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 lifetime 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 radius of an individual ion and neutral buffer gas molecules. 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 210 mM ammonium acetate.

CDMS experiments were performed using Direct Mass Technology mode on a Thermo Scientific™ Orbitrap™ E-NSI hybrid quadrupole-Orbitrap™ Mass Spectrometer. All ions were produced by electrospray ionization using myoglobin and ubiquitin ~1 µM in an ion flow source and a spray voltage ~1.7 kV in a Nanospray Flex source.

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

\[ c = \frac{\Delta \sigma}{\Delta \phi} \]

where \( \Delta \sigma \) is Boltzmann’s constant, \( \theta \) is the ion frequency along the central axis, \( \Delta \phi \) is the average ion path length for one oscillation, \( c_0 \) is the constant, and \( c \) is the ion decay constant. The temperature and the ion temperature were determined from the Raw NSI scan header, and for a sample estimated as ~5.31 mm, Ion Velocism obtained from STORIboard (www.proteinaceous.net/storiboard), a software package for analyzing Direct Mass Technology mode collected on a Q Exactive UHR was used to determine \( c \) according to

\[ c(\theta) = \frac{\Delta \sigma}{\Delta \phi} \]

(1)

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

RESULTS

Heating of Ubiquitin vs. nitric for denatured myoglobin 15-20+ from a one hour CDMS determination of Collision Cross Sections using Direct Mass Technology Mode analyzed using STORIboard.

Figure 1. Myoglobin 15-20+ Lifetimes

Heating of Ubiquitin vs. nitric for denatured ubiquitin 9+, the calibrator for determining collisional pressure, 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 from the Release Orbitrap Pressure measurement using Direct Mass Technology Mode analyzed using STORIboard.

Figure 3. Determining Orbitrap Pressure

Heatmap of ion lifetime vs. m/z from the Release Orbitrap Pressure measurement using Direct Mass Technology Mode analyzed using STORIboard.

Figure 4. GroEL Lifetimes

Heatmap of ion lifetime vs. m/z from the Release Orbitrap Pressure measurement using Direct Mass Technology Mode analyzed using STORIboard.

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

Plot of denatured myoglobin 15-20+ ion decay versus transient time compiled from individual ion measurements (blue)). The data according to Equation 1 (orange) allows for the determination of the ion decay constant \( c \), using the CCS at the CCS vs. cross section for denatured ubiquitin 9+, 3986 Å^2 and Equation 1, the pressure when the Orbitrap \( p \) can be calculated.

Figure 6. GroEL 14mer 66+ CCS

Heatmap of ion lifetime vs. m/z from the Release Orbitrap Pressure measurement using Direct Mass Technology Mode analyzed using STORIboard.

Figure 7. High-Pressure \( p \) Determination

Plot of denatured myoglobin 15-20+ ion decay versus transient time as an example compiled from individual ion measurements (blue)). The data according to Equation 1 (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 8. GroEL 14mer 66+ CCS

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 ion death and how well the pressure is determined with the Orbitrap pressure measurement. Several examples 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 CCSs need to be more clearly defined.

REFERENCES

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