ABSTRACT

Antimicrobial peptides (AMPs), known as host defense peptides are considered as promising candidates to combat emergence of multidrug-resistant bacteria. AMPs are a class of peptides that are efficient in killing most microbes and can be found in wide variety of eukaryotes. The mode of action adapted by many AMPs involves the disruption of bacterial membranes. This ability is derived from the shared fundamental motifs of AMPs: cationicity and amphipathicity. It is believed that the electrostatic interactions between the cationic AMPs and anionic bacterial membranes and AMP, followed by hydrophobic insertion, drives formation of pores which eventually leads to leakage of cellular components.

Since AMPs attack the fundamental nature of cell membranes it is difficult for bacteria to evolve resistance against them. Complex secondary and tertiary structures and difficulties in isolating AMPs, however, have limited their use as antibiotics. Short and unstructured peptides are, therefore, more desirable. It has been demonstrated that small peptides derived from full length antimicrobial peptides also show antibacterial activity. Here, we do a detailed characterization of the antibacterial activity of one such derivative of human beta defensin-3 (hBD-3): CHRG01 [*KSSTRGRKSSRRKK*]. CHRG01 is a 14 amino acid long peptide that is unstructured in solution yet shows antimicrobial activity but at much higher concentration.

To make such short peptides more effective, it is essential to understand the role played by individual amino acids within the sequence. A 'design rule' for AMPs has been studied that represents the fundamental motifs of cationicity & hydrophobicity in terms of Arginine and Lysine content, and hydrophobicity. It states that the number of arginine and lysine residues is strongly correlated to the average hydrophobicity of a peptide. Specifically, AMPs with high arginine

content require low hydrophobicity while those with low arginine content require high hydrophobicity. Using this design rule we designed two analogues of CHRG01: KSR [*KSSSRSRKSSRR*] and KLR [*KLLLRLRKLLRR*] and evaluated their antibacterial potency. We find that both these peptides are random coiled in solution and are active against bacteria. However, KLR is highly potent, displaying very low MIC values. Scanning electron microscopy and fluorescence spectroscopy results indicate that the mode of action for both peptides is by formation of membrane pores. In addition, both the customized peptides are non-cytotoxic. Being short and unstructured, KLR can potentially be mass produced relatively cheaply and its pore-forming mechanism renders it less susceptible to bacterial resistance. Therefore, KLR can be considered as a potential antibiotic for future commercial use.

For enhanced pharmacokinetic properties and chemical stability, it is desirable to develop synthetic polymer-based antibiotics that mimic AMPs. According to the design rule the arginine/lysine content and hydrophobicity of such AMPs are correlated, with lower arginine content necessitating increased lysine content and increased hydrophobicity. Therefore, we developed a series of polymers using lysine-mimicking amino ethyl methacrylamide with butyl methacrylamide to add hydrophobicity, and studied their antimicrobial activities. These polymers are found to be effective against both the bacteria at low concentrations with no toxicity to human blood cells, thereby showing significant potential to be used as antibiotics.