

## Affinity Purification of Crosslinked Peptides

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Chemical crosslinking is a tool for interrogating three-dimensional (3D) protein structure and protein-protein interactions through introduction of new covalent bonds [1]. The locations of these new bonds, along with the length and reactivity of the crosslinking reagent, provide low-resolution spatial information on the original 3D structure of the protein or complex [2]. However, determining the locations of the bonds introduced by the crosslinking reagent has proven to be a fairly difficult analytical task for several reasons. Crosslinking reaction yields are generally low. On digestion of a crosslinked protein, large numbers of peptides will not contain a crosslink. Side reactions to the crosslinking introduce non-informative species. The crosslinked peptides thus are minor components of a complex mixture. A selective method for isolating crosslinked peptides from such mixtures is desirable [3]. We describe here a model system for separating biotinylated, crosslinked peptides from tryptic digests of crosslinked proteins.

Neurotensin was crosslinked intramolecularly using sulfo-SBED, a heterobifunctional crosslinker with a biotin moiety. Appropriate volumes of neurotensin and sulfo-SBED solutions were combined in 96-well microtiter plates and maintained first under subdued light to allow the N-hydroxysuccinimide ester of the sulfo-SBED to attach to the lysine amine of neurotensin. A 365 nm UV lamp was then used to activate the arylazide portion of the crosslinker, which inserts non-specifically into the neurotensin. MALDI-TOF analysis revealed a mixture of products, most of which can be assigned as (1) complete or "one-sided" intramolecular crosslinking of neurotensin by sulfo-SBED, (2) crosslinking products that are oxidized, apparently at the biotin sulfur, or (3) unreacted neurotensin. The extent of oxidation could be decreased by exposing the samples to UV light for shorter times. The disulfide bond in the linker arm of sulfo-SBED allowed verification of some products via their *m/z* shifts on reduction with dithiothreitol and subsequent derivatization with iodoacetamide.

To determine whether peptide species containing an affinity-labeled crosslink, such as that introduced by reaction with sulfo-SBED, could be purified from non-labeled peptides, an affinity capture experiment was performed on a mixture containing sulfo-SBED-derivatized neurotensin and a tryptic digest of bovine hemoglobin. The affinity capture matrix was commercially-available immobilized monomeric avidin (Pierce). The avidin beads were prepared for use by blocking with biotin, then bovine serum albumin, to minimize non-specific binding. Beads and sample were incubated in a batch mode for 3 hours with gentle mixing. Several wash steps were performed before eluting biotinylated peptides from the beads using 50% CH<sub>3</sub>CN in aqueous 0.4% trifluoroacetic acid. MALDI-TOF mass spectra were obtained at several points during the affinity isolation (Figure 1). The final eluate contained predominantly biotin-bearing sulfo-

SBED-derivatized neurotensin peaks, marked by asterisks in Figure 1. These results suggest that the batch avidin bead separation enriches the biotin-containing peptide species fairly efficiently, with little non-specific binding.

This neurotensin/sulfo-SBED conjugate is a useful model system for elucidating side reactions associated with the crosslinking, and optimizing an affinity purification strategy that should enable the application of crosslinking and mass spectrometry to the more interesting insights into structure and geometry proteins and protein.

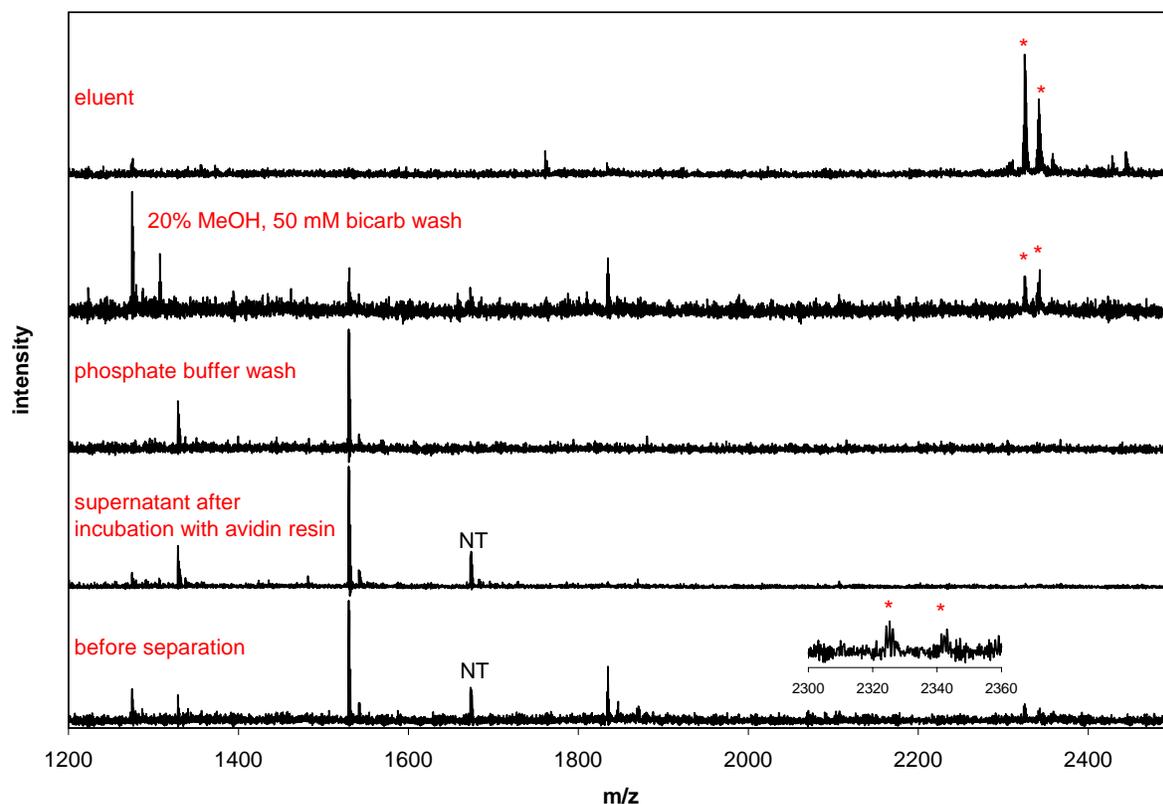


Figure 1. Avidin purification of sulfo-SBED crosslinked neurotensin. \*: biotinylated, crosslinked peptide. NT: unreacted neurotensin. Other peaks are hemoglobin tryptic peaks.

#### References

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This research sponsored by the Office of Biological and Environmental Research, U.S. Department of Energy, and the Laboratory Directed Research and Development Program of Oak Ridge National Laboratory (ORNL). ORNL is managed by UT-Battelle, LLC, for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.