Localized conformation analyses of mutated human IgG1 by hydrogen deuterium exchange mass spectrometry and differential scanning calorimetry

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Joomi Ahn [1], Xiaojun Lu[2], Colette Quinn[3], St John Skilton[1], Ying Qing Yu[1], Kenji Hirose[4], and Jihong Wang[2]
[1]Waters Corporation, [2] MedImmune, [3]TA Instruments, [4]Nihon Waters K.K.

Overview

This study investigates the localized conformational changes in mutated antibodies utilizing Hydrogen Deuterium Exchange Mass Spectrometry (HDX MS) and Differential Scanning Calorimetry (DSC).

Introduction

Hydrogen/deuterium exchange mass spectrometry (HDX MS) is a useful analytical tool for studying local changes in protein conformation. DSC provides insight into overall structure of folding/unfolding process. Both techniques can be used in the development and discovery of protein drugs.

A Human immunoglobulin G (IgG) was developed with a site-specific mutation. When the higher order structures of non-mutated and mutated IgGs were compared using alternate methods, the localized conformational changes at the substitution sites were not easily detected.

The HDX LC-MS system and the local HDX work-flow were deployed in this study to demonstrate the capability of detecting differences in deuterium uptake at the peptide level. The differences in deuterium uptake allowed us to see clear changes of conformation between the mutated and non-mutated forms. Time consuming HDX data processing was automated by an innovative HDX software tool, DynamX™. DynamX™ automatically calculates the deuterium uptake and these results are displayed in convenient comparative views: uptake curves, butterfly charts, and difference plots[1]. We report efficient HDX studies of a control and of two batches of mutated IgG1.

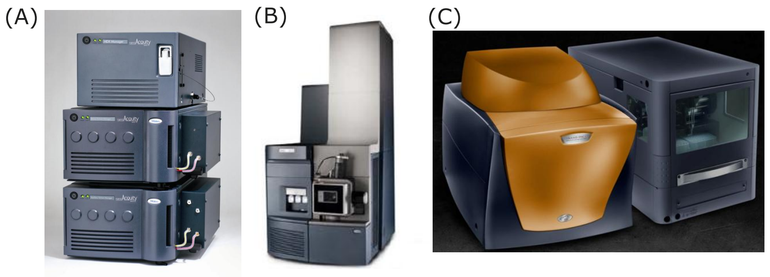

Figure 1. A complete HDX system solution used for this study: (A) nanoACQUITY UPLC system with HDX technology, (B) XEVO G2 Q-TOF MS, (C) Nano Differential Scanning Calorimetry Autosampler.

METHODS

.Samples: 3 batches of humanized IgG1: Control (non-mutated), mutated batch #A , and mutated batch #B.

.The IgG samples were digested online using a pepsin column. The digested peptides were separated in nanoACQUITY™ with HDX manager (Figure 1A) with the temperature set at 0 ˚ C to minimize back-exchange. The spectra were acquired with a XEVO G2 Q-TOF mass spectrometer. (Figure 1B)

.ProteinLynx Global Server (PLGS) for peptide ID and DynamX™ software (Figure 3) for automated deuterium calculations were used.

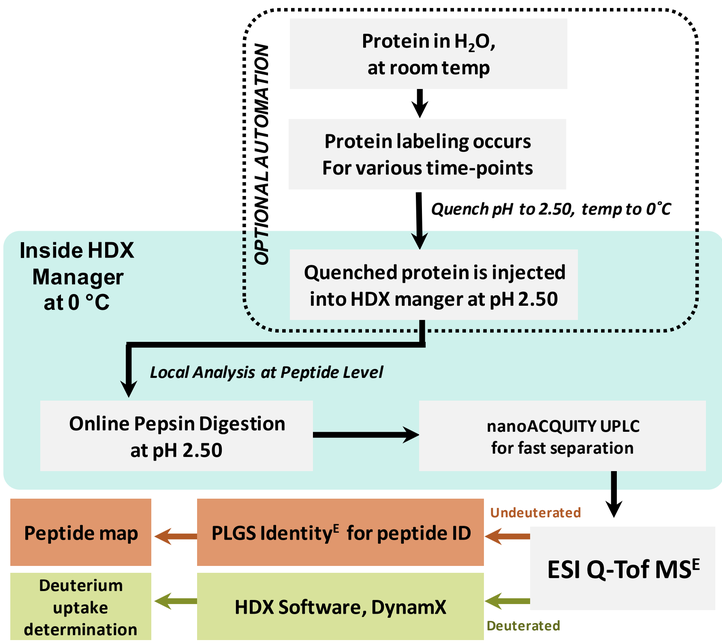

Figure 2. The HDX workflow at the peptide level. The peptide level analyses determined the location of conformational changes. Protein digestion was performed with an online pepsin column. The temperature inside of the HDX manager was set at 0˚ C as highlighted in the light blue area, where fast UPLC separation was performed within 10 min. Automated sample preparation such as labeling and quenching reactions can be performed using a Leap robotic system illustrated in the dotted line area. The non-deuterated peptides were identified in a PLGS data search to construct the peptide map and deuterated peptides were processed in DynamX™ software for deuterium uptake calculations.

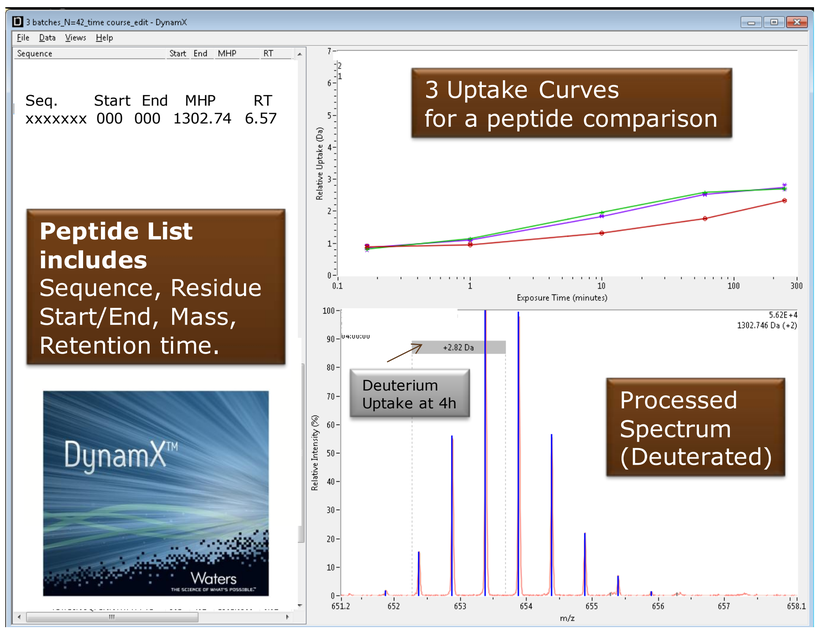

Figure 3. A screen-capture from DynamX™ software which automates HDX data processing. The peptide list, the uptake curves, and the processed spectrum are displayed in the left, the top right, and the bottom right panels, respectively. The peptide information and processed raw data are interactively linked and visualized. The 3 different uptake curves are overlaid for a comparison of the corresponding peptide.

Conclusion

•We have successfully used the Waters HDX MS and TA nano DSC technologies to localize conformational changes on mutated IgG batches in this comparability study.

•Differences in deuterium uptake were observed in the mutated regions. This finding was also confirmed by nano DSC data.

•DynamX™ software efficiently processes the large data sets required for this type of experiment.

References

. **Houde D, et al. J. Pharm. Sci. 6 (2011) 100, 2071-2086.**

RESULTs and Discussion

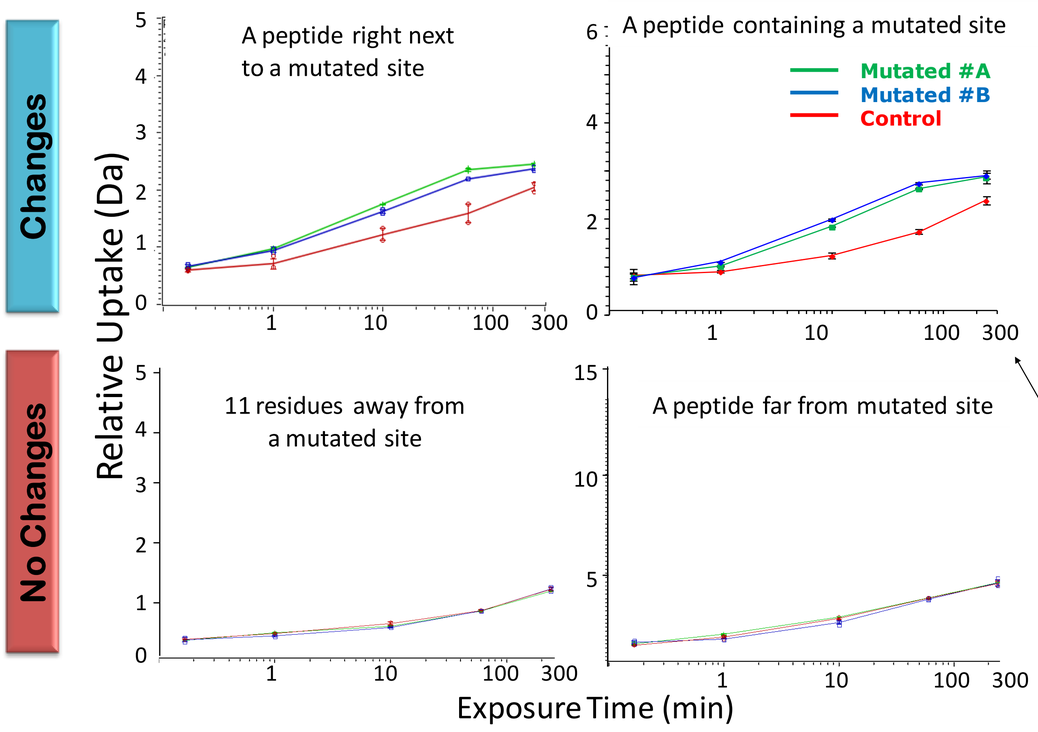

Figure 4. The uptake curves with deuteration changes (top two panels) and without changes (bottom two panels) comparing the control and the mutated batches. The key finding in this figure is the location at the peptide level of the local conformation that has been affected by the mutations.
Each panel shows the corresponding peptides from the Control, A and B batches. The deuterium uptakes of the peptide were measured as a function of deuterium exposure times at 0.17, 1, 10, 60, 240 min.
(A) The top two panels show that the peptides from mutated batches had faster exchange rates compared to the ones from the Control. These peptides were found nearby or at a mutated site. This indicates that the local effect related to the site-specific mutation has been determined by HDX. (B) However, the other two peptides located far from the mutated site showed the same deuteration uptake. This indicated that there were no structural changes at those locations. In both the heavy and light chains only the mutation sites showed changes.

Table 1. Relative deuterium uptakes of a peptide containing a mutated site. Each deuteration was calculated for control, mutated batch #A, and mutated #B at 0.17, 1, 10, 60, and 240 labeling times. 29.1% of deuteration is shown for this control peptide at 60 min whereas 44.1- 45.9% deuteration are shown for the same peptide containing a mutated site.

	Relative deuteration in Da					Relative deuteration %				
IgG batch	0.17 min	1 min	10 min	60 min	240 min	0.17 min	1 min	10 min	60 min	240 min
Control	0.838225	0.918925	1.243125	1.748175	2.393975	14.0%	15.3%	20.7%	29.1%	39.9%
Mut.#A	0.8104	1.03575	1.8511	2.64445	2.87445	13.5%	17.3%	30.9%	44.1%	47.9%
Mut.#B	0.79785	1.12075	2.00095	2.75145	2.91545	13.3%	18.7%	33.3%	45.9%	48.6%

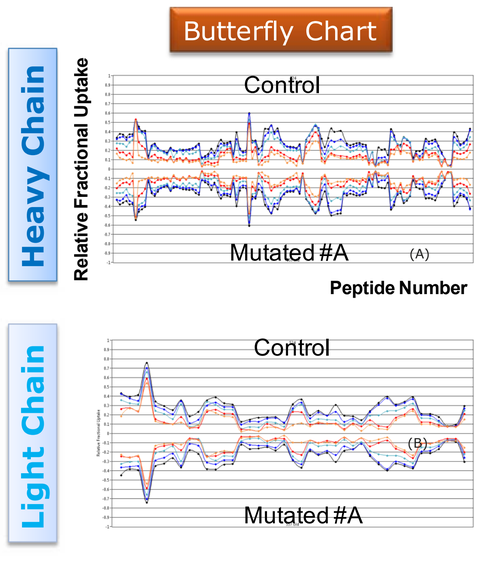

Figure 5. DynamX "Butterfly" charts comparing the control and the mutated batch #A. These charts make the data comparison convenient. Relative fractional uptake was plotted in the order of peptide number. Each data-point indicates the individual deuteration amount per identified peptide. 0, 17, 1, 10, 60, 240 minute time-points were color-coded in orange, red, light blue, dark blue, and black, respectively. (A) A heavy chain comparison. (B) A light chain comparison.

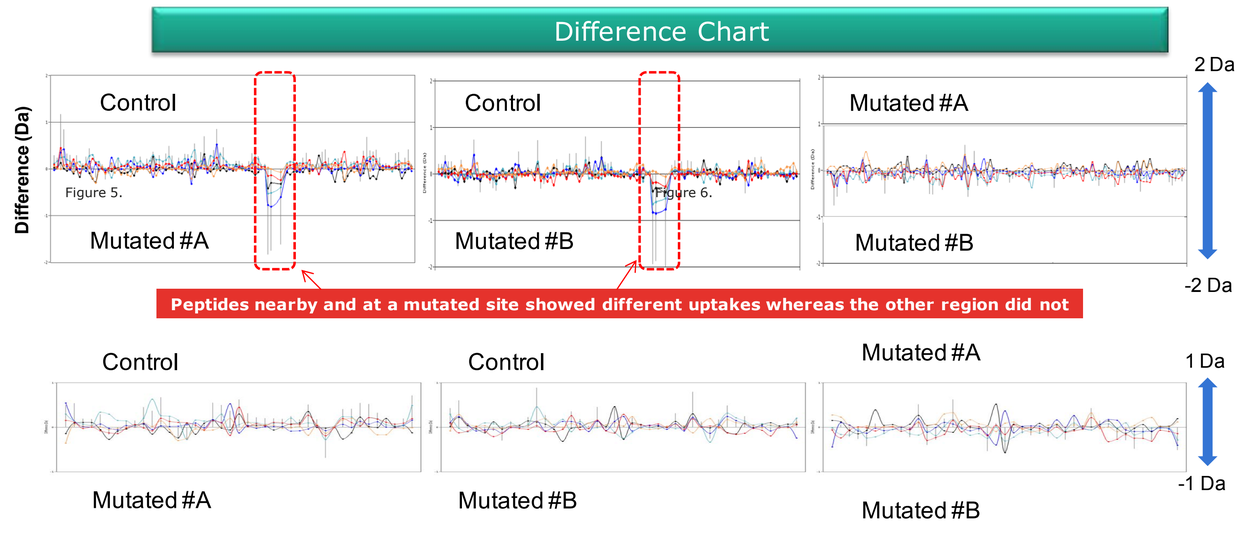

Figure 6. DynamX "Difference" charts comparing the control vs. batch #A vs. batch #B in the left, middle, and right panels, respectively. This visualization helps to locate which peptides contain different uptakes in comparative view. The red boxes highlight the peptides near and at a mutated site, revealing selected peptides from batch #A and batch #B which had higher uptakes compared to the corresponding Control peptide. Each data-point indicates the difference in uptake between the control and a mutated batch. The vertical bar represents the sum of the uptake differences across the time-points; the longer the vertical bar, the larger difference found between the control and mutated batches. Vertical bars less than +/- 1 Da difference in the other regions are considered to indicate insignificant changes. In the third panel Batch #A was directly compared to Batch #B, showing that the two forms were very similar, and that there were no significant differences in each of the heavy and light chains.

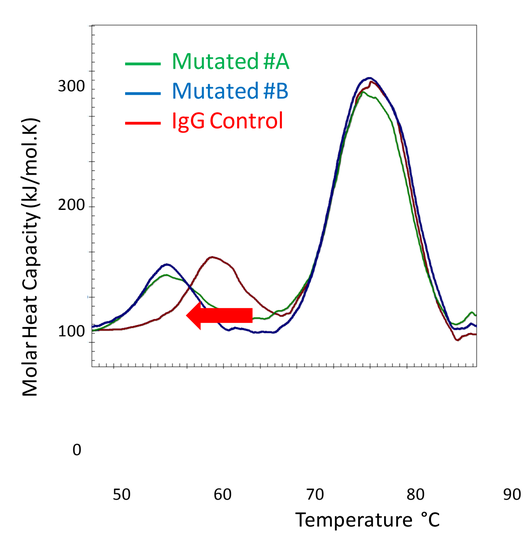

Figure 7. Background corrected DSC scan data of mutated (green and blue traces) and control (red trace). The major difference between the two scans is the low temperature transition indicating that the difference between the mutated samples and the control sample only affects a specific domain of the IgG. The shift to lower temperature (red arrow) of a portion of the trace indicates that the mutation has decreased the stability of this region. This DSC result is well agreed with the findings from the HDX analysis, which confirmed the attributed region included the mutated sites. (data not shown) Nano DSC is used to probe the global conformational changes with specific domain information whereas the HDX MS confirmed localized conformational changes at peptide level.