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Crystal Structure and Functional Studies Reveal that PAS Factor from *Vibrio vulnificus* is a Novel Member of the Saposin-Fold Family

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Introduction

Nearly all virulence factors are located on the bacterial surface or are secreted. In that regard, a small protein (76 amino acids) designated PAS factor has been cloned from the species of *Vibrio* and is thought to be a novel secretion factor that is expressed only in vivo after infection. The precise function of PAS factor in *Vibrio* remains unknown, however, though it may be involved with the secretion of such bacterial periplasmic proteins as alkaline phosphatase and β-lactamase. Consistent with that idea, ectopic expression of PAS factor in *Escherichia coli* cells makes the host cell membrane leaky, resulting in secretion of periplasmic proteins into the culture medium.

To better understand the biological function of PAS factor, in the present study we determined the crystal structure of the protein from *V. vulnificus* at 1.8 Å resolution. In addition, we investigated its interaction with phospholipid membranes by analyzing its CD spectra and conducting liposome leakage experiments. We found that, despite a lack of sequence homology, the three-dimensional structure of PAS factor is highly similar to amoebapore A, a 77-residue pore-forming toxin from the human pathogen *Entamoeba histolytica*, which is a member of the saposin-fold family of proteins that bind to membranes and lipids.

Results and Discussion

Overall structure of PAS factor

PAS factor is a single compact domain comprised of five α helices (α1, α2, α3, α4 and α5), the dimensions of which are approximately 33 Å x 27 Å x 40 Å (Figure 1). By grouping the α3 and α4 helices into one helix, the PAS factor fold can be described as a distorted four helix bundle with an up-and-down topology. Charged and polar residues are mostly exposed on the surface, while most of the hydrophobic residues are buried inside the hydrophobic core of the helical bundle.

Binding of PAS factor to liposomes

By analyzing tryptophan fluorescence emission from four single-tryptophan mutants (V10W, T22W, F35W, and L70W), we identified the putative phospholipids binding site of PAS factor. The resultant membrane destabilization likely mediates secretion of periplasmic proteins required for the in vivo survival and pathogenesis of *V. vulnificus* (Figure 2).

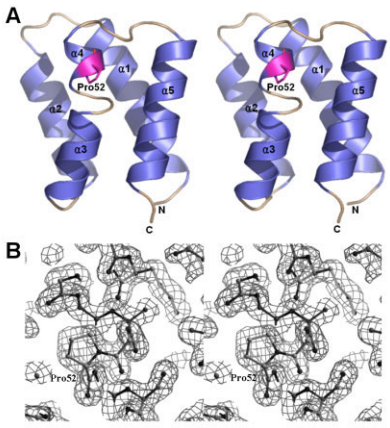

Figure 1. Structure of the PAS factor protein. *A*, Ribbon diagram showing the overall structure of the PAS factor protein. The α-helices (α1, α2, α3, α4 and α5) form an antiparallel five-helix bundle with an up-and-down topology. A sharp helix kink is caused by the presence of Pro52 residue between the α3 and α4 helices.

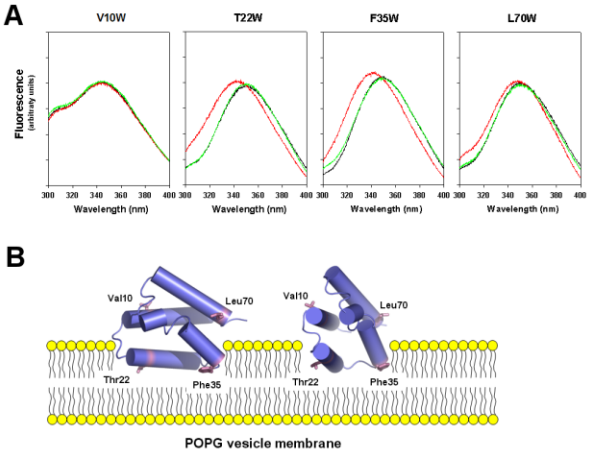

Figure 2. Model showing the possible interaction of PAS factor with a membrane.

References

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