Detection of Phase Transitions in Monolayers Using Retardation of Evaporation of Water

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Received October 22, 1987; accepted October 6, 1989

The role of cholesterol in inducing the phase transitions in mixed monolayers with arachidyl alcohol and oleic acid has been studied by surface pressure and evaporation resistance measurements of the monolayers. The resistance of the monolayers to water evaporation indicates the effect of molecular interactions in mixed monolayers. The evaporation resistance of monolayers has been found to be a more sensitive parameter for studying the molecular interactions in mixed monolayers than the average area/molecule. A minimum of 15–20 mol% of cholesterol is required in a cholesterol–arachidyl alcohol mixture to completely fluidize the two-dimensional solid alcohol film. The evaporation resistance sharply decreases to a lower value at a molar ratio of 20:80 for cholesterol:arachidyl alcohol due to the phase transition in the monolayer. Cholesterol fluidizes the arachidyl alcohol monolayer by disrupting the association of saturated hydrocarbon chains of the alcohol. In the case of oleic acid–cholesterol mixed monolayers, 75 mol% of cholesterol is required to show a maximum solidifying effect. The evaporation resistance has been found to be maximum at this particular molar mixture. It is proposed that cholesterol molecules occupy the molecular cavities present in two-dimensional liquid oleic acid monolayers and thus reduce the evaporation of water.

INTRODUCTION

Cholesterol is one of the most abundant lipid components in the biological membranes (1). The abundance of cholesterol in the biomembranes makes it relevant to study its behavior with other lipid components in monolayers or bilayers. An excellent review has summarized the role of cholesterol in phospholipid membranes (2). The monolayers behave in more or less the same manner as the lipids in biological membranes (3). Small modifications in the phospholipid composition or cholesterol content in biomembranes are sufficient to alter membrane fluidity and to affect a number of enzymatic and cellular functions. A review highlighting the outcome of such modifications in membranes has appeared recently (4). Cholesterol, being a spacer molecule, imparts fluidity to the monolayers as well as occupying the molecular cavities present in the lipid monolayer (5).

The fluidity of hydrocarbon chains is an essential requirement for the existence of a bilayer (6–8). The presence of a high content of cholesterol in the erythrocyte membrane suggests that the cholesterol may be playing the role of biological plasticizer by increasing the flexibility or fluidity of the membrane. On the other hand, the increased cholesterol to phospholipid ratio in the cell membrane has been found to be responsible for imparting a rigidity to the membrane which inhibits many cellular functions (9, 10). The rigidifying effect of cholesterol is measured by determining membrane viscosity (η) from fluorescence measurements (11). In one study, fluorescence measurements made with a probe indicated that increasing cholesterol content restricts molecular motion in the hydrophobic portion of the membrane lipid bilayer (12).

This paper presents our studies on the role

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0021-9797/90 $3.00

of cholesterol in inducing phase transitions in mixed monolayers as evidenced by surface pressure–area and evaporation resistance measurements. The water molecule, having a diameter of about 3 Å, promises to be an important probe for studying molecular packing and molecular interactions in mixed monolayers (13, 14).

MATERIALS

Cholesterol (purity = 99+%) was purchased from Sigma Chemical Co. Arachidyl alcohol and oleic acid were also Sigma products. All the chemicals and solvents used were of high purity grade and were used without any further purification. The cholesterol concentration was 1 mg/ml in the mixed solvent system of chloroform + methanol + *n*-hexane (1:1:3, v/v/v). Equimolar solutions of oleic acid and arachidyl alcohol, like that of cholesterol in the same solvent mixture, were prepared. Granular anhydrous $CaCl_2$, used for evaporation rate measurements, was an MC/B (Ohio) product. Twice-distilled water was used throughout the entire study.

METHODS

Surface Pressure Measurements

The Wilhelmy plate method was used with slight modifications (15). The surface pressure was measured with a sand-blasted rectangular platinum plate (5-cm perimeter) suspended from a pressure transducer connected to a universal transducer readout (Statham). The pressure transducer was mounted on an adjustable elevating stand. A Lucite trough of 570-ml capacity was used as a Langmuir trough. The temperature of the trough was maintained at 23 ± 1°C. The volume of the water in the tray was kept constant. The lipid solution was spread on the surface (initial area = 360 cm^2) by means of an "Agla" microsyringe (Burroughs Wellcome Laboratories, UK). In order to remove the possible surface-active impurities from the surface, the surface was cleaned by an external suction pump before spreading a monolayer. The area available per molecule in mixed monolayers was calculated as described elsewhere (5).

Evaporation Resistance Measurements

The rate of evaporation was measured (16) by the increase in weight of the desiccant, anhydrous $CaCl_2$, which was always kept at 3 mm above the water surface. By this technique, the rates of evaporation of water through the monolayers of fatty acids, esters, and alcohols were measured (17, 18). Evaporation resistances from these values were obtained from the diffusion theory utilizing Fick's Law, which predicts that the rate of mass transfer is proportional to the reciprocal of the thickness of the diffusion layer. The resistance values were calculated from

$$r = \left(A(w_w^0 - w_d^0)\frac{t}{M}\right)_{\text{film}} - \left(A(w_w^0 - w_d^0)\frac{t}{M}\right)_{\text{no film}},$$

where A is the area of the desiccant surface (cm^2), w_w^0 is the concentration of water vapor in equilibrium with the liquid water (g/cm^3), w_d^0 is the concentration of water vapor in equilibrium with the desiccant surface (g/cm^3), t is the time of absorption, and M is the mass of water vapor absorbed. The resistance is expressed in s/cm. A detailed account of the evaporation resistance calculations is described elsewhere (16). The various theories for evaporation resistances and their validity have been critically examined by Barnes in a recent review article (19).

RESULTS AND DISCUSSION

Cholesterol–Arachidyl Alcohol Monolayers

Arachidyl alcohol and cholesterol (5) are known to form condensed monolayers (i.e., steep surface pressure–area curves). Similarly the mixed monolayer of arachidyl alcohol and cholesterol is also condensed.

The limiting or collapse areas for arachidyl alcohol and cholesterol have been found to be

around 20 and 40 Å^2, respectively, which is close to their reported values in the literature (20, 5). Figure 1 shows the average area per molecule plotted against the mole fraction of arachidyl alcohol and cholesterol at various surface pressures. The experimental points are close to the simple additivity rule (broken line). The arachidyl alcohol monolayer is in a two-dimensional *solid state,* whereas the cholesterol monolayer is in a two-dimensional *liquid state* as inferred from the movement of talc particles sprinkled on the monolayer under a stream of air (5). The strong interactions between molecules in a solid monolayer allow less water molecules to escape than in a monolayer in the liquid state. Therefore the evaporation rate of water from the arachidyl alcohol is much lower than that from the cholesterol monolayer. This results in a high resistance to evaporation through arachidyl alcohol against the cholesterol monolayer (Fig. 2). It can be seen that a striking change in evaporation occurs at a 20:80 mixture. This shows that only 20 mol% of cholesterol completely *fluidizes* a two-dimensional solid film into a liquid film. The bar in the graph represents the uncertainty of the resistance values.

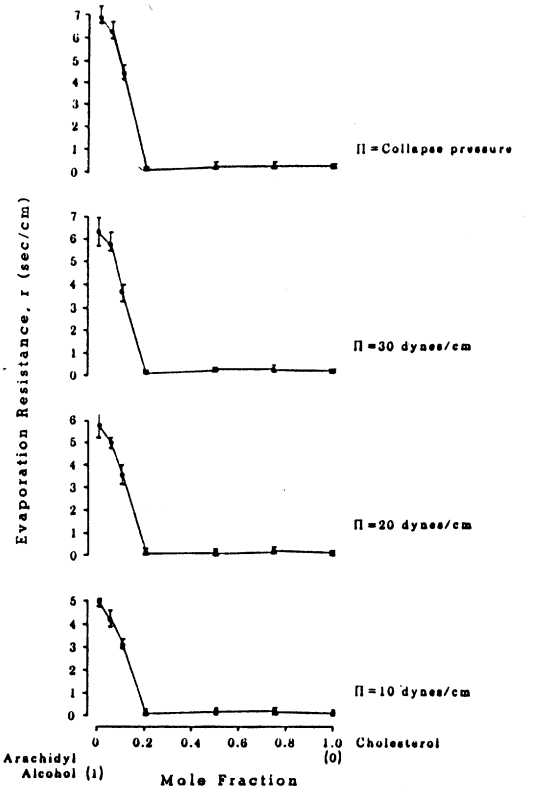

FIG. 2. Evaporation resistances for arachidyl alcohol–cholesterol monolayers at various surface pressures.

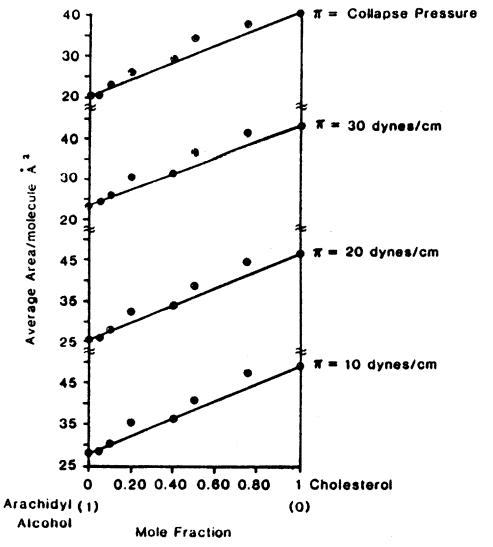

FIG. 1. Average area/molecule of arachidyl alcohol–cholesterol at various surface pressures.

Cholesterol molecules being asymmetric in shape (almost planar in structure) disrupt the ordered structure of arachidyl alcohol monolayers. The schematic representation of this effect is shown in Fig. 3. It should be emphasized that the average area/molecule does not show the phase transition (Fig. 1), which is clearly seen by evaporation data (Fig. 2). Thus the evaporation through a monolayer is a more sensitive parameter for detecting phase transitions in monolayers than the average area/molecule.

Cholesterol–Oleic Acid Monolayers

Oleic acid forms a highly expanded liquid monolayer (13). The surface pressure–area curves for the cholesterol–oleic acid mixture are also expanded. Most likely, this is due to the presence of *cis*-double bonds in the oleic

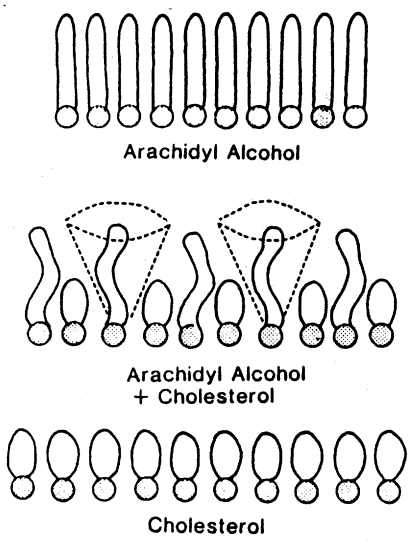

FIG. 3. Schematic representation of the molecular arrangement in arachidyl alcohol–cholesterol monolayers. The cones in the mixed monolayer represent the space occupied by the thermal motion of the hydrocarbon chains of alcohol.

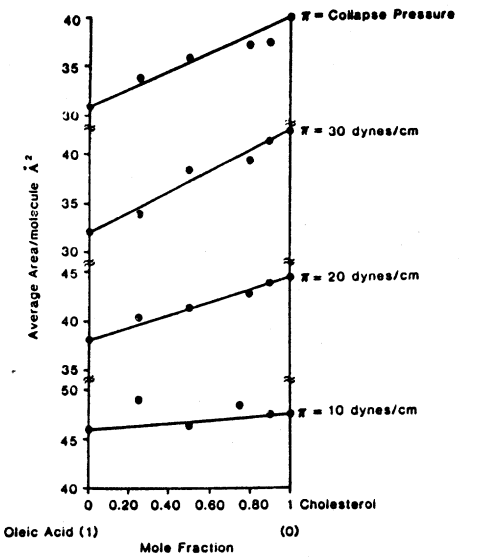

FIG. 4. Average area/molecule of oleic acid–cholesterol monolayers at various surface pressures.

acid monolayer which form compressible, highly expanded films.

The limiting or collapse area for oleic acid has been found to be around 32 $Å^2$/molecule. Figure 4 shows the average area/molecule, plotted against the mole fraction of oleic acid and cholesterol at various surface pressures. The area/molecule obeys the simple additivity rule in mixed monolayers.

Figure 5 shows the evaporation resistance values for mixed oleic acid–cholesterol monolayers plotted against the mole fraction of the components. Both oleic acid and cholesterol form two-dimensional *liquid* monolayers which allow a large number of water molecules to diffuse through them. From Fig. 5 it is very clear that as the molar ratio of cholesterol and oleic acid reaches 3:1, the tightest molecular packing (or the maximum lateral interaction in the hydrocarbon portion of the monolayers) is observed, as reflected by the maximum in evaporation resistance values. The bar in the graph represents the uncertainty of the resistance values. The concept of molecular cavities (5) in monolayers can be used to explain this tight packing in oleic acid–cholesterol mixed monolayers. Because of the presence of *cis*-double bonds and the thermal motion of the fatty acyl chains in the oleic acid monolayers,

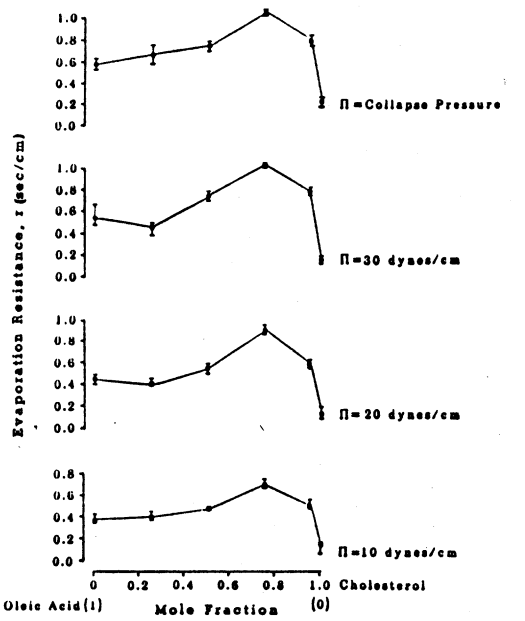

FIG. 5. Evaporation resistances of oleic acid–cholesterol monolayers at various surface pressures.

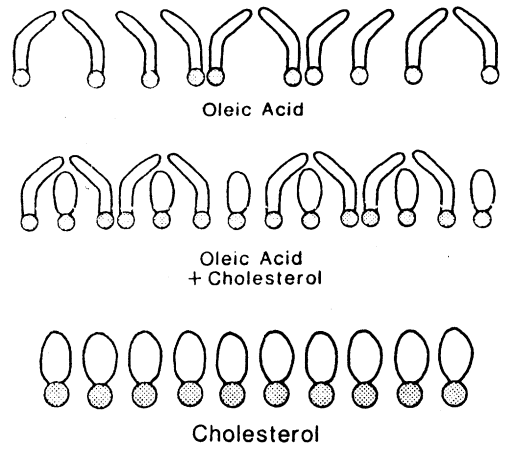

FIG. 6. Schematic representation of the molecular arrangement in oleic acid–cholesterol monolayers. Cholesterol occupies the molecular cavities present in the mixed monolayer.

a vacancy or "cavity" is created between the oleic acid molecules. The cholesterol molecules having a smaller height and an asymmetric structure thus partly occupy these molecular cavities, which results in a higher resistance of mixed monolayer toward the evaporation of water (Fig. 6). These results show the importance of the specific molar ratio of 1:3 for oleic acid–cholesterol in mixed monolayers for the maximum lateral interaction. It is interesting that the maximum condensation of egg lecithin–cholesterol monolayers was also observed at the 1:3 and 3:1 ratios (5, 22). Shah (23) has proposed a hexagonal geometrical arrangement of lipid molecules at the interface to explain the optimum behavior of several mixed surfactant systems at the 1:3 molar ratio.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Rotary International Foundation student exchange program which enabled Y.K.R. to conduct this research at the University of Florida. Y.K.R. also thanks the Center for Surface Science and Engineering for providing the laboratory facilities.

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