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Yi-Li Huang et al., J Proteomics Bioinform 2016, 9:11(Suppl)
http://dx.doi.org/10.4172/0974-276X.C1.092

Evaluation of disulfide scrambling at various pH on Avastin digestion by mass spectrometry

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Disulfide linkages play an important role in protein stability and activity. Thus, it is critical to characterize disulfide bonds to ensure quality and functions of protein drugs. Protein digestion procedures cannot be avoided for disulfide linkage analysis in conventional manner. In order to preserve enzyme activity during protein digestion, it is commonly carried out at basic environment which increases the possibilities of disulfide bond scrambling. However; when disulfide bond rearrangement occurs, it is not quite easy to differentiate whether by sample itself or digestion process cause the scrambling disulfide linkages. In this study, optimization on digestion pH was realized for the reduction of disulfide bond rearrangement. Three sets of proteases, including trypsin plus Glu-C, thermolysin and Lys-C were used, followed by dimethyl labeling and mass spectrometry for bevacizumab (Avastin) disulfide linkage analysis. There was no scrambled disulfide bond identified at pH 6 when using Lys-C or trypsin plus Glu-C as enzymes. When thermolysin was applied, there were still scrambled disulfide bonds identified either at pH 5, pH 6 or pH 7. Nevertheless, there was fewer scrambled disulfide bonds observed at low pH. All disulfide bonds on bevacizumab can be solved with this approach. The results demonstrated that by choosing the proper enzymes, using lower digestion pH environment could reduce the degree of scrambled disulfide linkages.

Biography

Yi-Li Huang is a Chemistry major master student at National Taiwan Normal University. His research interests focus on analytical chemistry, and proteomics-related mass spectrometry. His research thesis entitled “Evaluation of disulfide scrambling at various pH on Avastin digestion by mass spectrometry” will be presented in this proteomics conference.

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