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Review

Possible function of VIPP1 in maintaining chloroplast membranes☆

Lingang Zhang, Wataru Sakamoto *

Institute of Plant Science and Resources, Okayama University, Kurashiki, Okayama 710-0046, Japan

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ABSTRACT

A protein designated as VIPP1 is found widely in organisms performing oxygenic photosynthesis, but its precise role in chloroplasts has remained somewhat mysterious. Based on its structural similarity, it presumably has evolved from bacterial Phage shock protein A (PspA) with a C-terminal extension of approximately 40 amino acids. Both VIPP1 and PspA are membrane-associated despite the lack of transmembrane helices. They form an extremely large homo-complex that consists of an oligomeric ring unit. Although PspA is known to respond to membrane stress and although it acts in maintaining proton motive force through membrane repair, the multiple function of VIPP1, such as vesicle budding from inner envelope to deliver lipids to thylakoids, maintenance of photosynthetic complexes in thylakoid membranes, biogenesis of Photosystem I, and protective role of inner envelope against osmotic stress, has been proposed. Whatever its precise function in chloroplasts, it is an important protein because depletion of VIPP1 in mutants severely affects photoautotrophic growth. Recent reports of the relevant literature describe that VIPP1 becomes highly mobile when chloroplasts receive hypotonic stress, and that VIPP1 is tightly bound to lipids, which implies a crucial role of VIPP1 in membrane repair through lipid transfer. This review presents a summary of our current knowledge related to VIPP1, particularly addressing the dynamic behavior of complexes against stress and its property of lipid binding. Those data altogether suggest that VIPP1 acts *a priori* in chloroplast membrane maintenance through its activity to transfer lipids rather than in thylakoid formation through vesicles. This article is part of a Special Issue titled: Chloroplast Biogenesis.

1. Introduction

More than a decade ago, vesicle-inducing protein in plastids 1 (VIPP1) was characterized and proposed to be involved in thylakoid formation in *Arabidopsis* chloroplasts and in cyanobacteria [1,2]. Apparently, VIPP1 emerged from a bacterial protein Phage shock protein A (PspA) that is involved in the response to membrane stress. The emergence of VIPP1 was specific to organisms performing oxygenic photosynthesis and was correlated with the emergence of thylakoids. Therefore, it was inferred to play a direct role in thylakoid membrane formation through vesicles. Nevertheless, recent studies conducted by several other groups have suggested that the thylakoid biogenesis is indirectly regulated by VIPP1 through its impact on the thylakoid membrane [3,4]. Moreover, our group recently provided evidence that VIPP1 is crucial to maintaining the envelope rather than thylakoid membranes [5]. These reports collectively suggest that VIPP1 is a multi-functional protein related to the biogenesis and maintenance of chloroplast membranes (Fig. 1). What is the actual function of VIPP1?

In addition to these observations [1–5], several properties of VIPP1, such as forming an extremely large complex and association with the inner envelope despite the lack of no obvious transmembrane domains, raises the question of how this protein exerts its function. Recently, highly mobile property of VIPP1 shed light on its dynamic response to stress [5,6]. No matter what the function is in plastids, VIPP1 is an essential protein in plastids. It acts not only in thylakoid membranes but also in the inner envelope. Consequently, 'VIPP' can be interpreted as a 'Very Important Protein in Plastids'. This review surveys our current knowledge related to VIPP1, with extra emphasis placed on the association and dynamics of VIPP1 individual particles and its lipid-binding character. Wherever possible, comparison between PspA and VIPP1 is made for better understanding. Previous reviews of VIPP1 have been presented by Bultema et al. [7] and Vothknecht et al. [8].

2. Overview of VIPP1 studies and proposed function in chloroplasts

2.1. Discovery and subsequent history of VIPP1 studies

Two decades ago, VIPP1 was first described biochemically as a protein termed M30, which has been localized at the inner envelope as well as the thylakoid membranes of pea chloroplasts [9]. Later, *VIPP1* gene was identified based on the characterization of a knockdown mutant in *Arabidopsis* and *Synechocystis* sp. PCC6803 [1,2]. *Arabidopsis* VIPP1-knockdown (approximately 20% of original levels) and knockout lines caused seedling lethality. In *Synechocystis*, complete disruption of *VIPP1* gene seems impossible, suggesting that VIPP1 is essential. The

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* Corresponding author at: Institute of Plant Science and Resources, 2-20-1 Chuo, Kurashiki, Okayama 710-0046, Japan. Tel./fax: +81 86 434 1206.
E-mail address: saka@okayama-u.ac.jp (W. Sakamoto).

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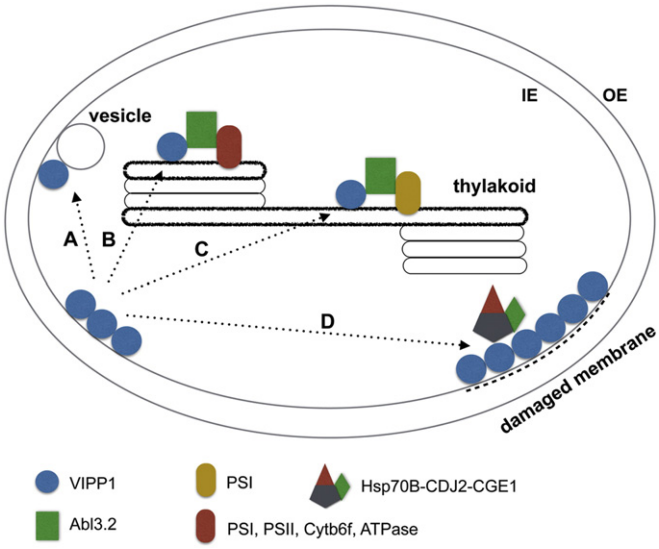

Fig. 1. Multiple functions proposed for VIPP1 in chloroplasts. (A) Vesicle induction: VIPP1 is possibly involved in the budding of vesicles from the inner envelope, which in turn is necessary for the maintenance of structurally and functionally intact thylakoid membranes [1]. (B) Photosynthetic protein supercomplex formation: VIPP1 provides structural lipids through Alb3.2 for the biogenesis of some protein complexes of the thylakoid membrane, such as PSI and PSII, as well as the cytochrome b_6f complex and ATP synthase in *Chlamydomonas* [3]. (C) PSI biogenesis: VIPP1 is necessary for the biogenesis of PSI, possibly by participating in the insertion of PSI polypeptides into thylakoid membrane mediated by Alb3.2. Furthermore, the absence of PSI inhibits the biogenesis of 'normal' thylakoid membrane in *Synechococcus* sp. PCC 7002. [4]. However, the marked difference in their results in *Chlamydomonas* and *Synechococcus* suggests strongly that VIPP1 plays differing roles in prokaryotes and eukaryotes. (D) Envelope membrane protection: When the envelope membrane integrity of *Arabidopsis* chloroplast was disturbed, the VIPP1 particles detached from the inner envelope and started moving inside the stroma. Some reassemble into a filament-like structure along with the damaged region of inner envelope membrane and become involved in the membrane repair [5].

mutants in both organisms showed comparable loss of thylakoid membrane content and structure, as well as reduced photosynthetic activity [1,2]. Furthermore, vesicular structures at the inner envelope of chloroplasts, which are presumed to mediate the thylakoid biogenesis [10,11], can no longer be observed in *Arabidopsis* mutants, indicating that VIPP1 is necessary for vesicle budding. Consequently, the protein was designated as a 'vesicle-inducing protein in plastids 1' [1].

Despite this nomenclature, evidence supporting the function of VIPP1 in vesicle formation has remained scarce since the first report, except for the defective vesicle-like structures of *vipp1* mutants observed in electron microscopy [1]. Consequently, although its involvement in vesicle induction cannot be fully excluded, the name VIPP1 itself should be regarded with caution: VIPP1 rather seems to have many features in common with lipid transfer proteins (see below). In addition to these observations [1,2], the idea of VIPP1 being involved in thylakoid formation derived from the initial finding that VIPP1 seems to have evolved from PspA of cyanobacteria by gene duplication [2]. Observations suggest that the evolution of thylakoids in photosynthetic organisms coincides with the acquisition of VIPP1, whereas PspA has been lost in land plants and VIPP1 only remains. Although the circumstances were regarded as supporting this idea, accumulating genome information from many cyanobacteria shows that the appearance of *VIPP1* gene does not necessarily coincide with the acquisition of thylakoid membranes. It is noteworthy that VIPP1 has diverse roles by interacting with membranes. Its role is not limited to thylakoid formation.

2.2. Proposed VIPP1's function in chloroplasts

VIPP1 was first proposed to participate in thylakoid biogenesis via membrane vesicles (Fig. 1A) [1]. However, this functional assignment is questionable, as described previously [5]. More importantly, VIPP1 shows no similarity to known proteins of the cytoplasmic vesicular transport system. Consequently, the precise role of VIPP1 in the cycle of vesicle budding, migration and fusion remains enigmatic. In contrast to these earlier reports [1,2], Nordhues et al. [3] found neither a marked reduction of thylakoid membrane nor changes in the number of membranes per granum in VIPP1-RNAi/amiRNA strains of *Chlamydomonas*. This RNAi line has no apparent limitation of thylakoid membranes for housing protein complexes, which points to the role of VIPP1 in the biogenesis and assembly of core complexes. One possibility to account for such a role of VIPP1 is delivery of structural lipids into thylakoid membranes (Fig. 1B). Similarly, Lo and Theg [12] demonstrated a stimulatory role of VIPP1 in cpTat transport pathway by enhancement of substrate binding, possibly through condensation of the thylakoid membrane to enhance productive cpTat substrate binding and transport. More recently, Zhang et al. [4] reported that VIPP1 is not necessary for thylakoid formation but that it is necessary for the biogenesis of photosystem I in *Synechococcus* sp. PCC 7002 (Fig. 1C). The link between thylakoid biogenesis and photosystem I accumulation has also been reported [13].

Prior works have examined thylakoid membranes, but our recent work has unraveled a distinct role of VIPP1 in chloroplast envelopes (Fig. 1D) [5]. We performed thorough characterization of *Arabidopsis vipp1* knockdown and knockout mutants, which revealed that chloroplasts and plastids in these mutants exhibit a unique morphology, forming balloon-like structures (Fig. 2A). Several lines of evidence show that the balloon-like chloroplasts result from chloroplast swelling related to osmotic stress: under hypotonic conditions, chloroplasts in vivo tend to show round chloroplasts with swollen stroma. In fact, swollen chloroplasts purified from *vipp1* knockdown mutant returned to normal when treated with hyperosmotic solution. These results suggest strongly that VIPP1 is necessary to maintain plastid envelopes to prevent membrane damage, such as the function proposed for PspA in bacteria. In fact, *Arabidopsis VIPP1* rescued defective proton leakage in an *Escherichia coli pspA* mutant, further confirming that VIPP1 can act in membrane repair [5].

Balloon-like chloroplasts such as those in *vipp1* are observed occasionally as a consequence of membrane damage, although it remains unclear why they are formed. They are also detected in wild-type *Arabidopsis* when placed under hypotonic stress. Other stresses aside from osmotic stress can cause membrane damage in chloroplast envelopes. For instance, swollen stroma have been observed in the tree leaves of *Taxus cuspidata* exposed to freezing (Fig. 2B) [14] and in leaves of maize that has been affected by drought and high temperatures [15]. We noted that some *Arabidopsis* mutants defective in galactolipid biosynthesis (e.g., *dgd1* mutant impaired in digalactosyldiacylglycerol [DGDG] accumulation) exhibit chloroplasts with swollen stroma [16,17]. From those observations, we infer that the balloon-like chloroplasts resulted from the weakened envelopes because of the lack of VIPP1.

2.3. Dynamics of VIPP1 and PspA

A notable observation in our study is that although the VIPP1 particles (see below) detected by VIPP1-GFP fusion in mature leaves were static in vivo (Fig. 3), they started moving rapidly when the chloroplast was under hypotonic stress [5]. Recently, VIPP1-GFP puncta in cyanobacteria showed dynamics under high light condition [6]. These results suggest that VIPP1 responds to envelope stress rapidly by supplying less-aggregated VIPP1 proteins to the swelling area. A filament-like structure along the envelope is assumed to represent a newly formed VIPP1 complex that is reassembled around the damaged inner envelope, which was supposed to be beneficial to stabilize the membrane and assist membrane resealing [5]. Experimental detection of membrane tension reduction is reported in cells undergoing recovery

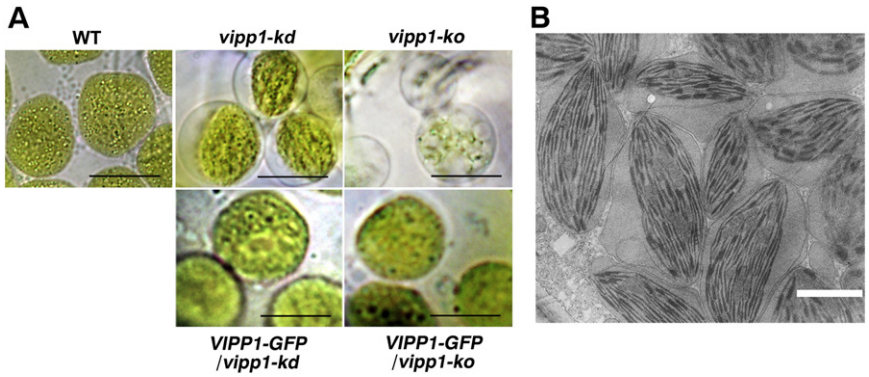

Fig. 2. Swollen chloroplasts of *Arabidopsis vipp1* mutants and winter needles of *Taxus cuspidata*. (A) Chloroplasts from unfixed leaf tissue of wild-type (WT), and *vipp1* mutants and their VIPP1-GFP complemented lines were examined under bright-field microscopy (bars = 10 μm). Photographs provided from the authors' laboratory. (B) Electron micrographs of chloroplasts in winter needles of *T. cuspidata* (bar = 2 μm). Courtesy of Ayumi Tanaka, Hokkaido University.

through membrane bilayer resealing [18]. *N*-ethymaleimide-sensitive factor attachment protein receptors (SNAREs) could form a tetrameric bundle of coiled helices to draw the membrane surface together for fusion [19,20]. Compared with discrete VIPP1 particles, the filament-like structure of VIPP1 is more suitable to be scaffold for membrane resealing. New VIPP1 can be supplied either by de novo synthesis of VIPP1 or by disassembly of preexisting complexes. Although both cases are possible, our live imaging implies that the latter case of the complex disassembly participates in this filament formation. How this movement is regulated in response to membrane stress remains as a subject for additional studies. Similarly to VIPP1, PspA-GFP particles are demonstrated to move inside *E. coli* cells. Real-time observations revealed that lateral PspA complexes are highly mobile. They are mediated by the MreB cytoskeleton to form lateral complexes properly and to move between the cell poles. Such a trafficking of PspA through MreB is necessary to maintain proton motive force across membranes under the pIV-secretin stress [21]. In chloroplasts, no MreB-like protein is found, implying that VIPP1 requires a distinct system to allow movement.

Furthermore, a recent study showed that PspB and PspC proteins share some common behaviors in mobility when detected as a GFP fusion. Under non-stress conditions, numerous mobile GFP signals have been widely distributed in the cell. PspBC are believed to sense stress and trigger activation of the Psp system by scanning the membrane for an inducing event [22,23]. In the case of stress conditions, however, PspB-GFP and PspC-GFP will aggregate into bright foci located around the pole, although some other dim foci were kept dynamics elsewhere. Similarly to PspA-GFP, the less mobile foci that formed by PspB-GFP and PspC-GFP become brighter, which implied that the size of complexes increased [24]. Consistent with these descriptions of PspA and PspBC dynamics, some VIPP1 particles in chloroplasts of *Arabidopsis* mutually re-associate to form filament-like supercomplexes after several minutes (around 3 min) of hypotonic stress. Once formed, they remain stationary along with the inner envelope membrane, even though other singular small foci continue moving inside swollen chloroplasts [5].

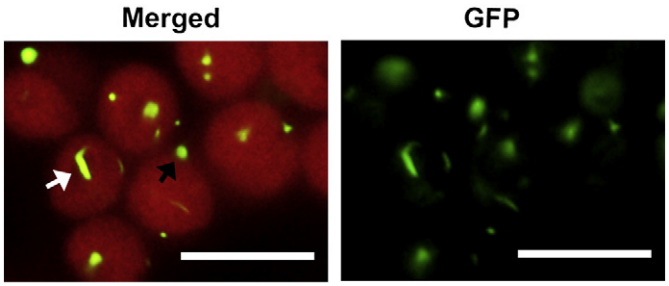

Fig. 3. Morphologies of VIPP1-GFP supercomplexes. Microscopic observations of chloroplasts in mature leaves were made with the transgenic *Arabidopsis* plants expressing VIPP1-GFP. Signals corresponding to GFP are shown on the right panel and the merged image with chlorophyll autofluorescence is shown in the left panel. The signals are detected on the surface of chloroplasts with variable size and morphology. The black arrow shows a round VIPP1-GFP particle. The white arrow indicates a rod-like structure of VIPP1-GFP that is presumed to be organized by individual particles (bars = 10 μm).

3. Structural insight of PspA and VIPP1 and their complex formation

3.1. VIPP1 and PspA: structural similarities and differences

In actuality, PspA is a member of a bacterial stress response system designated as the 'phage shock protein system' (Psp system) [25]. PspA supports the bacterial cell under stressful circumstances by mitigating proton leakage [26]. VIPP1 also shares significant structural similarity with their evolutionary ancestor PspA, those including a predominantly α-helical structure, the formation of oligomeric high molecular weight complexes and a tight association with membranes [7,27–29]. One interesting feature that is specific to VIPP1 is a C-terminal 38 amino acid extension of which the origin is unclear. Such a C-terminal extension is not apparent in PspA family. Therefore, it might have a specific role in photosynthetic membranes.

In contrast, secondary structures of PspA proteins from non-photosynthetic and photosynthetic organism show some differences. Based on several prediction programs, the amino acids between 21 and 156–158 of PspAs in non-photosynthetic bacteria are predicted to form a single long α-helix, whereas a short random coil is shown to interrupt this helix at position 80 in cyanobacterial PspAs. This type of interruption around 80 is also apparent in all VIPP1 proteins. Therefore, it seems likely that the random-coil structure at position 80 was acquired by PspA proteins in photosynthetic bacteria that were subsequently passed on to VIPP1 in chloroplasts [30].

In general, by introducing a loop (e.g., short random coil) into the long helix, the protein becomes more flexible. It can take different conformations by interacting with other molecules. Aside from the random-coil region at position 80, a longer random coil (17–40 amino acids) followed by a short C-terminal α-helix (6–9 amino acids) appeared at the C-terminus of VIPP1. Our preliminary result suggests that this extra fragment might also increase the VIPP1 flexibility compared to that of PspA, which might benefit the membrane maintenance of chloroplasts where a membrane system is more complicated than the photosynthetic bacteria [30].

Based on these observations [30], it is reasonable to infer that the difference in secondary structure allows VIPP1 to form more flexible association with membranes than PspA. One possibility to explain this evolutionary consequence is that photosynthetic microorganisms

require VIPP1 to cope with the drastic formation of thylakoid membranes that do not exist in non-photosynthetic bacteria. Alternatively, photosynthesis gives rise to extraordinary membranes emphasizing results from a series of light-driven redox reactions, in which VIPP1 that has more flexibility than PspA is necessary to prevent the organism from proton leakage across the membranes. The dynamic behavior of VIPP1 against hypotonic stress might be reflected by such flexibility.

3.2. Complex formation

VIPP1 and PspA form extremely large homo-oligomeric rings that are structurally varied but which putatively consist of a basic ring structure [7,27–29,31]. In fact, PspA from *E. coli* forms a single ring-like structure with nine-fold rotational symmetry. This PspA ring of calculated approximately 1 MDa mass has an outer diameter of approximately 20 nm, inner diameter of approximately 9.5 nm, and height of approximately 8.5 nm [28]. In contrast, VIPP1 proteins of *Arabidopsis* and *Synechocystis* organize into varied ring structures with higher molecular masses (up to approximately 2 MDa) [27,29]. *Synechocystis* VIPP1 has six ring structures composed of 12–17 internal rotational symmetry. These structures share the same height (22 nm), although their outer diameter varies: 25–33 nm [29].

In *E. coli* PspA, four helical domains (HDs, HD1–HD4) were predicted based on the secondary structure. The central helical regions (HD2 and HD3) are sufficient to form a PspA hexamer. The protein containing only HD1, HD2, and HD3 forms dimers. Apparently, HD4 is involved in forming a 36-mer ring [32,33]. Recently, two amphipathic helices, ahA and ahB, in PspA HD1 have been studied in detail. These amphipathic helices enable PspA to switch from a low-order gene regular into an inner membrane-bound high-order effector complex under membrane stress. A conserved residue proline at position 25 is involved in the sequential use of the amphipathic helices and ahA-inner membrane interaction [34]. Consistent with PspA, the 21 N-terminal amino acid residues of VIPP1, which form the first α-helix of the protein, are also essential for assembly of the 2 MDa super homo-oligomers as well as for the interaction of VIPP1 with the inner envelope membrane of chloroplasts [35]. By contrast, neither the VIPP1 specific C-terminal helix nor large parts of the C-terminus of the PspA-like domain are necessary for the formation of the annular particle of VIPP1 [27,31,35].

4. Lipid-binding activity through the complex formation

4.1. Lipid binding of VIPP1

Originally, PspA was shown to associate with the plasma membrane via two membrane-bound Psp proteins PspBC [36], but it can also interact directly with membranes [26], which suggests further that membrane association–disassociation is part of a regulatory cycle of PspA function and that it depends on its oligomerization state. Similarly, experimental evidence has shown that oligomerization is a prerequisite for VIPP1 function in thylakoid formation [37]. The N-terminal α-helix of VIPP1 consisting of 21 amino acids appeared to be essential for the assembly of the protein to form high molecular weight complexes, and for the interaction of VIPP1 with the inner envelope membrane of chloroplasts.

It is particularly interesting that attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) has shown that the recombinant VIPP1, expressed as a fusion protein in *E. coli*, can bind with bacterial lipids. This lipid binding is so tight that VIPP1 is always co-purified with lipids. However, several lines of evidence indicate that lipids are *a priori* unnecessary for forming VIPP1 complex formation. The ATR-FTIP spectra of VIPP1 high molecular weight complexes before and after lipase treatment were structurally distinguishable, which further implied that protein conformation has been slightly changed and that a minor part of VIPP1 α-helix structures might be converted into disordered structures (<10%). More importantly, the absorption bands of the lipid groups are reduced significantly [35]. These data suggest that conformation of VIPP1 individual particles is affected by its lipid-binding state: lipid-bound VIPP1 particle comprise high-level α-helices; lipid-free VIPP1 particles prefer a more random structure. Nordhue et al. [3] reported that VIPP1 affects the biogenesis and assembly of thylakoid membrane core complexes, particularly the two photosystems. They speculated that lipids might be loaded onto VIPP1 directly at their synthesis site at the inner envelope and might be released into a photosystem assembly complex at the thylakoid membrane, although not proved experimentally.

4.2. Similarity to lipid transfer protein

It is particularly interesting that VIPP1 appears to include a region (72–288 amino acid) named 'Apolipophorin-III like' according to its NCBI annotation, which is almost identical to the PspA-like domain of VIPP1 (72–290 amino acid). Apolipophorin-III (apoLp-III) is a prototypical exchangeable apolipoprotein found in many insect species. It functions in transport of diacylglycerol (DAG) from the fat body lipid storage depot to flight muscles in the adult life stage [38,39]. The protein includes five long, amphipathic α-helices that form an up-and-down helix bundle with short loops connecting the helices. In the bundle, the hydrophobic faces orient toward each other and create a hydrophobic core, whereas the hydrophilic surfaces are directed toward the solvent. This molecular organization ensures high protein solubility in the hemolymph [40,41].

The similarity of VIPP1 with apoLp-III induces us to analyze each helix of VIPP1 and to examine whether these α-helices exhibit the features of amphipathicity or not. Fig. 4 portrays the helix wheel projections corresponding to each α-helix of VIPP1 predicted using online software (PSIPRED, Psi-blast based secondary structure prediction, http://bioinf.cs.ucl.ac.uk/psipred/) [42,43]. Blue dots represent the hydrophobic amino acids that are distributed to one side of the helix, whereas the opposite side is occupied by basic and polar residues, demonstrating that VIPP1 is a typical amphipathic protein that is well suited for membrane binding. As revealed by X-ray diffraction [44], a model amphipathic α-helix adopts an orientation parallel to the membrane plane, with its central axis positioned at the level of the lipid glycerol group: hydrophobic residues are inserted between fatty acyl-chains, whereas polar residues face lipid polar heads (Fig. 4C).

A similar analysis was also conducted with the consensus sequence of the VIPP1 C-terminus. A hydrophobic face on one side of the amphipathic α-helix comprises four hydrophobic residues [8]. Here, it is apparent that not only the α-helix of C-terminus, but also the other six α-helix structures all include the hydrophobic face on one side (Fig. 4B). Just like the apoLp-III, these amphipathic α-helices of monomer VIPP1 can potentially form a helix structure through up-and-down folding of different helical segments. Twelve to seventeen helix units are organized into one ring particle [7]. Native staining of VIPP1 annular particles purified from *Arabidopsis* or expressed in *E. coli* both confirmed the hole in the central part of the particle. The size is estimated at about 120 Å [27,29,31,35].

A similar structure was also found in the other homologous proteins of apoLp-III, such as apolipoprotein A-I (apoA-I) and apolipoprotein E (apoE) of animals [45–48]. Both are characterized by segments of amphipathic α-helices, which is responsible for lipid binding [49]. Insect apoLp-III is a single-domain protein composed of a 5-helix bundle. In the case of apoE and apoA-I, one or two additional helices at the C-terminal end complement the 4-helix bundle and serve to anchor the protein to the lipoprotein particle [50,51]. The C-terminal helices might have evolved to fulfill the more complex role in vertebrate lipid transport processes. It is plausible that the fifth helix in insect apoLp-III provides further instability, thereby facilitating a rapid switch between a lipid-free to a lipid-bound state and allowing apoLp-III to be recycled efficiently [48]. The lipid-binding character is unquestionably

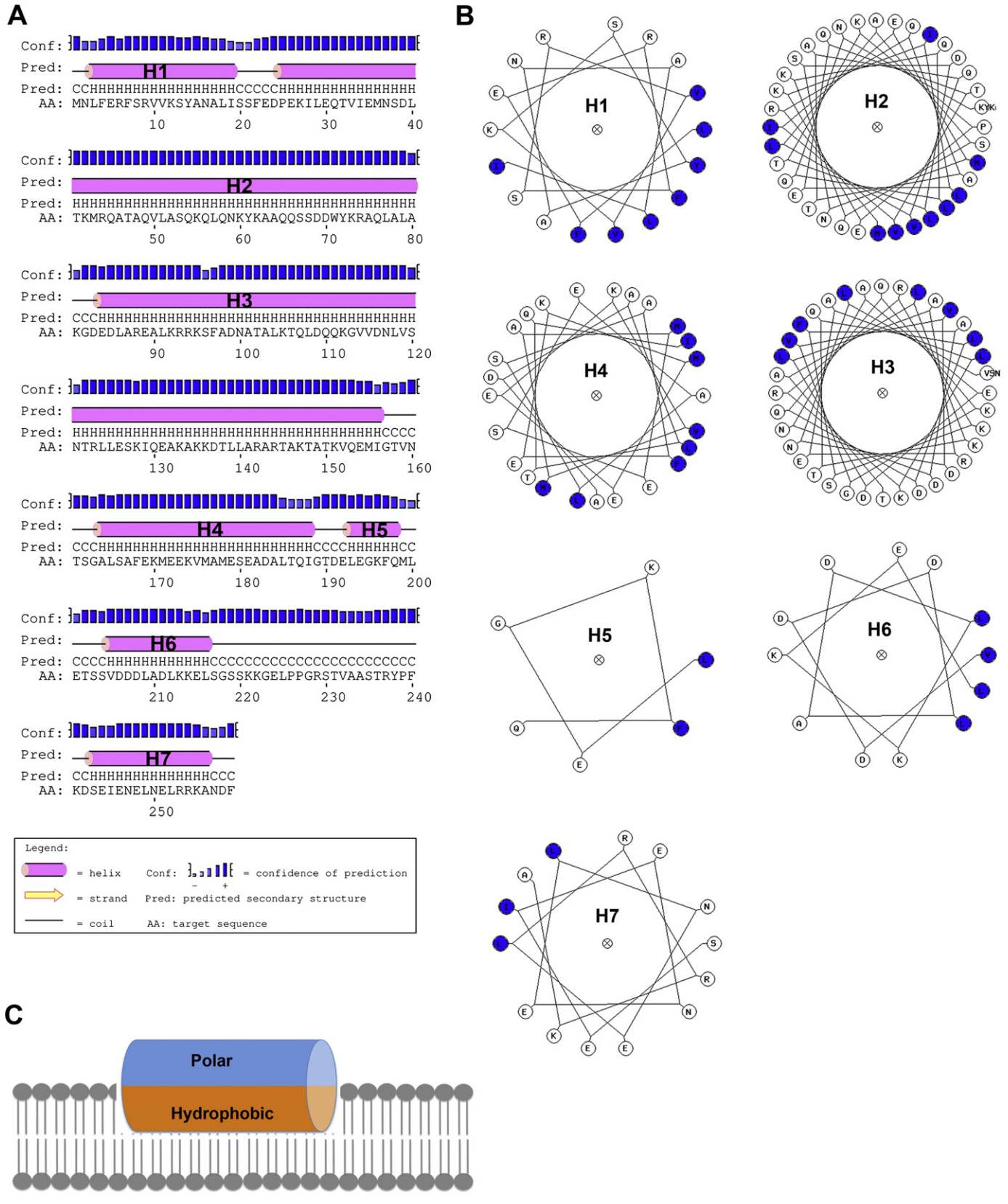

Fig. 4. Amphipathic helices of VIPP1. (A) Distribution of α-helix and random-coil regions within *Arabidopsis* VIPP1 amino acids. The amino acid sequence of mature VIPP1 (without its transit peptide, 1–72 amino acid) was used to perform the analysis with software PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/). H1–H7 denote the different α-helices of VIPP1 and correspond to those described by Otters et al. [35]. (B) Helical wheel projection of VIPP1. The hydrophobic amino acid is shown in blue. Others are polar or basic residues. Prediction was performed with software (Hexiator; http://www.tcdb.org/progs/helical_wheel.php). Each complete helix turn included 3.6 residues. (C) Schematic view of the interaction between an amphipathic α-helix and membrane lipids. Half of the amphipathic α-helix is embedded inside the lipid membrane with its hydrophobic residues. The polar residues face the lipid polar heads.

inherited by the presence of the amphipathic helices. Consequently, the simple molecular fold of a helix bundle is a signature, underlying theme that provides an excellent structural arrangement for various proteins to bind to lipid surfaces.

5. Clustering of VIPP1 individual particles

5.1. Detection of high-magnitude VIPP1/PspA cluster and interacting proteins

In *E. coli*, PspA reportedly forms a large and regular scaffold structure, which presumably stabilizes the stressed membranes physically through multiple interactions over large membrane surface areas [52]. Similarly, a lattice-like structure of VIPP1-GFP was observed in *Arabidopsis* leaves in a development-dependent manner [5]. In older leaves, the lattice-like structure of VIPP1 can promote the closure of transient holes or leaks formed in envelopes. Our live imaging implies that the filamentous structure in hypotonically stressed chloroplasts represents a newly formed VIPP1 complex that accumulates around the damaged inner envelope [5].

In *Chlamydomonas*, the large VIPP1 complex has been shown to interact with proteins such as CDJ2/HSP70B [53]. Moreover, VIPP1 rings can assemble into large, rod-shaped supercomplexes resembling a microtubule-like structure under the regulation of HSP70B-CDJ2-CGE1 chaperones [31]. The VIPP1 rods might act as a sort of scaffold for the transport of proteins and/or lipids to promote thylakoid formation. Alternatively, the VIPP1 rods might represent an inert storage form, from which VIPP1 might be supplied by the chaperones for a function in sustaining membrane integrity. VIPP1 interacts with alb3.2 [54], which is suggested to play a role in the maintenance and assembly of both photosystems in the thylakoid membrane. In *E. coli*, Kobayashi et al. [26] demonstrated that PspA exists as a high-order oligomer (36-mer) in vitro. They also demonstrated that these supercomplexes could bind membrane vesicles containing PG, and only high-order PspA oligomers are able to repair the membrane upon stress.

5.2. Lipid as a key factor for VIPP1 clustering: a proposed function

VIPP1 particles can cluster under some conditions in the absence of HSP70-CDE1-CDG1 chaperones. Aseeva et al. [27] reported that native VIPP1 purified from *Arabidopsis* can mutually associate when they undergo denature–renature cycles, but this process did not interfere with the formation of VIPP1 individual annular particles. Given that VIPP1 binds tightly with lipids, as suggested by Otters et al. [35], it is possible that the denature–renature process can remove the lipids from VIPP1 ring particles. Depletion of lipids might then allow VIPP1 particles to cluster mutually.

Based on the observations described previously, we hypothesize that VIPP1 comprises complexes of two distinct types that are mutually interchangeable through the lipid binding. The first type is a functional ring-like structure that is assembled from VIPP1 molecules. However, the precise structure of this ring remains elusive, probably because it is vulnerable to membrane stress. The second type is the high-magnitude stack of different ring-like particles that engenders the formation of rod–scaffold and lattice structures. Under a static state (i.e., non-stressed condition), it is possible that most VIPP1 is present as ring particles and that it is stably attached to the inner envelope surface and thylakoid membranes. Under a dynamic state (i.e., hypotonic stress or during leaf maturation), the ring particles become highly mobile, and associate mutually to form rod or scaffold structures, thereby sealing the damaged membranes. This model seems consistent with the proposed function of PspA for membrane protection in bacteria [26].

Similarly to the proposed function of VIPP1 described previously, apolipoprotein is another well-studied protein of which the high-order assembly is under the regulation of the lipid-binding state. The A class apolipoproteins (ApoA-I, ApoA-II, Apo A-IV and Apo A-V) act as detergents that emulsify lipids to form with lipoprotein particles. They change their states from a lipid-free to lipid-bound state, or vice versa. Early studies showed that lipid-free ApoA-I self-associates to form oligomers for which assembly takes place in a concentration-dependent manner [55–57]. These cuboidal particles can stack into characteristic structures [58]. The particles become disassembled under the lipid-binding state, and binding of as few as two lipid molecules seems sufficient to disrupt apoA-I self-association in favor of monomeric lipid-poor species [57,59]. Overall similarity between apolipoprotein and VIPP1 implies that VIPP1 is an apolipoprotein-like protein in chloroplasts. Membrane-binding activity might allow VIPP1 to transfer lipids between the distant region of the envelope and thylakoid membranes.

6. Concluding remarks

Lipid binding is an essential characteristic of VIPP1 for its function of envelope membrane protection and/or thylakoid formation. The fact that the envelope acts as the site of lipid production raises the question of how the lipids of the thylakoid are transferred from its production site (envelope) to its destination (thylakoid). Even in the case of envelope protection, lipids are presumed to be transferred to its damaged site to carry out membrane repair according the models of plasma membrane resealing of animals. In fact, since it was first cloned in 2001, VIPP1 was presumed to undertake the function of lipid transfer in the process of thylakoid formation [1]. Nevertheless, until now, no direct evidence has supported that inference. The finding of VIPP1 dynamics highlights the role of VIPP1 in the process of lipid transfer inside the chloroplast. Another interesting feature is the preference of particular lipids for VIPP1 binding. For example, galactolipids such as monogalactosyldiacylglycerol (MGDG) and DGDG are the major lipids in photosynthetic membranes. It is possible to examine whether VIPP1 has any preference for binding to these lipids, or not, which might well explain why VIPP1 has evolved to adopt membrane damage in photosynthetic organisms.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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