ATOMIC FORCE MICROSCOPY OF DIMYRISTOYLPHOSPHATIDYLCHOLINE MEMBRANES

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We have observed the surface images of dimyristoylphosphatidylcholine (DMPC) monolayer and DMPC vesicles with an atomic force microscope (AFM). It was confirmed that the lattice structure of polar head groups of DMPC molecules in a Langmuir–Blodgett monolayer is similar to those of large unilamellar DMPC vesicles. AFM images of small unilamellar vesicles of DMPC showed larger lattice structures than those of the monolayer, reflecting differences of molecular packing in these membranes.

KEYWORDS atomic force microscope; dimyristoylphosphatidylcholine; Langmuir–Blodgett film; small unilamellar vesicle; lattice structure

The atomic force microscope (AFM) is a relatively new instrument for imaging the surface topography of biological specimens in an aqueous environment or in air at room temperature.1) We can obtain three-dimensional images with nanometer resolution under physiological conditions by the use of AFM. Thus, biological application of AFM is destined to be of great importance.2) Many biological specimens such as amino acids,3) proteins,4) DNA,5) membrane proteins6) and a variety of membranes7) have been imaged.

In the study of the relationship between the structures and functions of membranes or membrane-like systems and membrane proteins, surface information on them is quite important. AFM can provide a direct image of the surface structure at high resolution under various conditions. We report here the AFM imaging of three types of DMPC membranes including a Langmuir–Blodgett (LB) monolayer and bilayers of DMPC vesicles.

LB films were prepared by the usual method, as follows: DMPC was dissolved in
benzene–chloroform (8:2) and spread on the water surface of a film balance. The surface pressure was then increased until the spread monolayer reached a condensed crystalline phase (20 mN/m). The DMPC membrane was transferred by dipping (0.5 cm/min) to a quartz plate which had been treated with octadecyltrichlorosilane. Thus, polar groups of DMPC were facing the air. DMPC small unilamellar vesicles prepared by the sonication method were filtrated through a polycarbonate membrane of 0.2μm pore (Nuclepore). Multi–lamellar vesicles were used without further homogenization. An aliquot of vesicle suspensions was placed on a cleaned quartz plate and was air–dried at room temperature. AFM images were scanned in the constant force mode ( 5 nN) with a SFA–300 (Seiko Instrument, Inc.) at room temperature. The images in each case were obtained from different samples in duplicate. The distances between spots were calculated from 30-40 bright points.

Figure 1(a) shows a high resolution image of LB monolayer surface of DMPC. Figure 1(b) is a filtered–Fourier transform processed image. Three distinguished lattice orientations along lines AB, CD and EF were clearly observed. The individual bright spots along the rows correspond to the polar head groups of DMPC molecules. The

![AFM Images](image)

**Fig. 1.** AFM Images of the Polar Region of DMPC Monolayer on Quartz Plate Prepared by the Langmuir–Blodgett Technique

(a) Surface view of DMPC monolayer; (b) Top view of the same image as in (a); (c) Cross–sections along lines AB, CD and EF in (b). The unit is nm.
spots deviated slightly from a regular hexagonal lattice. The distortion of the two-dimensional molecular lattice should be due to a quiver motion of hydrocarbon chains between scans. The short distance along one direction is $0.44\pm0.03$ nm, which can be estimated from the cross section along line AB shown in Fig. 1(c). In the other two directions, CD and EF, the mean distances are $0.55\pm0.04$ and $0.58\pm0.05$ nm. Such a lattice structure for the head groups in DMPC monolayer is comparable with that of bilayers of large unilamellar vesicles of DMPC reported by Lal et al.$^9$)

Further, this lattice structure is similar to that of individual hydrocarbon chains of the double-chained phospholipid molecules reported by Hörber et al.$^9$), who observed bilayers of dimyristoylphosphatidic acid with STM (scanning tunneling microscope). These findings suggest that a fundamental property of the lattice structure in the L-B monolayer is similar to that in a bilayer. It can be said that the surface structure of the bilayer in large vesicles is similar to that of LB film.

AFM images of DMPC small unilamellar vesicles are displayed in Fig. 2. It is obvious from the cross sectional profile along the lines drawn in Fig. 2(c) that the spacing is large along each parallel row compared with LB monolayers. The short distance in

![AFM Images of Small Unilamellar DMPC Vesicles on Quartz Plate in Air](image)

(a) Surface view of DMPC unilamellar vesicles; (b) Top view of the same image as in (a); (c) Cross-sections along lines AB, CD and EF in (b). The unit is nm.
direction CD is 0.52±0.13 nm, and the long distances in the other two directions AB and EF are 0.85±0.11 and 0.92±0.14 nm, respectively.

The differences of these values for the lattice structures between large unilamellar and small unilamellar vesicles may reflect the difference in the curvature of these vesicles. These facts suggest that AFM can provide reliable information about the topographical condition of polar groups in lipid vesicles and that AFM use is promising in liposome technology. AFM images of DMPC membranes observed in the present study are the first ones that clearly show the lattice structures of the head groups in the various kinds of DMPC membranes.

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REFERENCES

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