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

Chengjie Ji, Qian Li, and Yankun Li
NovaBioAssays LLC, Woburn, MA

NovaBioAssays
Innovative Mass Spectrometric Solutions

Novel Aspect

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

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

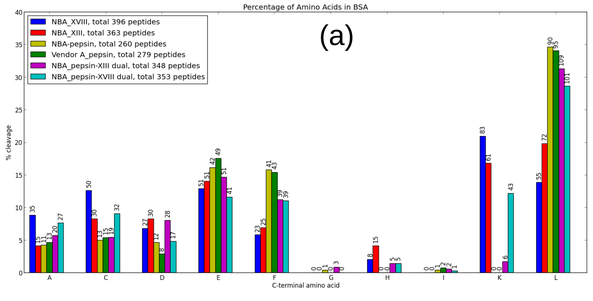
- **Enzyme column preparation:** protease type XVIII, type XIII, pepsin was immobilized onto POROS chromatography resins in house.
- The immobilized columns were evaluated using our in-house developed customized CTC-PAL-based HDX automation (refer to poster WP314)
- **MS:** Q Exactive MS.
- **MS/MS database search software**: Mascot.
- **Protein denaturation:** 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 before they were loading onto the immobilized enzyme columns.

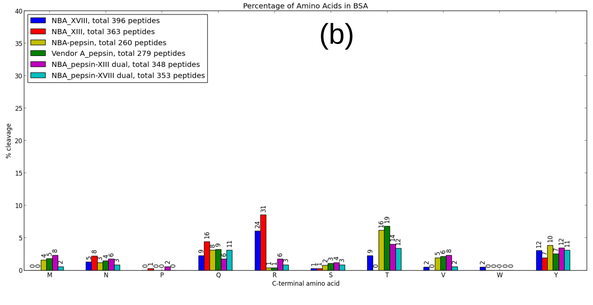
Results

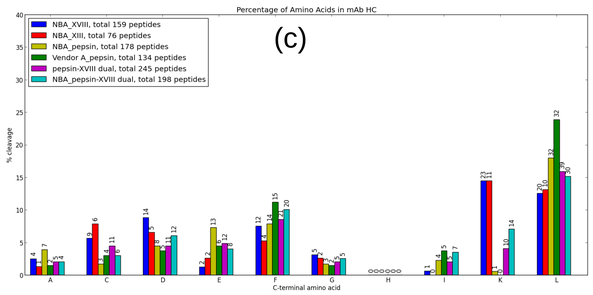
Table 1. Digestion performance summary for six tested enzyme columns.

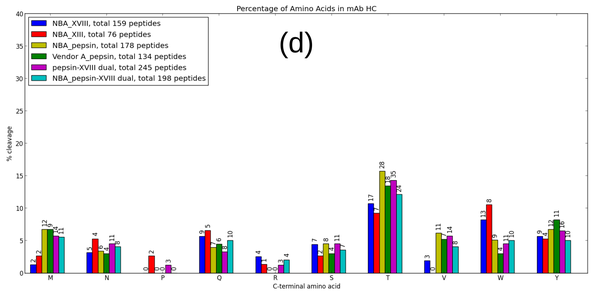
Protein name		NBA-XVIII	NBAXIII	NBA_pepsin	Vendor A_pepsin	NBA_pepsin-XIII	NBA_pepsin-XVIII
Bovine serum albumin	Top 3 C-terminal cleavage AA	K,L,C	L,K,E	L,E,F	L,E,F	L,E,F	L,K,E
	# of identified peptids	396	363	260	279	348	353
	Sequence Coverage (%)	98	90	97	98	98	98
mAb HC	Top 3 C-terminal cleavage AA	K,T,D	K,L,W	L,T,F	L,T,F	L,T,F	L,T,F
	# of identified peptids	159	76	178	134	245	198
	Sequence Coverage	95	82	93	84	97	97
mAb LC	Top 3 C-terminal cleavage AA	L,F,Y	R,K,(E,L)	L,V,(E,Y)	L,V,I	L,E,V	L,E,F
	# of identified peptids	118	78	121	94	223	144
	Sequence Coverage	100	94	99	99	99	99

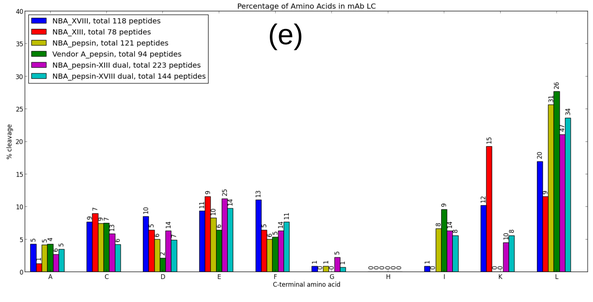
Figure 1. Individual enzyme and dual enzyme cleavage site preference at the C-terminal side in BSA (a) and (b), and in a commercial mAb HC (c) and (d) as well as its LC (e) and (f).











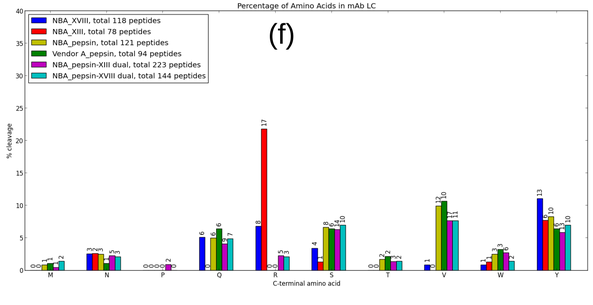
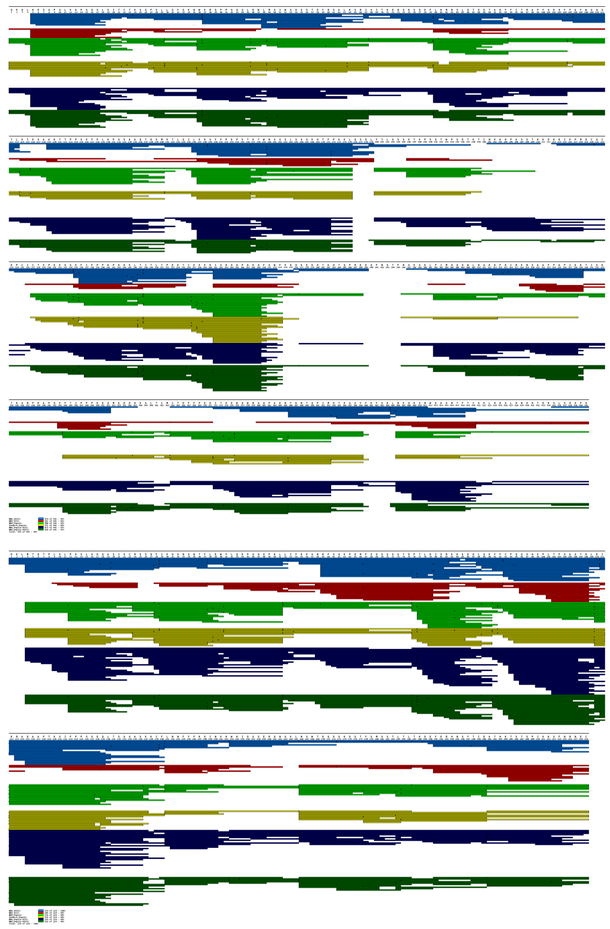


Figure 2. Sequence coverage for the HC and LC of the mAb digested using six different enzyme columns.



Conclusions

- ✓ Protease XVIII column shows better performance than pepsin or protease XIII columns in terms of # of the peptides identified and sequence coverage for the two tested proteins.
- ✓ Using the combination of different enzyme columns show increased sequence coverage and resolution for potential detection of differences in conformation between control and experimental samples, as well as increased sequence redundancy to obtain greater confidence in the results .