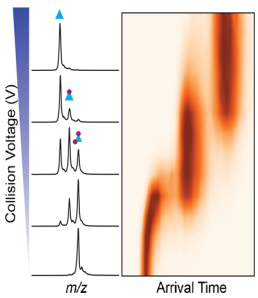
J. Am. Soc. Mass Spectrom. (2017) 28:579–586
DOI: 10.1007/s13361-016-1555-1

FOCUS: EMERGING INVESTIGATORS: RESEARCH ARTICLE

Characterization of Membrane Protein–Lipid Interactions by Mass Spectrometry Ion Mobility Mass Spectrometry

Yang Liu,[1] Xiao Cong,[1] Wen Liu,[1] Arthur Laganowsky[1,2,3]

[1]Center for Infectious and Inflammatory Diseases, Institute of Biosciences and Technology, Texas A&M University, Houston, TX 77030, USA
[2]Department of Chemistry, Texas A&M University, College Station, TX 77842, USA
[3]Department of Microbial Pathogenesis and Immunology, College of Medicine, Texas A&M University, Bryan, TX 77807, USA



Abstract. Lipids in the biological membrane can modulate the structure and function of integral and peripheral membrane proteins. Distinguishing individual lipids that bind selectively to membrane protein complexes from an ensemble of lipid-bound species remains a daunting task. Recently, ion mobility mass spectrometry (IM-MS) has proven to be invaluable for interrogating the interactions between protein and individual lipids, where the complex undergoes collision induced unfolding followed by quantification of the unfolding pathway to assess the effect of these interactions. However, gas-phase unfolding experiments for membrane proteins are typically performed on the entire ensemble (*apo* and lipid bound species), raising uncertainty to the contribution of individual lipids and the species that are ejected in the unfolding process. Here, we describe the application of mass spectrometry ion mobility mass spectrometry (MS-IM-MS) for isolating ions corresponding to lipid-bound states of a model integral membrane protein, ammonia channel (AmtB) from *Escherichia coli*. Free of ensemble effects, MS-IM-MS reveals that bound lipids are ejected as neutral species; however, no correlation was found between the lipid-induced stabilization of complex and their equilibrium binding constants. In comparison to data obtained by IM-MS, there are surprisingly limited differences in stability measurements from IM-MS and MS-IM-MS. The approach described here to isolate ions of membrane protein complexes will be useful for other MS methods, such as surface induced dissociation or collision induced dissociation to determine the stoichiometry of hetero-oligomeric membrane protein complexes.

Keywords: Native mass spectrometry, Mass spectrometry of intact membrane protein complexes, Membrane proteins, Lipids, Membrane protein lipid interactions, Collision induced unfolding, Ammonium channel

Received: 28 July 2016/Revised: 6 October 2016/Accepted: 3 November 2016/Published Online: 6 December 2016

Introduction

Membrane proteins interact intimately with the lipid bilayer in which they are embedded [1]. Many vital cellular processes rely on membrane proteins and their function, including trafficking and signal transduction [2–4]. Detailed understanding of how lipids affect the structure and function of proteins is therefore important for understanding fundamental biological processes. Recently, mass spectrometry (MS) approaches have emerged that can quantify individual binding events of protein–ligand complexes, and when performed in unison with an ion mobility device, ions are separated based on their shape and charge. This method, referred to as ion mobility mass spectrometry (IM-MS), provides insight into protein conformation by reporting on the rotationally averaged collision cross-section (CCS) [5, 6].

Nano electrospray ionization is routinely employed to introduce intact protein complexes into a mass spectrometer that is tuned to maximize desolvation and minimize activation of the ions [7, 8]. Depending on the instrument, desolvation, including activation, can be accomplished, for example, through adjusting source pressure, source temperature, and increasing the potential on specific elements, such as “cone” voltage (for review see [9–12]). More specifically, increasing source temperature, for example using a heated-capillary, can effectively desolvate ions through “in-source” collisional activation; however, elevated temperatures can lead to dissociation of noncovalent soluble protein complexes [13–18].

Mass spectra of membrane protein complexes recorded under instrument settings typically used for soluble proteins

Correspondence to: Arthur Laganowsky;
e-mail: ALaganowsky@ibt.tamhsc.edu

often result in a large, unresolved hump [7, 19, 20]. Thus, membrane proteins require a larger degree of activation, which is typically achieved in the trap or collision cell of the instrument, in order to desolvate, liberate them from the detergent micelles, and yield resolved mass spectra [7, 19, 21–23]. Although membrane proteins require more activation compared with their soluble counterparts, the excess detergent and lipid form a protective layer that protects them during activation such that native-like conformations can be observed by ion mobility post-detergent removal [19, 24–26]. Minimal increases in energy (above the threshold to strip detergent from the membrane protein complex) can perturb membrane protein structure, similarly to soluble proteins [25].

IM-MS is well suited for recording collision-induced unfolding (CIU) profiles of ions by measuring their mobility post collisions with neutral gas molecules in the trap, which can provide useful information for molecular analysis [27, 28] Such methods have been used to describe conformation and stability of large protein complexes and how events such as ligand binding can affect their structure [7, 28–31]. Unlike other biophysical approaches, where typically the observable is the ensemble of species (apo and ligand bound states) in solution, IM-MS is capable of resolving individual protein–ligand binding events and characterizing their structural and conformational effects. For example, Ruotolo and colleagues could distinguish the class of ligand bound to a protein kinase, a soluble protein, by their CIU profiles [32]. Of late, CIU-based quantitative IM-MS methods have been developed to determine how lipid-binding events stabilize membrane protein complexes [25, 33]. Using the software program Pulsar [33], one can easily generate CIU profiles for *apo* and lipid bound states and use algorithms to quantify the transitions in the unfolding pathway. The stability afforded by the bound lipid is calculated by the sum of the differences between transitions in the CIU profiles for *apo* and lipid bound states. Using this IM-MS method, we have previously demonstrated that different lipids can stabilize membrane proteins to varying degrees, with the most stabilizing lipids modulating the structure and function of membrane proteins [25]. As an example, we have shown that the activity of the bacterial water channel aquaporin Z can be modulated nearly 3-fold by cardiolipin, a lipid that significantly stabilized the channel in IM-MS studies. However, the molecular mechanism behind the increased activity remains unclear.

CIU profiles of membrane proteins are typically performed on the entire ensemble (*apo* and lipid bound species) that raises uncertainty to the contribution of individual lipids and the species that are ejected in the unfolding process. During the CIU process, where collision voltage is increased in a stepwise fashion, the bound ligand(s) may eject from the complex as neutral or charged species. If the ligand ejects as a neutral, the signal from remaining charged species will contribute to the signal minus the mass of the ejected ligand, giving rise to heterogeneity of distinct *apo* and ligand bound states. In contrast, a ligand ejected as charged species could alter the initial charge state of the ion, such as for collisionally activated dissociation of ribonucleases in complex with nucleotides [34], and the signal for charge-stripped ion would contribute to neighboring ion(s). As current CIU methods for membrane proteins do not isolate ions prior to collisional activation, the CIU profile for a given *apo* or lipid bound state can be compromised by overlapping ions that are the product of the protein complex minus lipid(s) ejected as either neutral or charged species.

To further develop IM-MS methods to probe membrane protein lipid interactions, we employed mass spectrometry ion mobility mass spectrometry (MS-IM-MS), which has been shown to allow for greater depth of information [35, 36]. We selected for study a model integral membrane protein, the trimeric ammonia channel (AmtB) from *Escherichia coli* in complex with lipids that we used in our previous studies [7, 25, 37]. In the Synapt G1 instrument [38], the quadrupole is located before the collision cell, where collisional activation is typically applied to membrane proteins [25], making it a necessity to have a resolved mass spectrum prior to entering the quadrupole in order to isolate specific ions. In this study, we investigated higher source temperatures as a basis of "in-source" collisional activation to release membrane proteins–lipid complexes from the detergent micelle. By this method, we could record resolved mass spectra prior to entrance into the quadrupole and for the first time isolate ions of AmtB–lipid complexes in the quadrupole. After successfully isolating specific lipid-bound states of AmtB, we generated CIU profiles by collisional activation in the collision cell, which is located after the quadrupole. These CIU profiles are free of ensemble effects, such as contributions from product ions that have ejected lipid(s). We then compare results from IM-MS and MS-IM-MS approaches to provide insight into how CIU profiles can be affected by lipid ejection and the relationship to measured biophysical parameters.

Experimental

Sample Preparation

The ammonia channel (AmtB) was expressed and purified from *Escherichia coli*, and prepared for IM-MS as previously described [25]. In brief, the purified protein was buffer exchanged into AA buffer (200 mM ammonium acetate, pH 7.3) supplemented with 0.5% tetraethylene glycol monooctyl ether (C_8E_4). Stock solutions of synthetic phospholipids with 1-palmitoyl-2-oleoyl (PO, 16:0-18:1) acyl chains (Avanti Polar Lipids Inc., Alabaster, AL, USA) were prepared as previously described [7]. Briefly, desiccated lipid was dissolved in AA buffer supplemented with 0.5% C8E4 and 5 mM 2-mercaptoethanol (β-ME); 4 uL of protein solution (80 nM) was mixed with 4 uL of lipid solution (8 uM), and the mixture back-filled into a gold-coated glass capillary tip. Samples were allowed to equilibrate at 26 °C for 5 min prior to recording data [37].

Protein and Lipid Quantification

Protein concentration was determined with the DC Protein Assay kit (Bio-Rad, Hercules, CA, USA) using bovine serum albumin as the standard. Phospholipid concentration was determined by phosphorous analysis [39, 40].

Ion Mobility-Mass Spectrometry (IM-MS)

IM-MS and MS-IM-MS were performed on a Synapt G1 HDMS instrument (Waters Corp., Milford, MA, USA) equipped with a radio frequency generator to isolate higher *m/z* species (up to 32 k) in the quadrupole, and a temperature-controlled source chamber as previously described [37]. Instrument parameters were tuned to maximize signal intensity for IM-MS and MS-IM-MS while preserving the native-like state of AmtB. The source temperature was set to 23 (ambient), 40, 80, or 120 °C, capillary voltage of 1.7 kV, sampling cone voltage of 200 V, extractor cone voltage of 10 V, argon flow rate in the trap was set to 7 mL/min (5.2×10^{-2} mbar), and transfer collision energy at 15 V. The T-wave settings were for trap (300 ms^{-1}/1.0 V), IMS (300 ms^{-1}/20 V), transfer (100 ms^{-1}/10 V), and trap DC bias (35 V). CIU was performed from 10 V to 200 V on trap collision energy in 10 V steps. For MS-IM-MS, the quadrupole LM resolution was set to 6. To minimize differences caused by variations in gold-coated glass capillary tips, each replicate was collected from one tip using the same preparation of protein-lipid mixture.

Data Processing and Analysis

IM-MS and MS-IM-MS data were processed with the software program Pulsar [33]. The intensities of protein and protein–lipid species were deconvoluted and converted to mole fraction using UniDec [41].

Results and Discussion

Activation of Membrane Protein Complexes for MS-IM-MS

As a step towards MS-IM-MS of membrane protein complexes, we set out to develop methods to activate membrane proteins in the source region such that resolved ions can confidently be isolated in the quadrupole prior to entering the collision cell. Starting with a modest trap collision voltage setting of 20 V and a maximum setting for the cone voltage (200 V), the mass spectrum we obtained at ambient source temperature (23 °C) was poorly resolved and not ideal for isolating apo and lipid bound states of AmtB (Figure 1). To further activate ions upstream in the instrument, we then explored raising the source temperature from ambient to 40, 80, and 120 °C. With increasing source temperatures we observed an overall increase in signals for resolved AmtB species, especially for the lower charge states of the protein complex (Figure 1). It is unclear why there is an increase in abundance of lower charge states at elevated source temperatures, but plausible explanations could be charge stripping in the source region, or improved desolvation for lower charge state ions. Notably, with a mild source temperature setting of 40 °C, we could obtain resolved mass spectra suitable for isolating ions using the quadrupole in our instrument. Interestingly, increasing source temperature under modest trap settings resulted in resolved mass spectra comparable to that collected at

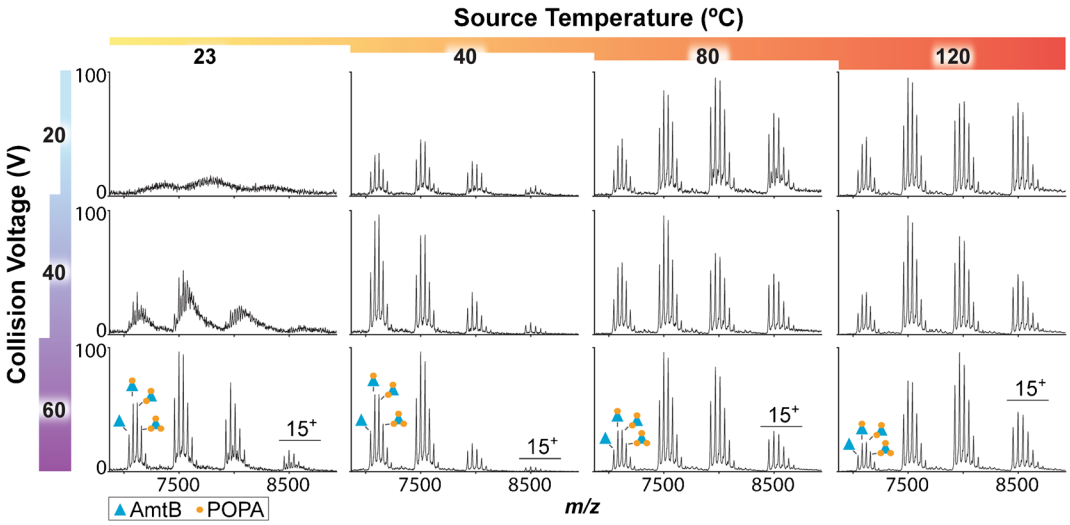


Figure 1. Representative mass spectra of AmtB bound to 1-palmitoyl-2-oleoyl phosphatidic acid (POPA) recorded at different source temperatures and collision voltages. The mass spectrum acquired at ambient source temperature (23 °C) produced an unresolved spectrum at the two lowest collision voltage settings. Given the configuration of the Synapt G1 instrument [38], these results demonstrate the necessity of "in-source" activation prior to entrance into the quadrupole such that resolved ions can be isolated. Increasing source temperature activates ions comparably to increased collision voltage settings at ambient source temperature

ambient source temperature with 60 V applied to the collision cell. Thus, elevated source temperature can provide sufficient "in-source" collisional activation to liberate AmtB from the detergent micelle.

CIU Profiles Acquired by IM-MS and MS-IM-MS

We then carried out a series of experiments at different source temperatures to understand the impact of elevated source temperature on CIU of AmtB bound to lipids (Figure 2). Herein we focus on the 15^+ charge state of AmtB bound to lipid(s) since we have previously characterized their CIU profiles [25]. Interestingly, the first transition from a native-like state, where the measured CCS agrees with the calculated CCS for AmtB [25], decreases roughly by 20 V with each 40 °C increase in source temperature (Figure 2a). A similar drop in collision voltage for the two subsequent transitions was observed as well. We speculate that the change in the collision voltage required to unfold the AmtB–lipid complexes to be the result of an overall increase in internal energy. This would be consistent with reports for soluble system, where increased "in-source" activation raises the internal energy of ions and lowers the collisional activation post-source to fragment or dissociate noncovalent complexes [15, 42]. Moreover, we also observed an increase in the width of the arrival time distribution (ATD) for all species with increasing source temperature and the native-like ATD nearly doubling in width. The cause behind this interesting observation is unclear, but one plausible explanation is that there are contributions from other ions that have either ejected bound lipids or charge-stripped in the collision cell.

Given our ability to isolate ions of AmtB–lipid complexes, we recorded CIU profiles of AmtB bound to two lipids by collisional activation in the collision cell, which is located after the quadrupole (Figure 2b). After isolating the ions corresponding to the 15^+

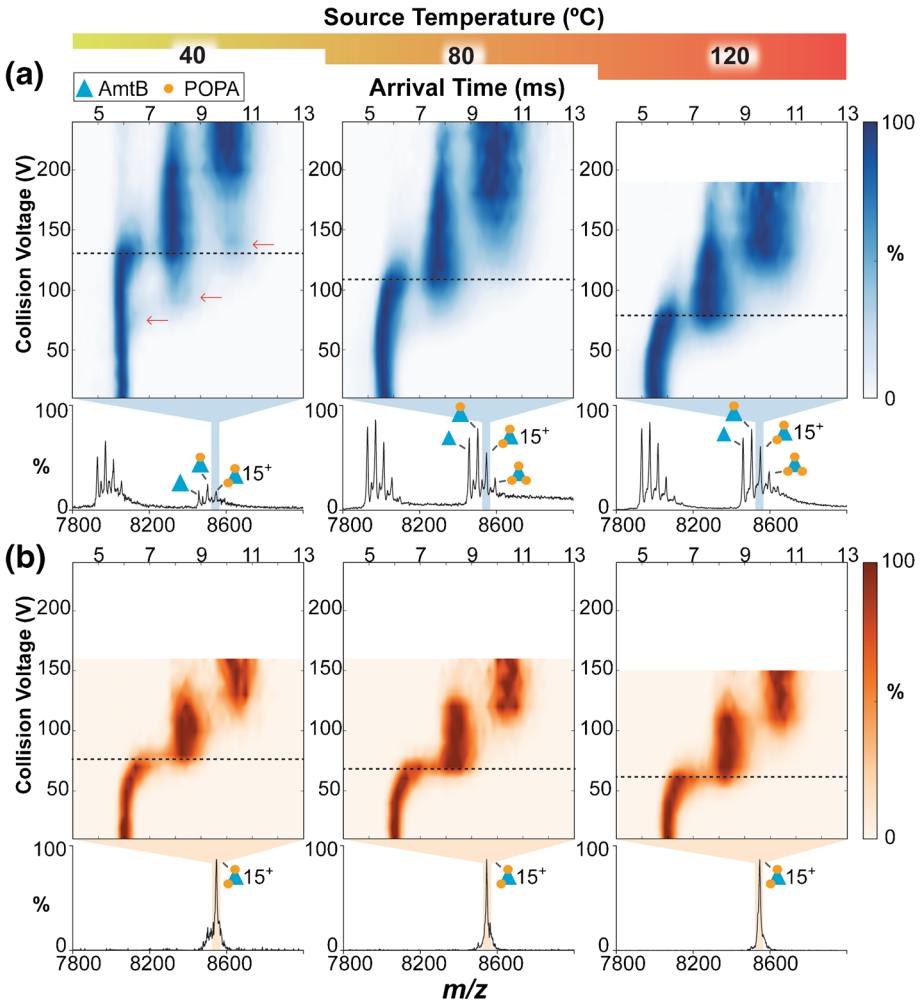


Figure 2. Collision induced unfolding (CIU) profile of the 15^+ charge state of AmtB bound to two 1-palmitoyl-2-oleoyl phosphatidic acid (POPA). Shown are CIU profiles acquired using either (**a**) IM-MS or (**b**) MS-IM-MS with source temperature set to 40, 80, or 120 °C. CIU profiles were generated using the software program, Pulsar [33]. Representative mass spectrum recorded at a collision voltage of 20 V is shown with respective CIU profiles recorded at different source temperatures. The first transition from a native-like to a partially unfolding intermediate, calculated by Pulsar, is shown as a dashed line. The faint overlay of MS-IM-MS CIU profile in the IM-MS CIU profile (**a**, **left panel**) is indicated by red arrows

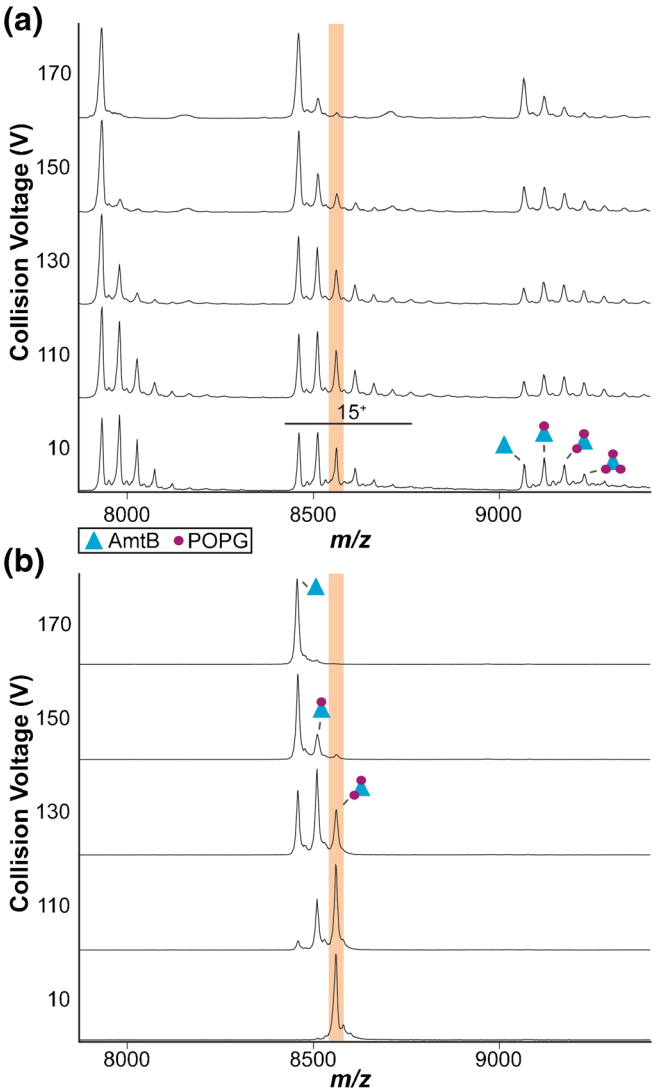


Figure 3. MS and MS-IM-MS of AmtB in complex with 1-palmitoyl-2-oleoyl phosphotidylglycerol (POPG) recorded at different collision voltages and source temperature of 120 °C. (**a**) Representative mass spectrum of AmtB bound to POPG without isolation in the quadrupole. (**b**) Isolation of the 15^+ charge state of AmtB$(POPG)_2$ prior to activation in the trap (bottom panel). Upon activation in the trap, bound POPG molecules are ejected as a neutral species with increasing trap collision voltage

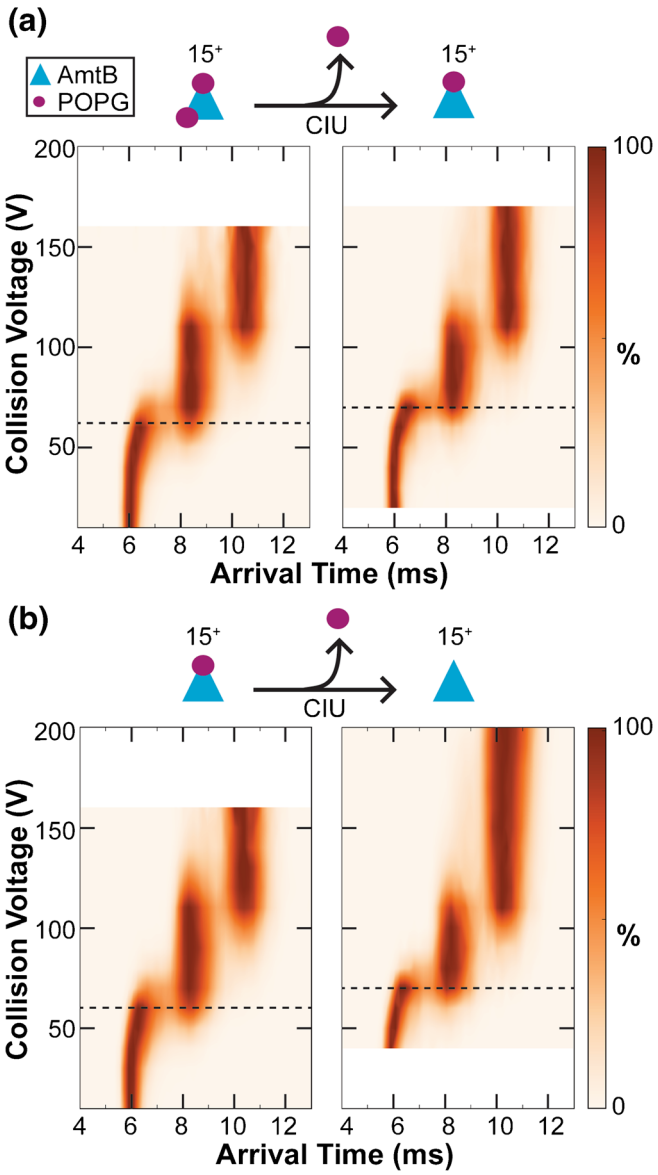


Figure 4. MS-IM-MS CIU profiles of AmtB bound to (**a**) two, or (**b**) one POPG molecule(s). MS-IM-MS data recorded at a source temperature of 120 °C. The CIU profile for the product AmtB species minus an ejected lipid is shown on the right. The first transition lines are shown as described in Figure 2

charge state of AmtB bound to two 1-palmitoyl-2-oleoyl (PO) phosphatidic acid (POPA) lipids, we subjected these ions to increasing trap collision voltage to record MS-IM-MS CIU profiles. In contrast to IM-MS CIU profiles, the unfolding transitions occurred at significanlty lower trap collision voltage. For example, at a source temperature of 40 °C the first transition was at 75 V versus 130 V for IM-MS and MS-IM-MS, respectively. In addition, the drop in transition collision voltage with increasing source temperature was roughly 7 V, approximately one-third of the value observed in IM-MS. Surprisingly, the width of the ATD was consistent among the source temperatures tested, implying source temperature is not responsible for ATD widening observed in IM-MS. Most interestingly, we noted a faint transition occurring in the IM-MS CIU profile around ~75 V that coincidentally matched the first transition observed in the MS-IM-MS CIU profile (Figure 2a, b, left panels). Upon closer examination, it appears there is an *apo* MS-IM-MS CIU profile that faintly underlies the profile acquired by IM-MS, specifically weak ATD distributions starting to appear at 8 and 10 ms and from collision voltages indicated by arrows. Taken together, our results provide evidence that IM-MS CIU profiles of protein–lipid complexes can be heterogeneous, which we hypothesize is due to the contribution from product ions after ejection of their bound lipid(s).

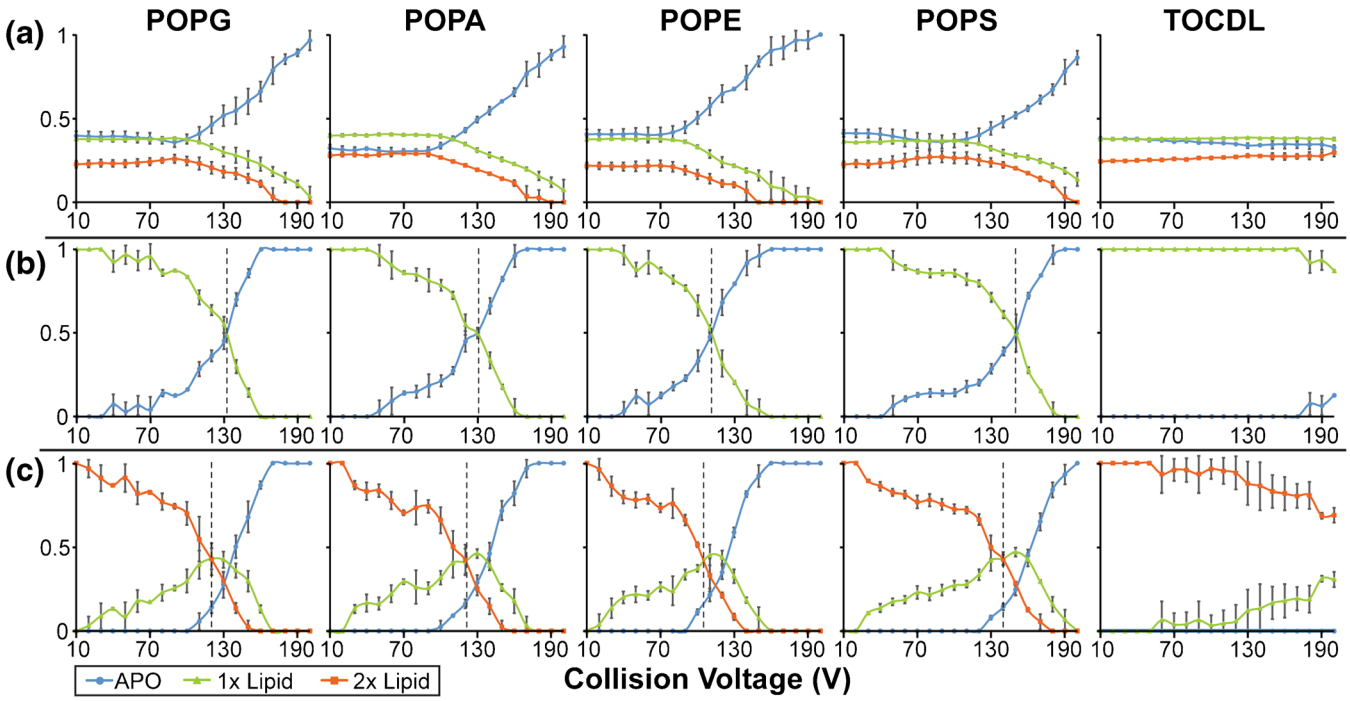


Figure 5. Mole fraction of AmtB lipid species across CIU profiles for (**a**) IM-MS and MS-IM-MS of AmtB bound to (**b**) one and (**c**) two lipid(s). Data was recorded at a source temperature of 120 °C. Dashed lines indicate the estimated collision voltage at which half of the bound lipid has been ejected. Phosphatidylserine (POPS), phosphatidylethanolamine (POPE), and tetra oleoyl (18:1) cardiolipin (TOCDL). Reported are average and standard deviation (n = 3)

Ejection of Bound Lipid(s) from AmtB During CIU

To understand how bound lipids eject from AmtB, we first examined the mass spectra in IM-MS CIU profiles. In general, we observed a gradual decrease in signals corresponding to AmtB bound to lipid with increased collision voltage (Figure 3a). We then investigated mass spectra in MS-IM-MS CIU profiles. The signal for the isolated 15^+ charge state of AmtB bound to two lipids was absent at a trap collision voltage above 160 V (Figure 3b), which is dramatically different from IM-MS profiles where lipids remained present even above collision voltage settings of 200 V (Figures 3a and 5). After examining mass spectra for MS-IM-MS profiles of AmtB bound to two PO phosphatidylglycerol (POPG) molecules, we observed that even though AmtB$(POPG)_2$ gradually lost both bound lipids with increased trap collision voltage, the charge state of AmtB remained the same (Figure 3b), indicating that the bound lipids ejected as neutral species. Interestingly, we could obtain a CIU profile for the product of AmtB$(POPG)_2$ minus one POPG, yielding AmtB$(POPG)_1$ (Figure 4a). Analysis of MS-IM-MS CIU profiles for AmtB bound to other lipids gave similar results, even when selecting ions corresponding to AmtB bound to one lipid (Figure 4b). Notably, all lipids investigated in this study ejected from AmtB as neutral species. Thus, the product ions after ejection of their bound lipid(s) in the CIU process compromise other ions of the same charge state but lower in mass [i.e., mass minus the ejected lipid(s)].

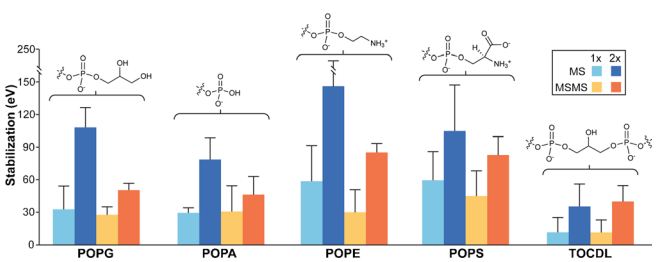


Figure 6. Stabilization of AmtB bound to lipid determined from IM-MS and MS-IM-MS. The head group structure of each lipid is shown. Stabilization was calculated by comparing the transitions in CIU profiles for apo and lipid-bound states in units of electron volts as previously described [25, 33]. Reported are the average and standard deviation (n = 3)

To gain further insight into the lipid ejection process during CIU, we plotted the mole fraction of AmtB species from IM-MS and MS-IM-MS CIU profiles (Figure 5). As seen by IM-MS CIU profiles, the PO-type lipids have similar trends but slightly differ in the initial transition point where lipids start to eject. In contrast, the mole fraction of bound tetra oleoyl (18:1) cardiolipin (TOCDL) is fairly constant, with a ~14% change in the fraction of apo AmtB across the CIU profile. We then plotted the mole fraction for AmtB species derived from MS-IM-MS CIU profiles for AmtB bound to one (1x) or two (2x) lipids (Figure 5b, c). Similar to IM-MS, all the PO-type lipids exhibited similar patterns. However, an initial lipid loss occurring early on in the CIU profile does not appear in the mole fraction plot from IM-MS. TOCDL was virtually constant with some ejection at higher collision voltage settings and an absence of apo AmtB for MS-IM-MS 2x. The discrepancy for

TOCDL dissociation is intriguing, but similar observations have been noticed for some soluble protein–ligand systems [10, 34]. The rapid ejection of lipid in general appears to happen at collision voltages where the third and fourth partially unfolded intermediates begin to appear in the CIU profile. It is also worth noting that these mole fraction plots can be a useful reference for quantifying lipid binding on other instruments, such as the Orbitrap [43].

IM-MS and MS-IM-MS Derived Stabilization of AmtB Lipid Binding

After establishing that lipids are ejected from AmtB as neutral species, we set out to determine the stabilization afforded by AmtB binding one and two lipids using IM-MS and MS-IM-MS CIU profiles (Figure 6). The calculated stabilization for AmtB binding one lipid determined from IM-MS and MS-IM-MS CIU profiles are similar to IM-MS giving slightly higher values. The stabilization values for 1x are statistically indistinguishable, which is in agreement with our recent report on the thermodynamics of these lipids binding AmtB [37]. There is slight deviation for the binding of two lipids, where stabilization for binding of two lipids is the greatest for IM-MS CIU profiles. The products of lipid ejection contributing to CIU profiles can in part explain the increase in stability. More specifically, this effect could potentially be enhanced for some lipids that readily dissociate from AmtB, providing a rationale for the increase in stabilization for the second lipid-binding event in some cases. In support of this idea, POPG and phosphatidylethanolamine (POPE) have the most pronounced increase in AmtB stabilization for the second lipid-binding event, and these lipids appear to dissociate more readily from AmtB (see Figure 5c). Furthermore, stabilization for AmtB binding TOCDL, a lipid that does not readily eject from the complex, is indistinguishable. In short, the effects of lipid ejection on CIU profiles are enhanced for lipids that readily dissociate, and IM-MS and MS-IM-MS yield similar ranking of lipids that stabilize AmtB.

As we used the same lipids in our recent study [37], we set out to compare stabilization values to equilibrium dissociation constants (K_D). We found no correlation between the stabilization values and K_D. This observation is directly in line with a recent report for concanavalin A, a tetrameric soluble protein, binding carbohydrates [44]. The discrepancy between these two values could be rationalized by the unfolded protein and unfolded protein–lipid (or protein–ligand) complex differing in energy, making the stabilization energy not equal to the binding energy [37].

Conclusions

Lipids have essential roles in the folding, structure, and function of membrane proteins [1–3, 25, 45–47], and the development of new methods to elucidate how lipids exert their effects are of great biological importance. Here, we describe the application of "in-source" collisional activation of membrane protein complexes to produce resolved ions that can be isolated in the quadrupole of a Waters Synapt G1 instrument while preserving their native-like structure in the gas phase. Using this method, we collected for the first time MS-IM-MS CIU profiles for a membrane protein in complex with lipid. This led to the finding that bound lipids eject from AmtB as neutrals, which gives rise to overlapping CIU profiles when using the IM-MS CIU method. We have also shown that the stability for AmtB bound to different lipids do not vary significantly between IM-MS and MS-IM-MS measurements, and no correlation to biophysical parameters, such as K_D, was found. Further study is warranted to decipher the connection between protein stability (CIU profiles), ease of lipid dissociation, and biophysical parameters. The ability to desolvate and isolate ions of membrane protein complexes will be advantageous for other MS methods, such as ultraviolet photodissociation [48] and surface induced dissociation [49, 50].

Acknowledgments

The authors thank Dr. David H. Russell (Texas A&M University) for useful discussion. This work was supported by new faculty startup funds from the Texas A&M University.

References

1. Singer, S.J., Nicolson, G.L.: The fluid mosaic model of the structure of cell membranes. Science **175**, 720–731 (1972)
2. Lee, A.G.: Biological membranes: the importance of molecular detail. Trends Biochem. Sci. **36**, 493–500 (2011)
3. Contreras, F.X., Ernst, A.M., Wieland, F., Brugger, B.: Specificity of intramembrane protein–lipid interactions. Cold Spring Harb. Perspect. Biol. **3**, 1-18 (2011)
4. Long, S.B., Tao, X., Campbell, E.B., MacKinnon, R.: Atomic structure of a voltage-dependent K+ channel in a lipid membrane-like environment. Nature **450**, 376–382 (2007)
5. Ruotolo, B.T., Giles, K., Campuzano, I., Sandercock, A.M., Bateman, R.H., Robinson, C.V.: Evidence for macromolecular protein rings in the absence of bulk water. Science **310**, 1658–1661 (2005)
6. Kanu, A.B., Dwivedi, P., Tam, M., Matz, L., Hill Jr., H.H.: Ion mobility-mass spectrometry. J. Mass Spectrom. **43**, 1–22 (2008)
7. Laganowsky, A., Reading, E., Hopper, J.T., Robinson, C.V.: Mass spectrometry of intact membrane protein complexes. Nat. Protoc. **8**, 639–651 (2013)
8. Hernandez, H., Robinson, C.V.: Determining the stoichiometry and interactions of macromolecular assemblies from mass spectrometry. Nat. Protoc. **2**, 715–726 (2007)
9. Benesch, J.L., Robinson, C.V.: Mass spectrometry of macromolecular assemblies: preservation and dissociation. Curr. Opin. Struct. Biol. **16**, 245–251 (2006)
10. Loo, J.A.: Studying noncovalent protein complexes by electrospray ionization mass spectrometry. Mass Spectrom. Rev. **16**, 1–23 (1997)
11. Lorenzen, K., van Duijn, E.: Native mass spectrometry as a tool in structural biology. Curr. Protoc. Protein Sci. **17**, Unit17 12 (2010)
12. Pedro, L., Quinn, R.J.: Native mass spectrometry in fragment-based drug discovery. Molecules. **21** 1-16 (2016)
13. Goodlelt, D.R., Ogorzalek Loo, R.R., Loo, J.A., Wahl, J.H., Udseth, H.R., Smith, R.D.: A study of the thermal denaturation of ribonuclease S by electrospray ionization mass spectrometry. J. Am. Soc. Mass Spectrom. **5**, 614–622 (1994)
14. Robinson, C.V., Chung, E.W., Kragelund, B.B., Knudsen, J., Aplin, R.T., Poulsen, F.M., Dobson, C.M.: Probing the nature of noncovalent interactions by mass spectrometry. A study of protein−CoA ligand binding and assembly. J. Am. Chem. Soc. **118**, 8646–8653 (1996)

15. Penn, S.G., Fei, H., Kirk Green, M., Lebrilla, C.B.: The use of heated capillary dissociation and collision-induced dissociation to determine the strength of noncovalent bonding interactions in gas-phase peptide-cyclodextrin complexes. J. Am. Soc. Mass Spectrom. **8**, 244–252 (1997)
16. He, F., Ramirez, J., Garcia, B.A., Lebrilla, C.B.: Differentially heated capillary for thermal dissociation of noncovalently bound complexes produced by electrospray ionization1. Int. J. Mass Spectrom. **182/183**, 261–273 (1999)
17. Gabelica, V., Rosu, F., Houssier, C., De Pauw, E.: Gas phase thermal denaturation of an oligonucleotide duplex and its complexes with minor groove binders. Rapid Commun. Mass Spectrom. **14**, 464–467 (2000)
18. Lippens, J.L., Mangrum, J.B., McIntyre, W., Redick, B., Fabris, D.: A simple heated-capillary modification improves the analysis of noncovalent complexes by Z-spray electrospray ionization. Rapid Commun. Mass Spectrom. **30**, 773–783 (2016)
19. Barrera, N.P., Di Bartolo, N., Booth, P.J., Robinson, C.V.: Micelles protect membrane complexes from solution to vacuum. Science **321**, 243–246 (2008)
20. Reading, E., Liko, I., Allison, T.M., Benesch, J.L., Laganowsky, A., Robinson, C.V.: The role of the detergent micelle in preserving the structure of membrane proteins in the gas phase. Angew. Chem. Int. Ed. Engl. **54**, 4577–4581 (2015)
21. Housden, N.G., Hopper, J.T., Lukoyanova, N., Rodriguez-Larrea, D., Wojdyla, J.A., Klein, A., Kaminska, R., Bayley, H., Saibil, H.R., Robinson, C.V., Kleanthous, C.: Intrinsically disordered protein threads through the bacterial outer-membrane porin OmpF. Science **340**, 1570–1574 (2013)
22. Reading, E., Liko, I., Allison, T.M., Benesch, J.L., Laganowsky, A., Robinson, C.V.: The role of the detergent micelle in preserving the structure of membrane proteins in the gas phase. Angew. Chem. Int. Ed. **54**, 4577–4581 (2015)
23. Landreh, M., Liko, I., Uzdavinys, P., Coincon, M., Hopper, J.T., Drew, D., Robinson, C.V.: Controlling release, unfolding and dissociation of membrane protein complexes in the gas phase through collisional cooling. Chem. Commun. (Camb.) **51**, 15582–15584 (2015)
24. Borysik, A.J., Hewitt, D.J., Robinson, C.V.: Detergent release prolongs the lifetime of native-like membrane protein conformations in the gas phase. J. Am. Chem. Soc. **135**, 6078–6083 (2013)
25. Laganowsky, A., Reading, E., Allison, T.M., Ulmschneider, M.B., Degiacomi, M.T., Baldwin, A.J., Robinson, C.V.: Membrane proteins bind lipids selectively to modulate their structure and function. Nature **510**, 172–175 (2014)
26. Konijnenberg, A., Yilmaz, D., Ingolfsson, H.I., Dimitrova, A., Marrink, S.J., Li, Z., Venien-Bryan, C., Sobott, F., Kocer, A.: Global structural changes of an ion channel during its gating are followed by ion mobility mass spectrometry. Proc. Natl. Acad. Sci. U. S. A. **111**, 17170–17175 (2014)
27. Ruotolo, B.T., Tate, C.C., Russell, D.H.: Ion mobility-mass spectrometry applied to cyclic peptide analysis: conformational preferences of gramicidin S and linear analogs in the gas phase. J. Am. Soc. Mass Spectrom. **15**, 870–878 (2004)
28. Zhu, F., Lee, S., Valentine, S.J., Reilly, J.P., Clemmer, D.E.: Mannose7 glycan isomer characterization by IMS-MS/MS analysis. J. Am. Soc. Mass Spectrom. **23**, 2158–2166 (2012)
29. Ruotolo, B.T., Hyung, S.J., Robinson, P.M., Giles, K., Bateman, R.H., Robinson, C.V.: Ion mobility-mass spectrometry reveals long-lived, unfolded intermediates in the dissociation of protein complexes. Angew. Chem. Int. Ed. Engl. **46**, 8001–8004 (2007)
30. Hyung, S.J., Robinson, C.V., Ruotolo, B.T.: Gas-phase unfolding and disassembly reveals stability differences in ligand-bound multiprotein complexes. Chem. Biol. **16**, 382–390 (2009)
31. Han, L., Hyung, S.J., Mayers, J.J., Ruotolo, B.T.: Bound anions differentially stabilize multiprotein complexes in the absence of bulk solvent. J. Am. Chem. Soc. **133**, 11358–11367 (2011)
32. Rabuck, J.N., Hyung, S.J., Ko, K.S., Fox, C.C., Soellner, M.B., Ruotolo, B.T.: Activation state-selective kinase inhibitor assay based on ion mobility-mass spectrometry. Anal. Chem. **85**, 6995–7002 (2013)
33. Allison, T.M., Reading, E., Liko, I., Baldwin, A.J., Laganowsky, A., Robinson, C.V.: Quantifying the stabilizing effects of protein-ligand interactions in the gas phase. Nat. Commun. **6**, 8551 (2015)
34. Yin, S., Xie, Y., Loo, J.A.: Mass spectrometry of protein-ligand complexes: enhanced gas-phase stability of ribonuclease-nucleotide complexes. J. Am. Soc. Mass Spectrom. **19**, 1199–1208 (2008)
35. Zinnel, N.F., Pai, P.J., Russell, D.H.: Ion mobility-mass spectrometry (IM-MS) for top-down proteomics: increased dynamic range affords increased sequence coverage. Anal. Chem. **84**, 3390–3397 (2012)
36. Zinnel, N.F., Russell, D.H.: Size-to-charge dispersion of collision-induced dissociation product ions for enhancement of structural information and product ion identification. Anal. Chem. **86**, 4791–4798 (2014)
37. Cong, X., Liu, Y., Liu, W., Liang, X., Russell, D.H., Laganowsky, A.: Determining membrane protein-lipid binding thermodynamics using native mass spectrometry. J. Am. Chem. Soc. **138**, 4346–4349 (2016)
38. Pringle, S.D., Giles, K., Wildgoose, J.L., Williams, J.P., Slade, S.E., Thalassinos, K., Bateman, R.H., Bowers, M.T., Scrivens, J.H.: An investigation of the mobility separation of some peptide and protein ions using a new hybrid quadrupole/travelling wave IMS/oa-ToF instrument. Int. J. Mass Spectrom. **261**, 1–12 (2007)
39. Chen, P.S., Toribara, T.Y., Warner, H.: Microdetermination of phosphorus. Anal. Chem. **28**, 3 (1956)
40. Subbarow, C.H.F.A.Y.: The colorimetric determination of phosphorus. J. Biol. Chem. **66**, 26 (1925)
41. Marty, M.T., Baldwin, A.J., Marklund, E.G., Hochberg, G.K., Benesch, J.L., Robinson, C.V.: Bayesian deconvolution of mass and ion mobility spectra: from binary interactions to polydisperse ensembles. Anal. Chem. **87**, 4370–4376 (2015)
42. Smith, R.D., Barinaga, C.J.: Internal energy effects in the collision-induced dissociation of large biopolymer molecular ions produced by electrospray ionization tandem mass spectrometry of cytochrome *c*. Rapid Commun. Mass Spectrom. **4**, 54–57 (1990)
43. Gault, J., Donlan, J.A., Liko, I., Hopper, J.T., Gupta, K., Housden, N.G., Struwe, W.B., Marty, M.T., Mize, T., Bechara, C., Zhu, Y., Wu, B., Kleanthous, C., Belov, M., Damoc, E., Makarov, A., Robinson, C.V.: High-resolution mass spectrometry of small molecules bound to membrane proteins. Nat. Methods **13**, 333–336 (2016)
44. Niu, S., Ruotolo, B.T.: Collisional unfolding of multiprotein complexes reveals cooperative stabilization upon ligand binding. Protein Sci. **24**, 1272–1281 (2015)
45. Bogdanov, M., Dowhan, W., Vitrac, H.: Lipids and topological rules governing membrane protein assembly. Biochim. Biophys. Acta **1843**, 1475–1488 (2014)
46. Poveda, J.A., Giudici, A.M., Renart, M.L., Molina, M.L., Montoya, E., Fernandez-Carvajal, A., Fernandez-Ballester, G., Encinar, J.A., Gonzalez-Ros, J.M.: Lipid modulation of ion channels through specific binding sites. Biochim. Biophys. Acta **1838**, 1560–1567 (2014)
47. Jiang, Q.X., Gonen, T.: The influence of lipids on voltage-gated ion channels. Curr. Opin. Struct. Biol. **22**, 529–536 (2012)
48. Brodbelt, J.S.: Ion activation methods for peptides and proteins. Anal. Chem. **88**, 30–51 (2016)
49. Zhou, M., Dagan, S., Wysocki, V.H.: Protein subunits released by surface collisions of noncovalent complexes: nativelike compact structures revealed by ion mobility mass spectrometry. Angew. Chem. Int. Ed. **51**, 4336–4339 (2012)
50. Quintyn, R.S., Yan, J., Wysocki, V.H.: Surface-induced dissociation of homotetramers with D 2 symmetry yields their assembly pathways and characterizes the effect of ligand binding. Chem. Biol. **22**, 583–592 (2015)