ANTIMICROBIAL PEPTIDES FROM PLANTS AS MECHANISM OF DEFENSE

PEPTIDOS ANTIMICROBIANOS DE PLANTAS COMO MECANISMOS DE DEFENSA

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Abstract

Over the past decades numerous membrane-lytic peptides have been isolated from insects, amphibians, fungi, bacterias, mammals, and plants. The main group of antimicrobial peptides found in plants are thionins, defensins, and lipid transfers proteins. These peptides are present in most, if not all plant species, and so far contain an even number of cysteines (4, 6 or 8), which are all pair wise connected by disulfide bridges, thus providing high stability. This could constitute interesting candidates in order to engineer disease resistances in plants. This review article presents some examples of these molecules and discusses their structural properties and mechanisms of action.

Key words: antimicrobial peptides, antifungal, antibacterial, plant, defensins, thionins, fungi, innate immunity, AMP.

Resumen

En el curso de las pasadas décadas numerosos péptidos que ocasionan lisis de membranas se han aislado de insectos, anfibios, hongos, bacterias, mamíferos y plantas. El principal grupo de péptidos antimicrobianos encontrado en plantas lo constituyen las tioninas, defensinas y proteinas de transferencia de lipidos. Estos péptidos se encuentran en la mayoría, sino en todas las especies de plantas y hasta donde se conoce contienen un número par de cisteinas (4, 6 u 8), las cuales están formando puentes disulfuro, lo que le provee alta estabilidad. Por esta razón los péptidos antimicrobianos son excelentes candidatos para diseñar plantas resistentes a enfermedades. Esta revisión presenta algunos ejemplos de estas moléculas y discute sus propiedades estructurales y mecanismos de acción.

Palabras claves: péptidos antimicrobianos, antifúngico, antibacterial, planta, defensinas, tioninas, hongos, inmunidad innata, AMP

INTRODUCTION

Gene-encode antimicrobial peptides (**AMPs**) are an ancient and pervasive component of plants and animals innate defense mechanisms, they have been developed in order to control the natural flora and combat pathogens, and because of their mechanism of action, that is not receptor-mediated, AMPs have an excellent potential for development as a novel therapeutic agents that could overcome the antibiotic resistance problem. These peptides show a marked degree of variability, having evolved to act against distinct microbial targets in different physiological contexts.

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AMPs encompass a wide variety of structural motifs, but most of them are cationic and amphipathic with α-helical structure. However, there are hydrophobic AMPs with α-helical and β-sheet motifs (Epand and Vogel, 1999).

The AMPs may be classified, according to their structure, into linear peptides, mostly helical, such as cecropin, magainin, and melittin; disulfide-linked peptides, β-sheet or both β-sheet and α-helical, such as the well known defensins, tachyplesins, and peptides from the lantibiotic family containing posttranslational modified amino acids (Wu et al., 1999).

Numerous structure/activity relationship studies have indicated at least seven parameters that can influence the potency and spectrum of activity of α-helical AMPs: the size, the sequence, the degree of secondary structure (% helical content), the charge, the overall hydrophobicity, and the respective widths of the hydrophobic and hydrophilic faces of the helix (Tossi et al., 2000). It has been well established that these peptides act by enhancing the permeability of membranes, thus perturbing their barrier function. In the first stage of the membrane-permeabilizing process, the cationic peptides bind to membranes by adopting an amphipathic, mostly α-helical secondary structure with a polar helix face exposed to the lipid head groups, that are negatively charged, and the hydrophobic face somewhat embedded into the lipid acyl chain region forming conductance events, which are proposed to lead to the leakage of cell contents and cell death. There is ample evidence that membrane disruption can occur in model membrane systems, although it has been correctly pointed out that this occurs at very high peptide-to-lipid ratios (Wieprecht et al., 1997; Wu et al., 1999).

There are hundreds of known antimicrobial peptides and proteins that have been isolated from all the living species: insects, amphibians, bacterias, fungi, mammals and plants (Epand and Vogel, 1999). Particularly, plants produce a number of cysteine-rich AMPs, that are based on amino acid sequence homology and which are commonly found in the seeds (De Lucca et al., 2005). They are both inducible and constitutive and differ in size and structure. Plant AMPs have been classified and named on the basis of their mechanism of action (e.g. glucanases), structure (e.g., cysteine-rich), or their similarity to a known "type" protein. Some of them are antifungal proteins like pathogenesis related (**PR**) proteins, glucanases, chitinases, chitin-binding proteins, thaumatin-like (**TL**) proteins, cyclophilin-like proteins, glycine/histidine-rich proteins, ribosome-inactivating proteins (**RIPs**), killer proteins, protease inhibitors, defensins, thionins, lipid-transfer proteins (**LTPs**), hevein- and knottin-type peptides, cyclopeptide alkaloids and other unique peptide groups. Since the list of AMPs from plants is large and daunting this brief review will focus only on antimicrobial peptides from plants (less than 10 KDa, about 100 amino acid in length) as defense components, we will briefly highlight some of the well-studied mechanism of action of AMPs.

MECHANISMS OF ACTION

Interactions between bioactive peptides and cell membranes play a key role in a number of cellular processes, including insertion and folding of membrane proteins as well as the formation and structure of ion channels, and interaction of hormones with membrane receptors. A number of physicochemical properties, such as peptide charge, hydrophobicity, amphipathicity and the degree of secondary structure angle subtended by the polar face, have been shown to be critical in the cell-lytic properties of these peptides (Mozsolits et al., 2001).

Several studies have been done using model membrane systems aimed to characterize the mechanism of AMP membrane permeabilization, some of the main mechanisms of action proposed are listed below.

The Barrel-Stave Model. The Barrel-stave model involves three steps. In the first step, AMP monomers dissolved in solution are bound to the membrane. Next, these monomers penetrate into the lipid bilayer. Finally, the monomers aggregate into a barrel-like structure in which a central aqueous pore surrounded by proteins is formed. Therefore, peptides that act *via* the barrel-stave mechanism should kill bacteria below the experimentally observed micro molar concentrations, which are required to cover all the outer surfaces of the bacterial membrane (Steiner et al., 1988).

Theoretically, peptides that act through this mechanism have been found to have several distinct properties: first, they should be very hydrophobic in order to penetrate into the hydrophobic core of the lipid bilayers. Second, the peptides must recognize each other in the membrane bound state in order to form bundles of transmembrane pores. Third, the peptides should have a minimal length of ~ 22 amino acids in the case of α-helical peptides, or ~8 amino acids for a β-sheeted structure in order to transverse the lipid bilayers. Fourth, a transmembrane pore can be formed with as few as 3 molecules (figure 1a), (Shai, 2002).

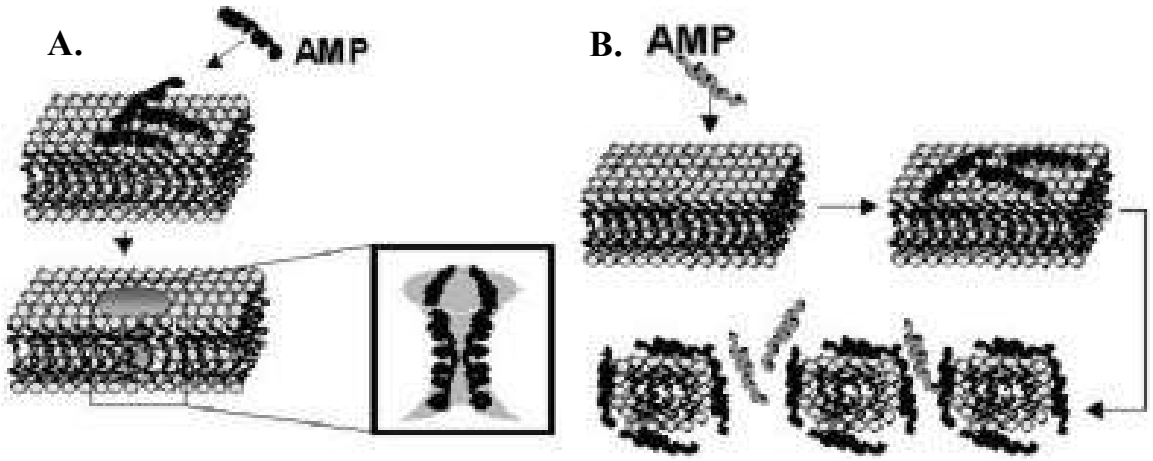

Figure 1. Mechanisms of Action. A. Barrel Stave Model. Peptides bind on the surface of the membrane until a threshold concentration is reached. Then peptide organization occurs in order to allow the permeation pathway. **B.** Carpet model. Peptide monomer binds preferential to the phospholipids head groups; then the alignment of the monomer on the surface occurs facing the phospholipids head groups or water molecules. Rotation of the peptide molecule leads to the reorientation of the hydrophobic residues towards the hydrophobic core of the membrane, thus disintegrating of the membrane occurs by disrupting the bilayer curvature.

The Non-ion Channel Formation Mechanism. The most likely site to be the target of membrane-permeating antibacterial peptides, is the inner bacteria membrane, which typically contains the electron transport chain and the enzymatic apparatus necessary for oxidative phosphorylation (Westerhoff et al., 1989). To reach this membrane, the peptides have to cross the bacterial cell wall, in which the outer surface contains lipopolysaccharides (**LPS**) in Gram-negative bacteria and acidic polysaccharides (teichoic acids) in Gram-positive bacteria. These components give the membrane surface of the bacterias a negative charge, therefore, the net positive charge of the antibacterial peptides should facilitate their binding to bacteria membranes. If high cooperation in binding occurs, the majority of the peptides should remain in the outer surface and then they will form large aggregates. Such aggregates would impede the diffusion of

monomer into the inner membrane. On the contrary, the presence of monomeric forms of the peptides on the outer surface of bacteria, which is characteristic of simple adhesion, should allow them to readily diffuse into the inner membrane and permeate it (Gazit et al., 1994).

The "Carpet" Mechanism. This model describes a situation in which lytic peptides are in contact with the phospholipid head groups throughout the entire process of membrane permeation. According to this model, lytic peptides initially bind onto the surface of the target membrane and cover it in a carpet-like manner. Membrane permeation occurs only if there is a high local concentration of membrane-bound peptides. This high concentration can happen, either after all the surface of the membrane is covered with peptide monomers, or after an association between membrane-bound peptides, forming, in both cases, a localized "carpet". There are four steps proposed for this model: **i**) preferential binding of peptide monomer to the phospholipids head groups, the initial interaction with the negatively charged target membrane is electrostatically driven, and therefore peptides are positively charged; **ii**) alignment of the peptide monomer on the surface facing the phospholipids head groups or water molecules; **iii**) rotation of the molecule leading to reorientation of the hydrophobic residues toward the hydrophobic core of the membrane; and **iv**) disintegrating the membrane by disrupting the bilayer curvature (figure 1B). In the "carpet" model, contrary to the "barrel-stave" model, peptides are not inserted into the hydrophobic core of the membrane, neither do they assembled with their hydrophilic surfaces facing each other. Furthermore, a peptide does not necessarily require the adoption of a specific structure upon its binding to the membrane (Shai, 1999).

Plant AMPs. There is not an organism in the world that is or has not been under the influence of a fungus. They are an amazing group, with one hundred thousand species world-wide, all of them are heterotrophes and eukariotic organisms with a protecting cell wall that affords a clear difference between them and their plant or animal host, providing an experimental target for antifungal antibiotics. The cell surface of a fungus is composed of three contiguous interconnected matrixes: an exocelullar or capsular component made by β glucans and mannoproteins, a wall component made by chitin, cellulose and some proteins, and the plasmalemma (figure 2).

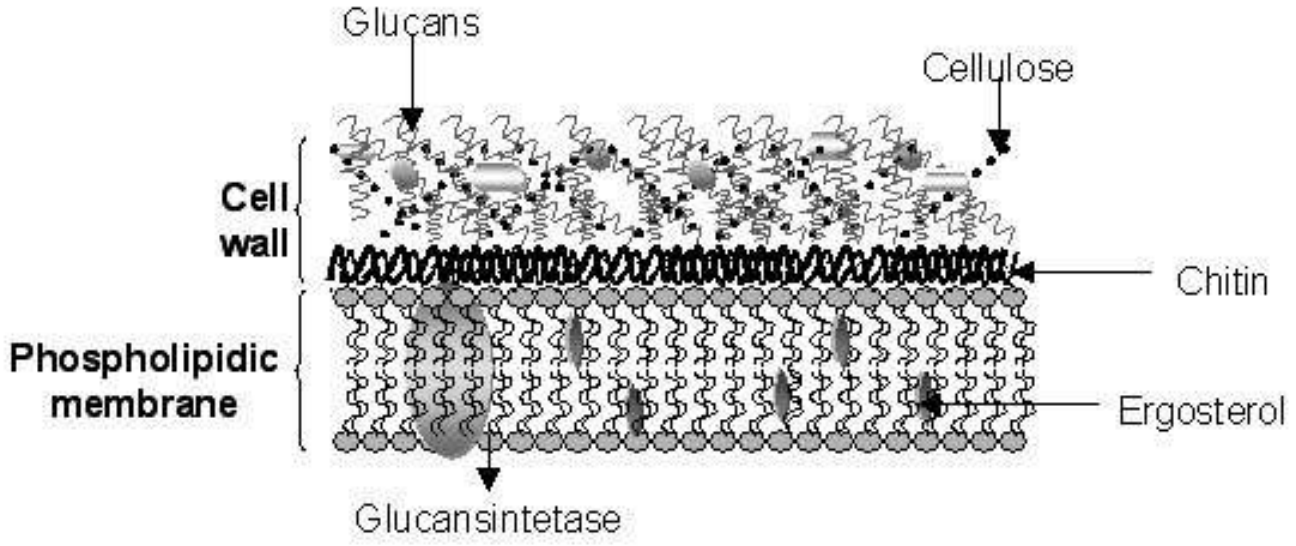

Figure 2. Schematic drawing of fungi and yeasts cell wall. It is composed, normally, of chitin, (1-3)β-D-glucan, (1-6)β-glucans, cellulose, lipids and peptides embedded in a protein matrix.

Plants are exposed to a large number of pathogenic fungi and bacteria; although they do not have an immune system, they have evolved a variety of potent defense mechanisms, including the synthesis of low-molecular-weight compounds, proteins, and peptides with antimicrobial activity. The plant AMPs are mostly antifungal, there are hundreds of antifungal peptides and proteins known, with more being discovered everyday. All plant antimicrobial peptides isolated so far contain even numbers of cysteines (4, 6 or 8), which are all pair wise connected by disulfide bridges. Their mechanisms of action are as varied as their sources and include fungal cell wall polymer degradation, membrane channel and pore formation, damage to cellular ribosome, inhibition of DNA synthesis, and inhibition of the cell cycle (Selitrennikoff, 2001). The most common and studied types of antimicrobial peptides from plants, their structural features and mechanisms of action are explained below.

Thionins. The thionin peptide family, usually known as α/β, are antimicrobial peptides, which have been isolated from a wide range of plant species, including both monocots and dicots. Traditionally, α/β-thionins were subdivided into five different classes (I, II, III, IV and V) (Bohlmann and Apel, 1991). *Type I* thionins are present in the endosperm of grains, are highly basic, and consist of 45 amino acids, 8 of which are cysteines (Egorov et al, 2005). *Type II* thionins were isolated from leaves and nuts of the parasitic plant *Pyrularia pubera* (Vernon, 1992) and from the leaves of barley *Hordeum vulgare* (Rodriguez-Palenzuela et al., 1988). They are slightly less basic than type I (reduced positive charge from +10 to +7) and consist of 46-47 amino acids. Both type I and II thionins have four disulfide bonds. *Type III* thionins were extracted from leaves and stems of mistletoe species, such as *Dendrophthora clavata, Phoradendron tomentosum, P. liga,* and *Viscum album* and consist of 45-46 amino acids. These thionins have tree disulfide bridges, and are basic as type II thionins. *Type IV* thionins are neutral in charge, consist of 46 amino acids with three disulfide bonds, and have been extracted from seeds of *Abyssinian cabbage* (van Etten et al., 1965). Finally, *type V* thionins are truncated forms of regular thionins found in some grains like wheat, but they are lacking of antimicrobial activity (Castangaro et al., 1994).

Modern structural analysis has shown that all five classes exhibit the same structural motif and α- and β-thionins share the same three-dimensional structure. The structure of thionins have been determinated either by X Ray diffraction or NMR, and the studies have revealed a global fold characterized by a L shape, where the long arm is formed by two antiparallel α-helices and the short arm by a β-sheet consisting of two short antiparallel β-strands, the architecture of this sheet is additionally strengthened by two disulfides bridges. After a short stretch of extended conformation, there is a helix-turn motif (figure 3A) (Stec, 2006). They have an amphipathic structure; the hydrophobic residues are clustered at the outer surface of the long arm of the L, whereas hydrophilic residues mainly occur at the inner surface of the L and at the outer surface of the corner of the L.

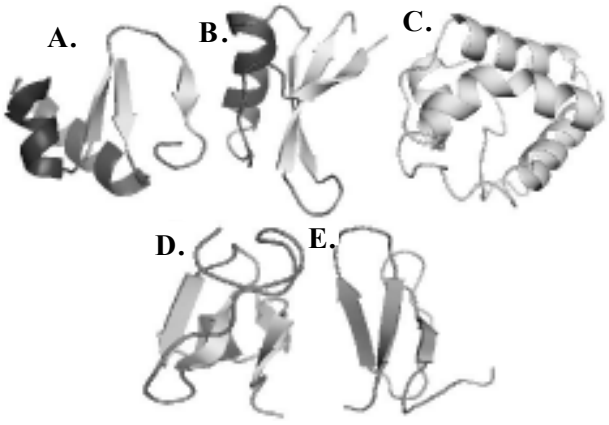

Figure 3. Schematic drawing of the AMPs three-dimensional structures. **A.** Thionin from seeds of *Crambe abyssinica* (Teeter et al., 1993), **B.** Plant defensin Ah-Amp1 from seeds of *Aesculus hippocastanum* (Fant and Borremans, 1999), **C.** LTP from seeds of *Oryza sativa* (Poznanski et al., 1999), **D.** Hevein-type from *Hevea brasiliensis* (Andersen et al., 1993), **E.** Knottin-type from the seeds of *Phytolacca americana* (Gao et al., 2001).

There are several independently proposed functions for these small cysteine-rich peptides. Several experiments suggest an important role in defense against pathogenic invaders strongly supported by thionin synthesis in response to bacterial invasion (Bohlmann et al., 1998), its accumulation in vulnerable tissues (Orru et al., 1997), its toxicity to different organisms and cell lines (Molina et al., 1993), and the improved resistance observed when they are expressed transgenetically (Iwai et al., 2002). On the other hand, their high concentration in the endosperm (in some species up to 10% of the seed mass) and high cysteine content suggest that they also serve as storage proteins (Stec, 2006).

Thionins inhibit the growth of Gram-positive and Gram-negative bacteria, fungi *in vitro* and some mammalian cell lines (Stec, 2006). Indirect experimental evidence has been accumulated indicating membrane disintegration upon thionin application. Only a few direct studies, in well-controlled conditions, have been carried out, though the precise mechanism of permeabilization remains unknown. Nonetheless, *in vivo* and *in vitro* studies suggest three different possible mechanisms to explain the primary lytic effect of toxin action on the membrane. The first mechanism proposes that the thionins arrange themselves at the surface of a bilayer and form an ion channel, like the "Barrel stave Model" described above (figure 1A). Experiments with artificial membranes show that membrane destruction occurs within 1 hour of treatment, which corresponds well with *in vivo* studies. These results suggest ion-channel formation mechanism (Llanos et al., 2004). However, the artificial membrane is destroyed (*i.e*. ion channel activity disappears) in the same time interval as reported for *in vivo* toxicity. Therefore, the proposition that, even with artificial membranes, membrane integrity is invariably lost at similar concentration and time thresholds argues against stable ion channel formation (Stec, 2006).

The second proposal contends that thionins modify the surface of the bilayer by forming patches or carpets (figure 1B). Several studies carried out with thionins provide arguments supporting this type of model. The crystal packing of all four active thionins clearly suggests potential arrangements that would promote patch formation (Papo and Shai, 2003).

The third mechanism proposes that the highly positively charged toxins form dimers bridged by phosphate ions. When they bind to the membrane, the dimers dissociate. In order to extract the phospholipids, the thionins must bind to individual phospholipids molecules stochiometrically and solubilize them. Binding of thionins to individual phospholipid head groups promotes formation of negatively charged patches of phospholipid molecules, that fluidify the membranes and facilitates phospholipid withdrawal by lowering the energy penalty for the phospholipid membrane separation. The withdrawal produces additional membrane instability and irreparable lysis. This initial lysis is even followed by extensive multidirectional damage done by toxins and other agents to the cytoplasmic and nuclear components of cell (Stec et al., 2004).

In fungi and yeasts, it was found that the thionins causes permeabilization of the fungus cell as evidenced by leakage into the culture medium of K^+, PO_4^{3+} and cellular components. Using artificial planar lipid bilayers, it was shown that thionins could alter electrical and physicochemical properties of the membrane at relatively low concentrations (1 mg/ml). This process does not involve the formation of ion channels by the thionins, since current-time plots showed highly irregular current spikes, but not square-like fluctuations as usually observed for channel-forming proteins (Thevissen et al., 1996).

Defensins. In innate immune response, only one class of antimicrobial peptides, the defensins, seems to be conserved between plants, invertebrates and vertebrates. This suggests that defensins are ancient peptides conserved across the eukaryotic kingdom, originated before the evolutionary divergence of plants and animals. Possibly, defensins have evolved from a single precursor, being a molecule with an overall structure resembling that of plant defensins

(Zhang, 2003). At the beginning, plant defensins were called γ-thionins since their size and cysteine content were found to be similar. However, subsequent structure analysis has demonstrated that γ-thionins are not related to thionins. Because of their structural similarity to mammalian and insect defensins, γ-thionins were renamed plant defensins (Terras et al., 1995). They have now been isolated from over a dozen of plant species and most probably occur in all species. The tissue in which they occur range from leaves (Kragh et al., 1995), tubers (Moreno et al., 1994), flower organs (Kragh et al., 1995), pods (Chiang and Hadwiger, 1991), and seeds (Osborn et al., 1995).

Plant defensins are small (45-54 amino acids), highly basic, amphipathic, cysteine-rich peptides. All plant defensins identified so far have eight cysteines. Structural studies have shown that defensin architecture comprises a triple-stranded β-sheet with and α-helix in parallel shape (figure 3B). In insect defensins, an α-helix is combined with a double-stranded β-sheet, stabilized by three disulfide bridges between the six cysteine residues. The core of the global fold of plant defensins as well as invertebrate defensins, includes a cysteine-stabilized α-helix β-sheet (CSab) motif consisting of an a-helix and a triple-stranded β-sheet, organized in a babb architecture and stabilized by four disulfide bridges (Thevissen et al., 2003). In this motif two cysteine residues, located one turn apart in the α-helix, form two disulfide bridges with two cysteine residues separated by a single amino acid in the last strand of the β-sheet (Broekaert et al., 1995).

Plant defensins are known for their effectiveness against bacteria, showing this biological activity with extreme specificity and frequently lacking of toxicity against other organisms (Villa-Perelló et al., 2003). Furthermore, these proteins appear to be specific to a certain group of bacteria. Defensins that are able to inhibit Gram-negative bacteria growth rarely decrease Gram-positive bacteria growth, and the opposite is also valid (García-Olmedo et al., 1998). However, a spinach defensin, contrary to most antimicrobial defensins, is an important exception for present lethal activity against both, Gram-negative bacteria, such as *Ralstonia solanacearum,* and Gram-positive bacteria, such as *Clavibacter michiganensis*. Likewise, this peptide has enhanced activity against phytopathogenic fungi, such as *Bipolaris maydis, Colletotrichum laganerium, Fusarium culmorum,* and *F. solani* (Segura et al., 1998).

As was said before, plant defensins are amphipathic, and this feature is closely related to disruption of microbial membranes and phospholipid liposomes. The antibacterial molecular mechanism of defense remains unclear and not completely elucidated, but some mechanisms have been suggested. One hypothesis for the role of defensins on antibacterial activity is inferred on the way that positively charged peptides interact with negatively charged membrane phospholipids, electrostatic interaction, following a membrane permeability modification (Pelegrini and Franco, 2005). In bacteria, permeabilization coincided with the inhibition of RNA, DNA and protein synthesis and decreased bacterial viability as assessed by the colony-forming assay. Conditions that interfered with permeabilization also prevented the loss of bacterial viability, indicating that permeabilization is essential for bacterial killing. In general the activity of defensins is diminished in the presence of increased salt concentrations, supporting the importance of electrostatic forces (Broekaert et al., 1997).

Based on the antimicrobial effects observed on fungi, at least two groups of plant defensins can be distinguished. The "morphogenic" plant defensins cause reduced hyphal elongation with a concomitant increasing in hyphal branching, whereas the "nonmorphogenic" plant defensins only slow down hyphal elongation but do not induce marked morphological distortions. The antifungal activity of plant defensins, whether morphogenic or not, is reduced by increasing the ionic strength (cations,

divalent at least one order of magnitude more potent than monovalent) of the fungal growth assay medium. (Lay et al., 2003). How defensins causes severe membrane damages after interaction with surface fungal cell is a real mystery and continues to be poorly understood. One possibility was described, where defensins might bind to glycolipids at fungal membrane surface. In this case, glycolipids will work as membrane receptors, despite two or more proteins could also be involved and consequently a pore will be formed, leading to ion influx/efflux (Thevissen et al., 2004). This mechanism, which blocked ion Ca^{2+} influx through fungi cell was observed (Spelbrink et al., 2004).

Lipid Transfer Proteins (LTPs). LTPs have the ability to transfer phospholipids between membranes. They are small proteins with 90 to 93 amino acids residues, and have a striking homology, (between 37 to 90% identity) conserved positions include those of the eight cysteines as well as 12 positions, which are invariably occupied by hydrophobic or aromatic residues. Tertiary structure determination from some plant species have revealed a similar folding pattern stabilized by four disulfide bonds and consisting of a bundle of four α-helices linked by flexible loops and a central tunnel-like hydrophobic cavity which can accommodate a fatty acid (figure 3C). LTPs have been isolated from a large number of sources, including mammals, plant, fungi and bacteria. In plants, they have been isolated from a wide range of species and have been found to be expressed in a variety of plant organs including embryos, cotyledons, leaves, stems, siliques, and various flower organs (Cammue et al., 1995). Localization studies by immunocytochemical electron microscopy have revealed that LTPs are located in the cell walls, at least in various *Arabidopsis* organs and in broccoli leaves (Thoma et al., 1994). The preferred occurrence of LTPs in outer cell layer can be interpreted as an argument in favor of a role of LTPs in repulsion or suppression of microorganisms invading from outside (Broekaert et al., 1997). One of these LTP-like proteins, namely, an antimicrobial protein from onion seed, does not transfer phospholipids in the classical LTP bioassay. Indicating that this activity may not be shared by all members of the LTP family (Cammue et al., 1995). It has become clear that different LTPs from different plant species can exert different antimicrobial activities. For instance, an onion seed LTP is highly active against a broad range of fungi, whereas a radish seed LTP is only moderately active and maize and wheat seed LTPs are inactive against most fungi. These AMPs have also shown to posses antibacterial activity against Gram-positive and Gram-negative bacterias (Cammue et al., 1995). The mechanism of action is not known, but it may be that these peptides insert themselves into the fungal cell membrane, and the central hydrophobic cavity forms a pore, allowing efflux of intracellular ions and thus leading to microbial cell death (Selitrennikoff, 2001).

Hevein- and Knottin-Type Antimicrobial Peptides. Hevein, a small 4.7kDa, cysteine-rich, chitin-binding peptide, is present in the rubber tree (*Hevea brasiliensis*) latex (Archer et al., 1969). Many chitin-binding peptides have multidomain structure, sharing a cysteine-rich domain of about 40 residues that has been shown to harbor the site responsible for chitin binding (Lee et al., 1991). Hevein, as well as the hevein-type antimicrobial peptides contains eight cysteines that are all linked by disulfide bonds, except the hevein-type peptides from amaranth seeds that contain only six cysteines. The three-dimensional structure of hevein has a triple-stranded β-sheet and a short single turn α-helix connecting the second to the third β-strand (figure 3D) (Andersen et al., 1993). Hevein-type peptides are small (43 residues) chitin-binding peptides. While hevein is a rather weak antifungal, the hevein-like peptides inhibit the growth of *Alternaria brassicicola, Ascochyta pisi,* and *Fusarium culmorum* (Broekaert et al., 1992). Their mechanism of action is the inhibition of the hyphal growth of fungi by binding to chitin (Van Parijs et al., 1991) and they also can cause leakage of cytoplasmic material by attaching to hyphal tips and septum (Koo et al., 1998).

The knottin-type peptides were first isolated from *Mirabilis jalapa,* and contain six disulfide-linked cysteines. The cysteines of these peptides can be relatively well aligned with those of a group of peptides named "knottins" (Chagolla-López et al., 1994), that includes α-amylase inhibitor from amaranth seeds, calcium channel-binding toxins from *Conus* snails, and a sweet-taste modifying peptide from *Gymnema sylvestre*. Knottins have a knot-like fold characterized by a compact triple-stranded β-sheet and a long loop connecting the first to the second β-strand, (figure 3E). From a structural point of view, knottin-type peptides are distantly related to hevein-type peptides in that their cysteine motif (C-C-CC-C-C) and cysteine conectivities are identical to those found in the hevein portion encompassing the first six cysteines (Broekaert et al., 1997).

The hevein is present in leaves, and stems, but not in roots. RNA blot analysis of different tissues of amaranth probed that, in hevein and knottin-type peptides, the expression was restricted to the seeds (Broekaert et al., 1997). Hevein-and knottin-type peptides inhibit a whole range of fungi and Gram-positive bacteria at concentrations below 10 mg/ml, but in presence of divalent cations the antimicrobial activity is lost (Broekaert et al., 1997).

Other AMPs. *Impatiens antimicrobial peptides.* The plant seeds of *Impatiens balsamina* contain a group of antifungal basic peptides, 20 amino acids in length that contain four cysteine residues forming two intramolecular disulfide bonds, they do not have significant homology with any peptide or protein, and are nontoxic to human, plant, and insect cells. They may include a β-turn, but does not show evidence for either helical or β-sheet structure (Broekaert et al., 1997). They inhibit up to 50% of the growth of phytopathogens, such as *Alternaria longipes* and *Fusarium culmorum*. Their mechanism of action is unknown, though it is known that at even high concentrations (500 mg/mL) no visible cell lysis occurs (De Lucca et al., 2005).

Cyclopeptide alkaloids. They are produced by members of *Rhamnaceae* and other plant families. Frangufoline is one of these peptides and have significant growth inhibition activity against *Aspergillus niger*. Though frangufoline is know to bind to calmodulin, a Ca^{2+}-binding protein that mediates calcium-driven metabolic reactions, the mode of action of these molecules is unknown (De Lucca et al., 2005).

Other plant peptides include the 5 kDa Pseudothionin-St1 present in potato tubers and found active against *Fusarum solani* (Moreno et al., 1994). Another potato antifungal peptide is sankin-1, a 63 amino acid residue, cysteine-rich molecule (Segura et al., 1999). Sankin-1 is constitutive and active at low concentrations against potato fungal pathogens. Maize (*Zea mays*) seeds produce the antifungal peptide MBP-1 with 33 amino acids and no free cysteines, and is mostly helical (Duvick et al., 1992). It shows no homology with thionins, and inhibits the hyphal elongation of several phytopathogenic fungi, such as *Fusarium moniliforme* and *F. graminearum*.

IWF6 is present in sugar beet leaves, and is active against *C. beticola.* It has no homology to any known antifungal protein. However, it has, less than 26% homology with agelenin, a neurotoxin from the venom of the spider *Agelene opulenta* that acts as calcium blocker. Though the IWF6 mode of action is unknown, this homology suggests that may act in a similar manner (Kristensen et al., 2000).

Application of antimicrobial peptides from plants. Several applications of natural occurring AMPs have been discussed during the last two decades. They are an attractive alternative for chemical food additives, for the treatment of fungal and bacterial infections, and the most common, for crop protection. One of the most promising tools for crop protection is the use of these peptides to produce transgenic plants and some examples are listed in table 1. However, several aspects have to be thoroughly examined prior a possible application, like activity under physiological conditions, selectivity and synergistic effects, cross-reactions, etc. (Theis and Stahl, 2004).

Table 1. AMPs and their potential application in agricultural development.

AMP	Source	Recipient plant(s)	Increased resistance against test organism(s)	References
AlfAFP (Defensin)	Alfalfa	Potato	*Verticillium dahliae*	(Gao et al., 2000)
MsrA1 (chimera)	Synthetic	Potato	*Erwinia* sp., *Fusarium* sp., *Phytophthora* sp.	(Osusky et al., 2000)
Defensin	Radish	Tomato	*Alternaria solani*	(Parashina et al., 2000)
Defensin	Pea	Canola	*Leptosphaeria maculans*	(Wang et al., 1999)
Defensin	Oat	Rice	*Burkholderia plantarri* and *B. glumaeby*	(Segura et al., 1998)
Defensin	Radish	Tobacco	*Alternaria longipes*	
α-hortodothionin (Defensin)	Barley	• Tobacco • *A. thaliana*	• *Pseudomona syringae* • *Fusarium oxysporum*	• (Carmona et al., 1993) • (Epple et al., 1997)
Defensin	Pea	Tobacco	*Fusarium oxysporum, Ascochyta pinodes, Trichoderma reesei, Ascochyta lentis, F. solani, L. maculans, Ascochyta pisi, Alternaria alternata*	(Lai et al., 2002)
Defensin	Chinese cabbage	Tobacco	*Phytophthora parasitica*	(Park et al., 2002)
Defensin	Wasabi	Rice	*Magnaporthe grisea*	(Kanzaki et al., 2002)

SUMMARY

AMPs have been studied for many years and have been noticed how significant they are in innate immune system of insects, plants and vertebrates. They are an amazing weapon against many kinds of microorganisms and have specific biochemical characteristics that are important to their mechanisms of action. Understanding the biology of AMPs should allow the development of novel therapeutics for infectious or inflammatory diseases, food protection, transgenic plants and clinical treatment of pathogens. However, there are some aspects that have to be examined, like the activity under physiological conditions, resistance, selectivity and synergistic effects, prior to possible applications. That is why is or high-priority to study AMPs mechanisms of action and its structure-activity relationship, because this could answer a lot of questions. We would like to apologize to anyone who find our description of his or her work inadequate or whose work we have accidentally omitted.

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