Tandem Digestion of Monoclonal Antibodies Using Novel Cysteine Proteases

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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 high resolution, and ability to separation of hydrophobic variants such as oxidation, glycoforms, and lysine variants. Another utility of reversed phase chromatography can be used when analyzing fragments while 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. Two common approaches to fragment generation are using the site-specific proteases IdeS and IgdE, which cleave below and above the hinge of IgG1 antibodies, respectively. This process allows for the isolation or characterization of specific regions of the antibody. Although analytical characterization by RPLC at the subunit level will provide more insight into sample heterogeneity, often further chemical digestion can be performed to obtain more detail. However, other variants - namely, disulfide variants - cannot be identified if this chemical reduction is performed.

As such, Faid and colleagues demonstrated the capabilities of digesting mAbs using both IgdE and IdeS as a method to identify free sulfhydryls.[1] Indeed, identification of free sulfhydryls has been limited mainly to colorimetric techniques; this tandem digestion allows for this identification of free sulfhydryls along with other variants commonly observed in subunit analysis. **Figure 1** shows NIST mAb that has been digested with IgdE and IdeS. Resulting fragments are observed, namely the Fc/2 and Fab, the former which gives insight on oxidation, glycoforms, and lysine variants, and the latter which gives insight on the hypervariable region. **Figure 1** inset shows the putative free sulfhydryl variants, which are later eluting to the Fc/2 fragment. It is important to note that there are some earlier eluting impurities, which are thought to be artifacts of the digestion itself.

Figure 2 shows a similar profile for trastuzumab, with free sulfhydryls also present in the sample. Later eluting variants may be deamidated variants, which have been observed with trastuzumab Fab. However, these might also be free sulfhydryl variants, which are also later eluting with the Fab fragment. Identification by high resolution mass spectrometry would be necessary to identify each variant. Once identified, the LC-UV method might then be appropriate for monitoring appropriately.

In summary, the use of cysteine proteases is an increasingly useful sample preparation technique to gain insight on mAb heterogeneity in conjunction with a wide pore core-shell LC column, bioZen™ 2.6 µm WidePore C4. The strategic partial proteolysis allows for characterization of specific regions of the mAb. By combining cysteine proteases IgdE and IdeS, one can gain insight on both conserved and hypervariable regions of the antibody, as well as identification of other variants, such as free sulfhydryls, all while gaining resolution and ruggedness associated with bioZen core-shell LC columns.

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:	NIST, IdeS/IgdE Digested (0.5 mg/mL), Figure 1 Trastuzumab, IdeS/IgdE Digested (0.5 mg/mL), Figure 2

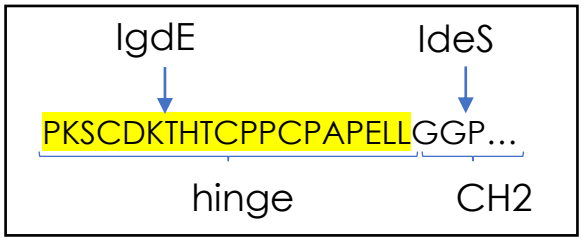


Figure 1. NIST mAb, IdeS and IgdE Digested

Separation of mAb fragments generated by IdeS and IgdE digested NIST mAb (RM 8671). Inset shows the early eluting hinge, as well as putative sulfhydryl variants. *Impurity peaks generated as a result of digestion.

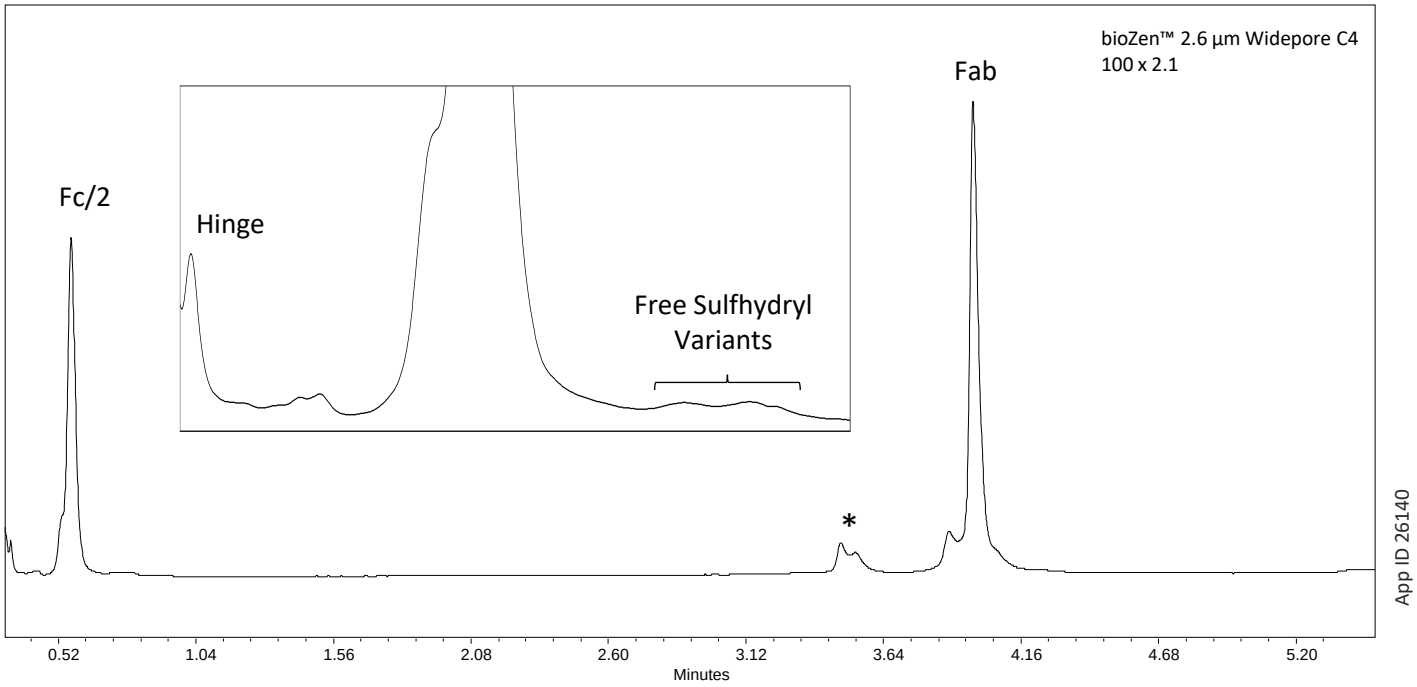
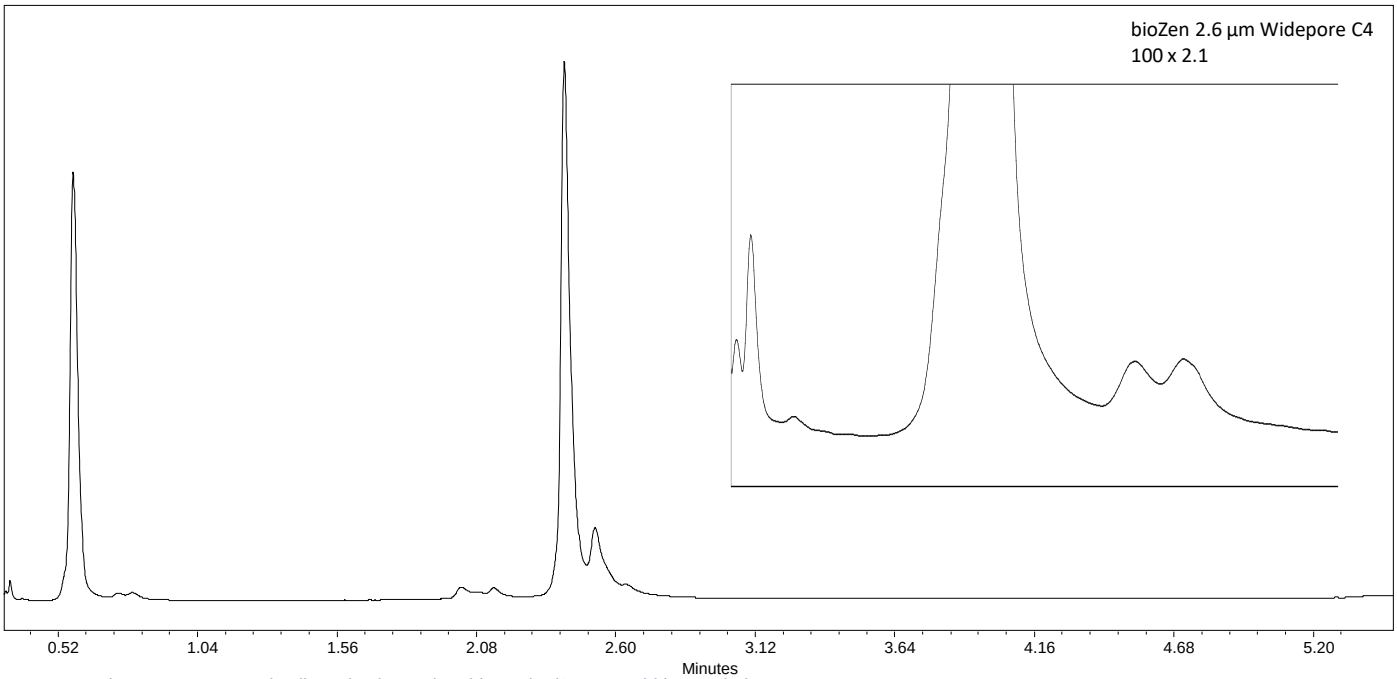


Figure 2. Trastuzumab, IdeS and IgdE Digested

Separation of mAb fragments generated by IdeS and IgdE digested trastuzumab. Inset shows separation of free sulfhydryl variants. Fab fragment also shows deamidated variants associated with trastuzumab Fab but may also contain potential sulfhydryl variants.



Reference

[1]Faid, Valegh et al. "Middle-up analysis of monoclonal antibodies after combined IgdE and IdeS hinge proteolysis: Investigation of free sulfhydryls." *Journal of pharmaceutical and biomedical analysis* vol. 149 (2018): 541-546. doi:10.1016/j.jpba.2017.11.046

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