Characterization of the Conformation of Therapeutic Antibody Oxidation Variants with Optimized Hydrogen/Deuterium Exchange Mass Spectrometry

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Poster Note 64465

Overview

Purpose: Probe the conformation of herceptin and its oxidation variants.

Methods: Fully automatic hydrogen/deuterium exchange mass spectrometry

Results: There are no significant conformational changes for most regions of herceptin and its oxidation variants. However, local solvent exposure differences in the vicinity of the peptides containing methionine oxidation were observed.

Introduction

Monoclonal antibodies (mAbs) have been increasingly used for detection and treatment of diseases. Characterization of chemical degradation of mAb-based drugs is a primary concern for biopharmaceutical development due to the subtle but critical local conformational changes that may impact safety and efficacy.[1,2] It is thus important to have an analytical tool that can detect these minor conformational changes. Hydrogen/deuterium exchange mass spectrometry (HDX) has emerged as a powerful tool to investigate the conformation of intact proteins, including mAbs. In this study, an optimized HDX workflow was developed and used to probe the conformation of Herceptin and its oxidation variants.

Methods

Therapeutic antibody, Herceptin, was partially oxidized with 0.01% H_2O_2 overnight. Both non-oxidized and oxidized mAb were diluted (1 to 9 ratio) with labeling buffer and incubated for multiple time points. The samples were then quenched with 4M guanidine, 200mM citric acid (pH 2.7) at 0.5 °C and subject to online pepsin digest at 8 °C for three minutes at 50 µL/min flow rate in a fully automated manner using H/D-X PAL™ (LEAP Technology). The digested peptides were injected into a Thermo Scientific™ PepMap™ trapping column washed for one minute and eluted to a Thermo Scientific™ Hypersil™ Gold C18 reverse phase column. A Thermo Scientific™ Ultimate™ 3000 nano pump system was employed to separate the digested peptides with 5% to 40% mobile phase B in 6 minutes gradient at flow rate of 40 µL/min. The separated peptides MS analysis was performed with Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer. The data dependent MS/MS HCD spectra were collected using undeuterated protein for peptides identification first. And MS full scan at 60K was collected for HDX analysis. Figure 1 is the HDX work station set up. Figure 2 is HDX experimental workflow.

Liquid Chromatography

Thermo Scientific™ online pepsin Column: 2.1 x 3 mm

Thermo Scientific™ Dionex™ trapping Column: 500 µm x 15mm, C18 PepMap300, 5µm

Hypersil Gold analytical Column: 0.5 mm x 100mm, 3µm

Data Analysis

Data was processed with Proteome Discoverer 1.4™ software for peptide identification. Peptide mapping and PTM analysis was performed with PepFinder 2.0™ software. HDX experimental data were analyzed with HDExaminer and the Mass Analyzer HDX algorithm.[3,4]

FIGURE 1. HDX Work Station

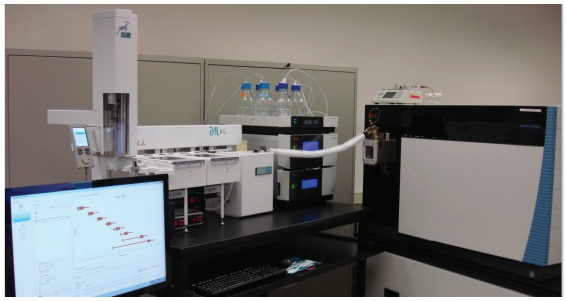
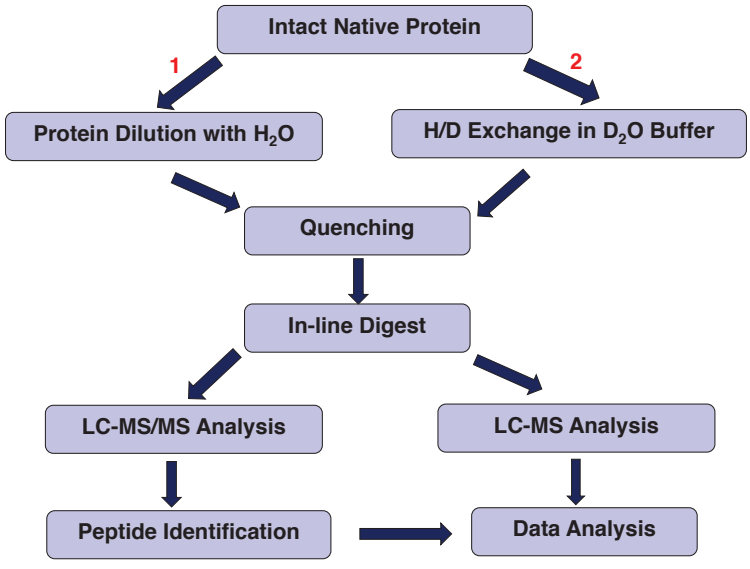


FIGURE 2. HDX experimental workflow



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Results

Peptide mapping of Hercetin

MS/MS experiments were first performed using non-deuterated Herceptin for peptide identification. Nearly 100% sequence coverage was achieved for both the Herceptin and oxidized Herceptin samples. Figure 3 is the peptide map of Herceptin generated by the Pepfinder software. More than 200 peptides generated by online pepsin digestion from the optimized HDX workflow were identified. These were subsequently used to probe the conformation of the two samples by HDX.

FIGURE 3. Peptide map of Herceptin

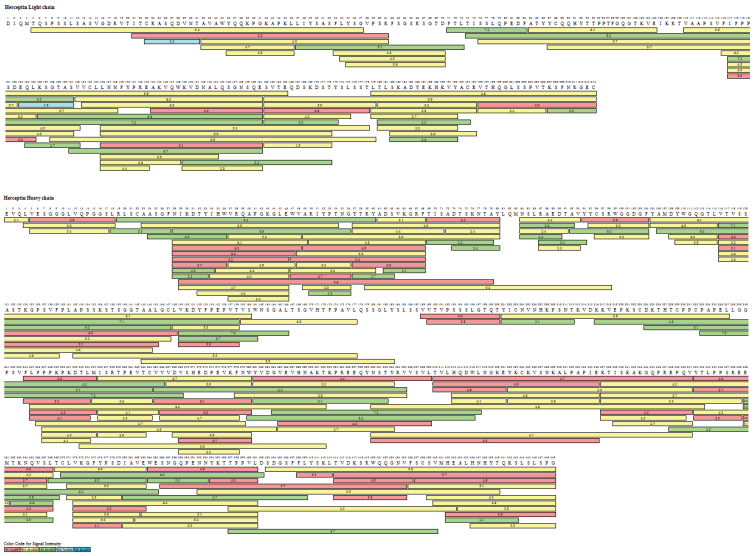
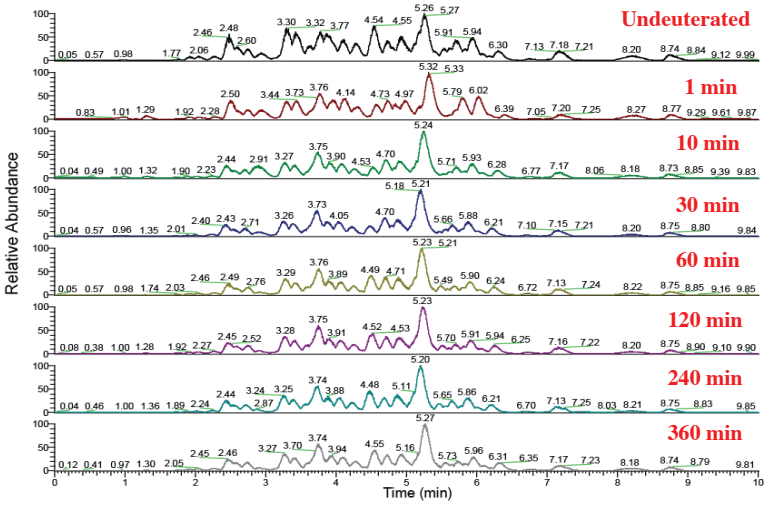


FIGURE 4. Herceptin HDX experimental base peak chromatogram



Conformation of Herceptin and its oxidized variants

Multiple time points of HDX experiment were performed for both Herceptin and oxidized Herceptin samples. Highly reproducible chromatograms were obtained for the various experimental time points (Figure 4). MS full scan spectra were collected to measure the deuterium uptake to probe the conformation of the therapeutic antibody and its variants. The deuterium uptake information were processed by HDExaminer.

FIGURE 5. a) Light chain deuterium uptake mirror plot of Herceptin and Herceptin variants. b) Deuterium uptake information modeled to Herceptin light chain crystal structure (PDB 1N8Z). The relative percent deuterium incorporation is shown at 30, 600, 3600 and 7200 seconds respectively.

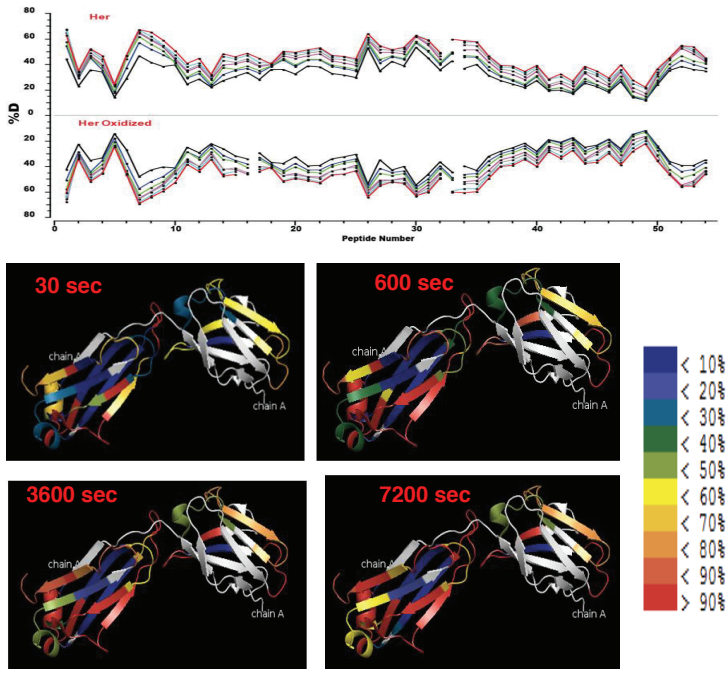
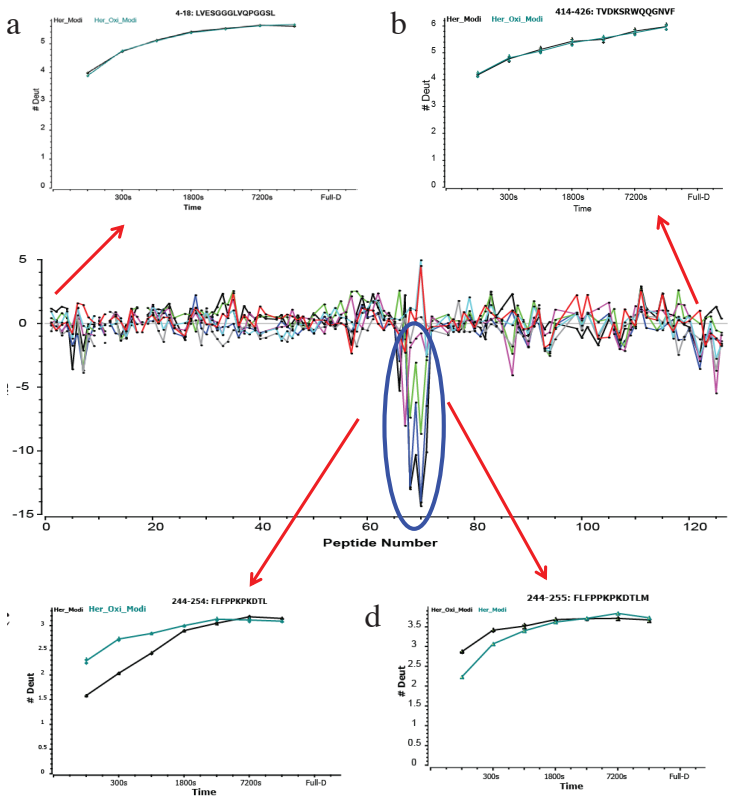


Figure 5 shows the light chain deuterium uptake measurement information. The very similar deuterium uptake patterns of the two samples indicate that there is no significant light chain conformational differences between Herceptin and Herceptin variants samples as shown in Figure 5a). The deuterium uptake measurements were exported to PMOL software and incorporated with the available Herceptin crystal structure as shown in Figure 5 b). The deuterium incorporation difference provides the information understanding the conformation dynamics of the light chain.

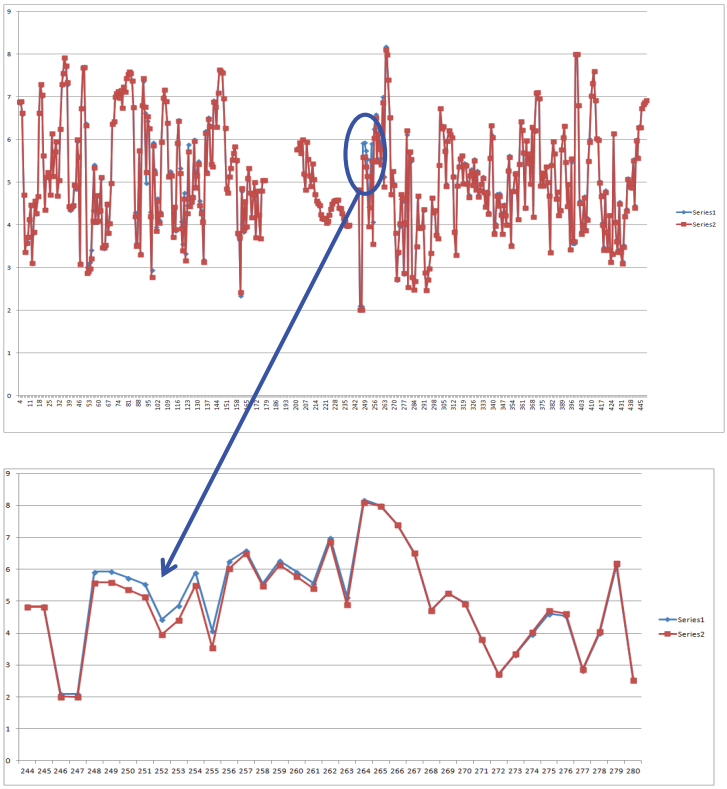
FIGURE 6. Herceptin versus Herceptin variants heavy chain deuterium uptake residual plot. Inserts a, b, c d are the specific peptides deuterium uptake plots of Herceptin and its variants



In Figure 6, heavy chain deuterium uptake difference between Herceptin and its oxidized variants is plotted vs. peptide number. The data were obtained with HDExaminer from MS full scan at various deuterium exchange time points. At most regions the difference is minimal (inserts a, b), except at the specific region where in the vicinity of methionine (residue 255), which is the amino acid that is oxidized. The inserts (c, d) of Figure 6, deuterium uptake plots of peptide FLFPPKPKDTL and FLFPPKPKDTLM, show the different kinetic behavior of deuterium uptake of Herceptin and its oxidized forms; after oxidation, the deuterium uptake is faster. Structurally, it is more sterically accessible for solvent exchange when methionine's SCH_3 terminal is oxidized to $SOCH_3$ or SO_2CH_3.

In Figure 7 protection factors for each residue in heavy chain of Herceptin and its variants were plotted. Mass Analyzer HDX algorithm was used to calculate the protection factor at the amino acid level. HDX model is built to simulate the whole deuterium labeling and back exchange processed during the digestion and analysis. The HDX model utilized the maximal information of the entire HDX MS data set (both the HDX kinetics and the labeling information from all overlapping peptides)[4]. 400 simulation was employed for this data set. Similar to findings shown in Figure 6, the protection factors are identical for most of the residues except in the region where methionine (residue 255) is involved. The oxidized variants have lower values compared to the original form, consistent with results obtained with HDExaminer.

FIGURE 7. (top): the average value of protection factors for each residue in heavy chain of Herceptin and its variants. (bottom): zoom in of the specific region with significant protection factor change



Conclusion

•A fully automated HDX workflow was developed and successfully applied to the study of conformational changes of Herceptin upon oxidation.

•The workflow was reliable and able to pinpoint the subtle but significant changes in the methionine region.

•The MS data were analyzed by two independent packages HDExaminer and Mass Analyzer and the conclusions are consistent.

References

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Acknowledgements

We would like to thank Professor Mark Avdalovic from the University of California Davis for supplying the monoclonal antibodies.

PN64465-EN 0616S