PMIRRAS Studies of Electric Field Driven Changes in Conformation and Orientation of Proteins in a Model Membrane Supported on a Au(111) Surface

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Using a combination of Lanmuir-Blodgett and Langmuir-Schaffer techniques we have built a phospholipid bilayers of 1,2-dimyristoyl-sn-3-phpsphatidylcholine (DMPC) that contained 10% of gramicidin D at a Au(111) electrode surface or a bilayer containing sphingolipids. This architecture allowed us to study the effect of an applied electric field on the orientation and conformation of membrane-spanning gramicidin (GD) channels and cholera toxin attached to the sphingolipid. Molecular level information regarding the orientation, conformation and hydration of the components of the bilayer have been obtained from PMIRRAS measurements. In the gel phase of the lipid, the presence of the peptide increases the mobility of the acyl chains in both the adsorbed and desorbed states. The orientation of the bilayer is a function of applied potential and the angle with respect to the surface normal decreases upon desorption, in parallel to the change of the tilt angle of acyl chains of DMPC molecules. The amount of hydrogen bonding involving the polar head group of the lipid increases in the DMPC:GD bilayer relative to the pure DMPC bilayer. The effect of the electric field on the helix of GD molecule has been followed by monitoring changes in the amide I band of the peptide. The electric field influences: (i) the conformation of the GD molecule, (ii) the hydrogen bonding pattern in the helix and (iii) changes the tilt angle of the molecule.

In addition a mixed phospholipid-cholestrol bilayer, with cholera toxin B (CTB) units attached to the monosialotetrahexosylganglioside (GM1) binding sites in the distal leaflet, was deposited at a Au(111) electrode surface. Polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS) measurements were also used to characterize structural and orientational changes in this model biological membrane upon binding of CTB and application of the electrode potential. The data show that binding of the toxin to the membrane decreases the number of *gauche* conformers in the acyl chains of the lipid and causes a marked decrease in the hydration of the ester group of the lipid, which is most likely due to water seclusion from the lipid bilayer upon protein binding. Our results show significant voltage-dependent changes in the orientation of the protein α-helices that may correspond to voltage-gated opening and closing of the helical pore within the B subunit of cholera toxin.