Identification of a CE-SDS shoulder peak as disulfide-linked fragments from common CH2 cleavages in IgGs and IgG-like bispecific antibodies

Conner Parthemore MS
Identification of a CE-SDS shoulder peak as disulfide-linked fragments from common C\textsubscript{H}2 cleavages in IgGs and IgG-like bispecific antibodies

Mingyan Cao\textsuperscript{a}, Yang Jiao\textsuperscript{a}, Conner Parthemore\textsuperscript{a}, Samuel Korman\textsuperscript{a}, Jiao Ma\textsuperscript{a}, Alan Hunter\textsuperscript{b}, Greg Kilby\textsuperscript{a}, and Xiaoyu Chen\textsuperscript{a}

\textsuperscript{a}Analytical Sciences, Biopharmaceutical Development, Biopharmaceuticals R&D, AstraZeneca, Gaithersburg, MD, USA; \textsuperscript{b}Purification Process Sciences, Biopharmaceutical Development, Biopharmaceuticals R&D, AstraZeneca, Gaithersburg, MD, USA
Introduction

• Therapeutic protein fragmentation is a critical quality attribute
• SEC is traditionally used to monitor aggregates, while CE-SDS is used to monitor fragments
• IgG1 HC CDR fragments typically appear as shoulder peaks in CE-SDS
• RPLC, intact mass, and top-down MS2 provide fundamental intrachain cysteine bonds in antibody folding domains, including the C_H2 domain and possible intrachain disulfide bond clippings
• In our study, we identify a non-reduced CE-SDS shoulder peak appearing in a bispecific antibody (bsAb-A) after heat stress
• Our study suggests that host cell proteases are the cause of C_H2 clipping in both mAb and bsAbs
Figure 1. Comparison of non-reduced and reduced CE-SDS profiles and degradation rates of three bsAb-A drug substance lots under accelerated stress conditions at 40°C up to three months. (A) nrCE-SDS overlay of heat-stressed Lot A, Lot B and Lot C; (B) rCE-SDS overlay of heat-stressed Lot A, Lot B and Lot C; (C) % purity of Lot A, Lot B and Lot C by nrCE-SDS; (D) % purity of Lot A, Lot B and Lot C by rCE-SDS.
**Figure 2** nrCE-SDS profiles of HIC fractions from 40°C 1 mon heat-stressed bsAb-A, in which size variant peaks are labeled from 1 to 11. (A) nrCE-SDS profiles of HIC prepeaks and main peak; (B) nrCE-SDS profiles of HIC post peaks.
Figure 2 nrCE-SDS profiles of HIC fractions from 40°C 1 mon heat-stressed bsAb-A, in which size variant peaks are labeled from 1 to 11 (A) nrCE-SDS profiles of HIC prepeaks and main peak; (B) nrCE-SDS profiles of HIC post peaks.
### Table 1. nrCE-SDS peak assignment based on HIC fractionation study and partial reduction study (for peak 10 assignment)

| nrCE-SDS pk # | Fragment | Mass (Da) | HIC enrichment | Complementarity fragment | CE-SDS pk # for complimentary Frag. |
|---------------|----------|-----------|----------------|--------------------------|-------------------------------------|
|               |          | Theo      | Detect         |                          |                                     |
| 1             | Hole HC 1-102 | 11,109    | 11,108         | HIC pre-pk 2, 3, 4, 5, 6 & post-pk 2 |                                     |
|               | Hole HC 1-101 | 10,992    | 10,992         | Post-pk 5, 6             |                                     |
|               | Hole HCI-109  | 12,106    | 12,105         | Pre-pk 4                 |                                     |
| 1'            | Knob HCI-99   | 11,019    | 11,019         | All HIC fractions        |                                     |
|               | Knob HCI-103  | 11,403    | 11,402         | HIC pre-pk 4, 5, 6       |                                     |
|               | Knob HCI-106  | 11,778    | 11,778         | HIC pre-pk 4, 5          |                                     |
|               | Knob HCI-107  | 11,943    | 11,943         | HIC pre-pk 4, 5          |                                     |
| 2             | x LC2-218    | 23,971    | 23,969         | HIC pre-pk 7, 6          |                                     |
| 3             | x LC + Cys   | 24,304    | 24,304         | All HIC fractions        |                                     |
| 3'            | λ LC + Cys   | 23,041    | 23041          | HIC pre-pk 5, 6          |                                     |
| 4             | Fab 2 fragment (λ LC-knob HC1-143, 144, 145) | 38,370 | 38,370 | HIC pre-pk 4, 5, 3 & post-pk 2 |                                     |
|               |              | 38,558    | 38,558         |                           |                                     |
|               |              | 38,702    | 38,701         |                           |                                     |
| 5             | Fab 1 (hole 1/Ab) | 48,738 | 48,737 | HIC pre-pk 7, 6          |                                     |
|               |              | 48,499    | 48,499         |                           |                                     |
|               |              | 48,256    | 48,256         |                           |                                     |
|               |              | 48,371    | 48,371         |                           |                                     |
|               |              | 48,353    | 48,352         |                           |                                     |
| 6             | Fab 2 (knob 1/Ab) | 47,875 | 47,873 | HIC pre-pk 4, 5 & post-pk 2 |                                     |
|               |              | 47,208    | 47,206         |                           |                                     |
|               |              | 47,637    | 47,639         |                           |                                     |

**Note:**
- HIC enrichment Complementary fragment
- CE-SDS pk # for complementary Frag.
- The table includes peak assignments based on HIC fractionation study and partial reduction study (for peak 10 assignment).
Figure 3. CE-SDS profiles of bsAb-A non-stressed control and 40°C stressed Lot A at 1, 2 and 3 mon.
Figure 4. RP-LC UV profiles of denatured and reduced bsAb-A at 40°C 3 month (A), zoom-in of RP-LC UV profiles of denatured and reduced bsAb-A at 40°C 3 mon. (B); and deconvoluted spectra of CH2 and CH1 clipped reduced fragments (C to G). Red: Lot A; Blue: Lot B; Green: Lot C.
Figure 5. Tandem mass (MS/MS) spectra of peptides (L)TVLHQDWLNGK resulting from CH2 clipping at L306 (top) and (L)HQDWLNGK resulting from CH2 clipping at L309.
Figure 6. Deconvoluted mass spectra of Fc in bsAb-A 40°C 3 mon heat-stressed Lot A, Lot B and Lot C and unstressed control (top), and deconvoluted mass spectra of deglycosylated Fc in bsAb-A 40°C 3 mon heat-stressed Lot A, Lot B and Lot C and unstressed Control (bottom).
Figure 7. Deconvoluted mass spectra of Oxi-Fc and Fc in bsAb-A 40°C 3 mon heat-stressed Lot A (top), and RP-LC UV profile of bsAb-A subunits of heat-stressed Lot A at 40°C 3mon (bottom).
**Figure 8.** Deconvoluted mass spectra of Fab 1 and Fab 2 in bsAb-A 40°C 3 mon heat-stressed Lot A, Lot B and Lot C and unstressed Control. Top: Knob ½ Ab Fab; bottom: Hole ½ Ab Fab
Figure 9. nrCE-SDS and reduced RP-LC UV overlay of SEC fractions from 40C 1mon heat-stressed bsAb-A and relative amount of the CH2 clippings at L306, L309 or D270 in SEC fractions. A: nrCE-SDS overlay; B: Reduced RP-LC UV overlay; C: Relative amount of the CH2 clippings at L306, L309 or D270 in SEC fractions.
Table 2. Sequences in the vicinity of CH2 cleavage sites L306 and L309

| EU # | 301 | 302 | 303 | 304 | 305 | 306 | 307 | 308 | 309 | 310 | 311 | 312 | 313 | 314 | 315 | 316 | 317 |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| IgG1 | R   | V   | V   | S   | V   | L   | T   | V   | L   | H   | Q   | D   | W   | L   | N   | G   | K   |
| IgG2 | R   | V   | V   | S   | V   | L   | T   | V   | V   | H   | Q   | D   | W   | L   | N   | G   | K   |
| IgG4 | R   | V   | V   | S   | V   | L   | T   | V   | L   | H   | Q   | D   | W   | L   | N   | G   | K   |
Figure 10. Overlays of nrCE-SDS (A), rCE-SDS (B) and reduced RP-LC UV (C) and zoom-in overlay of reduced RP-LC UV (D) of 40°C 1M bsAb-A Lot A incubated with protease inhibitors and without protease inhibitors.
Discussion

• Shoulder peak is related to protease activity in mAbs and bsAbs, and can be separated from the previously reported IgG missing HC N-terminal 100 amino acids

• C_{H2} clipping can be resolved in non-reduced CE-SDS profiles as a shoulder peak on the intact IgG

• The C_{H2} domain of IgGs is the most cleaved region and understanding these cleavages could help us better understand cleavages in other subclasses

• Identification of these clipping sites can lead to improve manufacturing and cell culture practices
Acknowledgements

• Mingyan Cao
• Yang Jiao
• Samuel Korman
• Jiao Ma
• Alan Hunter
• Greg Kilby
• Xiaoyu Chen
Questions
Confidentiality Notice

This file is private and may contain confidential and proprietary information. If you have received this file in error, please notify us and remove it from your system and note that you must not copy, distribute or take any action in reliance on it. Any unauthorized use or disclosure of the contents of this file is not permitted and may be unlawful. AstraZeneca PLC, 1 Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge, CB2 0AA, UK, T: +44(0)203 749 5000, www.astrazeneca.com