

Novel Isotopically-Coded Crosslinkers for Studying Protein-Protein Interactions in Proteomics

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INTRODUCTION

One of the ways to determine interacting proteins on the proteome-wide level is to apply crosslinking methodologies.

Crosslinked proteins can be proteolytically digested and the crosslinked peptides can be analyzed by mass spectrometry, thus providing information on interacting proteins.

One of the challenges of this approach is the complexity of the resulting peptide mixture, which makes it difficult to detect and identify crosslinked peptides.

Isotopically-coded crosslinkers help detect crosslinked peptides in the mass spectrum.

We previously reported the use of isotopically-coded cleavable crosslinkers (Petrotchenko E., Olkhovik V. and Borchers C., ASMS 2004) to facilitate discrimination between deadend, intrapeptide and interpeptide crosslinks, and to assist in identification of the individual peptides by MS/MS sequencing after chemical cleavage of the crosslinker.

Here, we present two new isotopically-coded crosslinkers, BiPS-D8 and TEABS-D12, which have additional features.

BiPS-D8 has a fluorescent group and 8 aliphatic deuterium atoms in the linker region.

TEABS-D12 has 12 deuterium atoms in the cleavable linker region as well as a biotin group. Cleaved crosslinked peptides still contain the isotopically-coded portion of the crosslinker which includes 4 deuterium atoms.

METHODS

Synthesis of the crosslinkers BiPSD8 and TEABSD12 was done using isotopically-labeled bromopropionic acid and succinic anhydride (C/D/N Isotopes Inc.), respectively.

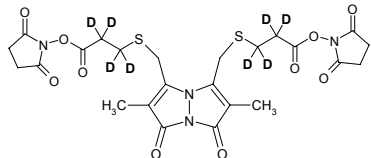
Crosslinking of the model peptides and the RNaseS heterodimer was done in phosphate buffered saline, pH 8.0, by adding a 10 mM stock solution of the crosslinkers in DMSO to give a final concentration of 0.5 mM.

Crosslinked RNaseS was isolated by SDS-PAGE and in-gel digested. Separation of the tryptic peptides was done on an Agilent 1100 HPLC system, equipped with fluorescent detector.

TEABS crosslinked peptides were affinity purified on monomeric avidin beads (Pierce) and eluted with 0.1% TFA, 50% acetonitrile solution.

MALDI-MS and MS/MS were performed on an ABI 4700 TOF/TOF mass spectrometer.

RESULTS



BiPS-D8

Figure 1. Structure of BiPS-D8, a fluorescent isotopically-coded crosslinker.

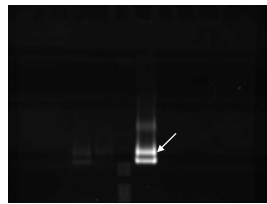


Figure 2. Gel image under UV illumination. The upper fluorescent band corresponds to the heterodimer of RNaseS crosslinked with BiPS.

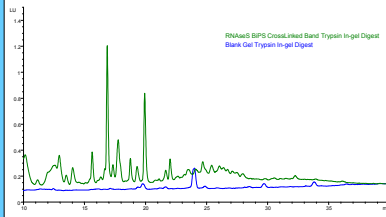


Figure 3. HPLC with fluorescent detection of an in-gel trypsin digest of the BiPS-crosslinked RNaseS complex. Ex=370 nm, Em=480nm.

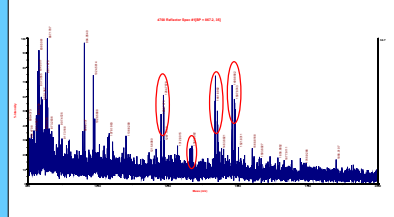


Figure 4. Mass spectrum of the fluorescent chromatographic fraction. The peaks showing D8 doublets correspond to BiPS crosslinked peptides.

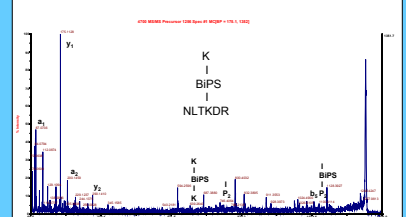
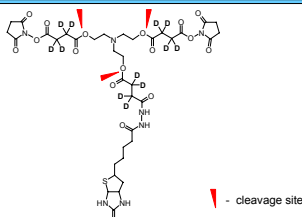


Figure 5. MS/MS spectrum of a BiPS-crosslinked peptide.



TEABS-D12

Figure 6. Structure of TEABS-D12, a biotinylated isotopically-coded cleavable crosslinker.

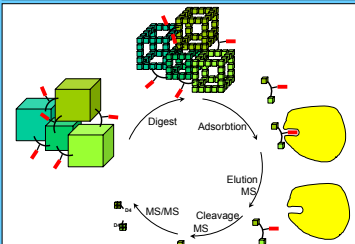


Figure 7. Analytical scheme of the TEABS-D12 crosslinking experiment.

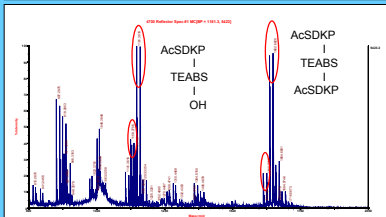


Figure 8. Mass spectrum of the elution fraction from the avidin column. D12 peak doublets correspond to TEABS-crosslinked peptides.

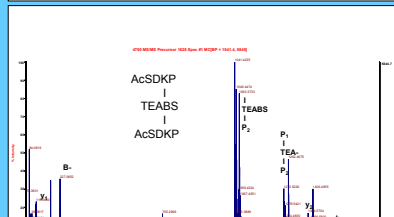


Figure 10. MS/MS spectrum of a crosslinked peptide.

CONCLUSIONS

The fluorescent moiety of the BiPS crosslinker allows image-guided isolation of the crosslinked protein complexes, the monitoring of the protease digestion procedure and fluorimetric detection of the crosslinker-containing peptides during HPLC separation.

The isotopically-coded biotinylated cleavable crosslinker TEABS allows affinity enrichment of the crosslinker-containing peptides, elution from affinity support and subsequent chemical cleavage to release individual peptides, which can be successfully sequenced by MS/MS, providing unambiguous identification of the interacting proteins.

ACKNOWLEDGEMENTS

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