OBSERVATION OF RIPPLE CONFORMATIONS OF P₃₀ PHASE OF DIMYRISTOYL PHOSPHATIDYLCHOLINE WITH THE SCANNING TUNNELING MICROSCOPE

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The P₃₀ or ripple phase of lecithin-water systems was first observed with x-ray diffraction by Tardieu, Luzzati and Reman in the early 1970's.¹ The ripple phase has been observed in numerous studies using x-ray diffraction, freeze-fracture electron microscopy, and other spectroscopies. Electron microscopy on lecithins far above full hydration showed the existence of a second ripple conformation nearly twice the wavelength of the ripples observed below full hydration leading to the designation of Λ and Λ/2 ripples. Yet none of these measurements have been able to provide a direct unambiguous measurement of the ripple amplitude and conformation. By modifying the technique of freeze-fracture replication to make it compatible with the scanning tunneling microscope (STM) we have observed both the Λ/2 ripple conformation and, for the first time with an STM, the Λ ripple conformation of dimyristoyl phosphatidylcholine (DMPC) in water.

A sample of 30% DMPC and 70% water was equilibrated at 20°C before plunge freezing in liquid propane. Replicas were prepared using standard freeze-fracture technique except that the 2nm platinum/carbon film was deposited vertically instead of at an angle since shadowing is not desirable for imaging with the STM.² After depositing a 30nm carbon backing the replicas were removed the chamber and an additional 200 nm of gold backing was added for extra mechanical support. The replicas were then cleaned in chromerase and water/ethanol, and mounted on a 200nm pore silver membrane.

Figure 1 shows an unfiltered STM image of the Λ/2 ripple phase. We found the Λ/2 phase has a ripple wavelength of 10.6±0.3 nm and an average peak to valley amplitude of 2.9±0.9 nm. Figure 2 shows an image of the Λ phase from the same sample. The Λ phase is known to resemble a triangle waveform with the peaks dented down (or the valleys dented up) so as to appear like an M (or W) as can be faintly seen in figure 2. The Λ phase wavelength was measured to be 20.4±0.3 nm. As can easily be seen in figure 3 the replica does not smoothly cover the ripples but is made up of small crystalites. It is these crystalites that are the primary source of our uncertainties as the STM can clearly image the gaps between the crystalites which are not indicative of the actual ripple conformation. We are currently studying the use of amorphous metals in the replicas as a way of reducing or eliminating the crystalline nature of the replica and providing a more accurate reproduction of the surface.

We had previously found that STM images of replicas in air could show significant amplifications of surface features so we built an environmental control chamber for the STM.³ This allowed us to evacuate the chamber to drive water and other contaminants off the replica surface then back fill with dry nitrogen during imaging. We have not observed any anomalous amplifications in images taken inside the chamber while we continue to see amplifications in images taken in air.⁴

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Figure 1) STM image of the A/2 ripple phase of DMPC in water. All units shown are nanometers.

Figure 2) STM image of the A ripple phase of DMPC in water.

Figure 3) STM image highlighting crystalline nature of the Pt/C replica.