5.09 Plant Peptide Toxins from Nonmarine Environments

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5.09.1 Introduction

Plants use a variety of approaches for their defense, ranging from physical means such as thorns and bark or visual deception of herbivores to biochemical substances that make the plant less attractive by smell or taste or act as toxins creating adverse effects on the attacker, ranging from mild illness to death. The molecules involved in biochemical plant defense range from small organic molecules, including examples such as atropine, digoxin, and terpenoids, to peptides and proteins. One could argue that because plants lack mobility and hence lack the fight-or-flight defensive options of animals their biochemical defenses must be particularly effective in terms of potency, speed of action, and diversity.

These plant defense molecules are thus of interest for a number of reasons, including their potential applications in the discovery of new pharmacological substances, their adaptation to nonproducing species, for example, for protection of crop plants from insect pests delivered either topically or via incorporation into transgenic plants, and as new structural scaffolds for protein engineering approaches.

In this review we focus on the toxic molecules from plants that are peptidic in nature. Peptides exhibit a wide range of biochemical activities and relative to classic organic chemicals, have the advantage of relatively easy synthetic access and analytical identification. Identification of peptides can be directly done from plant extracts, via nucleic acid sequences isolated from plant tissue, or from genomic data, as opposed to organic molecules, which are generally detected only at the molecular rather than genetic level. Knowledge of the gene coding for a plant defense peptide or protein also allows the identification of the expression pattern of the gene product as a response to an external challenge such as predation, injury, or infection.

We commence with some definitions. Although the word toxin is commonly understood to refer to any harmful substance, a formal definition of toxin is "a poisonous substance, especially one produced by a living organism." (*The American Heritage Science Dictionary*, 2005 by Houghton Mifflin Company.) The poisonous effect may be discomfort, disease, or death for a target organism and can be achieved via several mechanisms. Typically, peptide toxins elicit their poisonous potential by targeting receptors, cell membranes, or enzymes crucial to a cell's or organism's metabolism.

Peptides are defined less stringently. *The American Heritage Science Dictionary* (2005) defines a peptide as "a chemical compound that is composed of a chain of two or more amino acids and is usually smaller than a protein." There is no exact cutoff size when a peptide becomes a protein. For the purpose of this review, and reflecting the term peptide in its title, an arbitrary cutoff of 100 amino acid residues was chosen as the upper size limit of peptides that will be covered, that is, we will not describe examples of plant-based proteins that are larger than 100 amino acids, even though there are many examples in the plant kingdom. In some cases the literature cited in this review may refer to some of the discussed toxins as proteins, reflecting the fact that individual researchers have their own preferences on peptide/protein nomenclature, but in all cases we limit coverage to polypeptides of fewer than 100 amino acid residues.

Plants face assaults from a diverse range of pests and pathogens, including microbes such as fungi and bacteria, insects, or higher animals. **Figure 1** summarizes some of the classes of peptides that defend against these attacks. From these examples it is clear that plant peptide toxins feature a vast array of sizes, targets, and biological modes

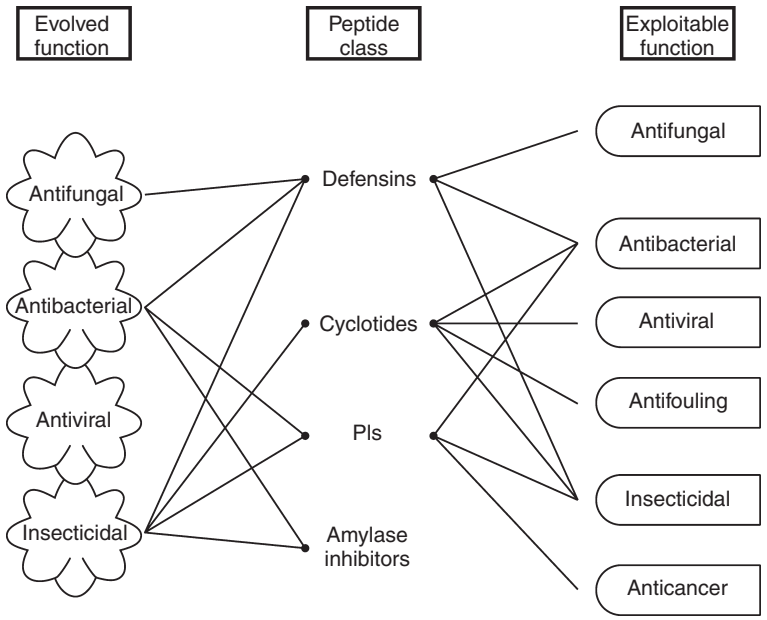
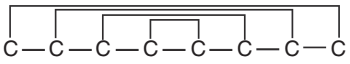
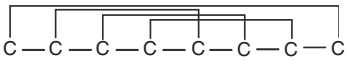
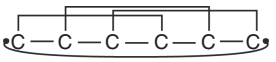
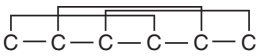
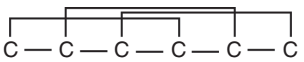


Figure 1 Examples of functions of different classes of plant defense peptides.

Table 1 Examples of plant defense peptides

Toxin	*Size (AA)*	*Source*	*Disulfide framework*	*Target*	*Mode of action*
α-Purothionin	46	*Triticum aestivum* (wheat)		Antimicrobial	Membrane binding
Rs-AFP2	50	*Raphanus sativus* (radish)		Antimicrobial	Membrane binding
Kalata B1	29	*Oldenlandia affinis* (kalata kalata)		Insecticidal	Membrane binding
CMCTI-1	29	*Cucumis melo*			Enzyme inhibitor
SFTI-1	14	*Helianthus annuus* (common sunflower)			Trypsin inhibitor
AAI	32	*Amaranthus hypochondriatus*		Insecticidal	α-Amylase inhibitor

The disulfide bridges and, if applicable, head-to-tail backbone cyclization are indicated on top and bottom, respectively, of the generic sequence.

of action, making them a broad field of research. Many of the peptide families discussed in this article are disulfide-rich peptides, as highlighted in **Table 1**. The occurrence of disulfide bridges in peptides often results in well-defined tertiary structures and many plant defense peptides have been structurally characterized by X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy. Reflecting our interests and expertise we thus place a substantial emphasis in this article on structures and structure–activity relationships of peptides.

In addition to the literature cited herein the reader might find it helpful to access publicly available databases on peptide and protein sequences to gain additional insight into the peptide families discussed here. These databases include PROSITE,[1] pfam,[2] and Swiss-Prot/TrEMBL.[3] Many of these databases and related analysis tools are accessible via the expert proteomics analysis system ExPASy.[4] As the number of peptide sequences grow as a result of discovery *in planta* or from genome screening, these resources will provide an ongoing update of knowledge on plant peptides and proteins.

The review is structured around major classes of toxic plant proteins. Each peptide family will be introduced, giving background information on discovery, distribution, and general biological activity. We then describe the structural features of the peptide families followed by a description of their biological activities with an emphasis on the mode of action to achieve their activity. First, the classes of plant defense peptides that are defined by a common architecture of the mature peptide, that is, thionins, plant defensins, and cyclotides will be described. These peptide families utilize different mechanisms of defense. We then describe examples of peptides that have defined toxic activity but originate from different peptide families. These examples include enzyme inhibitors targeting proteases or amylases. This review also covers the potential applications of plant-derived peptide toxins in crop protection and medical applications, either using transgenic or engineered and synthetic peptide approaches.

5.09.2 Plant Defense Peptides

5.09.2.1 Thionins

Thionins are cystine-rich, cationic small peptides (~5 kDa) found in monocots and eudicots.[5] They are divided into the families of α/β-thionins and γ-thionins. As is now generally accepted practice, we will refer to γ-thionins as plant defensins, as they are structurally more closely related to mammalian and insect defensins

(see Section 5.09.2.2) than to α/β-thionins. There are five classes of α/β-thionins (I–V) found in different tissues and different plants, and having different properties. Type I α/β-thionins comprise 45 amino acids and feature four disulfide bonds. Type II thionins are 46–47 amino acids in length. Type I and II thionins differ in the number of basic residues in their sequences, typically 10 for type I and 7 for type II. Type II thionins also have four disulfide bonds. Type III thionins feature three disulfide bonds and are 45–46 amino acids long. They are as basic as type II thionins. Thionins of type IV are approximately 46 amino acids long and of neutral charge. They have three disulfide bonds.[5,6] Type V thionins are reported to have evolved from type I thionins by a process of accelerated evolution.[7] They have shorter sequences, are of neutral charge, and their toxicity has not been fully elucidated.[5]

Thionins are expressed as precursor proteins that are processed to yield the mature peptide. The precursor peptide consists of an N-terminal signal sequence, the mature peptide sequence, and an acidic C-terminal protein.[6,8–10] Expression of thionins is inducible by external stimuli. For example, high levels of type II thionin mRNA are present in seedlings of barley grown in darkness. Upon exposure to light, levels of mRNA drop significantly but thionins expressed prior to light exposure remain stable.[11] Assault with fungal pathogens elicits transient expression of thionins under illumination[12,13] and chemical stress induces longer-lasting responses, as has been shown upon challenge with salts of magnesium, zinc, manganese, and cadmium.[14]

5.09.2.1.1 Structural aspects of thionins

Figure 2 shows a global alignment of selected thionin sequences. The background color for a given residue indicates the degree of conservation of that residue in a particular position in the sequence.[15,16] It is clear that thionins are highly conserved over different species. Based on their conserved sequences and similar three-dimensional structures it is reasonable to assume a common mode of action for all thionins.

Although thionins encompass a range of sequence diversity, and different types have different numbers of disulfide bridges, their overall structure is fairly similar, as revealed by either X-ray or NMR structures. The structural architecture consists of an N-terminal short β-strand linked to two antiparallel α-helices that are connected by a short random turn motif, followed by another β-strand forming an antiparallel β-sheet and a C-terminal coiled region. An overview of α/β-thionin structures published to date was reported in a recent review by B. Stec.[5] One of the best-studied peptides in the thionin family is crambin and very high-resolution structures of this peptide have been published with resolution as high as 0.54 Å.[17] Crambin differs from other thionins in its neutral charge, high hydrophobicity, and nontoxicity. **Figure 3** highlights the conserved structural features of thionins with the structures of α-purothionin,[18] β-purothionin,[19] crambin,[17] and viscotoxins A3[20] and B.[21]

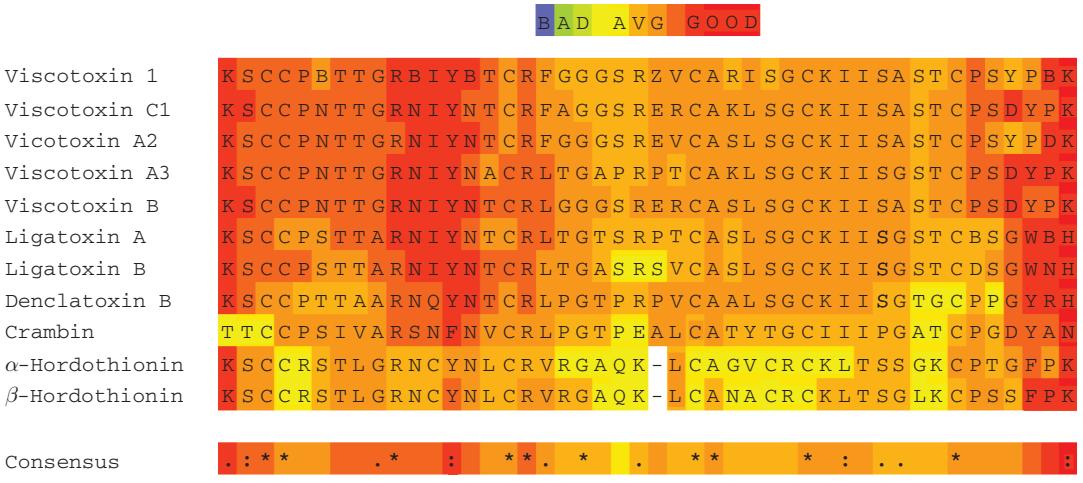


Figure 2 A global alignment of selected peptides from the thionin family reveals the similarities in their sequences. Increasing redness of the background indicates higher scores, that is, higher probability of the respective residue at that position (B: Asp or Asn, Z: Glu or Gln).