



Analysis of Intact and C-terminal Digested IgG1 on an Agilent Bio MAb 5 μ m Column

Application Note

BioPharma

Authors

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Abstract

Nearly all proteins undergo modifications after translation from mRNA. The modifications likely occur at the end of the variation in the presence or absence of C-terminal lysine on the heavy chain. Therefore, the consistency of monoclonal antibody production, particularly IgG1, should be monitored. Agilent Bio MAb columns are specifically designed to characterize the charge heterogeneity of monoclonal antibodies, including C-terminal lysine variance. The Agilent Bio MAb 5 μ m (non-porous, 5 μ m) 4.6 \times 250 mm PEEK column offers even higher resolution, enabling better peak identification and accurate quantification. IgG1 from two different sources was analyzed on an Agilent Bio MAb 5 μ m column on two different HPLC instruments, an Agilent 1260 Infinity Bio-inert Quaternary LC and an Agilent 1100 Series LC.



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Introduction

Monoclonal antibody/IgG1 (mAb) is an important type of bio-therapeutic that has gained popularity during the past decade. Due to its structural complexity, IgG1 usually demonstrates a high degree of heterogeneity, which includes various post-translational modifications. Among these, variable levels of C-terminal lysine on the heavy chain are commonly present on the therapeutic antibodies. Although their impact on bioactivity is not well understood, the degree of heterogeneity of C-terminal lysine variants indicates the manufacturing consistency and, therefore, should be assessed. Cation-exchange chromatography is an effective method to characterize IgG1 charge heterogeneity. The Agilent Bio MAb 5 μ m column offers the selectivity required to achieve high resolution for IgG1 analysis.

Experimental

Two different sources of Chinese hamster cell monoclonal antibody IgG1, both CHO-cell derived humanized, were used for the study. Carboxypeptidase B, sodium chloride, sodium monobasic phosphate, and sodium dibasic phosphate were purchased from Sigma-Aldrich, St. Louis, MO, USA.

C-terminal cleavage procedure

IgG1, 1 mg/mL in 10 mM pH 7.5 sodium phosphate buffer, was incubated with 12.5 units of carboxypeptidase B for 2 hours at 37 °C.

Conditions

Column: Agilent Bio MAb 5 μ m, 4.6 x 250 mm PEEK (p/n 5190-2407)
Mobile phase: A. 10 mM Na phosphate buffer, pH 5.5
B. A + 0.5 M NaCl
Flow rate: 0.85 mL/min
Gradient: 10 to 35% B from 0 to 25 minutes (unless otherwise stated)
Detection: UV 225 nm
Sample: 5 μ L of 1 mg/mL of intact or C-terminal digested IgG1
Instrument: Agilent 1260 Infinity Bio-inert Quaternary LC or Agilent 1100 Series LC

Results and Discussion

Carboxypeptidase B is an enzyme that cleaves basic amino acids, such as lysine and arginine, from the C-terminus of proteins. By comparing the chromatograms of intact and C-terminal digested IgG1, C-terminal lysine on IgG1 can be identified. Figure 1 shows the overlay of intact and C-digested IgG1 chromatograms from source A using an Agilent Bio MAb 5 μ m column, with the top panel showing the entire chromatogram and the bottom panel showing the enlarged area indicated by the box on the top panel. The early eluting peak at approximately 2 minutes corresponded to the enzyme carboxypeptidase B. There were four basic variant peaks on IgG1 from source A, whereas two of the peaks were from lysine variants, that is, lys-1 and lys-2, as indicated on the chromatogram.

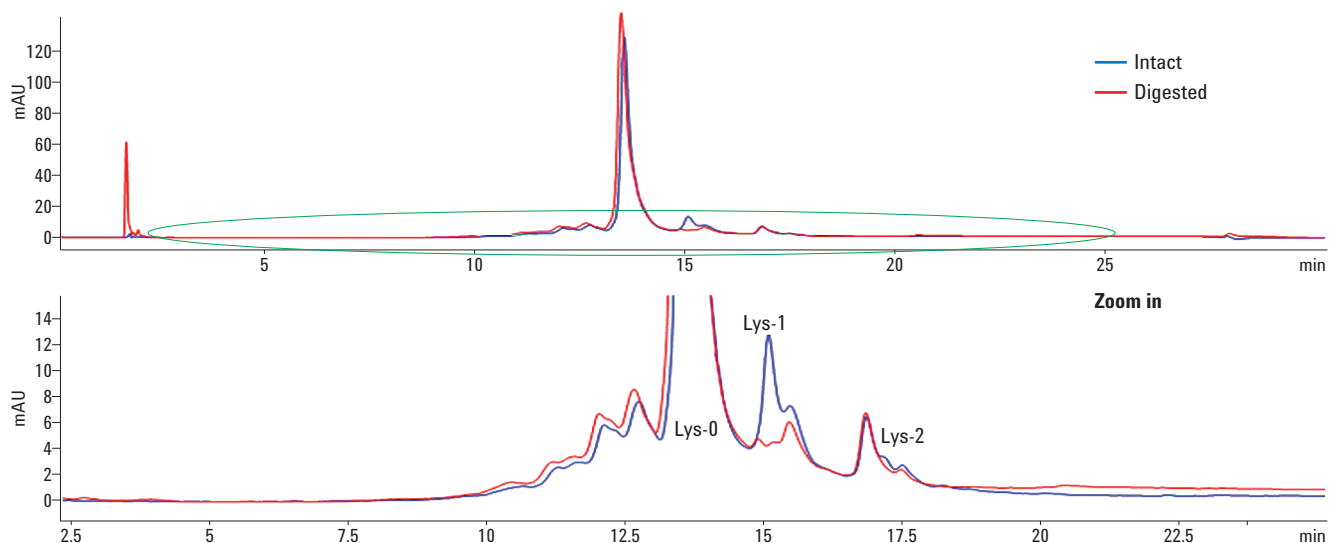


Figure 1. Separation of intact and C-terminal digested IgG1 from source A using an Agilent Bio MAb 5 μ m column. Gradient 10 to 30% B in 25 minutes.

Figure 2 refers to intact and digested IgG1 from source B. There were two basic variant peaks on IgG1 in this case, whereas two of the peaks were from lysine variants, that is, lys-1 and lys-2, as indicated on the chromatogram. By comparing the digested IgG1 peak, it was obvious that these two broad peaks contained information from lysine variants.

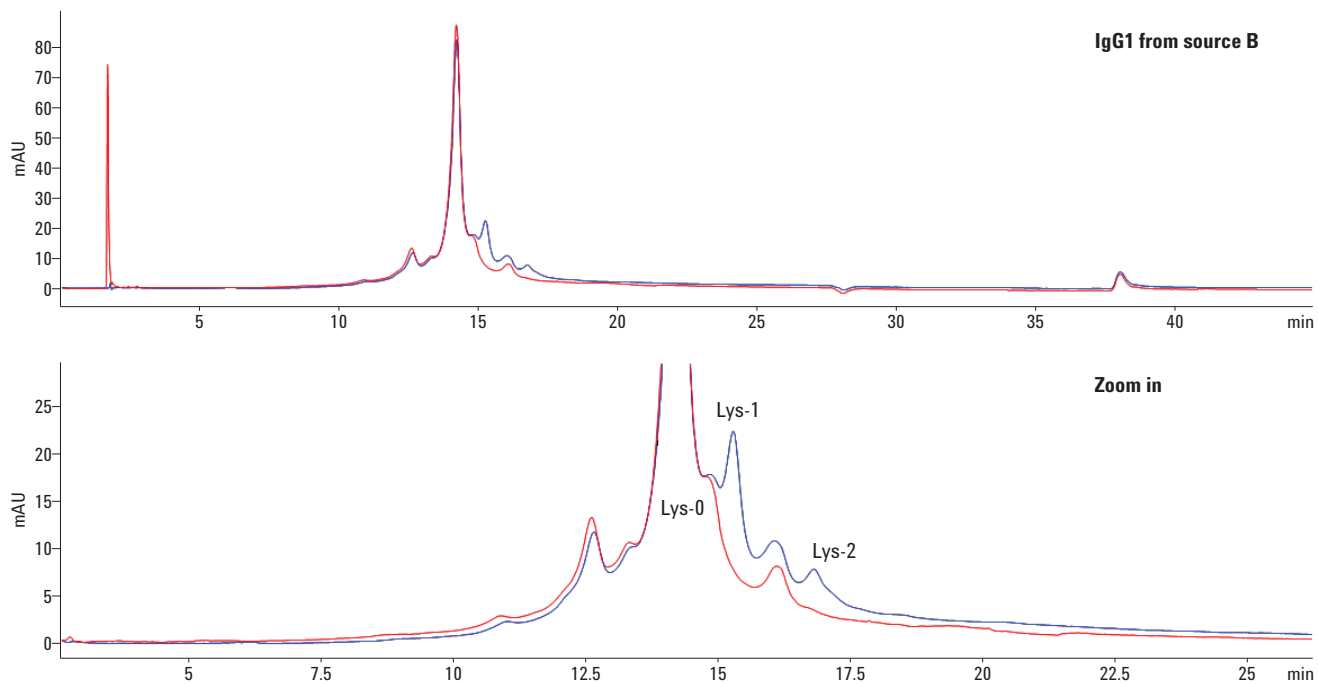


Figure 2. Separation of intact and C-terminal digested IgG1 from source B on an Agilent Bio MAb 5 μ m column.

With high resolution, the peak areas of the variants lys-1 and lys-2 from source A can be calculated (Figure 3). They possessed approximately 272 (8.64%) and 148 (1.34%) peak areas of the total peak area, respectively. The reproducibility of analysis was tested with 10 injections using IgG1 from

source A. It was evident that retention time, peak shape, and peak area were highly consistent. Calculation data for C-terminal peaks from source B was also analyzed (data not shown).

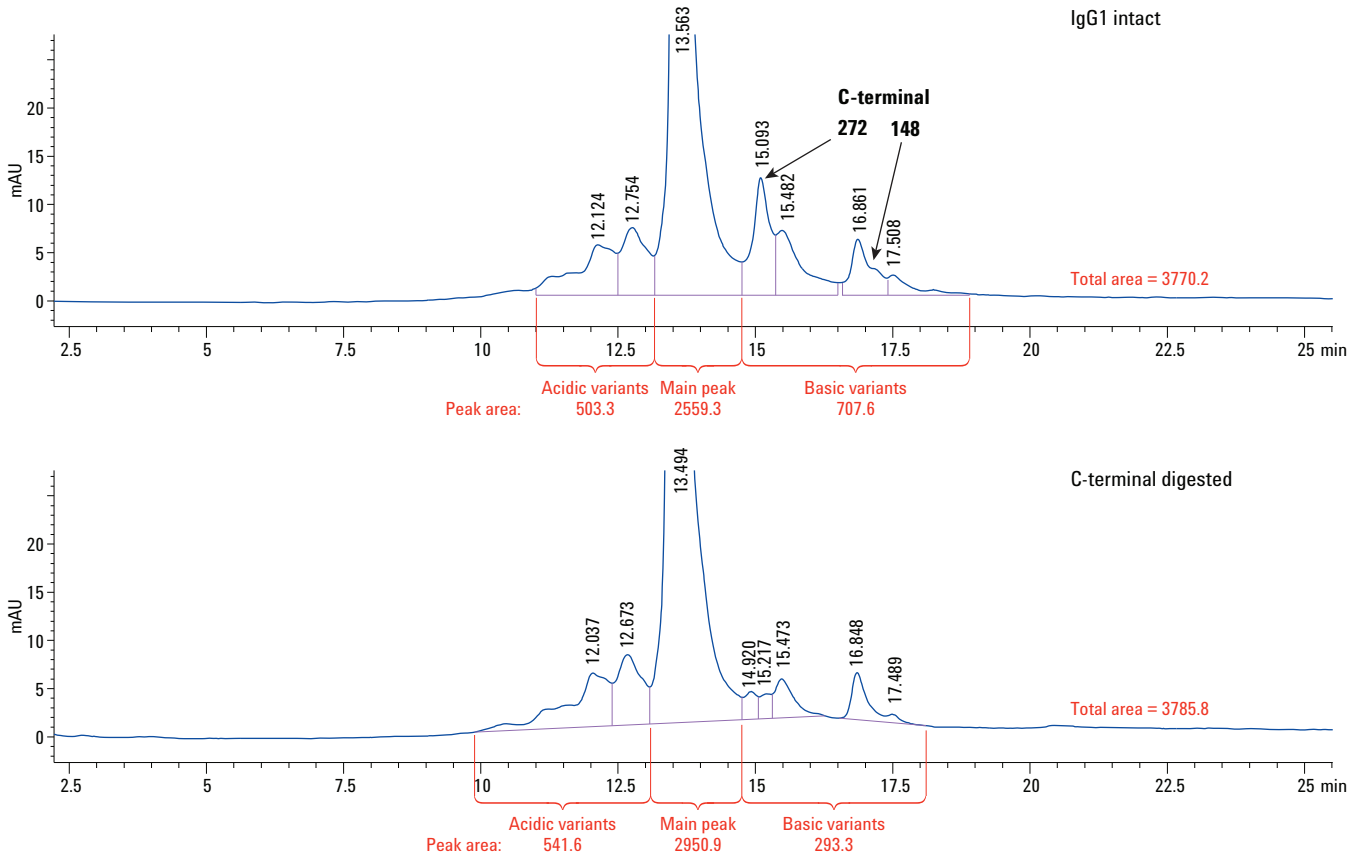


Figure 3. Calculation of C-terminal digested IgG1 from source A using an Agilent Bio MAb 5 µm column on the Agilent 1260 Infinity Bio-inert Quaternary LC.

The same experiments were repeated on an Agilent 1100 Series LC. Figure 4 compares the chromatogram of Agilent Bio MAB 5 μm , 4.6 x 250 mm PEEK column of intact (top panel) and C-terminal digested (bottom panel) IgG1 from source A. It was clear that the Agilent 1260 Infinity Bio-inert Quaternary LC (Panel B, right) provided better peak shape and higher sensitivity, as was reported elsewhere [1].

Conclusion

The Agilent Bio MAB 5 μm , 4.6 x 250 mm PEEK column, fitted to an Agilent 1260 Infinity Bio-inert Quaternary LC, delivered high resolution analysis of IgG1 monoclonal antibody. IgG1 from two different sources had different separation profiles as well as lysine variant percentages. Although both were derived from CHO-cell lines, different manufacturing processes accounted for the different degrees of lysine presence in the two IgG1s.

Reference

1. P. T. Duong, F. Ahmed, T. Clary. "HPLC Separation of Monoclonal Antibody Charge Variants: Comparison of the Agilent Bio MAB, 10 μm and a Competitor's 10 μm Particle WCX Column using the Agilent 1260 Infinity Bio-inert Quaternary LC System". Application Note, Agilent Technologies, Inc, Publication Number 5990-6844EN (2010).

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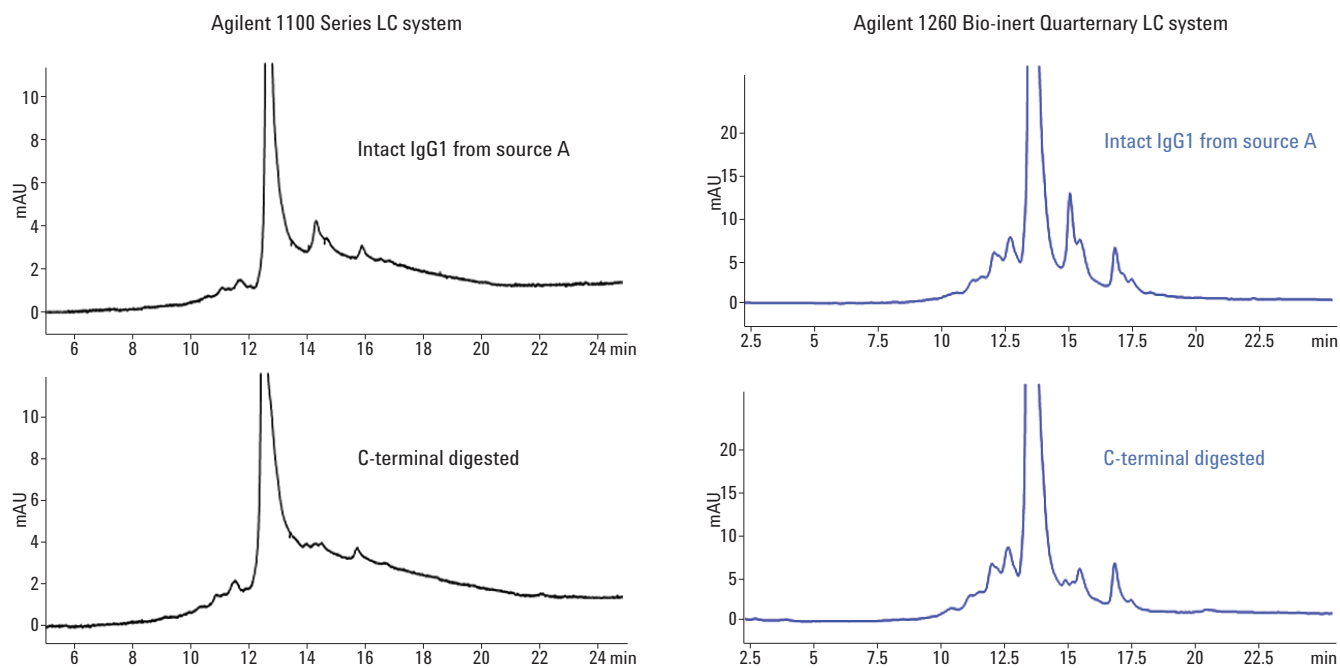


Figure 4. Comparison of separation of intact and C-terminal digested IgG1 from source A using an Agilent Bio MAB 5 μm PEEK column on an Agilent 1100 Series LC and an Agilent 1260 Infinity Bio-inert Quaternary LC.

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