INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title : Bactericidal Potency and Extended Plasma Half-life of Stereochemically Diversified Polypeptide Constructs

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SHORT ABSTRACT

Conventional peptide design only guarantees functional group constitution by opting specific amino acid sequence, and not their spatial orientation, because peptide molecules are very fluxional and its conformation is subjected to external flux. This is principally due to the isotactic stereochemistry of the peptide chain. Stereo-chemical engineering of peptide main chain offers expansion of design space of a typical sequence, possibly offering greater conformational rigidity by avoiding the 'roughness' of the folding funnel. In this thesis, we experiment with this conceptual possibility by designing amphipathic peptide systems of diversified stereochemistry, which can potentially act as bactericidal agents. We have designed three series of peptides, stepwise investigating the possibilities of such an enquiry, eventually resulting in at least half a dozen peptide molecules, qualified for future development as therapeutic agents. The principal objective of our first series of peptides was to verify, whether we can use the gramicidin helix as a template for AMP design. Gramicidin is a class of penta-decapeptides isolated from soil bacteria *Bacillus brevis*, but their utility as an antibiotic was limited to topical use due to high levels of hemo-toxicity. Activity profiles of the four de novo designed peptide variants designed in the first phase, show better efficiency in treating Gram-positive bacteria than Gram-negative variants. Significantly, our hemolytic assay confirms very low levels of hemo-toxicity for the re-designed peptides, unlike gramicidin. In the next series of peptides in the second phase, we put to test, this conceptual possibility already established in theoretical models, by designing amphipathic peptide systems and experimenting them on Gram-positive, Gram-negative and antibiotic-resistant bacteria. The unusual conformational rigidity and stability of syndiotactic peptides enable them to retain the designed electrostatic environment, while they encounter membrane surface. All the six designed systems exhibited bactericidal activity, pointing to the utility and specificity of stereo-engineered peptide systems for therapeutic applications. This phase of the work provided us important insights and useful directives in designing novel peptide systems with antimicrobial activity, by expanding the design space, incorporating D-amino acid as an additional design variable. We employed this knowledge-base in the design of next series of eight peptides, with varied electrostatic fingerprints. The phenomenal levels of anti-bacterial potency exhibited by half a dozen peptides in this series, high on specificity and less on toxicity, qualify them for next level of development as an effective therapeutic agent.

Tuberculosis is one of the leading causes of death, with an annual mortality rate of 2 million. The present treatment regimen for Mycobacterium species is strenuous, extending up to 12 months. Even then, rise of antibiotic resistance has limited the prognosis, with increased instances of multidrug resistant (MDR–TB) and extremely drug resistant (XDR–TB) cases reported. We demonstrated the bactericidal potency of cationic amphipathic peptides designed in phase two and three, as effective agents against *Mycobacterium smegmatis*. Potency of stereo-engineered LDLD or DLDL peptides have retained their potency, while their poly L variants rapidly lost their activity, when the experiment was repeated in human serum. Stability against enzymatic degradation was one of the main reasons for the underutilization of peptides, which could otherwise be a good therapeutic option with minimal side effects. Our assessment with alternating LDLD (or DLDL) stereo-chemical sequence of antimicrobial peptides, suggest that stereo-chemical engineering of designed peptide can address the most significant associated limitation of peptide based treatment regimen against bacterial infections.