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Review

Cysteine-free peptides in scorpion venom: geographical distribution, structure-function relationship and mode of action

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Scorpion venoms are well known sources of Na^+-channel, K^+-channel, Cl^--channel, Ca^{2+}-channel and ryanodine channel selective peptides. In 1993, the first cysteine-free peptide was isolated from scorpion venom. Within the last six years, cysteine-free peptides with and without antimicrobial activity have been isolated from scorpion venom. The first antimicrobial peptides being parabutoporin and hadrurin, after which nine more have followed. Characteristics of these peptides include pore-formation and/or antimicrobial activity. Six peptides of similar structures without antimicrobial activity have also been isolated. Two of these peptides have bradykinin-potentiating functions. The functions of the other four are unknown. These peptides have the potential to combat cancer, a variety of skin or wound bacterial and fungal infections. This review will focus on the primary and secondary structures as well as reported functions and applications of the cysteine-free peptides identified in scorpion venom.

Key words: cysteine-free peptides, scorpion venom.

INTRODUCTION

Scorpion venom is a complex mixture composed of a wide array of substances. It contains mucopolysaccharides, hyaluronidase, phopholipase, relative low molecular mass molecules like serotonin, histamine, protease inhibitors and histamine-releasers. It is also a rich source of toxic polypeptides that affect ion channel function of excitable and non-excitable cells (Simard and Watt, 1990; Possani et al., 2000). It has been proposed that 100 000 different peptides exist in the 1500 distinct species around the world, of which only 0.02% are known to interact with ion channels (Possani et al., 2000). To date there are references of 191 Na^+- channel (Rodríguez de la Vega and Possani, 2005), 121 K^+-channel (Rodríguez de la Vega and Possani, 2004), 5 Cl^--channel (Debin et al., 1993), 2 Ca^{2+}-channel (Possani et al., 2000) and 2 RYR channel selective peptides (Valdivia and Possani, 1998).

Cysteine-free antimicrobial peptides (AMP) have also been identified and characterized from the venom of six scorpion species. These peptides have the ability to integrate with mammalian and bacterial membranes and form transmembrane pores (Verdonck et al., 2000; Moerman et al., 2002).

Cysteine-free non-antimicrobial peptides (NAMPs) have also been isolated from scorpion venom. These peptides have either bradykinin-potentiating activity (Ferreira et al., 1993; Meki et al., 1995) or the activity of the peptides is simply unknown at present (Ali et al., 1998). Most of the effort in the discovery of new peptides in scorpion venom has been focused on ion channel tox-

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ins.

However, in the past few years, peptides with unique cysteine-free structures and antimicrobial activities have been isolated from the venom of various scorpion species found in areas of Africa, South America and Asia. The mode of action and structure-function relationship of AMPs and NAMPs isolated from different scorpion venom are discussed.

CYSTEINE-FREE Amps IN SCORPION VENOM

Parabutoporin (PP), from the venom of *Parabuthus schlechteri* (Verdonck et al., 2000) and hadrurin, from the venom of the scorpion *Hadrurus aztecus* (Torres-Larios et al., 2000) were the first cysteine-free AMP purified and characterized. To date eleven of these peptides have been identified and characterized (Table 1). The AMPs from scorpion venom consist of 13 to 50 amino acids and the molecular mass range from 1463.92 Da to >5030 Da. Six of these peptides include a common primary sequence of $Sx_3KxWxSx_5L$ and the common primary sequence of Gx_2Wx_2IKS has been reported in five of the peptides ("x" represents uncommon amino acids) (Moerman, 2002).

East and West African scorpions

Pandinus imperator (CL Kock, 1841) (family: Scorpionidae Latreille, 1802). Two antimicrobial peptides, namely the 44 amino acid pandinin 1 (Pin1; 4799.2 Da) and 24 amino acid pandinin 2 (Pin2; 2612.6 Da) have been isolated and characterized to date. In aqueous solutions these peptides have an unordered structure, but an α-helical structure is obtained experimentally in membrane-mimicking environment such as phosphate buffer solution (PBS) and dodecylphosphocholine (DPC). Pin1 was shown to contain 2 α-helical regions (residues 3-14 and 20-39), separated by a random coil region (WSSEP) including a proline residue at position 19 causing a break in the α-helical structure. Helical wheel diagrams of residues 3-14 and 20-39 of Pin1 and the entire Pin 2 have shown that both peptides are amphipathic, with hydrophobic and hydrophilic residues on opposite sides of the helices (Corzo et al., 2001).

The inhibition of gram-positive bacterial growth was greater than that of gram-negative bacteria (Corzo et al., 2001). Pin1 and Pin 2 showed MICs of 1.3 – 5.2 µM and 2.4–4.8 µM against the gram-positive bacteria tested, respectively. Against the gram-negative bacteria tested, Pin1 and Pin 2 showed MICs of >20.8µM and 19.1 – 38.2 µM, respectively (Corzo et al., 2001).

Only Pin 2 hemolysis of sheep erythrocytes, demonstrating its ability to interact not only with charged phospholipids, but also with zwitterionic membranes (Corzo et al., 2001; Belokoneva et al., 2003).

Pin1 and Pin 2 have also been reported to induce calcein leakage from small unilamellar vesicles (SUV) of different phosphatidylcholine (PC) / sphingomyelin (SM) ratios. SUVs of PC and a PC/SM ratio of 1 showed pore-formation induced by both peptides. Increasing the SM content to a PC/SM ratio of 0.25 decreased the pore-forming activity of Pin1. The proposed reasoning for this occurrence is that the increased SM caused a strengthening effect of the SUV and restricted the insertion of Pin1. This agrees with the hemolytic activity of Pin1 which is higher towards guinea pig erythrocytes, having a much higher PC/SM ratio (Belokonova et al., 2003). As Pin1 and Pin2 are of adequate length to span the entire membrane it has been proposed that transmembrane pores are formed via the 'barrel-stave' or 'toroidal-pore' model (Belokonova et al., 2003).

Southern African (South Africa and Madagascar) scorpions

Parabuthus schlectheri (Purcell, 1899) (family: Buthidae C.L. Koch, 1837)

Parabutoporin (PP) is an AMP isolated and characterized from the venom of *P. schlectheri*. It has a molecular mass of 5030 Da. The most remarkable characteristic is the high lysine content (11) and the amount of charged residues (17 in total). The positive (11 lysine and 1 arginine) and negative (1 aspartate and 4 glutamate) charges are located at opposite ends of the molecule (Verdonck et al., 2000). The peptide conforms to an α-helical secondary structure in the presence of secondary structural-promoting environment (Willems et al., 2002). The helix wheel projection also indicates an amphipathic α-helix character for a majority of the peptide that stretches across the membrane (residue 11-35), with the polar hydrophilic and apolar hydrophobic amino acid side chains positioned on opposite sides of the helix (Verdonck et al., 2000).

Activity of PP has been investigated on a variety of cell types (Verdonck et al., 2000; Willems et al., 2002; Moerman et al., 2002; Moerman et al., 2003). The cationic, amphipathic α-helix structure allows for easy interaction with lipopolysaccharides of eukaryotic cells and, therefore, PP was initially characterized as a pore-forming peptide. The existence of leak currents originating in rat dorsal root ganglion cells (Verdonck et al., 2000) and cardiac myocytes (Du Plessis, 1999) is indicative of this property. The pores formed by PP are between 1.38 nm and 1.78 nm in diameter, allowing for non-selective trafficking of cations and anions (Elgar et al., 2006a). This movement of ions causes depolarization of the membranes of cadiomyocytes and a neuroblastoma cell line (Elgar et al., 2006b). The cationic structure also allows for interaction with the negatively charged outer membranes of bacteria, thus acting as a novel class of antimicrobial peptides working at micromolar concentrations (Moerman et al., 2002; Willems et al., 2002).

Table 1. Primary and secondary structures of the cysteine-free (A) AMPs, (B) NAMPs and (C) peptides of unknown function isolated from scorpion venom.

A	Primary / secondary structure	MW	References
parabutoporin	FKLGSFLKKA$_{10}$WKSKLAKKLR$_{20}$AKGKEMLKDY$_{30}$AKGLLEGGSE$_{40}$EVPGQ #	5030.0 Da	Verdonck et al., 2000; Moerman et al., 2000
	cchhhhhhhh$_{10}$hhhhhhhhhh$_{20}$hhhhhhhhhh$_{30}$hhhhh?cccc$_{40}$ccccc		
opistoporin 1	GKVWDWIKST$_{10}$AKKLWNSEPV$_{20}$KELKNTALNA$_{30}$AKNLVAEKIG$_{40}$ATPS # ▲	4833.6 Da	Moerman et al., 2000
	cchhhhhhhh$_{10}$hhhhccccch$_{20}$hhhhhhhhhh$_{30}$hhhhhhhhhc$_{40}$cccc		
opistoporin 2	GKVWDWIKST$_{10}$AKKLWNSEPV$_{20}$KELKNTALNA$_{30}$AKNFVAEKIG$_{40}$ATPS # ▲	4870.0 Da	Moerman et al., 2000
	cchhhhhhhh$_{10}$hhhhccccch$_{20}$hhhhhhhhhh$_{30}$hhhhhhhhhc$_{40}$cccc		
hadrurin	GILDTIKSIA$_{10}$SKVWNSKTVQ$_{20}$DLKRKGINWV$_{30}$ANKLGVSPQA$_{40}$A #	4435.6 Da	Torres-Larios et al., 2000
	hhhhhhhhh$_{10}$h hh$_{20}$hhhhhhhhhh$_{30}$hhhhhhhhhh$_{40}$h		
pandinin 1	GKVWDWIKSA$_{10}$AKKIWSSEPV$_{20}$SQLKGQVLNA$_{30}$AKNYVAEKIG$_{40}$ATPT # ▲	4799.2 Da	Corzo et al., 2001
	ccchhhhhhh$_{10}$hhhhccccch$_{20}$hhhhhhhhhh$_{30}$hhhhhhhhhc$_{40}$cccc		
pandinin 2	FWGALAKGAL$_{10}$KLIPSLFSSF$_{20}$SKKD	2612.6 Da	Corzo et al., 2001
	chhhhhhhhh$_{10}$hhhhhhhhc?$_{20}$cccc		
IsCT	ILGKIWEGIK$_{10}$SLF ▲	1501.9 Da	Dai et al., 2001
	(>90% α-helical structure)		
IsCT2	IFGAIWNGIK$_{10}$SLF ▲	1463.9 Da	Dai et al., 2002
	(>90% α-helical structure)		
BmKbpp	FGSFLKKV$_{10}$WKSKLAKKLR$_{20}$SKGKQLLKDY$_{30}$ANKVLNGPEE$_{40}$EAAAPAERRR$_{50}$ # (proposed to be α-helical)	UN	Zeng et al., 2000
BmKn2	FIGAIARLLS$_{10}$KIF	UN	Zeng et al., 2004
	chhhhhhhhh$_{10}$hcc		
BmKb1	FLFSLIPSAI$_{10}$SGLISAFK	UN	Zeng et al., 2004
	chhhhhhhhh$_{10}$hhhhhhcc		
B			
Peptide T	KKDGYPVEYD$_{10}$RAY	-	Ferreira et al., 1993
	(100% coiled structure)		
Peptide K12	LRDYANRVIN$_{10}$GGPVEAAGPP$_{20}$A	2137.3 Da	Meki et al., 1995
	(α-helical and random coiled structures at the N- and C-terminal respectively)		
C			
Bs10	VTMGYIKDGD$_{10}$GKKIAKKKNK$_{20}$NGRKHVEIDL$_{30}$NKVG	3784.9 Da	Ali et al., 1998
	(25% α-helical, 37% β-sheet and 38% coiled structure)		
BmKn1	FIGAVAGLLS$_{10}$KIF	UN	Zeng et al., 2004
	chhhhhhhhh$_{10}$hcc		
BmKa1	GESEENEEGS$_{10}$NESGKSTEAK$_{20}$NTDASVDNED$_{30}$SDIDGDSD	UN	Zeng et al., 2004
	ccccccccccc$_{10}$ccccccccccc$_{20}$ccccccccccc$_{30}$cccccccc		
BmKa2	ASMDNSDD$_{10}$ALEELDNLDL$_{20}$DDYFDLEPAD$_{30}$FVLLDMWANM$_{40}$LESSDFDDME$_{50}$	UN	Zeng et al., 2004
	cccccccchh$_{10}$hhhhhhcccc$_{20}$ccccccccccc$_{30}$chhhhhhhhhh$_{40}$hhcccccccc$_{50}$		
IsCTf	ILGKIWEGIK$_{10}$S	UN	Dai et al., 2002
	(randomly coiled structure)		
IsCT2f	IFGAIWNGIK$_{10}$S	UN	Dai et al., 2002
	(randomly coiled structure)		

Linear, α-helical peptides have the common sequencing of *Sx$_3$KxWxSx$_5$L* (#) and/or *Gx$_2$Wx$_2$IKS* (▲). (c- coil; h- helix; ?- uncertain; MW- molecular weight; UN- unknown).

PP is most active in inhibiting the growth of gram-negative bacteria (MIC 1.6-6.3 μM) over gram-positive bacteria (6.3 - >50 μM) (Moerman et al., 2002). The large polar portion of the helix, high positive charged and extended angle subtended by the positively charged residues could probably explain the profound activity towards gram-negative over gram-positive bacteria (Moerman et al., 2002).

PP has also an effect on the intracellular Ca^{2+} concentration of granulocytes in the presence and absence of extracellular Ca^{2+}, indicating that pore-formation as well as G-proteins are involved in the release of Ca^{2+} from intracellular stores (Moerman et al., 2003). This peptide has also been reported to inhibit (by suppression of NADPH oxidase via a Rac activation pathway) and activate human granulocytes (by stimulating exocytosis and chemotaxis) (Willems et al., 2002).

Opistophtalmus carinatus (Peter, 1861) (family: Scorpionidae Latreille, 1802)

Two 44 amino acid residue peptides, namely opistoporin 1 (OP1) and 2 (OP2) have been isolated from the southern African scorpion specie *O. carinatus* of molecular masses of 4833.6 and 4870 Da, respectively. The only difference being different in amino acid residues at position 34 (leucine in opistoporin 1 and phenylalanine in opistoporin 2) (Table 1) (Moerman et al., 2002). These peptides contain 12 charged residues (8 lysines, 3 glutamates and 1 aspartate) and have a +4 net charge. Both have α-helical secondary structure in a phosphorlipid-mimicking environment. Opistoporins contain 2 α-helical domains (residue 3-14 and 20-39) separated by a random coiled region (WNSEP). OP1 also possesses hydrophobic and hydrophilic residues on opposite sides of its helical wheel diagram (residues 20-37), indicating an amphipathic nature (Moerman et al., 2002).

Similar to PP, OP1 has a variety of functions including pore-formation, antimicrobial activity and interaction with G-proteins although higher concentrations are required (Elgar et al., 2006; Moerman et al., 2002; Willems et al., 2002; Moerman et al., 2003).

Opisthacanthus madagascariensis (Kraepelin, 1894) (family: Ischnuridae Pocock, 1896)

Two peptides, IsCT and IsCT2, were isolated and characterized from the crude venom of *O. madagascariensis*. The peptides have molecular masses of 1501.9 and 1463.9 Da, respectively. IsCT is composed of 13 amino acid residues and enriched with hydrophobic (3 isoleucine, 2 leucine) and basic amino acids (2 lysine). IsCT2 has 78% homology with IsCT and differs only by the replacement of lysine at position 4 with arginine and glutamate at position 7 with aspartate (Table 1). Both peptides have an amphipathic α-helical secondary structure in the presence of secondary structural promoting solution (60% TFE) and the arrangement of the hydrophobic and hydrophilic residues are on opposite sides of the α-helical structure (Dai et al., 2001; Dai et al., 2002).

Growth inhibition of gram-positive (MIC of 1 - 25 µg/ml) and gram-negative (MIC of 5 - 200 µg/ml) was observed with both peptides, although a slight preference towards gram-positive inhibition was seen (Dai et al., 2002). IsCT and IsCT2 are both hemolytic towards sheep erythrocytes, having a HD_{50} of 50 - 75 µM (Dai et al., 2001). IsCT2 is more hemolytic than IsCT, but less hemolytic than Pin2.

IsCT and IsCT2 showed the leakage of calcein from SUVs composed of PC and SM. Both peptides showed more efficacies in SUVs of PC/SM ratio of 1 than SUVs of only PC or SM (Belokonova et al., 2003). IsCT and IsCT2 are shorter peptides when compared to Pin1 and Pin2. Therefore a different pore-forming mechanism is proposed whereby the shorter peptides cause pore-formation in a 'detergent-like' ('carpet' model) manner. The shorter peptide monomers would be too short to span the entire membrane limiting the formation of transmembrane pores. Therefore, these peptides would have to cover the membrane surface in a 'carpet-like' manner causing membrane permeability in a 'detergent-like' manner (see 1. Introduction) (Belokonova et al., 2003).

South American (Mexico) scorpion

Hadrurus aztecus (Pocock, 1902) (family: Iuridae Thorell, 1876)

The venom of *H. aztecus* contains the peptide hadrurin which accounts for ~1.7% of the total venom protein. It has a molecular mass of 4435.6 Da and 41 amino acid residues in its primary sequence (Table 1). There are 7 basic amino acid residues, 3 of which are grouped as a triplet of sequence lysine-arginine-lysine. Amino acid residues 1 - 11 and 18 - 41 indicate α-helical structures with the hydrophobic and hydrophilic residues on opposite sides of the helix (Torrs-Larios et al., 2000).

Hadrurin inhibited gram-positive (MIC < 10 µM) and gram-negative bacteria (MIC > 40 µM). The peptide is hemolytic with a HD_{80} of 20 µM (Torres-Larios et al., 2000).

The lytic activity of hadrurin was also investigated against zwitterionic-mimicking phosphatidyl-choline (PtdCho) membranes and membranes of an acidic nature (phosphatidyl-serine (PtdSer) plus PtdCho). Hadrurin has the ability to lyse the zwitterionic membranes at low concentrations whereas a higher peptide/lipid ratio is required to achieve similar effects in the acidic membranes (Torres-Larios et al., 2000). It is postulated that hydrophobic interactions could be responsible for the interactions between hadrurin and the membrane, where the presence of the phosphocholine promoted the oligomerization of the peptide monomers (Torres-Larios et al., 2000).

Asian (China) scorpion

Buthus martensii (Karsch, 1879) (family: Buthidae C. L. Koch, 1837)

A full-length cDNA encoding the precursor of a peptide, BmKbpp, is found in the venom of *B. martensi* (Karsch,

1879) and is similar to the amino acid sequence of a bradykinin-potentiating peptide (peptide K-12) from the scorpion *B. occitanus* (Zeng et al., 2000). The cDNA encodes a precursor of 72 amino acid residues, including a signal peptide of 22 residues and an extra R-R-R tail at the C-terminal end of the precursor. During the cloning process these residues were removed to give rise to a 47 amino acid peptide (Table 1). The amino acid sequencing has 61.7% homology with PP (Moerman, 2002). Literature concerning this peptide is limited and no helical wheel or circular dichroism spectra exists at this stage, but it is predicted that this peptide will be highly α-helical and linear in nature because of the absence of cysteine residues.

The 18 amino acid residue BmKb1 and 13 amino acid residue BmKn2 show distinct hydrophobic and hydrophilic regions, making both peptides markedly amphipathic. BmKb1 has a +1 net charge and possesses one α-helical domain (residues 2-16) and two random coiled regions (residues 1 and 17 - 18) at both terminal ends. Similarly, BmKn2, of +2 net charges, possesses an α-helical region (residues 2 - 11) with two random coil regions at residue 1 and 12-13 (Zeng et al., 2004). Based on primary structures, BmKn1, 2, IsCT and IsCT2 forms a distinct group of peptides, with 89 and homology between BmKn1 and 2. The homology of IsCT's is less than 30%.

BmKb1 and BmKn2 were tested for antimicrobial activity against gram-positive and gram-negative bacteria. Both peptides inhibited the growth of gram-positive and gram-negative bacteria. When compared to the activity of IsCT and IsCT2, BmKn2 showed a two and three-fold higher activity against *P. aeroginosa* and *E. coli*, respectively. BmKb1 showed weaker growth inhibittory activity of gram-negative and gram-positive bacteria than BmKn2, IsCT and IsCT2 (Zeng et al., 2004).

CYSTEINE-FREE Namps PEPTIDES

Bradykinin, together with angiotensin II, is a crucial humoral factor for blood pressure regulation. The key enzyme in the angiotensin-bradykinin system is the angiotensin converting enzyme (ACE) that generates angiotensin II from angiotensin I and degrades bradykinin. In the past decade, two native bradykinin-potentiating peptides (BPPs), Peptide T and K_{12} were isolated from scorpions *T. serrulatus* and *B. occitanus*, respectively. This gives new insight into possibilities of blood pressure regulating drugs (Zeng et al., 2005). To date six cysteine-free peptides without antimicrobial activity have also been isolated from scorpion venom. Two of these peptides have a bradykinin-potentiating function, while the functions of the other four are unkown.

South American (Brazil) scorpion

Tityus serrulatus (Lutz and Mello, 1922) (family: Buthidae C. L. Koch, 1837)

The investigation of *T. serrulatus* crude venom gave rise to the first cysteine-free bradykinin-potentiating peptide to be isolated from the venom of a scorpion. Peptide T is a 13 amino acid residue peptide with a totally coiled secondary structure (Feirrera et al., 1993). Although this peptide has the same amount of amino acids as IsCT and IsCT2, no homology exists. Peptide T potentiated the contractile activity of bradykinin on the isolated guinea pig ileum, inhibited the conversion of angiotensin I to angiotensin II in guinea-pig ileum tissue and increased the depressor effect of bradykinin on arterial blood pressure in the anaesthetized rats (Feirrera et al., 1993).

North African (Egypt) scorpion

Buthus occitanus (Amoreux, 1789) (family: Buthidae C. L. Koch, 1837)

A 21 amino acid residue peptide, named Peptide K_{12} has been isolated and characterized from this scorpion venom (Meki et al., 1995). The uniqueness of this peptide is its cysteine-free primary sequence (Table 1), resulting in an α-helical region of the N-terminal, together with the randomly coiled C-terminal (Zeng et al., 2005). Peptide K_{12}, although structurally unrelated to Peptide T, exhibitted similar bradykinin-potentiating activities. The peptide was reported to potentiate the contractile activity of bradykinin in guinea pig ileum and rat uteruses as well as a decline in systemic blood pressure of rats (Meki et al., 1995).

Asian (China) scorpions

Buthus sindicus (Pocock, 1900) (family: Buthidae C. L. Koch, 1837)

The crude venom of *B. sindicus* was shown to contain a number of short-chain peptides of which Bs10 is one. Bs10 had a cysteine-free primary structure (Table 1) and showed α-helical (25%), β-sheet (37%) and random coils (38%) in its secondary structure (Ali et al., 1998). The function of this peptide is unknown at present (Ali et al., 1998; Zeng et al., 2005).

Buthus martensii (Karsch, 1879) (family: Buthidae C. L. Koch, 1837)

BmKa1 and 2 (highly acidic peptides) and BmKn1 (highly basic peptide) have also been identified from the cDNA library of the venom gland from *B. martensii* (Karsch1879) The synthetic 38 amino acid BmKa1 showed a 100% coil structure, whereas the 50 amino acid residue BmKa2 contained three random coiled regions (residues 1 - 8, 17 - 31 and 43 - 50) separated by two α-helical domains (residues 9 - 16 and 32 - 42). However, BmKa2 showed a distinct amphipathic nature (Zeng et al., 2004). BmKa1 and 2 did not inhibit the growth of bacteria,

probably due to the lack of α-helical regions and therefore does not target bacterial membranes.

Mode of Action

Several hypotheses have been proposed to explain the interaction between the membrane disrupting peptides and membranes. AMPs have the ability to form ordered three dimensional structures when in contact with mammalian or bacterial membranes at sufficient concentrations. Four models termed the 'barrel-stave', 'torroidal-pore', 'carpet' and Shai-Matsuzaki-Huang models have been described.

The 'barrel-stave' model was initially described for alamethicin, a peptide from the fungus *Trichoderma viride* (Bechinger, 1997). The formation of transmembrane pores by bundles of amphipathic α-helices are arranged so that their hydrophobic surfaces interact with the lipid core of the membrane and their hydrophilic surfaces line an aqueous pore. This model requires peptides to be of a certain length in order to traverse the hydrophobic core of the bilayer (Shai, 1999; Sato and Feix, 2006). The 'torroidal-pore' model was first proposed for magainin peptides from the skin of the frog species *Xenopus laevis* (Ludtke et al., 1996). It is similar to that of the 'barrel-stave' model however an AMP-induced expansion of the lipid head group region results in a bending of the bilayer back on itself thereby connecting of the outer and inner leaflets. The pore is composed of a mixture of peptide and phospholipids (Sato and Feix, 2006). The 'carpet' model was initially described for the peptide-lipid interaction of dermaseptin S, an antimicrobial peptide isolated from the skin secretions of the frog genus *Phyllomedusa*. Peptides conforming to the 'carpet' model are in contact with the phospholipids head group throughout the entire process of membrane permeation. Membrane permeation occurs either when the entire membrane surface is covered with peptide monomers, or alternatively, after there is an association between membrane-bound peptides, forming a localized 'carpet' (Shai, 1999; Tossi et al., 2000). The final model, namely the 'Shai-Matsuaki-Huang' model (Zasloff, 2002) incorporates the three above-mentioned models which include the interaction of the peptide with the membrane, disruption of the membrane, diffusion to the intracellular fluid and targeting of intracellular targets by the peptides.

Although the carpet and channel-forming ('barrel-stave' and 'toroidel-pore') models have a number of differences, they also share some common characteristics. Both types of mechanisms begin with AMP association parallel to the membrane surface, followed by perpendicular peptide insertion of accumulated peptides, which form transmembrane pores. In the carpet model, peptides remain associated with the phospholipids head groups throughout the membrane disintegration process. This is similar to the transition that occurs in the toroidal-pore model, and it has been suggested that the formation of "holes" or toroidal pores may occur as an early step in membrane disintegration due to the 'carpet' model. It seems likely that most AMPs, by virtue of their amphipathic character, will act as detergents at sufficiently high concentrations (Sato and Feix, 2006).

Irrespective of the model of interaction, AMPs kill bacteria and/or eukaryotic cells in various ways. It is proposed that;

(i) A net movement of ions causes a fatal depolarization.
(ii) Creation of pores cause cellular content to leak out.
(iii) The activation of deadly processes such as induction of hydrolases that degrade the cell membrane i.e. peptidoglycan autolysis.
(iv) The scrambling of the usual distribution of lipids between the leaflets of the bilayer, resulting in disturbances of the membrane function.
(v) The damaging of critical intracellular targets after internalization of the peptide (Tossi et al., 2000; Zasloff, 2002).

Role of Cysteine-Free Peptides In Scorpion Venom

AMPs have been isolated from a variety of animals and insects. These peptides are proposed to be an important part of the organism's innate immunity (Hancock and Lehrer, 1998; Zasloft, 2002). AMPs found in the blood, tissue and hemolymph of various animals, insects and scorpion species serve as protection against the invasion of pathogens. With regards to AMPs peptides in scorpion venom, consensus of the role of these peptides has yet to be reached although many functions have been postulated.

Venom is produced by 2 venom glands in the tail and stored in 2 venom sacs in the scorpion's telson. These are anatomical structures important for the survival of scorpions because they rely on their venom as offensive weapons in order to catch prey (neurotoxins). It is proposed that AMPs may protect the scorpion from bacterial infection via the venom glands and sacs that are externally exposed via 2 venom ducts. Furthermore, cysteine-free peptides could serve as effective defensive armory against predators as well as offensive artillery in prey capture. PP, OP1, Pin1, IsCT, IsCT2 and hadrurin in the respective venoms, all have pore-forming activity in either SUVs or mammalian cells. The peptide-induced pores result in ion trafficking across the membranes. This would lead to depolarization of neural cells which would induce the serve pain associated with scorpion strings which could deterring predators. Depolarization of neural cells would also trigger a cascade of events such as a sustained increase in intracellular Ca^{2+} causing hypercontracture of skeletal muscles and release of neurotransmitters associated with hypersensitivity, convulsions and immobilization of smaller animals allowing for the capturing of prey (Simard and Watt, 1990).

It is known that the binding of certain ion-channel toxins to their respective receptor sites on ion channels is

dependent of the membrane potential (Gilles et al., 2001). Therefore peptide-induced cell depolarization, as induced by pore-forming peptides, could facilitate the voltage-dependant binding of these toxins. Simply put, the pore-forming peptides could potentiate the action of other neurotoxins found in the venom.

Peptide T and K12 potentiate the function of bradykinin. Bradykinin is associated with a Ca^{2+}-dependant second-dary messenger system whereby changes in the vascular smooth muscle causes vasodilation and increased blood supply leading to localize heat production, redness and swelling of an effected area of the body (in this case a scorpion sting). Bradykinin is also associated with the release of nitric oxide and activation of cyclic GMP, as well as the inhibition of ACE (Stewart, 2005), with the result being vasodilation. This vascular effect of bradykinin could be associated with the decreased blood pressure associated with scorpion envenomations (Jain et al., 2006). Bradykinin plays an important role in inflammatory responses and pain (Stewart, 2005). BPPs found in scorpion venom may contribute to the intense pain experienced by scorpion stings (Chowell et al., 2006).

CONCLUSION

The resistance of a variety of bacterial micro-organisms against commercially available antibiotics is on the rise, creating a need for alternative methods in combating them. This sparked a wide spread interest in the search for “natural antibiotics”, found in animals and plants.

A particular class of AMPs, namely the magainins from the skin of the African clawed frog *Xenpus laevis*, has shown much promise in the search for applications of nature’s antibacterial therapy. As of late magainin II have shed light as novel anti-cancer and anti-tumoral therapies (Lehmann et al., 2006) and magainin-mimetic derivatives have shown to exhibit antibacterial activity against perio-dontal pathogens (Genco et al., 2003). Other studies have also shown the effect of several AMPs (defensins, protegrins, cathelicidins, cecropins, polyphemusins, magainins and melittins) against various sexually trans-mitted disease-causing pathogens and the HIV and herpes simplex virus (Yedery and Reddy, 2005). How-ever, a major drawback of AMPs is its non-specific binding to various membranes, leading to hemolysis of red blood cells as well as injury to normal functioning cells. Through amino acid substitutions studies these unwanted actions of the AMPs could be drastically reduced.

A standard battery of tests including hemolytic activity, antimicrobial activity (against predetermined gram-posi-tive and gram-negative bacteria, fungi, viruses) and pore-formation will not only make results comparable but might also bring us much closer to finding elusive natural anti-biotic capable of treating drug resistant micro-organisms.

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