

Title: Biophysical studies of the short antimicrobial peptide RIR interacting with membrane-mimetic systems

#### Abstract

The discovery of antimicrobial agents, including antibiotics, antivirals, antifungals, and antiparasitic drugs greatly enhanced our ability to prevent and treat infectious pathogens such as bacteria, fungi, viruses, and parasites. Today, there are increasing reports of bacterial infections, and the reduced efficacy of antibiotic therapy has inflated the clinical challenge of bacterial infections. The proliferation of superbugs that are microorganisms resistant to multiple antimicrobial agents has elevated antimicrobial resistance (AMR) to a critical global health crisis, resulting in a minimum of 700,000 annual fatalities, a number projected to reach 10 million by 2050. With the antibiotics reservoir cornered to the remaining few last-resort antibiotics, the treatment regime is witnessing a gradual shift toward alternative therapeutics such as antimicrobial peptides (AMPs). A short AMP RIR has been identified in water buffaloes.

Water buffaloes are acclimatized to thrive in harsh tropical and subtropical climates, wet grasslands, and marshy and swampy areas. Along with their excellent milk-producing ability, buffaloes are well-known for their exceptional disease resistance. The AMP RIR was studied and proved its antimicrobial activity, but its killing mechanism is still open to debate. The objective is to investigate the interaction of RIR with lipid bilayers mimicking model membranes. Therefore, zwitterionic dipalmitoylphosphocholine (DPPC) and anionic palmitoyloleoylphosphoglycerol (POPG) lipids were chosen as models for, respectively, eukaryotic, and bacterial membranes. Infrared (IR), thermogravimetric analysis (TGA), scanning electron microscopy (SEM), and fluorescence techniques were employed. At very low peptide concentrations, IR spectra indicate that RIR adopts a major  $\alpha$ -helix structure without model membranes. An increase in the  $\beta$ -sheet structure content of RIR is observed in the presence of POPG while a random coils structure of RIR is observed in the presence of DPPC. TGA data indicates that the complex RIR-POPG is stronger than the complex RIR-DPPC. Fluorescence data indicate a decrease in fluorescence intensity and a blue shift in wavenumbers of RIR in the presence of POPG. These results indicate that RIR prefers the bacteria-mimicking negatively charged phospholipid; RIR promotes membrane disruption by forming pores in POPG. They also suggest that RIR has considerable potential for future development as a novel antibiotic drug.