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Insect Antimicrobial Peptides and Their Applications

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Abstract

Insects are one of the major sources of antimicrobial peptides/proteins (AMPs). Since observation of antimicrobial activity in the hemolymph of pupae from the giant silk moths *Samia Cynthia* and *Hyalophora cecropia* in 1974 and purification of first insect AMP (cecropin) from *H. cecropia* pupae in 1980, over 150 insect AMPs have been purified or identified. Most insect AMPs are small and cationic, and they show activities against bacteria and/or fungi, as well as some parasites and viruses. Insect AMPs can be classified into four families based on their structures or unique sequences: the α -helical peptides (cecropin and moricin), cysteine-rich peptides (insect defensin and drosomycin), proline-rich peptides (apidaecin, drosocin and lebocin), and glycine-rich peptides/proteins (attacin and gloverin). Among insect AMPs, defensins, cecropins, proline-rich peptides and attacins are common, while gloverins and moricins have been identified only in *Lepidoptera*. Most active AMPs are small peptides of 20–50 residues, which are generated from larger inactive precursor proteins or pro-proteins, but gloverins (~14 kDa) and attacins (~20 kDa) are large antimicrobial proteins. In this mini-review, we will discuss current knowledge and recent progress in several classes of insect AMPs, including insect defensins, cecropins, attacins, lebocins and other proline-rich peptides, gloverins, and moricins, with a focus on structural-functional relationships and their potential applications.

Keywords

alpha-helical peptide; cysteine-rich peptide; glycine-rich peptide; proline-rich peptide; lipopolysaccharide; conformational changes

Introduction

Insects can produce a variety of antimicrobial peptides/proteins (AMPs). Antibacterial activity in insects was first observed in the bacteria-immunized pupae of the giant silk moths *Samia Cynthia* and *Hyalophora cecropia* (Boman et al., 1974; Faye et al., 1975), and later on in the bacteria-induced *Drosophila melanogaster* adult flies (Robertson and Postlethwait, 1986). The first insect AMP (cecropin) was purified from the pupae of *H. cecropia* in 1980 (Hultmark et al., 1980; Steiner et al., 1981), and since then over 150 insect AMPs have been

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identified. Most insect AMPs are small and cationic/basic with activities against bacteria and/or fungi, and some AMPs also show activities against some parasites and viruses. Insect AMPs can adopt certain structures or contain unique sequences and thus can be classified into four groups: the α -helical peptides (e.g., cecropin and moricin), cysteine-rich peptides (e.g., insect defensin and drosomycin), proline-rich peptides (e.g., apidaecin, drosocin and lebecin), and glycine-rich proteins (e.g., attacin and gloverin) (Bulet and Stocklin, 2005; Otvos, 2000).

The majority of insect AMPs, such as insect defensins, cecropins, proline-rich peptides and attacins, have been found in more than two insect orders, but moricin and gloverin have been identified only in *Lepidoptera*. Most AMPs are synthesized as inactive precursor proteins or pro-proteins, and active peptides (20–50 residues) are generated by limited proteolysis. But active gloverins (~14 kDa) and attacins (~20 kDa) are large proteins. In *D. melanogaster*, seven classes of AMPs (cecropin, attacin, defensin, drosomycin, diptericin, drosocin and metchnikowin) have been identified and regulation of *Drosophila* AMP genes by the Toll and IMD (immune deficiency) signaling pathways has been well studied. There have been many reports on AMPs from various insect species, but very few reviews on insect AMPs (Imler and Bulet, 2005; Li et al., 2006). Several reviews related to insect AMPs are mainly from *Drosophila* with a focus on activation of AMPs in response to various infections or regulation of AMP gene expressions by the Toll and IMD signaling pathways (Fullaondo and Lee, 2012; Hetru and Hoffmann, 2009; Lazzaro, 2008; Lemaitre and Hoffmann, 2007; Levitin and Whiteway, 2008; Moy and Cherry, 2013). Antiparasitic peptides and antimalarial peptides have been reviewed recently (Bell, 2011; Pretzel et al., 2013). Thus, in this mini-review, we will discuss current knowledge, recent progress, structural-functional relationships, and potential applications of insect defensins, cecropins, attacins, lebecins and other proline-rich peptides, gloverins and moricins.

Insect defensins

Defensins are small (~4 kDa) cationic/basic AMPs with six conserved cysteine residues that form three intramolecular disulfide bridges, and they have been identified in nearly all living organisms. Based on the structural characteristics, defensins can be classified into three families: “classical” defensins, beta-defensins and insect defensins (Ganz and Lehrer, 1994). There are many reviews on defensins, including vertebrate defensins in innate immunity (Ding et al., 2009; Jarczak et al., 2013; Lehrer and Lu, 2012; Wilson et al., 2013; Zhao and Lu, 2014; Zhu and Gao, 2013), plant defensins (Carvalho Ade and Gomes, 2011), mode of action and structure of defensins from different kingdoms (Wilmes et al., 2011), mode of action of plant, insect and human defensins in anti-fungal response (Aerts et al., 2008), and defensins as novel leads of antifungal therapeutics (Thevissen et al., 2007). In this mini-review, we will focus on insect defensins.

Insect defensins are small cationic peptides of 34–51 residues with 6 conserved cysteines. Many cysteine-rich peptides with different names, such as sapecins (Matsuyama and Natori, 1988b; Yamada and Natori, 1993), royalisin (Fujiwara et al., 1990), tenecin-1 (Moon et al., 1994), holotricin-1 (Lee et al., 1995), heliomicin (Lamberty et al., 1999), spodoptericon (Volkoff et al., 2003), gallerimycin (Schuhmann et al., 2003), coprisin (Hwang et al., 2009),

and lucifensin (Cerovsky et al., 2010), may all belong to the insect defensin family (Table 1). Insect defensins have been identified in the orders of *Diptera*, *Hymenoptera*, *Hemiptera*, *Coleoptera*, and *Lepidoptera*, and defensin is also present in the ancient insect order of *Odonata* (Bulet et al., 1992), suggesting that insect defensins may derive from a common ancestor gene.

Insect defensins were first reported as sapecins (40 residues) containing 6 cysteines in the flesh fly *Sarcophaga peregrina* (Matsuyama and Natori, 1988a, b), and *Phormia terranova* (*Diptera*) defensins were isolated as cationic peptides (40 residues) from the hemolymph of bacteria-immunized larvae (Lambert et al., 1989). Since these peptides show significant homology to mammalian defensins, and hence named insect defensins. *S. peregrina* sapecin and *P. terranova* defensin-A are synthesized as pre-pro-proteins composed of a signal peptide, a pre-peptide and a mature defensin peptide of 40 residues (Dimarcq et al., 1990; Matsuyama and Natori, 1988a), and the three disulfide bonds in *P. terranova* defensin-A are formed between Cys³-Cys³⁰, Cys¹⁶-Cys³⁶, and Cys²⁰-Cys³⁸ (Lepage et al., 1991). Pre-pro-defensins have been confirmed in the mosquito *Aedes aegypti* (Cho et al., 1996), the blood-sucking fly *Stomoxys calcitrans* (Lehane et al., 1997), the fall armyworm *Spodoptera frugiperda* (Volkoff et al., 2003), the silkworm *Bombyx mori* (Kaneko et al., 2008), and the cotton leafworm *Spodoptera littoralis* (Seufi et al., 2011).

The structure of insect defensins is composed of an N-terminal loop, an α -helix, followed by an antiparallel β -sheet (Fig. 1A) (Bonmatin et al., 1992; Cornet et al., 1995; Hanzawa et al., 1990). The α -helix and β -sheet are linked by two intramolecular disulfide bonds, forming a “cysteine-stabilized alpha beta (CS $\alpha\beta$)” or “loop-helix-beta-sheet” structure (Cornet et al., 1995). The CS $\alpha\beta$ motif has been confirmed in other insect defensin members (tenecin-1 and heliomicin) (Lamberty et al., 2001; Lee et al., 1998), and is also present in the antifungal peptide drosomycin from *D. melanogaster* (Landon et al., 1997), the antimicrobial peptide termicin from the termite *Pseudacanthotermes spiniger* (Da Silva et al., 2003), and a scorpion toxin charybdotoxin (Bonmatin et al., 1992). But drosomycin contains eight cysteines and an extra β -strand at the N-terminus, which is linked to the C-terminus by the extra disulfide bond (Fig. 1B). Thus, drosomycin and termicin may also belong to the insect defensin family. A new protein family (named X-tox) contains 11 imperfectly conserved CS $\alpha\beta$ motifs, but loses antimicrobial activity (Destoumieux-Garzon et al., 2009; Girard et al., 2008). X-tox proteins have been identified only in *Lepidoptera* (d'Alencon et al., 2013; Girard et al., 2008). Most insect defensins are cationic and about 40 residues long (Table 1), but two bee defensins, *Bombus pascuorum* defensin (Rees et al., 1997) and *Apis mellifera* royalisin (Fujiwara et al., 1990), are 51 residues long with an additional C-terminal loop after the last cysteine (Rees et al., 1997). *B. mori* defensin (36 residues) and *S. frugiperda* spodopteracin (36 residues) are anionic (pI 4.12 and 4.35, respectively) (Kaneko et al., 2008; Volkoff et al., 2003), whereas *S. littoralis* defensin is anionic and 50 residues long (Seufi et al., 2011).

Insect defensins are active mainly against Gram-positive bacteria, including *Micrococcus luteus*, *Aerococcus viridians*, *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus thuringiensis*, and *Staphylococcus aureus*. Some insect defensins are also active against Gram-negative *Escherichia coli* and some fungi (Lee et al., 2004; Lowenberger et al., 1995; Rees et al.,

1997; Seufi et al., 2011; Ueda et al., 2005; Vizioli et al., 2001; Yamada and Natori, 1993). Insect defensins may kill bacteria by formation of channels in the cytoplasmic membrane of bacteria (Cociancich et al., 1993), because sapecin has high affinity for cardiolipin, a major phospholipid of *S. aureus* (Matsuyama and Natori, 1990), and defensins can interact with phospholipid to induce microheterogeneity in the lipid membrane, which may be related to formation of channels responsible for the biological activity (Maget-Dana and Ptak, 1997). *E. coli* strains with rough mutants of lipopolysaccharide (LPS) are more sensitive to insect defensins than those with smooth LPS (Vizioli et al., 2001), suggesting that LPS may be a barrier for the antibacterial activity of insect defensins. A hendecapeptide derived from the helix region of sapecin-B has antibacterial activity comparable with that of sapecin-B but with a much broader spectrum of activity against *S. aureus*, *E. coli* and some yeasts, including *Candida albicans* (Yamada and Natori, 1994), suggesting that the conserved helical structure is key for the activity of insect defensins while other regions may contribute to binding to microorganisms. Interestingly, sapecin can also stimulate proliferation of *S. peregrina* embryonic cells (Komano et al., 1991).

Cecropins

Cecropins are a family of cationic antimicrobial peptides of 31–39 residues (Table 2), first isolated from the immunized hemolymph of *H. cecropia* pupae (Hultmark et al., 1982; Steiner et al., 1981), and have since been identified in *Lepidopteran*, *Dipteran* and *Coleopteran* insects. Members of the cecropin family also include those with different names, such as sarcotoxin-I (Okada and Natori, 1985), papiliocin (Kim et al., 2010), stomoxyn (Boulanger et al., 2002b; Landon et al., 2006), hinnavin (Yoe et al., 2006), SB-37 and Shiva (synthetic derivatives of cecropins) (Jaynes et al., 1988).

Cecropins are synthesized as secreted proteins and mature active cecropins are generated after removal of signal peptides. Cecropins have a broad spectrum of activity against Gram-negative and Gram-positive bacteria, as well as fungi (Cavallarin et al., 1998; DeLucca et al., 1997; Ekengren and Hultmark, 1999; Hultmark et al., 1982; Moore et al., 1996; Samakovlis et al., 1990; Vizioli et al., 2000). Most cecropins are amidated at the C-terminus, and amidation is important for interaction of cecropins with liposomes (Nakajima et al., 1987) and may contribute to the broad antimicrobial activity (Li et al., 1988). The two N-terminal residues (Gly¹-Trp²) of sarcotoxin-IA are important for the activity against *E. coli* since the two residues are required for binding of sarcotoxin-IA to the lipid A of LPS (Okamoto et al., 2002). Two N-terminal residues (Trp² and Phe⁵) of papiliocin and *H. cecropia* cecropin-A are also important for interaction of cecropins with negatively charged bacterial cell membrane (Lee et al., 2013a), and N-terminal Lys⁴ and Lys⁵ of sarcotoxin-IA are key residues for the interaction with lipid A and antimicrobial activity (Yagi-Utsumi et al., 2013). In addition to antimicrobial activity, cecropins and cecropin derivatives (SB-37 and Shiva) are also active against parasites, including *Plasmodium* and *Trypanosome* (Arrowood et al., 1991; Barr et al., 1995; Boisbouvier et al., 1998; Boulanger et al., 2002a; Gwadz et al., 1989; Jaynes et al., 1988; Rodriguez et al., 1995), and can inhibit replication of HIV-1 virus (Wachinger et al., 1998) and proliferation of cancer cells (Chen et al., 1997; Suttman et al., 2008). Papiliocin also has anti-inflammatory activity (Kim et al., 2011) and can induce apoptosis of *C. albicans* (Hwang et al., 2011), sarcotoxin-IA may play a role in

S. peregrina development (Nanbu et al., 1988), SB-37 and Shiva can enhance growth of murine fibroblast cells (Reed et al., 1992).

Cecropins adopt a random coil structure in aqueous solution but convert to α -helical structure in the hydrophobic environments. *H. cecropia* cecropin-A (37 residues) contains two helical regions in residues 5–21 and 24–37 (Holak et al., 1988), and sarcotoxin-IA (39 residues) exhibits an N-terminal amphipathic α -helix (residues 3–23) and a more hydrophobic C-terminal α -helix (residues 28–38) connected by a hinge region (residues 24–27) (Iwai et al., 1993). The N-terminal amphiphilic α -helix (residues 3–18) of sarcotoxin-IA is formed upon interaction with micelles, and amino acids in this α -helix are involved in specific interaction with lipid A (Yagi-Utsumi et al., 2013). Papiliocin (37 residues) adopts an unordered structure in aqueous solution but converts to α -helical structure in the presence of SDS (sodium dodecyl sulfate), DPC (dodecylphosphocholine), HFIP (1,1,1,3,3,3-hexafluoro-2-propanol) and LPS micelles, with two amphipathic α -helices (residues 3–21 and 25–36) linked by a hinge (Ala²²-Gly²³-Pro²⁴) (Fig. 1C) (Kim et al., 2011). Presence of Gly and Pro residues in the hinge region is important for the flexibility of the hinge (Oh et al., 2000).

Attacins

Attacins were first purified from the hemolymph of bacteria-immunized *H. cecropia* pupae with molecular masses of 20–23 kDa and isoelectric points (*pI*) of 5.7–8.3, and these attacin isoforms can be divided into two groups, the basic attacins (A–D) and acidic attacins (E and F) (Hultmark et al., 1983). *H. cecropia* attacin F (acidic, *pI* 9) is derived from attacin E (neutral, *pI* 7) by proteolysis (Engstrom et al., 1984a). Basic and acidic attacins are highly similar in amino acid sequences, only with higher contents of Asp residues in the acidic attacin, but they are encoded by two separate genes (Kockum et al., 1984; Sun et al., 1991). Attacins are synthesized as pre-pro-proteins containing a signal peptide, a pro-peptide (P domain), an N-terminal attacin domain, followed by two glycine-rich domains (G1 and G2 domains) (Hedengren et al., 2000; Sun et al., 1991). A conserved RXXR motif, which can be recognized by furin-like enzymes (Devi, 1991; Veenstra, 2000), is present at the N-terminal pro-peptide of attacins (Gunne et al., 1990; Kockum et al., 1984), indicating that mature attacins are produced by processing of pro-attacins by furin-like enzymes. The pro-peptide of attacin is required for secretion of pro-attacin and is removed at or after the trans-Golgi compartment, and pro-attacin does not have biological activity (Gunne and Steiner, 1993). Interestingly, the pro-peptide (P domain) of *D. melanogaster* attacin-C is longer and proline-rich, which is similar to small proline-rich peptides (see below), and this pro-peptide is active against Gram-negative bacteria (Rabel et al., 2004). Attacins have been identified in many lepidopteran species, including *H. cecropia* (Hultmark et al., 1983), *Heliothis virescens* (Ourth et al., 1994), *B. mori* (Sugiyama et al., 1995), *Trichoplusia ni* (Kang et al., 1996; Tamez-Guerra et al., 2008), *Hyphantria cunea* (Kwon et al., 2008), *S. cynthia* (Kishimoto et al., 2002), *Manduca sexta* (Rao and Yu, 2010), *Helicoverpa armigera* (Wang et al., 2010b), and *Spodoptera exigua* (Bang et al., 2012), as well as in some dipteran species like *D. melanogaster* (Asling et al., 1995; Dushay et al., 2000) and the Tsetse fly *Glossina morsitans* (Hao et al., 2001; Wang et al., 2008).

Most attacins are active against *E. coli* and some selected Gram-negative bacteria (Hultmark et al., 1983). *G. morsitans* attacin-A1 is active against *E. coli* and the protozoan parasite *Trypanosoma brucei* in vitro (Hu and Aksoy, 2005), *H. cunea* attacin-B is active against Gram-negative *E. coli* and *Citrobacter freundii* as well as the fungus *C. albicans* (Kwon et al., 2008), *S. exigua* attacin is active against Gram-negative *E. coli* and *Pseudomonas cichorii*, and Gram-positive *B. subtilis* and *Listeria monocytogenes* (Bang et al., 2012), and recombinant *Drosophila* attacin-A is active against *E. coli* with minor hemolysis against porcine red blood cells (Wang et al., 2010a). Interestingly, the hybrid protein, attacin-coleopteracin, has enhanced antibacterial activity against *E. coli*, *Burkholderia glumae* and *B. subtilis* compared to either attacin or coleopteracin alone (Lee et al., 2013b). But some members of attacins are leucine-rich, and they do not exhibit antimicrobial activity. For example, *H. cunea* attacin-A is leucine-rich and does not have antimicrobial activity (Kwon et al., 2008). Sarcotoxin-IIA from *S. peregrina* may be an attacin-like protein (Ando et al., 1987), since its C-terminal half is similar to that of basic attacin (Ando and Natori, 1988).

Attacins can inhibit growth of *E. coli* cells by directly targeting bacterial outer membrane to increase permeability (Engstrom et al., 1984b), and they can inhibit synthesis of several bacterial outer membrane proteins, including OmpC, OmpF, OmpA, and LamB (Carlsson et al., 1991) by binding to LPS even without entering the inner membrane or cytoplasm (Carlsson et al., 1998). CD (circular dichroism) spectrum showed that *H. cecropia* attacin-F adopts mainly random coil structure in aqueous solution (pH 6.4), but converts to a much more helical structure in the presence of a hydrophobic solvent HFIP (Gunne et al., 1990). Conversion of random coil structure in aqueous solution to more helical structure in the hydrophobic environment of attacins is similar to that of cecropins (discussed above) and gloverins, another family of glycine-rich antimicrobial proteins (see below). Thus, the helical structure of attacins may account for the antimicrobial activities. Attacins contain two glycine-rich domains (G1 and G2 domains) at the C-terminus. When adopting a random coil structure in the hemolymph, attacins are more susceptible to proteinases. Thus, it is interesting to know whether some of the small (7–10 kDa) glycine-rich antimicrobial peptides (see below) may be the proteolytic products of attacin-like proteins.

Lebocins and other small proline-rich peptides

Lebocins were first isolated from the hemolymph of *E. coli*-immunized silkworm, *B. mori*, as proline-rich and O-glycosylated 32-residue peptides (Hara and Yamakawa, 1995b). They share 41% identity in amino acid sequences to honeybee proline-rich peptide abaecin (34 residues), which is not O-glycosylated (Casteels et al., 1990). A cDNA clone for *B. mori* lebocin showed that lebocin was synthesized as a precursor protein of 179 residues, and the active 32-residue peptide is located closed to the C-terminus of the precursor (Chowdhury et al., 1995).

cDNAs encoding lebocin precursors have also been identified in other *Lepidopteran* species, including *M. sexta* (Rao et al., 2012; Rayaprolu et al., 2010), *T. ni* (Liu et al., 2000; Tamez-Guerra et al., 2008), *Pseudoplusia includens* (Lavine et al., 2005), *S. cynthia* (Bao et al., 2005), *Pieris rapae* (Genbank accession number: JN587806), *H. virescens* (Genbank accession number: FJ546346), and *Antheraea pernyi* (Genbank accession numbers:

EU557311, EU557312 and DQ666499). Interestingly, in all lebocin precursors, including *B. mori* precursors, the proline-rich peptides, ranging from 22 to 28 residues with 4–6 prolines, are located at the N-termini of the mature precursor proteins, and only *B. mori* lebocin precursors contain additional 32-residue peptides with 7 prolines that are closed to the C-termini (Rao et al., 2012). These results indicate that active lebocins are generated by proteolytic cleavage of the precursor proteins. In the lebocin precursors, there are several conserved RXXR motifs that can be recognized by furin-like enzymes (Devi, 1991; Veenstra, 2000). Our study showed that several truncated recombinant *M. sexta* lebocin-B and lebocin-C precursors can be processed by proteinases in the larval hemolymph, and three cleavage sites in both precursors have been determined by Edman degradation of the recovered cleavage products (Rao et al., 2012). Cleavage products of *M. sexta* lebocin-A precursor, which contains three conserved RXXR motifs, have also been confirmed in the hemolymph of *M. sexta* larvae by Mass spectrometry (Rayaprolu et al., 2010), further supporting the idea that active lebocin peptides are generated by proteolysis from precursor proteins.

Lebocins are active against Gram-negative and Gram-positive bacteria and some fungi (Table 3). *B. mori* lebocins are active against Gram-negative *Acinetobacter* sp. and *E. coli*, and the O-glycosylation is required for the full activity (Hara and Yamakawa, 1995b). Synthetic N-terminal proline-rich peptides of *M. sexta* lebocin-B (28 residues) and lebocin-C (27 residues) are active against Gram-negative *Serratia marcescens* and *Salmonella typhimurium*, Gram-positive *S. aureus* and *Bacillus cereus*, as well as the fungus *Cryptococcus neoformans* (Rao et al., 2012). These results suggest that the N-terminal proline-rich peptides from other lepidopteran species including *B. mori* may also be active. Interestingly, synthetic *M. sexta* lebocin-B peptide, but not lebocin-C, has agglutinating activity against *E. coli* (Rao et al., 2012). Synthetic peptides of other fragments (non-proline rich) based on the cleavage products of *M. sexta* lebocin-A, -B and -C precursors, also have some activity against bacteria (Rao et al., 2012; Rayaprolu et al., 2010), indicating diversity of antimicrobial peptides in insects.

In several other insects, proline-rich antimicrobial peptides of 16–34 residues with different names have been identified (Table 3). These peptides include drosocin (Bulet et al., 1993) and metchnikowin (Levashina et al., 1995) from *D. melanogaster*, pyrrhocoricin from the sap-sucking bug *Pyrrhocoris apterus* (Cociancich et al., 1994), formaecin from an ant *Myrmecia gulosa* (Mackintosh et al., 1998b), apidaecin (Casteels et al., 1989) and abaecin from honeybees (Casteels et al., 1990), and they are active against Gram-negative bacteria, Gram-positive bacteria, and some fungi (Table 3). Thus, small proline-rich peptides have been identified in the orders of *Diptera*, *Hymenoptera*, *Hemiptera* and *Lepidoptera*. They may not have high similarities in amino acid sequences, but are all proline-rich, and therefore should belong to the same family of proline-rich antimicrobial peptides.

The structure and function relationship and mode of action of apidaecin-type peptides have already been reviewed (Li et al., 2006), and mammalian and insect proline-rich peptides have also been reviewed (Scocchi et al., 2011). Some insect proline-rich peptides are O-glycosylated, while some others are not, but O-glycosylation clearly enhances antimicrobial activity. A common name is more suitable for this family of insect proline-rich antimicrobial

peptides (PR-AMPs) (Scocchi et al., 2011), as they are identified in at least 4 orders of insects but not just in *Lepidoptera*. PR-AMPs from different insect orders may differ in the amino acid sequences of precursors and/or in the processing processes, as some precursors are shorter and do not contain the conserved RXXR motifs like those in the lebecin precursors, but the active peptides all contain high contents of prolines. Whether a member of the PR-AMP family is active against Gram-negative or Gram-positive bacteria or fungi may depend on binding of a PR-AMP to microbial surface and the structural and/or conformational changes of the PR-AMP upon binding to microorganisms.

Moricins

Moricin was first isolated from the hemolymph of *E. coli*-immunized *B. mori* larvae as a highly basic 42-residue peptide (Hara and Yamakawa, 1995a). Moricin has been found only in *Lepidopteran* insects so far, and cDNAs encoding moricins have been identified in *M. sexta* (Zhu et al., 2003), *Spodoptera litura* (Oizumi et al., 2005), *G. mellonella* (Brown et al., 2008), *H. armigera* (Wang et al., 2010b), *S. exigua*, *H. virescens*, and *Hyblaea pueria* (cDNA sequences are available in the NCBI database). Unlike lebecins that are generated from larger precursor proteins by proteolysis, moricins are synthesized as secreted proteins after cleavage of the signal peptides.

Moricins have activity against Gram-negative and Gram-positive bacteria, and *G. mellonella* moricins also show high activity against filamentous fungi and yeast (Brown et al., 2008; Dai et al., 2008; Hara and Yamakawa, 1995a). The tertiary solution structures of moricins show a long α -helix with 8 turns along nearly the full length peptide, except for a few residues at the N- and C-terminal regions (Dai et al., 2008; Hemmi et al., 2002; Oizumi et al., 2005) (Fig. 1D). The N-terminal segment of the α -helix (residues 5–22) is amphipathic and is responsible for increase in membrane permeability for killing of bacteria, and the C-terminal segment of the α -helix (residues 23–36) is hydrophobic and is critical for antimicrobial activity of moricin (Hemmi et al., 2002). This structure is similar to that of cecropins (discussed above), except lacking a hinge region in moricins.

Expression of insect AMP genes is regulated by nuclear factor- κ B (NF- κ B)/Rel and GATA transcription factors. NF- κ B and GATA binding sites have been identified in the promoter regions of several classes of AMP genes, including attacin genes (Sun et al., 1991; Taniai et al., 1996a; Taniai et al., 1996b) and moricin genes (Cheng et al., 2006). We have identified a 22-bp NF- κ B-GATA *cis*-element, which can enhance the activity of *D. melanogaster* and *M. sexta* AMP gene promoters when inserted into the promoter regions (Rao et al., 2011). More importantly, we have identified a 140-bp *cis*-element, named moricin promoter activating element (MPAE), which may contain binding sites for transcription factors specific for *Lepidopteran* insects, as insertion of MPAE into *Drosophila* drosomycin promoter can enhance the promoter activity specifically in *S. frugiperda* Sf9 cells (lepidopteran cell line) but not in *Drosophila* S2 cells (dipteran cell line) (Rao et al., 2011). Expression of moricin can be regulated by the Toll-Spätzle pathway (Zhong et al., 2012).

Gloverins

Gloverin is a basic, glycine-rich and heat-stable antibacterial protein of ~14-kDa, first purified from the hemolymph of *Hyalophora gloveri* pupae (Axen et al., 1997). So far, gloverins have been identified only in *Lepidoptera*, including *H. armigera* (Mackintosh et al., 1998a), *T. ni* (Lundstrom et al., 2002), *G. mellonella* (Seitz et al., 2003), *Antheraea mylitta* (Gandhe et al., 2006), *M. sexta* (Abdel-latif and Hilker, 2008; Xu et al., 2012), *Diatraea saccharialis* (Silva et al., 2010), *Plutella xylostella* (Etebari et al., 2011; Eum et al., 2007), *S. exigua* (Hwang and Kim, 2011), and *B. mori* (Kawaoka et al., 2008; Mrinal and Nagaraju, 2008). Gloverins are synthesized as pre-pro-proteins, and the N-terminal regions of pro-gloverins contain a conserved RXXR motif (Xu et al., 2012). Thus, it is likely that mature gloverins are produced after removal of the N-terminal pro-regions (~22–26 residues) by furin-like enzymes. But recombinant *T. ni* pro-gloverin is also active against *E. coli* with comparable activity to that of *H. gloveri* mature gloverin (Lundstrom et al., 2002), suggesting that removal of pro-regions may not be necessary for some pro-gloverins to be active.

Gloverins are active mainly against *E. coli*, with higher activity against *E. coli* mutant strains (D21f2, D21 and D22) containing rough mutants of LPS (Axen et al., 1997; Kawaoka et al., 2008; Mackintosh et al., 1998a; Mrinal and Nagaraju, 2008; Lundstrom et al., 2002; Moreno-Habel et al., 2012). But *T. ni* gloverins are also active against a virus (Moreno-Habel et al., 2012), *S. exigua* gloverin is active against a Gram-positive bacterium (*Flavobacterium* sp.) but inactive against *E. coli* strain with smooth LPS (Hwang and Kim, 2011), and *M. sexta* gloverin is active against a Gram-positive *B. cereus* and two fungi (*Saccharomyces cerevisiae* and *C. neoformans*) but inactive against *E. coli* strain with smooth LPS (Xu et al., 2012). It is not clear how gloverins from different lepidopteran species have activity against *E. coli*, Gram-positive bacteria, fungi or even a virus. It was originally suggested that basic gloverins may interact with LPS via chargecharge interaction with negatively charged lipid A since LPS can inhibit the activity of gloverin (Axen et al., 1997). However, our study showed that recombinant *M. sexta* gloverin (*pI* ~9.3) can bind to the O-specific antigen and outer core carbohydrate moieties of LPS, Gram-positive bacterial lipoteichoic acid (LTA) and peptidoglycan (PG), and laminarin (beta-1, 3-glucan), but does not bind to lipid A (Xu et al., 2012), suggesting that gloverins may interact with LPS by binding to different moieties of LPS. Gloverins could be active against *E. coli*, Gram-positive bacteria or fungi if they can bind to bacterial or fungal surface through interactions with LPS, LTA, PG or laminarin.

Most gloverins are basic or highly basic (*pI* ~8.3 or *pI* > 9.0) and contain high content (>18%) of glycine residues (Xu et al., 2012). Gloverin adopts random coil structure in aqueous solution but undergoes conformational transition to α -helical structure in the hydrophobic environment (Axen et al., 1997). Binding of basic gloverin to LPS may suppress the growth of *E. coli* by inhibiting synthesis of bacterial outer membrane proteins and increasing permeability of the membrane (Axen et al., 1997). But four *B. mori* gloverins (*pI* ~5.5, 7.0, 6.3 and 7.0 for *B. mori* gloverins 1–4, respectively) (Kawaoka et al., 2008), *H. virescens* gloverin (*pI* ~7.2) (Genbank accession number: ACR78446), and *A. mylitta* gloverin-2 (*pI* ~6.8) (Genbank accession number: ABG72700) have acidic to neutral *pI*. *B.*

mori gloverins are active against *E. coli* (Kawaoka et al., 2008; Mrinal and Nagaraju, 2008), but whether acidic/neutral gloverins can interact with LPS through charge-charge interaction is unknown.

Our study with four recombinant *B. mori* gloverins showed that *B. mori* gloverins at pH 5.0 (positively charged) bound to rough mutants of LPS and lipid A but not smooth LPS, and these gloverins at pH 8.0 (negatively charged) do not bind to rough LPS or lipid A (Yi et al., 2013), indicating that charge-charge interaction is required for binding of gloverins to LPS. However, the four *B. mori* gloverins mainly adopt random coil structure in aqueous solution from pH 3–8, but contain α -helical structure in the presence of organic solvent HFIP, smooth and rough LPS, or lipid A (Yi et al., 2013), indicating that hydrophobic environment (HFIP or LPS), but not charge-charge interaction, is required for conformational conversion of gloverins from random coil to α -helical structure. Our antibacterial activity assay showed that positively charged *B. mori* gloverins (at pH 5.0), but not negatively charged gloverins (at pH 8.0), are active against *E. coli* mutant strains containing rough LPS but inactive against *E. coli* strain with smooth LPS (Yi et al., 2013). Together, our results suggest that binding of gloverins to LPS (or other microbial surface molecules) is the prerequisite, and conformational conversion of gloverins from random coil to α -helical structure upon binding to microbial surface is the key for the activity of gloverins against *E. coli* (or other microorganisms). This mechanism may also apply to cecropins, moricins and attacins since they all undergo conformational changes in the hydrophobic environment upon binding to microbial surface.

B. mori gloverin-1 gene is the ancestral one among the four genes, and *gloverin* genes 2–4 are derived from duplication (Mrinal and Nagaraju, 2008). Knockdown expression of *B. mori gloverin-2* gene by RNAi in the embryos reduces hatching rate (Mrinal and Nagaraju, 2008), and RNAi of *S. exigua gloverin* gene in larvae also reduces pupation and prolongs larval period (Hwang and Kim, 2011). *B. mori gloverin-1* is expressed in larval but not in adult gonads, while *gloverins* 2–4 are expressed in adult but not in larval gonads (Mrinal and Nagaraju, 2008). Our study showed that *M. sexta gloverin* is expressed at a higher level in the testis of naïve larvae (Xu et al., 2012). These results suggest that, in addition to have antimicrobial activity, gloverins may also play a role in development and/or reproduction.

Gloverins among lepidopteran species share high similarities, for example, *M. sexta gloverin* is 48–74% identical to gloverins from known *Lepidopteran* species (Xu et al., 2012). There are two glycine-rich proteins of ~14 kDa from *Hemipteran* and *Coleopteran* insects, hemiptericin (133 residues, 14.7 kDa) from *P. apterus* (Cociancich et al., 1994) and tenecin-4 (120 residues, 13.1 kDa) from *T. molitor* (Chae et al., 2012). Both tenecin-4 and hemiptericin share ~22% identity to *H. gloveri gloverin*, but tenecin-4 has higher identity (22–24%) to *H. virescens* and *T. ni gloverins* than hemiptericin (15–17% identity). Other glycine-rich peptides are 7–10 kDa, such as *P. terranova* and *D. melanogaster* dipterocins (82 residues) (Dimarcq et al., 1988; Reichhart et al., 1989; Wicker et al., 1990), *S. peregrina* sarcotoxin-III (7 kDa) (Baba et al., 1987), *Zophobas atratus* and *Allomyrina dichotoma* coleopterocins (72–74 residues) (Bulet et al., 1991; Sagisaka et al., 2001), *H. diomphalia* holotricin-2 (72 residues) (Lee et al., 1994), *Acalolepta luxuriosa* acaloleptins (~8 kDa) (Imamura et al., 1999), and honeybee hymenoptaecin (93 residues) (Casteels et al., 1993).

These glycine-rich peptides have activities against Gram-negative and Gram-positive bacteria. They are not gloverin homologs, but are similar to glycine-rich domains (G1 and/or G2 domains) of attacins, and they may have similar mechanisms to gloverins in killing of microorganisms. Interestingly, *A. dichotoma* coleopteracin precursors also contain a conserved RXXR motif (Sagisaka et al., 2001), and active coleopteracins may be produced by proteolysis.

Potential applications of insect AMPs

Insect AMPs may have potential applications in agriculture, disease vector control and medicine. Use of recombinant AMPs in paratransgenic control system (Hurwitz et al., 2012) and paratransgenic control of vector borne diseases (Hurwitz et al., 2011), potential therapeutic applications of AMPs and clinical development of AMPs (Ahmad et al., 2012; Andres, 2012; Eckert, 2011; Korting et al., 2012; Seo et al., 2012), functional and structural characteristics of plant and animal AMPs (Sarika et al., 2012) have been reviewed elsewhere. We will focus on application of insect AMPs in agriculture and control of disease vectors.

AMPs have been engineered in plants to confer resistance to bacterial and fungal pathogens. Plant defensins have been expressed in transgenic rice (Choi et al., 2009; Jha and Chattoo, 2010), wheat (Li et al., 2011), banana (Ghag et al., 2012), tomato (Abdallah et al., 2010; Portieles et al., 2010), “Egusi” melon (Ntui et al., 2010), peanut (Swathi Anuradha et al., 2008), tobacco (Portieles et al., 2010; Swathi Anuradha et al., 2008), and *Arabidopsis* (Kaur et al., 2012). Transgenic expression of an insect defensin (*G. mellonella* gallerimycin) and cecropin (sarcotoxin-IA) in tobacco also confers resistance to pathogenic fungi (Mitsuhashi et al., 2000; Ohshima et al., 1999). Expression of cecropins in transgenic plants, including rice and tomato, can confer resistance to bacterial and fungal pathogens (Coca et al., 2006; Jan et al., 2010; Oard and Enright, 2006; Sharma et al., 2000). Metchnikowin (proline-rich peptide) has been expressed in transgenic barley to enhance resistance ((Rahnamaeian et al., 2009; Rahnamaeian and Vilcinskis, 2012). Chimeric peptides by combining active regions of two AMPs have also been used in transgenic plants to enhance resistance or broaden spectrum of resistance to pathogens (Osusky et al., 2000; Yevtushenko et al., 2005). However, expression of AMPs in transgenic plants may have an impact on host gene expression (Campo et al., 2008) or host plant fitness (Nadal et al., 2012).

Insect AMPs exhibit activity against some parasites, including *Plasmodium*, filarial nematode, and *Trypanosome*. Two recent reviews summarize current progress about antimalarial and antiparasitic peptides (Bell, 2011; Pretzel et al., 2013). Cecropins and defensins have been shown to be active against parasites (Chalk et al., 1995; Fieck et al., 2010; McGwire et al., 2003; Rodriguez et al., 1995; Shahabuddin et al., 1998). Expression of AMPs in transgenic vectors such as mosquitoes is a new approach for killing parasites or blocking parasite transmission. Defensin-A gene has been engineered in the yellow fever mosquito, *A. aegypti*, under the control of the vitellogenin (Vg) promoter and its expression can be activated by blood meal (Kokoza et al., 2000). Expression of cecropin-A in transgenic *Anopheles gambiae*, a vector for human malaria parasites, can reduce the number of *Plasmodium berghei* oocysts (Kim et al., 2004), and co-expression of cecropin-A and

defensin-A in transgenic *A. aegypti* can cooperatively block *Plasmodium* transmission (Kokoza et al., 2010).

Concluding remarks

Insect AMPs were originally discovered by purification of active peptides/proteins from bacteria-induced hemolymph. This approach is limited, since only AMPs that are present at relative high concentrations in the hemolymph can be purified and identified. Orthologous AMP genes in different insect species can also be identified by analysis of genomic sequences. However, whole genome analysis may not identify small AMPs, particularly small peptides that are generated from precursor proteins by proteolytic processing such as proline-rich peptides, because precursor proteins in different insects may not have high similarities. Thus, there may be a large number of insect AMPs in hemolymph that have not been purified or identified. Most insect AMPs, including insect defensins, cecropins, gloverins and basic attacins, are basic (cationic). Moricins also contain a long amphipathic α -helix. Thus, these insect AMPs are either positively charged or contain positively charged surface (even anionic AMPs contain amphipathic α -helix) under physiological pH, which can facilitate binding of AMPs to negatively charged microbial surface via charge-charge interaction. Binding of insect AMPs to microbial surface is prerequisite for antimicrobial activity. Cecropins, moricins, gloverins and attacins adopt unordered structures in aqueous solutions, but convert to more helical structures in the presence of hydrophobic environment such as LPS. Therefore, upon binding to microbial surface, insect AMPs can convert to more helical structures, which are the key for antimicrobial activity.

Insect AMPs have a broad spectrum of activity against bacteria, fungi, some parasites and viruses. Even AMPs from the same class but different insect species may have activity against different microorganisms. This may be because AMPs from different insect species may differ in the ability of binding to microorganisms. Whether an AMP is active or not against a microorganism depends on its binding ability to the microorganism and the conformational conversion to more helical structure. Single insect AMP may not have strong activity against microorganisms; however, the overall activity of all the AMPs in the hemolymph could be very strong and significant. Insect AMPs may have potential applications in agriculture, disease vector control and medicine. To express AMPs in transgenic plants or vector insects, it is necessary to consider a strong and tissue-specific (or pathogen-specific or inducible) promoter for stable expression of AMPs. Also, expression of two different AMPs may confer stronger or broader resistance. For potential application of insect AMPs in medicine, small peptides may be more suitable candidates and chemical modifications of small peptides are necessary to generate more potent and stable peptides.

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Fig. 1. Structures of an insect defensin, drosomycin, cecropin and moricin

Pdb files of *P. terraenovae* defensin (PDB: 1ICA) (A), *D. melanogaster* drosomycin (PDB: 1MYN) (B), *P. xuthus* papiliocin (cecropin) (PDB: 2LA2) (C), and *B. mori* moricin (PDB: 1KV4) (D) were downloaded from <http://www.rcsb.org/pdb> website and the 3-dimensional structures were displayed by Pymol program. N and C indicate N-terminus and C-terminus of the peptides, respectively, and disulfide bonds are indicated by sticks. An extra disulfide bond that linked the N-terminal β -strand to the C-terminus of drosomycin is boxed (B).

Table 1

Insect defensins and defensin-like peptides

Insect Species		Peptide Name	Size (aa)	Activity	References
Diptera	<i>Phormia terranova</i>	Defensin A, B	40	G+	Lambert <i>et al.</i> , 1989
	<i>Sarcophaga peregrina</i>	Sapecin A, C	40	G+, G−	Matsuyama and Natori, 1988a, 1988b Yamada and Natori, 1993
		Sapecin B	34		
	<i>Aedes aegypti</i>	Defensin A, B, C	40	G+, G−	Lowenberger <i>et al.</i> , 1995
	<i>Stomoxys calcitrans</i>	Smd 1	46	N/A	Lehane <i>et al.</i> , 1997
		Smd 2	40		
	<i>Lucilia sericata</i>	Lucifensin	40	G+	Cerovsky <i>et al.</i> , 2010
	<i>Anopheles gambiae</i>	Defensin	40	G+, G−, fungi	Vizioli <i>et al.</i> , 2001
	<i>Chironomus plumosus</i>	Defensin A, B	36	G+	Lauth <i>et al.</i> , 1998 [#]
	<i>Eristalis tenax</i>	Defensin	40	N/A	Altincicek and Vilcinskas, 2007 [#]
	<i>Drosophila melanogaster</i>	Drosomycin	44	fungi	Landon <i>et al.</i> , 1997
		Defensin	40	G+	Imler and Bulet, 2005
	<i>Apis mellifera</i>	Royalisin	51	G+	Fujiwara <i>et al.</i> , 1990
	<i>Bombus pascuorum</i>	Defensin	51	G+, G−, fungi	Rees <i>et al.</i> , 1997
Hymenoptera	<i>Nasonia vitripennis</i>	Navidefensin2-2	42	G+	Gao and Zhu, 2010 [#]
	<i>Formica rufa</i>	Defensin	40	G+	Taguchi <i>et al.</i> , 1998 [#]
	<i>Tenebrio molitor</i>	Tenecin-1	43	G+	Moon <i>et al.</i> , 1994
	<i>Holotrichia diomphalia</i>	Holotricin-1	43	G−	Lee <i>et al.</i> , 1995
	<i>Copris tripartitus</i>	Coprisin	43	G+, G−, fungi	Hwang <i>et al.</i> , 2009
	<i>Zophob atratus</i>	Defensin B, C	43	G+, G−	Bulet <i>et al.</i> , 1991
	<i>Anomala cuprea</i>	Defensin A, B	43	G+, G−	Yamauchi, 2001 [#]
	<i>Oryctes rhinoceros</i>	Defensin	43	G+	Ishibashi <i>et al.</i> , 1999 [#]
	<i>Allomyrina dichotoma</i>	Defensin	43	G+	Miyanoshita <i>et al.</i> , 1996 [#]
	<i>Acalolepta luxuriosa</i>	Defensin 1	43	G+, G−	Ueda <i>et al.</i> , 2005
	<i>Spodoptera frugiperda</i>	Spodoptericin	36	N/A	Volkoff <i>et al.</i> , 2003

Insect Species	Peptide Name	Size (aa)	Activity	References
	<i>Sl</i> -gallerimycin	75 ^a		
<i>Samia cynthia ricini</i>	<i>Scr</i> -gallerimycin	74 ^a	N/A	Hashimoto <i>et al.</i> , 2008 [#]
<i>Spodoptera litura</i>	<i>Sl</i> -gallerimycin	75 ^a	N/A	Wang <i>et al.</i> , 2012 [#]
<i>Bombyx mori</i>	Defensin A	36	N/A	Kaneko <i>et al.</i> , 2008
	Defensin B	38		
<i>Spodoptera littoralis</i>	<i>Sp</i> liDef	50	G+, G–	Seufi <i>et al.</i> , 2011
<i>Galleria mellonella</i>	Defensin	43	fungi	Lee <i>et al.</i> , 2004
	Gallerimycin	76 ^a	fungi	Schuhmann <i>et al.</i> , 2003
<i>Heliothis virescens</i>	Helioicinin	44	fungi	Lamberty <i>et al.</i> , 1999
<i>Mamestra brassicae</i>	Defensin	98 ^a	G+, G–	Mandrioli <i>et al.</i> , 2003 [#]
<i>Archeoprepone demophaon</i>	ARD1	44	fungi	Landon <i>et al.</i> , 2004 [#]
<i>Pyrthocoris apterus</i>	Defensin	43	G+, G–	Cociancich <i>et al.</i> , 1994
<i>Rhodnius prolixus</i>	Defensin A, B, C	43	G+	Lopez <i>et al.</i> , 2003 [#]
<i>Pseudacanthotermes spiniger</i>	Termicin	36	fungi	Da Silva <i>et al.</i> , 2003
<i>Aeschna cyanea</i>	Defensin	38	G+, G–	Bulet <i>et al.</i> , 1992

G+: Gram-positive bacteria; G–: Gram-negative bacteria;

^a : pre-pro-protein sequence; N/A: not available

[#] : these references have not been cited in this mini-review

Table 2

Cecropins and cecropin-like peptides

Insect Species	Peptide Name	Size (aa)	Activity	References
<i>Diptera</i>	<i>Sarcophaga peregrina</i>	39	G ⁻ , G ⁺	Okada and Natori, 1985
	<i>Stomoxys calcitrans</i>	42	G ⁻ , G ⁺ , fungi, <i>trypanosome</i>	Boulanger <i>et al.</i> , 2002b
	<i>Anopheles gambiae</i>	35	G ⁻ , G ⁺ , fungi	Vizioli <i>et al.</i> , 2000
	<i>Aedes aegypti</i>	34	G ⁻ , G ⁺ , fungi	Lowenberger <i>et al.</i> , 1999 [#]
	<i>Aedes albopictus</i>	35	<i>E. coli</i> D31	Sun <i>et al.</i> , 1999 [#]
	<i>Drosophila melanogaster</i>	34	G ⁺ , G ⁻ , fungi	Ekengren and Hultmark, 1999
<i>Coleoptera</i>	<i>Paederus dermatitis</i>	34	G ⁻ , G ⁺ , fungi	Memarpoor-Yazdi <i>et al.</i> , 2013 [#]
	<i>Acalolepta luxuriosa</i>	35	<i>E. coli</i> , <i>M. luteus</i>	Saito <i>et al.</i> , 2005 [#]
<i>Lepidoptera</i>	<i>Hyalophora cecropia</i>	Cecropin A	G ⁻ , G ⁺ , fungi	Hultmark <i>et al.</i> , 1982
		Cecropin B	G ⁻ , G ⁺	
		Cecropin C	G ⁻ , G ⁺	
		Cecropin D	G ⁻ , G ⁺	
		Cecropin E	G ⁻ , G ⁺	
		Cecropin F	G ⁻ , G ⁺	
	<i>Papilio xuthus</i>	Papiliocin	G ⁻ , G ⁺ , fungi	Kim <i>et al.</i> , 2010
	<i>Artogeia rapae</i>	Hinnavin I	G ⁻ , G ⁺	Yoe <i>et al.</i> , 2006
		Hinnavin II		
	<i>Manduca sexta</i>	Cecropin D	N/A	Dickinson <i>et al.</i> , 1998 [#]
	<i>Spodoptera litura</i>	Spodopsin Ia, Ib	G ⁻ , G ⁺	Choi <i>et al.</i> , 1997 [#]
	<i>Bombyx mori</i>	Cecropin A	G ⁻ , G ⁺	Yamano <i>et al.</i> , 1998 [#] Taniai <i>et al.</i> , 1995 [#] Yang <i>et al.</i> , 1999 [#]
		Cecropin B		
		Cecropin D		
	<i>Agrius convolvuli</i>	Cecropin D	G ⁻ , G ⁺	Kim <i>et al.</i> , 2000 [#]
	<i>Helicoverpa armigera</i>	Cecropin D	G ⁻ , G ⁺	Wang <i>et al.</i> , 2007 [#]
	<i>Heliothis virescens</i>	Cecropin B	G ⁻	Lockey and Ourth, 1996 [#]

Insect Species		Peptide Name	Size (aa)	Activity	References
		<i>Hyphantria cunea</i>	35	G ⁺ , G ⁺	Park <i>et al.</i> , 1997 [#]
		<i>Spodoptera littoralis</i>	38	N/A	Seufi, 2011
<i>Isoptera</i>		<i>Pseudacanthotermes spiniger</i>	42	N/A	Landom <i>et al.</i> , 2006
Synthetic derivatives		SB-37	37	<i>Plasmodium, Trypanosome</i>	Jaynes <i>et al.</i> , 1988
		Shiva	37		

G⁺: Gram-positive bacteria; G⁻: Gram-negative bacteria; N/A: not available

[#]: these references have not been cited in this mini-review

Table 3

Proline-rich antimicrobial peptides

Insect Species		Peptide Name	# of proline/ O-glycosylated	Size (aa)	Activity	References
<i>Hemiptera</i>	<i>Pyrrhocoris apterus</i>	Pyrrhocoricin	5 / yes	20	G−, G+	Cocianich <i>et al.</i> , 1994
	<i>Oncopeltus fasciatus</i>	Pyrrhocoricin	6 / yes	20	G−, G+	Schneider and Dom, 2001 [#]
	<i>Palomena prasina</i>	Metalnikowin I, IIA	5 / no	15	G−	Chernysh <i>et al.</i> , 1996 [#]
		Metalnikowin IIB	4 / no	16		
		Metalnikowin III	5 / no			
<i>Hymenoptera</i>	<i>Apis mellifera</i>	Apidaecin Ia, Ib, II	6 / yes	18	G−	Casteels <i>et al.</i> , 1989
		Abaecin	10 / no	34	G−	Casteels <i>et al.</i> , 1990
	<i>Myrmecia gulosa</i>	Formaecin	5 / yes	16	<i>E. coli</i>	Mackintosh <i>et al.</i> , 1998b
	<i>Bombus pascuorum</i>	Apidaecin	7 / no	17	G−	Rees <i>et al.</i> , 1997
		Abaecin	13 / no	39	G+, G−	Rees <i>et al.</i> , 1997
	<i>Pteromalus puparum</i>	Abaecin-like	7 / no	19	G−, G+	Shen <i>et al.</i> , 2010 [#]
	<i>Bombyx mori</i>	Lebocins 1, 2	8 / yes	32	G−	Hara and Yamakawa, 1995b Furukawa <i>et al.</i> , 1997 [#]
		Lebocin 3, 4	7 / yes			
<i>Lepidoptera</i>	<i>Trichoplusia ni</i>	Lebocin	10 / yes	32	N/A	Liu <i>et al.</i> , 2000; Tamez-Guerra <i>et al.</i> , 2008
	<i>Manduca sexta</i>	Lebocin-A ^a	2 / no	22	G−, G+	Rayaprolu <i>et al.</i> , 2010; Rao <i>et al.</i> , 2012
		Lebocin-B ^a	5 / no	27	G−, G+, fungi	
		Lebocin-C ^a	5 / no	28		
		Lebocin	2–3 / N/A	31–32		
	<i>Samia cynthia</i>	Lebocin	8 / N/A	32	N/A	Bao <i>et al.</i> , 2005
<i>Pseudoplusia includens</i>	Drosocin	6 / yes	19	<i>E. coli</i>	Lavine <i>et al.</i> , 2005	
<i>Diptera</i>	<i>Drosophila melanogaster</i>	Melchikowin	7 / no	26	G+, fungi	Bulet <i>et al.</i> , 1993 Levashina <i>et al.</i> , 1995

G+: Gram-positive bacteria; G-: Gram-negative bacteria;

^a: synthetic peptides; N/A: not available[#]: these references have not been cited in this mini-review