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REVIEW

Host defence peptides from invertebrates – emerging antimicrobial strategies

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Abstract

Cationic antimicrobial (host defence) peptides are found as potent components of the innate immune system of all invertebrates in which they have been investigated. They vary substantially in their amino acid sequences, secondary structures, inducibility, potency and antimicrobial activity spectra. This enormous diversity is providing templates for the design and development of both antibiotic peptides and peptides that selectively modulate innate immunity to increase protection against infections and sepsis.

Keywords: Antibiotics; Antimicrobial peptides; Cationic peptides; Invertebrate immunity; Innate immunity

Contents

Properties of cationic antimicrobial (host defence) peptides

Cationic amphiphilic peptides are found as a component of the innate immune system of all species of life, including invertebrates and vertebrates (Hancock and Diamond, 2000; Zasloff, 2002; Bulet et al., 2004; Iwanaga and Lee, 2005). Collectively they have been termed (cationic) antimicrobial peptides due to the observation that many have direct antimicrobial activity under physiological conditions, or cationic host defence peptides, reflecting their broader involvement in mammalian immunity, including an ability to neutralize bacterial endotoxins such as LPS (a property shared by many invertebrate peptides) and to modulate the activities of the innate and adaptive immune systems.

Cationic antimicrobial (host defence) peptides in nature comprise short sequences of amino acids ranging from around 12 to 50 amino acids in length. They have

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net positive charges of +2 to +9 due to their lack or small number of acidic residues (glutamate or aspartate) and excess number of cationic (arginine or lysine and/or histidine) residues, and around 30–50% hydrophobic residues. Table 1 demonstrates a modest number of invertebrate peptides while Fig. 1 demonstrates some representative structures. A variety of modifications are observed in different peptides, but this is not common. Some peptides pre-form their secondary and tertiary structures in free solution, most notably those with two to four disulphide bridges, stabilizing a predominantly β-sheet or β-hairpin structure (although many of the larger β-stranded peptides have an additional α-helix). On the other hand, a large number of peptides have little or no structure in free solution, folding into an amphipathic or amphiphilic structure upon contact with a membrane target (Powers and Hancock, 2004). The most common of these are those which fold into α-helical structures, while other peptides which have an over-representation of particular amino acids (e.g. proline, tryptophan, histidine) can form more extended structures. While their three-dimensional structures might vary, most peptides in their final folded conformations have patches of hydrophobic and charged residues that permit the peptides to interact strongly with membranes. These interactions can be relatively selective for bacterial membranes due to the high proportion of anionic lipids at their membrane surface, a substantial electrical potential gradient oriented internal negative and a lack of rigidifying lipids like cholesterol. However, it is worth pointing out that there is a spectrum of toxicities ranging from relatively benign to extremely toxic and some of the most potent toxins, including scorpion charybdotoxin, bee venom melittin and wasp venom mastoparan, have strong antimicrobial activity and overlapping physical properties with the host defence peptides.

Antimicrobial peptides are an essential component of invertebrate innate immunity and consequently are found in every invertebrate in which they have been looked for. A full and detailed account of these peptides is beyond the scope of this review, and we refer the reader to the following reviews in which insect (Hetru et al., 2000) and marine invertebrates (Tincu and Taylor, 2004) are discussed in detail.

Functions of cationic peptides in invertebrate immunity

Invertebrates are devoid of adaptive immune responses which appeared in evolution some 450 million years ago (Hoffmann, 2004); in particular, invertebrates lack such critical elements of adaptive immunity as antibodies and lymphocytes, immunological memory and true self vs. non-self discrimination. Nevertheless, invertebrates can resist infections and can also transmit lethal diseases to humans and livestock, without necessarily succumbing to these infections. Thus, innate immunity, the most ancient first line of immune defence, is vital in invertebrate host defences, and indeed has become conserved throughout the animal kingdom. In their quest to understand how invertebrates survive without adaptive immunity, researchers discovered several aspects of immune responses which play a critical role in animal innate immunity, including the family of pathogen recognition receptors (Toll-like receptors; TLR), production of toxic oxygen and metabolites, and antimicrobial peptides. It is now understood that the immune system of invertebrates is complex and heterogeneous (Iwanaga and Lee, 2005), even though there are several elements in common, especially within related invertebrates. For example, microbial challenge of insects results in haemocyte-mediated cellular immune response including phagocytosis and encapsulation, and humoral defences which include production of antimicrobial peptides (Iwanaga and Lee, 2005).

Antimicrobial peptides in invertebrates came into focus in the 1980s (Steiner et al., 1981) and are now described as key effector molecules in antimicrobial host defences. The last decade has seen the identification of numerous antimicrobial peptides in invertebrates (Bulet et al., 2004). Gene-encoded, naturally occurring antimicrobial peptides found in the haemolymph, both in the plasma and haemocyte cells, are involved in both systemic as well as site-specific protection against microbial pathogens in invertebrates. They are produced in the phagocytic cells of invertebrates (Mitta et al., 1999; Iwanaga et al., 1998; Dimarcq et al., 1988), with tissues such as insect fat bodies being the main site of synthesis (Engstrom, 1998). Individual antimicrobial peptides are expressed either constitutively, for example in the haemocytes of shrimp, oysters or horseshoe crab (Bachère et al., 2004; Iwanaga and Kawabata, 1998), or are induced upon pathogenic challenge, such as in *Drosophila* where antifungal peptide synthesis is mediated by the Toll receptor (Lemaitre et al., 1996; Imler and Hoffmann, 2000). Upon entry of pathogens, the circulating haemocytes of invertebrates migrate through chemotaxis to the site of injury and release antimicrobial peptides, which in turn exert their antimicrobial effects, as well as potentially eliciting responses that modulate inflammation, thus creating a parallel scenario to that which is observed with the mammalian innate immune response.

One of the dramatic discoveries in insects that led to a paradigm shift in thinking about innate immunity was the discovery of pattern recognition receptors (TLR) in *Drosophila melanogaster* (Hoffmann, 2003). The use of similar receptors and pathways for innate immune