

are capable of assembling in two strikingly different membranes: mammalian membranes that contain up to 40% of cholesterol and insects membranes that contain larger fraction of shorter unsaturated lipids and no cholesterol. Recently, it was shown that mutations in the transmembrane domain of the Sindbis virus E2 protein produce differential alterations in the protein association with the lipid bilayer: some mutants were able to grow in insect cells, but not in mammalian cells [1,2]. The Sindbis virus with STM-16 deletion mutation of the E2 transmembrane domain shows the most pronounced differential growth in mammal and insect cells while STM-18 shows almost wild-type behaviour. We have investigated the interaction of synthetic peptides mimicking E2 domain mutants with lipid bilayers with the goal to understand constraints placed upon membrane spanning domains for correct integration into the bilayer. The phospholipid composition was chosen to represent mammalian and insects' membranes. Results of EPR spin-labeling experiments show that both STM-16 and STM-18 peptides adopt a transmembrane configuration in bilayers with lipid composition mimicking that of insects. In mammalian cell mimicking membranes and containing cholesterol the STM-16 peptide aggregates at the surface of the bilayer. Both peptides exhibit transmembrane orientation in bilayers consisting of "mammalian" lipid mixture but without cholesterol. Thus, we show that cholesterol content of the lipid mixture modulates insertion of the peptides into bilayer mimicking mammalian cell membrane. Supported by NSF grant MCB-0451510 to TIS.

[1] Hernandez, R., *et. al. J Virol* **2003** 77(23), 12710-9.

[2] West, J., *et. al. J. Virol.*, **2006** 80:4458-4468.

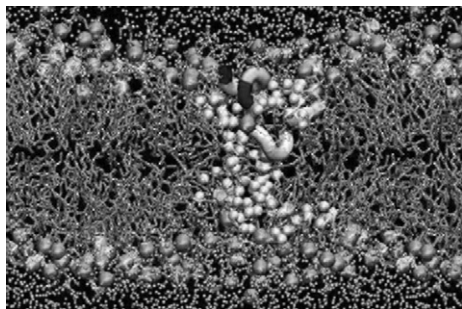
2011-Plat

Disordered Pore Formation At Rigid/Fluid Boundary Zones As A New Mechanism For Peptide-Membrane Interaction With The Beta-sheeted Antimicrobial Peptide Cateslytin

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The peptide cateslytin (RSMRLSFRARGYGFR) produced by enzymatic degradation of stress proteins is remarkably active against a large number of microorganisms including *Plasmodium Falciparum* responsible for Malaria. Its mode of action on membranes mimicking the external negatively charged membrane of these microorganisms has been studied by circular dichroism, infra-red (attenuated total reflection), high-resolution magic angle sample spinning and wide line solid state NMR, patch clamp and molecular dynamics. The peptide, which is unstructured in solution, adopts an aggregated beta sheet structure upon interacting with negatively charged membranes. Rigid membrane domains are formed upon interaction and separate from more fluid zwitterionic membrane zones. No macroscopic membrane lysis is observed, suggesting that crossing through membranes occurs at phase boundary defects. Patch clamp and all atom molecular dynamics of these zones indicate that disordered pores of ca. 10 Angstrom are formed by a mechanism that is analogous to molecular electroporation (Jean-François et al., *Biochemistry & Biophysical J.*, 2008).



2012-Plat

High-Resolution Structure of Piscidin in Aligned Lipid Bilayers: Implications for Antimicrobial Mode of Action

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Piscidin is an amphipathic cationic antimicrobial peptide that belongs to a large family of versatile host-defense peptides, which interact, at least initially, with cell membranes in order to perform their function. In the research presented here, we characterize the secondary structure and dynamics of piscidin in order to identify factors optimizing specific molecular interactions that are directly related to its function and mode of action. Our long term goal is to identify common principles that will facilitate the design of pharmaceuticals with broad-

spectrum antibacterial activity, minimum induction of bacterial resistance, and low toxicity for mammalian cells.

Previously, we demonstrated that membrane-bound piscidin 1 (p1) adopts an alpha-helical structure, which lies in the plane of the bilayer and experiences fast motions. Here, high-resolution solid-state NMR spectra have been obtained from multiply ¹⁵N-backbone-labeled p1 aligned in hydrated lipid bilayers. Analysis of data from twenty sites of this 22-mer reveals two helical segments separated by a kink at Gly₁₃. This kink may help one portion of the peptide insert more deeply in the hydrophobic lipid bilayer, which may be related to the mechanism of membrane disruption. To characterize water exposure, hydrogen-deuterium exchange experiments have been performed on ¹⁵N-backbone labeled samples. In addition, solid-state NMR was applied to ¹⁵N-side-chain-labeled His-17 p1 to titrate this side chain, which resides at the interface between the hydrophilic and hydrophobic domains of p1. Overall, our atomic-level investigation of the structure, dynamics, and water exposure of membrane-bound piscidin provides new insights into its mode of action and thus help understand how antimicrobial peptides recognize membranes and initiate their activities on microbial cells.

2013-Plat

Membrane Thinning Is Not A Unique Signal Of Pore Formation By Antimicrobial Peptides

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We have observed a hydrocarbon chain length dependent perturbation of saturated acyl chain phosphatidylglycerol bilayers by the antimicrobial peptide peptidyl-glycylleucine-carboxamide (PGLa) using X-ray diffraction, solid-state ²H-NMR, differential scanning calorimetry and dilatometry. In the gel phase, PGLa assumes a surface alignment and induces a quasi-interdigitated phase, previously reported also for other peptides. This effect is most pronounced for C18 phosphatidylglycerol. Above the lipid chain melting transition, in the fluid phase, the PGLa helix inserts into the membrane above a certain threshold concentration. In this case we found an increase of the membrane thickness and NMR order parameter for C14 and C16 phosphatidylglycerol bilayers, though not for C18. The data is best understood in terms of a close hydrophobic match between the C18 bilayer core and the peptide length when PGLa is inserted with its helical axis normal to the bilayer surface. The C16 acyl chains appear to stretch in order to accommodate PGLa, whereas tilting within the bilayer seems to be energetically favorable for the peptide when inserted into bilayers of C14 phosphatidylglycerol. In contrast to the commonly accepted membrane thinning effect of antimicrobial peptides, the data demonstrate that pore formation does not necessarily relate to changes in the overall bilayer structure.

2014-Plat

Basis For The Broad-spectrum Antimicrobial Activity Of Interfacially-active Peptides

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Recently, we rationally designed and screened a library of small, membrane pore-forming beta-strand peptides based on interfacial activity: "the ability of a molecule to partition into the membrane-water interface and to alter the packing and organization of lipids". From this library we selected ten soluble and highly potent pore-forming peptides using a function based high-throughput screen. Many natural antimicrobial peptides (AMP) act directly on microbes and permeabilize their membranes. Our library peptides were rationally designed to disrupt membranes which were similar to those of microbial membranes. Accordingly, *in vitro* experiments showed that the selected peptides permeabilize live microbial cell membranes and all ten have potent, broad-spectrum activity against many different species bacteria and fungi. These potent, biologically-active peptides apparently undergo nonspecific interactions with membranes. Here we explore the basis for broad-spectrum antimicrobial which is a very poorly understood phenomenon. We find that many members of the library have potent activity against some microbes, however broad-spectrum against multiple classes of microbes is rare overall. Similarly, subtle changes in peptide structure propensity cause the loss of activity against some microbes, but not others. Comparison of structure-function relation of these peptides in lipid vesicles to their activity in biological membrane suggests that "interfacial activity" is the basis for biological activity. A very specific and narrow range of interfacial activity gives rise to broad-spectrum antimicrobial activity.