Alzheimer's Association, NIGMS, and the NIA for their financial support of this work.

2506-Pos

The Interaction with β-Amyloid Impairs the Mechanical Stability of Polymer Cushioned Membrane

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The mechanism of neurodegeneration caused by β-amyloid (Aβ) in Alzheimer's disease is still controversial. Neuronal toxicity is exerted mostly by various species of soluble Aβ oligomers. Recent data depict membranes as the main sites where proteins/peptides are recruited and concentrated, misfold, and nucleate amyloids; at the same time, membranes are considered key triggers of amyloid toxicity.

We demonstrated the capability of Aβ to penetrate and destabilize stacked lipid bilayers in a previous work. In this study, in order to maintain the natural fluidity of the membrane, polymer cushioned lipid bilayers have been used as a model for neuronal membrane. Layer-by-layer technique was used for the fabrication of the polymer cushion of charged poly-electrolytes, the lipid membrane is built on the polymer film by unilamellar vesicle fusion. Neutron reflectivity was used to monitor the kinetics of adsorption of the lipid bilayer onto the polymer surface; the conditions for the best surface coverage have been determined. The structure of the lipid bilayers is modified by the interaction with Aβ1-42; Neutron reflectivity showed a change of the scattering density profile in the direction perpendicular to the membrane plane, suggesting penetration of Aβ in the double layer. Atomic force microscope (AFM) has been used to test the lipid packing of the membrane through film rupture experiments and to compare the bilayer morphology in the presence or in the absence of Aβ. We demonstrated that the presence of Aβ weakens the lipid packing in the model membranes.

We compared the results obtained on polymer cushioned lipid bilayers with those obtained using a rigid substrate (freshly cleaved mica) for membrane preparation.

2507-Pos

The Effect of Mutant Aβ Peptide Aggregation on the Stability of Model Lipid Bilayers

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A hallmark of Alzheimer's disease (AD) is the rearrangement of the β-amyloid (Aβ) peptide to a non-native conformation that promotes the formation of toxic, nanoscale aggregates. One of many potential pathways for Aβ toxicity may be modulation of lipid membrane function on cellular surfaces. There are five mutations clustered around the central hydrophobic core of Aβ near the α-secretase cleavage site (the A21G Flemish mutation, E22K Italian mutation, E22G Arctic mutation, E22Q Dutch mutation and the D23N Iowa mutation). These point mutations are associated with hereditary diseases ranging from almost pure cerebral amyloid angiopathy (CAA) to typical Alzheimer's disease pathology with plaques and tangles. We hypothesizethat these point mutations alter the Aβ aggregation pathway and its interaction with cellular lipid membranes, resulting in altered disease progression and phenotypes. Brain lipid extract was used to form bilayers that are physiologically relevant models of neuronal cell surface. Intact lipid bilayers are exposed to different mutant forms of Aβ, and Atomic Force Microscopy was used to follow the aggregation of Aβ and membrane integrity over a 24 hour period. The goal of this study was to determine how point mutations in Aβ alter electrostatic interactions between the Aβ and the lipid surface. These interactions may affect aggregation, morphological characteristics, and bilayer disruption of Aβ on the model lipid membranes which may play a role in Aβ-related toxicity.

2508-Pos

A Molecular Dynamics Study of Amyloid-β (1-42) Peptide Dimer Formation on the Surface of Phospholipid Bilayers

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The Amyloid-β (Aβ) peptide is an integral aggregate species in the progression and neurotoxicity of Alzheimer's disease. While Aβ fibrils were historically considered the toxic species in Alzheimer's disease, recent evidence has shifted the focus towards oligomers as the most dangerous aggregate structure for neurons. In this aggregation process, the conversion of monomeric Aβ into a dimer constitutes the first step in oligomer formation. Further work has shown that cell membranes may play a substantial role in promoting aggregation through facilitating the protein-protein interactions that drive aggregation. We have used extensive replica exchange molecular dynamics simulations to demonstrate that monomeric Aβ does not adopt stable secondary structure over timescales that have allowed for significant structure formation in solution. Further, to characterize dimer formation on the surface of a model lipid bilayer, we have used a thermodynamic cycle to indirectly calculate free energies of dimerization on the bilayer surface. Use of a thermodynamic cycle helps to decrease bias due to initial conditions that would occur through directly calculating a dimerization free energy. We have calculated the free energies of dimerization for a preformed dimer containing a single antiparallel β-sheet or a pair of β-hairpin monomers. While these structures are representative of predicted fibril structures, comparison of dimerization free energies provides insight into the effect of the bilayer on the dimerization process. We have found that the bilayer does affect dimerization free energy depending on the dimer structure and bilayer surface charge. Our work demonstrates that a lipid bilayer is able to substantially hinder Aβ monomer structure formation and influence Aβ dimer formation.

2509-Pos

Protective Role of 17-β-Estradiol in LDL Amyloidogenesis

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In early atherogenesis, subendothelial retention of lipidic droplets is associated with an inflammatory response-to-injury, culminating in the formation of foam cells and plaque. Low density lipoprotein (LDL) is the main constituent of subendothelial lipidic droplets. LDL can be sketched as an inner lipidic core surrounded by a phospholipid monolayer, with the protein (apoB-100) wrapped around the particles' surface and partly seeping into the phospholipid monolayer and the inner cholesterol core.

We found that in a naturally occurring subpopulation of LDL (electronegative LDL-), the apoB-100 is misfolded and is capable of triggering the formation of aggregated, amyloid-like LDL structures. LDL- can be produced in human plasma by secretory phospholipases A2.

Both protein misfolding and LDL amyloids can well represent modifications able to transform this cholesterol carrier into a trigger for a response-to-injury in the artery wall.

Furthermore, by using Small Angle X-ray Scattering we furnish further evidences that the hormone 17-β-estradiol (E2) binds to a single highly specific site in apoB-100 and stabilizes its structure, even if the formation of LDL- is not altered by E2 binding. This results in an increased ellipticity of apoB-100, an overall volume shrinkage with modifications both in the outer shell and lipidic core, and an increased resistance to structural and conformational loss. Notably, also the formation of LDL amyloid aggregates is hindered by E2. Our findings converge to a picture where a possible explanation of the beneficial effect of E2 in the protection against the vascular response-to-injury can find its mechanism.

In addition, our results add arguments to the stringent lipid-protein structural interplay in LDL, with modifications in lipids being paralleled with apoB-100 structural and functional modifications, and vice versa.

2510-Pos

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Increasing evidence implicates interactions between Abeta peptide and lipid in the development of Alzheimer's disease. More generally, Abeta peptide interactions with membranes seem to depend on the composition of the lipid bilayer and the structural features of the peptide. One key parameter should be pH since one site of intracellular Abeta peptide production and/or accumulation is likely to be endosomes. This intracellular endosomal accumulation was suggested to contribute to disease progression.

In this workr, we report a study on the 11-22 amphiphilic domain of Abeta in interaction with model membrane; this region contains most of the charged residues of the N-terminal domain of Abeta. We show that the peptide charge, and more precisely the protonation state of histidines 13 and/or 14 is important for the interaction with lipids. Hence, it is only at endosomal pH that a conformational change of the peptide is observed in the presence of negatively charged lipid vesicles, i.e. when both lipid headgroups and histidines can interact through electrostatic interactions. Specific interactions of the fragment with phosphatidylserine and to a lesser extent with phosphatidylcholine, but not

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 Aβ peptide with membranes.

2511-Pos
Regulation of Apoptosis at the Mitochondrial Level by Bcl-2 Proteins
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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
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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.