The Application of Ion Mobility Spectrometry and Ultrahigh Resolution Spectrometry for Analysis of Anti-Microbial Peptides from Frog Skin Secretions

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Frog skin secretions of antimicrobial peptides (AMPs) are of increasing interest due to the protection they impart against microbes. Such peptides offer the prospect of new and efficient sources of bacterial, fungal, and viral treatments. Recently, an epizootic of chytridiomycosis from the fungal pathogen *Batrachochytrium dendrobatidis* (Bd) caused the death of many amphibian species in the Andes. However, certain species of frogs such as *Hypsiboas gladiator* were able to survive. It is speculated that the survival of *Hypsiboas gladiator* is due to AMPs secreted from their skin.

The identification and characterization of these peptides is necessary for understanding the antimicrobial function of AMPs. However, despite recent advances in peptide sequencing technology, complex biological systems still pose many challenges for analysis. In the present work, peptide extracts from amphibian skin secretions were sequenced using liquid-chromatography (LC) coupled to trapped ion mobility spectrometry (TIMS) and mass spectrometry (MS), and isomeric species were further analyzed and elucidated using TIMS coupled to ultrahigh-resolution MS fragmentation.

The analysis of three samples from frog skin extracts using LC-TIMS-MS yielded a large number of peptide sequence candidates. Following this, TIMS coupled to ultrahigh-resolution MS fragmentation was used to analyze *m/z* values with multiple sequence candidates to provide further identification. Additionally, ultrahigh-resolution MS fragmentation was conducted using two different techniques: collision induced dissociation (CID) and electron capture dissociation (ECD). The two techniques were compared for multiple peptides, with CID providing higher sequence coverage than ECD.

The current work presents the complementary use of multiple peptide sequencing techniques and MS fragmentation for the rapid identification of thousands of peptide sequences in a novel matrix. This research expands upon previous analyses of complex biological matrices with advantages in speed, sensitivity and comprehensive coverage over traditional techniques.