Interaction of Lipid Monolayers with Poloxamer 188: An X-Ray Reflectivity and Diffraction Study

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Victims of electrical trauma suffer extensive loss of structural integrity of cell membranes in skeletal and nerve tissue [1,2]. When the lipid bilayer structure is damaged, protein ion pumps cannot keep pace with the increased diffusion of ions across the membrane. Under these circumstances, the metabolic energy of the cell is quickly exhausted, which leads to biochemical arrest and necrosis. Stable structural defects – "pores" in the range of 0.1 μm – have been demonstrated in electroporated cell membranes [3]. Poloxamer 188, a triblock copolymer of the form (ethylene oxide)$_{76}$-(propylene oxide)$_{29}$-(ethylene oxide)$_{76}$ (pEO-pPO-pEO) is known to help seal electroporated cell membranes, arresting the leakage of intracellular materials of the damaged cell [4,5]. However, the interaction mechanism between the cell membrane and poloxamer is still not clearly defined. Using a Langmuir monolayer to mimic the outer leaflet of the cell membrane, with P188 at sub-CMC concentration injected into the aqueous subphase after monolayer spreading, we have examined the interaction between the poloxamer and dipalmitoylphosphatidylcholine (DPPC) or dipalmitoylphosphatidylglycerol (DPPG) monolayers.

With synchrotron x-ray reflectivity and grazing-incidence x-ray diffraction at HASYLAB's beamline BW1, both the out-of-plane and in-plane structure of mixed phospholipid-poloxamer 188 monolayers was investigated at the air-water interface. X-ray reflectivity shows that at high surface pressures P188 is squeezed out from both DPPC and DPPG monolayers. Grazing-incidence X-ray diffraction reveals that P188 doesn't change the size of unit cell of ordered lipid molecules, indicating that P188 only inserts into the disordered phase of the monolayer. At low surface pressure, P188 goes to the air-water interface and physically occupies the available area, which forces lipid molecules to pack tightly to form 2-D crystallites even at high area per molecule (Fig. 1).

These results suggest that poloxamer 188 only inserts into the lipid film where the local lipid packing density is reduced, and is effectively excluded from the film when the lipid packing density of a normal membrane is re-established. The incapability of P188 to sustain its involvement in the system at high surface pressures can be beneficial in terms of its application. After electroporation, cells may activate a self-healing process, restoring the structural integrity of the bilayer. Consequently, as the cell heals and the lipid packing of the membrane is regained, P188 can be easily removed from the cell membrane.

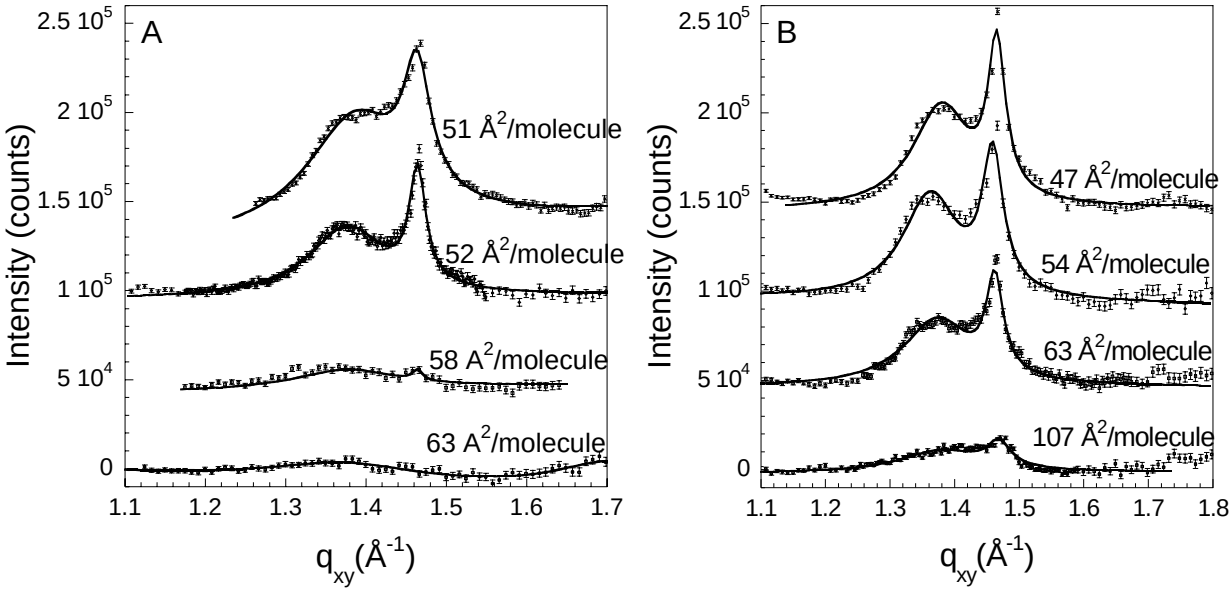

Figure 1: Bragg peaks – vertically integrated scattered intensity as function of the horizontal scattering vector component, q_{xy} – from GIXD on water subphase at 30 °C of (A) pure DPPC, and (B) P188-treated DPPC films. For clarity, the data have been offset vertically. The observed GIXD Bragg peaks indicate a centered rectangular packing of the lipid tails in a 2-D unit cell with a=5.4 Å, b=8.6 Å, A=46.4 Å^2. In (B), the diffraction peak observed at 107 Å^2/molecule corresponds to this highly condensed lipid phase despite the large area per DPPC molecule.

References

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