Ion Mobility-Mass Spectrometry Driven Natural Product Discovery

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A method for rapid analysis of extracts of hypogean microorganisms for the purpose of natural product discovery is presented. In this method, complex cellular extracts were analyzed using nano-electrospray ionization (nESI) and matrix-assisted laser desorption/ionization (MALDI)-ion mobility-mass spectrometry (IM-MS) for the determination of potential unique biomolecules. This method allows for the analysis of complex cellular extracts with little sample preparation as a result of the ability of IM-MS to separate isobaric species based upon chemical and conformational differences prior to mass analysis. Therefore, this method proposes to utilize the structural properties of natural products as a means of identification.

Keywords **— ion mobility-mass spectrometry, natural product discovery, cyclic peptide**

I. Purpose

THIS study proposes a method for the rapid interrogation of complex biological extracts for the purpose of natural product discovery. A significant caveat to natural product discovery is the idea that the metabolic profile of an organism is dynamic. This results in the production of specific secondary metabolites only under certain conditions, such as stress or competition[1]. As a result, a means to rapidly analyze an extract with little sample preparation allows for the conservation of resources, time, and sample quantities. The most common methods of mass spectrometry metabolite detection couple condensed phase separations (gas chromatography, two dimensional gas chromatography, liquid chromatography, and capillary electrophoresis) as pre-ionization separations methods[2]. These separations, however, occur on the order of minutes to hours for a single sample[3]. We propose the use of an ion mobility post-ionization separation technique with mass spectrometry as a method for the analysis of hypogean organism extracts, which occurs on the order of milliseconds.

II. Methods

The research described utilizes the unique structural properties common to natural product biomolecules, such as large numbers of chiral centers, low numbers of rotatable bonds, macrocyclic structures, large degrees of ring fusion, and non-standard amino acids [4], as a means of separating unique natural product compounds from the complex cellular matrix. Gas phase packing trends for biomolecules have been observed in ion mobility separations performed under low field conditions based upon prevailing intramolecular forces, monomer subunit identities, and degree of branching [5]. There exists, therefore, a metric to which these unique structures may be compared.

For this study, cyclic peptide natural products were first studied using MALDI-IM-MS in an attempt to confirm that the unique structural properties would beget deviation from these packing trends. It was shown that cyclic peptides generally fall significantly below the predicted densities of linear peptides, thereby allotting differentiation based upon this deviation. Molecular dynamics simulations were then performed to better understand this result. Conformational space analyses were performed on a number of cyclic peptide natural products, generating *c.a.* 24,000-30,000 conformations for a given structure. These data were then discriminated against using experimentally derived collision cross section values, which correspond to the gas phase conformation observed. Finally, crude extracts were analyzed using nESI-IM-MS as a means of rapidly analyzing for the presence of cyclic peptides.

III. Conclusion

Cyclic peptide natural products were found to generally adopt more dense gas phase conformations when analyzed using MALDI and nESI-IM-MS methods. Molecular dynamic simulations were implemented to gain chemical understanding of the observed densities. This method was then applied to complex cellular extracts as a means of rapid analysis. Future experiments will investigate the effects of external stimuli to biomolecule production, using IM-MS and IM-MS/MS as a means of rapid analyte identification.

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