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应用纪要

Gas Phase Protein Structure is Consistent with Structural Measurements Made by X-Ray Crystallography

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Abstract

Here, we investigate and present the use of charge-reduced (proton transfer) myoglobin, cytochrome C, and lysozyme as T-Wave ion mobility calibration standards and their use determines the collisional cross-sections (CCSs) of biological protein complexes.

Introduction

It is important for structural biologists to know that the native protein complex they are analyzing by mass spectrometry has the same stoichiometry and shape as those determined by other techniques, such as X-ray crystallography or nuclear magnetic resonance (NMR). The Waters SYNAPT High Definition MS (HDMS) System and nanoelectrospray sources lend themselves particularly well to preserving non-covalent interactions, thus allowing one to analyze compounds in their native conformation and stoichiometry[1].

The structure of a protein or a protein complex is critical to its function. Utilizing the T-Wave[2] ion mobility capability of the SYNAPT HDMS System allows you to measure the intact mass of the biological complex and infer the shape of the intact complex; this provides some insight into the function of the protein complex.

Here, we investigate and present the use of charge-reduced[7] (proton transfer) myoglobin, cytochrome C, and lysozyme as T-Wave ion mobility calibration standards and their use determines the collisional cross-sections (CCSs) of biological protein complexes.

We also compare the T-Wave derived CCSs to the theoretically calculated CCSs, which use the open source code MOBCAL[3,4]. MOBCAL allows you to input a coordinate file, which in this case is an RSCB Protein Data Bank (PDB) (//www.rcsb.org/pdb/home/home.do < https://www.rcsb.org/pdb/home/home.do>) coordinate file of a protein of interest. Using the Projection Approximation[1,3] algorithm, the orientation-averaged CCS is reported for the PDB structure of interest.[1]

Experimental

The instrument used in this study was a SYNAPT HDMS System, which combines high-efficiency ion mobility-based measurements and separations with a hybrid quadrupole orthogonal acceleration time-of-flight (oa-TOF) mass spectrometer, as shown in Figure 1. Samples were introduced with a borosilcate glass nano-electrospray tip.

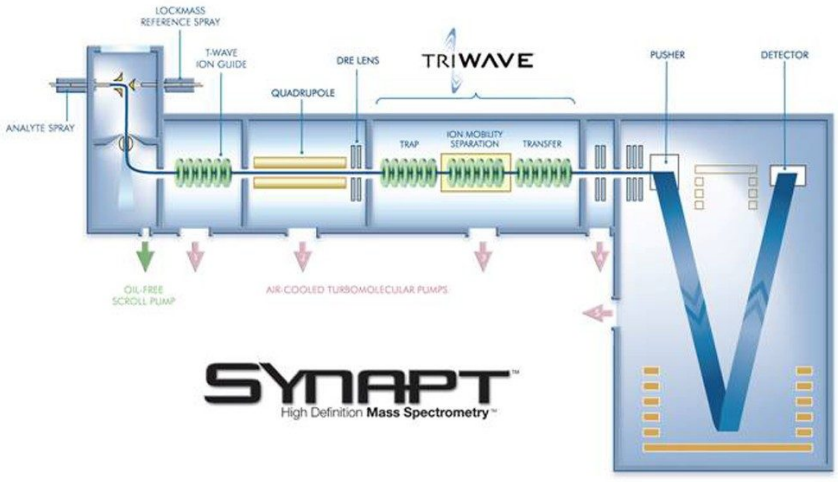

Figure 1. Schematic of the SYNAPT HDMS System.

The protein standards Myoglobin, Cytochrome-C, and Lysozyme were dissolved in acetonitrile 50% (v/v), formic acid 0.1% (v/v), and 1,8-diazabicycloundec-7ene (DBU)5 0.1% (v/v). For example, the presence of DBU 0.1% (v/v) reduced the average charge state of myoglobin from +18 to +10.

T-Wave ion mobility calibration was carried out using a modification of an existing protocol6, which utilizes charge-reduced protein standards. The protein multiply charged ions, of known collisional cross section[7], used for IMS calibration were: Myoglobin +20 to +4 and Cytochrome-C +16 to +3. The IMS calibration was validated using the multiply-charged ions of the protein lysozyme, whose CCSs have previously been determined on a standard IMS drift tube[7].

Yeast alcohol dehydrogenase and bovine carbonic anhydrase were infused into the mass spectrometer at a concentration of 1 µM in an aqueous 50 mM ammonium acetate solution.

MS and IMS Conditions

MS system:	SYNAPT HDMS System
Ionization mode:	nanoESI+

Capillary voltage:	1 kV
Cone voltage:	30 V
Trap CE:	8 V
Trans CE:	4 V
IMS bias:	20 V
Acquisition range:	*m/z* 1000 to 32,000 (256 µsec)
Trap/trans gas:	SF6
IMS gas:	N_2
MS T-Wave speed:	250 m/sec
IMS T-Wave height:	7.5 to 8.5 V

Results and Discussion

Upon infusion of an aqueous 1.0 µM solution of ADH into the mass spectrometer, a narrow, well-defined multiply-charged envelope was observed at *m/z* 6,000, corresponding to intact ADH tetramer of mass 147.7 kDa, as shown in Figure 2. A narrow multiply-charged envelope was also observed at *m/z* 3,000, corresponding to the ADH monomer (37 kDa). For carbonic anhydrase, only a monomer (29 kDa) was observed.

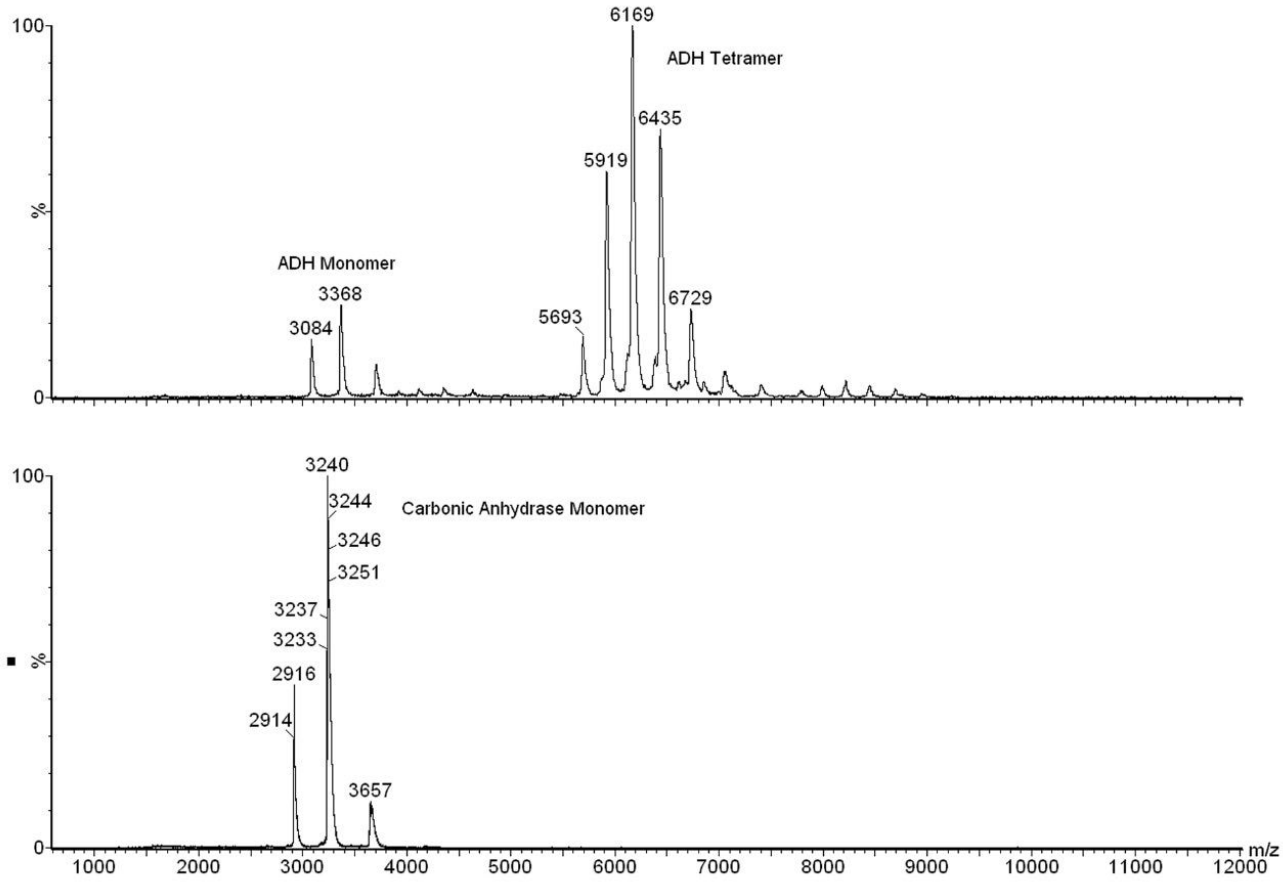

Figure 2. Nanoelectrospray spectrum of yeast alcohol dehydrogenase (upper spectra) and carbonic anhydrase (lower spectra) acquired over the m/z range 600 to 12,000.

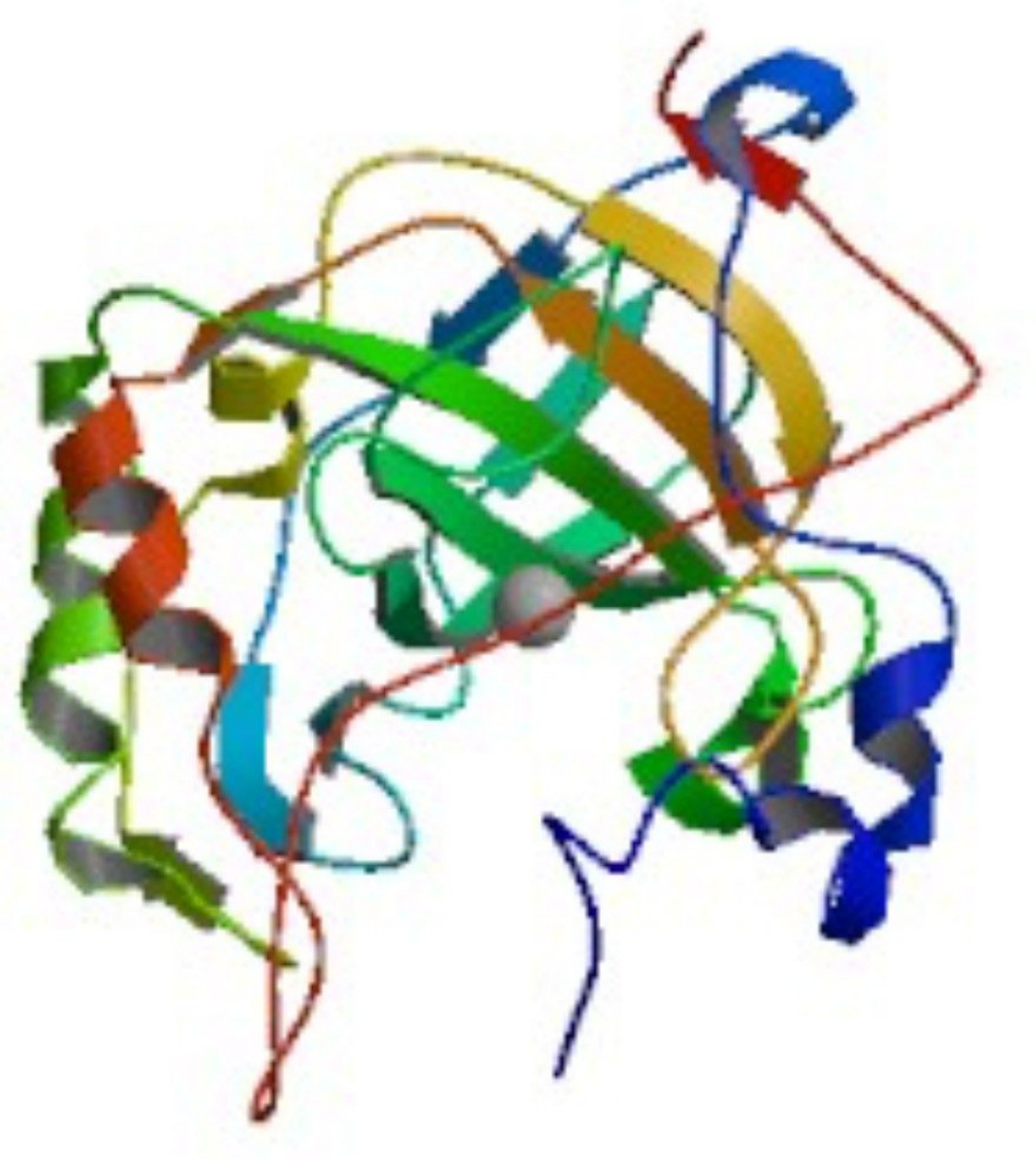

Figure 3. Structure of carbonic anhydrase (1V9E) acquired from the RSCB Protein Data Bank.

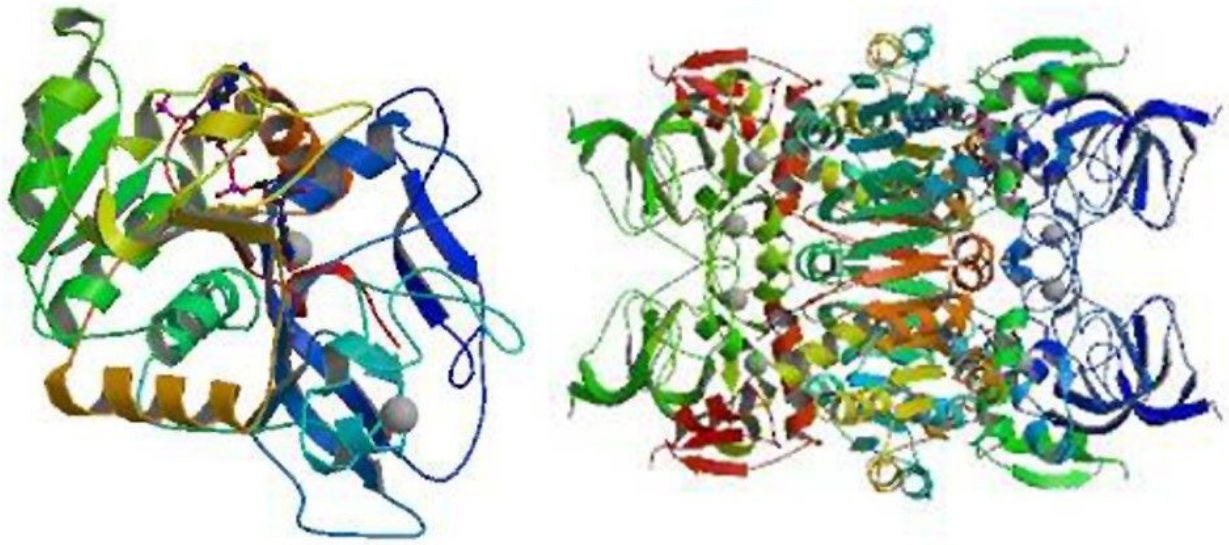

Figure 4. Structures of the ADH monomer (1PSO) and tetramer (2HCY) acquired from the RSCB Protein Data Bank.

The native mass spectral stoichiometric information obtained for carbonic anhydrase and alcohol dehydrogenase are consistent with the stoichiometry reported by the available X-ray crystallographic data of the proteins of interest. This demonstrates that within a mass spectrometer you can retain native non-covalent interaction, which is observed in other biophysical characterization techniques, such as X-ray crystallogrpahy or NMR. Electrospray ionization and mass spectrometry, therefore, do not disrupt any important non-covalent subunit interactions that may be present within the protein complex

The MOBCAL program can be used to calculate the collisional cross-section of any biological or inorganic molecule that has a published 3-dimensional structure. The T-Wave derived CCSs for the ADH monomer, ADH tetramer, and carbonic anhydrase all agree with the MOBCAL projection approximation derived CCSs, as shown in Tables 1, 2a, and 2b.

Z	*m/z*	T-Wave CSS Å^2
10	2910	2230 +/- 16
9	3233	2218 +/- 13
8	3637	2215 +/- 16

Table 1. T-Wave IMS measured CCSs for the individual carbonic anhydrase charge states. The MOBCAL Projection Approximation CCS values for PDB file 1V9E are 1877 Å2.

Z	*m/z*	T-Wave CSS Å^2
12	3085	2531 +/- 13
11	3367	2515 +/- 19
10	3708	2530 +/- 28

Table 2a. T-Wave IMS measured CCSs for the individual ADH monomer charge states. The MOBCAL Projection Approximation CCS values for PDB file 1PSO is 2371 Å^2.

Z	*m/z*	T-Wave CSS Å^2
26	5689	7388 +/- 35
25	5917	7428 +/- 22
24	6164	7457 +/- 41
23	6432	7545 +/- 60
22	6724	7696 +/- 44

Table 2b. T-Wave IMS measured CCSs for the individual ADH tetramer charge states. The MOBCAL Projection Approximation CCS values for PDB file 2HCY is 7269 Å^2.

This provides more supporting evidence that the gas phase stoichiometry of protein complexes and their shape are consistent between mass spectrometry and X-ray crystallography, two very different biophysical characterization techniques. The advantage of mass spectrometry coupled to ion mobility is that they provide mass, stoichiometric, and size information on the protein complex of interest very rapidly in the order of a few minutes.

Conclusion

- Native gas phase stoichiometry for protein structures are consistent with structures determined by X-ray crystallography
- The T-Wave measured CCSs for the ADH monomer, ADH tetramer, and carbonic anhydrase consistently match the theoretical calculated CCSs generated by the MOBCAL program
- Solution-phase charge reduction is an efficient way of accessing the lower charge state of proteins, which are not accessible under normal electrospray conditions

- Ions with a lower overall charge display extended ion mobility drift-times, which can be used to extend the existing T-Wave ion mobility calibration routine
- The extended IMS calibration was successfully validated with known published CCSs of the protein lysozyme

References

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