Platform: Membrane-Active Peptides and Toxins

2228-Plat
Lipid-Mediated Polypeptide Interactions in Membranes: Case Study on the Synergism Between Linear Cationic Antimicrobial Peptides
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Biophysical investigations, that aim to explain the antimicrobial activities of cationic linear peptides will be presented (1,2). In particular we aim to understand how some mixtures of these peptides exhibit synergistic activities. Therefore, the structure, topology and dynamics of PGLa and magainin 2 were investigated in oriented phospholipid bilayers using solid-state NMR in the presence or absence of the other peptide and as a function of the membrane lipid composition (2,3). Furthermore, fluorescence spectroscopy was used to investigate how the peptides interact with each other within the lipid bilayer environment.
Whereas, magainin 2 exhibits stable in-planar alignments under all conditions investigated PGLa adopts a number of different membrane topologies with considerable tilt angle variations (2,3). Notably, the hydrophobic thickness modulates the alignment of PGLa (1). In equimolar mixtures of PGLa and magainin 2 the former adopts transmembrane orientations in DMPC when at the same time magainin 2 remains associated with the surface (1). In contrast in bilayers, which represents better the natural membrane composition (1-palmitoyl-2-oleoyl-phospholipids), both peptides adopt a surface oriented topology. Therefore, lipid-mediated interactions play a fundamental role in determining the topology of membrane peptides and proteins (3) and thereby potentially also the regulation of their activities. These results have important consequences for the mechanistic models explaining synergistic activities of the peptide mixtures and will be discussed in the context of unpublished data where membrane structure and interactions are correlated with biological activities. Furthermore, recent results obtained with DNP enhanced solid-state NMR spectroscopy of supported lipid bilayer samples using prototype equipment will be presented.
References:
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2229-Plat
Multiscale Simulations of Diphtheria Toxin T-Domain Membrane Association
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Diphtheria toxin translocation (T) domain is an alpha-helical bundle at neutral pH, which upon acidification refolds, inserts into the lipid bilayer and translocates the attached catalytic domain across the endosomal membrane. Recently, we have used a combination of computational and experimental approaches to characterize the conformation of the membrane-competent state of the T-domain, formed in solution as a result of histidine protonation [1]. Here, we model the T-domain membrane binding using a MARTINI coarse-grained (CG) model of the membrane-competent state of the protein and POPC/POPG lipid bilayers. Multi-microsecond coarse-grained molecular dynamics (MD) simulations have shown two preferred orientations of T-domain bound to the membrane interface. We will also present the results of atomic resolution refinement of these protein-lipid assemblies through approximately 10 μs-long MD simulations. An improved lipid force field model has been designed for these simulations. In both membrane bound models, T-domain slightly inserted into a POPG lipid bilayer while maintaining its orientation relative to the membrane normal. We focused our studies in a membrane bound model where partially unfolded helices TH1-4 are facing the lipid bilayer interface. Approximately 5.5 μs-long MD simulations showed little change of the T-domain orientation. However, neutralization of all acidic residues Asp and Glu followed by 3.5 μs-long MD simulations showed T-domain reorientation with helices TH8-9 aligned to the membrane plane and further protein insertion into the lipid bilayer. This suggests that protonation of acidic residues on the membrane interface may facilitate protein conformational changes leading to the final transmembrane state of helices TH8-9.
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[1] Kurnikov et al. (2013) JMB. 425:2752-2764.

2230-Plat
Peptide:Lipid Ratio and Membrane Surface Charge Modulate the Mechanism of Action of the Antimicrobial Peptide BP100
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We present a comprehensive study of the conformational and functional properties of BP100 (H-KKLFKKILKYL-NH_2) upon interaction with large unilamellar vesicles, LUVs, and giant unilamellar vesicles, GUVs, containing variable proportions of zwitterionic PC and negatively charged PG. Theoretical prediction and calculation of hydrophobic moment indicate that BP100 is a surface-seeking amphipathic helix. Accordingly, CD spectra showed that, upon binding to PG-containing LUVs, BP100 acquires α-helical conformation. NMR data showed that the helix spans residues 3-11. Moreover, CD spectra evinced peptide aggregation in the membrane and/or vesicle aggregation, as a function of peptide:lipid ratio and PG content. Dynamic light scattering confirmed vesicle aggregation under conditions of electroneutralization. BP100 decreased the absolute value of the zeta (ζ)potential of LUVs with lower PGcontents. For higher PG contents, the ζ potential remained initially constant, a decrease occurring when *ca.* 80% of the negatively charged lipids were neutralized. This effect was rationalized in terms of an ion exchange process. Higher peptide concentrations were required for leakage from LUVs aqueous inner compartment at high ionic strength. Moreover, while a gradual release took place at low peptide:lipid ratios, instantaneous lossoccurred at high ratios, suggesting vesicle disruption. Optical microscopy of GUVs also evinced BP100-promoted disruption of negatively charged membranes. We propose that the mechanism of action of BP100 is a function of both peptide:lipid ratio and negatively charged lipid content. While the gradual release of inner contents is a consequence of membrane perturbation by a small number of peptide molecules (monomers or small aggregates), membrane disruption results from a sequence of events: peptide aggregation on the membrane surface, leading to lipid clustering, andgiving rise to peptide-lipid patches that eventually would leave the membrane in a carpet-like mechanism.

2231-Plat
The Use of Surface Plasmon-Based Infrared Spectroscopy to Detect Intercellular Junctions Alterations in Inflamed Intestinal Cells
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Intoduction: A novel surface plasmon resonance (SPR) apparatus, based on surface plasmon and waveguide mode spectroscopy, was used to study cellular dynamics with high resolution. In this method, the infrared light produced by the Fouier Transform Infrared (FTIR) generates a surface plasmon wave that propagates on a gold-sample interface and penetrates deep into the sample covering most of the cell height. When monolayer of epithelial cells reaches full confluence a waveguide mode is generated, propagating inside the cell monolayer. Analysis of SPR and waveguide minima provide exact information regarding cell coverage of the surface, cell layer integrity, average cell height and quality of cell-cell contacts.
Results: We conducted our experiments utilizing different intestinal cells and demonstrated the systems' ability to monitor real time changes in monolayer formation in different conditions including: (a) high and low cell concentrations, (b) exposure to stress conditions mimicking the development of a specific inflammatory bowel disease and (c) the differences between normal and cancer cell lines. We found that Lipopolysachride (LPS) and Hypoxic conditions caused altered junctional formation, a phenomenon that was dramatically down regulated when we silenced a specific cellular pathway by siRNA methodology. Additionally, we found differences in the monolayer formation-kinetics between normal and cancer cell lines.
Conclusions: Our results show the ability of the novel SPR system to detect junctional changes within monolayer of living intestinal cells. Taking into consideration the label free and high resolution features of the SPR, we propose