HYDROGEN/DEUTERIUM EXCHANGE MASS SPECTROMETRY (HX MS) SYSTEM FOR CAPTURING CONFORMATIONAL CHANGES OF BIOTHERAPEUTICS

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INTRODUCTION

Hydrogen/deuterium exchange mass spectrometry (HX MS) has proven to be a useful analytical method for the study of protein dynamics and changes to protein conformation. The applications in HX MS require a system that can perform rapid chromatographic separations at 0 °C and accurate mass measurements of labeled proteins and peptides with small quantities of material.[1] Recent improvements in LC-MS have made HX MS an indispensable tool for biotherapeutic proteins. In this study, we describe comprehensive HX MS workflow recently adopted by a biopharmaceutical laboratory. This workflow utilizes a nanoACQUITY / Synapt HDMS interfaced with a newly developed chiller module. In this system, online pepsin digestion was coupled to highly reproducible UPLC performed at low temperature. MS^E analyses show high confidence peptide identification, up to 100% linear sequence coverage, and reproducible peptic peptide peak area (less than 10 % RSD) in large scale replicate analyses of phosphorylase b. To illustrate how these data are useful for HX MS, we describe a recent study of conformational changes in recombinant monoclonal IgG1 antibody upon post-translational modifications.[2]

METHODS

LC / MS system:
Waters nanoACQUITY UPLC®
Chiller Module (CM) and Electric Module (EM)
Binary Solvent Manager (BSM)
Auxillary Solvent Manger (ASM)
Waters Synapt™ HDMS™
ESI positive mode
Capillary / Cone : 3.0 kV / 37 V
Source / Desolvation : 80 °C / 175 °C
Desolvation gas : 800 L/h

Chromatography
Peptides: 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.
Intact Protein : The analytical column was an ACQUITY UPLC® BEH C4 1.7 µm 2.1 x 50 mm. The desalting column was used a MassPREP Desalting cartridge column 2.1 x 5 mm.

On-line pepsin digestion : 2.1 x 50 mm immobilized pepsin columns packed in-house[3] and 2.1 x 30 mm pepsin column purchased from Applied Biosystems

MS Data Collection and Processing:
Undeuterated peptides as control: MS^E data were collected for all analyses. Data were processed using ProteinLynx Global Server(PLGS) 2.4 with Identity^E
Deuterated peptides : HX-Express was used to measure the mass shift and peak width upon the deuterium uptake of peptides.

RESULTS & DISCUSSION

Label & Quench

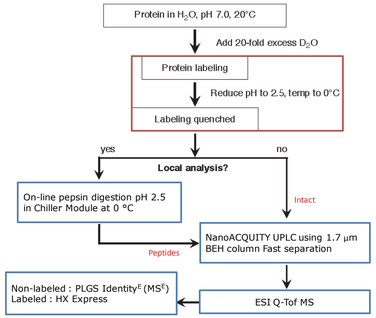


Figure 1. HX MS Workflow from protein labeling to data analysis.[5]

NanoACQUITY UPLC with Chiller Module

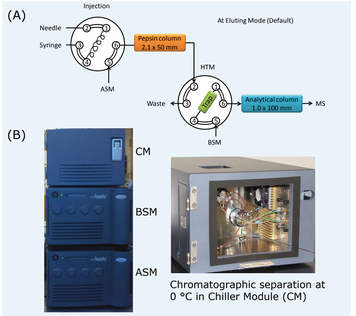


Figure 2. (A) Fluidic schematic for online pepsin digestion in nanoACQUITY. (B) NanoACQUITY UPLC with Chiller Module (left). Inside view of Chiller Module (right)

Waters ESI Q-TOF MS

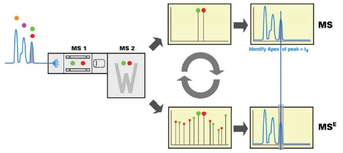


Figure 3. MS^E schematic. The peptide precursor ions were time-aligned with all of their MS^E fragments.

Data Analysis

Undeuterated peptides

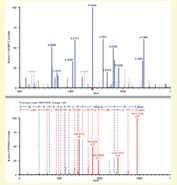
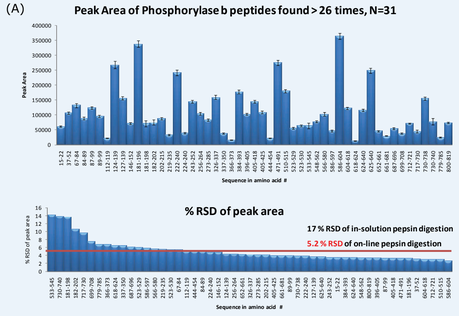


Figure 4. Accurate peptide identification based on MS^E. First, the undeuterated (control) peptides were identified in this manner, then deuterated peptides of same species were confirmed with retention time.

Online Pepsin Digestion



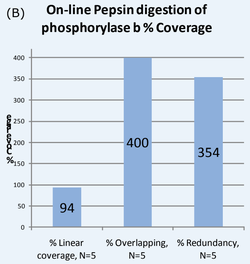


Figure 5. (A) Reproducibility of peak area for peptic peptides in replicates of phosphorylase b in nanoACQUITY system. This indicated that the majority of peptides produced from online digestion were well below 17 % RSD peak area in in-solution off-line digestion.

(B) The % Coverage of on-line pepsin digestion of phosphorylase b. The linear coverage was 94 % and the overlapping coverage was 400 %, which was calculated by the normalized number of amino acid residues that repeatedly found from the overlapped peptides. The redundancy score was calculated by the normalized sum of amino acid count that repeatedly found in the sequence. (C) A part of phosphorylase b sequence map was shown to illustrate the overlapping coverage and redundancy score.

Deuterated peptides

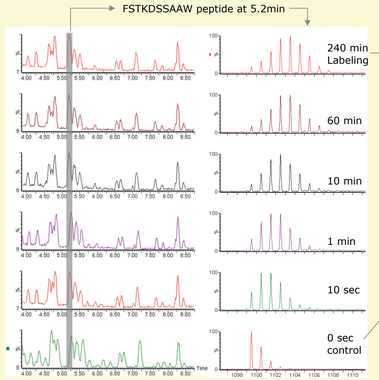


Figure 6. BPI chromatograms and spectra of interferon alpha-2b. The reproducible chromatograms with undeuterated (0 sec) and deuterated (10 sec, 1, 10, 60, and 240 min. D2O labeling, respectively) peptides were shown. The higher mass shift was observed upon deuterium uptake at longer time of continuous labeling.

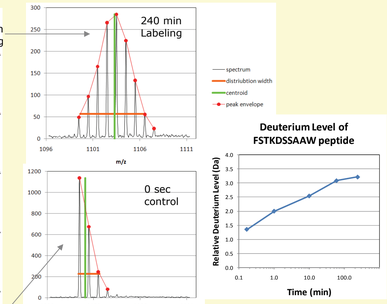


Figure 7. Deuterium incorporation calculation in HX-Express.[4] The relative deuterium level (in Da) was plotted in right panel. The data shows the relative mass difference between control and each time point, which was determined by centroid masses in green bar.

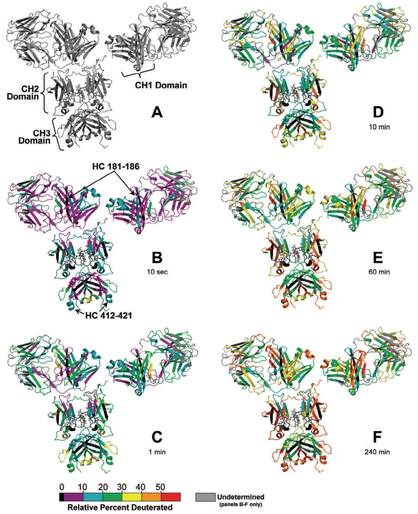


Figure 8 Deuterium incorporation information modeled to the structure of IgG1. Panel A shows the model structure of IgG1 illustrating the location of CH1, CH2, and CH3 domains. The relative percent deuterium incorporation is shown at 10 sec, 1, 10, 60, 240 min. (B-F, respectively) [2]

CONCLUSIONS

- **NanoACQUITY UPLC with newly improved Chiller Module was used in HD exchange MS.**
- **HD exchange MS analysis is a useful analytical method for protein conformational studies.**

References

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