Temperature Dependence Of Dipalmitoyl Phosphatidylcholine/
Dihexanoyl Phosphatidylcholine Aqueous Mixtures

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There is great interest in the process of lipid self-assembly, especially the microstructure formed from lipid mixtures. Spontaneous unilamellar vesicles have been reported by Roberts from mixtures of long-chain lecithins and short-chain lecithins (1). Alone, dipalmitoyl phosphatidylcholine (DPPC) forms normal multilamellar phases, a solid Lα phase up to about 37°C, a Ps- or ripple phase from 37 to 43°C, and a fluid or Lα phase above 43°C. Dihexanoyl phosphatidylcholine (DHPC) by itself generally aggregates as spherical micelles over this temperature range. At temperatures below 40°C the two lipids, DPPC and DHPC, phase separate, leading to the possibility of physical segregation of the two lipid species. As a result of this separation, the DHPC might migrate to the boundaries of the DPPC-rich bilayers where it could stabilize the bilayer edge to allow the formation of bilayer discs, as first described by Fromherz and Rüppel (2).

We have investigated the microstructure of these mixtures as a function of temperature using freeze-fracture replication and transmission electron microscopy. Samples are prepared by mixing DPPC and DHPC (a generous gift from G. Riedey) at 4/1 mole ratio with water to form a total lipid concentration of 25 mM. Thin films of the sample trapped between Balzer's planchettes were then rapidly frozen from a controlled temperature and humidity by plunging into liquid propane cooled by liquid nitrogen. Platinum-carbon replicas were then made in a Balzer 400 freeze-etch device at -170°C (and 15°C) and cleaned by standard procedures (3).

Using transmission electron microscopy, we find that the dilute solutions of short-chain and long-chain phospholipids show discoid structures above Tm ~ 38°C and two ripple structures of the Ps- phase below the main transition temperature (see figs. 1 and 2). Above Tm = 38°C, we found primarily flat, circular unilamellar discs. Fig. 1 shows a population of such discs, the discs marked by arrows are parallel to the fracture surface and show a bilayer structure about 50 nm in diameter. The discs are also often perpendicular to the fracture surface in the image but the orientations are more difficult to understand. At temperature below Tm, we see extended rippled bilayer fragments with angled edges. Two ripple types are observed as in single component DPPC bilayers, one of wavelength 12 nm, the second of wavelength 24 nm. We have not observed closed vesicles at either regime, above or below Tm; it appears that flat discs or rippled structures are stable at all temperatures. This is consistent with the "edge actant" role of DHPC to stabilize the bilayer edge against closing into vesicles.

Fig. 1) DPPC/DHPC (4:1) at 40°C. Discoidal aggregates (arrows) are observed above the main transition temperature. Diameter: 50 nm. X132,000.

Fig. 2) DPPC and DHPC mixtures at 25°C show ripple structures of the Pβ' phase. Wavelength: 20 nm. X90,000.