Plant Host Defense Peptides: Snakins

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Plant host defense peptides (HDPs), also known as antimicrobial peptides (AMPs), are regarded as one of the most prevalent barriers elaborated by plants to combat various infective agents. HDPs are arbitrarily referred to as small, thermal stable, and positively charged peptides, generally comprising peptides of less than 100 amino acid residues with an overall net charge of +2 to +9, and molecular weight of 4 to 9 kDa. Plant HDPs can be classified into at least eight major families, including defensins, thionins, non-specific lipid-transfer proteins (LTPs), hevein-like peptides, Snakins, knottins, α-hairpinins, and cyclotides, among which the Snakin family peptide has been reported to be the most abundant potential AMP that is active against fungal and bacterial pathogens. The Snakin/GASA family peptides consist of 12 cysteine residues, being the foremost cysteine-rich peptides among different classes of plant HDPs.

Keywords: snakin ; gibberellic acid stimulated in arabidopsis (GASA) ; plant host defense peptides (HDPs) ; antimicrobial peptides (AMPs)

1. Introduction

Plants as sessile organisms are constantly facing threats from a wide spectrum of microorganisms, including bacteria, fungi, viruses, and protozoa, as well as herbivores and insects; they have evolved highly effective mechanisms to defend against invaders that are harmful to their life. Plant host defense peptides (HDPs), also known as antimicrobial peptides (AMPs) are regarded as one of the most prevalent barriers elaborated by plants to combat these microorganisms in a rapid, direct, and durable way [1]. Plant HDPs are ancient weapons of defense, constituting essential components of plant innate immune systems [1][2][3]. They possess a considerable proportion of hydrophobic amino acids (>30%) within a linear or cyclic structure[4]. HDPs have a broad spectrum of antifungal, antibacterial, antiviral, and anticancer activities (see reviews by[5][6][7][8][9][10]. While most HDPs function in host defense as direct microbicides, others act as modulators that indirectly regulate the host immune response [11]. HDPs can restrain or kill pathogenic organisms at micro-molar concentrations, commonly by non-specific mechanisms [12]. This potentially helps HDPs to evade resistance development in target organisms, which makes HDPs a promising alternative to conventional antibiotics [13].

In past decades, many studies that attempted to explore the mode of action of HDPs were described. It has been demonstrated that the general activity mechanism of HDPs is associated with disruption of the cell membrane. Many HDPs are polycationic, whereas the lipids on the surface of bacteria are mainly anionic; thus, disruption or clustering of anionic lipids occurs when cationic peptide binds to the surface of the microbial membrane. In fungal or yeasts cell walls/membranes, the interaction with HDPs involves negatively charged components such as glycophospholipids (GPLs), sphingolipids, and mannoproteins[13]. The interaction consequently can cause membrane disruption, membrane permeation, pore formation, ion channel modification, dissipation of electrochemical gradients, leakage, and/or eventual cell death. Many reviews were published on this subject[4][9][12][13][14][15]. Although membrane interactions are the main contributor to the activity of most HDPs, there are accumulating reports of HDPs that act on intracellular targets [16]. So far, at least four types of action mechanism of HDPs have been reported[10]: (1) HDPs directly interact with microbial membranes, resulting in membrane fluctuation, pores formation and/or membrane depolarization, finally dysfunction of membranes and cell death; (2) HDPs impede the biosynthesis of intra- and/or extra-cellular biomolecules, such as proteins, nucleic acid lipoteichoci acids and galcopeptides, causing metabolism abnormalities; (3) HDPs increase the K^+ efflux and disrupt Ca^{2+} homeostasis and reactive oxygen species (ROS) production, leading to cell apoptosis; (4) HDPs inhibit adenosine triphosphate (ATP) synthesis, resulting in blocking ATP-dependent cellular processes.

The antifungal mechanisms of antifungal peptides/proteins (AFPs) have been well characterized [13]. AFPs can either interact with cell receptors inducing signaling cascades accompanied by Ca^{2+} influx and/or translocation to the fungal membrane via transporters [17]. The antifungal activity of AFPs is generally peptide- and species-dependent. For instance, although the radish defensins strongly inhibit the growth of certain fungi, it has no influence on other species [18]. The

antifungal potency is determined by several parameters, such as the (cationicity) net charge, the hydrophobicity, the distribution of the residues and associated structure, length, and amphipathicity[19][20]. Moreover, HDPs can penetrate the cells and interact with inner cell compartments like mitochondria and stimulate cell apoptosis.

In plants, the majority of HDPs are cysteine-rich, and the number of cysteine residues is even, for example, 2, 4, 6, 8, 10, and 12 [21]. This feature elicits the formation of multiple disulfide bonds, conferring plant HDPs with extraordinary high thermal, chemical, and proteolytic stability which is crucial for them to act as a chemical shield against multiple pests and pathogens[22][23]. The distinctive topological configuration facilitates HDPs to sustain their conformation and activity even in harsh environments, such as low pH (e.g., plant vacuole) or proteolytic (e.g., the digestive systems of herbivores) conditions (for reviews see[23]). HDPs are scattered widely in nature, including mammals, birds, insects, and plants[2]]. In plants, the number of genes encoding peptides is higher than that in animals [24]. According to the record on the PlantPepDB database (http://www.nipgr.ac.in/PlantPepDB/), to date, there have been 3848 plant-derived peptides identified from 443 plant species including two cryptogams (i.e., algae and bryophyte) plus 17 gymnosperm and 424 angiosperm, and this number is still increasing[6]. In model plants, such as rice, *Arabidopsis,* and alfalfa, HDPs have been estimated to account for at least 3% of the expressed proteins[1][23][25]. The widespread occurrence and dynamic activity of HDPs underscored their critical role in plant innate immunity [26].

HDPs show a wide diversity in size, sequence, structure, and antimicrobial mechanisms. The plant HDPs are apt to evolve dynamically, which often results in the presence of multiple HDPs gene families [26]. Based on the sequence and structure similarity, as well as the number and arrangement of cysteine residues in the primary sequence, plant HDPs can be classified into at least eight major families, including defensins, thionins, non-specific lipid-transfer proteins (LTPs), hevein-like peptides, Snakins, knottins, α-hairpinins, and cyclotides[1][23]. A search for potential AMPs in 1267 plant transcriptomes by a computational pipeline resulted in 4849 sequences assigned to the Snakin family, showing that the Snakin family peptide is the most abundant potential AMP that is active against fungal and bacterial pathogens [27].

2. Molecular Characterization of Snakins

Snakins are generally small (~ 7 kDa), positively charged and cysteine-rich proteins [28] involved in plant defense responses, such as antimicrobial activity against a wide range of phytopathogens[29][30][31][32][33][34][35] and animal pathogens[36][37], as well as in a variety of plant development processes[38][39][40][41]. The first defined Snakin peptide, Snakin-1 (StSN1) was purified from potato tubers by Segura et al., (1999), who reported that it had some sequence motifs in common with snake venoms and named it Snakin[35]. Thereafter, accumulated studies have been implemented for Snakins, and now it is known that the Snakin family peptides are characterized by having 12 cysteine residues at constant positions in a conserved domain called GASA (Gibberellic Acid Stimulated in *Arabidopsis*) at the C-terminal region. They also have a putative signal peptide at the N-terminus, and a variable region in the middle of their sequences. The amino acid sequence of GASA domain in Snakins consist of a peculiar Cys-motif "$XnCX_3CX_2RCX_{8(9)}CX_3CX_2CCX_2CXCVPXGX_2GNX_3CPCYX_{10(14)}KCP$" (where X is any of 20 proteinogenic amino acid residue except for cysteine, R is arginine, V is valine, P is proline, G is glycine, Y is tyrosine and K is lysine), in which the number and arrangement of cysteine residues is highly conserved[6][23][25][28][42]. As these features are also shared by GASA gene family members, thereafter we use Snakin/GASA instead of Snakin in this review. Comparison with the other major families of the aforementioned plant HDPs which have less than ten cysteine residues, the Snakin/GASA family peptides represent the foremost cysteine-rich peptides among different class of plant antimicrobial peptides. HPLC-ESI-QTOF and crystallography analyses show that the 12 highly conserved cysteine residues are involved in the formation of up to six disulfide bonds [43][44]. The 3D structure by X-ray and mass spectrometry data unravels a helix-turn-helix (HTH) motif conserved in the Snakin peptides [44][45]. These results suggest that the disulfide bonds and the HTH motif are necessary for the spatial structure of Snakin/GASA and might be critical for the Snakin's interactions with its target (e.g., cell membrane, protein, and DNA).

The Snakin/GASA peptides comprise a multigene family and are distributed in a vast number of plants, yet they are not present in animals. Although the homologous gene sequences can also be found in a few bacteria, including *Escherichia coli*, *Klebsiella pneumoniae*, *Nitriliruptoraceae bacterium*, *Acinetobacter baumannii*, *Soehngenia saccharolytica*, *Glycocaulis profundi,* and *Staphylococcus warneri* (https://www.ncbi.nlm.nih.gov/), whether or not these genes code for Snakin/GASA peptides requires future investigation. In an early study, by comprehensive genome sequence analysis, approximately 445 genes coding for Snakin/GASA proteins have been discovered in 33 plant species[25]. Further bioinformatics mining data reveals that the Snakin/GASA genes are present in all well-characterized sequenced plant species, but are completely absent in moss and green algae, implying that the emergence of Snakin/GASA could be an

adaptation of ancestral plants to land[46]. An overview of the Snakin/GASA family members in some selected plant species (Table 1) reveals that the Snakin/GASA peptides exhibit significant diversity in many aspects, such as the number of family members, protein length, and pI values (Table 1).

Table 1. Overview of the Snakin/GASA gene family in some selected plant species.

Plant Species	Family Members	Length (aa)	pI	References
Petunia (*Petunia hybrida*)	5	104–112	9.08–9.40	[47]
Arabidopsis thaliana	15	87–275	7.41–9.98	[41][48][49]
Rice (*Orazy sativa*)	9	92–152	8.77–9.28	[41]
Maize (*Zea mays*)	10	80–129	8.26–9.30	[41]
Potato (*Solanum tuberosum*)	18	88–143	6.01–9.72	[50]
Apple (*Malus domestica*)	26	88–305	4.11–10.14	[48]
The rubber tree (*Hevea brasiliensis*)	16	88–241	8.75–10.00	[51]
Common wheat (*Triticum aestivum L.*)	37	261–1099	4.99–5.27	[52]
Soybean (*Glycine max*)	37	66–198	5.65–9.54	[53]
Grapevine (*Vitis vinifera* L.)	14	64–298	8.50–9.64	[54]

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