Structural and Functional Studies of Membrane-Interacting Antimicrobial and Neuroimmune Peptides: Insights Gained from Investigating Piscidin and Orexin

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Abstract

This research focuses on multifunctional peptides, which interact with lipid bilayers as well as perform functions at the interface of the immune and nervous systems. Host survival depends on molecules that adopt structures related to multiple functions. Studying the structure-function relationships of these peptides are helpful for discovering new ideas to treat diverse diseases, such as infectious diseases, neurological disorders, and inflammation. Two families of peptides are featured as archetypes: piscidin, a host defense peptide that was first isolated from the mast cells of hybrid striped bass and later reported to appear in neuroepithelial cells; orexin, which is able to activate G-protein coupled receptors that are involved in wakefulness and appetite and also participate in the host defense function.

The first set of experiments focus on Piscidin 1 (P1) and Piscidin 3 (P3), which were optimized by evolution to display different but efficient potencies against pathogens despite being only 32% heterologous in sequence. The main mechanistic action of host defense peptides relies on the disruption of pathogenic membranes. Numerous mutants are designed to study not only the metal-carrying sequence of P1 and P3 but also the key structural differences between them. Half maximal effective concentration generated from dye leakage assays is used to quantify and compare the permeabilization capability of the peptides in relation to their membrane activity on pathogens. Membrane composition was chosen to reproduce bacteria dangerous to humans, including Escherichia coli and Vibrio species. P1 and P3 keep high activity against Vibrio species even though bacteria have the special ability to use polyunsaturated fatty acids to enhance virulence. Biological activity was characterized on these species by a collaborator. The lipid oxidation assay was used to test our hypothesis that the metallopeptides can generate reactive oxygen species and bring oxidative stress to the polyunsaturated fatty acids. On the structural side, circular dichroism was used to study the peptide secondary structure and establish the correlation between peptide α-helical content and membrane activity. Solid-state NMR was applied to obtain high-resolution structures at the atomic level. In another set of experiments, the focus is on orexin A and its receptor. The synthesis of the disulfide bonded peptide while also labeling it for NMR was successfully attempted by a collaborator, enabling structural studies by CD and NMR. The receptor is cloned into bacteria and the expression is tested, leading to promising data and paving the way for

further research.

In summary, we have investigated structure-function relationships in antimicrobial and neuroimmune peptides. This helps us better understand the diverse modes of action by host defense molecules in the immune and nervous systems, and thus may provide new ideas for developing peptide-based drugs for clinical applications.