Studying the Conformational and Functional Impact of Chemical Degradations in the Antibody Complementarity-Determining Regions Using Hydrogen Deuterium Exchange Mass Spectrometry and ELISA

H. Wei 1, Y. Yan 2, Y. Fu 1, M. Zeng 1, R. Ludwig 1, L. Tao 1, T. Das 1
1 Bristol-Myers Squibb Company, 2 Washington University

Purpose

Chemical degradations in mAbs can potentially change their conformation dynamics, impacting their efficacy as therapeutic drugs. Conformational modifications affecting the complementarity-determining regions (CDRs) may impair target binding affinity and therefore drug potency. In order to better understand the interplay between changes in protein conformation and mAb attributes induced by chemical modifications, we utilized hydrogen deuterium exchange mass spectrometry (HDX MS) to interrogate the conformational impact of Asp isomerization and Met oxidation in the CDRs of an IgG1 antibody (mAb1). Evaluating the HDX results in combination with an antigen binding ELISA assay we aimed to reveal the correlation between modification, structure, and function for the therapeutic antibody.

Methods

Native, isomerized and oxidized mAb1 were purified from a naturally degraded mAb1 sample using hydrophobic interaction chromatography (HIC). HDX experiments of the native, isomerized and oxidized mAb1 samples were performed on a HDX manager system with automatic sample handling, online digestion and separation (Waters, Milford, MA). Continuous labeling with deuterium was initiated by diluting each protein stock solution into deuterated buffer (pD 7.0). HDX control samples (non-deuterated) were prepared by diluting each of the protein stock solution into aqueous buffer. HDX reactions were maintained at 23 °C and quenched at 20 s, 2 min, 15 min, 1 h and 4 hs, followed by online pepsin digestion for generating the peptic peptides and ultra high performance liquid chromatography (UHPLC) for separating the peptic peptides at 0oC. MS detection was immediately followed after the separation on a Synapt G2-Si mass spectrometer (Waters) in the MSE mode for measuring the accurate mass of labeled/non-labeled peptic peptides at both precursor ion and product ion levels. The MSE data were analyzed using the ProteinLynx Global Server 3.0 (Waters). The uptake levels and HDX kinetic curves were generated using the DynamX software (Waters). Binding activity of the antibody variants was analyzed by a 96-well format sandwich ELISA method that can detect a dose-dependent binding activity of antibody.

Results

From the non-deuterated experiments 210 peptic mAb1 peptides were identified reproducibly, covering >99% of the mAb1 linear sequence. The HDX levels of these peptides were then measured and the deuterium incorporation differences between the native state and either modified state were calculated. MAb1 contains one Asp in the CDR region which is susceptible to isomerization. We observed a significant increase in deuterium labeling in the peptide containing the isomerized Asp compared to the native peptide, indicating that isomerization leads to a more dynamic and solvent exposed conformation in the local region. MAb1 also contains one Met in the CDR region which is susceptible to oxidation. Data indicates a slightly more protected local region in CDR as a result of Met oxidation, which is opposite to the effect of isomerization.

In addition to inducing local conformational changes, post-translational modifications on antibodies can sometimes lead to allosteric structural changes and impact on the antibody’s overall conformation. In assessing the overall conformational changes between the native, isomerized and oxidized mAb1, the HDX levels of all the peptic peptides were measured and compared between the native mAb1 and either modified species. No significant HDX differences between the native state and the isomerized or oxidized states were detected for most regions in mAb1. However, a few regions with overlapping peptides consistently showed significantly increased or decreased deuterium uptake levels in the modified samples, suggesting conformational changes.

Conformational changes may lead to functional differences. Therefore, the binding activity of each of the purified mAb1 modified forms was determined using an ELISA assay. The relative binding affinity to the mAb1 target protein was measured for the native, isomerized and oxidized mAb1 sample. The isomerized sample showed greatly reduced binding compared to the native sample. On the other hand, the isolated oxidized fraction showed full activity compared to the native sample, indicating no impact on the antibody’s potency caused by Met oxidation.

Conclusion

We have applied HDX MS to examine both the local and global structural impact of two common modifications, Asp isomerization and Met oxidation, at CDR of mAb1. Asp isomerization induced local and global structural changes, leading to largely impaired binding. On the contrary, Met oxidation results in a much smaller structural perturbation to the local CDR region and the allosteric regions, and no significant impact on binding affinity to target protein. This study can serve as an important reference to future studies on correlations between CDRs isomerization and antibody attributes, especially for IgG1 mAbs. Future studies in this area could involve studying more mAbs from the IgG families on structure-modification correlations, gaining more insight into the antibody modification-structure-function relations.