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IR Spectroscopy of Protein and Peptide–Membrane Interactions: In Situ Strategies for Tracking Fusion, Remodelling, and Refolding
Jessica Li, Michael W. Wong, Lauren Lin, Vanessa Bianchi, Michelle Edwards, Christopher M. Yap.

Attenuated total reflection-infrared spectroscopy is a powerful technique for characterizing protein and peptide–membrane interactions. Using a real-time, flow-through design and a single bounce diamond ATR element, we have examined how three different families of proteins interact with model supported lipid bilayers. These include (1) a lipoprotein from the saposin family; (2) the dynamin-related GTPase Mgm1p; (3) a series of model membrane-antibacterial peptides designed to adopt a helical motif in bacterial membranes. Our results provide compelling evidence of the interaction of these proteins, including membrane remodeling, GTPase activity, and peptide aggregation.

We will describe the integration of laser-based fluorescence spectroscopy with ATR-IR, which, when coupled with correlated in situ atomic force microscopy, will afford a uniquely capable multimodal imaging and characterization platform.

Structure-Function Relationship Investigations of Neuropeptide Y Bound to Hydrated Lipid Bilayers

Neuropeptide Y (NPY) is a neuroendocrinic peptide which belongs to the neuropeptide Y family. It has bactericidal effects on Gram-negative bacteria and higher affinity for lipopolysaccharide (LPS), neutralizing its effect. NPY promotes the aggregation of negatively charged large unilamellar vesicles (LUV) and LPS aggregates, by dynamic light scattering, while for zwitterionic phosphatidylcholine (POPC) LUV the size remains unchanged. The aggregation increases with peptide concentration until promotion of massive aggregation followed by sample flocculation/precipitation [1,2]. Through a Förster resonance energy transfer (FRET) assay, the aggregation is followed by (hemifusion of the negatively charged vesicles, culminating at their leakage (also assessed by fluorescence spectroscopy). With the RβP12-1 lipid interaction, there is a progressive change in the zeta-potential of the LUV systems and LPS aggregates [2]. LUV systems composed of phosphatidylglycerol (POPG) and POPC:POPG mixtures have higher zeta-potential variations than POPC LUV. For the LPS aggregates, RβP12 neutralizes the surface charge and, at higher peptide concentrations, overcompensates it [2]. The results demonstrate that RβP12 mechanism of action at the molecular level involves the interaction with the LPS of the outer membrane of Gram-negative bacteria, followed by internalization and leakage induction through the (hemifusion of the bacterial outer and inner membranes, both enriched in phosphatidylglycerol.

[1] Dominguez, MM et al., Biophys. J. 2009, 96, 987-996.
[2] Dominguez, MM et al., PLoS ONE 2009, 4, e3835.

Significance of Stereochimistry in Short Antimicrobial Peptides

Virginia F. Smith, Barney M. Bishop, E. Andrew Papanastasiou, Myra Jehangir, Hannah Choi, Monique L. van Hoek.

Cationic antimicrobial peptides (CAPs) are essential elements of immunity in higher organisms. These peptides are capable of exerting a direct antimicrobial effect through a process that appears to at least in part involve interaction between the peptides and the bacterial membrane, which ultimately leads to membrane disruption. The lipids and other components that comprise cellular membranes contain chiral centers with defined stereochemistry that CAPs may encounter as they interact with the membrane. The significance of membrane and peptide chirality is not well defined. Helical CAPs provide an attractive tool for addressing these questions. They adopt a helical conformation when they interact with bacterial membranes, but in the absence of the influence of negatively charged bacterial membranes, they assume a relatively unstructured random coil. Formation of an amphiphilic helix is usually essential to the antimicrobial activity of CAPs. The results of these studies could be significant in understanding the modes of action of NPY as a multifunctional peptide active at various bacterial and mammalian lipid membranes.

References:
[1] Dominguez, MM et al., Biophys. J. 2009, 96, 987-996.
[2] Dominguez, MM et al., PLoS ONE 2009, 4, e3835.