Determining Collision Cross Sections Using Orbitrap Charge Detection Mass Spectrometry

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ABSTRACT

Collision cross sections (CCS) have typically been measured using ion mobility spectrometers. However, the measured ion signal decay rate in a Fourier transform mass analyzer due to collisions with the background gas can also be used. The Selective Temporal Overview of Resonant Ions (STORI) method in charge detection mass spectrometry (CDMS) provides precise lifetimes for individual ions which can be used to calculate CCSs. An overview of the process with initial CCS results for individual charge states of myoglobin compared to other methodologies will be presented. Furthermore, limitations of the methodology will be discussed using GroEL as an example.

INTRODUCTION

Collision cross sections are widely considered useful in determining protein size and structure, parameters relevant to many fields and applications. In general terms, the CCS is the rotationally averaged combined radii of an individual ion and neutral buffer gas atom/molecule. Methods to determine CCSs include theoretical calculations and several experimental approaches, such as ion mobility (IM) spectrometry and various Fourier transform mass spectrometry techniques. This work intends to demonstrate the feasibility of using Direct Mass Technology mode, a recently-released Orbitrap-based charge detection mass spectrometry (CDMS) technique, to determine protein CCSs.

MATERIALS AND METHODS

Myoglobin from equine heart and ubiquitin from Sigma were diluted using 50:50 methanol/water with 0.1% formic acid to concentrations of 0.6 µM and 2 µM, respectively. The *E. Coli* GroEL 14mer was purified at a concentration of 2.6 µM in 200 mM ammonium acetate.

CDMS experiments were performed using Direct Mass Technology mode on a Thermo Scientific™ Q Exactive™ UHMR Hybrid Quadrupole-Orbitrap™ Mass Spectrometer. All ions were produced by electrospray ionization; myoglobin and ubiquitin at 3 µL/min using an Ion Max source and a spray voltage between 3.5 - 4.0 kV, GroEL using borosilicate emitters and a spray voltage ~1.7 kV in a Nanospray Flex source.

Collision cross sections (σ) were determined from the following relation, as described in [1],

$$\sigma = \frac{kTc}{flp}, \quad (1)$$

where k is Boltzmann's constant, T is temperature, f is the ion frequency along the central electrode, l is the average ion path length for one oscillation, p is pressure, and c is the ion decay constant. Temperature and the ion frequency were determined from the RAW file scan header, and it was estimated $l = 65.1$ mm[1]. Ion lifetimes obtained from STORIboard (www.proteinaceous.net/storiboard), a software package for analyzing Direct Mass Technology mode collected on a Q Exactive UHMR were used to determine c according to

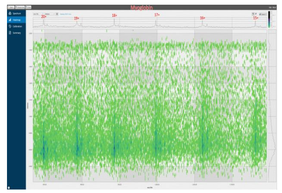
$$N(t) = N_0 e^{-ct}. \quad (2)$$

A species with a known collision cross section[2], ubiquitin 9+, was used to determine p from Equations 1-2. A key assumption of this methodology is that collisions with background gas result in ion death.

Python code to calculate CCSs from STORIboard analysis files (.dmt) is available upon request.

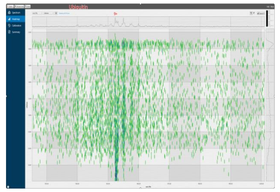
RESULTS

Figure 1. Myoglobin 15-20+ Lifetimes



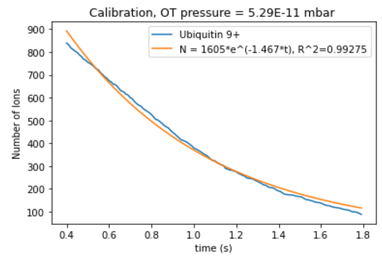
Heatmap of ion lifetime vs. *m/z* for denatured myoglobin 15-20+ from a one-hour CDMS measurement using Direct Mass Technology Mode analyzed using STORIboard

Figure 2. Ubiquitin 9+ Lifetimes



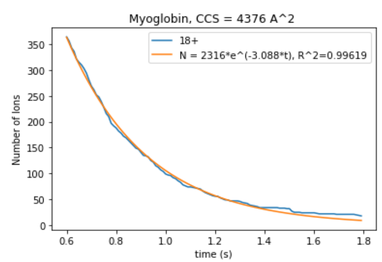
Heatmap of ion lifetime vs. *m/z* for denatured ubiquitin 9+, the calibrant for determining orbitrap pressure, from a one-hour CDMS measurement using Direct Mass Technology Mode analyzed using STORIboard

Figure 3. Determining Orbitrap Pressure



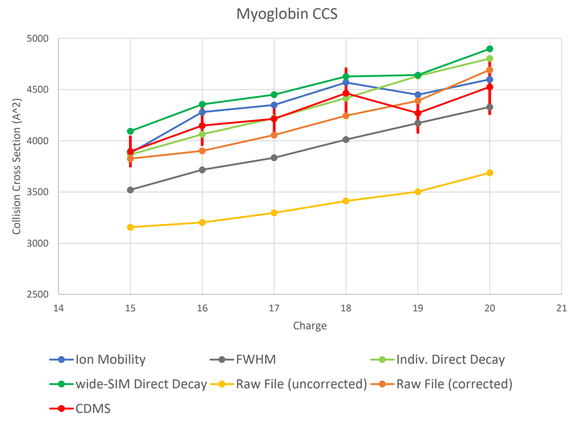
Plot of denatured ubiquitin 9+ ion decay versus transient time compiled from individual ion measurements (blue). Fitting the data according to Equation 2 (orange) allows for the determination of the ion decay constant c. Using the known collision cross section[2] for ubiquitin 9+, $\sigma = 2090$ Å^2 and Equation 1, the pressure within the Orbitrap, p, can be calculated.

Figure 4. Determining Myoglobin CCS



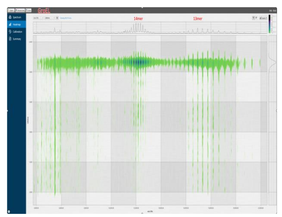
Plot of denatured myoglobin 18+ ion decay versus transient time as an example compiled from individual ion measurements (blue). Fitting the data according to Equation 2 (orange), combined with the Orbitrap pressure , p, determined using denatured ubiquitin 9+ (see Figure 3) allows for the calculation of the collision cross section

Figure 5. Myoglobin 15-20+ CCS Determined Using Different Methods



The collision cross section in Å^2 for the 15-20+ charge states of denatured myoglobin, as determined using various methods. For a description of each method, please see the Supplemental Information in [2]. The method described here, labeled 'CDMS', is in good agreement with other methods for determining CCSs. Error bars represent the standard deviation for multiple replicate CDMS measurements.

Figure 6. GroEL Lifetimes



Heatmap of ion lifetime vs. *m/z* for the GroEL 14mer and 13mer from a 45-minute CDMS measurement using Direct Mass Technology Mode analyzed using STORIboard. Charges on the 14mer ranged from 55-75+ and 35–50+ for the 13mer. Unlike myoglobin or ubiquitin, very few ions die within the full acquisition time.

Figure 7. Higher-Pressure p Determination

Same as Figure 3, but for the experimental conditions necessary to collect GroEL spectra, including higher N_2 trapping gas pressures. The relatively-low CCS for denatured ubiquitin 9+ results in much shorter ion lifetimes, ultimately increasing the uncertainty in determining the Orbitrap pressure, p.

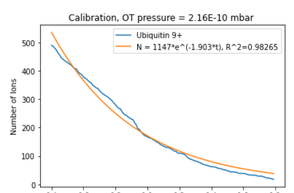
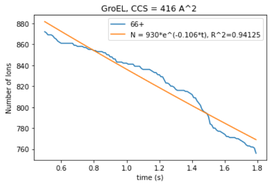
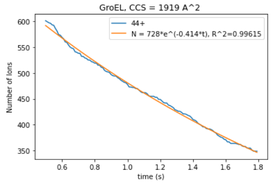


Figure 8. GroEL 14mer 66+ CCS



Same as Figure 4, but for GroEL 14mer 66+ using the Orbitrap pressure , p, determined in Figure 7. The combination of so few GroEL ions decaying with the uncertainty in p results in a seemingly inaccurate CCS.

Figure 9. GroEL 13mer 44+ CCS



Same as Figure 8, but for GroEL 13mer 44+. Again, the CCS determined using this method appears inaccurate.

CONCLUSIONS

Collision cross sections for individual charge states can be calculated using data readily available from the CDMS STORI method. The accuracy of the method depends upon the validity of the assumption that collisions with the background gas result in ions dying and how well the pressure within the Orbitrap can de determined. For species such as denatured myoglobin, the CCS determined using the CDMS STORI method is in good agreement with other methods. However, for GroEL CCS results imply the 14mer is ~10% the rotationally-averaged size of denatured myoglobin, despite being ~50x the mass. Therefore, the constraints of the CDMS STORI method for determining CCS need to be more clearly defined.

REFERENCES

1. Sanders, J.D., Grinfield, D., Aizikov, K., Makarov, A., Holden, D.D., Brodbelt, J.S.: Determination of Collision Cross-Sections of protein Ions in an Orbitrap Mass Analyzer. *Anal. Chem.* **90** 5896-5902 (2018)

2. James, V.K., Sanders, J.D., Aizikov, K., Fort, K.L., Grinfield, D., Makarov, A., Brodbelt, J.S.: Advancing Orbitrap Measurements of Collision Cross Sections to Multiple Species for Broad Applications. *Anal. Chem.* **94** 15613-15620 (2022)

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TRADEMARKS/LICENSING

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