Monoclonal antibodies primary structure and biosimilarity assessment in a single analysis using transient isotachophoresis sheathless capillary electrophoresis-tandem mass spectrometry

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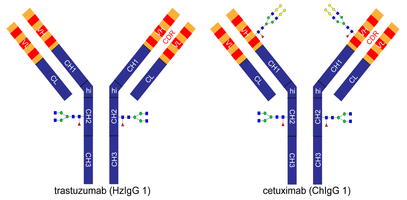
Overview

Monoclonal antibodies (mAbs) represent currently the therapeutic agent category experiencing the most important progression due to their therapeutic potency and specificity. Some patents regarding mAbs are going to end in the near future, giving the opportunity to alternative actors to produce and market the same protein which is defined in biopharmaceuticals as biosimilars. Here, capillary zone electrophoresis coupled to tandem mass spectrometry by the intermediate of an ultra-low flow interface (CESI-MS/MS) was used to characterize marketed mAbs and their respective candidate biosimilar simultaneously over different facets of their primary structure **using a sole sample injection of 200 fmol of digested peptides**. CESI-MS/MS data enabled to obtain simultaneously **100% sequence coverage**, structures of **15 glycoforms** and the characterization of all PTMs hot-spots present on the studied mAbs samples. CESI-MS/MS allowed to conclude regarding the biosimilarity study between approved mAbs and biosimilar candidate. Characterization results allowed to specifically point out the facets of the candidate which were not complying to be considered as a biosimilar.

Introduction

Monoclonal antibodies (mAbs) have taken a major market share in the pharmaceutical industry and their development is constantly increasing. Several patents pending upon early approved mAbs are going to end in the next few months, giving the opportunity to different companies to produce "copies" of the concerned antibody, those copies are referred as **biosimilars** or **follow-on biologics**. Since a few years, regulatory agencies have been working toward establishing guidelines designed to determine critical criteria that must be common between an innovator mAb and its biosimilar. mAbs are highly complex proteins that display a wide range of micro heterogeneities that requires multiple analytical methods for full structure assessment and quality control [1]. As a consequence, the characterization of mAbs on different level is particularly product and time-consuming.

In this work, we have developed a transient isotachophoresis CE-ESI-MS/MS methodology, by mean of the CESI-MS system, in order to obtain in the first place the characterization of several mAbs. This characterization, over different level of the protein, was performed in a single analysis of each sample. In a second time, each mAb was compared to a candidate biosimilar in order to establish if the methodology developed could be used to assess the biosimilarity between two samples.



Methods

Studied samples. Trastuzumab, cetuximab in their final formulation and respective candidate biosimilar trastuzumab-B and cetuximab-B were characterized by CESI-MS/MS and the obtained data confronted for biosimilarity assessment

Samples treatment. Tryptic digestion in-solution of mAbs. Digestion buffer: 50mM bicarbonate ammonium, reducing reagent: dithiothreitol 100mM, alkylation reagent: iodoacetamide 100mM. Sample peptide final concentration : 0.3 µg/µL

CE conditions. Instrument: Sciex separation CESI-MS prototype (Brea, CA, US) equipped with a bare-fused silica capillary cartridge (90 cm, 30 µm i.d.), injection volume: 100 nL (220 fmol of digest), BGE: 10% acetic acid, separation voltage: +20 kV, duration: 50 min. CESI-MS interface for MS coupling

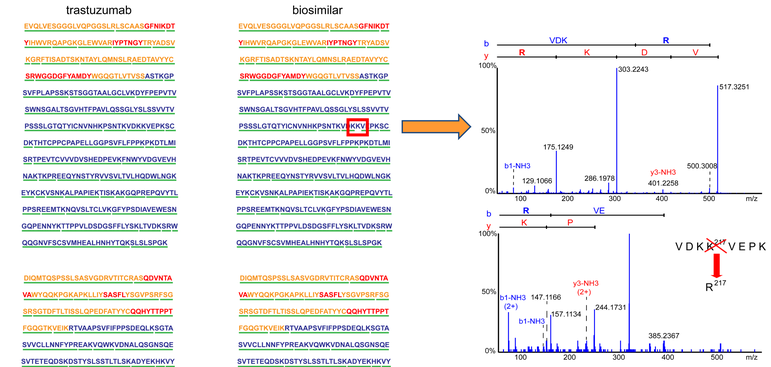
Mass spectrometry. MS/MS was performed using an ESI-Qq-TOF 5600 tripleTOF® (AB Sciex, San Francisco, CA) MS/MS method: IDA (top15 precursor ions), dynamic exclusion (0.15 min after 2 spectra), mass range 100-2000 m/z, duty cycle 1.75 sec, capillary voltage -1.75 kV, curtain gain 5, Source temperature 160°C.

[1] Beck A, Sanglier-Cianférani S, Van Dorsselaer A. , " Biosimilar, biobetter, and next generation antibody characterization by mass spectrometry." Anal Chem 2012, 84,4637-4646

[2] Gahoual R., Biacchi M., Chicher J., Kuhn L., Hammann P., Beck A., François Y-N., Leize-Wagner E., " Monoclonal antibodies biosimilarity assessment using transient isotachophoresis preconcentration-capillary zone electrophoresis-tandem mass spectrometry " 2014, submitted.

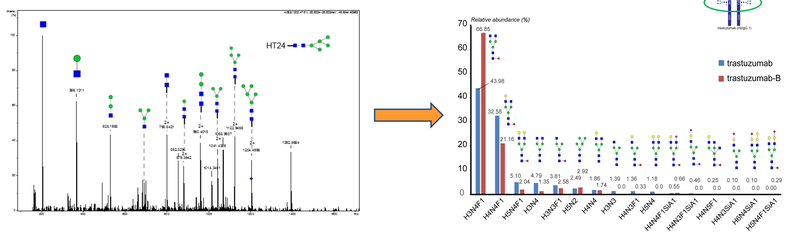
Results

Amino acids sequence characterization / comparison



100 % sequence coverage (no miscleavages / PTMs) → **1 AA difference characterized for trastuzumab biosimilar**

Trastuzumab glycoprofiling / profiles comparison

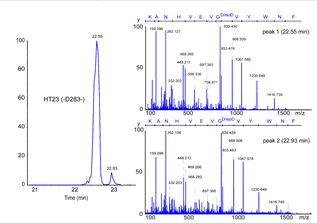


Structures deduced from MS/MS spectra

Glycoforms relative abundances established

15 glycosylations structures characterized → **Glycoforms distribution comparison for biosimilarity**

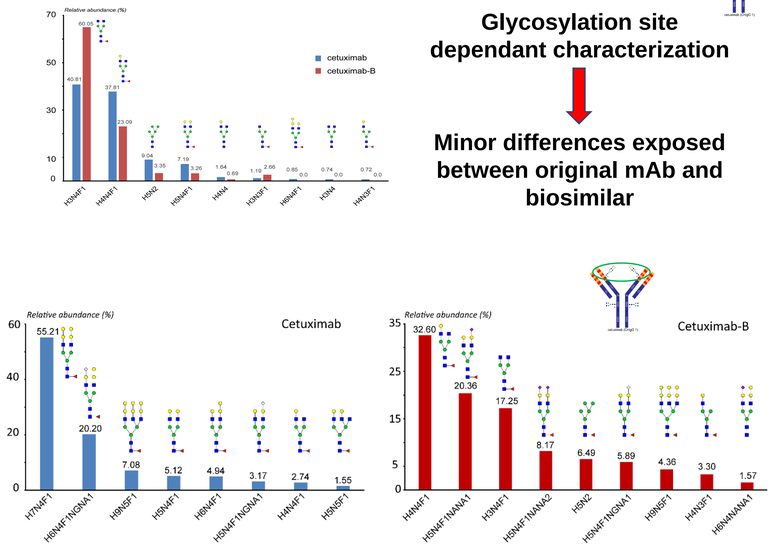
PTMs hot-spots characterization / abundance comparison



CESI allowed separation of peptides with PTMs

Relative quantitation of each PTMs hot-spots obtained

Cetuximab glycoprofiling / profiles comparison



Glycosylation site dependant characterization

Minor differences exposed between original mAb and biosimilar

Different glycoforms characterized between cetuximab and biosimilar (glycans containing murine sialic acids)

Conclusions

	Trastuzumab	Trastuzumab-B	Cetuximab	Cetuximab-B
sequence coverage	100%	100%	100%	100%
identified glycosylations	15	10	10	16
other PTMs hotspots				
glutamic acid cyclization	1 / 1	1 / 1	1 / 1	1 / 1
methionine oxidation	2 / 2	2 / 2	0 / 0	0 / 0
asparagine deamidation	4 / 4	2 / 2	4 / 4	4 / 4
aspartic acid isomerization	6 / 6	3 / 3	2 / 2	2 / 2

Data obtained from solely one injection of each sample tryptic digest allowed to characterize each mAbs simultaneously over several levels defining their primary structure. **Biosimilarity study results indicated that in both cases, candidate biosimilars exhibited minor differences compared to the originator mAb.** In the case of trastuzumab-B, one AA difference compared to the original mAb leaded to this conclusion while in the case of cetuximab-B uncontrolled expression of murine glycoforms toxic to human metabolism ended up in the rejection candidate biosimilar.

Acknowledgements

Authors would like to thank AB Sciex for the loan of a prototype CESI system and a 5600 TripleTOF® mass spec. The authors would like to express their gratitude to Dr. M. Anselme (Sciex, Les Ullis, France) for his support and Dr. Joseph Vlasak (Merck, West Point, PA) for critical discussions around antibody structural characterization.