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Protease Type XVIII Columns for Enhanced Digestion Efficiency and Sequence Resolution for Protein HDX Monitored by Q Exactive MS

Introduction

Recent advancements in LC/MS, automation, and informatics technologies have made HDX-MS becoming a robust and indispensable tool not only for complex academic research exercise but also for discovery and development of protein drugs in pharmaceutical industries. However, the selection of enzymatic columns to perform automated HDX-MS experiments is very limited. Pepsin column is almost the only commercially available column and it provides low digestion efficiency and low sequence resolution for some proteins due to its preferred cleavage sites. Protease type XVIII has been tested in solution but has shown poor digestion efficiency under the test conditions in the literature. Here we present data generated from protease type XVIII columns for automated HDX-MS experiments demonstrating surprisingly high efficiency.

Methods

Protease type XVIII was immobilized onto POROS chromatography resins in house. The immobilized columns were evaluated using our in-house developed customized CTC-PAL-based automated HDX platform and the resultant peptides were separated using a customized C8 column and monitored by Q Exactive MS. The MS/MS raw data were searched using PepFinder software. All the tested proteins were denatured either in 4 M urea/0.425 M TCEP or in 2 M guanidine HCl/0.425 M TCEP (pH2.5) for 3 min at 4 degree before they were loading onto the immobilized enzyme columns.

Preliminary Data

Our initial results show that protease type XIII prefers to cleave on the C-terminal end of basic amino acids such as K and R and produced the highest number of fragments and the best sequence coverage compared to pepsin. The number of peptides identified for BSA and a commercial monoclonal antibody using protease type XVIII column were 1195 and 788, respectively, while the number of peptides identified for BSA and a commercial monoclonal antibody using a commercially available pepsin column were 1006 and 677, respectively. In addition, in real applications of HDX MS for protein higher order structure characterization, detecting the increased number of peptides for the proteins of interest has resulted in multiple overlapping peptides in many regions of the sequence, which has subsequently led to increased resolution for potential detection of differences in conformation between control and experimental samples, as well as increased sequence redundancy to obtain higher confidence in the results.

Novel Aspect

The first application of the Protease XVIII column to HDX-MS significantly improves digestion efficiency and the sequence coverage and resolution.