Trapped Ion Mobility Spectrometry (TIMS) enables differentiation of isobaric N-glycan isomers by specific collisional cross sections

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Introduction

Protein-based therapeutics undergo strict quality control throughout their entire developmental and production phase. There are several critical quality characteristics for therapeutic proteins. The impact on the quality of these biological products due to minor variations can be serious. A critical quality attribute which is challenging to control is the variation in the N-glycosylation pattern, as many N-glycan isomer structures can be attached. The most difficult species to analyze are the isobaric linkage isomers. Since the chromatographic separation of these species is difficult and time-consuming, ion mobility separation offers an attractive analytical alternative to distinguish these isomeric forms. In this study we determined specific (collisional cross sections) CCS values of two different isobaric N-glycan structures using a TIMS-MS system.

Methods

N-glycan reference standards were prepared at Alvotech Hannover using in-house methods.

Two isobaric pairs of 2-AB labelled N-glycan structures were analyzed. A neutral isobaric N-glycan pair as mixture of the C3 and C6 isomers of the biantennary complex-type N-glycan with proximal α1,6-linked fucose lacking one terminal ß-galactose residue. And a sialylated isobaric N-glycan pair consisting of a mixture of the α2,3- and α2,6-Neu5Ac linkage isomers of the disialylated, biantennary complex-type N-glycan with proximal α1,6-linked fucose.

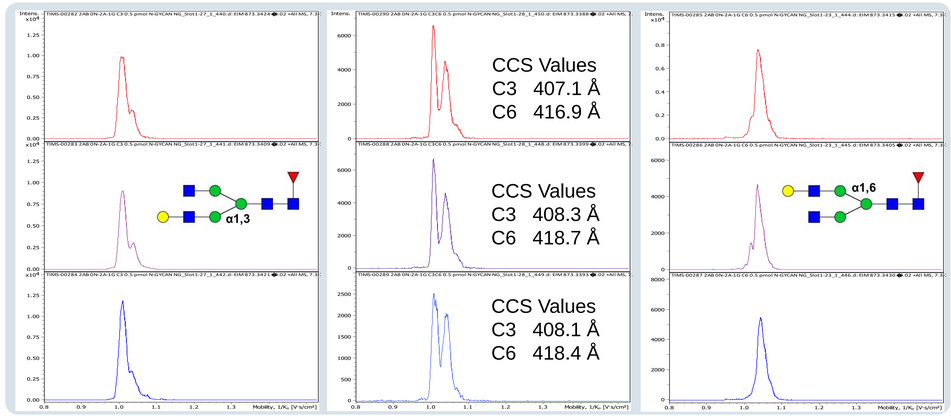

Fig. 1 Mobilogram traces from triplicate injections of 2AB 0N-2A-1G C3 (left), 2AB 0N-2A-1G C6 (right) and of a mixture of 2AB 0N-2A-1G C3/C6 (middle). Values are showing the CCS values of the two signals in the mixture.

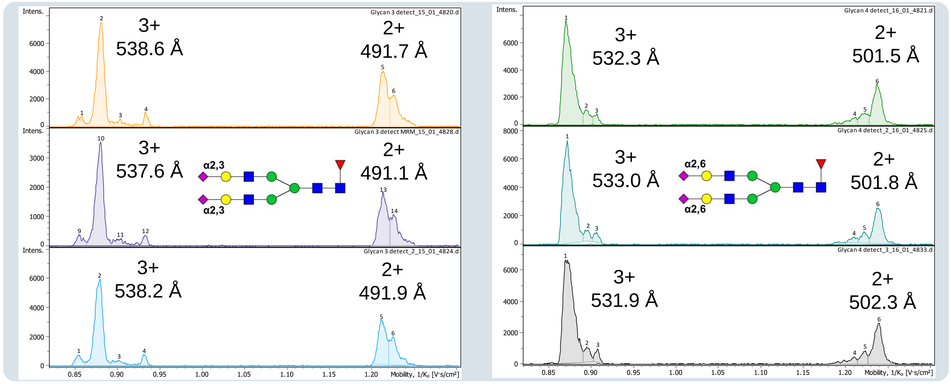

Fig. 2 Mobilogram traces from triplicate injections of 2AB 2N(α2,3-Neu5Ac)-2A (left) and of 2AB 2N(α2,6-Neu5Ac)-2A (right), showing charge states 2+ and 3+. Values are showing CCS values of the signal with highest intensity of each charge state.

For LC-MS measurements, N-glycan reference standards were labelled with 2-AB according to Bigge et al., 1995 and diluted to 1 pmol/µl.

Using an Elute UPLC system (Bruker Daltonics) equipped with a BEH C4 column (2.1x100 mm, 1.7 µm, Waters) and coupled to a timsTOF Pro (Bruker Daltonics) mass spectrometer we injected 1 µl of the prepared solution utilizing a 12 minutes run with 0.1% formic acid in water and 0.1% formic acid in acetonitrile as mobile phases. Samples were measured in triplicate to proof reproducibility of obtained trapped ion mobility separation (TIMS) CCS values.

Results

- Isobaric oligosaccharide chains could be distinguished by trapped ion mobility separation (TIMS) due to slight structural changes
- Individual collision cross section (CCS) values for the different isobaric molecules could be obtained with excellent reproducibility
- Standard Deviation of CCS values was 0.65 for the doubly charged neutral biantennary N-glycan signals and 0.55 (3^+ signal) and 0.41 (2^+ signal) resp. for sialylated biantennary N-glycans

Summary

Trapped ion mobility separation (TIMS) is a promising tool for biopharmaceutical product characterization. We presented differentiation of two different pairs of 2-AB labelled isobaric N-glycans with excellent reproducibility of obtained CCS values. The obtained characteristic CCS values of the analysed molecules are showing the potential for unambiguous identification of glycans with terminal α1,3-galactose (Galili epitope) or terminal N-glycolylneuraminic acid (Neu5Gc) structure motifs playing in important role in adverse immune reactions.

Conclusions

- Very consistent ion mobility values observed across entire LC peak
- Highly reproducible CCS values enable LC-independent differentiation of isobaric N-glycan structures
- Powerful determination of critical quality attributes like N-glycosylation of biopharmaceutical products by trapped ion mobility separation is demonstrated
- Potential shown for important role of TIMS for unambiguous identification of N-glycan motifs which might be involved in adverse immune reactions

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