Impact of crosslinker chemistry on peptide fragmentation spectra of crosslinked peptides

Randy J. Arnold[1]; Suraj Saraswat[1]; Chao Ji[2]; Haixu Tang[2]; Predrag Radivojac[2]; James P. Reilly[1]

[1]Department of Chemistry, [2]School of Informatics and Computing, Indiana University, Bloomington, IN 47405

Introduction

The study of protein-protein interactions is aided greatly by mass spectrometry-based identification of cross-linked peptides. However, cross-linked peptides present several challenges. First, these structures tend to form higher charge state precursors with as many as five or six charges common when modest-sized peptides are cross-linked, producing fragments that can have from one to five charges. Second, with their higher charges, the same normalized collision energy that can induce breakage of a single bond for doubly- or triply-charged peptides can produce multiple fragmentation events in the same cross-linked species, producing double-fragmentation product ions. Third, the structure of the cross-linker itself can have an impact on the fragmentation of the cross-linked peptide.

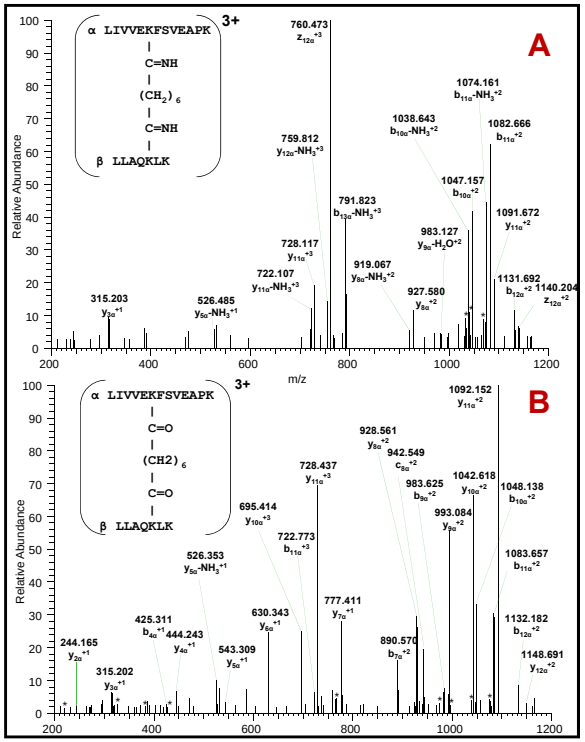
Methods

In our efforts to understand, and ultimately predict with reasonable accuracy, the fragmentation of cross-linked peptides, this work investigates the influence of two different lysine-to-lysine crosslinkers: DEST and BS3. DEST, or diethyl suberthioimidate, was developed in our lab[1] and incorporates two easily ionized amidinated groups into the cross-linked peptide. BS3 (Bis[sulfosuccinimidyl] suberate) is a commercially- available crosslinker with two N-hydroxysulfosuccinimide esters (BS3) that produce two amide groups upon reaction.

Data sets

E. coli ribosomes are macromolecular complexes that contain 55 proteins and three large RNA molecules. Because x-ray crystal structure data have been recorded for ribosomes of a few different bacteria, these complexes provide excellent prototype samples for cross-linking studies that probe protein-protein interactions. In the present work, ribosomal proteins isolated from *E. coli* are labeled with each of the two crosslinkers, and LC-MS/MS data are acquired using reversed-phase nano-LC coupled to an LTQ-Orbitrap. Tandem mass spectra for the same two peptides crosslinked with each of the two crosslinkers are shown in the figure on the right to demonstrate the similar and unique characteristics of the fragmentation patterns. Peptides crosslinked with the commercial molecule BS3 provide fragmentation patterns with charges presumably associated with the peptide sequences. In contrast, the DEST crosslinked peptides display some unique fragmentation (when fragmenting the same precursor charge state) presumably due to the two amidination sites where the crosslinker binds to the peptides. It has previously been demonstrated that these two sites are readily protonated. We have previously demonstrated that machine learning models can be used to predict peptide ion fragmentation characteristics. Data obtained in the present study will be used to extend this computational modeling to the significantly more challenging case of crosslinked peptides.

Figure 1 (right). MS/MS spectra for the peptides LIVVEKFSVEAPK / LLAQKLK crosslinked with **A**) DEST and **B**) BS3 and fragmented by CID of the 3+ charge state (precursor m/z 803.44 and 804.16 respectively). Strong peaks that can be assigned to the sequence are labeled with the assignment and weaker peaks that can be assigned are labeled with an asterisk (*). Fragmentation of peptide LIVVEKFSVEAPK is designated as alpha (α) while fragmentation of peptide LLAQKLK is designated as beta (β). Notice that all labeled peaks correspond to fragmentation of peptide LIVVEKFSVEAPK .



Results and Observations

DEST and BS3 produce chemically different crosslinks that fragment in unique ways, as shown in Figure 1. Also, both Figures 1 and 2 support the observation that crosslinked peptides tend to preferentially fragment on one, but not the other, peptide backbone. In addition to the lysine-to-lysine crosslinkers shown here, we have also utilized synthetic peptide libraries containing cysteine to create disulfide crosslinked peptide fragmentation. These spectra demonstrate similar features, including a preference for fragmentation of just one of the two peptides. This result is important in developing algorithms to identify MSMS fragmentation spectra of crosslinked peptides.

Future Directions

- 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

The authors acknowledge Matt Lauber for sharing data. Funding was provided by NIH R01 RR024236-01A1 to PR.

References

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

Figure 2 (below). Ratio of fragment ion intensity from the two peptides in a DEST-crosslinked peptide MS/MS spectrum when the smaller intensity peptide is divided by the larger intensity peptide for **A**) all 98 peptides identified from *E. coli* ribosomal proteins, **B**) 35 peptides with difference in length less than 3, **C**) 35 peptides with difference in length between 3 and 7, and **D**) 28 peptides with difference in length greater than 7.