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HYDROPHOBIC INTERACTIONS OF YELLOW WASP TOXIN PEPTIDES WITH ION CHANNELS AND ITS EFFECT ON INTEGRITY OF BIOLOGICAL MEMBRANES

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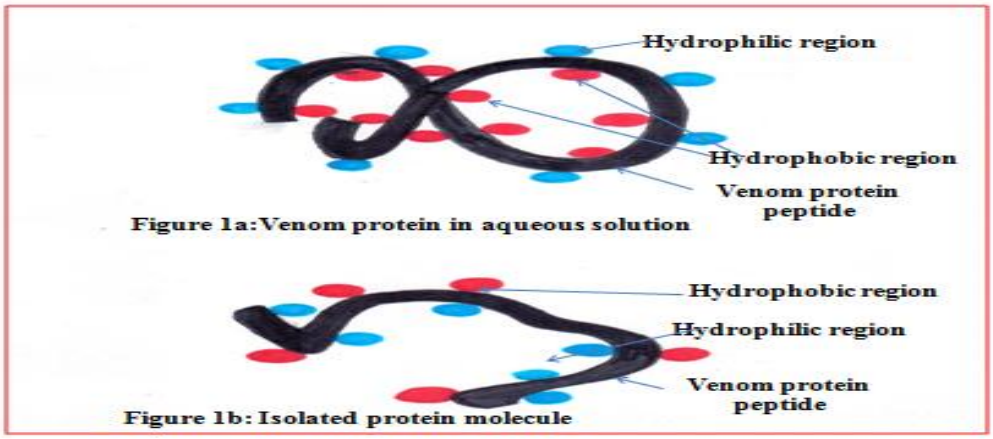
ABSTRACT

Present review article explains hydrophobic interactions that form during binding of toxin or toxin peptides to ion channels or activated ionic pumps which actively maintain ionic concentration across the plasma membranes. Wasps venom toxins mainly, anoplin a short peptides after binding with a receptor proteins, changes its confirmatory structure. Wasp toxin peptides maintain hydrophobic versus electrostatic interactions with binding to lipid bilayer. These structural changes increase antimicrobial and hemolytic activity, and lipid interactions. Peptides of the mastoparans family showed antimicrobial effects due to presence of net positive charge, amphipathicity and hydrophobicity. Other factors such as peptides length, amino acid substitutions at different positions of the peptide chain, N-terminal acylation and C-terminal deamidation also contribute its interaction with ion channels. Wasp venom toxins such as melittin, phospholipases-A_2, hyaluronidases and other bioactive substances directly interacts with cell membranes and disrupt its integrity and generate immune allergic responses. These biological interactions of membrane-peptides complexes are important to analyze both the dynamic properties and pharmacological activities of toxins in solution.

KEYWORDS: Hydrophobic interactions, amphipathicity, transportans-10, Mastoparan-X, G-proteins, *Polistes flavus*, *Anoplius samariensis* and Anoplin.

INTRODUCTION

Hydrophobic interactions display relationship between water and hydrophobes (e.g. low water soluble molecules). Hydrophobic interactions are important for the folding of proteins and other macromolecules. This is important in keeping a protein alive and biologically active because it allowed protein to decrease its surface area and reduce the undesirable interactions with water (Figure 1a and 1b). Hydrophobic interaction is due to the action of Host-Guest mechanism by forming the hydrophobic cavity (Figure 2). There are five different types of bonds are responsible for folding the protein molecules viz., hydrophobic interaction, ionic bonds, hydrogen bonds, disulphide bonds and peptides bonds. These all bonds are responsible for the folding of protein molecules and functionally become active the protein as well as other macromolecules (Figure 3a & 3b). Besides, proteins there are many other biological substances that rely on hydrophobic interactions for its survival and functions, like the phospholipids bilayer in every cell of the body. Their binding depends on carbon chain branching. Antibacterial susceptibility of toxin peptides also depends on hydrophobicity and position of various amino acids substituted.



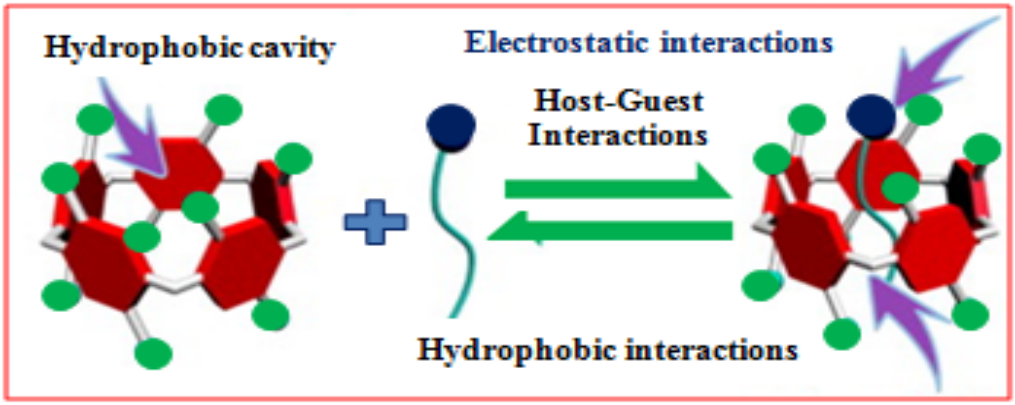


Figure 2: Host-Guest interaction.

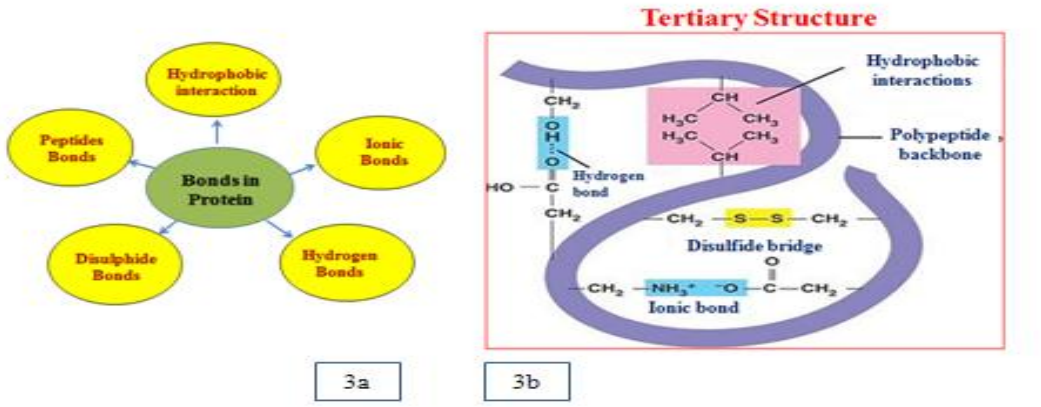


Figure 3a and 3b: There are five different types of bonds viz., hydrophobic interactions, ionic bonds, hydrogen bonds, disulphide bonds and peptides bonds helps of folding the protein molecules provide tertiary structure.

Anoplin, the short peptide works against bacterial membranes similar to hemolytic activity occurs in blood cells.[1] Slight modifications in cationic α-helical antimicrobial peptides (AMPs) structure enhance its activity and selectivity.[2] Such modifications or structural changes increase antimicrobial and hemolytic activity of toxins. It promotes lipid interactions which finally improve therapeutic index of toxin peptides against so many strains of bacteria.[3] For framing good interaction of toxin with the bacterial membranes all possible substitutions are to be made in the active site region.[2] Thus, strong hemolytic activity could be achieved by subtle increase in the hydrophobicity of the hydrophobic face or increase in the polarity of the hydrophilic face of the helixes or-most efficiently a combination of both are possible. Few toxins peptides such as PepFect3 (PF3) and PepFect6 (PF6), together with their parental (CPP) cell penetrating peptides transportan-10 show major interactions with lipid membranes.[4] Transportan-10 (Tp10) preferentially binds to the membrane surface with its hydrophobic side facing the hydrophobic lipid core. Such orientation allows Lys residues, with positively charged long side chains, to stay in the polar environment during the insertion process.[5]

Four new peptides of the mastoparan family are characterized recently in the venom of three neotropical social wasps, *Polistes major major, Polistes dorsalis dorsalis* and *Mischocyttarus phthisicus.* These peptides have shown hemolytic, mast cell degranulation activities and antimicrobial potency against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli (E.*

coli), *Pseudomonas aeruginosa*, *Salmonella typhi* and *Vibrio cholera*.[6] Toxin peptide analogues also showed strong antimicrobial activity due to their positive charge, hydrophobicity, amphipathicity, peptide length, amino acid substitutions at different positions of the peptide chain, N-terminal acylation and C-terminal deamidation.[7]

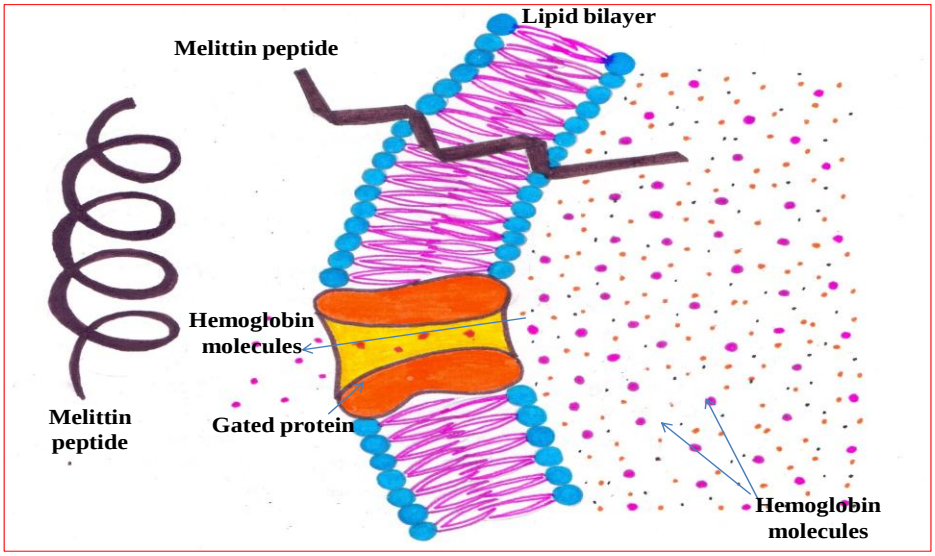


Figure 4: Hydrophobic interaction of the melittin peptide with the RBCs membrane.

Yellow wasp *Polistes flavus* venom contains melittin peptides which are alkaline in nature and exhibits amphipathic properties which allow it to interact with lipid membranes and causes the increases the permeability in RBCs and other cell membranes.[8] Wasp venom peptides melittin strongly acts upon bacterial cell membranes and interacts with RBCs cell membranes and disrupts its integrity and increases the diffusion of molecules from the cells.[9] Wasp venom melittin, phospholipases-A_2, hyaluronidases and other bioactive substances directly interacts with cell membranes and disrupt its integrity and generate immune allergic responses (Figure 4). The wasp venom phospholipase-A_2 is a membrane bound phospholipids containing enzymes that are important for production of the arachidonic acid.[10]

Interaction of toxins with protein receptors or protein-protein

Mastoparan are low molecular weight tetradecapeptides, are rich in hydrophobic and basic residues. After interaction, mastoparan peptide shows antimicrobial, mast cell degranulation

and hemolytic activities.[11] Some mastoparans peptide extracted from social wasps venom display antimicrobial activity and some are hemolytic and cytotoxic. These peptides possess net charges and their hydrophobicity contributes to modulate their biological activities. Mastoparan peptide interacts with the lipid bilayer by using its hydrophobic side; located within the lipid bilayer. Cross-saturation method is useful for determining the interface of not only protein-protein but also membrane-peptide complexes.[12] These also evoke catecholamine release, and inhibit secretory response upon nicotinic stimulation in adrenal chromaffin cells. MP fragments selectively inhibit the secretory response to nicotinic stimulation by attacking nAChR on the sites made up of hydrophobic and acidic amino acids but other than ACh-binding sites. These MP fragments inhibited catecholamine release induced by nicotinic stimulation in a noncompetitive manners.[13] Mastoparan-X, a tetradecapeptides isolated from wasp venom, activate GTP-binding regulatory proteins (G-proteins) that couple to phospholipase-C. It is only possible after interaction with membrane-bound G-proteins and their activation.[14] Mastoparan-X found in the presence of perdeuterated phospholipids vesicles, its analogs work as activators of G-proteins.[15] It also activates G-protein mediated mechanisms which stimulate the phospholipase enzymes that affects the mitochondrial membrane permeability and Ca^{++} mobilization from the mitochondrial matrix (Figure 5). Mastoparan induce the apoptosis by Ca^{++} release from intracellular release stored via PLC and IP3 and disruption of plasma membrane integrity occur secondary.[16]

By using computational mutagenesis, new mastoparan analogs are obtained, which show significant antimicrobial activity compared to the parent compounds.[17] Some mastoparans peptides extracted from social wasps display antimicrobial activity and some are hemolytic and cytotoxic in nature. More specifically, acidic residues play an important role in modulating the peptides that has increased the lytic and biological activities.[11] Activation of mast cells by mastoparan, at least two positively charged side chains are required on the hydrophilic side of the amphiphilic structure of the peptides.[18]

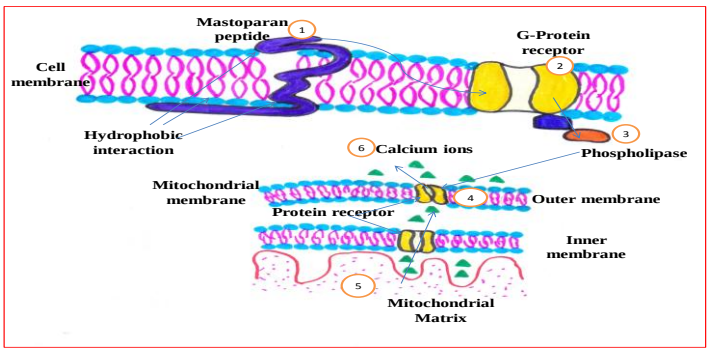


Figure 5: Mastoparan peptide hydrophobically interact with biological membrane and induce their action of mechanism.

More specially, net charges and hydrophobicity found in toxin peptides contribute to modulate their biological activities. The positively charged residues as well as that of the hydrophobic side chains appear to be of fundamental importance.[19] The role of hydrophobic interactions in the binding process has also been examined by using 8-anilino-1-naphthalene-sulphonic acid (ANS). These results have been employed to rationalize the energetic consequences of the binding reactions.[20]

Membrane-peptide interactions are involved in many crucial biological and pharmacological activities. The modes of interactions of membrane-peptides complexes are important to analyze both the dynamic properties and the contact residues of the membrane-bound peptide. For investigation of dynamic properties of a peptide bound to a lipid bilayer, using relaxation and amide-water exchange analyses, are used these directly determined the membrane-peptide interface, using the cross-saturation method.[7] For the models of a lipid bilayer and a peptide, isotropic bicelles and mastoparans were used, respectively. The results Mastoparansmastoparans show a heterogeneous distribution of motion over various time scales and interacted with the lipid bilayer by using its hydrophobic side.[12]

Antimicrobial peptides (AMPs) have shown a very high antimicrobial potential against multi-resistant bacteria. Anoplin is a decapeptide amide, GLLKRIKTLL-NH2 derived from the venom sac of the solitary spider wasp, *Anoplius samariensis* and are active against Gram-positive and Gram-negative bacteria and is not hemolytic towards human erythrocytes. The antibacterial activity and selectivity of the analogues against *S. aureus* and *E. coli* varied considerably, depending on the hydrophobicity and position of the various substituted amino acids. In certain cases the selectivity for Gram-positive and Gram-negative bacteria was

either reversed or altogether eliminated. In addition, it was generally found that antibacterial activity coincided with hemolytic activity.[1] Amino acid substitutions are made for improvement of antimicrobial activity without concomitant introduction of strong hemolytic activity. This could be achieved through subtle increases in the hydrophobicity of the hydrophobic face or through subtle increases in the polarity of the hydrophilic face of the helix, or-most efficiently-a combination of both.[2]

Decapeptide anoplin and 19 analogs showed antimicrobial and hemolytic activities against methicillin-resistant *Staphylococcus aureus* ATCC 33591 (MRSA), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), vancomycin-resistant *Enterococcus faecium* (ATCC 700221) (VRE), and *Candida albicans* (ATCC 200955). The anoplin analogs contain substitutions in amino acid positions 2, 3, 5, 6, 8, 9, and 10. Both 2Nal(6) and Cha(6) show improved therapeutic index against all strains tested.[3] The lipophilic amino acids, (S)-2-aminoundecanoic acid, the incorporation of a lipophilic amino acid residue into anoplin enhanced the antimicrobial activity, while selectivity towards microbial membranes was retained.[21]

Mastoparans analogs and their derivatives showed antimicrobial activity against *Bacillus subtilis* due to hydrophobicity, hydrogen bond donor and steric properties in membrane environment (Sodium, Potassium, Chloride)[17] and few of them are chemo-tactic peptides. Mastoparans, tachykinins, kinins, antibiotic peptides and a group of long peptides with one or two disulfide bonds. The partial overlap between the mastoparans group and the chemo-tactic peptides, tachykinins, kinins and antibiotic peptides show unique matching in activities in biological systems.[22] The PepFect family of cell-penetrating peptides (CPPs) is used to deliver nucleic acids across plasma membranes.[23] Here, Lys-phosphate salt bridge is a key factor in determining the orientation of the peptide in the interfacial region as well as in stabilizing the peptide-membrane interaction. The electrostatic attraction between Lys and phosphate groups is found to be main bottle neck for the translocation of Tp10 across the membrane.[5]

Mastoparan-X, a tetradecapeptides isolated from wasp venom, interact to G-proteins (GTP-binding regulatory proteins) that couple to phospholipase-C.[14] Mastoparan analogs show receptor-G-protein interactions.[15] In this, process hydrophobic interactions play important role.[20] Mastoparan peptides adopt alpha-helical conformation in anisotropic environments.[7]

Their cytoplasmic domains of multi spanning receptors interact with guanine nucleotide that binds to G-proteins. Moreover, differential regulation of G-protein activity in cellular functions.[19,15] The relative orientation of the positively charged residues as well as that of the hydrophobic side chains appear to be of fundamental importance. Few amphiphilic peptides completely inhibit the G-proteins intrinsic GTPase activity. Toxin peptide when bound to a lipid bilayer by using its hydrophobic side.[7] Protonectin is a (ILGTILGLLKGL-NH2), peptide extracted from the venom of the wasp *Agelaia pallipes pallipes.* It promotes mast cell degranulation activity, antibiosis against Gram-positive and -negative bacteria, and chemo-taxis in polymorpho-nucleated leukocytes.[24] Cross-saturation method is used for determining the interface of not only protein-protein but also membrane-peptide complexes.[12]

Toxin and lipid interactions

For membrane interaction toxins peptides need aqueous environment for aggregation. These peptide complexes are held together by hydrophobic interactions and hydrogen bonds. Molecular determinants of the peptide/lipid inter-relationships and structural intrinsic properties of tilted peptides can explain the influence of CPP on the bilayer organization.[25] Mere changes in efflux rate reflect the differences in the thermodynamics of binding and insertion of the free and amidated peptide groups.[26] Further, interactions between peptide antimicrobials and phospholipids bilayer can elucidate role of the lipids in cell-penetrating and antimicrobial peptides.[27] Membrane-peptide interactions are involving in many crucial biological and pharmacological activities. Their modes of interactions of membrane-peptides complexes are important to analyze both the dynamic properties and the contact residues of the membrane-bound peptide. Ves v 5 is a major allergens found in yellow-jacket venom which shows varying extents of cross-reactivity in patients if bitten by other insect species. It is highly useful for the development of a vaccine for treatment of insect allergy.[28]

The Physical properties of membranes, such as fluidity, charge or curvature influence their function. Proteins and peptides can modulate those properties and conversely, the lipids can affect the activity and/or the structure of the former. Crystals of troponin-C are stabilized by an intermolecular interaction that involves the packing of helix-A from the N-terminal domain of one molecule onto the exposed hydrophobic cleft of the C-terminal domain of a symmetry related molecule. The contact region of mastoparan as it might be bound to the two Ca^{++} binding proteins. Interaction in troponin-C suggests a possible mode for the binding of

amphiphilic helical molecules to troponin-C and to calmodulin. A possible binding mode of melittin to calmodulin is also proposed. Although some of the characteristics of binding are similar for the two amphiphilic peptides, the increased length of melittin requires a significant bend in the calmodulin central helix similar to that suggested recently for the myosin light chain kinase calmodulin binding peptides.[29] Not only hydrophobic interactions are important in this model, but there are several favorable electrostatic interactions that are predicted as a result of the molecular modeling. The regions of troponin-C and calmodulin to which amphiphilic helices binds are similar to the regions to which the neuroleptic drugs such as trifluoperazine have been predicted to bind.[30] The interaction between PDI and the bound peptide therefore is enhanced by the formation of mixed disulphide bonds.[31]

CONCLUSION

In wasp venom, peptides of the mastoparan family showed biological activities, especially antimicrobial, with the net positive charge, hydrophobicity, amphipathicity, peptide length, amino acid substitutions at different positions of the peptide chain, N-terminal acylation and C-terminal deamidation. Wasp venom toxins interact with the lipid bilayer by using its hydrophobic side; of Gram-positive and Gram-negative bacteria. Epitopic interactions of toxin peptides take place to different amino acids with a different force of attraction that make diverse biological functions of the peptides found in yellow jackets, hornets, and paper wasps. These act as different antigens and show cross reactivity in biological. Interaction in troponin-C suggests a possible mode for the binding of amphiphilic helical molecules to troponin-C and to calmodulin. In antimicrobial activity of peptide toxins from wasps is due their molecular properties such as hydrophobicity, hydrogen bond donor and steric. These are correlated with contributions from the membrane environment maintained due to sodium, potassium, chloride ions whose presence specify action of channels, receptor binding and ligand gated transport of toxins through membrane. Hydrophobic interactions play important role in action of wasp toxins on biological membranes which affect their integrity and permeability.

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