negative lipid in the membrane can bind to a positive arginine or lysine group on MBP.

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H/D Exchange Provides Insights Into The Orientation Of Bacillus Thuringiensis Pi-phospholipase C Binding To Mixed Component Vesicles

Xiaomeng Shi[1], Mary F. Roberts[1], Robert Meklemburg[2], Patrick L. Wintrode[2].
[1]Boston College, Chestnut Hill, MA, USA, [2]Case Western Reserve University, Cleveland, OH, USA.

Determining the orientation and conformation of peripheral membrane proteins when they are docked to target membranes is difficult. In many cases, flexible regions of the proteins provide the major contacts with the membranes. *B. thuringiensis* PI-PLC has two discrete binding sites for phospholipids - the active site for binding PI (or substrate competitors such as PMe or PG), and an activator site that is specific for phosphatidylcholine molecules. We have examined H/D exchange of the protein by Mass Spectrometry for the protein binding to 1:1 and 1:9 PG/PC small unilamellar vesicles (SUVs), conditions chosen for tight binding of the protein. In the absence of SUVs most of the protein amide groups are easily exchanged with D_2O. However, with SUVs are present, the regions helix B and helix C show a reduced exchange rate consistent with protection by the membrane. This is consistent with how this protein is thought to bind to membranes based on mutagenesis studies. Other interesting observations are that the beta strand E exchanges more slowly after binding to membrane while helix E exhibits faster exchange. To further explore how this enzyme is interacting with lipids, the H/D exchange rates of a constructed covalent dimer (disulfide-linked W242C), which is thought to mimic the membrane-induced dimer structure of w.t. PI-PLC in solution, are also examined with or without lipids present. Further study of a mutant protein, W47A/W242A, with very low affinity for vesicles and which is thought to exist as a dimer in solution, also provides some insights into self association of PI-PLC monomers. Clearly, for peripheral membrane protein dynamics, protein/protein, and protein/membrane interactions, H/D exchange experiments coupled with MS can provide information on structural changes not accessible by other structural methods.

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Molecular Dynamics Simulation of the ENTH Domain on Lipid Bilayer

Chun-Liang Lai, Gary S. Ayton, Gregory A. Voth.
Department of Chemistry and Center for Biophysical Modeling and Simulation, University of Utah, Salt Lake City, UT, USA.

Clathrin-mediated endocytosis is necessary for a number of cellular phenomena, and the adaptor protein, epsin, has been indicated to play a role during the pathways of these processes. The amino terminal region of epsin is characterized by a highly evolutionary conserved region known as the ENTH (epsin NH_2-terminal homology) domain, which is critical for membrane deformation, although the exact mechanism of this process is still elusive. In order to unravel the behavior of this domain when it interacts with lipid bilayer, atomistic molecular dynamics simulations have been performed for both the wild type and mutants on lipid bilayer. The stability of these ENTH domains and their possible role in membrane deformation are reported.

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FCS of Mutated Phosphatidylinositol-specific Phospholipase C Enzymes Monitors the Interplay of Substrate and Activator Lipid Binding

Mingming Pu[1], Mary F. Roberts[1], Anne Gershenson[2].
[1]Boston College, Chestnut Hill, MA, USA, [2]Brandeis University, Waltham, MA, USA.

Phosphatidylinositol-specific phospholipase C (PI-PLC) enzymes are activated on the surface of vesicles by nonsubstrate phospholipids with the extent of activation tuned by the lipid composition. *Bacillus thuringiensis* PI-PLC can be activated by a small amount of phosphatidylcholine (PC) towards its substrate PI. Fluorescence correlation spectroscopy (FCS) has been used to study fluorescently labeled PI-PLC and a series of mutants (P42G, K44A, K44E, Y88A, and Y246/247/248S) binding to small vesicles of a substrate analogue (phosphatidylglycerol) and PC as a function of the vesicle composition. For PI-PLC and most of the mutant proteins there is a synergistic effect of the two types of phospholipids in anchoring the enzyme to a vesicle. If a mutation alters the affinity of substrate at the active site, binding should vary with X_{PC} and be enhanced in PC-rich region; on the other hand, if a mutation alters the affinity of activator, K_d will increase with increased X_{PC}. In this way, careful determination of the apparent K_d helps to sort out effects of enzyme mutations on activator and substrate sites. FCS is particularly useful in exploring the contribution of K_d to PI cleavage by mutant PI-PLC enzymes. For a fixed X_{PC}, if the bulk concentration of vesicles is above K_d, enzymatic activities should be similar, as long as vesicle binding is the critical step. However, it should drop dramatically when the bulk concentration is below K_d measured by FCS (a long as the substrate analogue is a good mimic for substrate). The results provide a direct analysis of vesicle binding and catalytic activity and shed light into how occupation of the activator site enhances activity. Currently, we are extending the analysis of this system with tethered small unilamellar vesicles and single-molecule methods.

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X-ray reflectivity Structural Study of PKCα-C2 Domain Binding to SOPC/SOPS Lipid Monolayers

Chiu-Hao Chen[1], Sarka Malkova[1], Fei Long[2], Wonhwa Cho[2], Mark L. Schlossman[1].
[1]Department of Physics,University of Illinois at Chicago, Chicago, IL, USA, [2]Department of Chemistry, University of Illinois at Chicago, Chicago, IL, USA.

An understanding of signal transduction mechanisms is vital to investigate the causes of diseases. The C2 domain is a conserved protein signaling motif and membrane-targeting domain widely found in signaling proteins. In this work, we study the interaction of the C2 domain of protein kinase Cα (PKCα) with a lipid monolayer of a mixture of SOPC (1-stearoyl-2-oleyl-sn-glycero-3-phosphocholine) and SOPS (1-stearoyl-2-oleoyl-sn-glycero-3-phosphoserine). Recent results from crystallography and EPR studies indicate that PKCα-C2 is likely to orient parallel to the membrane. In this work, we use x-ray reflectivity to directly determine that the PKCα-C2 domain is perpendicular to the membrane. Our new analysis method allows us to test all orientations and demonstrates that our data is inconsistent with the parallel orientation. To carry out this experiment, the PKCα-C2 was injected into the subphase under an SOPC/SOPS (7:3) mixture supported on a buffered aqueous solution. X-ray reflectivity was used to determine the orientation and penetration depth of PKCα-C2 bound to the SOPC/SOPS monolayer. The reflectivity is analyzed in terms of the known crystallographic structure of PKCα-C2 and a slab model that represents the lipid layer, yielding an electron density profile of the lipid layer and bound C2 domain. The orientation of PC/PS-bound PKCα-C2 is described by two angles, $\theta = 35^0$ and $\varphi = 210^0$, and the domain penetrates 7.6 Å into the lipid layer. The structure that we determined is consistent with many observations from mutational studies. The perpendicular model further suggests how PKCα-C2 interacts with other lipid components such as phosphatidylinositol, other domains within PKC such as the C1 domain, and the receptor for activated C-kinase.

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Influence of Lipid Modifications of NRas on the Interaction with different Model Biomembranes and their Orientation at the Lipid Interface

Andrea Gohlke[1], Gemma Triola[2], Herbert Waldmann[2], Annette Meister[3], Alfred Blume[3], Roland Winter[1].
[1]TU Dortmund University, Dortmund, Germany, [2]Max-Planck-Institute of Molecular Physiology, Dortmund, Germany, [3]Martin-Luther-University Halle-Wittenberg, Halle/ Saale, Germany.

The membrane associated protein NRas is a member of the Ras-superfamily of GTPases and one of the main regulators of the MAP signal cascade, being responsible, among others, for cell growth and differentiation. Its capability of binding to the membrane is enabled by posttranslational modification with farnesyl and palmitoyl residues. To determine the influence of the nature of these modifications and to investigate the difference between active (GTP) and inactive (GDP) NRas, five different NRas constructs with different lipid anchors and nucleotides (Far/Far (GDP), Hd/Far (GDP), Hd/Hd (GDP), Stbut/Far (GDP) and Hd/Far (GPPNHP)) have been synthesized. By using surface plasmon resonance spectroscopy, we were able to follow the insertion and dissociation process of the lipidated proteins into and out of the membrane. We show that the binding kinetics of the different NRas proteins are markedly influenced by the lipid composition of the membrane, by the nature of the lipid anchors as well as by the nucleotide loading of the protein. Furthermore, we studied the correlation of the insertion process with the orientation of the protein at the lipid interface, using infrared reflection absorption spectroscopy. The results show that the properties of the lipid anchors have a major influence not only on the insertion process, but also on the orientation of the protein at the lipid interface.