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How Membrane Perturbations Modulate the Activity and Selectivity of Antimicrobial Peptides

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Membrane-active peptides are of growing interest due to their potential antimicrobial, antifungal, and cell-penetrating properties. We investigate two cyclic antimicrobial hexapeptides, cR_3W_3 and $c(RW)_3$, composed of cationic arginine and aromatic tryptophan. Despite their similar composition and structure, their sequence differences lead to subtle but distinct amphiphilic properties that alter their induced membrane perturbation.

In order to explore their mechanism of action and selectivity, extensive biophysical analyses were conducted using model membranes containing either POPE and POPG, two lipids abundant in bacterial membranes, or POPC, a major component of mammalian membranes. We studied binding and adsorption of the peptides using monolayers and ITC experiments, observing a strong preference for negatively charged model membranes over zwitterionic ones. Both peptides altered the lipid order and packing, as determined by fluorescence spectroscopy of membrane-embedded Laurdan. Such membrane perturbations are likely to contribute to the antimicrobial killing process by initiating the dissociation of membrane proteins.

Furthermore, DSC and Laurdan fluorescence spectroscopy revealed pronounced lipid demixing and the formation of large PG-rich clusters promoted by the peptides in lipid mixtures that exhibit non-ideal mixing. Membrane permeabilization was determined by calcein release. We observed fast, all-or-none leakage for cR_3W_3, while $c(RW)_3$ exhibited only marginal leakage activity. It appears that the observed leakage is not related to the formation of large PG-rich lipid clusters, as previously described for both peptides. Instead, a significant portion of the leakage seemed to be related to vesicle fusion. Importantly, fusion is highly unlikely in microbes, but PE-containing model membranes are biased for it.

In summary, both peptides induce changes in lateral order lipid upon binding, which is considered a selective mechanism of antimicrobial killing. However, cR_3W_3 has a larger hydrophobic molecular surface and induces additional membrane leakage, which may come at the expense of selectivity compared to $c(RW)_3$.
These findings highlight the importance of achieving an optimal balance of different types of membrane perturbation when designing antimicrobial peptides. Fine-tuning amphipathicity could be a key strategy to enhance both the efficacy and selectivity of the peptide.