amino acid side chains and have the potential to provide a wealth of biological information.
- Current understanding of how crosslinked peptides fragment in tandem mass spectrometry is limited.
- We propose to create libraries of crosslinked peptides that can be analyzed by standard proteomics methodology.
- Analysis of these samples can potentially improve our understanding of crosslinked peptide fragmentation and increase the accuracy of their identification.

Methods

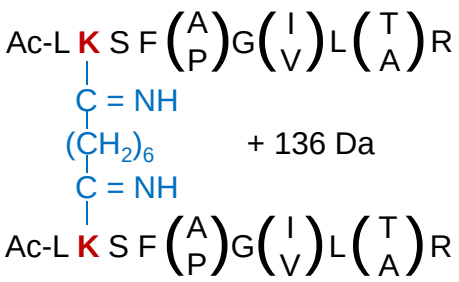
- Peptide libraries are synthesized using standard Boc-protected amino acids and resin-based split-and-mix combinatorial synthesis[2]
- Small 8-sequence libraries have three different positions that each contains one of two different amino acid residues
- Within the error of our ability to split the sample during synthesis, the two different residues at a given position are in equimolar abundance in the library
- When reacted to form cross-linked species, each 8-sequence library will produce 36 unique crosslinked peptides.
- Disulfide and DEST chemistries are illustrated here, but others are readily incorporated.

Crosslinked Peptides from Peptide Libraries

Disulfide crosslinker at C



DEST crosslinker at K[1]

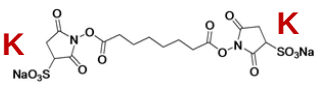


One library = 36 crosslinked peptides

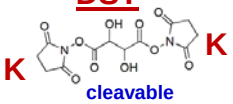
8 unique sequences, lettered A-H: AA, BB, CC, DD, EE, FF, GG, HH, AB, AC, AD, AE, AF, AG, AH, BC, BD, BE, BF, BG, BH, CD, CE, CF, CG, CH, DE, DF, DG, DH, EF, EG, EH, FG, FH, GH [CL = (US^2 + US)/2]

Other crosslinkers[2]

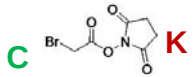
BS3



DST



SBA



Opportunities / Future Directions

- Synthetic peptide libraries allow for systematic study of various parameters of crosslinked peptide fragmentation, including sequence length, position of crosslinking, and crosslinking chemistry.
- Any crosslinking chemistry can be used, since peptides can be dissolved in any solvent and appropriate residues can be incorporated at any location.
- MS/MS spectra of peptides can be predicted by machine learning models – a similar strategy that incorporates prior knowledge (fragmentation pattern of isolated peptides) has great potential for crosslinked peptide fragmentation spectra.
- Tendency of a peptide bond to fragment and amount of charge remaining on a fragment can be modeled using a non-linear regression model.
- Prediction of crosslinked peptide fragmentation spectra should facilitate more robust identification of these species in biological data.

Acknowledgements

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References

1. Lauber, M. A.; Reilly, J. P. *Anal. Chem.* **2010**, *82*, 7736-7743.
2. Bohrer, et al. *Anal. Chem.* **2010**, *82*, 6559-6568.
3. http://www.proteochem.com/proteincrosslinkers-c-1.html

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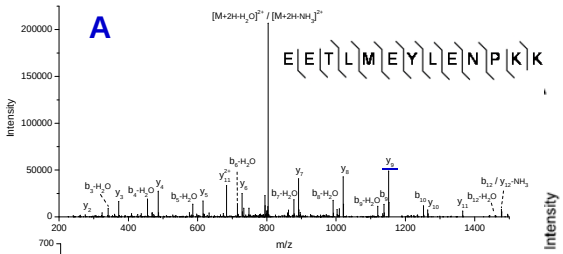
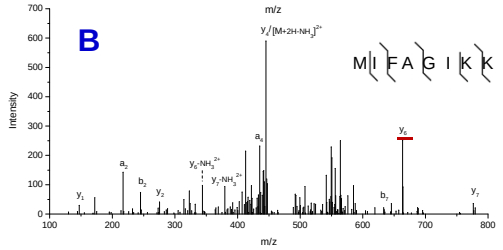
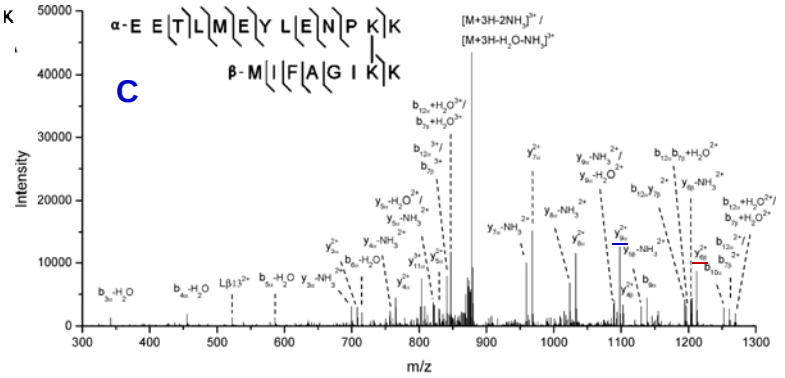
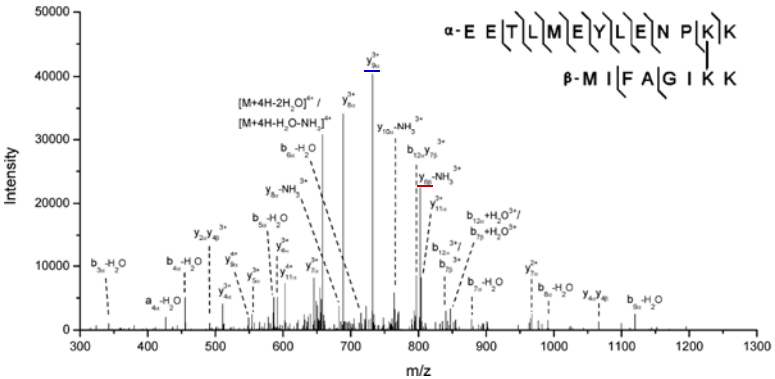
Example of DEST crosslinker [1]

MS/MS spectra for **A**) peptide EETLMEYLENPKK^{2+}, **B**) peptide MIFAGIKK^{2+}, **C**) DEST crosslinked EETLMEYLENPK*K/MIFAGIK*K^{3+}, and **D**) crosslinked EETLMEYLENPK*K/MIFAGIK*K^{4+}. Peptides were crosslinked in native cytochrome C and released upon digestion with trypsin. Note that prominent fragments y_9 from EETLMEYLENPKK and y_6 from MIFAGIKK also occur in the crosslinked peptide and that the charge states depend on the precursor charge state. K* indicates the location of the DEST crosslinking.









DEST = diethyl suberthioimidate