Tailoring Peptide Conformational Space with organic gas modifiers in TIMS-MS

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Abstract

Recently, we showed the advantages of Trapped Ion Mobility Spectrometry for the study of kinetic intermediates of biomolecules as a function of the starting solvent composition (e.g., organic content and pH) and collisional induced activation. In the present work, we further characterize the influence of the bath composition (e.g., organic content) on the conformational space of an intrinsically disordered, DNA binding peptide: AT-hook 3 (Lys-Arg-Pro-Arg-Gly-Arg-Pro-Arg-Lys-Trp). Results show the dependence of the charge state distribution and mobility profiles by doping the solution and the bath gas with organic modifiers (e.g., methanol and acetone). The high resolving power of the TIMS analyzer allowed the separation of multiple IMS band per charge state, and their relative abundances are described as a function of the experimental conditions. The use of gas modifiers resulted in larger ion-neutral collision cross sections, with a direct correlation between the size of the modifier and the CCS differences. Conformational isomer inter-conversion rates were observed as a function of the trapping time. Different from solution experiments, a larger variety of organic gas modifiers can be used to tailor the peptide conformational space, since peptide precipitation is not a problem.

Keywords. Trapped ion mobility mass spectrometry, intrinsically disordered protein, HMGA2, ATHP

Introduction

Mass spectrometry-based methods have increasingly become a complementary or alternative research tool for investigating the conformational space of biomolecules under a variety of conditions, including biologically relevant conditions.(Feng et al. 2008; Loo 1997; Miranker et al. 1993; Simoneit 2005; Winston and Fitzgerald 1997) Specifically, ion mobility spectrometry combined with mass spectrometry (IMS-MS) has the capability to perform separation and selection of gas-phase ions, from heterogeneous solutions. It provides insight into both stable and intermediate structures, allowing for a more dynamic view and native-like folding information,(Pi and Sael 2013) while resembling solution structures (memory effect).(Chen and Russell 2015; Liu et al. 2016; Meyer et al. 2013; Pierson et al. 2011; Rosu et al. 2010; Seo et al. 2016; Shi et al. 2016; Silveira et al. 2013; Voronina et al. 2016) Previous studies from our group showed the advantages of ESI-TIMS-MS for the study of kinetically trapped intermediates of biomolecules.(Adams et al. 2016; Benigni and Fernandez-Lima 2016; Benigni et al. 2015; Benigni et al. 2016; Frost et al. 2015; Gonzalez et al. 2016; Liu et al. 2016; Molano-Arevalo et al. 2014; Ridgeway et al. 2015; Schenk et al. 2015; Schenk et al. 2014a; Schenk et al. 2014c) Relevant to this study, we presented the folding pathways between local, free energy minima of AT-hook peptide 3 (ATHP3) leading to multiple, stabilized conformations.(Schenk et al. 2013) Protonation site, backbone relaxation and side-chain orientations were implicated in defining each structure. We have shown that the conformational space can be altered by introducing dopants into the TIMS cell for the case of flavin adenine dinucleotide.(Molano-Arevalo et al. 2014) Different from other experiments where gas modifiers are used to increase the analytical power of IMS by increasing the size of the collision partner or inducing higher order multi-pole interactions,(Fernández-Maestre et al. 2012; Kafle et al. 2014; Levin et al. 2006; Porta et al. 2013; Schneider et al. 2013; Waraksa et al. 2016) in this project we focused on the influence of the microenvironment on the stabilization of the conformational space of biomolecules.

In the present work, a ten amino acid intrinsically disordered peptide, Lys-Arg-Pro-Arg-Gly-Arg-Pro-Arg-Lys-Trp, was studied using nanoESI-TIMS-MS as a function of starting solvent (e.g. organic content and pH), bath gas collision partner and time after desolvation. This study is the first to report on the use of TIMS gas modifiers to tailor the peptide conformational space.

Experimental Methods

Materials and reagents. AT-hook peptides 3 (Lys-Arg-Pro-Arg-Gly-Arg-Pro-Arg-Lys-Trp) were purchased from Advanced ChemTech Inc. (Louisville, KY) and used as received. Methanol and acetone solvents, and ammonium

acetate salts utilized in this study were analytical grade or better and purchased from Fisher Scientific (Pittsburgh, PA). A Tuning Mix calibration standard (G24221A) was obtained from Agilent Technologies (Santa Clara, CA) and used as received.

Trapped Ion Mobility Spectrometry – Mass Spectrometry Analysis (TIMS-MS). Details regarding the TIMS operation and specifics compared to traditional IMS can be found elsewhere. (Fernandez-Lima et al. 2011a; Fernandez-Lima et al. 2011b; Hernandez et al. 2014; Schenk et al. 2014b; Schenk et al. 2014d) Briefly, mobility separation in TIMS is based on holding the ions stationary against a moving gas using an electric field. The separation in a TIMS device can be described in the center of the mass reference frame using the same principles as in a conventional IMS drift tube.(McDaniel and Mason 1973) Since mobility separation is related to the number of ion-neutral collisions (or drift time in traditional drift tube cells), the mobility separation in a TIMS device depends on the bath gas drift velocity, ion confinement and ion elution parameters. The reduced mobility, *K*, of an ion in a TIMS cell is described by:

$$K = \frac{V_g}{E} \approx \frac{A}{(V_{elution} - V_{out})}$$

where v_g and E are the velocity of the gas and the applied electric field across the TIMS analyzer region. $V_{elution}$ is the voltage when the ions elute in the V_{ramp} sweep and V_{out} is the voltage applied at the end of the TIMS analyzer region. A is a constant that relates to the velocity of the bath gas and electric field axial distribution and can be calculated using mobility standards. Notice that, once A is calculated for a given bath gas (e.g., Tuning Mix as calibrants for N_2 bath gas), it will not change when using gas modifiers since the pressure difference between P1 and P2 are kept the same (see details in Figure 1).

A custom-built, pulled capillary nanoESI source was utilized for all the experiments. Quartz glass capillaries (O.D.: 1.0 mm and I.D.: 0.70 mm) were pulled utilizing a P-2000 micropipette laser puller (Sutter Instruments, Novato, CA) and loaded with 10 μL aliquot of the sample solution. A typical nanoESI source voltage of +/- 600-1200 V was applied between the pulled capillary tips and the TIMS-MS instrument inlet. Ions were introduced via a stainless steel tube (1/16 x 0.020'', IDEX Health Science, Oak Harbor, WA) held at room temperature into the TIMS cell. It should be noted that all solvent studies were performed with nitrogen as the bath gas, and that all dopant experiments were conducted with peptides sprayed from 10 mM NH_4AC.

Mobility calibration was performed using the Tuning Mix calibration standard (G24221A, Agilent Technologies, Santa Clara, CA) in positive ion mode (e.g., *m/z* = 322, K_0 = 1.376 cm^2 V^{-1} s^{-1} and *m/z* = 622, K_0 = 1.013 cm^2 V^{-1} s^{-1}).(Hernandez et al. 2014) The TIMS operation was controlled using in-house software, written in National Instruments Lab VIEW, and synchronized with the maXis Impact Q-ToF acquisition program.(Fernandez-Lima et al. 2011a; Fernandez-Lima et al. 2011b) Gas modifiers were introduced at the entrance of the TIMS cell via vaporization of the respective solvents (e.g., methanol or acetone) at a ratio of 2:1 air:air modified mix (scheme shown in Figure 1). For simplified mobility calibration, the gas velocity was kept constant in all experiments (P1 and P2 values).

Results and Discussion

The analysis of ATHP3 peptide using nES-TIMS-MS resulted in a charge state distribution of $[M+H]^+$ to $[M+3H]^{+3}$. The ATHP3 motif is mainly comprised of basic residues with seven potential locations for protonation (e.g., N-terminus, four arginines and two lysines), however, the most abundant ion under all experimental conditions was the $[M+2H]^{2+}$ charge state (Figure 2a). The mobility distributions observed from our previous study of ATHP 3 $[M+2H]^{+2}$ using ESI-TIMS-MS are consistent with the current analysis by nanoESI-TIMS-MS.(Schenk et al. 2013)

At native conditions (Figure 2b, pink mobility bands) ATHP3 $[M+2H]^{2+}$ populates four conformers (A-D). The mobility bands are also conserved across the different organic solvent conditions (Figure 2c, pink mobility bands in blue panel). At higher trapping times (e.g., 500 ms), a kinetically trapped structure, and what we consider a more stable "desolvated" conformer, appears between bands B and C of the native. The presence of methanol in the TIMS cell did not alter the $[M+2H]^{2+}$ conformers, while acetone significantly changed the relative abundance and distribution of structures (Figure 2d, pink mobility band in pink panel). Three structures (A, B and C) were observed for ATHP3 $[M+3H]^{3+}$ over the range of starting solvent solutions (Figure 2b and 2c, green mobility bands). Conformer A was the major structure present in all experiments, followed by conformer B and C. The abundance of conformer B, however, increased in the presence of acetone in the TIMS cell. The mobility profiles of ATHP3 $[M+H]^+$ showed the presence of two structures (A and B) which were observed over the range of experimental conditions (Figure 2b, 2c and 2d, blue mobility bands). The distribution of ATHP 3 structures using acetone solvent cannot be recreated using acetone in the TIMS cell. One possible explanation is that the differences in conformational space is due to interaction with the ketone functional group of acetone. Unlike methanol's alcohol group, acetone's ketone group can form various interactions with the peptide, including 1) hydrogen bonding with the amide of the peptide backbone, 2)

disruption of hydrogen bonding networks or, more likely, 3) dipole-dipole interactions with the charged residues of the peptide. Confirming this explanation will be the subject of future studies.

Changes in the conformational space as a function of the trapping showed stabilization towards more energetically favored structures as a function of the trapping time for $[M+H]^{+2}$ charge state (Figure 3). While our measurements are only sensitive to the 50-500 ms time scale, potential rearrangements are possible in the first 50 ms after desolvation(Shelimov and Jarrold 1997). In the case of varying the starting solution (10 mM ammonium acetate, and with methanol and acetone), a common trend in the gas phase kinetics in nitrogen is the increase of the band E, which corresponds to the largest CCS (and largest $1/K_0$) for this charge state. However, in the case of gas modifiers, band E is not observed, and the trends are best characterized by a decrease of band B and band A for methanol and acetone, respectively, which correspond to the smallest CCSs. We interpret these results as the most stable gas-phase structures tending to have larger CCSs that those initially observed in solution. These effects may be a consequence of the absence of the solvent, since in the gas-phase the lack of water molecules promotes long range interactions. In the case of the $[M+H]^{+}$ and $[M+H]^{+3}$ charge states, similar trends were observed regarding the increase of larger CCS bands as a function of the trapping time (see Figure 2).

Conclusions

The results presented here displayed the utility of gas modifiers in TIMS-MS for investigating and monitoring solution versus gas-phase microenvironment contribution to the peptide conformational space. When ionized from native conditions (10 mM NH_4Ac and nitrogen bath gas), the mobility profiles of ATHP 3 show an ensemble of conformers, which were preserved as a function of increasing organic content (methanol and acetone). Although the overall IMS profiles were maintained, changes in the relative abundance of conformers (e.g., conformational isomerization to the more stable gas-phase structure) were observed and recorded. The interconversion of structures, however, was small and often did not exceed growth or decay abundances of ~10%. Comparison between starting solvent and bath gas with the same organic modifier showed that acetone as a dopant consistently changed the original IMS profiles. Overall, we find evidence for multiple stable conformers of these “disordered” motifs as a function of starting solvent (e.g. organic content), bath gas collision partner and time after desolvation. The sensitivity of TIMS-MS allows for the observation of many low abundant conformers, separation of closely related structures and tracking of gas-phase stable structures via isomerization kinetics. This methodology opens new avenues for the study of

biomolecules in the presence of gas modifiers that are not accessible during solution experiments, due to the typical precipitation of biomolecules during non-native conditions.

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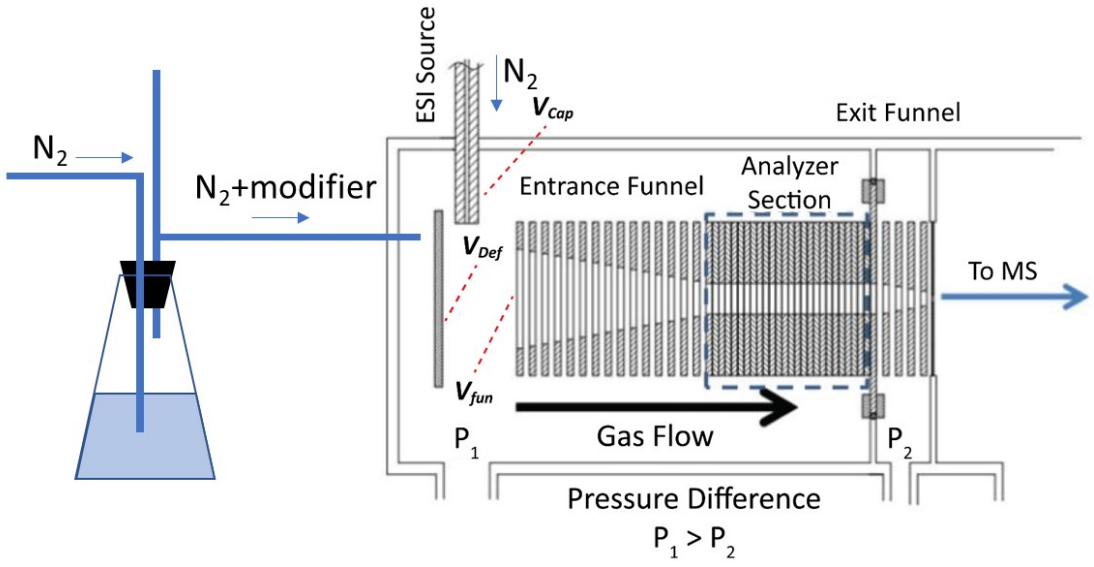

Figure 1. Scheme utilized for the nESI-TIMS-MS experiments with organic gas modifiers. Notice that the gas velocity in the TIMS analyzer is kept constant.

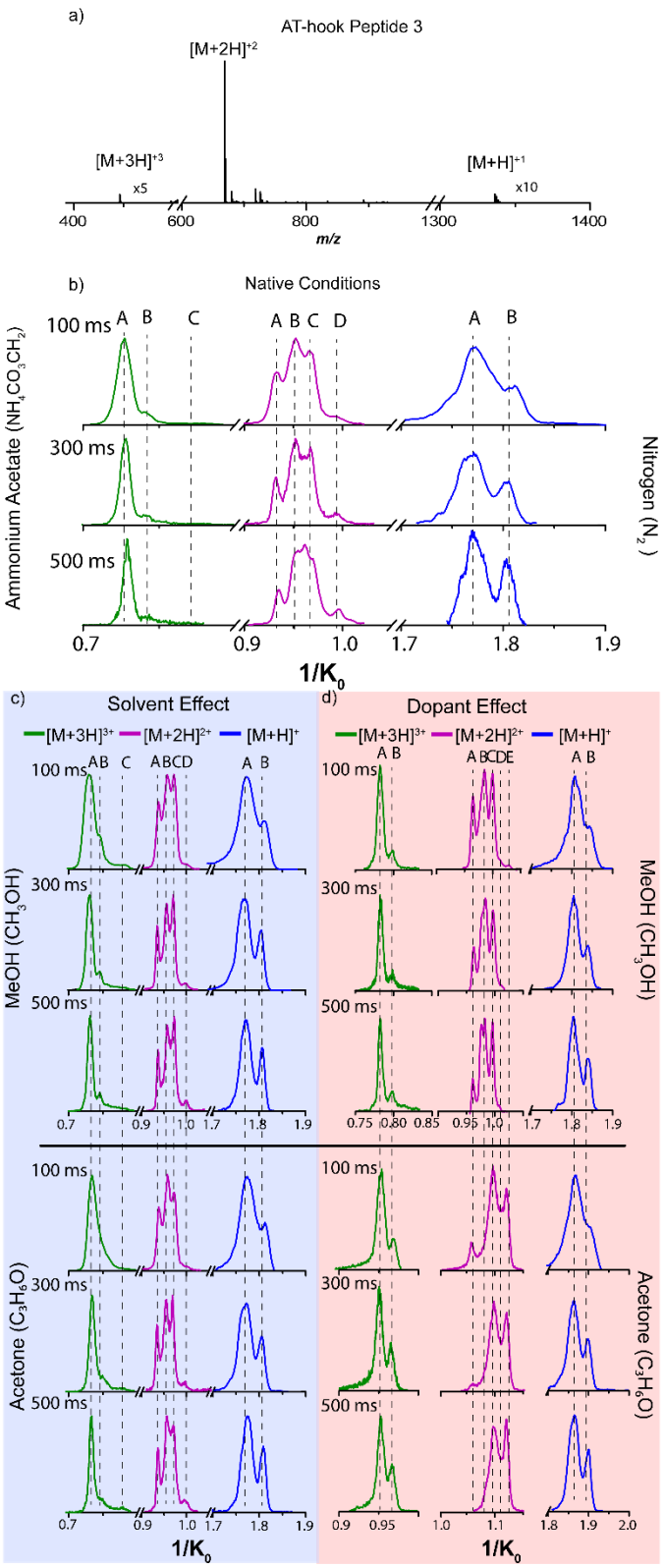

Figure 2. a) Typical mass spectra and b) native IMS spectra of ATHP3 as a function of c) starting solvent (methanol:H_2O or acetone:H_2O) or d) dopant bath gas (methanol or acetone).

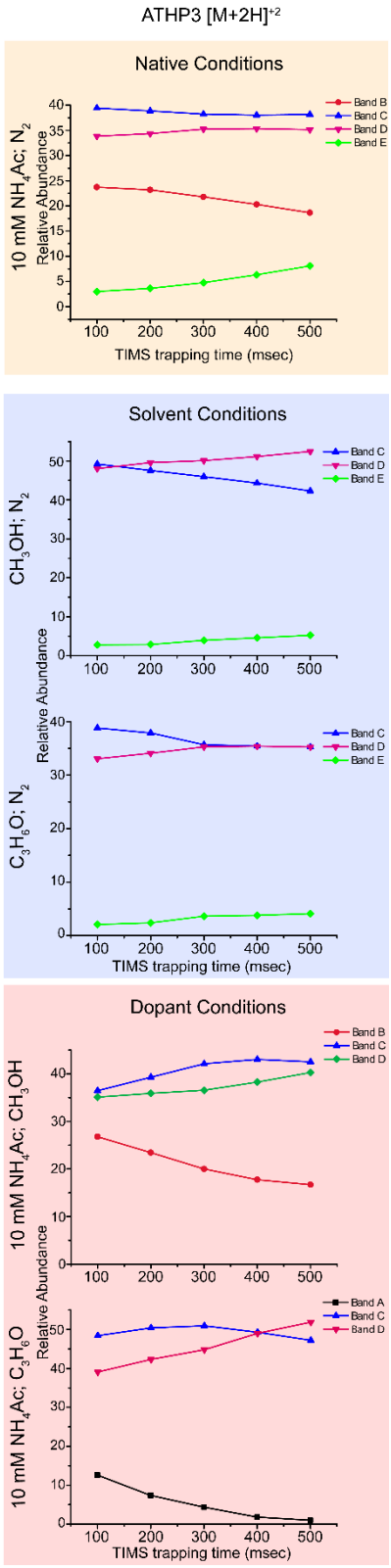

Figure 3. The relative abundances of ATHP 3 $[M+2H]^{+2}$ conformers as a function of the trapping time, stating solvent conditions and bath gas composition. Starting solvent and bath gas are listed to the left of the graphs

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