Abstract

Title: Biophysical Studies of *de-novo* Designed Antimicrobial peptides: Correlation with Biological membranes

Index no.- 93/19/Life Sc./26

The fast rise in drug-resistant infections has posed a significant challenge to antimicrobial treatments. The need for new antibiotics has driven researchers to find new ways to combat pathogenic microorganism, resulting in a boom of research focusing on antimicrobial peptides (AMPs) and their therapeutic potential. While these AMPs have a few basic characteristics and structural motifs, their sequences, activity, and targets differ significantly. AMPs, in fact, are less vulnerable to bacterial resistance than standard antibiotics. These diverse functions have sparked tremendous interest in research aimed at understanding AMP activity, and various protocols have been described to assess different aspects of AMP function, such as screening and evaluating the activities of natural and synthetic AMPs, measuring interactions with membranes, optimizing peptide function, and delivering the AMPs to their target sites. NMR spectroscopy has revealed a strong association between the structure and biological properties of distinct classes of AMPs, which is being used to build novel peptide-based antibiotics. In addition to its ability to offer high-resolution structures, NMR is particularly effective in investigating dynamics, opening the door to understanding the mechanism of complicated *in situ* physiological processes. Despite substantial development over the last decade, notably in AMP optimization, their routes of action, particularly *in vivo* as well as drug delivery, remain unknown because the peptides either undergo enzymatic destruction or exhibit high toxicity towards the host cells.

In this context, the objectives of this work are divided into six chapters. (I) The first chapter covers the brief introduction of the AMPs and its therapeutic potentials in combination with nanoparticles. (II) The second chapter is focused on the structure-activity relationship (SAR) of the AMPs derived from the human autophagy 16 (Atg16) polypeptide (K5). This study looked at the influence of various N-terminal fatty acids on the activity of a truncated counterpart of human Atg16. To further test and validate the peptide's potential to enter into and interact with the bacterial membrane, an analogue with the best therapeutic index (K30) was chosen for in-depth biophysical experiments. Following that, numerous biophysical approaches are used to study the impact of newly created N-trifluoroacetyl lysine and N-thioacetyl lysine peptides (KP 13, KP 15, and KP 24) and their cell-penetrating peptide conjugates as inhibitors of bacterial sirtuins. The conjugated peptides were efficiently absorbed and shown bacterial transcription inhibition, resulting in increased antibacterial effectiveness against Gram-negative and Gram-positive pathogens. (III) The subsequent studies focused on the fungal membrane specificity and interaction of VG16KRKP, a broad spectrum, nontoxic and non-hemolytic AMP derived from dengue virus fusion protein. Generation of mutant analogues and the high-resolution NMR studies revealed that the wild type peptide solely interacts with the membranes that contains ergosterol as major sterol component along with phosphatidylethanolamine (PE) as lipid moiety. (IV-V) In order to generate more potent AMPs with anti-pseudomonas activity, a bacterial lipopolysaccharide binding "**KNKSR motif**" was introduced in a shorter analog of VG16KRKP either at N- or C-terminal to generate KG18 and VR18 peptides, respectively. These two peptides showed excellent antifungal as well as antipseudomonal activity while keeping the low cytotoxicity and hemolytic profile. The VR18 peptide showed spectacular activity against invasive *Pseudomonas* strains in both *ex-vivo* and *in vivo* set up which led this peptide a suitable candidate to be used as a potent anti-keratitis agent in future. Additionally, both the peptides showed higher efficiency in combination with tungsten disulfide quantum dots (WS_2-QD) nanoparticles. The structure-function relationship of QD conjugates was further analyzed by low-resolution spectroscopic techniques and high-resolution NMR methods. (VI) Lastly, the innate immune system's ubiquitous antimicrobial enzyme lysozyme exhibits hereditary autosomal dominant amyloidosis, which causes multi-organ dysfunction. Designing of small synthetic molecules called HK compounds showed great efficacy to inhibit the aggregation propensity of lysozyme as revealed by NMR and biophysical techniques. This study paves the way to generate new anti-amyloidosis drugs to treat neurodegenerative disorders like Alzheimer's or Parkinson's disease in future.

Sk. Abdul Mohid. 26/7/22

(Sk Abdul Mohid)

26/7/22

(Sign of the Supervisor with Seal and Date)

DR. ANIRBAN BHUNIA
Professor
Dept. of Biophysics
BOSE INSTITUTE
Kolkata, India