Interfacial Protein-Lipid Interactions II

2501-Pos

Lipid Targeting of Synaptotagmin I C2 Domains on Asymmetric Two-Phase Planar Bilayers

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We showed previously that cholesterol-rich liquid-ordered domains with lipid compositions typically found in the outer leaflet of plasma membranes could induce liquid-ordered domains in adjacent regions of asymmetric lipid bilayers with apposed leaflets composed of typical inner leaflet lipid mixtures (Biophys.J. 91:3313-26 [2006]). The lipid requirements for this transbilayer coupling in asymmetric cholesterol-rich two-phase lipid bilayers were further investigated and found to be roughly correlated with their chain-melting phase transition temperatures (Biochemistry 47: 2190-8 [2008]). Bilayers containing brain PC (bPC), brain sphingomyelin (bSM) and cholesterol in the outer leaflet and bPC, bPE, POPS and Chol in the inner leaflet, form stable asymmetric two-phase bilayers that are thought to mimic mammalian plasma membranes. In the current work we have studied the calcium-dependent binding and lipid targeting of C2 domains of the presynaptic fusion calcium sensor protein synaptotagmin on asymmetric two-phase bilayers. C2A domains favor disordered over ordered inner leaflet lipid domains. Domain preference does not depend on the PS content in these membranes, but domain preference is more distinct when bPE is left out from the inner leaflet lipid mixture. C2AB tandem domains distinguish less between ordered and disordered lipid domains. Targeting to bilayers containing various amounts of PIP2 in the inner leaflet is also being investigated.

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2502-Pos

Synaptotagmin Perturbs Lipid Structure of Membrane Bilayers

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The perturbation of lipid acyl chain order by fusion proteins is widely reported in the membrane of viral entry and fertilization process. Synaptotagmin is the Ca2+ trigger for membrane fusion in neuronal exocytosis, and it may act by modulating lipid packing or membrane curvature strain. The effects of soluble synaptotagmin (C2AB) and the individual C2 domains (C2A and C2B) on the lipid order of POPC:POPS (3:1) membrane bilayer were examined with attenuated total reflection Fourier transformed infrared spectroscopy (ATR-FTIR). Our results show that C2AB and C2B decrease lipid order while C2A increases the lipid order. However, at higher protein concentrations a threshold is reached where, the effect on order is reduced or even reversed. The presence of 1% PIP2 in the lipid bilayer lowers these threshold concentrations. This change in lipid order is largely due to POPS and suggests that the effect on lipid order is due to the demixing of POPS. Interestingly, this effect is not seen in another negatively changed lipid POPG. The effect of synaptotagmin on demixing in PC:PS membrane bilayers was further investigated by fluorescence quenching. Our results show that the C2AB and C2B demix the PS in the presence of Ca2+, while C2A has little effect. Natural abundance 13-C HSQC NMR experiment on POPC:POPS (3:1) vesicles shows that the binding of synaptotagmin changes the chemical shift of PS in the presence of Ca2+, indicating an interaction between the protein and lipid. Taken together, these data suggest that synaptotagmin induces a PS-specific modulation of the acyl chain lipid order as a result of PS-demixing, which may play a role in the mechanism of Ca2+-mediated fusion in the central nervous system.

2503-Pos

Ratiometric Fluorescent ESIPT Probe Characterizes Binding of Alpha-Synuclein to Membranes

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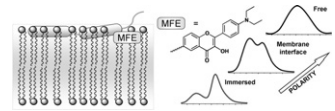
The aggregation of the protein α-synuclein (AS) is involved in the pathogenesis of Parkinson's disease. Evidence suggests that neurotoxicity may originate from the binding of oligomeric AS to cellular membranes, resulting in disruption and cell leakage. Defining the interactions of AS with membranes is thus essential for understanding its physiological and pathological functions.

For such studies, we developed a cysteine-reactive label (MFE) that senses protein microenvironment via the ratio of two emission bands resulting from Excited State Intramolecular Proton Transfer (ESIPT) [1]. We labeled AS at different positions (ala-to-cysteine mutations) and compared the binding to model membranes and the immersion level of its domains. AS has a greater affinity for membranes with high curvature (SUVs) than to LUVs and for negatively-charged than to neutral membranes. We are currently studying the binding of AS oligomers and the impact of membranes on AS aggregation. For other related studies see refs. [2-4].

[1] Demchenko et al (2009) Biophys J 96: 3461; [2] Celej et al. (2009) Biochemistry 48: 7465; [3] Caarls et al. (2009) J Fluor DOI 10.1007/s10895-009-0536-1; [4] posters by Yushchenko et al. and Fauerbach et al.



2504-Pos

Intermolecular and Intramolecular Interactions and their Role in Lamin a Accumulation at the Nuclear Membrane in Human Aging and Premature Aging Disease

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Hutchison-Gilford progeria syndrome (HGPS) is a premature aging syndrome causing systemic defects. It shows close analogies with normal aging at the molecular and cellular level. It was reported that Δ50 lamin A, the mutant form of an intermediate filament protein in the nucleus, causes lamin accumulation at the nuclear membrane resulting in mechanical anomalies.[1] It is unknown if this membrane accumulation is primarily caused by the deletion of a 50 AA exon or the retention of a post-translational farnesylation on the mutant. We use purified protein fragments, the lamin A tail domain that lacks interactions with other lamins in the structural protein network that supports the nuclear membrane, to quantify changes in protein stability and protein-membrane interactions in the cell and with synthetic membrane models. Over-expressed, labeled Δ50 lamin A tail domains show the same pathologic accumulation at the nuclear membrane as the full-length protein. Circular dichroism indicates no gross structural difference between the *wt* and Δ50 lamin A tail domains, but the unfolding temperature is significantly increased in the latter. This suggests topological differences between the Δ50 lamin A and the *wt* protein which may be responsible for the aging pathology observed *in vivo*. SPR indicates that the binding affinity of the unfarnesylated Δ50 lamin A tail domain to solid-supported, acidic membranes is higher than that of the *wt* lamin A tail domain. This suggests that other consequences of the 50 AA deletion than farnesyl retention may also play a role in the pathological accumulation of the full length protein at the nuclear membrane.

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[1]Dahl, K.N., et al. 2006. PNAS. U.S.A. 103:10271-210276.

2505-Pos

Structure and Cholesterol Binding Properties of the Amyloid Precursor Protein (APP)

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We describe the structure of C99 in model membranes. C99 is the 99-residue transmembrane C-terminal domain of the human amyloid precursor protein (APP), which is liberated by β-secretase cleavage of APP. We also show that cholesterol, an agent that promotes the amyloidogenic pathway associated with Alzheimer's disease, specifically binds to this protein. C99 was purified into model membranes where it was observed to homodimerize. The transmembrane domain of C99 was seen to be an α-helix that is flanked on both sides by mostly-disordered extramembrane domains, with two exceptions. First, there is a short extracellular surface-associated helix located just after the site of non-amyloidogenic α-secretase cleavage that helps to organize the connecting loop to the transmembrane domain, which is known to be essential for amyloid-β production. Second, there is a surface-associated helix located at the cytosolic C-terminus that plays critical roles in APP trafficking and in protein-protein interactions. Cholesterol was seen to associate in a saturable manner with C99, with the binding site being centered at the loop connecting the extracellular helix to the transmembrane domain. Binding of cholesterol to C99/APP may be critical for the trafficking of these proteins to cholesterol-rich membrane domains, which leads to cleavage by β- and γ-secretase and resulting amyloid-β production. These results suggest that APP may serve as a cellular cholesterol sensor that is linked to mechanisms for suppressing cellular cholesterol biosynthesis and uptake. *This work follows up on Beel-AJ et al. (2008) Structural Studies of the Transmembrane C-Terminal Domain of the Amyloid Precursor Protein: Does APP Function as a Cholesterol Sensor? BIOCHEMISTRY, 47, 9428-9446. We thank the*

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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