

# DIRECT EVIDENCE FOR THE FORMATION OF A MONOLAYER FROM A BILAYER

## An Ellipsometric Study at the Nitrogen–Water Interface

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**ABSTRACT** Direct evidence for the formation of a monolayer from a bilayer was measured by ellipsometry after spreading unilamellar vesicles of dioleoyl phosphatidylcholine (DOPC) at the nitrogen–water interface. The ellipsometric isotherms of DOPC vesicles and DOPC spread from an organic solvent were compared and found similar. From the observed ellipsometric angle ( $\delta\Delta$ ) in the plateau region ( $-1.04^\circ$ ) and literature data for refractive indices of an anisotropic film similar to DOPC, we have calculated a thickness of  $20 \pm 1 \text{ \AA}$ . These results strongly suggest that, similarly to DOPC spread from an organic solvent, DOPC vesicles form a monolayer when spread at the nitrogen–water interface.

### INTRODUCTION

The measurement of interactions between membrane components at the gas–water interface is usually made by examining the surface pressure–area isotherms of these components. Before compression, the components are spread on a water-containing subphase, using an organic solvent. In attempts to improve this model system, liposomes (1) or whole biomembranes (2–6) have been spread from aqueous solutions. This new approach is quite attractive but raises the question of how the films are organized at the gas–water interface.

Ellipsometry is a powerful method for studying the organization of surfaces covered by thin films (7). Because of its high sensitivity in the submonolayer region (8), ellipsometry can be an extremely useful tool for the investigation of the molecular organization of membrane components at a nitrogen–water interface.

The present study was undertaken to examine the organization of unilamellar vesicles of dioleoyl phosphatidylcholine (DOPC) spread at the nitrogen–water interface. The ellipsometric isotherm of DOPC vesicles was shown to be indistinguishable from the isotherm of DOPC spread from an organic solvent.

### MATERIALS AND METHODS

The DOPC (P.L. Biochemicals, Milwaukee, WI) was checked for purity by thin-layer chromatography (9) and was found to have a major spot and

a faint, faster moving component. Fatty acid analysis of DOPC by gas chromatography (10) showed 98.5% purity.

Vesicles were prepared by the dilution and dialysis method (11). Briefly, DOPC was dried under a stream of argon and placed under vacuum ( $10^{-3}$  Torr) for 3 h. Then, solid octyl glucoside (Sigma Chemical Co., St. Louis, MO) and buffer (50 mM Tris and 50 mM sodium acetate, pH 7.0) were added so that the final micellar detergent–lipid ratio was at least 10:1. This ratio insured that only single-walled vesicles (12) would be produced by the subsequent dilution and dialysis. The sample was vortexed to completely solubilize, and then allowed to equilibrate at  $4^\circ\text{C}$  for 4 h. This octyl glucoside/DOPC solution was then added dropwise (20 ml/h) to the same buffer during rapid stirring until the final octyl glucoside concentration reached 10 mM. The detergent was removed by dialysis at  $4^\circ\text{C}$  against a 100-fold excess of the same buffer for 36 h with three changes of buffer. The dialysis medium was continuously deoxygenated by bubbling with pure nitrogen.

The reconstituted membrane sample was then loaded onto 0–50% (wt/wt) continuous sucrose density gradients and centrifuged at 80,000 g overnight. The single band was collected from the gradients and the sucrose removed by dialysis as described above. The concentration of phospholipid was then determined by phosphate analysis (13).

The DOPC vesicles were spread at the nitrogen–water interface using a modification of Trurnit's method (14). Surface pressure, surface potential, and ellipsometric isotherms were measured as previously described (15). DOPC vesicles lost in the subphase during spreading were prevented from diffusing behind the Langmuir surface pressure measuring device, by a modification in trough design.

### RESULTS AND DISCUSSION

The surface pressure ( $\pi$ ), surface potential ( $\Delta V$ ), and ellipsometric ( $\delta\Delta$ ) isotherms of DOPC vesicles are presented in Fig. 1. The molecular area is given in  $\text{cm}^2$  since some DOPC vesicles are lost in the subphase during spreading.

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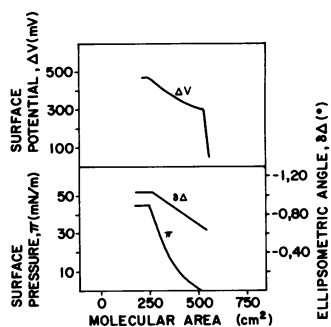


FIGURE 1 Surface pressure ( $\pi$ ), surface potential ( $\Delta V$ ), and ellipsometric ( $\delta\Delta$ ) isotherms of unilamellar vesicles of DOPC at the nitrogen-phosphate buffer ( $10^{-3}$  M, pH 7.2) containing  $10^{-1}$  M NaCl. NaCl was purified by multiple chloroform extractions. The water used was deionized and prepurified by filtration (NANOpure filter system; Barnstead Co., Division of Sybron Corp., Boston, MA) and

twice distilled in a quartz still (model Bi-18; Amersil, Inc., Sayreville, NJ). Its specific resistivity was  $18 \text{ M}\Omega \text{ cm}$  and its surface tension  $\geq 71 \text{ mN/m}$ . The temperature was maintained at  $19.5 \pm 0.5^\circ\text{C}$ .

The surface pressure, surface potential, and ellipsometric isotherms each exhibited a plateau region which occurred at a similar molecular area. In this region, values of  $45 \text{ mN/m}$  surface pressure,  $475 \text{ mV}$  surface potential, and ellipsometric angle of  $-1.04^\circ$  were measured. These values correspond very closely to those obtained from the isotherms of DOPC spread from an organic solvent (15).

Fig. 1 shows that slightly before the onset of the surface pressure, a jump in both  $\delta\Delta$  and  $\Delta V$  was detected. At this area per molecule, the values of  $\delta\Delta$  ( $-0.64^\circ$ ) and  $\Delta V$  ( $305 \text{ mV}$ ) were the same as those of the corresponding DOPC isotherms spread from an organic solvent (15). Furthermore, the shape of the isotherms of DOPC vesicles presented in Fig. 1, was indistinguishable from our previously reported isotherm of DOPC spread from an organic solvent (15).

Film thickness cannot be determined from the sole measurement of  $\delta\Delta$  because refractive indices are unknown. However, the use of literature refractive indices (16, 17) has been justified in the calculation of film thickness of DOPC spread from an organic solvent (15). Using the same justification, a film thickness of  $20 \pm 1 \text{ \AA}$  can be calculated for DOPC vesicles in the plateau region ( $-1.04^\circ$ ), since the present DOPC vesicle isotherms are nearly identical to the previous ones (15). This thickness corresponds to only the fatty acid chain region of a DOPC molecule because the polar head region cannot be detected by ellipsometry, because of its index of refraction being too close to that of the subphase.

Thus, this is direct evidence that DOPC vesicles form a monolayer at the nitrogen-water interface when spread from an aqueous solvent. The only previous suggestion for the formation of monolayers from bilayers comes from the work of Pattus et al. (1). They concluded that it is likely that spreading implies a drastic alteration of the phospholipid bilayer structure. Ellipsometry allowed us to determine the type of alteration, i.e., the formation of a monolayer.

However, one cannot predict what would happen with a

biomembrane containing membrane proteins. We are therefore investigating a more complex system, i.e., the discal membrane of the visual photoreceptor.

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