ASSESSING THE VARIABILITY OF PEPTIDE PEAK AREAS IN ONLINE PEPSIN DIGESTION

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OVERVIEW

This study is to assess the reproducibility of pepsin online digestion utilizing nanoACQUITY® with Synapt™ HDMS™ for LC/MS^E analyses.

INTRODUCTION

Hydrogen/deuterium exchange mass spectrometry (HXMS) has proven to be a useful tool for the study of protein conformation. Proteins studied with HXMS can be digested online with the acid protease pepsin.[1,2] One challenge in online digestion is the reproducibility of the peptide peak area. In this study, a variety of proteins were digested online with pepsin and the peptide peak areas were measured in large scale replicate analyses. Precursor and fragment ions were acquired in a data-independent manner by alternating the collision energy between a low and elevated energy state on a Q-Tof Mass Spectrometer. Time alignment was used to correlate precursor ions from the low energy with product ions from the elevated-energy data prior to a database search. Accurate precursor and product ion mass measurement showed high confidence peptide identification as well as reproducible peptide peak area produced from pepsin online digestion.

METHODS

LC / MS system:
Waters nanoACQUITY UPLC® and Synapt™ HDMS™
Binary Solvent Manager (BSM)
Eluent A: 0.1 % formic acid in water
Eluent B: 0.1 % formic acid in acetonitrile
Auxillary Solvent Manger (ASM)
Eluent A: 0.1 % formic acid in water
Eluent B: 100 fmol GFP in 50% A/ 50% B

Chromatography : The analytical column was an ACQUITY UPLC® BEH C18 1.7 µm 1.0 x 150 mm. The trap column was an ACQUITY VanGuard® Pre-column, BEH C18, 1.7 µm 2.1 x 5 mm.

On-line pepsin digestion : 2.1 x 50 mm immoblized pepsin columns packed in-house[3] and purchased from Applied Biosystems

Sample Preparation: phosphorylase b was dissolved in 10 mM potassium phosphate pH 7.0 then diluted with 100 mM potassium phosphate pH 2.5. 50 pmol were injected per run. Bovine serum albumin, cytochrome C, interferon α-2b, and monoclonal antibody (IgG) were prepared in the same manor.

MS Data Collection and Processing: MS^E data were collected for all analyses. Data were processed using ProteinLynx Global Server(PLGS) 2.4 (beta version) with Identity^E

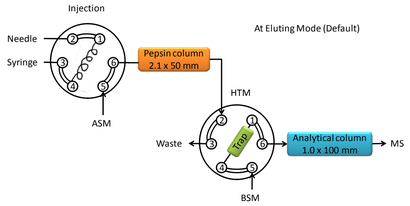


Figure 1. Fluid schematic for nanoACQUITY for online pepsin digestion. The protein loaded onto a pepsin column was digested for approximately 30 seconds.

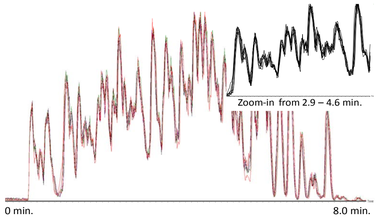


Figure 2. Overlay of every 5th chromatogram (BPI) of phosphorylase b in total of 31 injections. The fast separation in 6 min. gradient and MS scan at 0.2 scan/sec. were performed.

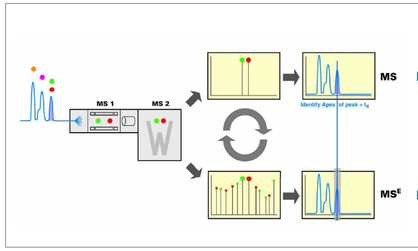


Figure 3. MS^E schematic. The peptide precursor ions were time-aligned with all of their MS^E fragments.[4]

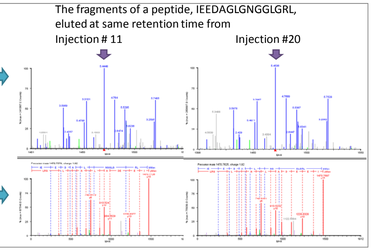


Figure 4. The accurate peptide identification based on MS^E. The peptides in replicates were accurately identified.

Protein	Aver. % RSD peak area	N=
Phos. b #1, col.#1	6.6	31
Phos. b #1, col.#1	6.1	5
Phos. b #2, col. #2	5.4	5
Phos. b #1, col. #2	6.2	5
Cytochrome C	8.3	3
BSA	8.2	3
Interferon α-2b	7.1	4
IgG, denatured	9.6	3

Figure 5. Summary of peak area % RSD for protein samples. The online pepsin digestion showed overall less than 10 % RSD in peak area, which is comparable to offline digestion (15 %) performed in nanoACQUITY system. Same concentration of phosphorylase b batch #1 and #2 were separately prepared. Column #1: AB commercial column; column #2: packed in house

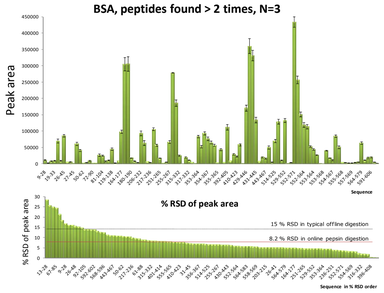
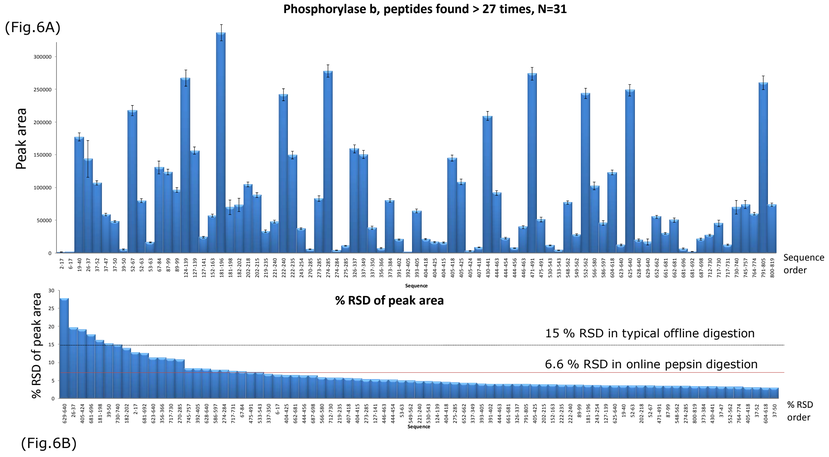


Figure 7. Reproducibility of peptide peak areas for BSA.

RESULTS & DISCUSSION

(Fig.6A)



(Fig.6B)

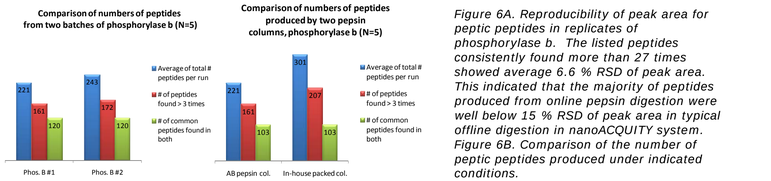


Figure 6A. Reproducibility of peak area for peptic peptides in replicates of phosphorylase b. The listed peptides consistently found more than 27 times showed average 6.6 % RSD of peak area. This indicated that the majority of peptides produced from online pepsin digestion were well below 15 % RSD of peak area in typical offline digestion in nanoACQUITY system. Figure 6B. Comparison of the number of peptic peptides produced under indicated conditions.

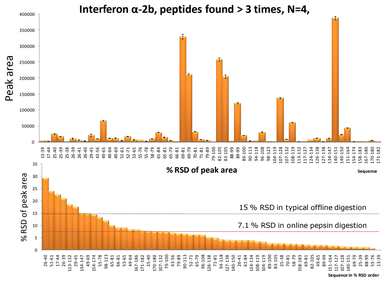


Figure 8. Reproducibility of peptide peak areas for interferon

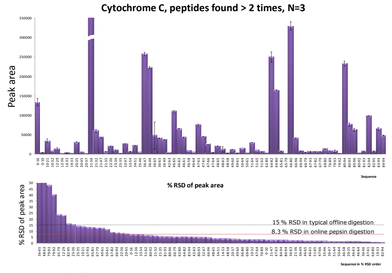


Figure 9. Reproducibility of peptide peak areas for cytochrome C

Figure 10. Comparison of peptide map for phosphorylase b, produced from AB commercial pepsin column (#1) and packed in house column (#2). 86 % and 93 % of peptide coverage were found from column #1 and #2 respectively.

CONCLUSION

- **High confidence peptide identification and accurate peak area measurements show the low variability of pepsin digestion during data analyses.**
- **The online pepsin digestion of protein in fast UPLC separation has proven to be reproducible.**
- **Such information is useful for HD exchange MS analysis for protein conformational study.**

Thanks to Dr. Thomas Wales for helpful comments.

References

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