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Structure and function of PspA and Vipp1 N-terminal peptides: Insights into the membrane stress sensing and mitigation

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ABSTRACT

The phage shock protein (Psp) response maintains integrity of the inner membrane (IM) in response to extracytoplasmic stress conditions and is widely distributed amongst enterobacteria. Its central component PspA, a member of the IM30 peripheral membrane protein family, acts as a major effector of the system through its direct association with the IM. Under non-stress conditions PspA also negatively regulates its own expression via direct interaction with the AAA+ ATPase PspF. PspA has a counterpart in cyanobacteria called Vipp1, which is implicated in protection of the thylakoid membranes. PspA's and Vipp1's conserved N-terminal regions contain a putative amphipathic helix a (AHa) required for membrane binding. An adjacent amphipathic helix b (AHb) in PspA is required for imposing negative control upon PspF. Here, purified peptides derived from the putative AH regions of PspA and Vipp1 were used to directly probe their effector and regulatory functions. We observed direct membrane-binding of AHa derived peptides and an accompanying change in secondary structure from unstructured to alpha-helical establishing them as *bona fide* membrane-sensing AH's. The peptide-binding specificities and their effects on membrane stability depend on membrane anionic lipid content and stored curvature elastic stress, in agreement with full length PspA and Vipp1 protein functionalities. AHb of PspA inhibited the ATPase activity of PspF demonstrating its direct regulatory role. These findings provide new insight into the membrane binding and function of PspA and Vipp1 and establish that synthetic peptides can be used to probe the structure-function of the IM30 protein family.

1. Introduction

The cell envelope provides protection from the environment and gives structural integrity to the cell in all organisms. Homeostasis of the plasma membrane is vital for functioning of the cell and the bacterial phage shock protein (Psp) response protects the bacterial membrane under various extracytoplasmic stress conditions. Although many different stimuli trigger induction of the Psp response, the common theme is disruption of the plasma membrane and consequently loss of the (trans)-membrane potential and dissipation of the proton motive force (pmf) [1–4]. By an unknown mechanism, the Psp response rescues the proton gradient and conserves the pmf by protecting the plasma membrane integrity. The central component of the Psp system is the peripheral plasma membrane binding protein PspA belonging to the IM30 family of proteins found in many organisms. In bacteria, the Psp response and PspA-like proteins are implicated in protein translocation, virulence and resistance to antimicrobials that target the cell wall or reorganise the membrane architecture [3,5–9].

In enterobacteria, PspA is a dual function protein responsible for both the negative regulation and effector function of the Psp response. Under non-stress conditions PspA directly interacts with the subunits of the *psp* transcription activator, the hexameric bacterial enhancer binding protein PspF, imparting negative regulation by inhibiting PspF's ATPase activity and the subsequent sigma54-dependent transcription of *psp* genes [3,10]. PspA-PspF interactions occur *via* the PspF W56 loop, a surface exposed hydrophobic region on each PspF subunit [11–13]. Under inner membrane (IM) stress conditions, PspF is released from the PspA-F inhibitory complex leading to induction of the Psp response. Stresses such as defects in protein translocation systems or mislocalisation of outer membrane secretins into the IM are sensed by the IM proteins PspB and PspC [13,14] resulting in a direct interactions

Abbreviations: Psp, Phage shock protein; pmf, proton motive force; IM, inner membrane; TLE, total lipid extract; PG, phosphatidylglycerol; PS, phosphatidylserine; SCE, stored curvature elastic; AH, amphipathic helix; ITC, isothermal titration calorimetry; CD, circular dichroism; SUV, small unilamellar vesicle; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; DOPG, 1,2-dioleoyl-sn-glycero-3-phospho-(1′-rac-glycerol); LUV, large unilamellar vesicle; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; DOPS, 1,2-dioleoyl-sn-glycero-3-phosphoserine; TFE, trifluoroethanol.

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between PspB-C and the PspA subunits of the PspA-F inhibitory complex that release PspF. The PspB-C dependent signalling is conditional and severe stresses such as extreme temperature, hyperosmotic or ethanol shock cause partial or complete PspB-C-independent induction of the Psp response [1,3,4]. Following release of PspF, the PspA binds to the IM and forms high-order oligomeric (up to 36mer) effectors complexes [13,15,16] able to repair IM damage and conserve the pmf [17] but unable to stably interact with PspF to impose negative control [18].

Characterising PspA IM-binding is key to understanding the mechanism by which PspA repairs the membrane. PspA as a high-order oligomer binds to vesicles made from *E. coli* total lipid extracts (TLE) and vesicles containing the anionic lipids phosphatidylglycerol (PG) or phosphatidylserine (PS). PspA was also shown to prevent proton leakage form *E. coli* TLE vesicles *in vitro* [17]. McDonald et al. [18] showed that both anionic lipids and accumulation of the membrane Stored Curvature Elastic (SCE) stress from type II lipids drive vesicle association of PspA. *Psp*-inducing extracytoplasmic stress stimuli may well lead to the accumulation of SCE stress and associated lipid packing defects within the IM which disrupt membrane integrity and so stimulate PspA binding. Anionic lipids promote PspA binding to vesicles with low membrane SCE stress; the higher the membrane SCE stress, the less anionic lipids contribute to membrane association [18] suggesting the SCE stress may be the primary signature of the damaged membrane to be repaired by PspA.

The N-terminal region of PspA consists of two putative amphipathic helixes (AHs) (see Fig. 1), AHb required for negative control, and AHa required for effector function [19]. The N-terminal AHa (ahA; residues 2–19) is responsible for IM binding and effector function *in vivo*. The lack of AHa or amino acid substitutions in the hydrophobic face of the helix abolishes PspA IM-binding and high-order oligomer formation *in vivo* and *in vitro*. The adjacent AHb (ahB; residues 25–42) is implicated in PspA negative control. The lack of AHb or amino acid substitution on the hydrophobic face of the helix abolishes PspA-PspF interaction and PspA negative control function but does not affect the IM-binding and effector function of PspA [19]. Notably, in the absence of PspF and lipids, PspA is able to form high-order oligomers *in vitro* [10,20] suggesting interaction with PspF *via* AHb is critical for preventing PspA to oligomerise. The conserved P25 helix-breaking residue separates AHa and AHb and is important for both the negative regulatory and effector function of PspA and thus might establish a mutually exclusive use of two AHs [19]. A monomeric PspA fragment 1–144 ($PspA_{1-144}$) is sufficient for PspF negative control, dependent upon residue E37 within the AHb. Importantly, in the crystal structure of $PspA_{1-144}$ the putative AHa region is unordered [21]. A transition from unordered-to-ordered alpha-helical structure upon membrane association would establish the region as a *bona fide* AH. If the PspA AHa is indeed a typical membrane-sensing AH, a structural transition following binding to the IM may cause the switch in function of the PspA. Indeed, a conformational change of PspA AHa upon IM interaction has been inferred from other functional and structural studies [19,21].

The PspA homologue Vipp1 which is implicated in thylakoid membrane biogenesis and protection in cyanobacteria, green algae and higher plants [22–25] also carries a putative N-terminal AHa (see Fig. 1). Notably, Vipp1 can substitute for PspA in *E. coli* [26,27] and the presence of its N-terminal region is required for binding to lipid vesicles and high-order oligomer formation [18,28]. Vipp1's functional similarity to PspA for vesicle stress recognition is high, except that the role of anionic lipids in Vipp1-membrane binding is more pronounced [18] in accordance with suggested function in thylakoid membrane fusion [29].

It appears that the interactions of the putative AH regions of PspA and Vipp1 are a critical aspect of the mechanisms by which the Psp response is regulated and IM or thylakoid membrane stress is ameliorated. However, there is currently no experimental evidence to show the direct functionality of these regions or characterise their behaviour as typical AHs. In this work we used synthetic peptides based on PspA and Vipp1 N-terminal AHs and vesicles of well-defined size and lipid composition to determine a direct AHa-membrane interaction and inhibition of PspF ATPase activity by PspA AHb. The quantifications of AHa membrane-binding and structural transition as well as its effect on vesicles stability offer novel insight to a possible mechanism of stress mitigation by the PspA and related effector complexes.

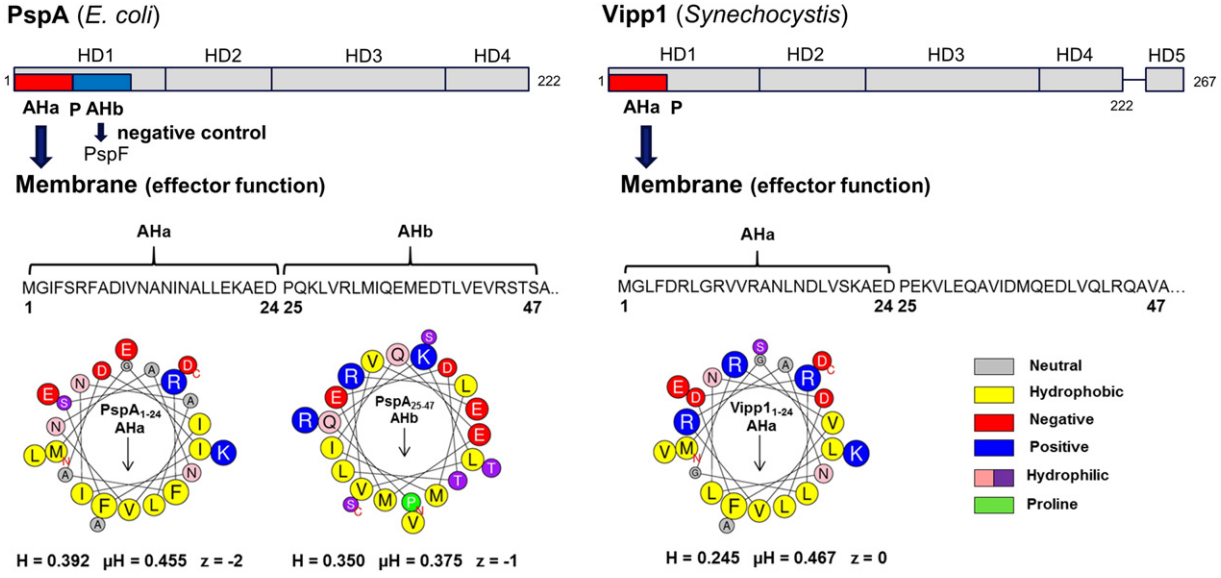

Fig. 1. N-terminal sequences of PspA and Vipp1. Schematics of PspA from *Escherichia coli* and Vipp1 from *Synechocystis* (top). Residues 1–222 of both proteins share sequence similarity and have 4 predicted alpha-helical domains labelled HD1 to HD4. Vipp1 has an extra C-terminal helical domain labelled HD5 separated from HD4 by a flexible linker region. Both proteins have an N-terminal putative AHa up to the strictly conserved P25 residue that is responsible for their membrane binding function [18,19,28]. PspA has a second putative AHb sequence after the P25 residue that causes negative regulation of PspF through interaction with the W56 hydrophobic loop [12]. The amino acid sequences and helical-wheel representations of the constitutive putative AH regions are shown below each schematic. The physiochemical parameters [50] of hydrophobicity (H), hydrophobic moment (μH) and net charge (z) are given for each AH. Arrows within each representation show the direction of the hydrophobic moment and residues are colour coded by their properties.