tyrosine fluorescence, from quenching experiments with spin labelled phospholipids using A1NT Trp.

814-Pos Board B693
Interaction Of Human Islet Amyloid Poly Peptide With Phospholipid Membrane Vesicles
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Amylin, also known as Human Islet Amyloid Polypeptide (hIAPP), is a 37-residue peptide, suspected to play a major role in the malfunction of insulin secretion in diabetes mellitus type II. Co-secreted with insulin in the beta cells, hIAPP, in higher rates destroys the barrier function of the beta-cells, leading to a failure in insulin production. Because of its amyloidogenity, aggregates of fibrils can be observed in the islands of Langerhans due to its overexpression. We studied the physico chemical properties of hIAPP by observing changes in its structure depending on time and the surrounding media using MALDI-TOF-MS, ATR FT-IR- and fluorescence spectroscopy. In water, hIAPP fibrils grow slowly, after a 37°C incubation for 24 hours some alpha-helices are twisted, and after two weeks, no random coil is detected anymore. We determined membrane binding of dansyl-labeled hIAPP to phosphatidylserine (PS)/ phosphatidylcholine (PC) membranes. Additionally, using confocal laser scanning microscopy the binding of TAMRA labelled hIAPP to giant unilamellar vesicles could be observed. At physiological pH, hIAPP is positively charged and thus negative charges at the phospholipid membrane surface accelerate the process of peptide folding. Being random coil as initial state, a mixture of antiparallel beta-sheets and alpha-helices emerges in time. In the presence of negatively charged PS/PC membranes, hIAPP aggregates can be seen within a few minutes after titration. To understand the process of penetration into cells, we performed leakage measurements of carboxyfluoresceine (CF) filled phospholipid large unilamellar vesicles by means of fluorescence spectroscopy. Titration of hIAPP to CF filled PS/PC liposomes showed different results concerning equilibrium time and maximal extent of leakage depending on the age and preparation of the peptide. In particular the composition of the vesicles seems to determine their stability in the presence of hIAPP.

815-Pos Board B694
Single Particle Analysis of Liposome Leakage Induced by Islet Amyloid Polypeptide
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Islet Amyloid Polypeptide (IAPP) is a 37-residue peptide hormone co-secreated with insulin from pancreatic β-cells. In patients suffering from type-2 diabetes mellitus (T2D), IAPP forms amyloid fibers in the pancreas, which are associated with cell death and the progression of the disease. A possible mechanism of cytotoxicity in T2D is the permeablizing of membranes by oligomeric IAPP, followed by leakage of ions or other molecules. We are examining the IAPP-induced leakage of individual liposomes through the use of single particle methods. Individual fluorescently labeled liposomes are measured post-IAPP exposure through the use of single particle burst analysis to determine the distribution of leakage states. By determining the role of individual residues, solution conditions, and lipid composition in modulating leakage insights are made into the mechanism of oligomer-mediated membrane leakage.

816-Pos Board B695
Amyloid Oligomers Alter The Conductance Of The Gramicidin Channel
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Amyloid oligomers alter the conductance of the gramicidin channel.
Our previous data suggest that Aβ does not itself contribute a new intrinsic conductance such as ion channel to the membrane, but it does seem to alter its physical properties, specifically increasing the apparent dielectric constant of hydrocarbon region. This effect could in turn affect the properties of membrane ion channels.
In order to test this notion we compared the effects of amyloid oligomers on the single channel conductance of gramicidin in 2 M NaCl and CsCl. Amyloid oligomers increase the single channel conductance in NaCl from 13 to 16 pS, but the situation in CsCl is more complicated. In CsCl, the single channel conductance histogram shows two peaks, one with a conductance essentially the same as control (42 pS) and one with a conductance significantly less than control (28 pS). In terms of a simple three barrier two site model such as that used by Barnett et al., 1986 this suggests that amyloid oligomers lower the energies of both Cs and Na ions in the gramicidin channel, but at different critical locations relative to the barrier profile. For Na^+, amyloid oligomers lower the principal central barrier and thus increase the translocation rate of Na^+ at a given voltage. For Cs^+, amyloid oligomers act as if they lower the energy of the Cs ion in the channel, but in such a way as to increase the depth of one or both of the two wells in the barrier profile.
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The Insulin-sensitizers Troglitazone And Rosiglitazone Alter Lipid Bilayer Properties
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Thiazolidinediones are widely used to treat hyperglycemia in patients suffering from type 2 diabetes. Three thiazolinediones - troglitazone (Resulin), rosiglitazone (Avandia), and pioglitazone (Actos) - have been marketed; troglitazone was subsequently withdrawn due to hepatotoxicity. The thiazolidinediones are selective peroxizome-proliferator receptor gamma (PPARγ) agonists and they increase insulin sensitivity. They also have been found to have anti-oxidant, anti-inflammatory, anti-atherosclerotic and cardiovascular effects, but PPARγ activation alone does not account for all their actions. All three derivatives, with troglitazone being the most potent, modulate L-type calcium and delayed-rectifier potassium Kv1.3 channels by a seemingly PPARγ-independent mechanism. This could result from the adsorption of amphiphilic molecules to the membrane, which can alter bilayer properties such as thickness, intrinsic curvature and elastic moduli, and thus membrane protein function. We therefore set out to determine whether the amphiphilic troglitazone and rosiglitazone alter lipid bilayer properties. Using gramicidin channels as probes, where we monitor the changes in channel lifetime and rate of appearance, we tested and compared the effects of troglitazone and rosiglitazone on channels of different lengths in DOPC bilayers. Troglitazone or rosiglitazone did not alter gramicidin channel conductances, suggesting that direct interactions are not involved. In contrast, the lifetimes of both channels increased with similar relative changes for both the shorter and the longer channels. Consistent with their effects on calcium and potassium channels troglitazone is more potent than rosiglitazone. Our results show that both troglitazone and rosiglitazone affect bulk membrane properties at the concentrations where they modulate other ion channels.

818-Pos Board B697
The Antimicrobial Peptide Gramicidin S Permeabilizes Phospholipid Bilayer Membranes Without Forming Discrete Ion Channels
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We examined the permeabilization of lipid bilayers by the β-sheet, cyclic antimicrobial decapeptide gramicidin S (GS) in phospholipid bilayers formed either by mixtures of zwitterionic diphytanoylphosphatidylcholine and anionic diphytanoylphosphatidylglycerol or by single zwitterionic unsaturated phosphatidylcholines having various hydrocarbon chain lengths, with and without cholesterol. In the zwitterionic bilayers formed by the phosphatidylcholines, without or with cholesterol, the peptide concentrations and membrane potentials required to initiate membrane permeabilization vary little as function of bilayer thickness and cholesterol content. In all the systems tested, the GS-induced transient ion conductance events exhibit a broad range of conductances, which are little affected by the bilayer composition or thickness. In the zwitterionic phosphatidylcholine bilayers, the effect of GS does not depend on the polarity of the transmembrane potential; however, in bilayers formed from mixtures of phosphatidylcholines and anionic phospholipids, the polarity of the transmembrane potential becomes important, with the GS-induced conductance events being much more frequent when the GS-containing solution is positive relative to the GS-free solution. Overall, these results suggest that GS does not form discrete, well-defined, channel-like structures in phospholipid bilayers, but rather induces a wide variety of transient, differently sized defects which serve to compromise the bilayer barrier properties for small electrolytes.

819-Pos Board B698
The Superstructure of an Antimicrobial Peptide, Alamethicin, in Lipid Membranes
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In this work we investigate the effect of membrane hydration and hydrophobic mismatch on the Alm channel superstructure in an oriented multilayer sample by x-ray scattering. Wide angle x-ray scattering (WAXS) near 1.4 $Å^{-1}$ indicates that the lipid chain region is not much perturbed by the incorporation of up to 10 mole percent Alm. Low angle x-ray scattering (LAXS) indicates that when the sample is very dry, which promotes interactions between neighboring

bilayers, a body centered tetragonal crystal packing of Alm channels is formed. As the hydration level increases closer to biological conditions, the separation between bilayers increases, the interbilayer interactions weaken, and the crystalline order disappears while considerable diffuse scattering remains. The effect of hydrophobic mismatch is examined for two mono-unsaturated lipids, diC18:1PC and diC22:1PC, that differ in bilayer thickness by 7.3 Å. There is also additional in-plane scattering at a medium q of 0.7 $Å^{-1}$ that our analysis suggests may not be from the Alm channel structure.

820-Pos Board B699

Characterization of the Dynamic Structural Changes of Melittin - Lipid Bilayer Interactions

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Melittin, the soluble peptide of bee venom, has been demonstrated to induce lysis of phospholipid liposomes. We have previously explored the dependence of lysis on liposome composition and now explore the interaction in more structural detail at the level of the lipid bilayer. Supported Lipid Bilayers are probed optically using the waveguide technique Dual Polarization Interferometry (DPI) to obtain data on the mass and birefringence of the lipid bilayer structures. The birefringence is a measure of the degree of alignment and compression of the lipid tails and is highly sensitive to changes of ordering within the membrane. The interaction of the bilayers with Melittin is probed as a function of peptide concentration and the resultant mass and birefringence changes related to the interaction mechanism. For the zwitterionic phosphatidyl choline the lytic ability of melittin is dependent on the degree of acyl chain mobility, with melittin able to induce lysis of liposomes in the liquid crystalline state, whilst those in the gel state show strong resistance to lysis. Thus the interaction of Melittin with Dimyristoyl-Glycero-Phospho-choline (DMPC) lipid bilayers is probed both above and below its transition temperature.

821-Pos Board B700

Experiments Meet Hydrophobic Mismatch: A Re-evaluation Of The Orientation Of Model Transmembrane Peptides From Solid-State NMR

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The basic physical rules underlying the organization of biological membranes can be gathered under the simple, but powerful, concept of hydrophobic mismatch. For example, the mutual adjustment of the lipid and protein hydrophobic lengths can be related with the existence of lipid rafts and explain discrete secretory pathways in the Golgi apparatus. The orientation of membrane protein fragments is predicted to follow the same hydrophobic mismatch principles, as illustrated by some experiments and molecular dynamics simulations. However, this appears to be challenged by results of solid-state 2H NMR experiments on model transmembrane peptides, displaying tilt angle values unexpectedly small and weakly reacting to changes of the lipid bilayer thickness. Here we bridge theory and experiments to show that previous 2H NMR experimental data of model transmembrane peptides in membranes of different thickness can be re-interpreted by using alternative models which consider explicit rigid-body peptide fluctuations. The result is a new set of tilts which follows nicely the hydrophobic mismatch expectations, and is coherent with molecular dynamics simulations as well as with other mismatch studies conducted with natural protein fragments.

822-Pos Board B701

The Structural Plasticity Of Lung Surfactant Peptide KL4 In Lipid Membranes

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Surfactant protein B, SP-B, is critical to lung function and is particularly important in the trafficking of lipids within pulmonary surfactant and altering lipid properties at the air-water interface. The N- and C-terminal segments of SP-B have been identified as the most active domains in SP-B and remedial efforts in treating respiratory distress have focused on these domains and synthetic analogs of them. KL_4 is a 21-residue peptide mimetic of the C-terminus of SP-B. The periodicity of the lysine residues in KL_4 should prevent formation of a canonical amphipathic α-helix at lipid interfaces, yet upon partitioning into membranes CD measurements suggest formation of a helix. Using a suite of ssNMR experiments, in concert with circular dichroism spectroscopies, we are developing a molecular level understanding of the varied structure and function of KL_4 and its parent sequence, $SP\text{-}B_{59\text{-}80}$. In particular, our results highlight their lipid-dependent plasticity and unusual amphipathic helical secondary structures. We will present structural data obtained with solid-state NMR measurements which can resolve two helical conformations in KL_4 with backbone torsion angles that deviate from a traditional α-helix and highlight the adaptive structure of amphipathic helices. We will also present phosphorous and deuterium NMR lineshape data which demonstrate the concentration dependent effects of SP-B related peptides on lipid dynamics in POPC/POPG and DPPC/POPG lipid lamellae. Our observations suggest a means for the peptides to penetrate deeply into lipid environments containing a high percentage of saturated lipids and to bind more peripherally to vesicles containing higher levels of unsaturated lipids. The adaptive structure and penetration depth of SP-B related peptides could explain the mechanism of action of SP-B and demonstrate the structural variability possible for amphipathic helices in lipid bilayer environments

823-Pos Board B702

Structure Of Complexes Of Helix-5 From Bax With Lipid Membranes

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Bax is a proapoptotic protein implicated in the release of cell-death activating factors from the mitochondrial intermembrane space. Although the structure of the membrane-bound forms of Bax is unknown, it has been proposed to form proteolipidic pores. Studies with synthetic lipid vesicles have shown that fragments encompassing helix-5 of Bax retain a membrane permeabilization ability that is similar to that of the full-length protein. Here we report on the structure of peptide-membrane complexes formed by a Bax helix-5 peptide and lipid bilayers. The relative orientation of the peptide and the lipids are determined using site-specific infrared spectroscopy, assisted by isotopic labeling of backbone groups with the $^{13}C{=}^{18}O$ probe. The peptide is highly α-helical in all lipid membranes studied, and its orientation reveals different binding modes that depend on the bilayer phase state. In partially fluid POPC bilayers helix-5 of Bax lies almost parallel with respect to the membrane plane, most likely interacting at the level of the interface. However, in gel phase DMPC bilayers the peptide adopts a tilted orientation, which suggests a deeper insertion in the membrane. In turn infrared spectroscopy and X-ray diffraction data show that in some instances the Bax helix-5 peptide influences the phase transition properties of the lipids by increasing membrane fluidity. Taken together, these effects can be related with the membrane perturbation properties of the Bax helix-5 fragment and with its mechanism of action as a molecule inducing the formation of lipidic pores.

Membrane Physical Chemistry I

824-Pos Board B703

Lateral Stress Profiles In Lipid Monolayers

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We have used molecular dynamics simulations to study the lateral stress profiles in lipid monolayers at the air/water interface. From the calculations, we determined the ''surface of tension'' in the complex interfacial layer. We identified the factors for monolayer stability, which allows explaining the maximum surface pressure sustained by a selected lipid mixture (collapse pressure). This is relevant for understanding the function of biological interfaces, such as the surfactant-covered gas exchange interface in the lungs, and designing artificial/replacement surfactant mixtures.

We calculated the stress distributions for lipid monolayers of different composition under varying surface pressure, including both liquid-expanded and liquid-condensed phases. The stress distribution in the hydrocarbon chain region is most affected by the surface pressure. In the liquid-expanded phase, the stress becomes negative at the chain/air interface. In the liquid-condensed phase, the negative stress in the chains is partially compensated by positive pressure due to increased density, and the profile is characterized by multiple peaks originating from chain and head group ordering. The simulations were performed with both atomistic and coarse-grained molecular models, which led to qualitatively similar results. To test the estimated collapse pressures, the coarse-grained model was used to simulate monolayer collapse upon lateral compression. To induce 2D-3D transformations that require long time scales, small defects were introduced, which provided nucleation sites for monolayer folding.