Quantification of Monoclonal Antibody Stability Change After Forced Degradation Studies

Summary

- Forced degradation studies used throughout biologic development and is a requirement of regulators.
- Degradation of antibodies mimics observations seen when comparing different antibody constructs.
- Degradation studies of an IgG1 monoclonal antibody were performed and the change in stability was quantified via chemical denaturation.
- Antibody samples measured with SUPR-CM™ fluorescence plate reader and analyzed by fitting of a three-state function to the ratio of intensities versus denaturant concentration trends.
- Deamidation and glycation reduced antibody stability profile while oxidation only affected first transition region.

Introduction

The development of new antibody biologics requires maximizing the stability of antibodies so that functionality is not lost due to the product aggregating or degrading over time. Improving the stability of the antibody can be done via alteration of the antibody polypeptide sequence (antibody engineering), and by changing the solution conditions the antibody is suspended in (formulations).[1, 2]

Further insight into the stability of the antibody can be gained by looking at the products from degradation samples.[3,2] Within formulations, degradation studies are used to identify solution conditions that predict long term stability. The limits of instability can also be determined when using different solution conditions. Degradation studies are also used to mimic observations of a formal stability study under ICH (International Council for Harmonization) conditions.[3] Degradation studies are used throughout the development process and is a requirement for certain regulators.[1, 2]

Types of degradation study include agitation, photolytic, glycation, hydrolysis, thermal, and oxidation.[4, 2] A review of forced degradation studies has been done by Nowak, et al., where they focused on recombinant antibodies.[2]

In this study, the change in stability of a monoclonal antibody (mAb) is quantified after degradation via deamidation, glycation, and oxidation. Chemical denaturation experiments were performed on the antibody before and after degradation to assess the change in stability.

Along with investigating stability changes after degradation, the dataset illustrates the kinds of changes observed when comparing similar antibody constructs. For example, when selecting stable candidates amongst mutant variants of a parent antibody.[5]

[1] Klick S, et al. Toward a Generic Approach for Stress Testing of Drug Substances and Drug Products. Pharmaceutical Technology. 29 (2), 48-66. 2005.

[2] Nowak C, et al. Forced degradation of recombinant monoclonal antibodies: A practical guide. mAbs. 9(8), 1217-1230. 2017.

[3] Brummer H. How to approach a forced degradation study. SGS Life Science Services: Technical Bulletin. 31, 1–4. 2011.

[4] Blessy M, Ruchi D P, Prajesh N P, Agrawal Y K. Development of forced degradation and stability indicating studies of drugs—A review. Journal of Pharmaceutical Analysis. 4 (3), 159-165. 2014.

[5] Kerwin, B A, et al. Framework Mutations of the 10-1074 bnAb Increase Conformational Stability, Manufacturability, and Stability While Preserving Full Neutralization Activity. Journal of Pharmaceutical Sciences, 109 (1), 233-246. 2020.

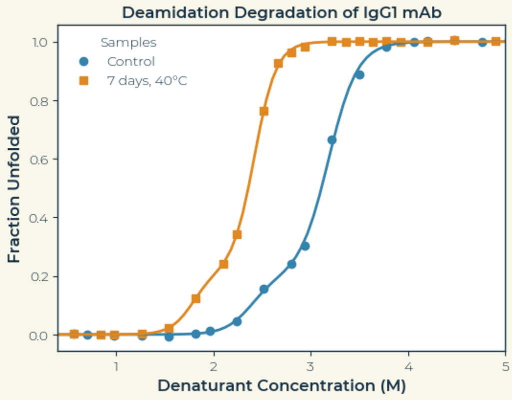

Figure 1 – Denaturation curves comparing the deamidation sample with the control sample. The trendline was fitted with a three-state function. Incubating the IgG1 mAb has significantly lowered the stability of the antibody as the transition regions appear at lower denaturant concentrations.

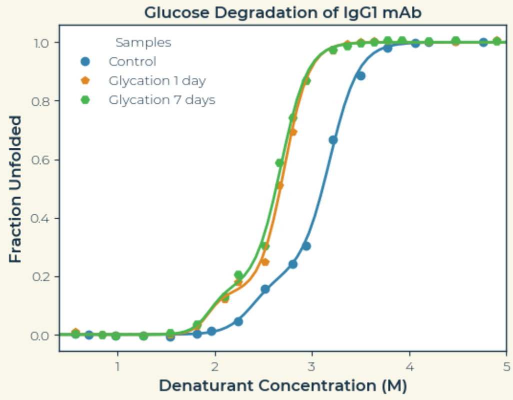

Figure 2 – Denaturation curves comparing the control sample with the 1 day and 7 days glycation samples. Both glycation curves show a significant decrease in stability, compared to the control sample. However, there is little change between the 1 day and 7 day samples, which implies that the glycation process was over shortly after 1 day.

Method

Preparation of Degradation Samples

Four samples of IgG_1 mAb were prepared in PBS to a final concentration of 1.5 mg/ml. One sample contained 2 M glucose, while another contained 50 mM glucose. The third sample contained 0.3% hydrogen peroxide. The unaltered sample and the 2 M glucose sample were incubated in a water bath, at 40°C, for 7 days. The 50 mM glucose sample was incubated at room temperature for 1 day, while the peroxide sample was incubated for 3 hours at room temperature. A control sample of 1.5 mg/ml IgG_1 mAb in PBS was made and underwent the same buffer exchange process as the other samples.

After incubation, the samples had their buffers exchanged via centrifugal filtration to remove the glucose and peroxide. All samples had their buffers exchanged with PBS to maintain consistency in the method of preparation. Amicon Ultra 4 centrifugation filter tubes (30 kDa) were used to exchange the buffers in five rounds of centrifugation. The filters were centrifuged at 2800 xg for 10 mins.

Dispensing and Measurement

Samples were dispensed into the wells of a 384-well microplate (Greiner) by the Mantis® liquid handler (Formulatrix®). The samples were prepared in triplicate and used 24 denaturant concentrations from 0 M to 6 M.

Microplates were measured with the SUPR-CM fluorescence plate reader (Protein Stable™) and used a 500 ms well measurement time. The total time to read the plate was 2.5 minutes. The intrinsic fluorescence spectra were used to generate the denaturation curve by calculating the ratio of intensities at 355 nm and 330 nm. Denaturation curves were fitted to a three-state function, with the Gibbs free energy (ΔG^{o}) and mid-point of inflection (C_m) being used to quantify the stability of the antibody.

Results

The denaturation curve for the deamidation sample is compared to the control sample in **figure 1**. It shows a significant reduction in stability highlighted by the transition regions having shifted to lower denaturant concentrations. The data is also of sufficient resolution to distinguish two transition regions. Fitting of a three-state model to the data confirmed the reduction in mAb stability as the C_m values for both transition regions, listed in **table 1**, dropped

Table 1 – Values for the Gibbs Free Energy and the midpoint of inflection for each sample in the degradation study.

Sample	$\Delta G°_1$	C_{m1}	$\Delta G°_2$	C_{m2}
Control	42.79	2.39	51.85	3.18
Deamid	39.73	1.79	52.02	2.42
Gly (1 day)	57.76	1.93	51.92	2.72
Gly (7 days)	50.67	1.94	47.44	2.68
H_2O_2	27.23	2.20	49.12	3.21

by 0.60 M and 0.76 M, after the deamidation process.

Incubating the mAb in a 50 mM glucose solution, for one day, has resulted in a reduction in the stability of the antibody as the transition regions (shown in **figure 2**) occur at lower denaturant concentrations than the control sample.

Comparing the 1 day and 7 days glycation samples show little change (0.01 M for C_{m1} and 0.04 M for C_{m2}). The glycation process for the 7 days sample would be near completion as this sample was incubated for longer than the 1 day sample, but also used 40 times higher concentration of glucose (2 M). Given the similarity between the 1 day and 7 days sample, the data implies that the glycation process was near complete for both samples.

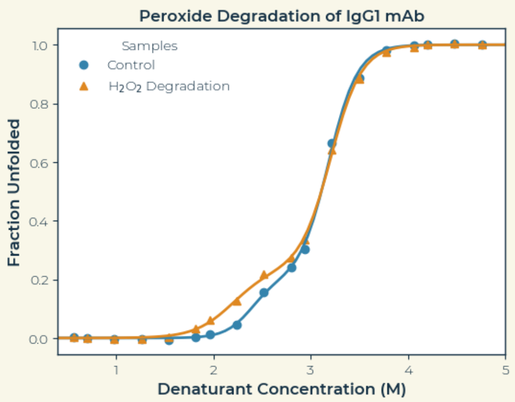

Figure 3 – Denaturation curves comparing the peroxide degradation sample with the control sample. The plot shows a significant change in stability for the first transition region with the second transition region remaining mostly unaffected.

Comparing the denaturation curve of the oxidized sample to the control sample (**figure 3**) reveals a more subtle change in mAb stability. The three-state behavior is still present, but only one of the transition regions has changed after treatment with hydrogen peroxide. The C_m value for the first transition region decreases by 0.19 M while the second transition region only changes by 0.03 M. The Gibbs free energy for the second transition region shows a change of 2.73 kJ mol^{-1} while $\Delta G°_1$ decreases by 15.56 kJ mol^{-1}.

Conclusion

Forced degradation studies are routinely employed during the development of new biologics. Along with providing evidence for the mechanisms by which a mAb can degrade, the change in stability mimics the observations when comparing different antibody constructs.

The change in stability of an IgG1 mAb was measured via chemical denaturation before and after deamidation, glycation, and oxidation studies. Samples prepared in 384-well microplates were measured with the SUPR-CM fluorescence plate reader.

Deamidation and glycation samples decreased the stability of the mAb significantly when compared to the control sample. However, the gradients of the transitions do not change greatly when compared to the control sample. This implies that both transition regions were affected to a similar extent. This contrasts with the oxidation sample, which showed a decrease to only the first transition region, which implies that the other transition region is the more stable of the two.

Table 2 – Values for the Gibbs Free Energy and the midpoint of inflection from three-state fitting of low sample usage data in **figure 4**.

$\Delta G°_1$	C_{m1}	$\Delta G°_2$	C_{m2}
27.13	2.45	91.02	3.04

The data acquired with only 300 ng of antibody per well is shown in **figure 4** and shows the fraction unfolded values from three repeat measurements of the plate. The consistency of the data points between the different scans is low, with a standard deviation averaging 0.015. Despite the small amount of antibody used, the fraction unfolded plot of **figure 4** still show the three-state behavior that is expected with this IgG_1 mAb. Fitting of a three-state model converged, and Gibbs free energy values and midpoint inflection points where obtain and shown in **table 2**.

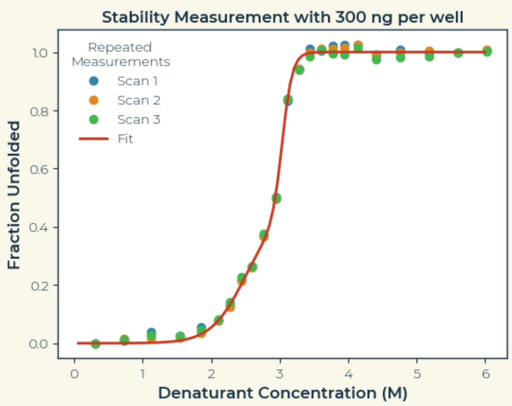

Figure 4 – Denaturation curve of IgG_1 mAb where each well only used 300 ng of antibody. Plate was measure three time and the plotted together along with the three-state function fitted trendline.

Finally, small sample consumption was demonstrated where only 300 ng of mAb was used per well. The data generated had low standard deviation between repeated scans and still showed three-state behavior that was successfully fitted to.

Intro

- Focus on degradation studies and how/why the pharmaceutical industry uses them during engineering and formulation processes.

Sources:

- Silke Klick, et al. Toward a Generic Approach for Stress Testing of Drug Substances and Drug Products. Pharmaceutical Technology. 29 (2), 48-66. 2005.
    - Pharmaceutical companies perform stress testing (also called forced-degradation studies) during preformulation to help select compounds and excipients for further development, to facilitate salt selection or formulation optimization, and to produce samples for developing stability-indicating analytical methods.
    - stress testing often is repeated when manufacturing processes, product composition, and analytical procedures are refined and reach a more final state.
- M Blessy, Ruchi D. Patel, Prajesh N. Prajapati, Y.K. Agrawal. Development of forced degradation and stability indicating studies of drugs—A review. Journal of Pharmaceutical Analysis. 4 (3), 159-165. 2014.
    - Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions.
- H. Brummer. How to approach a forced degradation study. SGS Life Science Services: Technical Bulletin. 31, 1–4. 2011.
    - Forced degradation studies are carried out for the following reasons (including): To develop and validate a stability indicating method; To generate more stable formulations.
- Christine Nowak, et al. Forced degradation of recombinant monoclonal antibodies: A practical guide. mAbs. 9(8), 1217-1230. 2017.
    - From early stage candidate selection to post approval, forced degradation studies are frequently performed to support manufacturability assessments, formulation development, establishment of stability-indicating methods and comparability.
    - Degradation of recombinant mAbs can negatively affect product quality, safety and efficacy and thus needs to be detected if it occurs.
    - Forced degradation studies have been commonly used by the industry to support the development of mAb therapeutics through-out the life cycle of the products for various purposes (Table 1)
    - It is also the expectation of agencies that forced degradation studies be used to understand the product degradation pathways, and establish stability indicating methods enabling monitoring degradation, if occurs, within the shelf life.
    - Table 1. Purposes and rationale of forced degradation studies: Formulation development Identification of conditions such as buffers, excipients, pH, and/or temperature to provide appropriate long-term stability; Intrinsic stability of the products Forced degradation can help define the boundary of instability under various environmental factors.
    - High Temp: The increase in acidic species is likely caused by multiple degradation mechanisms, including modifications such as deamidation.
    - While oxidation of methionine residues is the major

degradation pathway, hydrogen peroxide can also result in mAb fragmentation Oxidation causes formation of insoluble and soluble aggregates, which are probably due to oxidation-induced conformational changes.
    - Degradation can lead to aggregates.
    - Although non-reducing sugars are commonly used for formulation, it is possible for them to degrade into reducing sugars, and thus become reactive toward amine groups.

250 words needed.

- Stability important when developing new antibody biologics.
- Degradation studies used throughout development process and is expected by regulators.
- Forced degradation studies used within formulation to identify solution conditions that provide long term stability and help establish the limits of instability when using different conditions. Also used to mimic observations of a formal stability study under ICH (International Council for Harmonization) conditions.
- Types of degradation studies include agitation, thermal, photolytic, glycation, hydrolysis and oxidation. (Blessy, Nowak)
- Along with investigating stability impacts from degradation effects, degradation studies also mimic the kinds of stability changes observed when looking at different mutant strains of a parent antibody.
- The change in stability of a monoclonal antibody is quantified via chemical denaturation when subject to thermal, glycation and oxidation degradation studies.

Development of new antibody biologics require maximizing the stability of antibodies so that functionality is not lost due to the product aggregating or degrading over time. Improving the stability of the antibody can be done via alteration of the antibody polypeptide sequence (antibody engineering) and by changing the solution conditions the antibody is suspended in (formulations). [Klick2005, Nowak2017]

Further insight into the stability of the antibody can be gained by looking at the products from degradation samples. [Brummer2011, Nowak2017] Within formulations, degradation studies are used to identify solution conditions that provide long term stability. The limits of instability can also be determined when using different solution conditions. Degradation studies are also used to mimic observations of a formal stability study under ICH (International Council for Harmonization) conditions. [Brummer2011] Degradation studies are used throughout the development process and is a requirement for certain regulators. [Klick2005, Nowak2017]

Types of degradation study include agitation, photolytic, glycation, hydrolysis, thermal and oxidation. [Blessy2014, Nowak2017] Mechanisms for how each approach degrades the protein can be reviewed here [Nowak2017].

In this study, the change in stability of a monoclonal antibody (IgG1) is quantified after degradation via deamidation, glycation and oxidation. Chemical denaturation experiments were performed on the antibody before and after degradation to assess the change in stability.

Along with investigating stability changes after degradation, the dataset illustrates the kinds of changes that can be observed when comparing similar antibody constructs. For example, when selecting stable candidates amongst mutant variants of a parent antibody. [Kerwin2020]