Intact Protein Multi-Attribute Method (MAM) that Includes the Identification and Quantification of Protein Clipping Events

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Overview

- Proteolytic enzymes or chemical hydrolysis can cause clipping events in biopharmaceuticals.
- Clipping needs to be monitored as a quality attribute to develop processes yielding low levels of clipped side products.
- Identification of clipping variants is a first step to optimize the process minimizing side products.
- Specific workflows in the BioPharma Compass (BPC) software were applied to identify such protein clipping variants, which we applied to two antibody models:
  - NISTmAb IdeS digest: detection of production process related clipping variants.
  - Panitumumab (IgG2c) SpeB digest: elucidation of the unknown proteolytic activity of SpeB in IgG2.
  (Note: in IgG2 SpeB cleaves specifically near the hinge region)

Methods

Sample Handling

The mAbs were digested using IdeS or SpeB (both Genovis), denatured, reduced and separated by reversed phase chromatography. Intact subunit monoisotopic molecular weight (MW) and Middle-Down Sequencing (MDS) spectra were acquired on a maXis ETD ultra-high resolution QTOF and a rapifleX MALDI-TOF/TOF (all Bruker), respectively. Middle-Down sequencing spectra were acquired by MALDI-TOF/TOF (all Bruker), respectively. Down sequencing spectra were acquired by MALDI-ISD and Middle-Down-Sequencing. These were used in a 2nd round search to find multiple unspecific clipping sites in all subunits and, again, P/D as major chemical hydrolysis site (Fig.2).

Data Analysis

Based on the LC and HC sequences, all possible clipping variants were computed using BioPharma Compass 2021 (Bruker) in an automatic workflow. Experimental data based on intact masses or MDS were automatically matched to the corresponding N- or C-termini of the clipping products.

Results

LC-ESI-MS based screening for clipping variants in NISTmAb IdeS digest

The dataset was screened for clipping variants using the NISTmAb subunit sequences with variable modifications (Fig.1). Major clipping sites were found at Fd: D188(P)89 and Fc: D34(P)35; a minor site at Fc2: CBS(KH)6. The MW based screening allowed to detect internal cleavage products directly, while the MDS screening requires one terminus to be included to reduce analysis time.

LC-MALDI-1-ISO based MDS screening of clipping sites of SpeB in panitumumab

All MALDI-1-ISO spectra were acquired against all possible truncation products and include either the N- or the C-terminus. The major SpeB cleavage site HC: E224(C225) was identified amid the 4 cysteine crosslinks of the hinge region, thus establishing the Fc2 and Fd termini (Fig.3) of the primary SpeB cleavage products. These were used in a 2nd round search to find multiple unspecific clipping sites in all subunits and, again, P/D as major chemical hydrolysis site (Fig.2).

Conclusions

- Protein clipping variants were identified by intact mass as well as by Middle-Down-Sequencing.
- Detection of clipping events is the basis to reduce clipping variant-related side activities in biopharmaceuticals.
- The clipping analysis workflows in BioPharma Compass were also used to identify unspecific cleavage products of SpeB digestion in IgG2 – in contrast, IgG1 hinge is cleaved very specifically.

BioPharma