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 explore the S3b helix of Kev-1 retained voltage-dependent gating. Our work provides a template for investigating mechanisms by which PFTs target cell membranes with high-affinity.

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Physiochemical Membrane Properties Reveal a Structural Element Involved in the Adaptation of Actinoporins to Cholesterol-Rich Membranes
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The plasma membrane houses a complex mixture of membrane proteins and lipids, and is also the specific target of amphiprotic proteins such as pore-forming toxins. The activity of all of these proteins is exclusively 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 fragmenta, 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 mutants 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
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In this study, we investigated the interaction of three types of cationic peptides, QARR (Rev), QERRR (R8), and QAKK (Kev) with heparin which was used as a GAG model. Isothermal titration calorimetry demonstrated that the favorable enthalpy in binding of the Arginine-Glycosaminoglycan Interaction Regulates Penetration Efficiency of Arginine-Rich Cell-Penetrating Peptides in Biological Membrane Yuki Takechi1,2, Yu Koyama3,4, Kazushika Nishitsuji1, Kenji Uchimura1, Toru Kawakami5, Kohsaku Kawakami6, Keiichiro Okuhira1, Hiroyuki Saito7.
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Cell-penetrating peptides (CPPs) comprise a group of peptides that consist of cationic 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 (QARRRRRRRRRRRRRR), R8 (QERRRRRRRRRRRRR), and Kev (QAKKRRRRRRRRRRRR), 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 6 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. In 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 z-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 z-helix formation of the peptides upon binding to the biological membrane surface.