Utilizing N-glycans to develop aggregation resistant monoclonal antibodies.

Micah Castillo[1], Mehmet Şen[1], Veysel Kayser [2]

University of Houston, Department of Biology and Biochemistry[1], Houston, TX; University of Sydney School of Pharmacy [2]

Introduction

Alteration of an antibody's glycosylation pattern can increase its stability, and therefore viability as a candidate in the biotherapeutics market. The advantage of the introduction of an N-linked glycan is the ability to target a specific region of a monoclonal antibody (mAb), which does not have a role in antigen recognition and effector function such as complement dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC). This study focuses on the two antibodies Herceptin® (trastuzumab) and Humira® (adalimumab) which are monoclonal immunoglobin G1 (IgG1) commercial antibodies involved in blocking HER2 receptors in breast cancer and dampening inflammatory pathways, respectively.

Objective

To enhance stability by introducing N-linked glycosylation sites into computationally determined aggregation-prone regions of mAbs. The stability of mutant variants of the commercial antibodies, Herceptin® (trastuzumab) and Humira® (adalimumab), will be assayed with novel mutations at the 117th and 118th amino acids of their heavy chains, respectively.

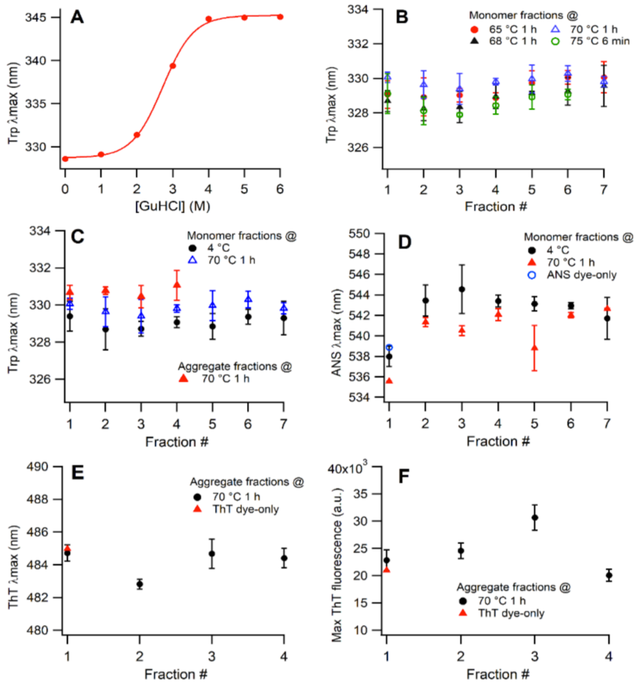
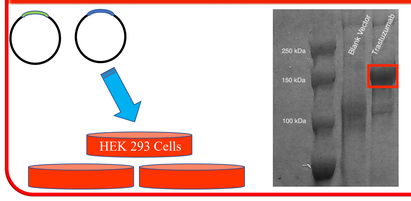


Figure 1: Characterization of trastuzumab monomer and aggregate fractions from SE-HPLC by fluorescence spectroscopy; A) Trp max emission wavelength shift of trastuzumab with increasing concentrations of GuHCl; B) Trp max emission wavelength of monomer fractions from different sections of the chromatogram peak stressed at elevated temperatures; C) Trp max emission wavelength of monomer and aggregate fractions stressed at 70 °C compared with 4 °C; D) Max emission wavelength of ANS bound to monomer fractions stressed at 70 °C compared with fractions at 4 °C; E) Max emission wavelength of ThT bound to aggregate fractions stressed at 70 °C; F) Max fluorescence of ThT bound to aggregate fractions stressed at 70 °C. Error bars represent the SD.

Transfection and Generation of Stable Cell Lines



Fourier transform infrared (FTIR) spectroscopy and Intrinsic fluorescence

Assessing affect of glycosylation on the secondary and tertiary structure.

Resistance to Guanidine·HCl Induced Protein Unfolding

Glycosylation has been shown to change antibody resistance to unfolding under increasing concentrations of Guanidine·HCl.

The absorbance spectra will be collected using UV spectroscopy. The second derivative of each spectrum will be calculated to monitor the changes in the microenvironment of Trp residues

Dynamic Light Scattering (DLS)

DLS will be used as an alternative method for determining the distribution profile of of aggregates, fragments, and monomers in solution.

Differential scanning calorimetry and fluorimetry (DSC and DSF).

Evaluate the thermal stability of both glycosylated and de-glycosylated antibodies.

Antibodies normally have three thermal transitions in DSC

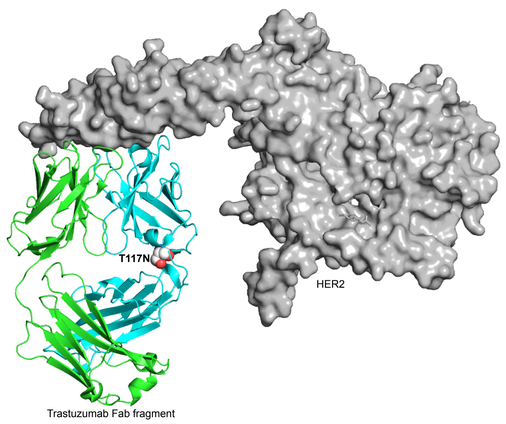


Figure 2: Crystal structure 1N8Z of extracellular domain of human HER2 complexed with Herceptin Fab. Sufarce exposed amino acids mutated for substitution to an N-linked glycosylation site.

Accelerated Stability Test

Assess stability of both glycosylated and de-glycosylated antibodies under thermally challenging conditions for up to three months.

Size Exclusion Chromatography (SEC) is used to determine the percentage of aggregates, fragments, and monomers.

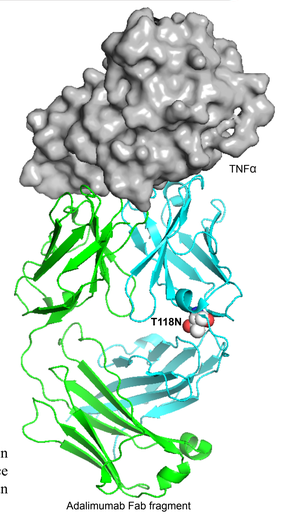


Figure 3: Crystal structure 3WD5 of TNFalpha in complex with Adalimumab Fab fragment. Surface exposed amino acids mutated for substitution to an N-linked glycosylation site (green)

Spatial Aggregation Propensity

Analysis of the aggregation prone regions of trastuzumab and adalimumab was accomplished using a tool known as 'Spacial Aggregation Propensity' (SAP). Previous studies have shown through molecular simulations and protein engineering that the peaks derived from SAP correspond to dynamically exposed hydrophobic regions commonly leading to aggregation[2].

$$\left(\begin{matrix}Spatial-aggregation-\\propensity\ (SAP)\end{matrix}\right)_{atom\ i} = \sum_{\substack{\text{Stimulatic}\\\text{Average}}} \left\{ \sum_{\substack{\text{Residues with atleast}\\\text{one side chain atom}\\\text{within R from atom i}}} \left(\frac{\begin{matrix}\text{SAA of side chain atoms}\\\text{within radius R}\end{matrix}}{\begin{matrix}\text{SAA of side chain atoms}\\\text{of fully exposed residue}\end{matrix}} \times \text{Residue Hydrophobicity} \right) \right\}$$

Spatial Aggregation Propensity Formula: SAA = "solvent accessible area" of side chain atoms contained within radius R from the central atom. "SAA of side chain atoms of fully exposed residue": obtained by calculating the SAA of side chains of the middle residue in the fully extended conformation of tripeptide Ala-X-Ala. 'Residue hydrophobicity' was obtained from the hydrophobicity scale of Black and Mould[3]. Normalized to a glycine hydrophobicity of zero.

Thermal Stability

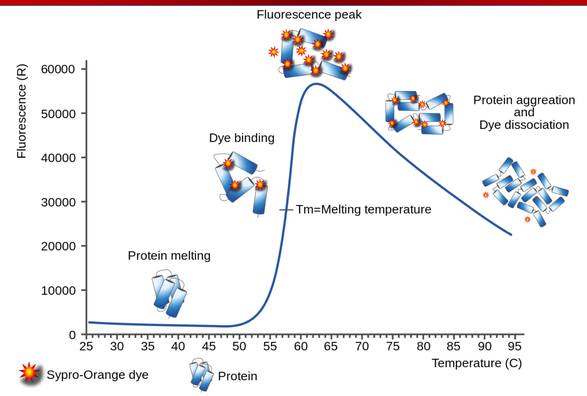


Figure 4: Protein unfolding and aggregation can be measured by the intensity of fluorescence from a dye that will bind as the antibody unfolds.

References

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3. Black, S. D., & Mould, D. R. (1991). Development of hydrophobicity parameters to analyze proteins which bear post- or cotranslational modifications. *Analytical Biochemistry*. https://doi.org/10.1016/0003-2697(91)90045-U

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