



PSI relieves the pressure of membrane fusion

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Some plant proteases contain a latent sequence known as the plant-specific insert (PSI) that, upon release from the full protease sequence, initiates membrane fusion to defend from pathogens. However, the mechanism by which it exerts its effects has been unclear. Zhao *et al.* report an elegant integration of biophysical experiments and molecular dynamics simulations to reveal events leading up to PSI-mediated membrane fusion. Their results demonstrate a pH-dependent monomer-to-dimer transition, clear evidence of membrane association, and probable structures of prefusion intermediates. These data expand our understanding of the elusive PSIs and may provide new directions for antimicrobial development.

Aspartic proteases (APs) are thought to have multiple functions in plants, many of which are not fully understood. However, their role in providing defense from pathogens is well-recognized. Their most famous role in this respect revolves around a curious structural domain, known as the plant-specific insert (PSI), that protrudes from the fold of the proteases. Nascent APs migrate from the endoplasmic reticulum to the vacuole, where the PSI is thought to be proteolytically excised. Free PSI protein can permeabilize the membranes of microbial pathogens (1). For example, the *Solanum tuberosum* (potato) AP kills the spores of the common soil fungus *Fusarium solani* and those of the causative agent of potato blight, *Phytophthora infestans*, through membrane permeabilization. However, how the PSI performs this role is less clear. Zhao *et al.* (2) now create a unified model for the events preceding PSI-mediated membrane fusion, providing a new framework to study these fascinating sequences.

PSIs are structurally similar to saposins and saposin-like proteins, which bind, transport, and mediate the catabolism of lipids, so it is not surprising that PSIs also interact with membranes. PSIs, however, have a distinct architecture where the N- and C-terminal domains are inverted relative to authentic saposins, earning them the moniker “swaposins,” even though they do adopt the characteristic saposin helix-loop-helix fold (3, 4) (Fig. 1A). The PSI surface includes a large number of basic and acidic residues (Fig. 1B), and because environmental pH gradually decreases as plant APs are trafficked to the vacuole, there has been significant interest in how pH change impacts PSI function. Previous biophysical studies mimicking the vacuolar environment suggest that acidic pH promotes helicity in the PSI structure and favors homodimerization, which is also supported by crystallographic data (4, 5). Acidic pH was also required for the PSI to induce leakage from lipid vesicles composed of anionic lipids, suggesting that electro-

statics are relevant to the PSI-lipid interactions. Consistent with a causative role in membrane distortion and fusion, PSI dramatically increases the size and morphology of lipid vesicles (5). Taken together, these lines of evidence support that variations in pH modulate PSI function. The relationship between pH, lipid interactions, and dimerization, however, was unclear.

To gain a more holistic view of the PSI-catalyzed membrane fusion mechanism, Zhao *et al.* report an elegant integration of biophysical experiments, notably NMR, and molecular dynamics simulations to reveal the cascade of events leading up to PSI-mediated membrane fusion (2). They used a clever approach that directly links vesicle fusion to fluorescence to investigate pH variations on the rate and extent of PSI-mediated fusion. No fusion was observed with PSI at neutral pH; however, a sharp transition toward increased fusion efficiency was observed near pH 4.5, the same value as PSI's pI. Lowering of the pH neutralizes acidic residues, giving the protein a net positive charge that favors interaction with phospholipids. (Fig. 1B). The authors nicely demonstrate a monomer-to-dimer transition at the same pH: Using solution NMR, they showed that a single state of the PSI is present at neutral pH, and a second state emerged as the pH was lowered. ¹⁵N relaxation parameters of the low-pH state were twice that of those of the neutral pH state, consistent with dimerization. Dimerization had minimal impact on the helical content of the PSI. Chemical shifts reporting on the chemical environment of the α -helical backbone amides underwent the greatest changes on dimerization, suggesting that they constitute the interface between the monomers. NMR also demonstrated a pH-dependent resonance broadening when PSI was in the presence of liposomes. No such broadening was observed at neutral pH, consistent with an acidic pH-driven interaction. Hydrogen-deuterium exchange solid-state NMR confirmed that liposomes protected several PSI amide protons from exchange, demonstrating that the protein was partially, but persistently, in the membrane.

Finally, the authors used several coarse-grained molecular dynamics simulations of PSI/lipid mixtures to gain more detailed insight into the topology of the PSI-membrane interaction. These kinds of simulations are a useful approach to follow the assembly of such nanoscale systems that are otherwise intractable with conventional molecular dynamics simulations. Performing these simulations at low lipid density revealed association of the PSI with the bilayer, although not insertion. Increasing the lipid density led to the first views of possible intermediates in the membrane fusion sequence, where the PSI dimer symmetrically bridged two bilayers (Fig. 1C). Simulations confirmed that the symmetrical interaction could likewise

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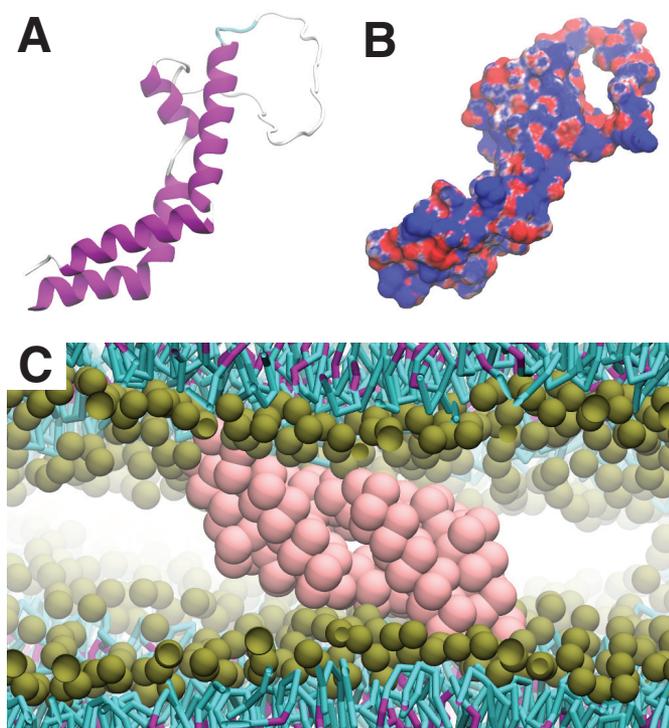


Figure 1. A, crystal structure of the potato PSI monomer (PDB code 3RF1) (4) with a modeled loop connecting the second and third helices. B, electrostatic potential (blue, positive; red, negative) mapped onto the solvent-accessible surface of the PSI monomer at pH 4.5. C, coarse-grained representation of the PSI dimer symmetrically bridging two phospholipid bilayers. Protein backbone atoms are depicted with pink spheres, lipid phosphates are depicted with yellow spheres, and the phospholipid tails are depicted with cyan sticks.

bridge two complete liposomes. Although fusion was not captured in the liposome simulations, their result was nevertheless satisfying because a similar bridged configuration was observed in coarse-grained simulations of vesicle fusion catalyzed by the saposin-like lung surfactant protein B (6). In total, these data lead to a new model in which the drop in pH promotes membrane association and dimerization, which brings two membranes together in a favorable way to promote fusion.

The sophisticated approach used by Zhao *et al.* illuminates a promising path forward in our understanding of PSIs, yet several unanswered questions remain. PSIs have been identified in several plant species with variations in their amino acid sequences, yet the relationships between structure and function are unknown. PSIs can use different pathways to target their protein cargo to the vacuole, but we do not know what interactions between the PSI and the cellular environment plots the course. Sequence also impacts the architecture of PSI-membrane interactions. Specifically, whether the result will be membrane fusion or permeabilization is unpredictable. Glycosylation of the PSI also impacts the mechanism of vacuolar targeting (7), yet little is known about how these complex polysaccharides guide protein transport. All of these questions warrant investigation, especially for protein engineering efforts.

Expanding our understanding of PSI structure and function and its interaction with biological membranes can be exploited for a variety of applications, especially in agriculture and medi-

cine. The observation that PSI confers plant resistance to microbial pathogens holds promise as a transgenic strategy to improve crop security (8). Membrane disruption is a proven strategy to treat systemic fungal infections (*i.e.* with polyenes). Intriguingly, the cytotoxic effects of the potato PSI extend to both Gram-positive and Gram-negative bacteria (9), so the possibility that PSIs may serve as a starting point for the development of more broad-spectrum antimicrobials deserves consideration. Finally, additional knowledge regarding the parallels between vacuolar and lysosomal sorting could be translated to targeted delivery of biologic drugs.

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Abbreviations—The abbreviations used are: AP, aspartic protease; PSI, plant-specific insert; PDB, Protein Data Bank.

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