Transmembrane transport is of special importance for all biological processes. In particular, the transmembrane transport of small ions, for which the lipid membrane is impermeable, plays an essential role. To provide a passage for these ions through the lipid bilayer, a variety of integral membrane proteins & peptides exist and function as ion carriers, channels and pumps [1]. Ionophores are a diverse group of components which increase the permeability of membranes to ions. Cellular viability depends both on the asymmetric distribution of ions—Na⁺, K⁺, Ca²⁺ and Cl⁻—across the membrane bilayer. Ion channels in cell membranes are therefore, important for cellular functions such as controlling the internal environment, maintaining a membrane potential in nerve cells and propagating an action potential.

The bulk of work on the use of peptides to study protein-ligand interactions has been focused on the naturally occurring membrane active peptides—gramicidin A, alamethacin and mellitin. Unlike gramicidin A and alamethacin, mellitin is water soluble. The peptide antibiotics gramicidin A and alamethacin form channels across the biological membranes. Other ionophores form stoichiometric complexes with cations and act as carriers to facilitate ion transport across the lipid bilayer. These are particularly useful tools for membrane research, especially in biogenetic systems or in other cases where ion gradients are important. The ionophore-cation complexes can be specific for particular ions and can be used to manipulate the ion and voltage gradients across the membranes.

Gramicidins are linear pentadecapeptides with uncharged and hydrophobic amino acids of alternating chirality which is produced by the aerobic soil bacterium Bacillus brevis [2-4]. The amino acid sequence of the peptide is HCO-L-Val¹-Gly²-L-Ala³-D- Leu⁴-L-Ala⁵-D-Val⁶-L-Val⁷-D-Val⁸-L-Trp⁹-D-Leu¹⁰-L-Trp¹¹-D-Leu¹²-L-Trp¹³-D-Leu¹⁴-L-Trp¹⁵-NHCH₂CH₂OH. This antibiotic forms
transmembrane channels specific for monovalent alkali metal and small organic cations [5]. The simplicity of its structure makes it an experimentally approachable system for understanding the molecular basis for ion transport across biological membranes.

Structural studies of gramicidin A have demonstrated considerable environmental dependence of its conformation as well as its conformational heterogeneity in a given environment [6]. Two of the species observed in dioxane are left handed, antiparallel and right handed parallel \( \beta^{5,6} \) double helix [7]. Gramicidin A in 1:1 (methanol/chloroform) is conformationally heterogeneous but on addition of Cs\(^+\) converges to single predominant conformation, right handed antiparallel \( \beta^{7,2} \) double helix with two ions bound [8]. In ethanol, four species are seen by two dimensional NMR, two double helices differing only in hydrogen bonding register are left handed antiparallel \( \beta^{5,6} \) double helix and right handed parallel \( \beta^{5,6} \) double helix [9]. X-ray analysis of gramicidin crystallized from ethanol or methanol shows double stranded helical structures [10,11]. Till date, no crystal structure of gramicidin A in bilayer is available. Only spectroscopic methods have been used to determine its conformation in lipid environments. Urry et al. [12] has shown that ion binding site is located near Trp\(^{11}\) and Trp\(^{13}\) in \( \beta^{5,6} \) head to head dimer and argued that the channel structure is left handed \( \beta^{6,3} \) head to head dimer. Solid state NMR study of gramicidin in oriented bilayer shows high resolution structure with right handed single stranded head to head \( \beta \)-helical structure with 6-7 amino acid residues per turn. \( ^{19}\)F NMR resonance of C-terminal fluorinated gramicidin A analogs in phospholipid vesicles and those of N-fluorinated analogs can only be explained by N-terminal to N-terminal dimers [13], of right handed single stranded \( \beta^{6,3} \) was found by NOSEY evaluation of gramicidin A in SDS micelles [8]. In spite of large number of extensive studies
what conformation gramicidin adopts in forming the channel still continues to be an active area of research.

A careful look at amino acid sequence of gramicidin A reveals that

(i) all leucine residues are in D-form and only the amino acid residues with branching at $\beta$ or $\gamma$ positions are in D-form,
(ii) N-terminal contains amino acid residues with smaller side chains,
(iii) C-terminal contain repeated sequence of D-Leu-Trp.

Therefore, this study deals with the detailed conformational study of gramicidin starting from the individual amino acid residues to di-, penta-, hexa-, hepta-, octa and pentadecapeptides maintaining the alternating chirality of amino acid residues. From the knowledge of the global, local and low energy minima in the $\phi, \psi$ energy maps of respective amino acid residues, various conformational states have been generated and their energies have been computed. Computations have also been carried out for $\phi, \psi$ values corresponding to various helices. The computational studies have been carried out for the following model peptides.

(i) for model dipeptides of the form Ac-X-D-Leu-NMe (where X is Gly, Ala, Abu, Aib, Val, Leu, Ile, Phe, Tyr and Trp); also for Ac-Ala-D-X-NMe in which X is Ala, Val, Leu & Ile and Ac-Val-D-Val-NMe.

(ii) for model tripeptides of the form

a. Ac-(X-D-Leu)$_3$ (where X is Gly, Ala, Aib, Val, Leu, Ile, Phe, Tyr and Trp);

b. HCO-(Aia-D-X)$_3$-AJa-NMe (where X is Ala, Val and Ile);
c. Ac-(D-Ala-X)$_3$-NMe in which X is Phe, Tyr & Trp and
d. Ac-(Val-D-Val)$_3$-NMe.
The basis for variation of L- and D- amino acid residues is to see an appropriate combination of amino acids which may eventually help in designing of gramicidins i.e. to look for the reason why the N-terminal contains amino acid residue with smaller side chains & why the amino acid with branching at β or γ positions are accommodated in the D-form only.

It is interesting to note that the pentapeptide, HCO-Val- Gly-Ala-D-Leu-Ala-NMe corresponding to N-terminal contains only one D-amino acid residue. Therefore, its conformation and the conformation of octapeptide HCO-Val-Gly-Ala-D-Leu-Ala-D-Val-Val- D-Val-NMe have been studied separately to further assess the role of D-amino acid residues. Utilizing the knowledge of these model hexapeptides, the conformation of N-terminal of gramicidin has also been studied by making following computational mutagenesis

(i) Gly\(^2\) with D-Ala\(^2\)
(ii) Val\(^7\) with Ala\(^7\)
(iii) Gly\(^2\) and Val\(^7\) with D-Ala\(^2\) and Ala\(^7\), respectively
(iv) Val\(^1\), Gly\(^2\) and Val\(^7\) with Ala\(^1\), D-Val\(^2\) and Ala\(^7\), respectively.

The dimer formation of gramicidin has been studied by taking its most stable conformation i.e. left handed helical form for the following geometries.

(i) Head to Head
(ii) Head to Tail
(iii) Tail to Tail

The interaction of potassium ion with gramicidin A has been studied by keeping K\(^+\) at both the mouths (N- and C-terminal) separately and also by varying the position of K\(^+\) along the helical axis. It has been shown that K\(^+\) interact preferably at C- terminal. The interaction energy of gramicidin with potassium has been calculated in the presence of two K\(^+\) ions by keeping one K\(^+\) ion at C-
terminal mouth of helix and varying other K⁺ ion along the helical axis with respect to the first. Further, the pore dimensions suggest that the ions move in a single file through the helical axis.

Dimerization of gramicidin has also been studied in the presence of K⁺ ion by keeping one K⁺ at C-terminal mouth of the helix and other ion at a distance of 15Å from the first potassium ion.

The reason why PCILO results are at variance with molecular mechanics results has been looked into.

References


