Characterizing the Effects of Membrane Fluidity and Lipid Chain Length on the Antimicrobial Activity of Protegrin-1

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Antimicrobial peptides are naturally occurring short amphipathic proteins, innate to the immune system and shown to induce selective lytic activity towards microbial pathogens. Protegrin-1 is an 18-residue, catemere, β-sheet antimicrobial peptide stabilized by two disulfide bridges. Concentration-dependent structural transformations of supported lipid bilayer patches as a result of peptide-membrane interactions have been visualized through the use of atomic force microscopy. A three-stage concentration-dependent transformation has been characterized, which begins with edge instability, followed by pore formation and worm-like micelle formation. This suggests that protegrin-1 acts to lower the line-energy at the edge of the bilayer. Membrane and lipid characteristics, including fluidity, charge and acyl chain length, can alter the activity of antimicrobial peptides. To identify the importance of both acyl-chain length and fluidity on the activity of protegrin-1, these two variables were decoupled. When the bilayers are examined at the same relative fluidity levels, they demonstrate the three-stage transformation observed on a fluid control bilayer, in contrast to the structural transformations that were observed in the gel phase bilayers. This suggests that fluidity exhibits a large influence on the transformations that occur as a result of protegrin-1. To examine the importance of acyl-chain length, the activity of antimicrobial peptides was studied using unsaturated bilayers. Our results indicate that the longer chain bilayers are less susceptible to disruption. This could be due to the hydrophobic mismatch between protegrin-1 and the thicker hydrophobic portion of longer chain lipid bilayers. These results highlight the importance of subtle membrane characteristics in the activity of antimicrobial peptides towards bacterial cells. Lipid bilayers with cholesterol are more accurate eukaryotic cell mimics and will allow examination of the selective preference of antimicrobial peptide activity.

A Systematic Approach Towards Elucidation of the Mode of Action of a Bacterial Thermosensor

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The membrane sensor and signalling protein DesK is involved in detecting the bacterium Bacillus subtilis by controlling the expression of the des gene cluster. This so-called Minimal Sensor (MS) fully retains the ability to respond to changes in temperature. Progressive deletions of TM segments revealed that only the first TM segment (TM1) is essential to regulate the kinase activity. Therefore, our engineered MS combines the N-terminal 17-residue portion of TM1 with the C-terminal 14-residue portion of TM5 which is naturally fused to the cytosolic catalytic domain. The MS N-terminus contains three hydrophobic aminoacids near the lipid-water interface creating an instability hot spot. This boundary-sensitive motif controls the sensing and transmission activity. Accordingly, we hypothesize that membrane thickness is the temperature agent that determines the signaling state of the cold sensor by dictating the hydration level of the meta-stable hydrophilic spot. This hypothesis is supported through the study of the sensing behavior of MS variants purposely constructed.

Membrane-Associated Folding and Unfolding

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1Institute of Biomembranes and Bijvoet Center for Biomolecular Research, Utrecht University, Utrecht, Netherlands, 2Instituto de Biologia Celular y Molecular de Rosario-CONICET, Universidad Nacional de Rosario, Rosario, Argentina. The membrane sensor and signalling protein DesK is involved in detecting temperature changes in the bacterium Bacillus subtilis. At low temperatures it triggers expression of a desaturation, which introduces double bonds into pre-existing peptide chains by regulating membrane fluidity. Recently it was discovered [1] that both sensing and transmission of DesK, which has five transmembrane segments, can be captured into one single chimerical transmembrane segment, the so-called ‘minimal sensor’. It was hypothesized that a group of hydrophilic amino acids flanking this transmembrane segment represent the molecular switch responsible for turning on and off the kinase state of DesK. This switch would be regulated by the extent of exposure of this group on the bilayer surface, which in turn would depend on membrane thickness. Here we tested this hypothesis by employing different biophysical approaches, using synthetic peptides corresponding to functional and non-functional mutants of the minimal sensor in artificial model membranes of phosphatidylcholines of varying thickness and at different temperatures. The results of these studies will be reported.

Engineering a Thermosensor To Dissect a Transmembrane Signaling System

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1Instituto de Biología Molecular y Celular de Rosario, Rosario, Argentina, 2Department of Bioengineering, Rice University, Houston, TX, USA. The DesK-DesR two-component system regulates the order of membrane lipids in the bacterium Bacillus subtilis by controlling the expression of the des gene coding for the delta 5-acyl-lipid desaturase. In this work, we address the process by which DesK transmembrane segments (TMS) transmit temperature signals across the membrane by engineering the 5 TMS domain of the DesK into a single-TMS chimeric sensor. This so-called Minimal Sensor (MS) fully retains in vivo and in vitro the sensing input and transmission output of the parental system. Progressive deletions of TM segments revealed that only the first TM segment (TM1) is essential to regulate the kinase activity. Therefore, our
than 95% of apoA-I is lipid-bound and associated with plasma mature HDL. To study the mechanisms that promote the production of lipid-free/ lipid-poor (cholesterol esterless capable) apoA-1 in the arterial wall, we developed an apoA-1 variant capable of reporting the lipidation-state of apoA-1 in real-time.

We employed fluorescence resonance energy transfer (FRET) to generate an apoA-1 reporter with lipidation-state specific fluorescence. ApoA-1’s four endogenous tryptophans (Trp) were substituted with phenylalanines and a single Trp was substituted in at position 19, as the FRET donor. A cysteine residue substituted in at position 136 was labeled with the fluorophore AEDANS, as the FRET acceptor. The resultant apoA-1 variant, apoA-I-W19-AED136, was lipitated to varying degrees producing rHDL of different sizes. The fluorescence emission spectrum of lipid free apoA-I-W19-AED136 and each of the rHDL particles was collected. Structural differences in the conformation of lipid-free apoA-I and apoA-I associated with different rHDL sizes altered the relative positions of the FRET donor-acceptor pair, leading to lipidation state specific fluorescence “fingerprint”. Lipid-free apoA-I-W19-AED136 showed the highest degree of energy transfer (E=0.571), and apoA-I exhibited decreasing levels of energy transfer with increasing rHDL particle size (7.8 nm (E=0.387), 8.4 nm (E=0.0780), and 9.6 nm (E=0.0334)). ApoA-I-W19-AED136 was successfully used to measure the transition rate of apoA-I between lipid-associated and lipid-free states, potentially, the rate limiting step of macrophage cholesterol efflux in the atherosclerotic plaque.

474-Pos  
**FVIIIa Binding to Phosphatidylserine-Membranes and Its Influence by Annexin V**  
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Binding of Factor VIII (FVIII) to Phosphatidylserine (PS)-expressing platelets is a key process in the intravascular pathway of the blood coagulation cascade. Deficiency of FVIII leads to a severe disease, hemophilia. In the human blood system binding of FVIII to platelets is influenced by many cofactors. One important cofactor is Annexin V, a protein that binds to PS-containing membranes in a Calcium-dependent manner. Annexin is known to inhibit binding of activated Factor VIII to membranes while it does not interfere with binding of inactivated FVIII to membranes in the absence of other cofactors. We investigate the binding behaviour of FVIII, activated FVIII and Annexin to PS/PC model membranes using Fluorescence Correlation Spectroscopy. Based on the understanding of the binding mechanism of each protein, we analyse their mutual inhibition behaviour. Finally, we perform the binding experiments [1] in blood plasma to measure in a more natural environment compared to buffer solution.  

475-Pos  
**Anionic Polymers Reverse Serum Inhibition of Pulmonary Surfactant by Promoting Accumulation of Surfactant Near the Air-Liquid Interface**  
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Acute respiratory distress syndrome (ARDS) is a common pathology, including the spectrum of respiratory diseases associated with lung injury, and exhibiting a high overall mortality and morbidity rate. Inactivation of surfactant by serum and inflammatory components leaked into the alveolar spaces is considered as an important pathogenic factor within ARDS. The mechanism by which inhibition is taking place depends on the nature of the inhibitory substance and could affect either the ability of surfactant to adsorb into the air-water interface or the ability of surfactant films themselves to reach the lowest surface tensions along the compression-expansion breathing cycles. Up to now, different polymers have proven to be useful to reverse or prevent inactivation of surfactant. We have explored the performance of inhibited surfactant and potential reactivating conditions using a fluorescent high-throughput method that detects and quantitates accumulation of surfactant near the air-liquid interface. This accumulation can be correlated in a first step with the concomitant decrease in surface tension that occurs when surface active lipids are transferred into the air-exposed side. Using this method we have evaluated inhibition of native porcine surfactant and of several clinical surfactants by serum, and the ability of hyaluronic acid (HA) to reverse or prevent this inhibition. A comparison was also made with the effect of other polymers. In general terms, presence of polymers in the subphase increases significantly the amount of surfactant associated with interfacial regions and seems to overcome, at least partially, the barrier to adsorption imposed by serum. Results obtained from a massive number of samples showed a very high reproducibility and a high correlation with data obtained using traditional methods to assess surfactant activity, such as surface balances or the Captive Bubble Surfactometer.

476-Pos  
**Confocal Microscopy and Competitive Adsorption: A New Look At Polymer-Enhanced Lung Surfactant Adsorption**  
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Lung surfactant (LS) is a mixture of lipids and proteins that lines the air-liquid interface of the alveolar walls and modulates the surface tension in the lungs. It therefore greatly reduces the mechanical work of breathing as well as prevents alveolar collapse upon expiration. Blood serum leaking into the alveoli as a result of trauma can lead to LS inhibition, which is one characteristic of acute respiratory distress syndrome (ARDS). The competitive adsorption of serum proteins, such as albumin, to the air-liquid interface of the alveoli blocks LS from forming a functional monolayer during ARDS. The addition of hydrophilic polymers, such as polyethylene glycol and chitosan, to the liquid subphase has shown to enhance functional LS adsorption in vivo. Optimal amounts of polymer allow LS to form a functional monolayer on the presence of albumin, thus reversing inhibition. Albumin must be displaced from the air-liquid interface in order for a functional monolayer of LS to form. Imaging of the competitive adsorption process with confocal microscopy has allowed us to better understand the mechanisms behind forming an functional LS monolayer under inhibitory conditions. We can simultaneously track LS, polymer, and albumin, as well as separately visualize phenomena occurring at the interface from those occurring in the bulk. As a result of these capabilities, we have studied how various parameters affect the transport of LS to the interface and the displacement of albumin in order to form a functional surfactant monolayer.

477-Pos  
**Surface Rheological and Morphological Studies of Peptoid Mimics of Lung Surfactant Protein C**  
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Surfactant protein C (SP-C) is a lipoprotein secreted by alveolar type II cells that has been implicated in surface-associated activities thought to facilitate breathing and to prevent alveolar collapse. The N-terminal cysteine residues of SP-C are palmitoylated, which is thought to be critical in stabilizing the helical structure and maintaining a surface-associated surfactant reservoir. However, the exact function of the two palmitoyl chains is not yet fully understood. In the current study, poly-L-substituted glycines or “peptoids”, a class of novel bio-based foldamers, have been employed to study the effects of N-terminal alkylation of a peptoid-based mimic of SP-C. Langmuir isotherms were performed to examine the reversibility of non-alkylated and di-alkylated SP-C mimic-containing lipid films during compression and expansion cycles at the air/liquid interface. Atomic force microscopy (AFM) of Langmuir-Blodgett films revealed extensive multilayer formation at high compression for a lipid