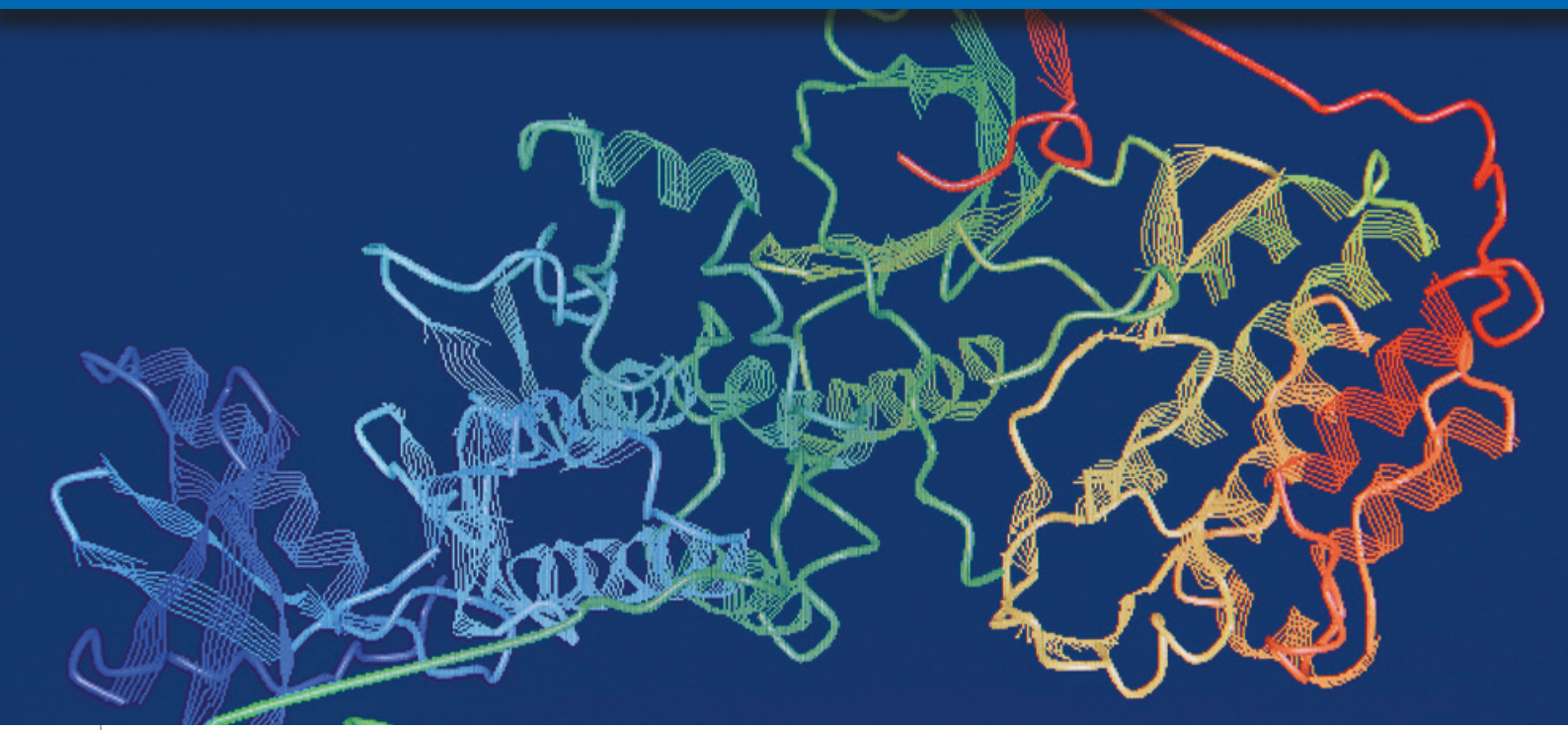

Characterization of Cysteine dioxygenase variants by trapped ion mobility with timsTOF Pro

Cysteine dioxygenase plays an essential role in sulfur metabolism by regulating homeostatic levels of cysteine. Human CDO contains a post-translationally generated Cys93–Tyr157 crosslinked cofactor.

Abstract

Understanding the mechanism of the Cys-Tyr cross-link biogenesis in CDO is difficult due to closely related isoforms in the starting material for studying cofactor biogenesis. For this reason, a method using trapped ion mobility separation (TIMS) was developed to separate crosslinked and uncrosslinked variants. A simple direct infusion in the timsTOF Pro enables this characterization in under 2 minutes. This assay could easily be converted to a high-throughput flow injection approach for projects with larger number of samples.

Keywords: Separation of intact proteins by ion mobility, Intact proteins, Top down, Protein ion mobility, TIMS, timsTOF Pro, Protein structure

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Introduction

Mass spectrometry is extensively used for the characterization of intact proteins' molecular weight. Separation in gas phase by trapped ion mobility measurements can facilitate these studies by reducing the sample complexity. In addition, it can provide insights in gas phase conformations and possibly structures. This is particularly interesting for the characterization of proteoforms of similar molecular weight.

Characterization of CDO variants [1] is indeed challenging, with each variant being just 2 Da apart from each other. Separation of the 2 forms can be obtained in denaturing SDS-PAGE gels and studied with traditional site-directed mutagenesis leading to the disruption of the formation of the Cys-Tyr cross-link and the inability of the protein variants to generate a mature enzyme active site. In this note, we address that challenge by harnessing the power of ion mobility with the Bruker timsTOF Pro to characterize the gas-phase structural differences between the crosslinked and uncrosslinked CDO variants, differing from each other by only 2 hydrogens.

Methods

CDO sample was received buffer exchanged into 20 mM ammonium acetate, at a concentration equal to 5644 µM (determined with UV-Vis). Sample was diluted 10x from original stock in 20 mM ammonium acetate and afterwards cleaned up with desalting columns (Bio-Rad, Micro Bio-Spin®).

After desalting, the sample was further diluted 100x with 50/49.9/0.1% methanol/water/acetic acid, with a final concentration equal to 5.6 µM (assuming no loss during the cleanup steps). The sample was then vortexed and allowed about 20 minutes or so to come to equilibrium prior to analysis.

Acquisition was performed via direct infusion at 1 uL/min on a timsTOF Pro equipped with an Apollo Source over a time frame of 2 minutes, by acquiring 3 technical replicates.

Results

An ion mobility heat map (Figure 1) was generated from the CDO infusion experiment. Two distinct charge distributions are observed at different mobilities indicating a clear separation of the 2 variants. Specifically, mobility values for the charge state +18 are circled in red, from which CCS values are derived. Calculated values are reported below:

Crosslinked CDO : 4472.29 Å
Uncrosslinked CDO : 4941.16 Å

The 3 technical replicates for the CCS values generate a statistical variation with %CV virtually equal to zero and for this reason are not reported. The significant difference between the 2 cross-sectional values shows the advantage of TIMS high resolution and specificity for the characterization of these variants just 2 Da apart (Figure 2). Without the TIMS separation, determining an accurate mass for the lower intensity variant would be virtually impossible, due to the overlap in the isotopic peak envelope. With these species, well separated and isotopic patterns measured with high resolution, the SNAP deisotoping algorithm can be used to determine the monoisotopic mass of each protein with high precision. We report excellent sub-ppm mass accuracy, namely 0.9 and 0.5 ppm for the crosslinked and native variant, respectively. Moreover, intensities of the variants enable relative quantification of the proteins, offering the opportunity to study biogenesis of cross-linking at different time frames in the biological system under investigation.

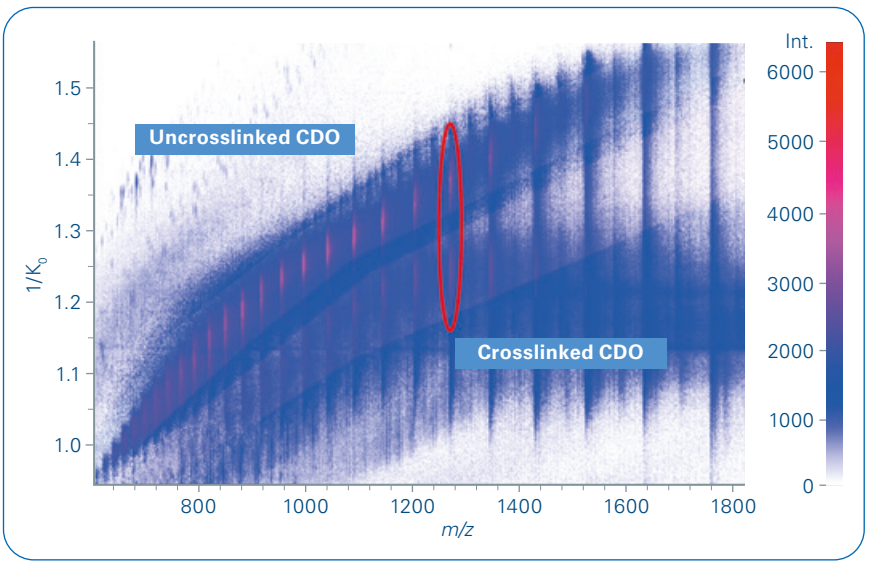

Figure 1. m/z *versus ion mobility heatmap for CDO.*

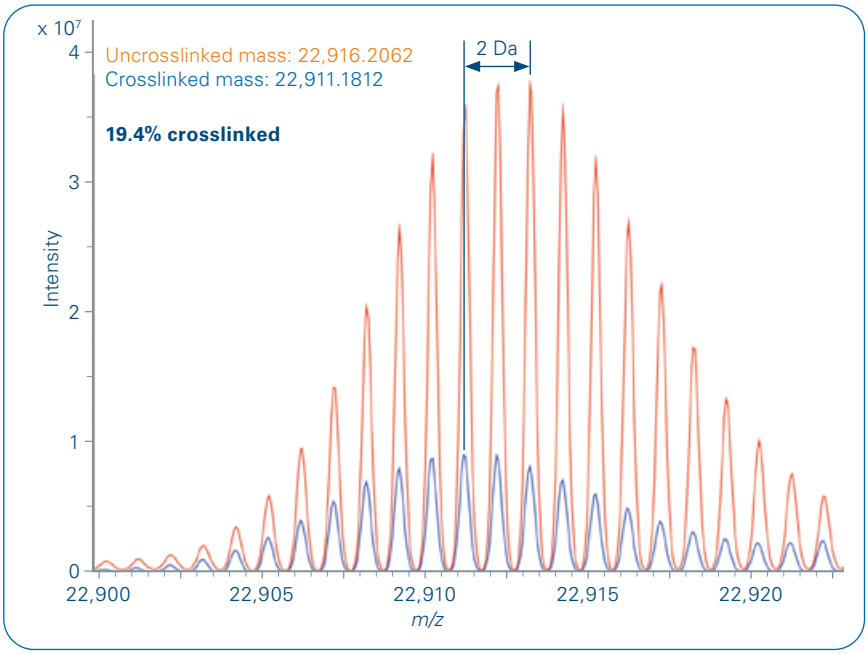

Figure 2. Deconvoluted Mass Spectra for IMS-resolved species.

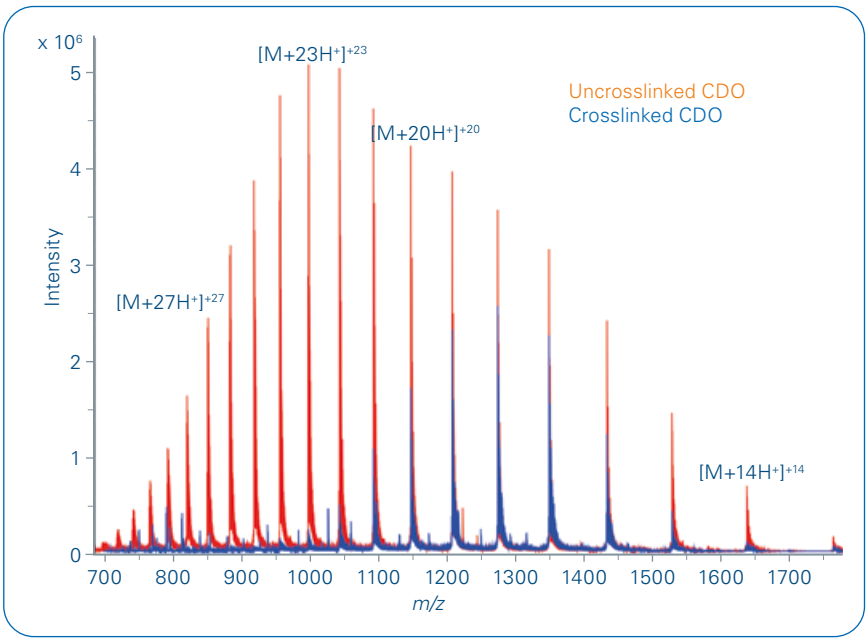

Figure 3. Extracted mass spectra from heatmap (highlighted areas).

Mass spectra extracted from the respective regions of interest for each CDO variant on the heat map (Figure 3) show different charge distribution implying a distinct spatial distribution of the three-dimensional shape of crosslinked versus uncrosslinked, consistent with the collision cross-sectional values. Predominance of higher charge state in the cross-linked CDO suggest a more folded and compact structure versus a more unfolded structure for the uncrosslinked variant.

Collectively, data show the excellent selectivity of the ion mobility on the Bruker timsTOF to distinctively characterize both CDO variants.

Conclusion

The selectivity of trapped ion mobility is a powerful separation tool for high resolution protein measurements. The improved specificity delivers higher mass spectra quality, including for closely related isoforms like in this example, and offers additional insights in protein biology.

Learn More

You are looking for further Information?
Check out the link or scan the QR code.

www.bruker.com/timstofpro

References

[1] Nature Chemical Biology, Vol 14 - september 2018 pages: 853–860

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