Title

MASS SPECTROMETRY BASED EXPERIMENTAL STRATEGIES TO CHARACTERIZE NATIVE AND NON-NATIVE DISULFIDE BONDS IN CYSTEINE-RICH PROTEIN THERAPEUTICS

Author

Adriana Z. Kita, *University of Massachusetts - Amherst* Follow

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Abstract

The impact post-translational modifications (PTMs) can have on the structure, function, and immunogenicity of protein therapeutics makes it especially important for these protein-based treatments to be well characterized. Mass spectrometry has become instrumental in the examination of enzymatic and non-enzymatic PTMs. Disulfide bonds fall into both of these categories in which native disulfide bonds are formed in the ER by disulfide mediated enzymes and non-native disulfide bonds are often formed by non-enzymatic reducing/oxidizing reactions. Disulfide bonds are particularly important for protein folding and reinforcing higher order structure, and are typically characterized by LC-MS of non-reduced peptides. However, characterizing the disulfide connectivity can be challenging when cysteine residues lie in close proximity within the primary sequence. Here we developed a dual proteolytic method with several gas-phase fragmentation techniques to map the cysteine-rich N-terminus of the protein therapeutic beta-glucocerebrosidase (GCase). We used this approach to map the native disulfide connectivity of GCase and also identified non-native disulfide bonds in a long-term stability sample.

Investigating non-native disulfide bonds can be challenging because they often exist at low levels and fully oxidized isoforms do not exhibit a change in molecular weight. Here we used lysozyme (LYZ) as a model to develop a rapid characterization strategy to monitor non-native disulfide conformers by electrospray ionization (ESI) MS. We demonstrate that this technique can be used to monitor large-scale conformational changes that often accompany disulfide scrambling. Initially, LYZ was subjected to disulfide scrambling and their disulfides were mapped. Then the charge state distribution of each scrambled species was monitored by ESI-MS. We show that we can distinguish non-native conformers from natively oxidized LYZ by comparing their extent and distribution of protonation during ESI-MS.

Here we demonstrate the application of mass spectrometry based experimental strategies that can be used to monitor large-scale conformational changes and characterize challenging native and non-native disulfide bonds in proteins. These methods are intended to improve the strategies that are currently used to characterize disulfide bonds in protein therapeutics and further detect non-native conformers.

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