phosphatidylethanolamine are further evidenced by the Langmuir monolayer technique.

From our results, we suggest that the protonation state of His residues could have a role in the pathogenic surface interaction of the whole $A\beta$ peptide with membranes.

2511-Pos

Regulation of Apoptosis at the Mitochondrial Level by Bcl-2 Proteins Marcus Wallgren¹, Anders Pedersen², Marc-Antoine Sani¹,

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During the life-time of a multicellular organism the high turnover of the cell mass has to be highly regulated to prevent unhealthy conditions. Redundant, damaged or infected cells are eliminated by apoptosis, which is one of the main types of programmed cell death. The anti-apoptotic protein Bcl-2 belongs to the Bcl-2 protein family, which functions as a major gatekeeper in the mitochondrial apoptotic pathway. Bcl-2 is found to a great extent in many breast cancers and is highly involved in the inherent resistance to anti-cancer drugs. This protein is mitochondrial membrane-associated and we will use different spectroscopic methods, mainly NMR spectroscopy, to provide structural information of the membrane-mediated mechanism underlying the action of Bcl-2 as a potent inhibitor of cell death. For this purpose we are aiming to work with the full-length Bcl-2 and study its interplay with membrane and another Bcl-2 protein, Bax. The pro-apoptotic Bax is the counterplayer of Bcl-2 and is upon activation translocated to the mitochondrial membrane where it forms oligomers, leading to pore formation, release of cytochrome c and cell death So far we have been working on the expression and purification of Bax and Bcl-2, and managed to obtain purified Bax using E. coli as expression system. For Bcl-2 we are working on a promising in vitro based protein expression strategy. We have made an initial characterization of the protein-membrane interaction for Bax using CD spectroscopy and we are now continuing with solid state 31P NMR experiments. By monitoring the change in phosphorous groups in model membranes in the presence of either inactivated or activated Bax, a better understanding of the role of membrane upon apoptotic induction can be gained.

2512-Pos

Mechanisms of Phosphatidylserine (PS) Recognition in Apoptotic Cell Clearance

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Studies have shown(1) that apoptotic cells (cells programmed for self destruction) undergo a loss of plasma membrane polarity causing PS lipids, which are normally confined to the cytosolic leaflet, to be exposed in the extracellular leaflet. Macrophage cells, a key component of the innate immune system, possess receptors which can detect the surface exposed PS and subsequently engulf and breakdown the apoptotic cells to prevent inflammation associated with cell death. Three unique PS receptors have recently been indentified and while their ability to recognize PS in a physiologically relevant manner has been confirmed, the exact nature of these receptors' interaction with PS containing membranes remains largely uncharacterized. Using recombinant expression in E. coli to produce soluble versions of all three receptors, we have investigated the interaction of these individual receptors with lipid monolayers of various compositions on a Langmuir trough setup. Specifically, we have employed fluorescent microscopy to monitor both the specific localization of the membrane bound protein, as well as morphological changes to the monolayer that occur as a result of protein/lipid interactions. In addition, constant pressure assays were also used to determine the degree of protein insertion into the monolayers. Considered collectively, these approaches allowed us to probe both the nature and specificity of these interactions by varying the subphase protein concentration, the percentage of PS in the membrane, and the anionic component of the membrane (substitution of Phosphatidylglycerol for PS). The results of these experiments allow us to begin to understand the molecular nature of these various interactions and subsequently shed light on how three distinct proteins, with three diverse recognition domains can all illicit a similar cellular response.

1. Reutelingsperger et al. J. Exp. Med. (1995) 182:1545-1556.

2513-Pos

Kinetic and Thermodynamic Studies of pH-Triggered Membrane Insertion of Diphtheria Toxin T-Domain

Alexander Kyrychenko, Mykola V. Rodnin, Yevgen O. Posokhov, Anna Thoma, Joshua Brettmann, **Alexey S. Ladokhin**. KUMC, Kansas City, KS, USA. The pH-triggered membrane insertion pathway of the T-domain of diphtheria toxin was studied using site-selective fluorescence labeling with subsequent application of several spectroscopic techniques (e.g., fluorescence correlation spectroscopy, FRET, lifetime quenching and kinetic fluorescence). FCS measurements indicate that pH-dependent formation of the membrane-competent form depends only slightly on the amount of anionic lipids in the membrane. The subsequent transbilayer insertion, however, is strongly favored by anionic lipids. Kinetic FRET measurements between donor-labeled T-domain and acceptor-labeled lipid vesicles demonstrate rapid membrane association at all pH values for which binding occurs. In contrast, the transmembrane insertion kinetics is significantly slower, and is also both pH- and lipid-dependent. Analysis of kinetic behavior of binding and insertion indicates the presence of several interfacial intermediates on the insertion pathway of the T-domain, from soluble W-state to transmembrane T-state. Intermediate interfacial I-state can be trapped in membranes with low content of anionic lipids (10%). In membranes of greater anionic lipid content, another pH-dependent transition results in the formation of the insertion-competent state and subsequent transmembrane insertion. Comparison of the results of various kinetic and equilibrium experiments suggests that the pH-dependences determining membrane association and transbilayer insertion transitions are different, but staggered. Anionic lipids not only assist in formation of the insertion competent form, but also lower the kinetic barrier for the final insertion. Supported by NIH GM069783(-04S1).

2514-Pos

Association of the Matrix Protein from Respiratory Syncytial Virus with Lipid Monolayers and Bilayers

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Enveloped viruses bear an outer envelope that is rich in sphingomyelin, cholesterol, phosphocholine lipids and phosphoethanolamine lipids. A number of proteins are intimately associated with the viral envelope, including matrix proteins, which are essential elements in controlling virion morphology and play key roles during budding of progeny virions from the plasma membrane. The association of the matrix protein from respiratory syncytical virus with membranes has been characterised by tensiometry, Brewster angle microscopy, and atomic force microscopy following deposition of Langmuir monolayers onto modified silicon-oxide substrates. Association of the protein with monolayers containing phosphocholines and cholesterol leads to the formation of materials with new properties that differ from those of either of the pure components. At all surface pressures, including the monolayer-bilayer equivalence pressure, the protein penetrates monolayers of phosphocholine/cholesterol or phosphocholine/phosphoethanolamine, with insertion between lipid molecules. In contrast, the behaviour of the protein in monolayers rich in cholesterol and sphingomyelin exhibits a different behaviour, with a simple partitioning behaviour (i.e. monolayer penetration) at low concentrations replaced by peripheral association at higher concentrations and pressures. Taken together, the evidence points to specific interactions between the protein and sphingomyelin. These findings are discussed in relation to the recently published structure of the protein (PNAS, 2009, 106, 4441-4446), the documented formation of viral filaments during key stages of the infection cycle and the isolation of the protein from detergent-resistant membrane fractions.

2515-Pos

A Role for Lipid-Protein Interaction in Intergral Membrane Protein Function

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The fukutins are a family of eukaryotic membrane proteins whose mis-localisation within the cell has been shown to be associated with the onset of Fukuyama muscular dystrophy. The interaction between the N-terminal transmembrane domain fukutin with the atypical lipid bilayer of the Golgi apparatus has been postulated to play a crucial role in the retention of the protein there.

To investigate the nature of these interactions we have developed a bacterial expression system for isolating the N-terminal transmembrane domain of Fukutin-I. We have characterised this hydrophobic peptide using molecular biology techniques, mass spectrometry, circular dichroism and nuclear magnetic resonance studies.

Currently, we are using solid state NMR and fluorescence studies to analyse how the oligomeric state, lateral segregation and structure of fukutin reconstituted into artificial bilayers varies as a function of varying lipid compositions. These studies will provide further insight into the role of lipid-protein interactions in membrane protein structure and assembly, segregation into lipid rafts and the role these properties may play in protein trafficking.