necessary for activity. Also, innate-immunity-like peptides were described that contained multiple histidine residues. Although these peptides consisted of 12 to 15 amino acids, these were less toxic to the host, and were lytic to numerous pathogens and cancer cells at slightly acidic environments. Here we report the design of an ultrashort histidine containing peptide whose antifungal activity could be significantly increased in a covalent trimeric form. Low micromolar activity was observed for Aspergillus fumigatus and Cryptococcus neoformans but not Candida albecans. Using transmission electron microscopy, we observed that this trimeric ultrashort histidine containing peptide formed distinct and differing nanostructures at pH 5 and 7, which could explain the activity differences. Since various organs or areas of the human body have a slightly acidic pH environment such as tumors, gastric lumen and lung-lining fluids in cystic fibrosis and asthma, understanding the importance of nanostructure-activity relationships of these pH dependent ultrashort peptides could lead to improvements in the delivery and administration of the peptides.

1459-Pos

Spectroscopic Studies of the Interaction of Native and TOAC-Labeled Peptide Hormones with Model Membranes: Angiotensin II

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The peptide hormone angiotensin II (DRVYIHPF, AII) plays an important role in the renin-angiotensin-aldosterone system. AII derivatives containing the paramagnetic amino acid 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid (TOAC) replacing residues 1 ($TOAC^1$-AII) and 3 ($TOAC^3$-AII) were synthesized and their conformational properties, as well as those of the native peptide, were examined in the presence of model membranes - micelles of 1-palmitoyl-2-hydroxy-phosphatidylcholine (LPC) and 1:1 mol:mol LPC: 1-palmitoyl-2-hydroxy-phosphatidylglycerol (LPG) and large unilamellar vesicles (LUV) of 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) and 1:1 mol:mol POPC:1-palmitoyl-2-oleoyl phosphatidylglycerol (POPG). Experiments were conducted at pH 4.0, 7.0, and 10.0 to evaluate the effect of peptide charge on peptide-membrane interaction. Fluorescence spectra showed that the peptides bound to negatively charged micelles to a much larger extent than to zwitterionic micelles. CD spectra of AII and $TOAC^1$-AII showed acquisition of secondary structure upon binding to LPC:LPG micelles at pH 4.0; the changes occurred to a lesser extent at the higher pHs. In the case of $TOAC^3$-AII, binding had a small effect on peptide conformation since the TOAC ring imposes a more constrained conformation already in solution. In the case of bilayers, the peptides interacted only with POPC:POPG LUV, especially at pH 4.0. Line broadening of EPR spectra of the labeled peptides also provided evidence for interaction of the labeled peptides with negatively charged micelles and bilayers. In several cases, two-component spectra were obtained, one due to the peptides in solution and the other to the bilayer-bound population, allowing for the calculation of partition coefficients. The rigidity of the TOAC-labeled analogue is very likely responsible for its inability to acquire the correct receptor-bound conformation, leading to loss of biological activity. These data show that spectroscopic studies can provide relevant information regarding peptide-membrane interaction.

1460-Pos

Unraveling the Molecular Basis of the Selectivity of the HIV-1 Fusion Inhibitor Sifuvirtide Towards Phosphatidylcholine-Rich Rigid Membranes

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Sifuvirtide, a 36 amino acid anionic peptide, is a novel HIV-1 fusion inhibitor with improved antiretroviral activity. The selective ability of this peptide to interact with lipid bilayers has already been identified (Franquelim et al, *J Am Chem Soc* 2008, **130**, 6215-23) and the aim of this work is to evaluate the interaction of sifuvirtide with several biomembrane model systems, retrieving details of its mode of action at the membrane level. Since this peptide has aromatic residues, fluorescence spectroscopy techniques were mostly used. The interaction was assessed by partition and fluorescence quenching experiments. Results showed no significant interaction with large unilamellar vesicles composed by sphingomyelin and ceramide. In contrast, sifuvirtide presented selectivity towards vesicles composed by phosphatidylcholines (PC) in the gel phase, in opposition to fluid phase PC vesicles. The interaction of this peptide with gel phase PC (zwitterionic) membranes ($K_p = 1.2\times10^2$) is dependent on the ionic strength, which indicates the mediation of electrostatic interactions at an interfacial level. The effects of sifuvirtide on the lipid membranes' structural properties were further evaluated using dipole potential membrane probes, zeta-potential, dynamic light scattering and atomic force microscopy measurements. The results show that sifuvirtide does not cause a noticeable effect on lipid bilayer structure. Altogether, one can conclude that sifuvirtide presents a specific affinity towards rigid PC membranes (in agreement with the adsorption model previously proposed), and the interaction is mediated by electrostatic factors, not affecting the membrane architecture. Because saturated PC lipids are found in high concentration in lipid rafts, but mainly in the viral envelope, the efficacy of sifuvirtide may be related to its screening ability towards those regions, allowing an increased concentration of this peptide drug near the fusion site.

1461-Pos

Fusion Peptide of Gp41 Self Associates in the Model Membrane and then Interacts with its Trans-Membrane Domain

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We have examined the peptide structure and membrane packing when the fusion peptide (FP) of gp41 was added either in the membrane alone or in the membrane containing gp41 the trans-membrane domain (TMD). Circular Dichroism (CD) measurements showed that FP is mostly in a beta sheet conformation independent of FP concentration. TMD has ~ 30% helical, ~25% beta sheet and the complex of TMD and FP has less alpha helix than the TMD itself, which indicates the TMD looses its alpha helical structure upon interacting with FP. DPH and TMA-DPH fluorescence anisotropy revealed that FP alone increased the interior packing of the membrane, but FP in the presence of TMD increased the interior packing at lower concentrations of FP and then decreased it at higher concentrations. FP alone increased membrane surface packing at lower concentrations but increased it at higher concentrations. In the presence of TMD, FP addition decreased surface packing cooperatively. From the lifetime of TMA-DPH in H2O and D2O we documented water penetration into the membrane. FP alone increases water penetration slightly whereas FP in presence of TMD significantly increased water penetration into the interface region of the membrane in a cooperative fashion. The fluorescence lifetime of C6NBDPC revealed that FP alone fills more space than the FP in the presence of TMD. In summary, our results clearly demonstrate that gp41 FP forms a complex with the gp41 TMD to alter both TMD structure and membrane structure. It remains to be seen whether this complex promotes membrane fusion. Supported by NIGMS grant 32707 to BRL.

1462-Pos

HIV Fusion Peptides Significantly Soften Lipid Bilayers

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The fusion peptide (FP) of the human immunodeficiency virus (HIV) is found on N-terminus of the viral envelope glycoprotein gp41 and is believed to play an important role in the virus entry process. In order to understand the immediate effect of this peptide on the cell membrane we have studied the influence of the synthetic fusion peptide residue FP-23 on the mechanical properties of model lipid bilayers. For this purpose, giant unilamellar vesicles (GUV) were prepared by electroformation from the unsaturated lipid dioleoylphosphatidylcholine mixed in various ratios with the fusion peptide. The bending stiffness of the vesicles was measured with two different methods: fluctuation analysis and aspiration with micropipettes. The data obtained from both of these approaches show that the bending stiffness of the membrane decreases gradually with increasing the concentration of the fusion peptide in the bilayer. Even low concentrations of only a few mol % FP-23 are sufficient to decrease the bending stiffness of the lipid bilayer by more than a factor of two. This observation is in agreement with previous results obtained with X-ray scattering on stacked lipid layers; see Tristram-Nagle and Nagle, Biophys. J. 93: 2048 (2007). Ongoing research is carried out to investigate the effect of FP-23 on the spontaneous fusion of GUVs.

1463-Pos

Augmentation of Single Channel Water Permeability by Modification of Membrane Anchoring

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Water transport through very narrow channels occurs according to the single file mechanism. While entering the channel, every water molecule loses most of its neighbouring water molecules. The energetic costs are thought to be

compensated, at least in part, by interactions with the channel wall and by interactions with the lipid headgroups at the channel mouth. Consequently, differences in single channel permeability (pf) measured for gramicidin A channels embedded into different lipids were interpreted in terms of differences in water dehydration costs. However, recent atomistic molecular dynamics simulations identified lipid headgroup interactions with the channel entrance leading to transient blocking of the channel. This observation suggests that the lipid environment affects the channel not only by changing the water energetics but also by mechanically blocking the entrance. To test this hypothesis we measured ion and water fluxes through acylated gramicidin-A derivatives, which were reconstituted into solvent free diphytanoyl-phosphatidyl-choline membranes. Ion conductance of channels with C9 and C10 acyl-chain anchors differed only by about 20 % from wild type gramicidin-A conductance. Similarly, the anchor had only a minor effect on dimer stability as indicated by a decrease in channel lifetime from 2.3 s to 2 s or 1.6 s for the C9 and C10 derivatives, respectively. As the gramicidin channels most of the time do not contain ions, the acyl-chain anchor affects water transport more efficiently. Two C9 anchors increased pf by a factor 2 or 3 depending on their position. In contrast, derivates with only one C9 or C10 acyl anchor showed no increase in pf. Taken together with data about the lipid dependency of pf, these results indicate that the lipid headgroups affect single file transport by both changing the solvation energy and by blocking the channel entrance.

1464-Pos

Amyloid Oligomers Increase the Lifetime and Single Channel Conductance of Gramicidin Channels

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Our previous data suggest that Aβ does not itself contribute a new intrinsic conductance to the membrane but instead alters physical properties of the membrane specifically increasing the apparent dielectric constant of hydrocarbon region. This change 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 and mean open time of gramicidin in 2 M NaCl and CsCl using DOPC and a series brominated lipids that change the dielectric properties of lipid bilayer at different depths into the membrane (11,12-bromo-16:0, 10,9- bromo-16:0 and 7,6- bromo-16:0, PC). Amyloid oligomers always increase the single channel conductance and mean open time both in 2 M NaCl and CsCl regardless of the nature of lipid used. The single channel conductance of gramicidin in brominated lipid membranes is always lower than that in DOPC membranes.

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. Brominated lipids apparently increase the depth of the wells at the ends of the channel consistent with their X-ray locations.

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1465-Pos

Observation of Beta-Amyloid Formation Via Membrane Binding

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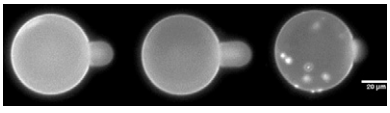
Alzheimer's Aβ-40 and penetratin exhibited the same conformation changes upon binding to membranes. Recently we have studied the thermodynamics of membrane-mediated β-aggregate formation in equilibrium experiments using penetratin-lipid mixtures. The results showed that penetratin bound to the membrane interface in the α-helical conformation at low peptide-to-lipid (P/L) ratios. As P/L exceeds a lipid dependent critical value P/L*, small β-aggregates were formed, which served as the nuclei for large β-aggregates. We tested this free energy description in a kinetic experiment using GUVs. A GUV made of 7:3 DOPC/DOPG and 0.2% lipid dye was aspirated by a micropipette and transferred to a solution of containing penetratin in various concentrations. As the peptides began to bind to the GUV, the membrane area initially expanded till it reached a maximum (this corresponds to P/L->P/L*). Then the area began to decrease from the maximum expanded value (corresponding to P/L exceeding P/L* where the membrane thinning decreases with increasing P/L). Concomitant with the area decrease, lipid aggregates began to appear on the surface of GUV and some of them came off the GUV surface.



1466-Pos

Membrane Mediated Peptide Conformation Change from Alpha-Monomers to Beta-Aggregates

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The major component of Alzheimer's disease amyloid plague, β-amyloid protein 1-40 and the peptide penetratin exhibited the same membrane mediated conformation changes. Both peptides are random coils in solution but change to α-helical or β-like conformations in the presence of negatively charged lipid membranes. Both peptides change from α to β conformations as the lipid charge increases or as the peptide concentration increases. Since the principle behind these phenomena might clarify the molecular mechanism of β-amyloid formation, we investigated the correlation between the peptide conformation of penetratin and its effect on the membrane thickness in four different lipids with varying degrees of chain unsaturation. The results revealed a new effect of membranes on penetratin , i.e., as the degree of chain saturation increased, the peptide changed from α-helical to β-like conformation. We found that penetratin in the helical conformation was bound to the interface and thinned the membrane. In contrast, penetratin in the β-conformation had little effect on the bilayer thickness, therefore it was most likely bound on the surface of lipid headgroups. From the systematic results we were able to deduce the molecular mechanism in terms of free energies that explains the effect of membrane binding on the secondary structure of penetratin. The mechanism could be the prototype for the membrane-mediated version of nucleation-dependent amyloid formation proposed by Jarrett and Lansbury. It might explain why membrane binding has been suspected as the catalyst for polymerization leading to amyloid formation.

1467-Pos

The Hydrophobic Surfactant Proteins Induce Cubic Phases Without Altering Spontaneous Curvature

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Prior evidence suggests that the hydrophobic surfactant proteins, SP-B and SP-C, promote adsorption of the surfactant lipids to the alveolar air/water interface by facilitating formation of a rate-limiting negatively curved stalk between the vesicular bilayer and the interface. In support of the proposed model, the physiological mixture of the surfactant proteins (SP), in amounts as low as 0.03% (w:w), induce 1-palmitoyl-2-oleoyl phosphatidylethanolamine (POPE) to form inverse bicontinuous cubic (Q_{II}) phases, in which each leaflet has the saddle-shaped net-negative curvature predicted for the hypothetical stalk. One mechanism by which the proteins might promote formation of the Q_{II} phases is by altering the spontaneous curvature of the lipid leaflets. If the lipid-protein mixtures form the inverse hexagonal (H_{II}) phase, then a shift in spontaneous curvature would change the dimensions of the unit cell. POPE forms H_{II} structures only above 71°C, and only for 0-0.03% SP. To obtain H_{II} structures with a wider range of protein contents, we substituted lipids that form H_{II} structures at lower temperatures. X-ray diffraction showed that 1,2-dioleoyl phosphatidylethanolamine and its stereoisomer 1,2-dielaidoyl phosphatidylethanolamine form Q_{II} phases with SP at or above 0.03%. During heating from 10 to 95°C, both lipids form H_{II} structures over the full range of protein concentrations from 0-3% SP. The dimensions of the H_{II} unit cell were unaffected by the content of protein. The lack of any effect of the surfactant proteins on the size of the H_{II} phase indicates that the proteins facilitate formation of the Q_{II} phases, and suggests that they promote adsorption, by a mechanism other than changing spontaneous curvature. (Studies conducted at the Stanford Synchrotron Radiation Lightsource).

1468-Pos

Modifications to Surfactant Protein B Structure and Lipid Interactions Under Ards Conditions: Consequences of Tryptophan Oxidation

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Oxidation of Surfactant Protein B (SP-B) is one of several mechanisms proposed to lead to inactivation of lung surfactant in patients with Acute Respiratory Distress Syndrome (ARDS). We have used solution NMR, circular dichroism and molecular dynamics simulation to explore the consequences of oxidation of the tryptophan residue in fragments of SP-B. These fragments include the N-terminal helix of SP-B, as well as Mini-B, a fragment that includes both the N-and C-terminal helices. The fragments were studied in a number of conditions including aqueous solution, organic solvent, zwitterionic and anionic micelles, as well as monolayers. Tryptophan oxidation was found to