Insights into the Properties of an Anticancer Peptide using Differential Mobility Spectrometry

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Project Motivation

Polybia-MP1 (IDWKKLLDAAKQIL-NH_2) is a bioactive host-defense peptide found in the venom of the Brazilian eusocial wasp Polybia Paulista, and exhibits selective anticancer and antimicrobial properties.[1] This targeted activity stems from the interaction of the amphipathic helix with negatively charged phospholipid head groups, which are found exclusively on the outer leaflet of invasive tissue, and ultimately leads to cell death by membrane poration and subsequent depolarization.[2] However, the mechanistic basis behind this process is not well understood, which introduces a major hurdle to developing MP1 as a novel chemotherapeutic or antibacterial agent. Utilization of differential mobility spectrometry (DMS) coupled with high level quantum chemical calculations could provide key insights which promote the implementation of bioactive peptides into clinical applications.

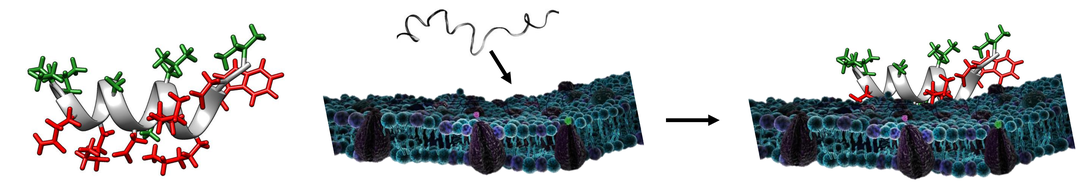


Figure 1. Polybia-MP1 (left) adopts an amphipathic helical structure, which localizes cationic residues to one particular face of the helix enabling interaction with negatively charged phospholipid head groups

Computational and Experimental Methods

We have mapped the potential energy surface (PES) and are currently conducting gas-phase microsolvation studies of $[MP1{\cdot}(H_2O)_n]^{2+/3+}$ to examine the evolution of conformational changes to peptide structure in increasingly aqueous media (n = 1 – 10 water). The PES searches, completed using a custom basin hopping algorithm, are coupled with high level electronic structure calculations to generate a series of two major motifs which are identified through DMS experiments on a SelexION 5500.

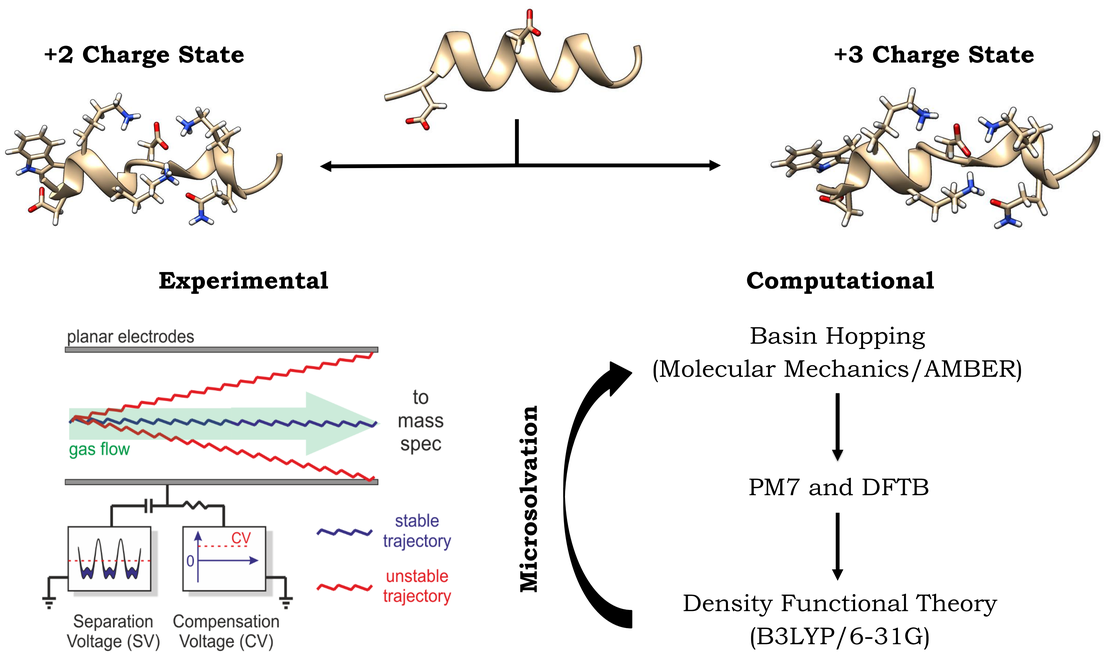
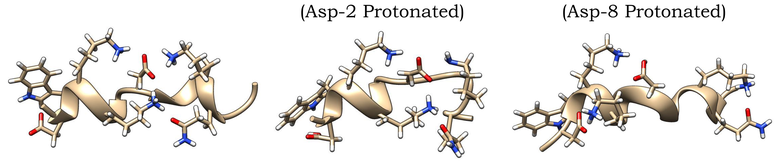


Figure 2. Schematic representation of DMS[3]

Results

DMS indicates the presence of at least two major conformers in both charge states

Bare Cluster Calculated Global Minimum



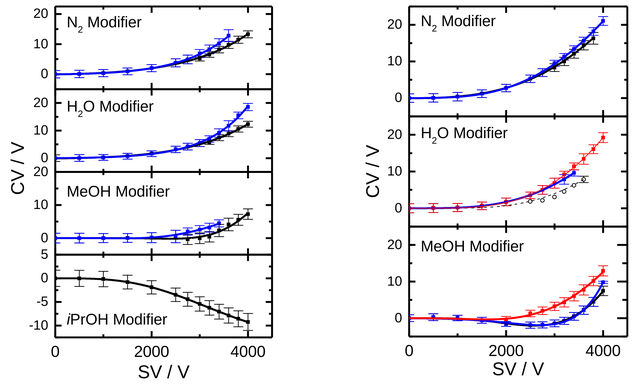
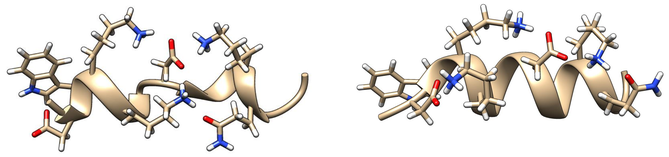


Figure 3. Global minima and dispersion plots of $[MP1 + 2H]^{2+}$ (left) and $[MP1 + 3H]^{3+}$ (right)

Two major conformers have been discovered through BH searches of $[MP1 + 2H]^{2+}$ and subsequent DFT optimization. Similar BH searches of $[MP1 + 3H]^{3+}$, whereby protonation occurs on Asp-2, favours Motif 1, while protonation on Asp-8 favours Motif 2. Given the high relative energies of other conformers (not shown), these are the most likely candidates for the two conformers identified by DMS.

$[MP1]^{2+}$ Calculated Isomers



Motif 1
0 kJ mol^{-1}
386.4Å^2

Motif 2
9.6 kJ mol^{-1}
374.3Å^2

Figure 4. Lowest energy conformers $[MP1 + 2H]^{2+}$ and their calculated collision cross sections (CCS)

***i*PrOH abstracts a proton from $[MP1+3H]^{3+}$ via a solvent bound network**

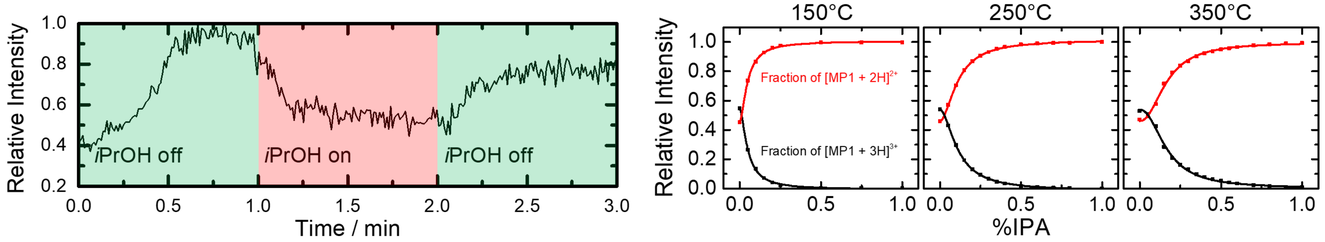


Figure 5. Modifier composition and temperature study on the deprotonation of $[MP1 + 3H]^{3+}$ by *i*PrOH

Conclusions

- Differential mobility spectrometry has identified at least two major conformers of MP1
- Major conformers involve two distinct binding motifs, mainly a tridendate coordination of Asp-8 by Lys-4, Lys-5, and Lys-11, or a bidentate coordination of Asp-2 by the N-terminus and Lys-5.
- Protonation on either Asp-2 or Asp-8 in $[MP1 + 3H]^{3+}$ heavily favours one conformer over the other
- Hydrogen deuterium exchange (HDX), collision induced dissociation (CID), and declustering potential studies support the presence of at least two unique conformers of MP1 (data not shown).

References and Acknowledgements

(1) Wang, K.; Yan, J.; Zhang, B.; Song, J.; Jia, P.; Wang, R. *Cancer Lett.* **2009**, *278* (1), 65–72.
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(3) Hopkins, W. S. *Mol. Phys.* **2015**, *113* (21), 3151–3158.

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