Mechanistic Studies of Histidine-Rich Host Defense Peptides

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Host defense peptides (HDPs) are important immune molecules involved in not only directly bacterial killing but also immunomodulatory effects on host cells. In 2001, fish HDPs called piscidins were discovered in the mast cell of fish. Since this important discovery that demonstrated the importance of mast cells in fighting infectious diseases, other HDPs evolutionary related to the hybrid striped seabass piscidins were identified in other fish species, including cod, tilapia, and mandarin fish. All members of the piscidin family differ from other HDPs due to their high content of histidines (~20% in piscidin versus 2% on average in HDPs).[1]

Remarkably, piscidins act at acidic pH in the phagosome of mast cells and granulocytes, as well as extracellularly at basic pH. Given that histidine side chains typically have pKa values near physiological pH but also are highly dependent on their surrounding environment, we postulated that the piscidins could be pH sensitive under the pH range that they encounter *in vivo*. Since piscidins are active under changing conditions, we also speculated that either individually or collectively, they exhibit pH resiliency.

To test our hypothesis, we have focused on four membrane-active piscidins: piscidin 1 (FFHHIFRGIVHVGK-TIHRLVTG) and piscidin 3 (FIHHIFRGIVHAGRSIGRFLTG) from hybrid striped seabass, and TP3 (FIH-HIIGGLFSVGKHIHSLIHGH) and TP4 (FIHHIIGGLFSAGKAIHRLIRRRRR) from tilapia. We previously used solid-state NMR to investigate the high resolution structures and orientations of piscidin 1 and piscidin 3 in the presence of bacterial cell mimics, 3:1 phosphatidylcholine-phosphoglycerol (PC/PG) (Fig. 1, top).[2] Here, we present results from NMR-monitored titrations of the histidine side chains of piscidin 1 and piscidin 3 in the presence of isotropic bicelles (Fig. 1, bottom). We also show the dye leakage assays that we performed using trapped calcein in large unilamellar vesicles in order to characterize the effect of pH on the permeabilization ability of the peptides (Fig. 1, middle). Our results indicate that piscidin 1 is more pH-resilient than piscidin 3 and its histidine side chains deprotonate at low pH than those of piscidin 3. This property is very significant since it allows the peptide to be as membrane effective at acidic than basic pH, as needed to kill bacteria in phagosomes and extracellularly.

Since TP3 and TP4 have not been structurally characterized,[3] we initiated circular dichroism and two dimensional ^{15}N-solid-state NMR studies to investigate their backbone structure and orientation in the presence of 3:1 PC/PG bilayers. Our results indicate that similarly to piscidins 1 and 3, TP3 and TP4 adopt strong helical content in this bilayer environment. Using ^{31}P solid-state NMR, we have characterized their membrane disruptive behavior and compared it to the hybrid striped seabass piscidins.

Overall, these investigations help us better understand structure-function relationships in a unique family of HDPs rich in histidines. Since these peptides have antimicrobial, wound-healing, and anesthetic properties, the principles derived from this research may lead to the development of novel immunotherapeutics.

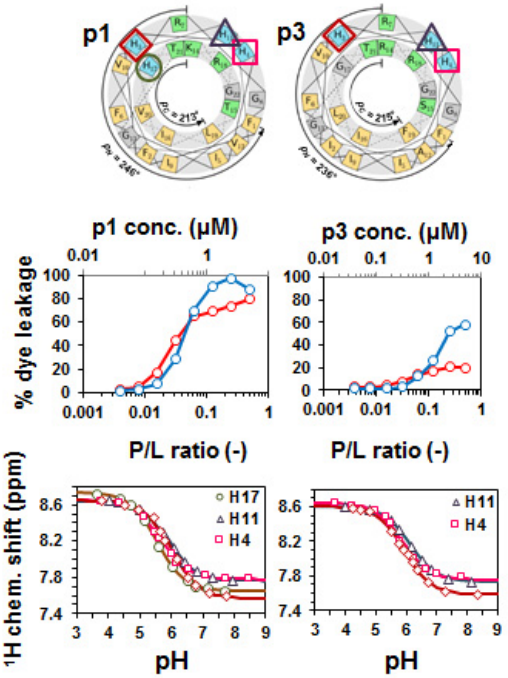

Figure 1. NMR-monitored titrations of histidine-rich host defense peptides from the piscidin family and permeabilization assays showing the pH resiliency of piscidin 1.

References

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