loss of glycan interactions between the protein and cell-surface receptors. Our results identify residues important in membrane binding and give us a starting point to understand the nature of these interactions. Our work provides a template for investigating mechanisms by which PFTs target cell membranes with high-affinity.

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Physicochemical Membrane Properties Reveal a Structural Element Involved in the Adaptation of Actinoporins to Cholesterol-Rich Membranes

Koldo Morante[1,2], José Manuel Martínez Caaveiro[1], Koji Tanaka[3], Juan Manuel González-Mañas[2], Kouhei Tsumoto[1,4].
[1]Department of Bioengineering, Graduate School of Engineering, The University of Tokyo, Tokyo, Japan, [2]Department of Biochemistry and Molecular Biology, University of the Basque Country, Leioa, Spain, [3]Department of Chemistry and Biotechnology, School of Engineering, The University of Tokyo, Tokyo, Japan, [4]Medical Proteomics Laboratory, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

The plasma membrane houses a complex mixture of membrane proteins and lipids, and is also the specific target of amphitropic proteins such as pore-forming toxins. The activity of all of these proteins is exquisitely regulated by the physicochemical landscape of the lipid bilayer. Actinoporins, cytolysins produced by sea anemones, are toxins that form transmembrane pores in membranes showing lipid phase separation, especially those containing sphingomyelin. Fragaceatoxin C (FraC), an actinoporin from Actinia fragacea, is activated by lipid-phase separation, but a description of the molecular basis behind the adaptation of the protein to membranes with different physicochemical properties and lipid composition is still obscure. In this work, we show that FraC contains a key conserved residue (Phe16) involved in cholesterol sensing. Mutations on Phe16 generated protein species that were not active in cholesterol-rich membranes regardless of the nature of the residue used in the substitution. In contrast, the lytic activity of wild-type FraC and the muteins was essentially identical. A detailed analysis shows that the lytic activity of the Phe16-defective toxin measured in raft-like model membranes was inversely correlated with the concentration of cholesterol in the membrane. This behavior can be explained by the segregation of liquid-ordered domains (cholesterol-rich) at the expense of liquid-disordered domains (rich in unsaturated phosphatidylcholines). These results describe complementary mechanisms of membrane recognition that have evolved in response to different membrane environments.

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Tarantula Toxins use Common Surfaces for Interacting with Kv and ASIC Ion Channels

Maryam Zamanian[1], Chanhyung Bae[1], **Kanchan Gupta**[1], Mirela Milescu[2], Dmitriy Krepkiy[1], Drew Tilley[3], Jon Sack[3], Vladimir Yarov-Yarovoy[3], Jae II Kim[4], Kenton Swartz[1].
[1]Molecular Physiology and Biophysics Section, NINDS, NIH, NINDS, Bethesda, MD, USA, [2]Division of Biological Sciences, University of Missouri, 205 Lefevre Hall, Columbia, MO, USA, [3]Department of Physiology and Membrane Biology, University of California, Davis, CA, USA, [4]Department of Life Science, Gwangju Institute of Science and Technology, Gwangju, Korea, Republic of.

Tarantula toxins such as Hanatoxin and GxTx1E target voltage-activated potassium (Kv) channels by partitioning into the lipid membrane and binding to their voltage-sensing domains to modify gating. While aspects of this inhibitory mechanism have been well-studied, no structures of the toxin-channel complex have thus far been solved. Recently, an acid sensing ion channel (ASIC) was crystalized in complex with PcTx1, a structurally related tarantula toxin that modifies activation of the channel by protons. That X-ray structure shows that the PcTx1 binding site is localized within the extracellular domain well above the membrane, where the toxin clamps onto helix-5 and inserts an Arg-finger into the subunit interface. Here we compare membrane interactions and channel binding surfaces of GxTx1E and PcTx1 to understand the relationship between these two classes of tarantula toxins. Our results demonstrate that PcTx1 can interact with membranes, albeit more weakly and superficially compared to toxins targeting voltage sensors. Chimeras in which helix-5 of ASIC replaced the S3b helix of Kv2.1 retained voltage-dependent gating, but did not transfer sensitivity to PcTx1. Remarkably, alanine-scanning mutagenesis of GxTx1E reveals that residues involved in binding to Kv channels overlap extensively with those PcTx1 uses to bind to ASIC. We conclude that tarantula toxins have evolved to use related surfaces to bind to structurally unrelated ion channels even when the detailed molecular interactions and physical environments are distinct.

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Translocation of Cationic Amphipathic Peptides Across Phospholipid Bilayers

Paulo F. Almeida.
Chemistry, Univ North Carolina Wilmington, Wilmington, NC, USA.

We have developed a method to measure translocation of amphipathic peptides across lipid membranes that uses giant unilamellar vesicles (GUVs) containing inner vesicles. When the GUVs are added to a peptide solution containing a water-soluble fluorescent dye, permeabilization of the membrane of the outer vesicle by the peptide results in the appearance of fluorescence in its lumen. The inner vesicles remain dark if the peptide does not translocate. However, the appearance of dye in the inner vesicles indicates peptide translocation across the outer membrane of the GUVs. This is because to cause dye flux into the inner vesicles, the peptide must have crossed the membrane of the outer vesicle. The method does not require that the peptide itself be fluorescently labeled; but if it is, this approach can be used to measure the kinetics of translocation. Initially we tested the method with three peptides derived from each class of antimicrobial, cytolytic, and cell-penetrating peptides. Those three peptides also had very different Gibbs energies of insertion into the bilayer, approximated by the difference between the Gibbs energy of binding to the membrane and the Gibbs energy of transfer from water to octanol. The initial measurements appeared to indicate that the probability of translocation was inversely correlated with the Gibbs energy of insertion. Now we extended this study to several variants of the original peptides. Two hypotheses were tested: (1) The probability of translocation simply decreases as the Gibbs energy of peptide insertion increases; (2) The probability of translocation increases if the distribution of positively charged residues along the peptide sequence is such that the inserted peptide has similar probabilities of returning the outside and crossing the membrane, to the inside of the vesicle. The results of these new tests are discussed.

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Arginine-Glycosaminoglycan Interaction Regulates Penetration Efficiency of Arginine-Rich Cell-Penetrating Peptides in Biological Membrane

Yuki Takechi[1], Yuto Yanagisawa[1], Kazuchika Nishitsuji[2], Kenji Uchimura[3], Toru Kawakami[4], Kohsaku Kawakami[5], Keiichiro Okuhira[1], Hiroyuki Saito[1].
[1]Institute of Health Biosciences, Graduate School of Pharmaceutical Sciences, The university of Tokushima, Tokushima, Japan, [2]Department of Human Pathology, Institute of Health Biosciences, The university of Tokushima, Tokushima, Japan, [3]Department of Biochemistry, Nagoya University Graduate School of Medicine, Nagoya, Japan, [4]Institute for Protein Research, Osaka University, Suita, Japan, [5]Japan World Premier International (WPI) Research Center for Materials Nanoarchitectonics (MANA), National Institute for Materials Science (NIMS), Tsukuba, Japan.

Cell-penetrating peptides (CPPs) comprise a group of peptides that consist of less than about 40 amino acids and traverse the cell membrane. Most of CPPs are generally cationic because they are enriched in arginine and/or lysine residues. It has been thought that the first step in the biological membrane penetration of CPPs is driven by electrostatic interactions between positive charges in CPPs and negative charges of cell surface glycosaminoglycans (GAGs). In this study, we investigated the interaction of three types of cationic peptides, Rev (TRQARRNRRRRWRERQR), Kev (TKQAKKNKKKKWKEKQK) and R8 (RRRRRRRRR) with heparin which was used as a GAG model. Isothermal titration calorimetry demonstrated that the favorable enthalpy in binding of the cationic peptides to heparin mainly arises from non-coulombic interaction. The heparin binding enthalpies of Rev, R8 and Kev were −14 kcal/mole, −10 kcal/mole and −0.5 kcal/mole respectively. Thus, it was indicated that binding of Rev and R8 to heparin was much more exothermic compared to that of Kev to heparin, and that GAGs have higher affinity to arginine-rich peptide than to lysine-rich peptide. ^{1}H NMR spectroscopy showed that proton signals of the Trp residue in Rev, not Kev, disappeared upon binding to heparin, demonstrating that Rev specifically interacts with heparin. The heparin binding induced α-helix structure in Rev whereas not in R8 and Kev. In addition, we demonstrated that Rev penetrates into Chinese hamster ovary cells using a membrane penetration assay of fluorescein labeled-CPPs, while subtle or no penetration was observed in the case of R8 and Kev. These results suggest that the penetration of CPPs across the cellular membrane is regulated by specific interaction of arginine residues in the CPP with GAGs and subsequent α-helix formation of the peptides upon binding to the biological membrane surface.