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Rapid Report

Electron diffraction study on two-dimensional domain structure of L-DPPC monolayer

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A spatial variation in the two-dimensional crystal lattice orientation of L-DPPC monolayer on a hydrophobic substrate was observed by use of an electron microscope with diffraction and dark-field techniques. It is shown that the monolayer has a mosaic structure with domains, each of which consists of a region with a homogeneously oriented lattice of L-DPPC molecules. The domain structure of L-DPPC monolayer on a hydrophilic surface is also discussed.

For a better understanding of biological processes on the surface of a lipid membrane, e.g., the proton transfer along the interface between lipid monolayer and pure water [1,2], it is necessary to know the detailed structure of the membrane including a lipid monolayer. Many investigations on structures of lipid monolayers have been reported recently based upon the results of fluorescence microscopic studies [3–5]. For the observation of a lipid monolayer by a fluorescence microscope, it is necessary to dope fluorescent probe molecules into the monolayer. A fluorescent probe, e.g., head-labeled NBD-DPPE (NBD, 7-nitrobenz-2-oxa-1,3-diazol-4-yl and DPPE, L-α-dipalmitoylphosphatidylethanolamine) has usually been doped into the lipid by 1–2 mol%. It is thought that the effect of probe molecules to the structure of monolayer can usually be neglected, since the amount of the probe doped into the monolayer is very small. However, it was shown that the structures of a pure L-DPPC (L-α-dipalmitoylphosphatidylcholine) monolayer and an L-DPPC monolayer doped with 2 mol% of L-DPPE differ with each other when observed by an electron microscope with dark-field and diffraction techniques [6]. Thus the doping of a small amount of another type of lipid molecules changes the molecular arrangement within a monolayer made up with pure lipid molecules. The molecular arrangement indicated by fluorescence microscopic observations, therefore, can be different from that of a pure lipid monolayer.

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L-DPPC was obtained from Sigma and used without further purification. A cylindrical glass vessel with an inner diameter of 2.7 cm was used as the cell for developing the monolayer. Purified water with specific resistivity of approx. $1.7 \cdot 10^7$ Ω cm was used as the bulk buffer medium for development. L-DPPC monolayer was obtained by placing a drop of 10 mg of L-DPPC/chloroform solution with concentration of 0.05 wt% on the water surface. The drop of solution spread swiftly on the surface resulting in an L-DPPC monolayer developed on the water surface. During this process, excess lipid molecules clustered into one particle and then went down to the bottom of the glass vessel as described before [1]. The lateral pressure of the monolayer obtained was around 40–43 dyne/cm. The monolayer was then transferred onto a hydrophobic surface of a micro-grid by horizontal lifting method from the water surface [7]. The hydrophobic surface was a thin carbon film of about 100 Å thick. The electron microscopic observation was carried out by dark-field and electron diffraction techniques on JEOL-200CX electron microscope operating at 200 kV accelerating voltage. No effect of the transferring and observation processes on the structure of monolayer had been proved by observations of monolayers at -180°C with use of a cryo-system [7]. The temperature of sample during the observation was below about 40°C, which was estimated through an observation on a sample with a known phase transition at about 40°C under the same experimental conditions including the electron beam radiation rate. To avoid the radiation damage of the sample by electron beam, we used as short exposure time as possible for taking photographs,

typically 0.5 s for diffraction patterns. The sample monolayer set in the electron microscope was thought to be in anhydrous state, since it was observed in the high vacuum of electron microscope, i.e., $\sim 10^{-7}$ Torr, and the diffraction pattern obtained after keeping the sample for several days in the vacuum gave the same pattern as that obtained within one hour after the insertion of the sample into the vacuum.

As shown in Fig. 1a, an inhomogeneous monolayer with holes of about 0.3 μm in diameter was observed by dark-field technique. A structure with holes is frequently observed not only for a lipid monolayer but also for a monolayer of an amphiphilic compound [8]. The size of a hole depends on the lateral pressure of a monolayer developed on the water surface [7]. In this photograph, white part is a carbon net supporting the thin carbon film, gray part the monolayer, and dark part a hole within the monolayer. Although the monolayer covers whole the area, the carbon support net prevents us from observing the part of monolayer above the support net, since the support net is more than an order of magnitude thicker than the monolayer. All L-DPPC monolayers prepared by the same way showed the same structure as shown in this photograph [7]. The monolayer gave an electron diffraction pattern with a hexagonal symmetry implying that it is in a two-dimensional crystalline state.

We next tried to get a series of electron diffraction

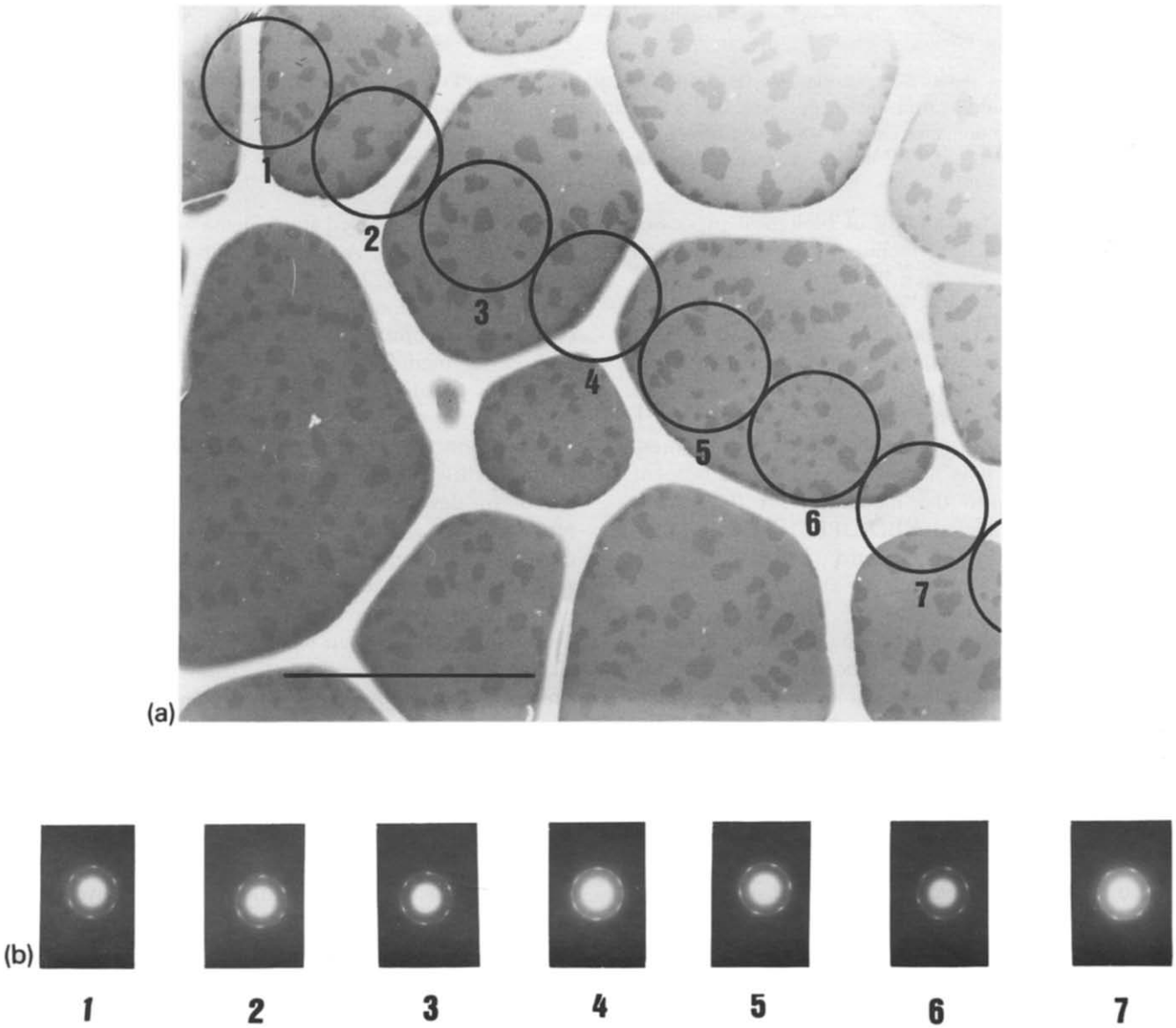

Fig. 1. (a) An electron microscopic image of an L-DPPC monolayer transferred onto the hydrophobic surface. The series of circles exemplify the areas from which electron diffraction patterns are obtained. Bar represents 4 μm. (b) Electron diffraction patterns obtained by shifting the observing area step by step as demonstrated in (a). The numbers for an encircled area in (a) and a diffraction pattern in (b) roughly correspond with each other.

patterns from selected areas of the monolayer to examine whether or not the structure of the monolayer is homogeneous. For that purpose electron diffraction data were obtained by the following procedures; (1) the beam current was set as low as possible for observation, (2) the magnification was set to $\times 10\,000$ or $\times 6000$, (3) the electron beam size was reduced down to 2 μm in diameter of the irradiation area, (4) then the measuring system was set to diffraction mode, and (5) finally by shifting the area for observation in the monolayer step by step toward one direction by 2 μm, the electron diffraction pattern from each area of the monolayer with diameter of 2 μm was photographed (Fig. 1b). After obtaining a series of photographs of electron diffraction patterns along one direction, the measuring system was changed from diffraction to image mode and the image of the monolayer was photographed by shifting back the area for observation on the monolayer to the original area (Fig. 1a) so as to check the monolayer structure from where the electron diffraction patterns were obtained.

At several areas of the successively selected areas, the set of six-points reflections thus obtained showed a sudden rotation by a finite angle on the circle with diameter of $(4.2\ \text{Å})^{-1}$ keeping the whole hexagonal six-points reflection unchanged (Fig. 1b). In other words, the rotation angle of the hexagonal reflections remained almost constant within several shift steps (i.e., within a shift distance) and changed abruptly to another rotation angle and again remains constant within another several shift steps. Fig. 2 shows a typical change in the rotation angle of the hexagonal pattern with the relative shift distance. This observation suggests that the monolayer on the hydrophobic substrate has a mosaic structure of crystalline domains with different lattice orientation. Small holes within a domain (see Fig. 1a) seem not to affect the average orientation of the lattice in a domain. The lattice may be locally deformed around the holes since the observed hexagonal spots show a weak arc-like tails in most of the cases observed (Fig. 1b). Since the hexagonal patterns are observed by shifting along a straight line, the longest persistent length (i.e., the length within which the rotation of reflectional spots is negligible) may correspond to a diameter of a domain of L-DPPC monolayer, within which the orientation of two-dimensional lattice does not change. The diameter of a domain thus evaluated from several sets of measurements was about 20 μm and shorter than the reported value of about 100 μm obtained from L-DPPC monolayer on water surface by use of a fluorescence microscope [3]. This difference in domain size can come from the difference in the method of observing a 'domain'. In the present method a domain means a region where all lipid molecules are arranged as in a two-dimensional crystal. On the other hand, in the reported fluorescence microscopic studies [3], a domain means a region where only the macroscopic tilt orientation of lipid molecules remains the same as in a 'smectic C' liquid crystal.

Preliminary electron diffraction measurements were also carried out on L-DPPC monolayers transferred onto a hydrophilic surface. The experimental details were the same as for the hydrophobic case above except for the transferring process, in which a monolayer was deposited on the hydrophilic substrate put under water by leveling down the water surface with the monolayer. It was indicated that the domain structure of the monolayer on the hydrophilic substrate was different from that on the hydrophobic substrate. The

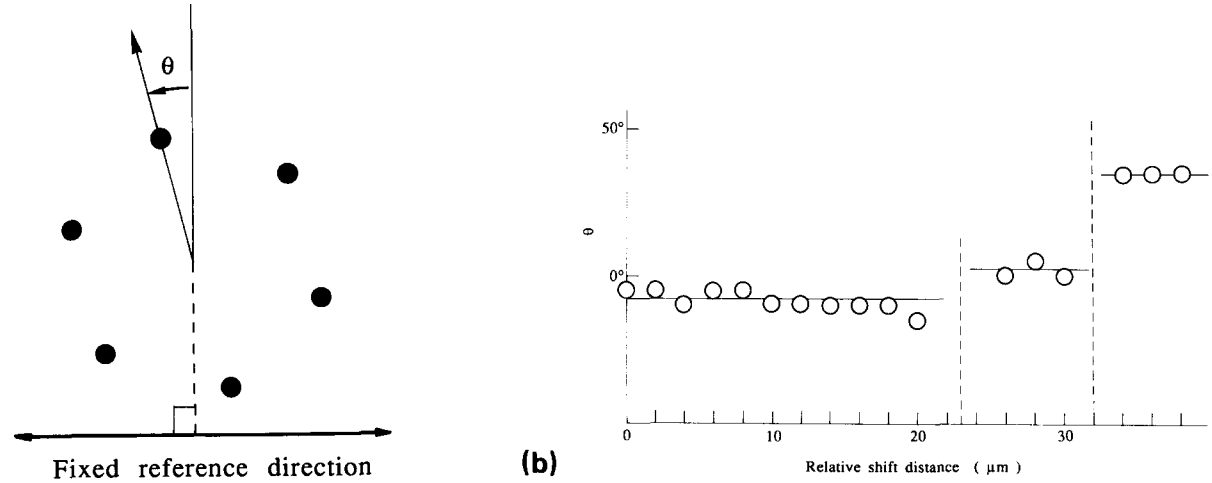

Fig. 2. Rotation angle θ of diffraction pattern in (a) plotted against the relative shift distance of the area giving rise to the diffraction pattern for monolayer transferred onto hydrophobic surface (b). θ was determined by use of a fixed reference direction printed on the film together with each diffraction pattern as shown in (a), and the distance was measured from the an area chosen arbitrary. Vertical broken lines in (b) show the area at which θ changes abruptly, where the diffraction pattern is a complex one composed of overlapped several sets of hexagonal reflections implying a 'domain boundary'.

diffraction pattern from the monolayer on the hydrophilic substrate was also a hexagonal one with the same dimension as for the monolayer on hydrophobic substrate. Although the monolayer again had a domain structure with the size of ≤ 10 μm, which was suggested by a discrete change in the lattice orientation, the orientation of crystal lattice (i.e., the rotation angle of the hexagonal reflection) changed slowly from place to place even within one domain. The rotation angle changed almost linearly with the relative shift distance by about 10 degrees at each shift distance of 2 μm. This means that the orientation of two-dimensional crystal lattice in one domain changes spatially. Therefore, a direction of the lipid crystal lattice in a domain may be represented by a set of lines with curvature for the case of the monolayer transferred onto the hydrophilic surface, whereas for the case of the monolayer on the hydrophobic substrate the direction remains constant within a domain. However, the transfer of monolayer onto the hydrophilic surface was very difficult for the system of pure water and lipid without doping. In the monolayer transferred onto a hydrophobic substrate from water surface, ends of hydrocarbon chains of lipid molecules, which form a monolayer on the water surface, may be anchored by the hydrophobic surface of substrate and the resulting molecular arrangement may not be changed in the course of water evaporation around polar head groups of lipid molecules. On the other hand, in a monolayer transferred onto a hydrophilic substrate from water surface, polar head groups of lipid molecules may be anchored onto the hydrophilic surface of substrate with some amount of water molecules attached. The water molecules seem to affect the molecular arrangement on the substrate during their evaporation. The resulting structures obtained here were mostly aggregations of lipid molecules or stacked layer structures restricting the homogeneous monolayer area rather small. The observed spatial change was obtained from the restricted areas of monolayer. As for the L-DPPC monolayer transferred onto a hydrophilic surface, therefore, further experiments are still necessary to clarify the detailed domain structure.

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