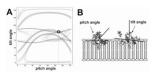
The N-terminal domain of huntingtin (Htt17), located immediately upstream of the decisive polyglutamine tract, strongly influences important properties of this large protein and thereby the development of Huntington's disease. Htt17 markedly increases polyglutamine aggregation rates and huntingtin's interactions with biological membranes. Here, an ensemble of low-energy conformations of the protein domain was identified by solution NMR in interfacial environments, and the structure was further refined using solid-state NMR spectroscopy on oriented phospholipid bilayers. The pronounced structural transitions of Htt17 upon membrane-association result in an inplane aligned α -helical conformation from K6 to F17. The membrane binding of Htt17 and the resulting permeability were quantitatively analyzed and are strongly dependent on lipid composition, whereas the helical tilt angle (~77 degrees) is nearly constant in all membranes investigated. The structure and lipid interactions of Htt17 have pivotal implications for membrane-

anchoring and functional properties of huntingtin and concomitantly the development **A** of the disease.

The Figure shows the solid-state NMR orientational restraints from three 15N and one 2H labelled sites (A) and the resulting alignment of the solution NMR structure in the lipid bilayer (B).



1859-Plat

Investigating the Mechanism by which Bcl-xL Regulates Ceramide Channels

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The level of ceramide, a sphingolipid, increases in mitochondria early in apoptosis resulting in the formation of ceramide channels. These channels are involved in the release of intermembrane space proteins, such as cytochrome c, into the cytosol. This release is a crucial and irreversible step in the apoptotic process. Formation of ceramide channels is inhibited by Bcl-xL, an antiapoptotic protein. Insights into the molecular basis for this regulation were obtained in a study of ceramide analogs (Perera, M. N. et al., Biochem. J. 445, 81, 2012). The results indicated that the effectiveness of Bcl-xL is very sensitive to changes in the hydrophobic regions of the ceramide channel. Furthermore, inhibitors (ABT-737, ABT-263 and antimycin A) that specifically bind to the hydrophobic groove of Bcl-xL interfere with this function of Bcl-xL. These results imply that the hydrophobic groove is important for Bcl-xL to inhibit channel formation or dissemble the channel. We have demonstrated direct binding of a ceramide molecule to Bcl-xL by a fluorescent ceramide competition technique. In addition, site-directed mutagenesis at a single residue in multiple locations in the hydrophobic groove has resulted in a reduction of the inhibitory action of Bcl-xL. These results support the conclusion that Bcl-xL regulates the ceramide channel through the hydrophobic pocket. This same feature is critical to the regulation of proapoptotic Bcl-2 family proteins, thus it inhibits comprehensively all the pro-apoptotic processes of the cell. (supported by NSF grant MCB-1023008)

1860-Plat

Elucidating the Molecular Details of Phosphatidylserine Membrane Recognition in Immune Response

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The immune system recognizes a vast array of chemical signatures as antigens although historically most research has focused more exclusively on protein/protein recognition. More recently it has been appreciated that lipid membranes can also provide important immunological signals as demonstrated in both phosphatidylserine (PS) recognition in apoptotic cell clearance and transient PS exposure in T Cell activation. Despite the clear immunological importance of PS exposure and recognition, there remain very few molecular details regarding the mechanisms of PS membrane recognition. Even more fundamentally, it remains unclear if all PS exposing membranes are immunologically equal or if there exists a sensitivity to additional membrane properties beyond simply the presence or absence of PS. To address this gap in our understanding we have made use of a novel combination of biophysical and biochemical techniques to elucidate the molecular mechanisms by which Tim4 (an immune related PS receptor) recognizes PS containing membranes. Tryptophan fluorescence binding assays have revealed that Tim4 binding is sensitive to membrane PS composition suggesting that there is more to the story than a single PS to single protein interaction. By utilizing a combination of x ray reflectivity measurements to determine membrane bound protein orientation and depth of penetration, as well as molecular dynamics simulations to support the experimental results, we have developed a protein/membrane binding model that provides structural evidence to explain the unique complexities of Tim4 mediated PS membrane recognition. Most significantly, these results provide a standard against which other immunologically related PS receptors can be compared, thereby allowing us to begin to address the more fundamental question of just how important lipid membrane recognition is for our bodies' immunological defense mechanisms.

1861-Plat

Investigating the Molecular Basis of cPLA2α Membrane Bending Katherine E. Ward¹, James P. Ropa¹, Emmanuel Adu-Gyamfi¹, Robert V. Stahelin^{1,2}.

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Signal transduction mediates disease through key molecular targets that initiate signaling networks. As protein-lipid interactions have been examined in the literature, their role in cellular signaling has become more prevalent as lipid-binding proteins have become high impact drug targets in cancer, inflammation and viral egress. One such target, termed cytosolic phospholipase $A_2 \alpha$ (cPLA₂ α), has been shown to play a key role in the production of the inflammatory mediators prostaglandins and leukotrienes. A novel function of the protein was recently discovered in our lab showing $cPLA_2\alpha$ bends zwitterionic membranes using model membranes, a process that is mediated by cPLA₂a's ability to deeply penetrate membranes. Others in the field have reported $cPLA_2\alpha$ to participate in Fc mediated phagocytosis, intra-Golgi trafficking and endosomal trafficking which further supports $cPLA_2\alpha$'s ability to bend membranes in biological processes. In addition, direct evidence has been reported using siRNA showing that $cPLA_2\alpha$ induced vesiculation in cells. These results translate into our cellular system as cells transfected with eGFP-cPLA2a form cytoplasmic vesicular structures. We have preliminary evidence showing cPLA2a membrane bending is mediated by oligomerization. The origin of oligomerization is currently under further investigation using both in vitro and cellular techniques.

1862-Plat

Probing for π -Cation Interactions in the Binding of B. Thuringiensis Phosphatidylinositol-Specific Phospholipase C Phosphatidylcholine-Rich Vesicles

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Bacillus thuringiensis phosphatidylinositol specific phospholipase C (PI-PLC) binds tightly to phosphatidylcholine (PC)-rich vesicles. A possible mechanism for tight binding to PC interfaces involves tyrosine π / choline cation complexes. With this in mind, we have mutated surface tyrosine residues (Y86A, Y88A, Y204S, Y246A, Y247A, Y248A, Y251A), located on the barrel rim and in two helices of this $(\alpha\beta)$ -barrel protein, to assess their contribution to vesicle binding. None of these mutations significantly alter the rate of PI cleavage in vesicles, as long as the PI concentration is > 4 mM. However, binding to PC-containing vesicles, as measured by fluorescence correlation spectroscopy, showed a loss of affinity. The loss-of-Tyr mutant proteins fall into two classes: (i) those where Kd(mut)/Kd(WT) < 5 (Y86A, Y247A) and (ii) those where the ratio of mutant Kd to that of the WT was 100-300 (Y88A, Y204S, Y246A, Y248A, Y251A). With the exception of Y204S/Y251A the effects of the mutations appear to be additive, We also attempted to enhance interactions with PC by introducing new Tyr or Trp residues on the surface, but these mutations either reduced membrane affinity or left it unchanged. Apparently, more specific interactions are needed to enhance binding. Estimating $\Delta\Delta G$ for these Tyr/PC interactions from the apparent Kd values, we find that the free energy associated with Tyr86 and Tyr247 is ~ 4 kJ/mol, comparable to the value predicted by the Wimley-White scale. In contrast, removal of the other surface Tyr is linked to a higher energy cost: 10-13 kJ/mol towards pure PC vesicles. These higher energies