CHAPTER

56

Insect Venom Peptides

MARIO SERGIO PALMA

ABSTRACT

The insects of the order Hymenoptera (bees, wasps, and ants) are classified in two groups, based on their life history: social and solitary. The venoms of the social Hymenoptera evolved to be used as defensive tools to protect the colonies of these insects from the attacks of predators. Generally they do not cause lethal effects but cause mainly inflammatory and/or immunological reactions in the victims of their stings. However, sometimes it is also possible to observe the occurrence of systemic effects like respiratory and/or kidney failure. Meanwhile, the venoms of solitary Hymenoptera evolved mainly to cause paralysis of the preys in order to permit egg laying on/within the prey's body; thus, some components of these venoms cause permanent/transient paralysis in the preys, while other components seem to act preventing infections of the food and future progenies. The peptide components of venoms from Hymenoptera are spread over the molar mass range of 1400 to 7000 Da and together comprise up to 70% of the weight of freeze-dried venoms. Most of these toxins are linear polycationic amphipatic peptides with a high content of α-helices in their secondary structures. These peptides generally account for cell lysis, hemolysis, antibiosis, and sometimes promote the delivery of cellular activators/mediators through interaction with the G-protein receptor, and perhaps some of them are even immunogenic components. In addition to these peptides, the Hymenopteran venoms also may contain a few neurotoxins that target Na^{+} and/or Ca^{+2} channels or even the nicotinic ACh receptor. This review summarizes current knowledge of the biologically active Hymenoptera venoms.

INTRODUCTION

The remarkable dominance of insects and other arthropods on land can be attributed, at least partially, to the extraordinary diversity of their chemical defense mechanisms [51]. In addition to the glandular defensive secretions, some arthropods developed sophisticated offensive/defensive chemical weaponry. In this regard, the development of venoms and its injection apparatus among the Insecta represented evolutionary attributes that contributed to adaptation of the insects to the many different terrestrial environments [51]. Thus, different orders of the Insecta developed their self-chemical weaponry, particularly the Hymenoptera (bees, wasps, and ants) that evolved into their venoms and stinging apparatuses according to their biology and behavior. The species with a solitary life history evolved their venoms to be used as paralytic tools in order to keep their prey alive for feeding and reproduction. The many wasp species taking this evolutionary way include the solitary aculeate wasps belonging to the superfamilies Bethyloidea, Scolioidea, Pompiloidea, Sphecoidea, and Vespoidea. This last superfamily is considered as a single family, the Vespinae, and contains the solitary families Massarinae and Eumeninae as well as the social Vespinae [37]. Members of this group of solitary wasps are seasonal, spending the cooler periods as diapausing larvae in nests provided by the mother-wasp. In the most cases, the food provided consists of arthropod prey paralyzed by injection of venom into their bodies. The immobilized prey is then carried to the nest, where the eggs are layed on the prey and the larval development takes place [37]. The constituents of these venoms are low-molecular-mass neurotoxins, such as polyamines, a cocktail of neurotransmitters, and a few peptides [26], which are discussed later in this chapter. Another group of solitary wasps, the Terebrant, which include the superfamilies Ichneumoidea, Cynipoidea, and Chalcicoidea evolved in the direction of parasitic behavior—that is, their venom evolved to promote short/long-lasting transient paralysis of the prey in order to permit egg-laying on/within the prey's body. In this case, after the egg laying, the prey recovers from the paralysis

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and carries the eggs of the parasitic wasps [37]. In this situation, the venom of these solitary wasps evolved to cause prey paralysis and seem to be constituted of high-molecular-mass proteins and low-molecular-mass compounds, generally presenting neurotoxicity. No peptide component is presently known for these venoms so that no further discussion about these components will be considered in the present chapter.

Meanwhile, those species that evolved in the direction of social behavior developed the formation of castes and established the hierarchic relationship among nestmates of different castes. Generally the social species built large nests containing many workers and larvae, in addition to storage of a reasonable amount of pollen, honey, or nectar dew, attracting many different types of predators to their nests [45]. These species evolved their venom to prevent the presence of predators, keeping them far from the colonies; the venom of these species is not used to promote lethal actions but to produce mneumonical actions on the victims of their stinging due to the uncomfortable effects of venoms such as pain, local burning, edema, swelling, bradycardia, tachycardia, headache, and, sometimes systemic effects like respiratory and/or kidney failure [32]. The venom of the social Hymenoptera consist of complex mixture of proteins, peptides, and low molecular mass compounds. The enzymes are responsible for the injury caused to tissues and frequently are immunogenic and therefore related to the allergy caused by these venoms in the victims of wasp and/or bee stings [13, 53]. Most abundant components of the venoms from social wasps and bees consist of peptide toxins.

Ants contain some groups of species that may be considered as the most specialized among the social insects, as well as contain species that may be considered as the least specialized ones. Some species form small and secretive cryptic colonies, preying only on a limited group of organisms, while other species live almost anywhere [5]. The ant venoms may be broadly classified as either predominantly consisting of protein and peptides or as a complex mixture of low-molecular-mass compounds. The ant species from the subfamilies Ponerinae, Myrmiciinae, Pseudomyrmecinae, and Ecitoninae generally contain venom rich in proteins and peptides.

This chapter summarizes the current knowledge concerning the biochemical and pharmacological properties of the most representative peptide toxins from insects of the order Hymenoptera, which are now thoroughly characterized. It is not easy to comprehensively review the extensive literature on all the major components of these venoms in the available space. Therefore, for the interest of readers the focus will be on the most relevant groups of biologically peptides from Hymenoptera venoms and the references mentioned will enable a more extensive search of the literature.

PEPTIDES FROM THE VENOMS OF SOCIAL HYMENOPTERA

The peptide components of venoms from social Hymenoptera are spread over the molar mass range of 1400 to 7000 kDa and together comprise up to 70% of the weight of freeze-dried Hymenoptera venoms [36]. Most of these peptides have polycationic amphipatic components, presenting a high content of α-helices in their secondary structures; these peptides generally account for cell lysis, hemolysis, antibiosis, and sometimes promote the delivery of cellular activators/mediators. In addition to this, the Hymenopteran venoms also may contain a few neurotoxic peptides.

Peptide Toxins from Honeybee Venom

Honeybee (*Apis mellifera*) venom contains well known peptides such as: melittin, apamin, tertiapin, secapin, and MCD-peptide. Some of these peptides present a detergent-like action on plasma membranes [8], causing cell lysis, while others are neurotoxins. Some aspects of the primary sequences, secondary structures and the biological actions will be emphasized.

Melittin

Melittin is the major component of honeybee venom, representing about 50% of the total honeybee venom. It consists of 26 amino acid residues, mostly with hydrophobic or at least uncharged side chains, except for the C-terminal region. Melittin can aggregates into a tetrameric form into the venom reservoir, being apparently inactive under this condition [4]. This peptide lowers the surface tension of water at the level of the plasma membrane, acting mainly by its natural detergent-like effect on the plasma membrane, causing cell lysis (specially of mast cells), followed by histamine delivery. Due to this lytic effect on cell membranes, this peptide may be considered as a venom diffusion factor, facilitating the entry of venom into the blood stream of the stung victims. X-ray crystallography of mellitin indicated that the residues 1 to 10 and residues 13 to 26 form α-helixes aligned about 120° to each other, while the proline in position 14 was suggested to be the cause of a bend in the middle of the rod structure [11]. Thus, a single polypeptide chain has the conformation of a bent alpha-helical cylinder [8]. When packed, the tetramer does so in a double planar layer. In order to achieve tight hydrophobic interactions, the hydrophobic residues of all four chains extend toward the center of the

tetramer. Due to its surfactant properties melittin is considered a direct hemolytic factor, acting synergistically with phospholipase A_2, activating this enzyme [4].

Apamin

The honey bee venom also contains apamin, a peptide of 18 amino acid residues. The CD spectroscopy study of apamin in solution is consistent with a peptide presenting as an alpha-helix an demonstrating a high degree of stability over a wide range of pH values [4]. This stability is a consequence of the rigidity imposed by two disulfide bridges. H^1-NMR studies of apamin in solution indicates a rigid, folded structure stabilized not only by the two disulfide bridges but by at least seven intra chain hydrogen bonds. The structure of apamin has been studied by the use of a number of spectroscopic techniques, such as H^1-NMR, CD, Raman, FT-IR, and structure prediction algorithms of energy minimization using NMR and CD data. At first, the most suitable interpretation of the experimental results was the proposal of the coexistence of both an alpha-helix and beta-turns [16]. However, until now, the best structure for apamin remains controversial, with several available structural models.

Apamin is permeable to the blood-brain barrier, causing its effects on the CNS by several routes of administration. It causes neurotoxic effects in the spinal cord of mammals, producing hyperactivity and convulsions in rats [15]. When peripherically applied, apamin seems to selectively and potently affect the potassium permeabilities of certain membranes, such as the smooth muscle of the gut. At very low levels, it appears that apamin treatment can convert the normal hyperpolarizing response to epinephrine into a calcium-dependent depolarization [9]. The peripheral effects of apamin suggest that the central action may be also due to decreased potassium fluxes, since this would broadly reduce inhibitory tone and increase the excitability.

Mast Cell Degranulating Peptide (MCD)

The MCD Peptide is a 22-residue-long toxin. Its secondary structure was studied by CD and ^{1}H-NMR [14, 50], which indicated a close resemblance to α-helical peptides from positions 13 to 19. It is stable over the pH range 2 to 8, stabilized by two disulfide bridges and six intrachain hydrogen bonds, which form a 28-atom ring structure, also observed in apamin [4]. It was suggested that this peptide may present two closely related conformations in solution, possibly differing by cys-trans isomerism involving the residues $Pro^{12}His^{13}$ [49].

It produces cytolysis, thus being considered a potent mast cell degranulator, causing the release of histamine [4]. This peptide is a facilitator of the response of honeybee venom, responsible for the reddening, swelling, and locally occurring pain at the site of honeybee stinging, typical of histamine-mediated responses.

Other Peptides

Secapin represents about 0.5% of total honey bee venom. It is a 25-amino-acid residue peptide, containing a single disulfide bridge and high proline content. Apparently, this peptide has no known physiological and/or pharmacological activity, probably because it was not sufficiently tested so far [4].

Tertiapin, which comprises about 0.1% of total honey bee venom, is a 21-amino-acid residue peptide, containing a single disulfide bridge and a C-terminal residue in an amidated form. This peptide is not particularly toxic on intravenous injection; it degranulates mast cells with low potency. It has been compared pharmacologically with prostaglandins under the same conditions [31]. Tertiapin has been proposed to have both alpha-helical and beta-sheet tendencies along the length of its sequence, raising the question of which conformational state the molecule is likely to be in. Considering the positions of both disulfide bridge as well as its CD spectrum, which has no indication of a beta-sheet, the structure of tertiapin is probably analogous to that of the MCD peptide [4].

Peptide Toxins from Bumblebee Venoms

Bombolitins

The bombolitins are a family of heptadecapeptides isolated from the venom of the bumblebee *Megabombus pennsyluanicus* [3]. These peptides are able to form amphiphilic α-helical structures either by self-association or by interaction with amphiphilic matrices, such as micelles or vesicles. The biological activity of these peptides seems then to be directly correlated to their capability to form an amphiphilic order structure (α-helix) that allows them to associate and penetrate cellular membranes. They are capable of lysing erythrocytes and lipossomes, in addition to increasing the activity of the enzyme phospholipase A_2. In more general terms the bombolitins share common biological properties with other "toxin" peptides such as melittin, crabrolin, and mastoparans.

PEPTIDE TOXINS FROM SOCIAL WASPS

The venoms of social wasps contain a series of polycationic amphipathic peptides, such as mastoparans,

chemotactic peptides, and wasp kinins, presenting a series of multifunctional pharmacological actions, which generally contribute to the occurrence of intense inflammatory processes.

Mastoparans

The most abundant peptide component from Vespide venoms is represented by the mastoparans, which involves a histamine-releasing principle, acting first as the MCD-peptide from honeybee venom. Mastoparans generally are polycationic, linear tetradecapeptide amides, rich in hydrophobic residues such as leucine, isoleucine, and alanine. The most representative molecules of species endemic from regions with a cold climate show characteristic lysine residues in positions 4, 11, and 12 [32], but novel peptide sequences showing a different pattern of lysine distribution have been identified recently in wasp species endemic from the tropical regions [29, 48]. Mastoparans are randonly coiled in water, while in methanolic solution and/or in the presence of lipid vesicles they are generally alpha helical peptides, causing their effects by interacting with plasma membranes due to their amphipaticity. According to their mode of action, these peptides may be classified into two groups: 1) those acting by cell lysis due to pore formation on cell membranes [1, 28, 32] and (2) those acting by interaction with G-protein coupled receptors, leading to the activation of degranulation mechanisms and resulting in many different types of secretions, depending on the type of target cell [18, 30]. In any situation the final action will be histamine delivered from mast cells, serotonin from platelets, or even insulin from pancreatic β-cells. In addition to the interaction with membrane components, mastoparans also activate phospholipases A [1]. Recently, antibacterial activities have been described for these peptides [27, 28].

Chemotactic Peptides

The second most important group of peptides among the Vespid are the chemotactic peptides, which generally are trideca-polycationic peptides, with primary sequences resembling the mastoparans in regards to the richness of hydrophobic amino acid residues and the amidation of the C-terminal residue [32]. These peptides induce chemotaxis in polymorphonuclear leukocytes and macrophages [52] and sometimes also release histamine from mast cells [32]. The result of such chemotactic activity is the development of a mild edema, accompanied by an inflammatory exudate around the site of stinging, containing mainly polymorphonuclear leucocytes. Thus, the chemotactic peptides do not cause pain directly but play an important role enhancing the inflammatory effect of wasp stings. A novel type of chemotactic peptide was recently reported in which the acetylation of the N-terminal residue seems to play part in the modulation of polymorphonuclear leukocytes attraction [44].

Kinin-Related Peptides (Wasp Kinins)

A third group of peptides biologically important among the Vespid toxins are the wasp kinins that generally are known as pain-producing peptides. These peptides consist of bradykinin molecules elongated at the N-terminus with some extra amino acid residues. Sometimes there is also an elongation from the original bradykinin C-terminal residue [32]. The general pharmacological properties are similar to those presented by human bradykinin; however, the effects of Vespidae kinins are much more pronounced and long lasting. These effects may be summarized as follows: When injected intravenously, they cause hypertension in rats, dogs, rabbits, and cats; hyppotension in chickens; brochoconstriction in guinea pigs; contraction of most isolated smooth muscle preparations; and relaxation of rat duodenum [42].

Sylverin

A fourth group of amphipathic polycationic peptides from Vespide venoms has been described, but the few characterized up to now are the sylverins [10]. These peptides contain from 21 to 25 amino acid residues in their primary sequences, presenting a single disulfide bridge, constituting the first example of this type of structure among the Vespid peptide toxins. These peptides are potent mast cell degranulators without known hemolytic activity.

Crabrolin

This is a 13-amino-acid residue polycationic peptide present in the venom of the hornet *Vespa crabro*, presenting a helical conformation [27]. This peptide produces potent hemolytic and antibacterial activity; however, it is a weak mast cell degranulator, with less than 20% the activity of mastoparan. Crabrolin is reported to be four times less active than mastoparan in guinea pig erythrocytes [2] and showed no effect in rat erythrocytes.

PEPTIDES FROM THE VENOMS OF SOLITARY WASPS

Contrary to social wasps, solitary wasps offensively use their venoms for prey capture; they inject their venoms into prey insects or spiders to paralyze and use

them to feed their larvae. Therefore, the solitary wasp venoms should contain some neurotoxins acting on nervous systems [37] and antimicrobial peptides to prevent their preys from being colonized by pathogenic microorganisms [25]. Recent investigations indicate that solitary wasp venoms may contain a variety of bioactive substances—in particular, amphipathic peptides with antibiotic and inflammatory actions besides neurotoxins [21–26].

Bradykinin-Related Peptides

Neurotoxic kinins have been reported in the venom of solitary wasps, such as threonine-bradykinin (Thr6-BK) and megascoliakinin (Thr6-BK-Lys-Ala) isolated from the venom of the European scolid wasp *Megascolia flavifrons* [38, 39]; Thr6-BK was also reported in the venom of the wasp *Colpa interrupta* [40, 41]. An analytical survey with the venom extracts of 27 species of solitary wasps from the families Pompilidae, Specidae, Eumenidae, and Scoliidae, using MALDI-TOF MS as the experimental tool, demonstrated that BK-related peptides were present in four of these species, with Thr6-BK as the major component in the venom of *Megacampsomeris prismatica* [26]. The wasp kinins are known to block the synaptic transmission of the nicotinic acetylcholine receptor in the insect central nervous system [19, 38–40].

Pompilidotoxins (PMTXs)

The pompilidotoxins (PMTXs) constitute a family of toxins consisting of 13 amino acid residues, isolated from the venoms of the pompilid wasps *Anoplius samariensis* and *Batozonellus maculifrons* [22]. PMTXs affect both the vertebrate and invertebrate nervous systems by blockade of sodium channel inactivation [46]. Most notably, this toxin discriminates between neuronal and cardiac sodium channels [21].

Eumenine Mastoparan-AF (EMP-AF)

EMP-AF is a tetradeca-polycationic peptide isolated from the solitary wasp *Anterhychium flavomargimatum micado*, with general characterisics of the mastoparans from social wasps venoms; however, the location of lysine residues in EMP-AF is a little bit different from the mastoparan, being present at positions 5, 8, and 12 instead of positions 4, 11, and 12 [6, 23]. NMR study of this peptide suggested that it consists of an amphipathic α-helix conformation stabilized by the C-terminal residue in the amidated form [47]. EMP-AF is mast cell degranulating peptide, which also affects the neumuscular transmission in the lobster walking leg preparation [23].

Anoplin

Anoplin is a linear polycationic decapeptide presenting an α-helical amphipathic conformation, isolated from the solitary wasp *Anoplius samariensi*. It is highly conserved in relation to crabrolin and mastoparan-X; therefore, it is also a mast cell degranulating component. This peptide shows antimicrobial activity, both against Gram-positive and Gram-negative bacteria, and is considered the smallest among the linear antimicrobial peptides yet found in nature [24].

PEPTIDES FROM ANT VENOMS

Most of the ants only present traces of proteins/peptides in their venoms; however, in those species producing peptides as components of their venoms, these toxins follow the same general pattern already observed for peptides from wasps and bees venoms—that is, short and linear polycationic peptides, presenting a high content of α-helices, which are responsible for cell lysis, hemolysis, histamine release from mast cells, and antimicrobial actions. A few neurotoxins are also known.

"Myr p" Peptides and Pilosulin

The venom of the Australian ant *Myrmecia pilosula* seems to contain a complex mixture of allergenic peptides, some of them forming heterodimers, apparently maintained by disulfide bridges. From this complex was identified Myr p 1, the major expressed allergenic product being a 56-residue peptide, while Myr p 2 is a 27-residue peptide; these peptides may dissociate or are cleaved to minor fragments, such as those previously identified as pilosulin-1 and -2, characterized as the fragments 57–112 from Myr p1 and 49–75 from Myr p2 [7]. Pilosulin-1 was characterized as a α-helical peptide with potent and broad spectrum antimicrobial activity, both against standard and multidrug-resistant Gram-positive and Gram-negative bacteria, and *Candida albicans* [54]. Other pilosulins have been identified and characterized as hemolysins and histamine release peptides [20].

Poneratoxins

Poneratoxin is a neuropeptide found in the venom of the ant *Paraponera clavata*. It is a peptide containing 25 amino acid residues, which affects the voltage-dependent sodium channels and blocks the synaptic transmission in the insect central nervous system in a concentration-dependent manner. The NMR structure shows the peptide in the form of two α-helices

connected by a β-turn; the helices have quite different characteristics from each other: One of them is apolar, whereas the second contains polar and charged amino acids. This will result in different interactions with cell membranes. The extremely hydrophobic N-terminal helix may interact with uncharged lipid bilayers, while the C-terminal helix, slightly positively charged and terminating with arginine, will be able to attach to negatively charged cell surfaces as previously found for other membrane interacting peptides. Such a toxin can thus use two different complementary modes of interaction to attain its target, cellular membranes [41].

Ponericins

Ponericins constitute a group of peptides isolated from the venom of the predatory ant *Pachycondyla goeldii*, exhibiting haemolysis, insecticidal activity against cricket larvae, and antimicrobial action against Gram-positive and Gram-negative bacteria. According to their primary structure similarities, they can be classified into three families: ponericin G, W, and L. Ponericins share high sequence similarities with known peptides: ponericins G with cecropin-like peptides, ponericins W with gaegurins and melittin, and ponericins L with dermaseptins. The comparison of the structural features of ponericins with those of well-studied peptides suggests that the ponericins may adopt an amphipathic α-helical structure in polar environments, such as cell membranes [34, 35].

Ectatomin

This peptide is a neurotoxin isolated from the venom of the *Ectatomma tuberculatum* ant and contains two highly homologous peptide chains (consisting of 37 and 34 amino acid residues) linked to each other by disulfide bonds [43]. Each chain consists of two alpha-helices and a hinge region of four residues; this forms a hairpin structure that is stabilized by disulfide bridges; the hinge regions of the two chains are connected together by a third disulfide bridge. Thus, ectatomin forms a four-alpha-helical bundle structure [33]. Ectatomin is a potent inhibitor of calcium currents after a latency of a few seconds in rat ventricular myocites [43].

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