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Leveraging Natural Biodefense Mechanisms: Method Development For Host Defense Peptide Discovery

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Antimicrobial host defense peptides (AMPs) provide a rich and diverse resource for the proactive development of broad-spectrum therapeutics against current and future bacterial and viral threats. They represent a natural biodefense mechanism that is used pervasively in nature, and they contribute to the ability of some species survive and thrive in pathogenically challenging environments. In additional to bactericidal activity, AMPs have also been shown to have antiviral properties.1 Despite the range of eukaryotic organisms that employ AMPs in defense against infection, this diversity is not well reflected in the set of known AMPs nor in those in clinical trials.2 This lack of species diversity reflects in part the analytical challenges associated with identifying novel AMPs. We have developed a bioprospecting process to provide a versatile platform for AMP discovery and characterization to inform and provide leads for therapeutic development. Well-established proteomics techniques typically rely on enzymatic digestion of proteins and a mass spectrometry analysis workflow in which peptides are fragmented via collisional dissociation methods and identified by reference to the known proteome of the species under study. Unfortunately, enzymatic digestion compromises the ability to infer the intact endogenous peptide sequences. Bioinformatics approaches may also be used for AMP gene prediction; however, peptide identification via database searches and gene prediction are limited by the quality of available sequence information and known AMP sequence patterns. We have developed a bioprospecting approach to AMP discovery which employs functionalized hydrogel particles to preferentially enrich AMPs and AMP-like peptides from biofluids followed by analysis of the captured peptides via tandem mass spectrometry. We successfully applied this process to identify peptides from American alligator and Komodo dragon plasma that exhibit antibacterial activity.3,4 More recently, we utilized lipid encapsulated hydrogel particles (lipobeads) as mimetics of healthy and virus-infected host cells to identify three peptides that exhibit a dose-dependent inhibition of Venezuelan equine encephalitis virus (under review). We have continued to refine our methodology, incorporating elements of traditional proteomics methods (solvent precipitation) and improved mass spectrometry parameters and techniques (increased ion injection time; supplemental activation).5–7 These efforts to refine our bioprospecting approach to AMP discovery combined with our focus on nontraditional species will facilitate our ability to leverage natural defense strategies toward development of novel therapeutics against new and emerging biological threats.

References

(1) Shartouny, J. R.; Jacob, J. Semin. Cell Dev. Biol. 2019, 88, 147–155.
(2) Lazzaro, B. P. et al. Science 2020, 368 (6490), eaau5480.
(3) Bishop, B. M. et al. PLOS ONE 2015, 10 (2), e0117394.
(4) Bishop, B. M. et al. J. Proteome Res. 2017, 16 (4), 1470–1482.
(5) Kay, R. G. et al. Rapid Commun. Mass Spectrom. 2018, 32 (16), 1414–1424.
(6) Azkargorta, M. et al. J. Proteome Res. 2015, 14 (6), 2649–2658.
(7) Cristobal, A. et al. Anal. Chem. 2017, 89 (6), 3318–3325.