Several studies suggest that tau in AD brains may exhibit abnormal interactions with the neuronal cell membrane. We hypothesize that the lipid membrane can mediate tau pathology by templating tau to misfold into an assembly-competent conformation and subsequently nucleating tau to aggregate into fibrils. We used lipid monolayers at the air/water interface as a model membrane to probe tau-membrane interactions. We found that although tau (hTau40) is highly soluble and charged, it is also highly surface active. hTau40 exhibits strong association with negative DMPG lipids, while exhibiting weaker interactions with the positive DMTAP and neutral DMPC lipids. Thus, tau-membrane interactions are strongly mediated by electrostatic interactions. To identify the hTau40 domain that is responsible for its interaction with membranes, we measured the interaction between different tau constructs (K18 and K32) and lipid membranes. Additionally, X-ray scattering experiments were carried out to elucidate the structural details of tau associated with lipid membranes. Our data show that tau's C-terminal, microtubule binding domain, is responsible for its association with the lipid membrane and that these binding events disrupts the ordering and structure of the membrane. Our study suggests that the ''soft'' nature of tau can give rise to rich dynamic behaviors at interfaces, such as the physiological lipid membrane interface. Our data implicate that the inner leaflet of the cell membrane, enriched in negatively charged lipids, can potentially recruit tau in the cytoplasm, which may be critical in initiating the cascade of pathogenic misfolding and aggregation events in AD.

1248-Pos

Global Bilayer Properties can Modulate Membrane Protein Oligomerization

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While sequence dependent oligomerization of individual transmembrane α-helices has been studied to some extent in the recent years, the influence of the lipid bilayer properties on defined helix-helix interactions remains largely uncharacterized. To study the potential impact of changing bilayer properties on a defined transmembrane helix-helix interaction we have followed association of fluorescently labeled glycophorin A transmembrane peptides in model membranes by fluorescence spectroscopy. Changes in Förster resonance energy transfer strongly suggest that the lipid bilayer thickness does significantly influence the monomer-dimer equilibrium of the transmembrane domain. Furthermore, the presence of cholesterol in model membranes promotes self-association of transmembrane helices by modulating the bilayer thickness and -more importantly- by affecting lipid acyl chain ordering. In addition, changes in the lipid composition, which modulate lipid bilayer curvature elasticity and the lateral pressure profile, affect GpA dimerization. In conclusion, the findings show that the physical state of a membrane can be critically involved in controlling specific and promiscuous interactions of α-helical transmembrane domains, as *e. g.* involved in membrane protein folding and assembly as well as in transmembrane signaling.

1249-Pos

Effects of Post-Translational Modifications on the Structure and Stability of Human LDL

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LDL remodeling in vivo (by hydrolysis, oxidation, glycosylation, lipid transfer, drugs, etc.) may affect LDL entrapment in the arterial wall, which causes inflammation and promotes atherosclerosis. The molecular basis underlying the pro- or anti-atherogenic effects of modified LDL is unclear. To test whether LDL modifications lead to changes in LDL structure and stability, we used (i) myeloperoxidase and Cu2+ to produce LDL oxidized to various stages, (ii) phospholipase A2 (PLA2) to hydrolyze LDL phospholipids, (iii) beta-glucose to glycosylate apoB in LDL. Earlier we showed that heat denaturation of LDL is a kinetically controlled reaction that involves partial unfolding of the beta-sheet structure in apoB, protein dissociation, and changes in LDL morphology such as fusion and rupture. Here we test the effects of LDL modifications on these structural transitions.

Our results show that LDL oxidation leads to a gradual unfolding of the secondary structure in apoB (observed by far-UV circular dichroism, CD) and inhibits heat-induced LDL fusion (observed by turbidity, near-UV CD and electron microscopy). We propose that fusion inhibition results from modifications that increase surface-to-core ratio (e.g., transfer of polar lipids to LDL or lipolysis of apolar lipids), and/or from protein cross-linking upon advanced oxidation.

To assess the effect of PC hydrolysis, we hydrolyzed LDL phospholipids by PLA2, removed free fatty acids by albumin, and analyzed the structure and stability of modified LDL. CD spectroscopy showed no significant changes in the apoB secondary structure. Turbidity and electron microscopy showed that PC hydrolysis promotes LDL fusion, an effect that is reversed by albumin treatment. Consequently, free fatty acids promote lipoprotein fusion. Interestingly, glycosylation of apoB and LDL treatment with niacin also promote lipoprotein fusion. These results help understand molecular basis for LDL fusion in vivo and in vitro.

1250-Pos

Thermodynamics of Gndhcl Induced Unfolding of A Helical Membrane Protein in Mixed Micelles

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Mechanisms of folding and stability of membrane proteins are poorly understood. This is linked to the known difficulties to establish reversible denaturation conditions for these proteins. In this work, we describe the equilibrium unfolding of CopA, an 804 residues Cu^{+}-transporting ATPase from *Archaeoglobus fulgidus*. Guanidinium hydrochloride induced a reversible decrease in fluorescence quantum yield, far UV ellipticity, and the loss of ATPase and phosphatase activities. Refolding of CopA from this unfolded state led to recovery of full biological activity and all the structural features characteristic of the native enzyme. The unfolding process showed typical characteristics of a two state process with ΔG_{ow} 13 kJ mol ^{1} and m 4 kJ·mol ^{1}·M ^{1}. These seemly atypical values suggest the existence of non-detectable unfolding intermediates. Moreover, the C_m was 3 M and the ΔCp_w° 0.93 kJ·mol ^{1}·K ^{1}, giving account of the thermophilic character of this protein. Circular dichroism spectroscopic analysis of the unfolded state shows that most of the secondary and tertiary structure was disrupted. The fraction of Trp fluorescence accessible to soluble quenchers shifted from 0.48 in the native state to 0.96 in the unfolded state with a significant red shift of fluorescence Trp spectra. Also, hydrophobic patches in CopA, mainly located in the transmembrane region, were disrupted as indicated by the lack of fluorescence from the 1-aniline-8-naphtalenesulfonate probe at high concentration of denaturant. Nevertheless, the unfolded state had a small but detectable amount of residual structure, which might play a key role in both CopA folding and adaptation for working at high temperatures.

Protein-Ligand Interactions II

1251-Pos

Thermodynamics of Binding Silver Ion to Jack Bean Urease

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Jack bean urease (JBU; E.C. 3.5.1.5) has six identical subunits, which each subunit consists of a single kind of polypeptide chain containing 840 amino acid residues with relative molecular mass of 90770, excluding the two nickel ions per subunit. Inhibition of urease by heavy metal ions is important special in view of heavy metal ion pollution. Silver ion nearly is always listed as one of the strongest inhibitors. Silver ions coordinate to nitrogen- (histidine) and possibly oxygen- (aspartic and glutamic acids) containing functional groups in urease. Here, a thermodynamic study of silver ions by JBU was carried out at two temperatures of 27 and 37°C in Tris buffer (30 mM; pH 7.0) using an isothermal titration calorimetry. There is a set of twelve identical and non-interacting binding sites for silver ions. The intrinsic dissociation equilibrium constant and the molar enthalpy of binding are 185 μM and 16.7 kJ/mol at 27°C and 229 μM and 16.3 kJ/mol at 37°C, respectively. The molar entropy of binding is +15.7 J K ^{1} mol ^{1}at 27°C and +17.1 J K ^{1} mol ^{1} at 37°C. Hence, the binding process of silver ion to HBU is not only enthalpy driven but also it is entropy driven, which the role of entropy driven should be more effective by increasing the temperature.

1252-Pos

A New ITC Assay for Measuring Ultratight and Low-Affinity Protein-Ligand Interactions

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Isothermal titration calorimetry (ITC) is the gold standard for the quantitative characterisation of protein-ligand and protein-protein interactions.[1] However, reliable determination of the dissociation constant (K_D) is typically limited to the range 100 μM > K_D > 1 nM. Nevertheless, interactions characterised by a higher or lower K_D can be assessed indirectly, provided that a suitable competitive ligand is available whose K_D falls within the directly accessible window.[2] Unfortunately, the established competitive ITC assay requires that the high-affinity ligand be soluble at high concentrations in aqueous buffer containing only minimal amounts of organic solvent. This poses serious problems