Analysis of IgdE Digested Monoclonal Antibody Fragments

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Overview

Monoclonal antibodies (mAbs) are well-established therapeutics, with a variety of different analytical methods associated with purity analysis. An analytical technique common for mAb purity is reversed phase LC (RPLC). This is a primary technique utilized for mAbs because of its relatively short analysis time, with being high resolution, and its ability to separate of hydrophobic variants such as oxidation, glycoforms, and lysine variants. Another utility of reversed phase is when analyzing fragments, with traditional methods using a reducing agent like dithiothreitol (DTT) to reduce interchain disulfides, yielding heavy chain and light chain.

Although the analysis of heavy and light chains is useful, the use of site-specific cysteine proteases is another approach that allows for better characterization of the antibody. One relatively recent breakthrough in protease development has been the use of IgdE proteases. Similar to IdeS, it is a site-specific protease that only cleaves at one site of IgG1 antibodies. However, unlike IdeS, it cleaves above the hinge, thus yielding an intact Fc and importantly, an intact Fab fragment that can be further isolated and characterized. It differs from other proteases such as papain in that IgdE can be used under non-denaturing conditions.

Figure 1 shows an overlay of intact trastuzumab and IgdE digested fragments, Fc and Fab. With the overlay, there does appear to be some undigested intact mAb present in the sample, indicating that the standard 1 unit/µg protocol may need some further optimization. Nonetheless, by utilizing the same chromatographic method for both intact and subunit analysis, one can determine if there is potentially undigested full-length antibody still present in the sample. Also interesting of note is how the Fab fragment elutes very closely to the full-length antibody, despite being 50 kD in molecular weight.

Figure 2 shows an overlay of Intact NIST and IgdE digestions. Interestingly, there do seem to be two clear distinct variants in the NIST fragment. Also, somewhat interesting is that both trastuzumab and NIST mAb Fc fragments elute at the same time (t=1.443 for both). Since both are humanized mAbs with similar glycosylation profiles (i.e. human IgG, complex, biantennary, neutral glycans), physicochemical properties are similar.

In summary, the analysis of antibody fragments can provide key insights into sample heterogeneity. By using the cysteine protease IgdE, further characterization of the Fab region can be obtained. Chromatographic separation can use the same analytical method performed on intact protein and other subunits such as IdeS generated fragments and heavy chain/light chain and optimized using a bioZen™ 2.6 µm WidePore C4 LC column. This allows for extensive characterization of the antibody, depending on the region of interest.

LC Conditions

Column:	bioZen 2.6 µm WidePore C4
Dimensions:	100 x 2.1 mm
Part No.:	00D-4786-AN
Mobile Phase A:	0.1 % TFA in Water
Mobile Phase B:	0.1 % TFA in Acetonitrile
Gradient Program:	30-40% B in 5 minutes (Fig 1-2)
Flow Rate:	0.8 mL/min
Temperature:	80°C
Detection:	UV @ 214 nm
Injection:	Trastuzumab, IgdE digested (0.5 mg/mL), Figure 1 NIST mAb, IgdE digested (0.5 mg/mL), Figure 2

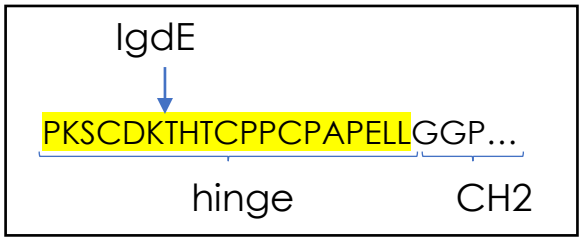


Figure 1. Trastuzumab, Intact and IgdE Digested

Overlay of intact trastuzumab (black trace) and fragments generated by IgdE digestion. Partial, undigested mAb is observed, indicating a possible need for digestion optimization.

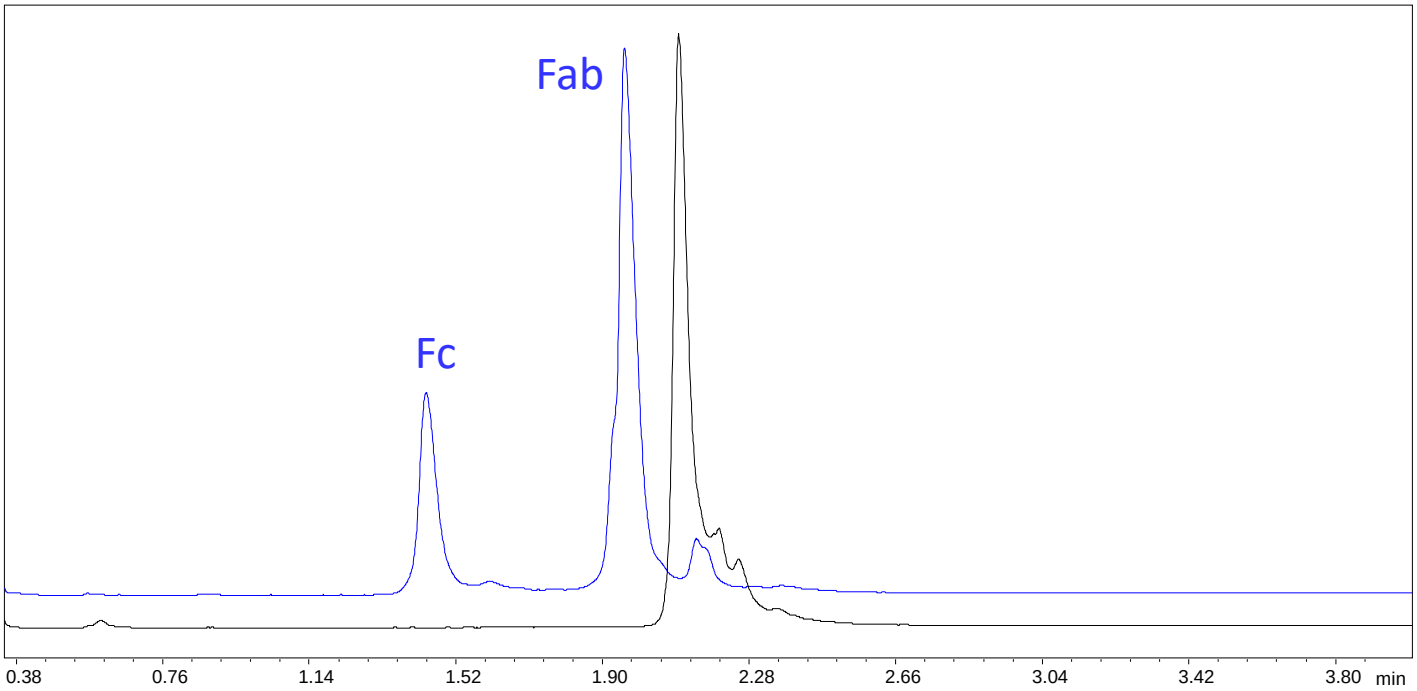
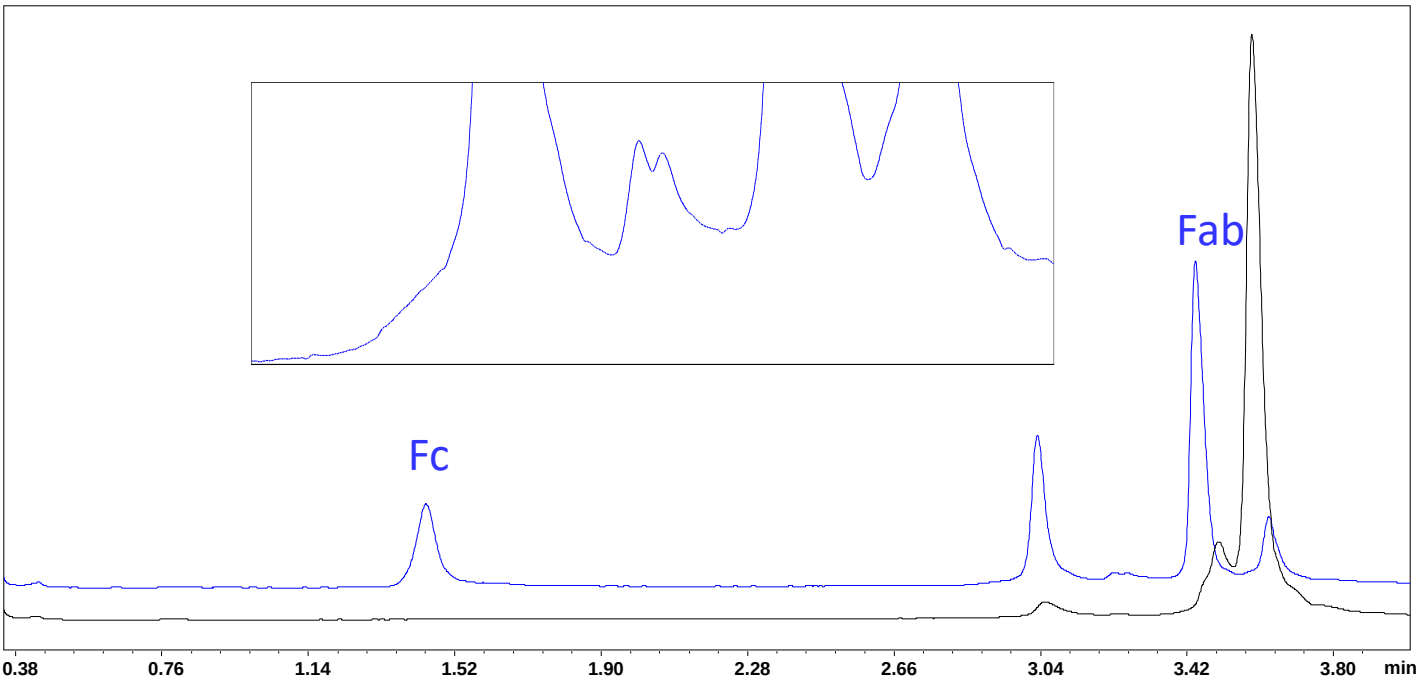


Figure 2. NIST mAb, Intact and IgdE Digested

Overlay of NIST mAb, showing variation in the Fab fragment. A distinct earlier eluting variant is observed, Fc retention is similar to trastuzumab, which shares the same conserved Fc of hIgG1, as well as similar glycoforms.



Reference

[1]Pisupati, Karthik et al. "Biosimilarity under stress: A forced degradation study of Remicade® and Remsima™." mAbs vol. 9,7 (2017): 1197-1209. doi:10.1080/19420862.2017.1347741

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