Investigation of Pore-Forming Transmembrane Toxins using Native Ion-Mobility Mass Spectrometry

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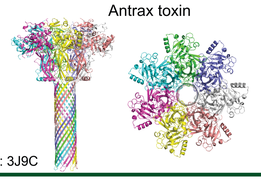
Abstract

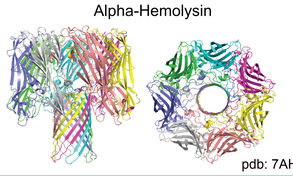
Anthrax toxin (ATX) and Alpha-Hemolysin (AHL) are examples of large transmembrane pore-forming toxins that are similar in structure and are proposed to have specific protein-lipid interactions. Due to the difficulty of studying these structures in solution, native mass spectrometry (native-MS) was used to examine the structure, stoichiometry, and lipid-binding of these membrane protein complexes and ion mobility (IM) analysis was used to further study the complexes' shape and size.

Antrax toxin



pdb: 3J9C

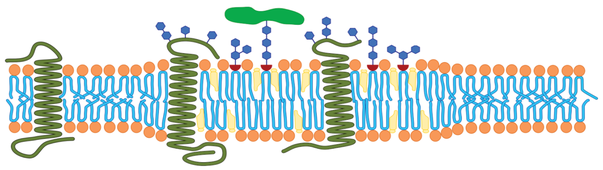
Alpha-Hemolysin



pdb: 7AHL

Introduction

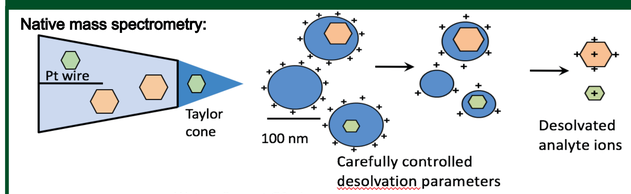
The lateral distribution of proteins and lipids in the plasma membrane remains elusive. Interactions between membrane proteins and lipids could have an effect on membrane distribution as well as protein function. ATX and AHL are both examples of pore membrane pore forming toxins. ATX is produced by the bacterium *Bacillus anthracis* and is a key virulent factor for the bacterium. When ATX binds a host cell, there are proposed to be specific protein-lipid interactions between ATX and lipid rafts that are enriched in sphingolipids and cholesterol. AHL, similarly, is proposed to have specific interactions between the rim domain of each subunit and phospocholine lipids. Studying these kinds of protein-lipid interactions are difficult using traditional solution-phase techniques. Native-MS can be used to examine the structure, mass, stoichiometry, and characterize conformational structures in the gas phase. Ion mobility (IM) analysis can be used to further study proteins shape and size based on its mobility through a buffer gas. Although studying these bacterial pore complexes presents many challenges, understanding the interaction between these pore complexes and lipids will further elucidate their mechanisms for treatment or to leverage these complexes as delivery vehicles for transporting large molecules into cells.



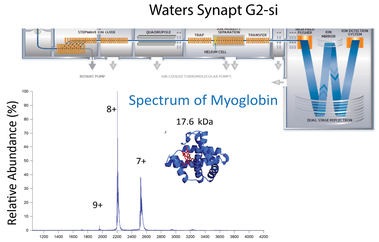
Steven White
Group UCI

Methods

Native mass spectrometry:



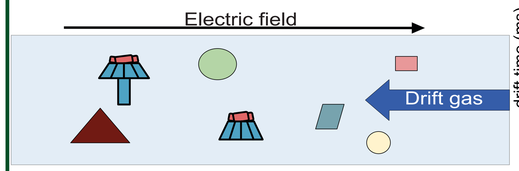
Waters Synapt G2-si



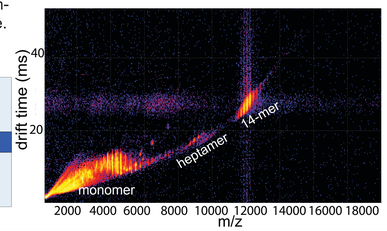
Native-MS uses electrospray ionization (ESI) to softly ionize and transfer analyte molecules from the solution-phase to the gas-phase while maintaining native-like high order structures. Desolvation parameters are carefully controlled to produce compact stable desolvated analyte ions that then enter the instrument. Instrument parameters such as capillary voltage and collisional energy are then tuned to produce optimal mass resolution.

Methods

Ion-Mobility (IM) uses weak electric fields to pull ions through a tube with a neutral buffer gas to create drag forces that separate ions based on their shape. For each ion, a drift time value is measured corresponding to the time an ion takes to traverse the mobility cell. From IM drift times, the shape and size of a complex can be determined by converting a proteins drift time into a collisional cross section (CCS) value.



The following is an ion-mobility spectrum of the protein complex, GroEL. GroEL is a 14-mer and is 800 kDa in size. From the spectrum, its final oligomeric and monomeric states are clearly resolved.



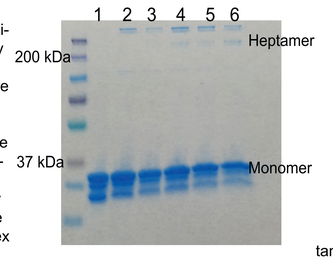
Surface Induced Dissociation (SID):

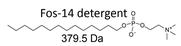
SID was used to characterize the prepore and pore form by fracturing the heptameric structure of protective antigen (PA) from ATX in the gas-phase. SID is a device inside our mass spectrometer where ions are directed to collide into a solid surface. SID can fragment protein complexes into smaller oligomeric pieces in one ultra fast collision, keeping the ensuing protein fragments folded. Ion-mobility was performed to illustrate the different oligomeric states and their abundance after SID.

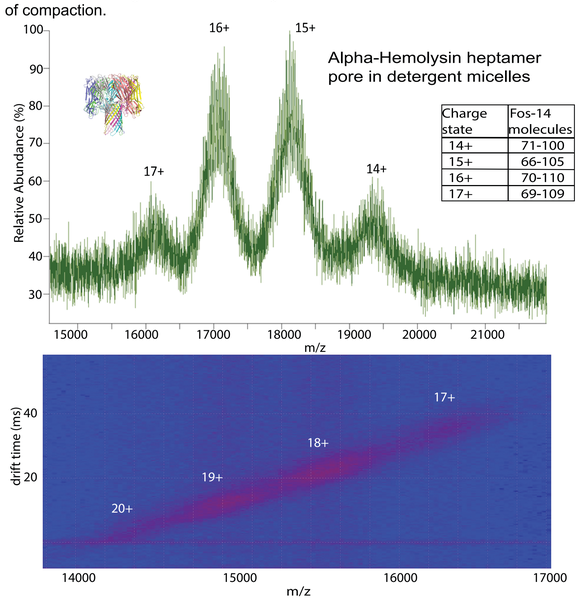
Alpha-Hemolysin Results

Alpha-hemolysin heptamer pores are prepared by concentrating toxin monomers in the presence of detergent using spin concentrators. For these studies we used the detergent tetradecylphosphocholine (FOS-14) for its ability to form stable toxin pores and for suitability in native-MS experiments. Samples of AHL underwent up to 5 rounds of concentration and dillution with Fos-14. As can be seen in the SDS-PAGE gel below the abundance of heptamer increases with each round.

A high resolution spectrum of the heptameric AHL pore complex in a detergent micelle was identified earlier in the summer by optimizing sample preparation and tuning parameters. High resolution of the toxin pore with its full compliment of noncovalently bound detergent molecules was seen. This is in contrast to previously reported literature results of similar native-MS studies of membrane proteins where the micelle could not be resolved and the protein was completely ejected from the micelle. We were also able to collect IM-MS spectra of the pore complex in a micelle and determine a CCS of 90.7 nm^2. This value is 15% smaller than the CCS value from the crystal structure of 105.2 nm^2 meaning we see a small degree of compaction.

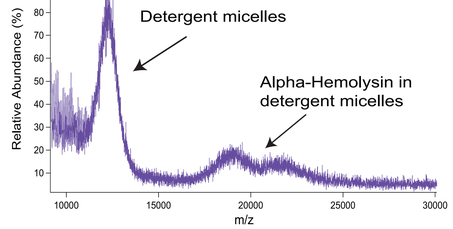




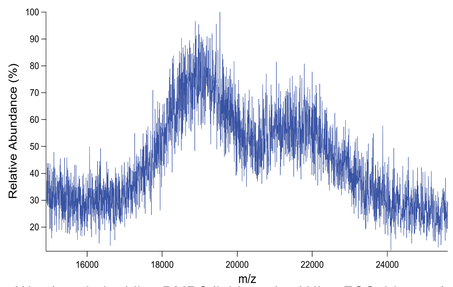


Charge state	Fos-14 molecules
14+	71-100
15+	66-105
16+	70-110
17+	69-109

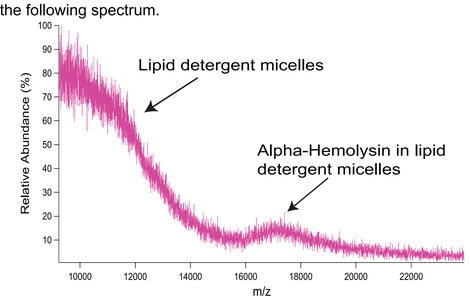
There have been difficulties in trying to replicate the spectrum even with following the same procedure. The mass spectra of heptameric AHL below were produced with similar sample preparation and tuning conditions.



The following spectrum illustrates what we believe to be the heptameric AHL complex. It is obvious that there is not a clear, narrow distribution of charge states, thus difficult to characterize the complex.

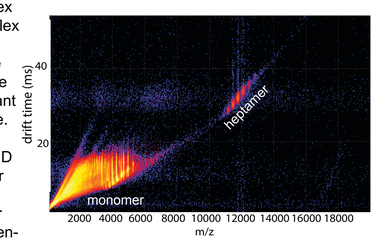


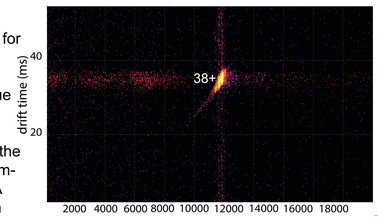
We also tried adding DMPC lipids to the AHL + FOS-14 sample to determine if we can observe any protein-lipid interactions. Similar sample preparation and tuning parameters were performed to achieve the following spectrum.

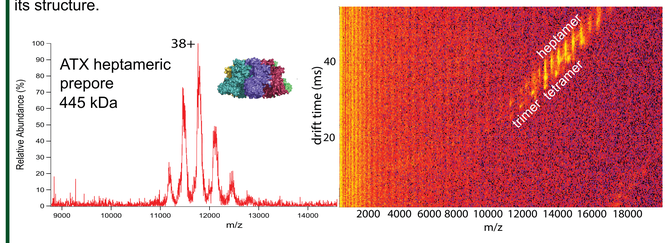


Anthrax Toxin Results

The native-MS spectrum of PA below confirms the heptameric prepore complex with a mass of 445 kDa. IM of the complex to the right shows each charge state is folded. We then isolated the 38+ charge state and performed SID. An SID voltage of 200 V was applied leading to significant fragmentation of the heptameric prepore. High abundance of trimer and tetramer fragments were produced suggesting SID may favor symmetrical fragmentation for this complex. There is also a significant abundance of charge stripped precursor heptamer which may overlap with the pentameric species. We also measured the CCS for the heptameric precursor and each of the fragments. The CCS values for the fragments show that these pieces remain folded after fragmentation of the complex. The precursor had a CCS value of 163.7 nm^2 compared to 88.4 nm^2 and 71.0 nm^2 for the tetramer and trimer, respectively. SID verifies the assembly of the native folded heptameric PA prepore complex. Further SID studies will use the PA pore complex and look for differences in SID fragmentation patterns to further study its structure.







Conclusion

Using native IM-MS presents the opportunity to understand the conformational landscape of non-covalent stoichiometry distributions for large membrane protein complexes. Performing SID to characterize ATX is unprecedented and can be used to identify native structure of other protein complexes in the future. Further optimization of native IM-MS techniques and sample preparation can lead to higher resolution spectra, ultimately leading to more information about these pore-forming toxins, such as identifying the specific lipid-protein interactions and how that affects membrane structure and toxin function. Although these complexes are challenging to study using traditional solution-phase techniques, these results illustrate that native IM-MS can be a powerful tool for characterizing structures and lipid-binding of large pore-forming toxin complexes.

Acknowledgments

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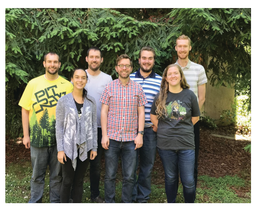

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