Interactions of Disruptive Materials with Phospholipid Bilayers

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The eukaryotic cell membrane is a complex mixture of lipids, cholesterol, and proteins. Direct study of the cell membrane must include these multiple variables, which significantly complicates their analysis. Model systems minimize the number of components used in a membrane study.[1] Atomic force microscopy (AFM) can be employed to study the morphology of model supported phospholipid bilayers (SLB).[2] Thermal and diffusive properties of membranes can be identified using differential scanning calorimetry (DSC)[3] and fluorescence correlation spectroscopy (FCS).[4] Attenuated total reflectance infrared spectroscopy (ATR-FTIR) can be applied to observe SLB disruption.[5] This suite of techniques is used to observe interactions of molecules interfering with phospholipid bilayers. Single- and multicomponent SLBs and phospholipid vesicles are employed as models of cell membranes.

The effects of the pore-forming peptide alamethicin (Alm) are shown for 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) bilayers with different compositions. A correlation is made between the presence of sphingomyelin (SM) or cholesterol (Chol) and increased vesicle leakage as well as formation of raft structures visible with AFM (Figure 1). ATR-FTIR shows that Alm disrupts all types of bilayers studied, further suggesting that the observed effects are directly related to Alm incorporation.

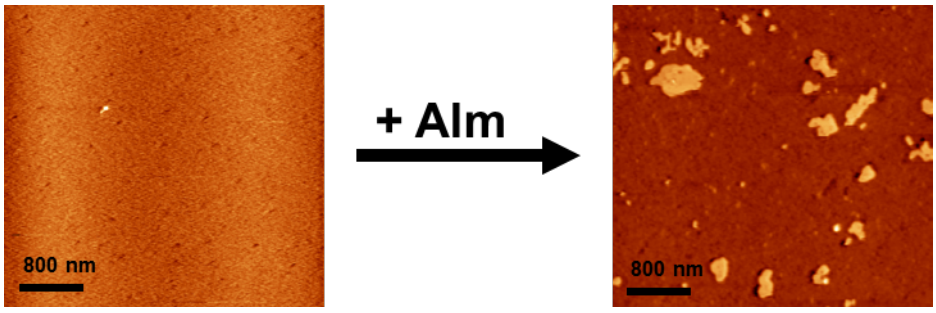

Figure 1: AFM images showing that the addition of alamethicin (Alm) to a SM:DMPC (60:40 mol:mol) generates phase-segregated domains protruding ca. 4 nm above the bilayer.

Previous work showed that the addition of Alm or melittin, another pore-forming peptide, can stabilize fusion and aggregation in vesicles incorporating Chol, leading to leakage without typical pepide-induced pores.[6] We measure changes in visible light scattering for each vesicle composition following addition of Alm, and show that turbidity increases in Chol-inclusive vesicles. We agree with previous conclusions for vesicles incorporating Chol. We propose that addition of SM enhances typical pore formation over neat DMPC.

A comparison is made between the interactions of two polychlorinated biphenyl (PCB) isomers and DMPC bilayers. Previous work showed that ortho PCBs, but not the planar isomers, cause cell death.[7] In this study, AFM images show multiple phase transitions in the presence of an ortho-substituted PCB, but only one phase transition in the presence of a planar PCB. Two

transitions are consistent with an uninterrupted bilayer, but one transition suggests that an intercalated molecule disrupts normal phase transition behavior. DSC results (Figure 2) show that the ortho PCB alters the thermal behavior of DMPC vesicles more than the planar isomer, which suggests that the ortho PCB is more strongly associated with the bilayer than the other isomer. This difference is related to polarization differences between the isomers, caused by their different geometries. FCS is employed to observe a PCB-lipid complex which highlights the different degrees of interaction between PCB isomers and lipids. A model is proposed where the ortho PCB inserts into the bilayer and strongly associates with the hydrophobic region. In contrast, the planar PCB isomer does not insert deeper than the hydrophilic region of the bilayer. The planar PCB interrupts the ordering interactions of water confined between the substrate and the lower bilayer leaflet.

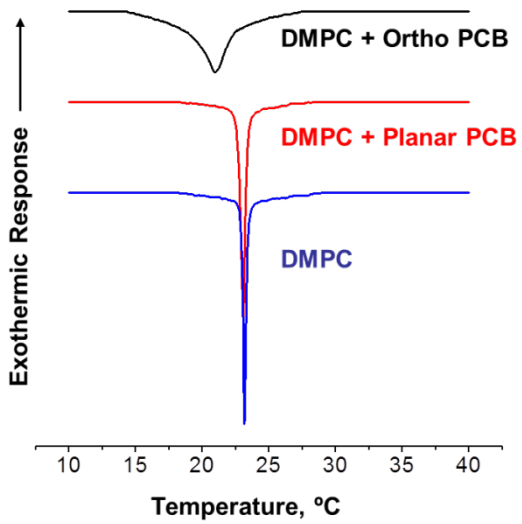

Figure 2: DSC thermograms comparing the behavior of DMPC vesicles with and without PCB.

References

1. (a) Sackmann, E., Supported membranes: scientific and practical applications. *Science* **1996,** *271*, 43-8; (b) Brown, R. E., Sphingolipid organization in biomembranes: what physical studies of model membranes reveal. *J. Cell Sci.* **1998,** *111*, 1-9.

2. (a) Kaasgaard, T.; Leidy, C.; Ipsen, J. H.; Mouritsen, O. G.; Jorgensen, K., In situ atomic force microscope imaging of supported lipid bilayers. *Single Mol.* **2001,** *2*, 105-108; (b) Xie, A. F.; Yamada, R.; Gewirth, A. A.; Granick, S., Materials Science of the Gel to Fluid Phase Transition in a Supported Phospholipid Bilayer. *Phys. Rev. Lett.* **2002,** *89*, 246103/1-246103/4; (c) Kaasgaard, T.; Leidy, C.; Crowe, J. H.; Mouritsen, O. G.; Jorgensen, K., Temperature-controlled structure and kinetics of ripple phases in one- and two-component supported lipid bilayers. *Biophys. J.* **2003,** *85*, 350-360; (d) Feng, Z. V.; Spurlin, T. A.; Gewirth, A. A., Direct visualization of asymmetric behavior in supported lipid bilayers at the gel-fluid phase transition. *Biophys. J.* **2005,** *88*, 2154-2164.

3. (a) Vist, M. R.; Davis, J. H., Phase equilibria of cholesterol/dipalmitoylphosphatidylcholine mixtures: deuterium nuclear magnetic resonance and differential scanning calorimetry. *Biochemistry* **1990,** *29*, 451-64; (b) McMullen, T. P. W.; Lewis, R. N. A. H.; McElhaney, R. N., Differential scanning calorimetric study of the effect of cholesterol on the thermotropic phase behavior of a homologous series of linear saturated phosphatidylcholines. *Biochemistry* **1993,** *32*, 516-22.

4. (a) Schwille, P.; Korlach, J.; Webb, W. W., Fluorescence correlation spectroscopy with single-molecule sensitivity on cell and model membranes. *Cytometry* **1999,** *36*, 176-182; (b) Benda, A.; Benes, M.; Marecek, V.; Lhotsky, A.; Hermens, W. T.; Hof, M., How to determine diffusion coefficients in planar phospholipid systems by confocal fluorescence correlation spectroscopy. *Langmuir* **2003,** *19*, 4120-4126.

5. (a) Reinl, H. M.; Bayerl, T. M., Interaction of Myelin Basic-Protein with Single Bilayers on a Solid Support - an NMR, DSC and Polarized Infrared ATR Study. *Biochim. Biophys. Acta* **1993,** *1151*, 127-136; (b) Fringeli, U. P.; Fringeli, M., Pore formation in lipid membranes by alamethicin. *Proc. Natl. Acad. Sci. U. S. A.* **1979,** *76*, 3852-3856.

6. (a) Wessman, P.; Stromstedt, A. A.; Malmsten, M.; Edwards, K., Melittin-Lipid Bilayer Interactions and the Role of Cholesterol. *Biophys. J.* **2008,** *95*, 4324-4336; (b) Wessman, P.; Morin, M.; Reijmar, K.; Edwards, K., Effect of alpha-helical peptides on liposome structure: A comparative study of melittin and alamethicin. *J. Colloid Interface Sci.* **2010,** *346*, 127-135.

7. (a) Tan, Y.; Li, D.; Song, R.; Lawrence, D.; Carpenter, D. O., Ortho-Substituted PCBs Kill Thymocytes. *Toxicol. Sci.* **2003,** *76*, 328-337; (b) Tan, Y.; Chen, C.-H.; Lawrence, D.; Carpenter, D. O., Ortho-Substituted PCBs Kill Cells by Altering Membrane Structure. *Toxicol. Sci.* **2004,** *80*, 54-59; (c) Tan, Y.; Song, R.; Lawrence, D.; Carpenter, D. O., Ortho-Substituted but Not Coplanar PCBs Rapidly Kill Cerebellar Granule Cells. *Toxicol. Sci.* **2004,** *79*, 147-156.