Thesis Title: *De novo* Design, Synthesis and Structure Activity Relation Studies on Antibiotic Peptides

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Introduction

(Abstract)

Antibiotics have played a major role towards increasing the average lifespan of humans in the early 20th century when diseases like tuberculosis plagued the human population. However, bacterial resistance mechanisms have undone the good work of these antibiotics and a significant amount of effort is now needed towards generating new classes of antibiotics. Cationic antimicrobial peptides (CAMPs) are ancient weapons of innate immunity, which are believed to act by destabilizing the bacterial membrane and affecting some intracellular targets. These peptides are found naturally throughout the living world from bacteria to humans (Bulet *et al*., 2004). Chances of bacterial resistance to these peptides are low, as restructuring the chemical composition of the bacterial membrane could prove to be a metabolically expensive process (Zasloff, 1998). For example, it takes 30 passages of *Pseudomonas aeruginosa* in sub-MIC concentrations of the antibacterial peptides like HB50, HB153 to increase resistance by two to four fold (Zhang *et al*., 2005), whereas under the same conditions, resistance to the aminoglycoside gentamycin can increase by 190 fold (Steinberg *et al*., 1997). Due to this and other beneficial features like a broad spectrum of action and rapid killing effects, antibacterial peptides serve as good templates for designing new antibiotics (Hancock and Sahl, 2006).

In addition to naturally occurring AMPs, *de novo* designed antibacterial peptides have been incorporated in the syntax of antimicrobial peptides to diversify the repertoire of arsenal against emerging pathogens. *De novo* design of antibacterial peptides represents an opportunity to generate new antibiotics (Fernandez-Lopez *et al*., 1998) and understand the physicochemical features that

define them. A limited number of *de novo* designed dimeric antibiotic peptides have also been described in literature. The dimeric design endows the peptide with a much higher antibiotic potency due to the increased effective molarity of the corresponding monomeric units (Tam *et al*., 2002).

Our laboratory has earlier *de novo* designed and synthesized, *ΔFd,* a 21 residues long lysine-branched dimeric antimicrobial peptide, which is highly potent against bacteria, broad spectrum in action and non-cytotoxic to mammalian cells (Chetal, 2005). Its dimeric design makes it 100 fold more potent and relatively more protease stable than its monomeric counterpart *ΔFm*. It includes in its sequence, α, β-didehydrophenylalanine (ΔF), a non-proteinogenic amino acid that restricts the conformational flexibility of peptides and induces the formation of 3_{10}-helical structures (Ramagopal *et al.,* 2001).

The aim of the present thesis is to elucidate the mode of action of *ΔFd* and the physicochemical requirements for its antibiotic action via structure activity relation studies. In addition, strategies for enhancing the clinical potential of antibacterial peptides by combining antibiotics with each other or by covalently linking them to other drugs have been investigated. The following is the rationale behind the work done in each chapter of this thesis:

a) Chapter 1: Till date, conventional antibiotics such as Kanamycin, Ampicillin and Vancomycin have been shown to have single targets within a bacterial cell. In contrast, antibacterial peptides have been termed “dirty drugs” as they are believed to have multiple targets in bacteria (Hale and Hancock, 2007). Previous studies from our laboratory (Chetal, 2005) (like membrane permeabilization, peptide uptake by bacteria, peptide-induced intracellular protein precipitation etc.) have suggested that *ΔFd* targets both the bacterial membrane and intracellular contents. To establish whether *ΔFd* has a membrane cum cytosolic site of action and to determine the nature of its

targets, we have further investigated the mode of action of *ΔFd* in Chapter 1. The results show that *ΔFd* targets the bacterial membrane, inhibits transcription and translation, and affects intracellular contents by binding to DNA and precipitation of cytosolic proteins in bacteria.

b) Chapter 2: Antibacterial peptides display extensive diversity in their size, sequence composition and secondary structure (Sitaram and Nagaraj, 2002). These factors have a profound impact on their antibacterial potency, selective toxicity, kinetics of action and stability against protease degradation. While a net positive charge and an amphipathic structure are considered essential for activity of CAMPs (Yeaman and Yount 2003), the complete physicochemical code for their action remains elusive. An increasing understanding of this code, has led to generation of improved analogs of naturally existing AMPs (Ahmad *et al*., 2006), however, more insights need to be gained. Chapter 2 is an endeavour to understand the physicochemical requirements for the activity of *ΔFd*. We have investigated the role of features like charge, helicity, aromaticity and method of dimerization in the activity of this peptide. The results clearly suggest that these factors not only influence antibacterial potency but also affect factors like spectrum of action, selective toxicity and killing kinetics.

c) Chapter 3: Factors like high cost of production, poor potency, toxicity and a poor half-life of these drugs have limited the success of AMPs at the clinic. Therefore, efforts must be made to optimize these peptides to make them good candidates for bacterial therapeutics. Chapter 3 explores strategies to augment the activity of antibacterial peptides by synergizing them with peptide and non-peptide antibiotics or modifying their N-terminus. In Part A we have found several benefits of synergizing peptide with other peptide and non-peptide antibiotics. This strategy is useful in lowering the dose of

the individual antibiotics, which could enhance the therapeutic index of antibiotics and make antibacterial peptide therapy more cost effective. Another strategy to augment the activity of antibacterial peptides is by the modification of their N-terminus. Previous studies have shown that the conjugation of fatty acids to the N-terminus can significantly enhance the activity of AMPs (Malina and Shai, 2005). Part B describes the design, synthesis and biological activity of *ArtR*^{3}*F*^{3}, a conjugate of Artesunate (a potent antimalarial drug) and a *de novo* designed antibacterial decapeptide *R*^{3}*F*^{3}. We have shown that while artesunate alone cannot inhibit bacterial growth, the coupling of artesunate to the N-terminus of *R*^{3}*F*^{3} enhances antibiotic activity and improves the killing kinetics of *R*^{3}*F*^{3} against *E.coli*.