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Interactions of antifungal plant defensins with fungal membrane components

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Abstract

Plant defensins are small, basic, cysteine-rich peptides that are generally active against a broad spectrum of fungal and yeast species at micromolar concentrations. Some of these defensins interact with fungal-specific lipid components in the plasmamembrane. Structural differences of these membrane components between fungal and plant cells probably account for the selective activity of plant defensins against fungal pathogens and their nonphytotoxic properties. This review will focus on different classes of complex lipids in fungal membranes and on the selective interaction of plant defensins with these complex lipids.

Keywords: Plant defensin; Sphingolipid; Glucosylceramide; *Neurospora crassa*; *Saccharomyces cerevisiae*; *Pichia pastoris*; *Candida albicans*; Mode of action

1. Plant defensins

To defend themselves against pathogen attack, plants produce a battery of antimicrobial compounds, including cationic antimicrobial peptides (AMPs) that generally have a broad activity spectrum. Since these peptides are simply produced by transcription and translation of a single gene, they can be delivered rapidly after infection with a limited input of energy and biomass. One family of such AMPs are plant defensins. Plant defensins are small (45–54 amino acids), highly basic, cysteine-rich peptides that are structurally related to defensins found in other types of organisms, including insects and humans. The three-dimensional structure of plant defensins comprises a cysteine-stabilized αβ motif (CSαβ motif) consisting of an α-helix and a triple-stranded β-sheet, organised in a βαββ architecture and stabilized by four disulfide bridges (reviewed in [11,65]). A molecular phylogeny tree based on the multiple alignments of over 80 defensin sequences from 38 different plant species has been composed [65]. Interestingly, there is no general pattern of clustering versus dispersal of the various defensins identified in a single species. The radish defensins, for example, are tightly grouped, suggesting recent diversification, whereas the *Arabidopsis* defensins are spread all over the tree [65]. The plant defensin family is quite diverse regarding amino acid composition as only the eight structure-stabilizing cysteines appear to be conserved among all plant defensins [65]. Possibly, this reflects diverse biological activities displayed by different plant defensins. Some plant defensins were not found to display any antimicrobial activity, while others were found to have antifungal and/or occasionally antibacterial activity in vitro. Among plant defensins that have antifungal activity, some cause hyperbranching of hyphae while others do not cause any morphological changes to filamentous fungi [65]. Interestingly, several plant defensins are active against human fungal pathogens such as *Candida albicans* [63,66]. So far, none of the plant defensins has been found to cause detrimental effects on cultured human or plant cells ([56]; Cammue BPA, unpublished results).

Several experiments point towards a role of the plant defensins in defending the host from fungal attack. It was shown that plant defensins play a role in the protection of seedling tissues during the early stage of emergence [55]. Plant defensin expression patterns in various tissues of different plant species, such as Chinese cabbage [44], pea [3], tobacco [31], potato [51], spinach [52], tomato [10] and Arabidopsis [46,64], were studied. From these studies, it can be concluded that plant defensins are not only present in seeds

Abbreviations: AFP, antifungal peptide; AMP, antimicrobial peptide; GlcCer, glucosylceramide; GIPC, glycosylinositolphosphorylceramide; Syr E, syringomycin E; IPC, inositolphosphorylceramide; MIPC, mannosylinositolphosphorylceramide; $M(IP)_2C$, mannosyldiinositolphosphorylceramide; SG, steryl glucoside

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but also in peripheral cell layers of fruits and floral organs, including the style, the ovary, the stamen filaments and anthers. This preferential location of plant defensins in peripheral cell layers of generative tissues is consistent with a role in protection of the organ against microbial challenge. In addition, some plant defensins were shown to be systemically induced upon fungal infection or wounding of vegetative tissues in different plant species, namely pea [13], radish [55], *Arabidopsis thaliana* [46,67], potato [8] and bell pepper [40]. All these experiments demonstrate that plant defensins are important components of the defense system in plants, namely as induced antimicrobial agents within vegetative tissues or as components of consitutitve defense barriers, especially those of storage and reproductive plant organs.

Direct evidence for a possible role of plant defensins in plant defense comes from the study of transgenic crops expressing plant defensins. It has been demonstrated that transgenic expression of plant defensins leads to protection of vegetative tissues against pathogen attack. For example, constitutive expression of a radish defensin gene in tobacco clearly enhanced its resistance to *Alternaria longipes* [55] and similarly, production of an alfalfa defensin in potato provided a robust resistance against *Verticillium dahliae* [19].

2. Mode of action of plant defensins

The physiological activities of many cationic antimicrobial peptides are generally related to their membranolytic properties. Human, insect and plant defensins contain amphipathic β-sheet structures, a feature that enables formation of ion channels in model lipid membranes [27]. In contrast to human and insect defensins, plant defensins have never been shown to induce ion-permeable pores in artificial membranes composed of phospholipids, nor change the electrical properties of artificial lipid bilayers. This demonstrates that a direct interaction between plant defensins and plasma membrane phospholipids is unlikely [12,59]. However, it has been shown that the plant defensins DmAMP1 and RsAFP2, isolated from *Dahlia merckii* and *Raphanus sativus*, respectively [43,56], induce an array of relatively rapid responses in fungal cells including increased potassium efflux and calcium uptake and membrane-permeabilization [59,62]. Using radiolabeled HsAFP1, a plant defensin isolated from *Heuchera sanguinea* [43], and radiolabeled DmAMP1, the existence of high-affinity binding sites for these plant defensins on fungal cells and membrane fractions was demonstrated [60,61]. Recently, these plant defensin-binding sites in the fungal plasma membrane were identified as complex lipids ([57]; Thevissen K, unpublished results). In this review, an overview of the different classes of lipids in fungal membranes will be presented. In addition, the interaction of plant defensins with some of the complex lipids will be discussed in detail. Structural differences of the complex lipids in fungal and plant cells probably account for the non-toxic properties of plant defensins for plant cells.

3. Fungal membrane components

The major classes of lipids found in biological membranes of eukaryotes are *phosphoglycerolipids*, *glycoglycerolipids*, *sphingolipids* and *sterols* (reviewed in [14]). A first class of eukaryotic membrane lipids are *phosphoglycerolipids*, which are regarded as the primary structural element of biological membranes. Phosphoglycerolipids consist of a glycerol backbone esterified with fatty acids in the *sn*-1 and *sn*-2 positions, and a phosphate group in the *sn*-3 position (Fig. 1A). One hydroxyl group of the phosphate is esterified

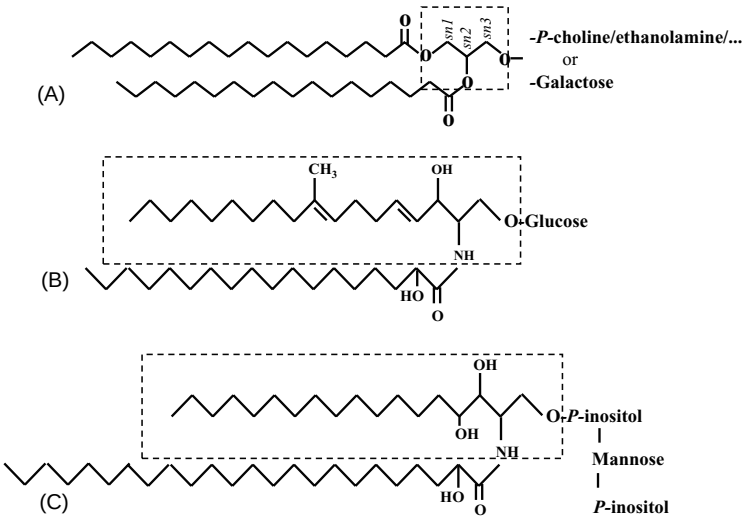

Fig. 1. Structure of different eukaryotic membrane components. (A) 1,2-Diacylglycerol with boxed glycerol-backbone (addition of choline-phosphate for phosphatidylcholine; addition of galactose for monogalactosyldiacylglycerol); (B) glucosylceramide (sphingoid backbone (9-methyl-4,8-sphingadienine) is boxed); (C) mannosyldiinositolphosphorylceramide $M(IP)_2C$ (sphingoid backbone (phytosphingosine) is boxed).

to the hydroxyl group of a polar head group, such as serine, ethanolamine, inositol or choline.

A second group of eukaryotic membrane lipids are *glycoglycerolipids*. The glycoglycerolipid structure consists of a sugar residue attached to either diacylglycerol or monoalkylmonoacylglycerol. Depending on the nature of the carbohydrate portion, the glycoglycerolipids can be classified into two broad categories: those containing galactose, referred to as galactoglycerolipids (such as monogalactosyldiacylglycerol) and those containing glucose, referred to as glucoglycerolipids (such as glucosyldiacylglycerol). There are only few reports on the presence of monogalactosyldiacylglyerols (MGDG) (Fig. 1A) in yeast membranes. In *C. albicans*, two reports describe the presence of MGDG in its membranes [2,20]. There are no reports on the occurrence of glucosyldiacylglycerols in fungi. In plants, MGDG are the major constituents of plastid membranes [26].

A third group of eukaryotic membrane lipids are *sphingolipids*, which have a backbone of ceramide rather than diacylglycerol. Ceramide is composed of a sphingoid backbone or long chain base (Figs. 1B and C) that is amide-linked to a fatty-acid. Ceramide can be further decorated by a polar head group at carbon atom C1 (reviewed in [39]). Based on the head groups, sphingolipids can be classified in phosphosphingolipids and glycosphingolipids. Glycosphingolipids can be further subdivided into neutral and acidic glycosphingolipids.

Neutral glycosphingolipids contain uncharged sugar residues such as glucose and galactose. The most common neutral glycosphingolipid found in fungi is glucosylceramide (GlcCer) (Fig. 1B). The last step in the biosynthesis of GlcCer is catalyzed by a UDP-glucose:ceramide glucosyltransferase (glucosylceramide synthase, encoded by *GCS* gene). GlcCer is present in membranes of many fungi, such as *Pichia pastoris* [49], *C. albicans* [38], *Pseudallescheria boydii* [47], *Cryptococcus neoformans* [33,48], *Aspergillus fumigatus* [9,68], *Sporothrix schenckii* [69] and *Neurospora crassa* [18]. This is in contrast with yeast species such as *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, that do not synthesize GlcCer [15,49]. In most fungal species, GlcCer is composed primarily of a 9-methyl-4,8-sphingadienine which is linked to a C16 or C18 α-hydroxy fatty acid [38,47–49]. While the fatty acid in yeast GlcCer are mostly saturated, the fatty acid in GlcCer from filamentous fungi belonging to Euascomycetes are Δ^3-unsaturated to varying extents, with the proportion of unsaturation depending on species, strain, morphology, and culture conditions [33,70]. This GlcCer unsaturation has never been reported in fungi belonging to Basidiomycetes, nor in Saccharomycetales. Interestingly, in the dimorphic fungus *Histoplasma capsulatum*, the presence of the unsaturation in GlcCer is correlated with the yeast-mycelium transition. It seems that this transition is accompanied by upregulation of an as yet uncharacterized ceramide 2-hydroxy fatty *N*-acyl (D)-Δ^3-desaturase activity [70].

In plants, GlcCer structures are diverse. GlcCer isolated from oat root cells is composed of a 4,8-sphingadienine linked to a C24 saturated α-hydroxy fatty acid [42]; GlcCer from soybean is primarily composed of the sphingoid backbone 4,8-sphingadienine linked to α-hydroxy palmitic acid (C16) [33,54]; wheat GlcCer has three major ceramide backbones, namely 4,8-sphingadienine linked to either α-hydroxy palmitic acid or α-hydroxy arachidic acid (C20) and 8-sphingenine linked to α-hydroxy palmitic acid [54]. So far, the main difference between fungal and plant GlcCer appears to be the addition of a branching 9-methyl group to the fungal sphingadienine base.

Acidic glycosphingolipids contain ionizable functional groups such as phosphate (phosphoglycosphingolipids) or sulphate (sulfatoglycosphingolipids) as well as charged sugar residues (such as glucuronic acid, neuraminic acid and sialic acid). Phosphoglycosphingolipids are mainly found in fungi, plants and protozoa but not in cells or tissues of mammals or other higher animals [35]. Phosphoglycosphingolipids are composed of a ceramide moiety, which is attached to an oligosaccharide via a phosphodiester linkage to myo-inositol. They are also termed glycoinositolphosphorylceramides (GIPCs).

In fungi, the ceramide moiety of GIPCs consists of the sphingobase phytosphingosine (Fig. 1C). In *S. cerevisiae*, there are only three major classes of GIPCs, namely inositolphosphorylceramide (IPC), mannosylinositolphosphorylceramide (MIPC) and mannosyldiinositolphosphorylceramide ($M(IP)_2C$) (Fig. 1C). These are formed by the sequential addition of two head group substituents: inositolphosphate and mannose. IPC is synthesized by transferring inositolphosphate from the phospholipid phosphatidylinositol onto ceramide. This reaction is catalyzed by IPC synthase (encoded by *AUR1* gene) [16,17]. Subsequently, mannose is transferred from GDP-mannose to IPC, forming MIPC [1]. This reaction requires the *SUR1* and *CSG2* genes [6,7]. Finally, MIPC is converted to $M(IP)_2C$ by transferring inositolphosphate from phosphatidylinositol to MIPC. $M(IP)_2C$ is the most complex sphingolipid in yeast [17]. This terminal step in *S. cerevisiae* sphingolipid synthesis requires the *IPT1* gene [17]. IPC, MIPC, $M(IP)_2C$, and related sphingolipids with variations in the hydroxylation pattern and chain length of the sphingoid base on one hand and variations in the chain length (C24, C26) and hydroxylation pattern of the very long chain fatty acid on the other hand make up a variety of more than 30 different sphingolipid molecular species in *S. cerevisiae*. These localize in the plasma membrane where they constitute 20–30% of all lipids [45]. Other fungi (besides *S. cerevisiae*) have been found to express GIPCs varying widely in glycosylinositol structure, depending on species and, in some cases, on morphological phase. According to the nature of the second sugar residue distal to inositol, fungal GIPC oligosaccharides glycan chain structures can be classified into several groups [5,25,34,36,70]. Recently, a novel type of glycosphingolipid was reported to occur in

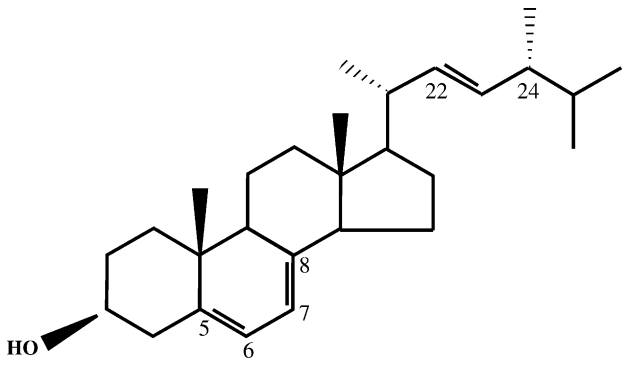

Fig. 2. Structure of ergosterol.

C. albicans [71]. Phospholipomannan (PLM) seems to be derived from MIPC by the addition of mannose phosphate. The glycan part of the molecule is an unbranched linear structure that may reach up to 19 mannose residues in length. The conferred hydrophilic properties allow PLM to diffuse into the cell wall. The biological significance of the structural diversity of the GIPC chains is unknown. However, it has recently been hypothesized that the production of more complex GIPC-glycan chains could be a characteristic of fungi exhibiting dimorphism between mycelial forms and a unicellular yeast stadium [34].

There are few reports on GIPC analyses using plant tissue material; most of this research has been performed between 1960 and 1980. Whereas membranes of yeast and fungal species contain MIPC and related IPC's, plants do not seem to have those types of mannosylated species of IPC. Instead, plant GIPCs contain glucuronic acid linked to the inositol. Glucosamine (*N*-acetylated or non-acetylated) is the next sugar linked to glucuronic acid [22,23,28]. So far, no glucuronic acid has been found in fungal GIPCs.

In contrast to *S. cerevisiae*, most other fungi produce besides inositolphosphoryl-containing sphingolipids, also glucosylceramides [15]. There is growing evidence that fungi maintain two separate pools of ceramides to be used for the synthesis of different sphingolipids. Ceramide backbones with C16 or C18 fatty acids linked to 9-methyl-4,8-sphingadienine are exclusively precursors for GlcCer synthesis, whereas ceramide backbones with very long chain C24 and C26 fatty acids bound to phytosphingosine are restricted to the synthesis of the inositol-containing phosphosphingolipids [32,68,69,70].

A fourth class of eukaryotic membrane lipids are *sterols*. These are essential lipid components of eukaryotic membranes and have been shown to be responsible for a number of important physical characteristics of membranes, such as membrane permeability and fluidity (reviewed in [14]). The fungal sterol, ergosterol (ergosta 5,7,22 tri-ene β-ol) (Fig. 2), comprises a 5,7 di-ene structure linked to a side-chain. Ergosterol differs from the plant sterols, sitosterol and stigmasterol, through the presence of unsaturations at C-7,8 in the ring structure and slight modifications of the side chain. Common to all sterols is the unsaturation at C-5,6 and the presence of the hydroxyl group at C-3. The latter provides the only hydrophilic component of the molecule and results in the proper orientation of the sterol molecule in the membrane. In many organisms, sterols are glycosylated at OH-3 giving rise to sterol glycosides. Steryl glycosides (SG) are widespread membrane lipids, occurring in all plants, several algae, fungi, slime molds, *Dictyostelium*, a few bacteria, and even animals [49,72]. Interestingly, the biosynthesis of SG in the yeast species *Pichia pastoris* depended on the culture conditions: the amount of steryl glucoside in cells grown in complete medium was much lower than in cells from minimal medium and a strong increase in the content of steryl glucoside was observed when cells were subjected to stress conditions such as heat shock or increased ethanol concentrations. Induction of steryl glucosides in cells from molds to humans in response to stress has been reported [29,41]. In mold cells, it was demonstrated that SG production is followed by activation of a protein kinase and induction of heat shock proteins [30]. These data point towards a possible role for steryl glucosides in stress resistance.

4. Interaction of antifungal plant defensins with fungal membrane components

The precise mode of action of plant defensins is still unclear and for most plant defensins molecular components involved in signaling and putative intracellular targets remain unknown. Only for DmAMP1 and RsAFP2, part of the molecular basis of their inhibitory activity towards the respective yeast species *Saccharomyces cerevisiae* and *Pichia pastoris* is known.

4.1. The dahlia plant defensin DmAMP1 interacts with M(IP)$_2$C

Via a genetic complementation approach, *IPT1* was identified as a gene determining sensitivity of *S. cerevisiae* towards DmAMP1 [57]. This gene encodes inositol phosphotransferase (Ipt1p), an enzyme involved in the last step of the synthesis of the sphingolipid M(IP)$_2$C (Fig. 1C) [17]. *S. cerevisiae* strains carrying a nonfunctional *IPT1* allele lack M(IP)$_2$C in their plasma membranes, bind significantly less DmAMP1 compared to wild-type strains, and are highly resistant to DmAMP1-mediated membrane permeabilization and growth inhibition [57]. All these phenotypic deviations can be restored by reintroduction of a functional *IPT1* gene. Under nutrient limitations, however, a *S. cerevisiae ipt1*-null mutant (Δ*ipt1*) was found to be as sensitive towards DmAMP1 as the corresponding wild-type yeast strain [24]. Interestingly, this yeast mutant still produces small amounts of M(IP)$_2$C via an alternative biosynthetic pathway [24]. Hence, it seems that DmAMP1-sensitivity is not necessarily dependent on the presence of a functional *IPT1* gene but instead, is dependent on the presence of M(IP)$_2$C. Therefore,

it was concluded that $M(IP)_2C$ and not Ipt1p is involved in constituting the binding site for DmAMP1 on the fungal plasma membrane. Moreover, studies using an ELISA-based binding assay revealed that DmAMP1 interacts directly with sphingolipids isolated from *S. cerevisiae* and that this interaction is enhanced in the presence of equimolar concentrations of ergosterol [58].

Binding of DmAMP1 could be competed for by the highly homologous defensins Ah-AMP1 and Ct-AMP1 [61], isolated from *Aesculus hippocastanum* and *Clitoria ternatea*, respectively [43,65]. This suggests that these defensins share the same binding site. Indeed, elimination of *IPT1* not only leads to DmAMP1 resistance but also to Ah-AMP1 and Ct-AMP1 resistance. A distantly related plant defensin, like the *Heuchera sanguinea* HsAFP1 [43,65], is not able to compete for the DmAMP1 binding site [61]. Accordingly, HsAFP1 is active on *S. cerevisiae* wild-type but elimination of *IPT1* in *S. cerevisiae* does not lead to enhanced resistance to HsAFP1. This demonstrates that HsAFP1 has binding sites on the plasma membrane distinct from these of DmAMP1, Ah-AMP1 and Ct-AMP1 [65].

4.2. The radish plant defensin RsAFP2 interacts with fungal glucosylceramides

The gene determining sensitivity of the yeasts species *P. pastoris* and *C. albicans* towards RsAFP2 was recently identified as *GCS* (Thevissen K, unpublished results). This gene encodes GlcCer synthase, an enzyme involved in the synthesis of glucosylceramides (Fig. 1B). *P. pastoris* and *C. albicans* Δ*gcs* null-mutants lacking the RsAFP2 sensitivity gene, were found to be at least 20-fold more resistant to RsAFP2 as compared to the corresponding wild-type strains. Remarkably, these Δ*gcs* mutants are also 20-fold more resistant to heliomicin, a plant defensin-like antifungal insect peptide as compared to the corresponding wild-type strains (Thevissen K, unpublished results). It has previously been suggested that defensins are ancient peptides conserved across the eukaroytic kingdom, originating 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 [65]. The observation that both plant and insect antifungal peptides are targeting similar structures in the fungal plasma membrane further supports the evolutional conservation of defensins as defense peptides between the plant and animal kingdom.

Using an ELISA-based binding assay, it was demonstrated that RsAFP2 only interacts with fungal GlcCer and not with human GlcCer nor soybean GlcCer. In addition, no interaction of RsAFP2 could be detected with structurally different complex lipid components such as MGDG from soybean. Differences in ceramide structure between fungal, plant and human GlcCer could account for the observed differential interaction of RsAFP2. GlcCer from various fungal and yeast species are identical (*N*-2′-hydroxyoctadecanoyl-1-*O*-β-D-glucopyranosyl-(4*E*,8*E*)-9-methyl-sphingadienine). Fungal GlcCer have a number of structural features that distinguish them from those found in plants, including the 9-methyl group branching of the sphingoid base, variable levels of unsaturation and length of the fatty acid chain (as reviewed above). These structural features of fungal GlcCer might be important determinants for interaction with antifungal defensins and subsequent cell growth inhibition.

4.3. Plant defensin-resistant Neurospora crassa mutants

The involvement of sphingolipids in the process leading to growth inhibition by plant defensins was also demonstrated for the filamentous fungus *Neurospora crassa*. Via chemical mutagenesis, *N. crassa* mutants that are resistant against the plant defensins RsAFP2, DmAMP1 and HsAFP1 were isolated [18]. These mutants were more resistant towards plant defensin-induced membrane permeabilization as compared to the *N. crassa* wild-type. Complex lipids of the plant defensin-resistant mutants were analyzed. Analysis of the acidic lipid fraction revealed a different, more highly glycosylated GIPC structure in the *N. crassa* mutants in comparison with the *N. crassa* WT GIPC [18]. Analysis of the neutral lipid fraction revealed structurally different GlcCer and increased levels of SG in the *N. crassa* mutants in comparison with the *N. crassa* WT. While the fatty-*N*-acyl moiety in the WT GlcCer was mostly *E*-Δ(3)-unsaturated (>90%) and composed of 2-hydroxy-3-(*E*)-octadecenoic acid (C18), those of the mutant GlcCer were essentially 100% saturated and composed of 2-hydroxy-hexadecanoic acid (C16) [18]. Although the amounts of GlcCer were similar in all three *N. crassa* strains, both mutants expressed considerably more SG than the WT. Steryl glucoside in the WT and mutants was characterized as ergosterol-β-D-glucopyranoside [18]. The observed clear differences in sphingolipid profiles of the *N. crassa* mutants could be linked to their resistance towards different plant defensins [18].

5. Model for the mode of action of plant defensins

All these findings are integrated in a tentative model for the mode of action of plant defensins (Fig. 3). The composition of the plasma membrane of fungal cells is asymmetric, which is typical of eukaryotic cells, with phosphoglycerolipids mainly in the inner leaflet, and sterols and sphingolipids in the outer leaflet [50]. The ergosterol to sphingolipid ratio of the yeast plasma membrane can be estimated at 1.4 [50]. Optimal DmAMP1 interaction was observed in an ELISA-based assay with an equimolar mixture of ergosterol and yeast sphingolipids, reflecting the in vivo yeast plasma membrane composition [58]. It has been shown that sphingolipids and sterols are enriched in specific domains in the outer plasma membrane, the so called membrane rafts [4,73]. Possibly, DmAMP1 interacts with fungal sphingolipids, which are concentrated in such specific rafts.

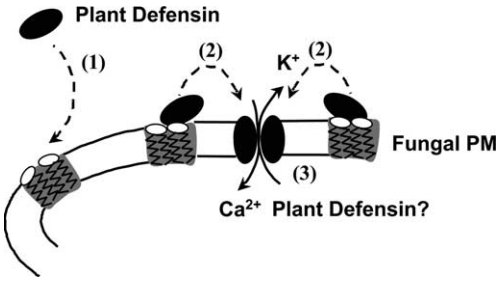

Fig. 3. Model for the mode of action of plant defensins: (1) plant defensins, represented by dark ovals; (2) bind to rafts composed of sphingolipids (represented by grey squares in the fungal plasma membrane (PM)), whereafter permeability of the membrane is altered; (3) resulting in increased Ca^{2+} uptake and K^+ efflux. Whether plant defensins are internalized and interact with intracellulair targets is currently not known.

Interaction of DmAMP1 with these rafts could result in high local concentrations of these membrane-bound defensins. In contrast to DmAMP1, interaction of RsAFP2 with fungal GlcCer was not enhanced in presence of different concentrations of ergosterol (Thevissen K, unpublished results), pointing towards different modes of interaction between different plant defensins and their fungal membrane target. After interaction of plant defensins with their respective membrane targets, fungal membrane permeability is altered, resulting in increased Ca^{2+} influx and K^+ efflux. Whether fungal growth arrest is a direct consequence of increased membrane permeability or results from interaction of plant defensins with an intracellular target is currently under investigation. To this end, electrophysiological experiments using lipid bilayers supplemented with purified fungal sphingolipids will be performed in the presence of plant defensins. Such experiments will give more insight in the link between interaction of plant defensins with sphingolipids and subsequent membrane-permeabilization. In addition, intracellular localization-experiments using fluorescently labeled plant defensins will be performed to investigate the existence of intracellular plant defensin-targets.

The yeast GIPC $M(IP)_2C$ is also required for fungicidal action of Syringomycin E (Syr E), a member of a large family of cyclic lipodepsipeptides produced by *Pseudomonas syringae* pv syringae [21,53]. Syr E is fungicidal and forms voltage-dependent pores in the fungal plasma membrane [37]. Recent findings with planar anionic lipid bilayers indicate that Syr E causes the formation of lipidic pores that are stabilized by this peptide [37], and it is conceivable that $M(IP)_2C$ promotes and becomes part of the structure of such pores in the yeast plasma membrane. In contrast, no such pore-forming capabilities could be demonstrated so far for DmAMP1, and it is difficult to speculate a similar role for $M(IP)_2C$ in DmAMP1 action.

In conclusion, the selective activity of plant defensins against fungal cells is most likely a result of differences at the level of the plasma membrane between fungal and plant cells. In contrast with most cationic α-helix containing antimicrobial peptides, plant defensins interact with specific components of the fungal plasma membrane before exerting their antifungal effect. Structural differences in these membrane components between fungal and plant cells probably result in their nonphytotoxic properties. Furthermore, none of the plant defensins tested so far have been found to be toxic on cultured human cells. Together with a recent observation that plant defensins are active against the human pathogen *C. albicans* at micromolar concentrations, plant defensins have an interesting potential with regard to the development of therapeutics [66]. To exploit this potential further, the mode of action of plant defensins needs to be unraveled in more detail. In our previous studies, we have demonstrated selective interaction of plant defensins with fungal-specific membrane components. Whether this interaction leads directly to growth inhibition or whether plant defensins are interacting with intracellular targets needs to be investigated. A putative selective interaction of plant defensins with intracellular targets would enlarge the potential of plant defensins considerably.

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