Poster Note 64771

Characterization of Conformation of Therapeutic Antibody Aggregation with Optimized Hydrogen/Deuterium Exchange Mass Spectrometer

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ABSTRACT

Protein aggregation is one of the major critical quality attributes (CQA). To determine the structural change of antibody in connection with aggregation is important. In this study, a therapeutic antibody, Herceptin®, was treated with different acidic solutions (pH 0.5, 1.5 and 2.5) to induce aggregation. The aggregation was quantified by UV 280 absorption. The conformation of Herceptin and its aggregation was characterized by an optimized hydrogen/deuterium exchange mass spectrometer (HDX). The HDX results revealed the more significant conformation change regions when aggregation was induced.

INTRODUCTION

Monoclonal antibodies (mAbs) have been increasingly used for detection and treatment of cancer and other diseases. Characterization of critical quality attributes (CQA) of mAb-based drugs is a primary concern for biopharmaceutical development. Structural characterization is used to assess the CQAs of biopharmaceutical products. Protein aggregation is a major CQA during monoclonal antibody (mAb) production. The formation of aggregates may impact safety and efficacy of mAbs. It is thus important to understand the mechanism of aggregation and the conformational changes of the aggregates. 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 used to probe the conformation of Herceptin and its aggregation.

MATERIALS AND METHODS

Instrument and Materials

A fully automated HDX platform, based on the H/D-X PAL™ system (LEAP Technologies) and the Thermo Scientific™ UltiMate 3000 pump system coupled online with an Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer, was used. Figure 1 shows the HDX work station. It is composed of an autosampler equipped with individually temperature-controlled sample plate and labeling plate. The syringe head and pepsin column can also be independently temperature-controlled. Chronos™ control software is fully integrated with the Thermo Scientific™ Xcalibur™ platform and the user interface is shown in Figure 2. Figure 3a) describes the three valve configurations at various stages of the experiment inside the cooling chamber. The flexibility allows for maximized productivity. Figure 3 b) shows the dual heads high pressure mixing pump on the left and ternary loading pump on the right. Flow rates and solvents can be changed on the fly during the experiment. The execution of the whole experiment was completely automated and controlled by Chronos™ software.

Therapeutic antibody (Herceptin) aggregation was induced by adjusting the pH to 0.5, 1.5 and 2.5 with HCl and by incubating each sample at room temperature for 30 min. The pH of the samples was subsequently adjusted to pH 9. Both non aggregated and aggregated mAbs were diluted with labeling buffer and incubated for multiple time points. The samples were then quenched and digested online with a pepsin column in a fully automated manner using the H/D-X PAL system. The digested peptides were injected into a Thermo Scientific™ Acclaim™ Pepmap™ C18 reverse phase column with a 7 min gradient. MS analysis was performed with the Orbitrap Fusion mass spectrometer.

Data Analysis

Peptide identification was performed with Thermo Scientific™ Proteome Discoverer™ 1.4 software . Peptide mapping and PTM analysis was performed with Thermo Scientific ™ BioPharma Finder™ 1.0 software. HDX experimental data were analyzed with HDExaminer™ software (Sierra Analytics) and the HDX tool in Mass Analyzer (licensed from Amgen).[1,2].

FIGURE 1. HDX work station coupled online to the Orbitrap Fusion mass spectrometer

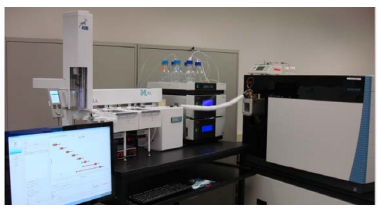


Figure 2. Chronos software interface, integrated with Xcalibur software

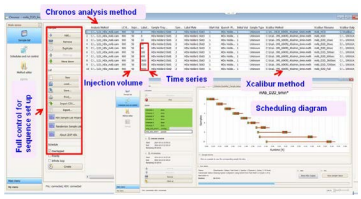


Figure 3. a). Flexible three valve configuration for carrying out different experimental tasks simultaneously in the cooling chamber, b). NCS-3500RS with binary rapid separation micro flow pump with ternary loading pump

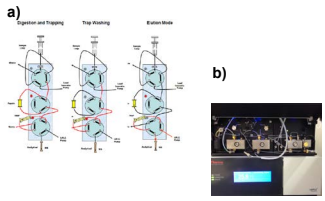


Figure 4. UV absorption spectrum of Herceptin and pH stressed samples

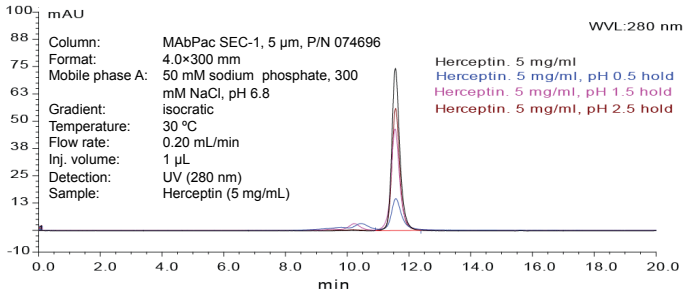
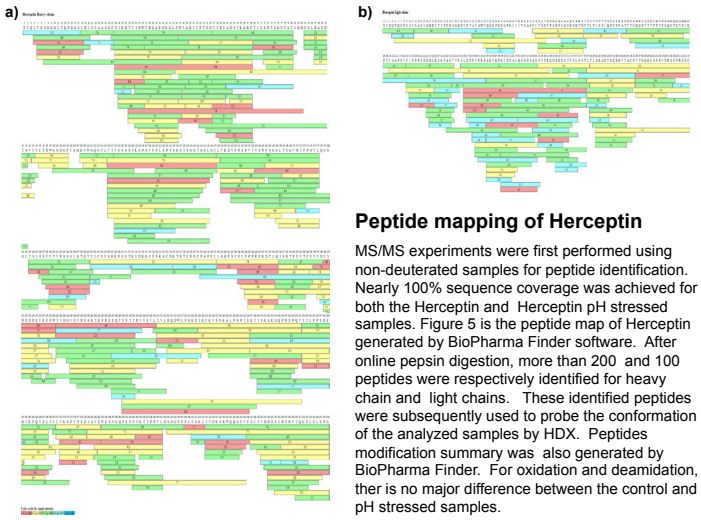


Figure 5. Peptide map of Herceptin by BioPharma Finder a) Heavy chain b) Light Chain



Peptide mapping of Herceptin

MS/MS experiments were first performed using non-deuterated samples for peptide identification. Nearly 100% sequence coverage was achieved for both the Herceptin and Herceptin pH stressed samples. Figure 5 is the peptide map of Herceptin generated by BioPharma Finder software. After online pepsin digestion, more than 200 and 100 peptides were respectively identified for heavy chain and light chains. These identified peptides were subsequently used to probe the conformation of the analyzed samples by HDX. Peptides modification summary was also generated by BioPharma Finder. For oxidation and deamidation, ther is no major difference between the control and pH stressed samples.

RESULTS

Herceptin and Herceptin Aggregation

Herceptin and its pH stressed samples were quantified by measuring UV 280 absorption. Figure 4 is the overlay absorption spectra of four samples. Aggregations around 25% for pH 0.5, 6% for pH 1.5 and 0% for pH 2.5 were observed.

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Herceptin and its Aggregation conformation characterization

Multiple time point HDX experiments were performed for both Herceptin and pH stressed Herceptin samples. Highly reproducible chromatograms were obtained for the various experimental time points. MS full scan spectra were collected to measure the deuterium uptake to probe the conformation.

Overall, the control and pH stressed samples had similar deuterium uptake profile but for most of the pH stressed sample peptides showed slightly more deuterium uptake, around 2 to 5%, than for the non-stressed sample. The differences could reach up to 10 to 15% for different regions for the light chain and could be even higher for the heavy chain. The sample stressed at pH = 0.5 showed more deuterium uptake than the pH = 1.5 sample. Finally, there was no significant deuterium uptake change between Herceptin and the stressed sample pH=1.5 (data not shown).

Figure 6. a): Herceptin and pH 0.5 stressed sample light chain deuterium uptake light chain residual plot. b), c): Example of specific peptides deuterium uptake plots of Herceptin and its pH 0.5 stressed sample

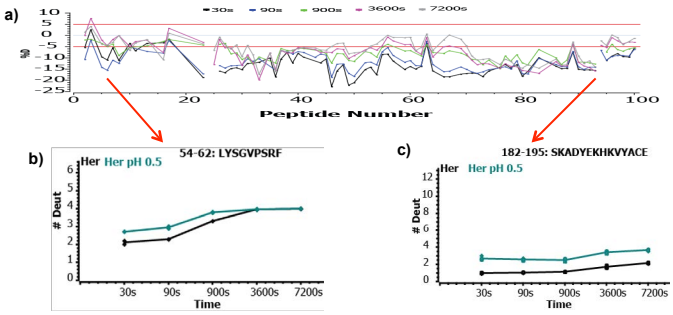


Figure 6 a) is the light chain deuterium uptake residual plot whereas figure b) and , c) are peptides uptake plots. In figure 6 b) peptide showed more differences for short labeling time but would reach to similar levels at longer time points, while in figure 6 c) peptide showed the significant differences persistent along all the time points. Protection factors were calculated and shown in figure 7. Certain regions have similar protection factors while other regions have significant differences, i.e., between amino acids160 and 190 for the heavy chain. These results were consistent with HDExaminer's analysis.

Figure 7. Herceptin and pH 0.5 stressed sample light chain protection factor comparison

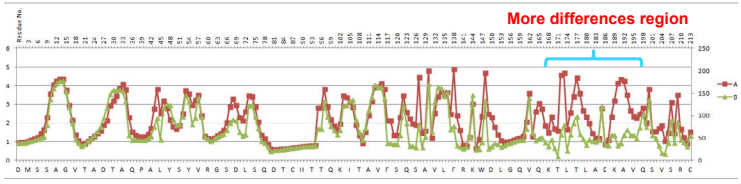
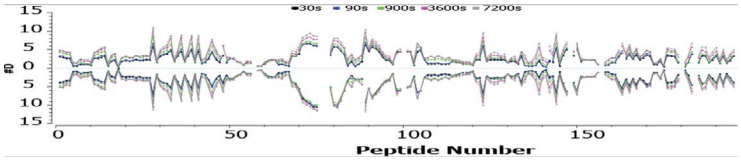


Figure 8. Herceptin and pH 0.5 stressed sample heavy chain deuterium uptake mirror plots



Similar deuterium uptake profiles of Herceptin and its pH stressed sample were observed for heavy chain and are shown in figure 8. In general, the stressed sample showed more deuterium uptake, and the amount of uptake increased with degree of aggregation. This implies that aggregation would impact the 3D structure of Herceptin and more exposed to solvents, i.e., more deuterium uptake. Furthermore, the increase in deuterium uptake was not uniform across all the peptides. Example deuterium uptake plots were shown in figure 9. In these examples, different peptides showed different deuterium uptake dynamics and levels. With mass spectrometry, the regions with such increase can be pinpointed. A pronounced deuterium uptake increase (up to 40%, see left panel of figure 10) was observed from amino acid 120 to 200 and 240 to 255 of the heavy chain for the sample stressed at pH=0.5. The differences could be up to 5 daltons, see figure 10, right panel. These results indicated that aggregation would cause more conformational change for the Fab region and smaller changes for the Fc region.

Figure 9. Herceptin and pH 0.5 stressed sample heavy chain peptides deuterium uptake plots

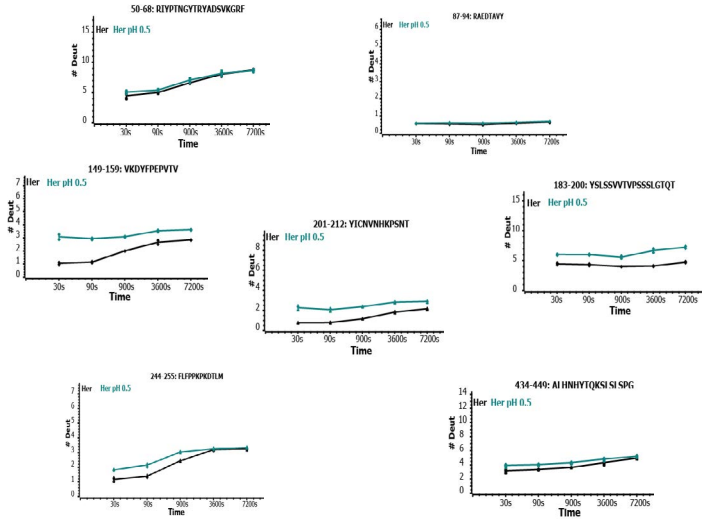


Figure 10. Herceptin and pH 0.5 stressed sample heavy chain deuterium uptake residual plots, Left: %D Right: #D

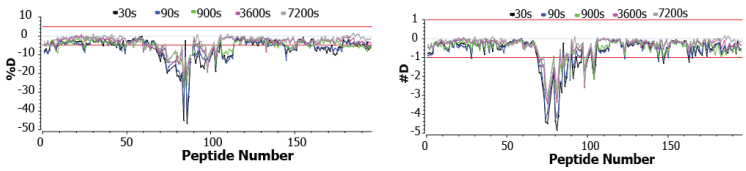
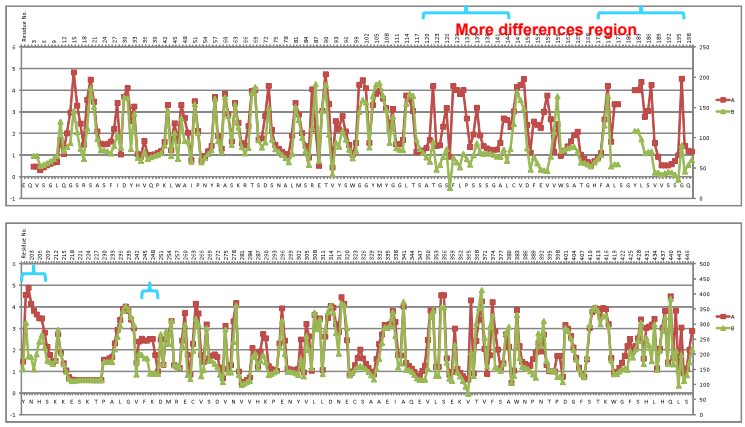
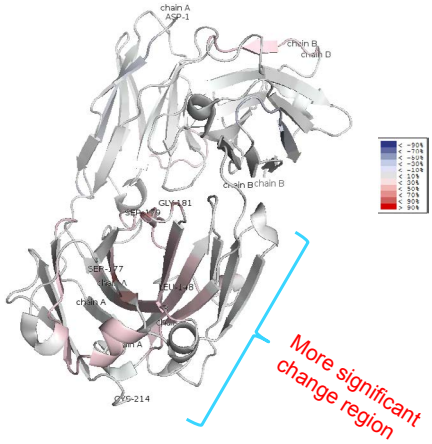


Figure 11. Herceptin and pH 0.5 stressed sample heavy chain protection factor comparison. Top: residual 1-200 Bottom: residual 201-449



To further understand the conformational dynamics, single amino acid residue level protection factors were calculated using Mass Analyzer HDX, (see figure 11). In most of the regions Herceptin showed slightly higher protection factor than the pH 0.5 stressed sample. From amino acid 110 to 210 and 240 to 255, Herceptin had a much higher protection factor, which is consistent with the deuterium uptake residual plots obtained from HDExaminer.

Figure 12. Herceptin and pH 0.5 stressed sample 1hr deuterium labeling Fab differential exchange map on crystal structure (PDB: 1N8Z)



Antibody aggregation is a complex, multistep process[3]. The conformation change with more deuterium uptake observed for the sample stressed at pH 0.5 agreed with the proposed aggregation mechanism[3,4] and the reported results[5] in the literature. The differential deuterium uptake of Herceptin and the sample stressed at pH 0.5 for1 hour labeling was mapped with Herceptin Fab crystal structure (PDB:1N8Z) and shown in figure 12. Most of the significant change regions were in the C_L, C_H and part of Fc C_H2 domains.

CONCLUSIONS

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

•In this study, we were able to pinpoint the subtle but significant changes around the C_L, C_H and C_H2 regions.

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

REFERENCES

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