A Structural Characterization of a Monoclonal Antibody Therapeutic (Trastuzumab) as Formulated and Under Multiple Conjugation Paradigms

Richard H. Huang[1], Christine S. Nervig[2], Valerie I. Collins[1], David J. Sloan[1], Shawn C. Owen[3]

[1] RedShiftBio, Inc. Burlington, MA [2] Department of Medicinal Chemistry, University of Utah

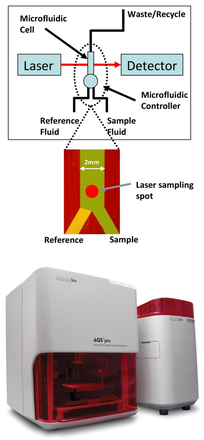
[3] Department of Pharmaceutics and Pharmaceutical Chemistry, Department of Medicinal Chemistry, Department of Biomedical Engineering, University of Utah

ABSTRACT

Antibody Drug Conjugates (ADCs) combine the targeting and specificity of a monoclonal antibody (mAb) with the cytotoxicity of a small molecule therapeutic. Biophysical characterization of the conformation and higher-order structure (HOS) of the mAb before and after drug conjugation is critical to understanding the stability and resulting efficacy of the ADC and requires the ability to detect subtle changes in secondary structure. Microfluidic Modulation Spectroscopy (MMS), developed and commercialized by RedShiftBio, is a novel infrared-based biophysical technique that combines a quantum cascade laser (QCL) with a modulated sample/buffer stream that feeds into a microfluidic optical flow cell. MMS provides robust, hands-free protein secondary structure analysis with much greater sensitivity than traditional technologies over a wide concentration range of 0.1 to > 200 mg/mL and overcomes conditions that are challenging for traditional spectroscopic techniques. In this study, the commercial mAb Trastuzumab was characterized by MMS directly in its formulation buffer to understand the stand-alone higher-order structure for this important mAb therapeutic. In addition, Trastuzumab was characterized at a variety of different drug-antibody ratios utilizing both non-specific labeling and click-chemistries for the drug conjugation to understand their effects on the mAb structure. Finally, the MMS results were compared to other biophysical techniques such as DSC.

INTRODUCTION

ADCs are an emerging class of biopharmaceutical drugs for cancer treatment due to their high specificity and targeting capability. Because drug delivery is directed to the cancer cells, dosages can be low and systemic toxicity is minimized. As a result, ADCs enable the use of more potent drugs than can be delivered by conventional chemotherapy with a more acceptable side effect profile for patients. Critical to the success of an ADC is maintenance of the native structure of the mAb within the conjugate since this underpins the targeting efficiency and stability of the full construct. Effective technology for the characterization of protein structure is therefore vital for development. In this study, trastuzumab and its ADCs are studied using MMS, a novel infrared-based biophysical technique highlighted in the diagrams on the right. ADCs produced by two different conjugation methods are structurally compared using the MMS data. Results reveal significant structural discrepancies from the different conjugation methods. These structural differences ultimately contribute to a change in thermal stability of the ADCs as measured by DSC.



METHODS

Trastuzumab (0.825 mg/mL), T-Cys-MMAE (0.842 mg/mL), T-Cys-Geld (0.736 mg/mL), and T-Lys-DM1 (0.785 mg/mL) in 5 mM histidine buffer at pH 6 with 2% w/v trehalose and 0.009% w/v polysorbate-20 were analyzed at room temperature using the 1st generation AQS^{3}pro MMS system. Samples were run in triplicate at a modulation rate of 1 Hz and backing pressure of 5 psi. Higher order structure (HOS) components of the samples were determined using the ***delta*** analytical software package. Thermal stability analysis was performed using the MicroCal VP-Capillary DSC (Malvern). All samples were analyzed at 0.5 mg/mL in the formulation buffer. The scan range of the analysis was 15-95°C at a heating rate of 60°C/hr. Data were buffer subtracted and baselined corrected and the temperature corresponding to the apex of endothermic transitions was defined as apparent T_m.

RESULTS

Antibody-Drug Conjugation Methods

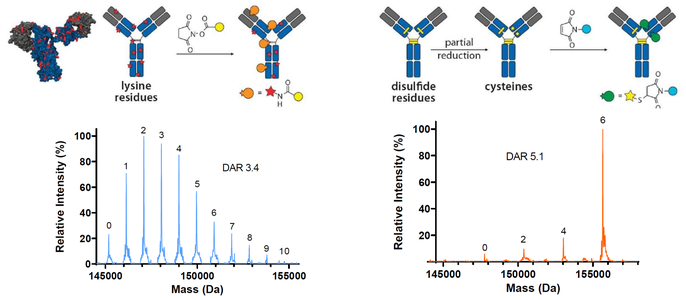


Figure 1. Two common antibody-drug conjugation methods and their resulting drug to antibody ratio (DAR) measured by LC-MS. Left: lysine-based ADC conjugation produces a heterogeneous population with a wide range of DAR. Right: cysteine-based ADC conjugation requires partial reduction of the inter-chain disulfide bonds and thus also produces a heterogeneous mixture of ADC species but with a narrower range of DAR.

MMS Characterization of ADCs by Different Conjugation Methods

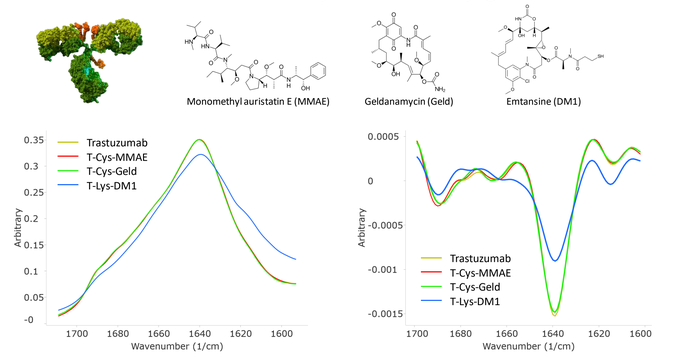


Figure 2. Top: crystal structure of an ADC (credit: Waters®) and the structures of the conjugated drugs used in this study. Bottom: absolute absorbance (left) and second derivative spectra (right) of the tested samples. Both cysteine-based ADCs, T-Cys-MMAE and T-Cys-Geld have very similar spectra compared to the unconjugated trastuzumab. The lysine-based ADC, T-Lys-DM1 shows significant differences in the full Amide-I range, specifically the 1620, 1640, and 1650 cm^{-1} regions.

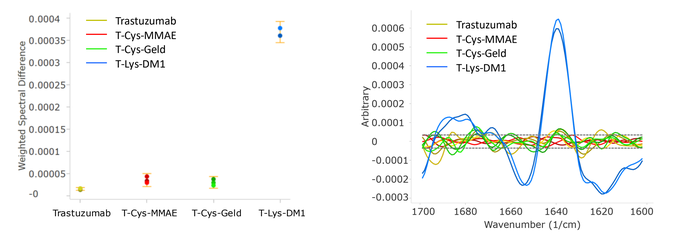
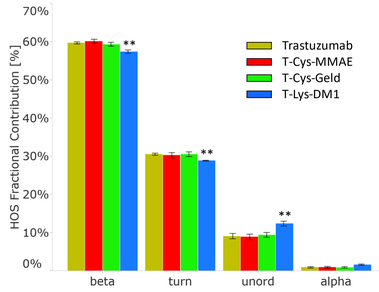


Figure 3. Spectral similarities of the tested samples are further compared by weighted spectral difference (WSD) (left) and delta (right) analyses. The unconjugated trastuzumab is the reference control in both plots. WSD shows numerically that the two Cys-based ADCs are much closer to the unconjugated mAb compared to the Lys-based ADC. Delta plot visually highlights the regions where the spectral changes are the most prominent.

RESULTS, cont'd

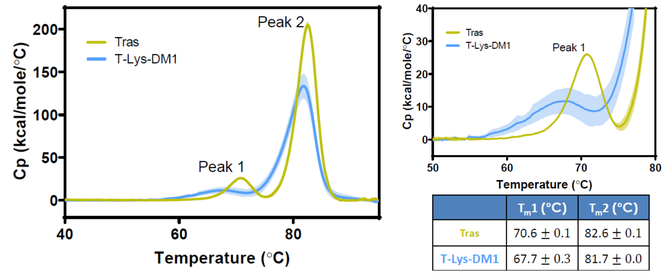
Higher Order Structure Analysis of ADCs



Samples	Beta	Turn	Unord	Alpha
Trastuzumab	59.6 ± 0.3	30.5 ± 0.3	9.0 ± 0.7	0.8 ± 0.2
T-Cys-MMAE	60.1 ± 0.5	30.2 ± 0.7	8.8 ± 0.7	0.9 ± 0.2
T-Cys-Geld	59.3 ± 0.5	30.5 ± 0.6	9.4 ± 0.6	0.8 ± 0.2
T-Lys-DM1	57.4 ± 0.4	28.8 ± 0.1	12.3 ± 0.6	1.5 ± 0.2

Figure 4. Higher order structure (HOS) analysis reveals that there is a significant difference in secondary structures of T-Lys-DM1 compared to the unconjugated mAb and the Cys-based ADCs. Specifically, there is a 2% drop in beta-sheet structure, 2% drop in turn structure, and 3% increase in unordered structure.

Thermal Stability Study of ADCs Using Differential Scanning Calorimetry



	T_m1 (°C)	T_m2 (°C)
Tras	70.6 ± 0.1	82.6 ± 0.1
T-Lys-DM1	67.7 ± 0.3	81.7 ± 0.0

Figure 5. Thermal stability of trastuzumab (Tras) and T-Lys-DM1 assessed by DSC ranging from 40°C to 90°C (left). The first melting point is highlighted showing a lower melting temperature of T-Lys-DM1 compared to Tras (right). The T_m1 peaks correspond to the unfolding of the C_H2 domain of the Fc region of the mAb. This result is a direct indication of a decreased stability in T-Lys-DM1 compared to the unconjugated trastuzumab.

CONCLUSIONS

- Cysteine-based conjugation of drug-antibody did not affect the secondary structure of trastuzumab.
- No structural difference was observed between the two cysteine-based ADCs with two different drugs, MMAE and Geld, which are different in both chemical structure and drug function.
- Lysine-based conjugation showed significant change in the secondary structure of trastuzumab. Specifically, beta-sheet and turn structures decreased and unordered structure increased.
- The decrease in beta-sheet and increase in unordered structure in T-Lys-DM1 resulted in a decrease in thermal stability compared to the unconjugated trastuzumab.