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Magnifying ion mobility spectrometry–mass spectrometry measurements for biomolecular structure studies

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Ion mobility spectrometry–mass spectrometry (IMS–MS) provides information about the structures of gas-phase ions in the form of a collision cross section (CCS) with a neutral buffer gas. Indicating relative ion size, a CCS value alone is of limited utility. Although such information can be used to propose different conformer types, finer details of structure are not captured. The increased accessibility of IMS–MS measurements with commercial instrumentation in recent years has ballooned its usage in combination with separate measurements to provide enhanced data from which greater structural inferences can be drawn. This short review presents recent outstanding developments in scientific research that employs complementary measurements that when combined with IMS–MS data are used to characterize the structures of a wide range of compounds.

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Introduction

Ion mobility spectrometry (IMS) experiments have traditionally employed a drift tube with stacked electrostatic rings used to establish a well-defined, constant electric field (E) [1,2]. The drift region is filled with an inert buffer gas where analyte ions are separated due to differences in their overall size and charge. The transit time (or drift time) of an ion in the drift region (t_D) can be related to its collision cross section (Ω) using [3]:

$$\Omega = \frac{(18\pi)^{1/2}}{16}\frac{ze}{(k_b T)^{1/2}}\left[\frac{1}{m_I}+\frac{1}{m_B}\right]^{1/2}\frac{t_D E}{L}\frac{760}{P}\frac{T}{273.2}\frac{1}{N} \quad (1)$$

The variables in Eq. (1) include: ion charge (ze), the Boltzmann constant (k_b), the reduced masses of the ion and the buffer gas (m_I and m_B), the length of the drift tube (L), the pressure and temperature of the buffer gas (T and P), and the neutral number density at STP (N). Essentially, from Eq. (1), biomolecular ion size differentiation becomes apparent because ions of a larger physical size experience a greater number of collisions with the buffer gas in the drift tube thus increasing t_D and ultimately the determined collision cross section (CCS).

Shortly after the introduction of soft ionization techniques for the production of biomolecular ions [4–6], IMS was combined with mass spectrometry (MS) measurements for the study of gas-phase biomolecular ions [7,8]. Such work led to considerations of the type and degree of structural information that could be garnered from IMS–MS measurements. Early studies focused on comparisons of an ion's drift time to that obtained for different ion conformer types often obtained by *in-silico* manipulation of established solution-phase structures [7,9]. This provided a similarity comparison relating the structures of ions to conformer types having different degrees of elongation and compactness. Although this seminal work laid the foundation for the use of IMS–MS in many ion structure studies, the limited structural details afforded by IMS–MS became readily apparent.

Early experiments were followed by notable advances in scientific instrumentation development that significantly expanded the reach of IMS–MS for biomolecular structure analysis. These efforts were primarily centered on improving the sensitivity and resolution of the measurement as well as the ability to manipulate ion structure. One of the most important developments with regard to sensitivity was the coupling of the dispersive IMS separation step with a dispersive MS analysis to improve ion utilization in IMS-MS measurements [10,11]. Development with regard to sensitivity also focused on the use of ion trapping devices that allowed the storage of continuously produced ions for the pulsed mobility measurement [12–14]. Instrumentation development centered on ion focusing and ion extraction was also pursued to improve measurement sensitivity [14–16]. With regard to resolution improvement, early efforts focused on devices that could sustain higher voltages for improved separations [17–19]. Finally, ion structure manipulation was demonstrated early

in the form of inducing precursor ion fragmentation or conformer transformation via collisional activation [20–22]. Although many other outstanding instrument advances were accomplished during the early development time period, arguably those highlighted above played a significant role in the commercialization of IMS–MS instruments. Commercialization of such instrumentation resulted in accelerated adoption of IMS techniques for all levels of biomolecular structure analysis.

Recent years have witnessed a significant expansion in the usage of IMS–MS in structure studies where collision cross section information is combined with results either from separate structural measurements or the observation of changes in IMS measurements resulting from instrument parameter or sample perturbation. The additional data afforded by the new approaches helps to refine details of ion structure.

Such experimental accoutrements can be broadly categorized into on-line and off-line approaches. The former category would represent any supplemental approach occuring as part of (or during) the IMS–MS measurement. An example would be the use of ion reactions that occur before, during, or after an ion's mobility measurement. Off-line approaches would include the use of separate measurements performed on or for the same analyte. An example would be the use of spectroscopic techniques to provide additional information about biomolecular structure. Below, a number of recent advances in these different areas is presented. Notably, an exhaustive review is not provided; rather, high impact studies for online and offline approaches are presented in that order.

On-line approaches (OnA): solution and ion source manipulations

The first area of discussion considers manipulations that are applied to the sample immediately prior to the production of ions or to the ion source that ultimately affect the information obtained from IMS–MS measurements. A recent study describing sample manipulation was performed by El-Baba et al. which showed the monitoring of distinct solution conformer populations of ubiquitin during protein unfolding induced by heating the electrospray solution at the needle [23••]. Remarkably, for the two-state cooperative process, multiple conformers for a number of ion charge states are observed along the transition path as shown in Figure 1a. The work challenges the long-held notion that along this pathway, only unstructured species should be encountered. Another study in this category coupled on-line solution-phase hydrogen/deuterium exchange (HDX) with mobility separation and MS analysis [24]. In separate work using temperature control immediately prior to and during the mobility separation, Servage et al. discovered that $[2M+14H]^{14+}$ ions could be stabilized by a very limited number of water adducts prior to dissociation into $[M+7H]^{7+}$ ions [25]. Finally, one study showed significant ability to alter gas-phase conformers and distinguish carbohydrate isomers with the addition of different metal cations [26].

OnA: gas-phase reactions

One area of research that has expanded in recent years uses higher-pressure regions of IMS–MS instrumentation to perform gas-phase hydrogen/deuterium exchange (HDX) reactions. Khakinejad et al. recently used IMS-HDX and tandem MS (employing electron transfer dissociation – ETD [27]) to more accurately estimate conformer populations comprising mobility features [28]. Here, the modeled reactivities of candidate ion conformers (see Figure 1b) from molecular dynamics (MD) trajectories were used to assign ion conformer contributions to overall conformer type abundance. In separate studies, Rashid et al. utilized a dual spray ion source to perform gas-phase HDX in the source region of a traveling wave ion mobility (TWIM) instrument [29]. The approach was used to study the conformations of peptide and protein ions. Others have used gas-phase HDX to distinguish isomeric carbohydrates [30] and other isobaric small molecules including peptides, metabolites, and lipids [31,32] according to differences in ion structure.

Other experimental work has utilized ion-ion reactions in downstream regions of IMS–MS instrumentation. Recently, Laszlo and Bush utilized cation-to-anion-proton-transfer reactions (CAPTR) [33] in the ion transfer region of a TWIM instrument to monitor the products resulting from the reaction of compact and elongated cytochrome *c* ions [34]. Product ions were observed to have very similar collision cross sections. In separate studies the approach was used to suggest that the ion charge should be considered carefully when attempting to relate collision cross sections to solution conformers for a number of different proteins (Figure 1c) [35•].

OnA: ion activation and dissociation

The use of collisional activation of biomolecular ions to induce structural transformations or ion fragmentation in a drift tube was first demonstrated more than a decade ago [36,37]. In recent years remarkable work has been conducted to develop the robust tool of collision-induced unfolding (CIU) for structural characterization [38]. Recently Eschweiler et al. have demonstrated the usage of CIU in which unfolding pathways of a homologous group of serum albumins were elucidated [39]. These experiments utilized the CIU patterns of protein–ligand species as well as non-covalent protein complexes to aid in the determination of unfolding pathways. Additionally, the high-reproducibility of CIU patterns was used recently to distinguish structural differences in a biosimilar as shown in Figure 2a [40]. In another seminal report, Allison et al. presented an experimental scheme involving the use of CIU for proteins and protein–ligand