Crosslinking Analysis by Reductive Amination with 1,3-Diformyl-5-Ethynylbenzene (DEB)

Michael J. Trnka & Alma L. Burlingame

Department of Pharmaceutical Chemistry, Mass Spectrometry Facility
University of California, San Francisco, CA 94158-2517
mtrnka@cgi.ucsf.edu

Featured of Crosslinking by Reductive Amination
1. Reacts with type 0 amino groups; which are well distributed as protein-protein interaction surfaces, to form secondary amides.
2. Preserves charge on modified residues leading to high charge density precursor ions, ideal for ETD; good fragment coverage from both peptides.
3. High resolution ETD yields diagnostic F, Q, X, L ions which confirm loss of individual peptides.
4. Small rigid structure of DEB provides more precise distance constraints. Discrimination between different conformational states of GroEL/GroES.
5. Type 0 modified (‘dead-end’) peptides contain an aldehyde chemical handle that can be used to discriminate these from Type 1, 2 (crosslinked) peptides.
6. DEB contains aldehyde moiety for post-labeling demonstration with biotin or fluoresceine.

Dead-end Modifications React with Hydrazides

Crosslinking of ADP-Bound GroEL/GroES Complex

Interatomic Distances of Crosslinked Residue Pairs

Concluding remarks: The high mass accuracy of the ion trap and mass spectrometer was instrumental in the identification of a new class of crosslinked peptides. In addition, low-resolution and high-resolution ETD experiments showed that crosslinked peptides are formed in both ETD experiments. The crosslinked peptides were identified using the crosslinking DEB and an ion-trap mass spectrometer. The results indicate that crosslinked peptides can be formed in both ETD experiments.