Analysis of IdeS 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 for mAb purity is reversed phase LC (RPLC). This is a primary technique utilized for mAbs because of its relatively short analysis time, with high resolution, and ability to separate hydrophobic variants such as oxidation, glycoforms, and lysine variants. Another utility of reversed phase is to analyze 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 also another approach that allows for better characterization of the antibody. Further, this can be combined with a reducing agent to further identify and quantitate mAb heterogeneity. One particularly useful partial digestion of mAbs is using IdeS, a cysteine protease that cleaves below the hinge region of the IgG. This enzyme generates a $(Fab')_2$ fragment and two Fc fragments (Fc/2). This allows for better characterization of the Fc fragment.

Figure 1 shows an overlay of intact and IdeS digested infliximab. Again, this digest yields the $(Fab')_2$ fragment and two single chain Fc fragments. At the intact level, there is not discrimination of lysine variants, which are more hydrophilic thus earlier eluting by reversed phase. After digestion, the lysine variant on the Fc of infliximab is clearly identified, as shown in the **Figure 1** inset. Interestingly, the $(Fab')_2$ fragment elutes later than the full-length antibody, which is somewhat counterintuitive as the fragment has a lower molar mass. Additionally, the chromatographic profile looks similar to the intact, with a partial post peak. This post peak could be deamidated variants in the heavy chain.[1]

In instances where IdeS digested fragments are not sufficient, one can perform a subsequent reduction after digestion, yielding smaller fragments. This process reduces $(Fab')_2$ into an Fd' and light chain. Again, these peaks might correspond to deamidated variants, though further analytical characterization by orthogonal methods, such as peptide mapping, would need to be performed to confirm peak identity.

In summary, the analysis of antibody fragments can provide key insights into sample heterogeneity. Separation of variants, such as lysine and deamidated variants, is possible by digestion using a site-specific cysteine protease, such as IdeS, and by using a bioZen™ 2.6 µm WidePore C4 LC column. By combining the enzymatic digestion with a subsequent reduction, further characterization of both Fc/2 and Fd' fragments allows for better characterization of both variable and conserved regions of the antibody.

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:	Infliximab, as indicated (0.5 mg/mL), Figures 1

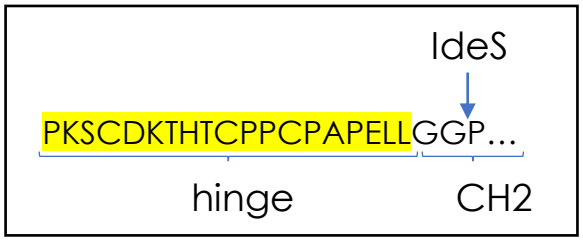


Figure 1. Infliximab, Intact and IdeS Digested

Overlay of intact infliximab (black trace) and fragments generated by IdeS digestion. Lysine variant (*) is shown as pre-peak in the Fc/2 fragment. Although smaller in molecular weight, the (Fab')2 fragment elutes later than full-length mAb.

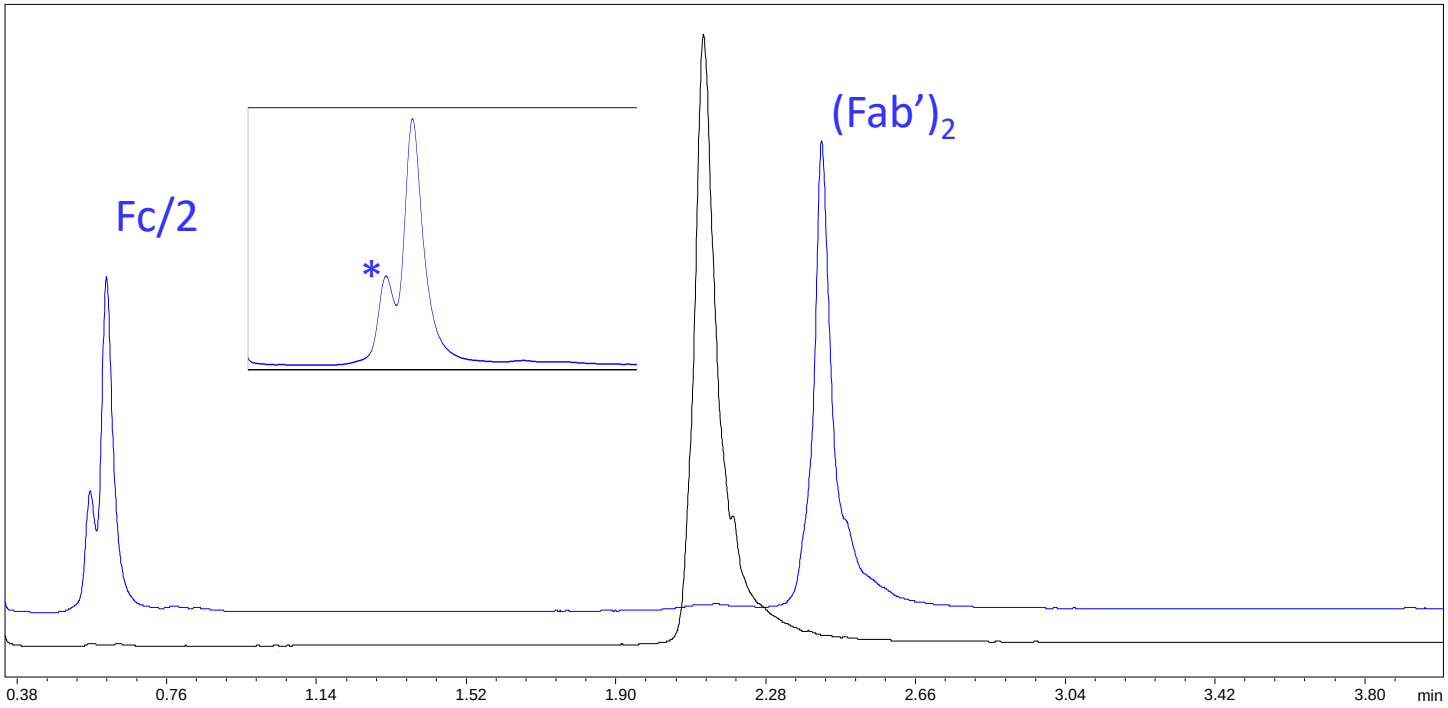
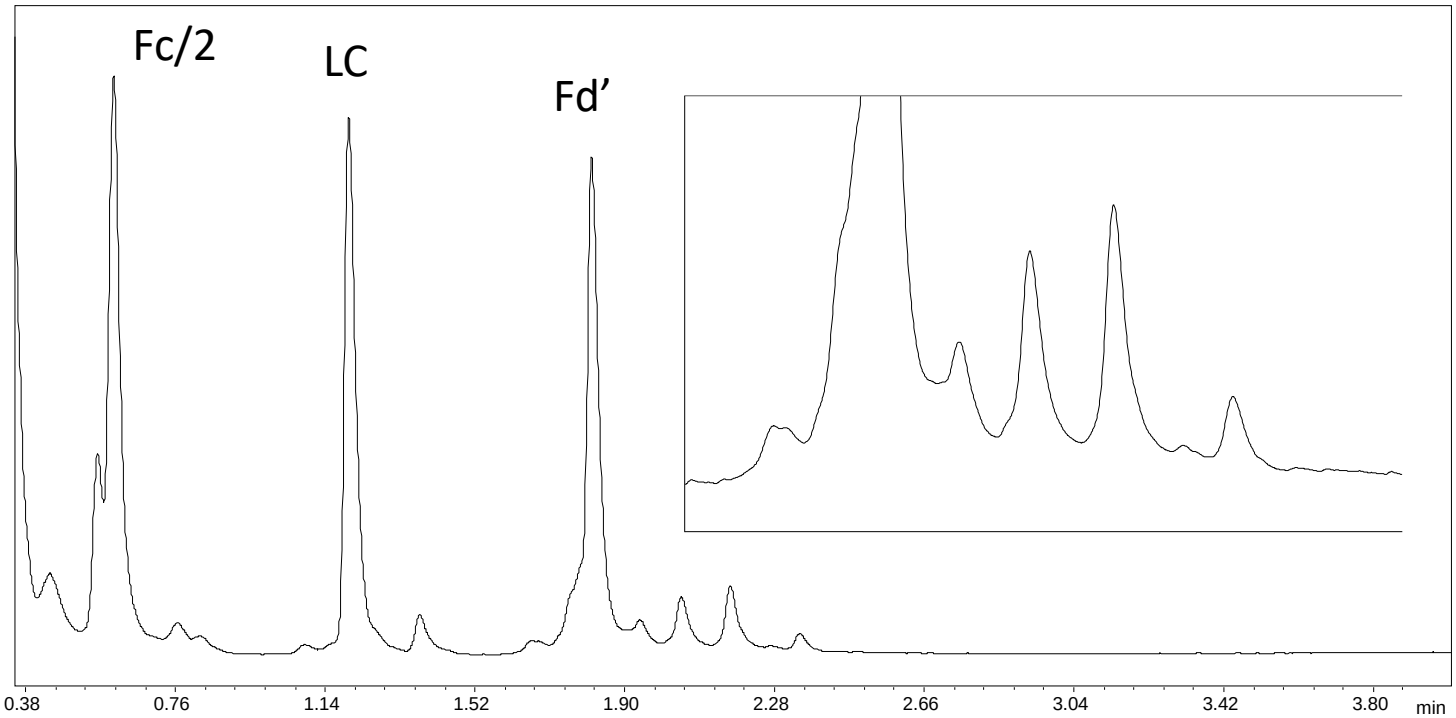


Figure 2. Infliximab, IdeS Digested and Reduced

After subsequent reduction of infliximab, fragments generated include Fc/2 as well as light chain (LC) and Fd', or the hinge containing variable region of the heavy chain. This provides insight on heterogeneity of the Fab region of the antibody that the protease digestion itself could not determine.



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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