Binding of antimicrobial peptides alters the thermotropic behaviour of model membranes

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The constantly growing antibiotic resistance emphasises the importance of alternatives to classical antibiotics, such as membrane-active antimicrobial peptides (AMPs). We examine the mechanism of action of the antimicrobial trivalent cyclic hexapeptide cR3W3. More precisely, we investigate the effects of the peptide on binary model membranes containing anionic and zwitterionic phospholipids. We use cardiolipin or phosphatidylglycerol as anionic and phosphatidylethanolamine or phosphatidylcholine as zwitterionic lipids, respectively.

Isothermal titration calorimetry (ITC) measurements show binding selectivity for negatively charged membranes over zwitterionic membranes. This agrees with the observed selectivity of the peptide for bacteria over mammalian cells. Differential scanning calorimetry (DSC) and Laurdan fluorescence spectroscopy reveal the influence of cR3W3 on the thermotropic membrane behaviour, such as lipid chain melting. Our findings enable the detection of electrostatic lipid clustering in saturated and unsaturated lipid membranes. We use the self-quenching dye Calcein to quantify vesicle membrane leakage induced by the peptide. On closer inspection, leakage turns out to be mainly caused by leaky fusion. Yet, the biological relevance of this mechanism should be carefully assed.

In conclusion, binding of cR3W3 to model membranes induces various effects: electrostatic lipid clustering, membrane fusion, vesicle aggregation, and vesicle leakage.