Interaction of Differently Oriented Lipids in Monolayer: Mixed Monolayers of 16-(9-Anthroyloxy)palmitic Acid with Phosphatidylcholine and Cholesterol

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ABSTRACT: 16-(9-Anthroyloxy)palmitic acid (16-AP) is a bifunctional molecule with carboxyl and 9-anthroyloxy groups attached at both ends of the hydrocarbon chain. At the air–water interface, in a monolayer, the 16-AP molecule has horizontal and vertical orientations, depending on the surface pressure of the monolayer. The miscibilities of 16-AP with dimyristoylphosphatidylcholine (DMPC), cholesterol (CH), and fatty acids in mixed monolayers were evaluated in investigations of monolayer phase transitions. Lipid molecules with flexible hydrocarbon chains, i.e., DMPC and fatty acids, formed homogeneous mixed monolayers with horizontally oriented 16-AP. On the other hand, the rigid molecule, CH, could not accommodate the horizontally oriented 16-AP in a monolayer, and there was a phase separation from 16-AP. In biological and reconstituted membranes, preferential binding of phospholipid to the integral protein and exclusion of cholesterol in close vicinity of the membrane protein have been recognized. On the basis of this work, it can be expected that flexible lipids readily accommodate the rough hydrophobic surface of integral proteins and stabilize the structure of the protein, while rigid lipids such as cholesterol are removed from the immediate environment of the membrane protein, if the protein does not interact specifically with the rigid lipids.

Phosphatidylcholine and cholesterol are flexible and rigid lipid components, respectively, constituting cell membranes. Cholesterol has a considerable influence on membrane fluidity and function (Demal & deKruyff, 1976). The enzymatic activity of membrane proteins seems to closely correlate with the fluidity (Kates & Kuksis, 1980; Shinizky & Henkart, 1979). The membrane viscosity is considered to determine the rate of membrane protein function (Yuli et al., 1981); however, when reconstituted membrane protein systems have been examined, little support has been obtained for this idea. In detailed investigations on the interaction of lipid and membrane protein, specific binding of lipid to membrane protein and the local environment around the protein were suggested to have important roles in protein activity (Warren et al., 1974; Johannsson et al., 1981; East & Lee, 1982; Simmonds et al., 1984). Jost et al. (1973) demonstrated the existence of motionally restricted lipid in the immediate vicinity of an integral protein. A variety of phospholipids and nonphospholipids have been investigated to elucidate the preferential interaction with the protein (Boggs et al., 1977; Griffith et al., 1982; Brophy et al., 1984; Knowles et al., 1981; Marsh et al., 1982; Warren et al., 1975; Silivius et al., 1984; Simmonds et al., 1984). These studies showed that the phospholipids and fatty acids had higher affinities for the membrane protein than did cholesterol and the steroids. The integral proteins in membranes have rigid structures and irregular rough hydrophobic surfaces that are assumed to influence the lipid–protein interaction.

To study the different behaviors of flexible and rigid lipids in membrane, mixed monolayer membranes of 16-(9-anthroyloxy)palmitic acid (16-AP) with dimyristoylphosphatidylcholine (DMPC) and with cholesterol (CH) were investigated at air/water interfaces. The monolayer is regarded to be half of the structure of the bilayer leaflet and can be manipulated, under controlled conditions. The 16-AP molecule is a bifunctional one with 9-anthroyloxy and carboxyl groups at both ends of a hydrocarbon chain, as shown in Figure 1. In the monolayer, this molecule has two different orientations, horizontal and vertical, depending upon the surface pressure. Phosphatidylcholine, cholesterol, and fatty acids have only vertical orientation in the monolayer. The miscibility and preferential interaction of monolayer components have been evaluated by investigations of phase transitions in the monolayer systems. Surface solution theory and the surface phase rule were considered to be potent tools for elucidating the interactions between monolayer components (Nakagaki & Funasaki, 1974; Handa & Nakagaki, 1979; Gershfeld & Pagano, 1972a,b; Pagano & Gershfeld, 1972).

Experimental Procedures

Materials. 16-(9-Anthroyloxy)palmitic acid (16-AP) was purchased from P-L Biochemicals Inc. L-α-Dimyristoylphosphatidylcholine (DMPC) and cholesterol (CH) were supplied by Sigma Chemical Co. Chloroform and benzene used in the spreading of lipid mixtures on the surface were redistilled.

Measurement. 16-AP and the mixtures of 16-AP and CH were dissolved in benzene. DMPC and the mixtures of DMPC and 16-AP were dissolved in the mixed solvent of benzene and chloroform (4:1). The solutions of lipid and lipid mixture were supplied from an Agla micrometer syringe on the double-distilled water with a pH of 5.8 in the Teflon-coated duralmin trough. The monolayer spread was left for 5 min for evaporating the solvent. The area per molecule of insoluble lipid or the average area per molecule of mixed lipid in monolayer, A (10^{-2} nm^2·molecule^{-1}) was calculated as $A = S/(nN_A)$, where S is the area of the aqueous surface between two movable barriers, n is the number of moles of insoluble lipid spread in the area S, and N_A is the Avogadro number. A is, therefore, changed by shifting the barrier. The surface pressure, F (mN/m), was measured by Whilhelm's plate method. The torsion balance used was Shimadzu T-NR. Temperature was kept constant at 25 °C by circulating the thermostated water through glass tubing immersed in the

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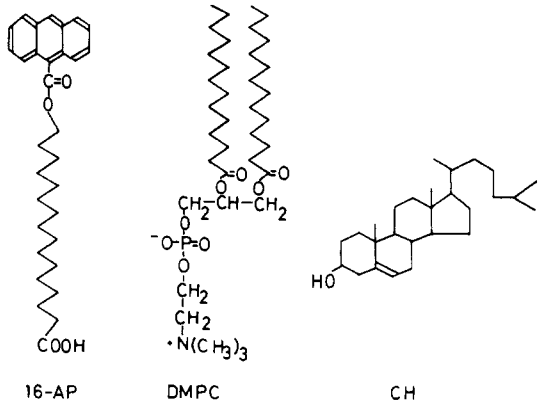

FIGURE 1: Chemical structures of 16-AP, DMPC, and CH.

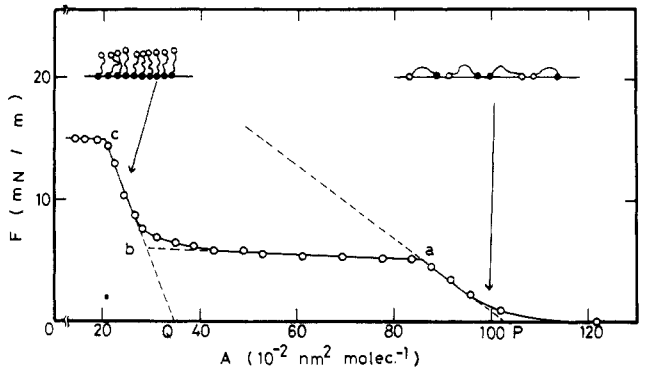

FIGURE 2: Surface pressure–area per molecule curve ($F - A$ curve) of 16-AP monolayer on double-distilled water at 25 °C. The orientations of 16-AP are schematically represented. The kink points indicate the phase transition (F = 4.8 mN/m) and the collapse (F = 14.8 mN/m) of the monolayer of 16-AP. The orientations of 16-AP are schematically represented. The open and closed circles represent the 9-anthroyloxy and carboxyl moieties, respectively.

trough. A ground plate of quartz was used after it had been cleaned in fuming nitric acid for more than one night and rinsed well with double-distilled water. In the monolayer containing an acidic lipid such as 16-AP and fatty acid, a negative surface potential developed (Fromherz & Masters, 1974). In distilled water, the surface potential is ca. −250 to −300 mV; thus, the surface has a pH of 4–5 units lower than the bulk value. The major part of 16-AP molecules in monolayer is, therefore, in a protonated state (–COOH) and gives a stable $F - A$ curve, as shown under Results. However, the PA monolayer on distilled water with a pH of 5.8 was not stable at a surface pressure above 20 mN/m, and the transitions of mixed monolayer of 16-AP and PA were observed at a surface pressure lower than 16 mN/m. Measurements were according to Gains (1966).

RESULTS

Monolayer of 16-AP. The surface pressure–area per molecule curve ($F - A$ curve) of 16-AP on distilled water is shown in Figure 2. The $F - A$ curve exhibited two kink points at 4.8 and 14.8 mN/m. It is noteworthy that at the lower surface pressure region ($F < 4.8$ mN/m), the area per molecule of 16-AP, A, is large, while in the region with $F > 4.8$ mN/m, the monolayer is considerably compressed. These findings suggested a significant phase transition at the first kink point. Further compression of the monolayer beyond the second kink point (at F = 14.8 mN/m) to the A value of less than 20×10^{-2} nm^2 molecule^{-1} did not alter the F value; therefore, the collapse of the monolayer is assumed to occur at the second kink point (Nakagaki & Funasaki, 1974).

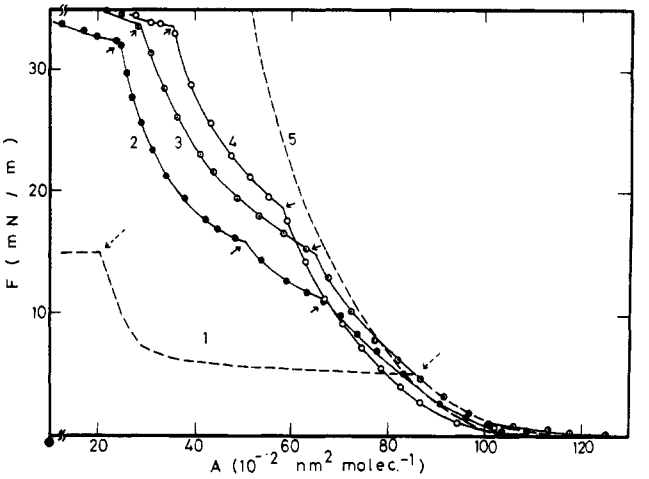

FIGURE 3: $F - A$ curves of mixed monolayer of 16-AP and DMPC at 25 °C. The 16-AP mole fraction: 1:1.0, 2:0.6, 3:0.5, 4:0.4, and 5:0. The arrows indicate the phase transition and collapse of the mixed monolayer.

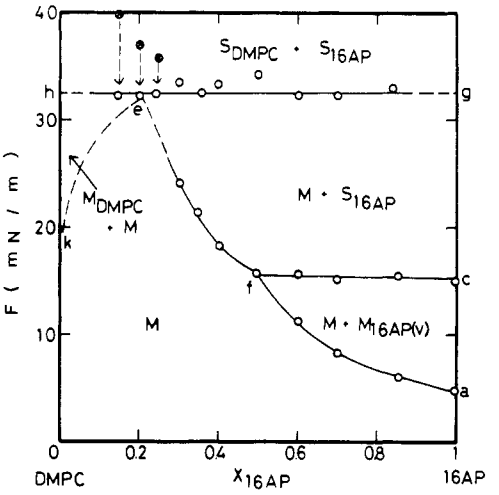

FIGURE 4: F values of kink point in mixed monolayer of 16-AP and DMPC are presented as a function of the mole fraction. The F values indicated by (⊗) were unstable and decreased to the stable values (○). The solid line af was theoretically calculated by eq 5 with f_{16AP} = 1 ($\Delta\delta$ = 0). M, mixed monolayer of DMPC and horizontally orienting 16-AP; $M_{16AP(v)}$, monolayer of 16-AP in vertically orienting state: S_{16AP}, solid phase of 16-AP formed by the collapse of $M_{16AP(v)}$; M_{DMPC}, monolayer of DMPC; S_{DMPC}, bulk lamellar phase (liquid-crystalline state) of DMPC.

Mixed Monolayer of 16-AP and DMPC. The surface pressure F of the mixed monolayers of 16-AP and DMPC of various compositions was measured, and some are shown in Figure 3. The mixed monolayers had two or three kink points on the $F - A$ curve. The F values of the lowest kink point were dependent on the monolayer composition, while the F values of the highest and second kink points (observed at F = 33 and 14.8 mN/m, respectively) were constant, irrespective of the monolayer composition. The F value of the second kink point was equal to the collapse pressure of the 16-AP monolayer. The relationships between the surface pressure of the kink point and the monolayer composition are shown in Figure 4. In Figure 5, the area per molecule of the mixed monolayer, A, is shown as a function of the mole fraction, X, at the F values of 4, 10, 20, and 30 mN/m. The kinks seen in this figure also indicate the significant phase transitions in the 16-AP–DMPC mixed monolayer.

Mixed Monolayer of 16-AP and CH. The $F - A$ curves of 16-AP and CH mixed monolayer were measured, and some are shown in Figure 6. The mixed monolayer had three kink

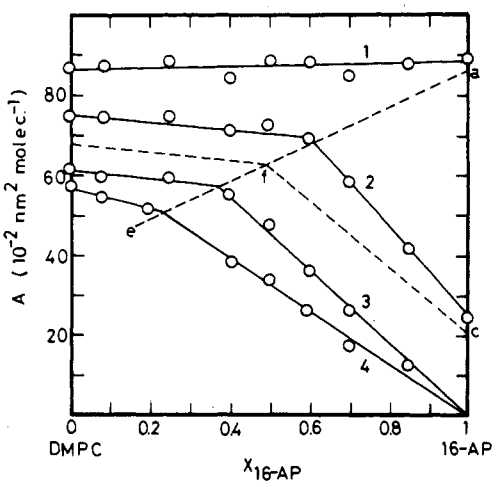

FIGURE 5: Surface area per molecule–composition correlations of 16-AP–DMPC mixed monolayer. Points a, c, and f on this diagram correspond to those in Figure 4. 1, F = 4 mN/m; 2, F = 10 mN/m; 3, F = 20 mN/m; 4, F = 30 mN/m.

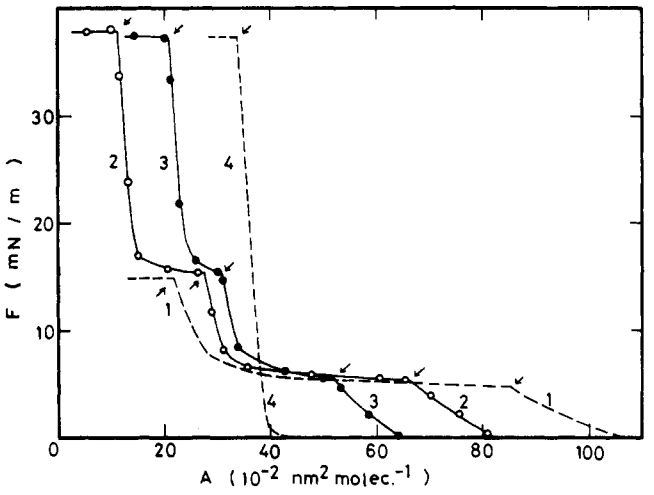

FIGURE 6: $F - A$ curves of mixed monolayer of 16-AP and CH at 25 °C. The 16-AP mole fraction: 1:1.0, 2:0.62, 3:0.35, and 4:0. The arrows indicate the phase transition (F = 4.8 mN/m) and the collapses (F = 14.8 and 38 mN/m) of monolayer.

points, all of which were completely independent of the monolayer composition. The F values of the lowest and middle kink points were equal to those of the phase transition and the monolayer collapse of pure 16-AP, respectively. The F value of the highest one was equal to the collapse pressure of the pure CH monolayer (F = 38 mN/m; Nakagaki & Handa, 1976). These results are summarized in Figure 7. In Figure 8, the areas per molecule of the mixed monolayer which was composed of 16-AP and CH are shown by F = 4 and 14 mN/m. The linear relations between A and X show that the additivity in area per molecule, A, of the mixed monolayer is retained in this mixture.

Discussion

Orientation of 16-AP in Monolayer. From the results in Figure 2, two states of the monolayer of 16-AP have to be considered. The compressibility of monolayer, β, which is usually defined as

$$\beta = -(1/A)(\partial A/\partial F)_{T,P} \quad (1)$$

is calculated. In the lower surface pressure (F < 4.8 mN/m) and the higher surface pressure (F = 5–14.8 mN/m) regions, β was found to be 0.033 ± 0.008 and 0.034 ± 0.003 m/mN, respectively. This would imply that almost all the intramolecular motions remain thawed during the phase transition at

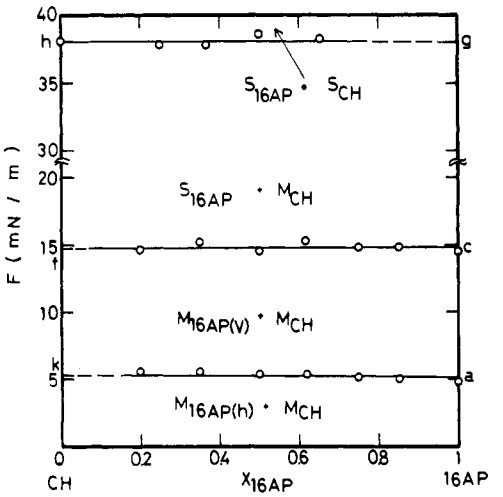

FIGURE 7: F values of kink point in mixed monolayer of 16-AP and CH are presented as a function of the composition. $M_{16AP(h)}$, monolayer of 16-AP in horizontally orienting state; $M_{16AP(v)}$, monolayer of 16-AP in vertically orienting state; S_{16AP}, solid phase of 16-AP; M_{CH}, monolayer of CH; S_{CH}, solid phase of CH formed by the collapse of M_{CH}. The diagram indicates that 16-AP in both horizontally and vertically orienting states does not mix with the CH molecule in the monolayer membrane.

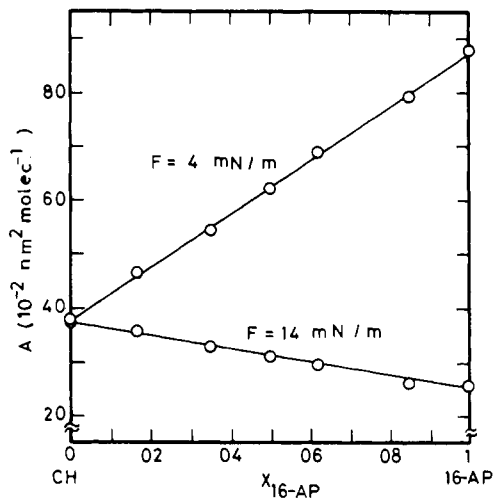

FIGURE 8: Surface area per molecule–composition correlations of the 16-AP–CH mixed monolayer.

4.8 mN/m. The difference in the A values between the two states is related to the difference in orientation of the bifunctional molecule in the monolayer, that is, horizontal and vertical. The extrapolated A values to F = 0 mN/m of the two states (P and Q in Figure 2) were 103 × 10^{-2} and 34.5 × 10^{-2} nm^2 molecule^{-1} and in accord with the horizontal and vertical orientations of 16-AP, respectively. Fluorescence quenching and the resonance energy transfer methods have been used to investigate the orientation of a series of *n*-(9-anthroyloxy) fatty acids in lipid bilayers. –COOH was thus shown to be located at the membrane surface (Haigh et al., 1979; Thulborn & Sawyer, 1978). These results suggest that in the vertical orientation of the 16-AP in the monolayer, –COOH is anchored in the aqueous subphase.

When the equilibrium spreading pressure of 16-AP was measured, we found that the value is equal to that of the second kink point in the $F - A$ curve of 16-AP (F = 14.8 mN/m). Thus, the equilibrium spreading pressure is the surface pressure of the lipid monolayer in equilibrium with its (three-dimensional) bulk phase (Nakagaki & Handa, 1976). This finding provides good evidence that the collapse of the 16-AP mono-

Table I: Compressibility of Monolayer, β, Molar Volume of Hydrocarbon Moiety, V_i, Surface Solubility Parameter, δ, Difference of δ, $\Delta\delta$, and Its Critical Value, $\Delta\delta_c$

	16-AP	DMPC	PA	SA	CH
β (mN/m) at F = 1–5 mN/m	3.4×10^{-2}	3.3×10^{-2}	7.5×10^{-3}	7.5×10^{-3}	4.2×10^{-3}
V_i (cm^3/mol)	279	492	279	311	361
$\Delta\delta_c$ ($cal^{1/2}$ $cm^{-3/2}$)		1.8	2.1	2.0	1.9
δ ($cal^{1/2}$ $cm^{-3/2}$)	6.0	6.0	7.0	6.9	4.9
$\Delta\delta$ ($cal^{1/2}$ $cm^{-3/2}$)		0.0	1.0	0.9	1.1
miscibility with 16-AP		miscible	miscible	miscible	immiscible

layer occurs at the second kink point of F = 14.8 mN/m.

Miscibility of Mixed Monolayer. The miscibility of insoluble lipid components in the mixed monolayer can be evaluated on the basis of the surface phase rule (Defay et al., 1966; Gershfeld & Pagano, 1972a,b; Pagano & Gershfeld, 1972; Handa & Nakagaki, 1979). When temperature and pressure (of the atmosphere) remain constant, the degree of freedom, as is defined in Gibbs' phase rule, is described as follows for the monolayer system:

$$f = c - p + 1 \quad (2)$$

Here, c is the number of insoluble components, and p is the number of phases (except air and aqueous phases). In the binary lipid mixture, c = 2 and $f = 3 - p$. Along the horizontal lines observed in the $F - X$ relations of Figures 4 and 7, the F value is independent of the monolayer composition, X, and therefore, f = 0. From eq 2, p is found to be 3; that is, three phases in equilibrium coexist on the horizontal lines in Figures 4 and 7.

Figure 4 is regarded to be the phase diagram representing the monolayer equilibrium of the 16-AP–DMPC mixture. In the region designated by M, there is mixed monolayer of DMPC and the horizontally oriented 16-AP. $M_{16AP(h)}$ and $M_{16AP(v)}$ indicate the monolayer of 16-AP in the horizontally and vertically orienting states, respectively. S_{16AP} is the solid phase of 16-AP and is formed by the collapse of $M_{16AP(v)}$. Coexisting in the region designated by M + S_{16AP} are the mixed monolayer and the collapsed solid phase of 16-AP. From the diagram shown in Figure 4, the components, DMPC and 16-AP, are miscible in the mixed monolayer of M. On the horizontal line heg, the system consists of the mixed monolayer e, which contains approximately 80% of DMPC, the solid phase of 16-AP, S_{16AP}, and the bulk phase of DMPC, S_{DMPC}. Point e is a point very similar to the eutectic point. The physical state of S_{DMPC} is lamellar in the liquid-crystalline state (T. Handa et al., unpublished results). The distinct kinks on the $A - X$ relations in Figure 5 also imply a significant change in the state of the mixed monolayer. The rapid decrease in the A value at the higher 16-AP mole fraction observed in line 2 is due to the separation of the monolayer of 16-AP in the vertical state from the mixed monolayer M as M → M + $M_{16AP(v)}$. A similar decrease in the A value seen on lines 3 and 4 arises from the collapse of 16-AP from the mixed monolayer as M → M + S_{16AP}. Here, the A values are linearly extrapolated to be zero at the 16-AP mole fraction of 1.0.

In the same manner, the surface phase rule was applied to the mixture of 16-AP and CH. On the horizontal lines in Figure 7, the three phases are in equilibrium. At the lowest link ak, the monolayer of pure 16-AP in the horizontal orientation, $M_{16AP(h)}$, is transformed into the monolayer of the vertically oriented 16-AP, $M_{16AP(v)}$. The constant surface pressure of this transition point, irrespective of the composition, clearly indicates that the 16-AP molecules (both horizontal and vertical orientations) are completely separated from the cholesterol molecules in the monolayer. On this horizontal line in Figure 7, three monolayers, that is, $M_{16AP(h)}$, $M_{16AP(v)}$, and the cholesterol monolayer M_{CH}, coexist on an air–water interface. The second transition at F = 14.8 mN/m is due to the collapse of $M_{16AP(v)}$ to S_{16AP}. The highest transition gh at 38 mN/m represents collapse of the cholesterol monolayer into the solid phase of CH, S_{CH}. The value of the surface pressure was equal to the equilibrium spreading pressure of cholesterol. The independence of these phase changes, irrespective of the monolayer composition, also implies that CH is completely immiscible with 16-AP in the monolayer. The simple additivity of the area per molecule of CH and 16-AP in the monolayer observed in Figure 8 is consistent with the formation of individual monolayers of these components (Gains, 1966). Thus, the bifunctional molecule, 16-AP, in horizontal orientation is miscible with phosphatidylcholine but immiscible with cholesterol, CH, in monolayer. These conclusions are schematically represented in Figure 9.

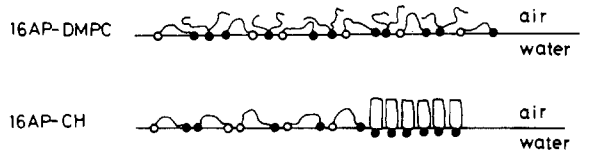

FIGURE 9: Schematic representation of the mixed monolayers of 16-AP–DMPC and 16-AP–CH. The 16-AP molecule is represented by open (anthroyloxy moiety) and closed (carboxyl moiety) circles attached at both ends of the solid line (hydrocarbon chain). In the 16-AP–CH mixed monolayer, the components are separated.

Surface Solution Theory. Gershfeld and Pagano (Gershfeld & Pagano, 1972a,b; Pagano & Gershfeld, 1972) introduced the surface regular solution theory in mixed monolayer to evaluate the miscibility of insoluble components, in the monolayer state. In this theory, the mixed monolayer was assumed to be a combination of the liquid hydrocarbon layer and the polar head group layer [Langmuir's duplex film model (1939)]. They concluded that when specific interactions between polar head groups [such as the Ca-induced association of acidic phospholipids (Ohnishi & Ito, 1974)] were not significant, the miscibility of insoluble components was determined by the interaction of the hydrocarbon moieties in the oily layer. The free energy was evaluated by using surface solubility parameters for the hydrocarbon moieties, δ. The δ value represents the strength of the intermolecular interaction between the hydrocarbon moieties in monolayer. The values were assessed to be 5.7–6.0 $cal^{1/2}$ $cm^{-3/2}$ for lipids displaying the liquid expanded monolayer and 6.9–7.0 $cal^{1/2}$ $cm^{-3/2}$ for those showing the liquid condensed monolayer, regardless of the chain length and the degree of saturation of the hydrophobic moieties. Cholesterol had the value of 4.9 $cal^{1/2}$ $cm^{-3/2}$ (Gershfeld & Pagano, 1972a,b; Pagano & Gershfeld, 1972).

Table I shows the compressibilities of monolayers investigated in this study (F = 1–5 mN/m). The β values indicate that 16-AP and DMPC form the liquid expanded monolayers, and palmitic acid (PA), stearic acid (SA), and cholesterol give the liquid-condensed monolayers. Cholesterol with the smaller

value of δ shows the condensed monolayer because of the limited intramolecular motions of this four-ring molecule. The molecules with a flexible hydrocarbon chain have large free volumes (areas) in the monolayer state and may be present as a liquid-expanded monolayer. The lipids with a remarkably long chain such as palmitic and stearic acids at 25 °C, however, have large cohesive forces exceeding the molecular motions, and liquid-condensed monolayers are formed (Gains, 1966).

The miscibility of lipid components in the mixed monolayer is estimated on the basis of the parameter $\Delta\delta$ as (Hildebrand & Scott, 1950)

$$\Delta\delta = |\delta_{16AP} - \delta_i| \quad (3)$$

Here, δ_{16AP} and δ_i are the solubility parameters of 16-AP and the i component, respectively, in monolayer. In the 16-AP monolayer of the horizontal state, $M_{16AP(h)}$, the carboxyl and 9-anthroyloxy groups are in contact with the aqueous phase, and only the hydrocarbon chain is considered to contribute to the formation of the liquid oily layer of the duplex film. This liquid-expanded monolayer has the value of 6.0 $cal^{1/2}$ $cm^{-3/2}$. The value of DMPC is the same as that of myristic acid in the liquid-expanded monolayer state, and δ_{DMPC} = 6.0 $cal^{1/2}$ $cm^{-3/2}$. The monolayers of PA and SA are in liquid-condensed states, and the δ values of these components are 7.0 and 6.9 $cal^{1/2}$ $cm^{-3/2}$, respectively (Gershfeld & Pagano, 1972a,b; Pagano & Gershfeld, 1972).

The critical value of $\Delta\delta$ for the stability of binary-mixed monolayer vs. the phase separation, $\Delta\delta_C$, is given according to (Hildebrand & Scott, 1950; Flory, 1953)

$$\Delta\delta_C = (RT/2)^{1/2}\left[\frac{V_{16AP}^{1/2} + V_i^{1/2}}{(V_{16AP}V_i)^{1/2}}\right] \quad (4)$$

Here, V_i is the molar volume of the hydrocarbon moiety of the i component and is estimated from the molar volume of the equivalent aliphatic hydrocarbon (Gershfeld & Pagano, 1972a,b; Pagano & Gershfeld, 1972). When the value of $\Delta\delta$ is larger than that of $\Delta\delta_C$, phase separation occurs. The $\Delta\delta$ and $\Delta\delta_C$ values calculated with eq 3 and 4 are shown in Table I. For mixed monolayers of 16-AP–DMPC, –PA, –SA, and –CH, the $\Delta\delta$ values are considerably lower than those of $\Delta\delta_C$, as seen in Table I.

These calculations indicate that the 16-AP molecule of horizontal orientation is miscible with DMPC, PA, SA, and also CH, in the mixed monolayer. Experimentally, however, CH is completely immiscible with 16-AP (see Figures 6 and 7). With this theory, the insoluble components in monolayer are assumed to constitute duplex films. Cholesterol is a rigid molecule and does not seem to fit the irregular surface of the horizontally oriented 16-AP molecule. CH, therefore, does not form the mixed oily liquid layer of the duplex film with 16-AP. On the other hand, PA, SA (data not shown), and DMPC have flexible long hydrocarbon chains to accommodate the horizontally oriented molecule, 16-AP, in the mixed oily liquid layer.

On the line af in Figure 4, the separation of $M_{16AP(v)}$ from the mixed monolayer M of 16-AP(h) and DMPC, M → M + $M_{16AP(v)}$, occurs. In this case, the surface pressure of transition, F, is presented according to (Defay et al., 1966; Nakagaki & Handa, 1976; Handa & Nakagaki, 1976)

$$F = F_0 - [kT/(A_h - A_v)] \times [\ln (f_{16AP}\,\phi_{16AP}) + (1 - V_{16AP}/V_{DMPC})(1 - \phi_{16AP})] \quad (5)$$

where f_{16AP} may be given by

$$\ln f_{16AP} = [V_{16AP}/(RT)]\Delta\delta^2(1 - \phi_{16AP})^2 \quad (6)$$

Here, F_0 is the surface pressure at the transition of pure 16-AP monolayer and is 4.8 mN/m. k is the Boltzmann constant, and A_h and A_v are the areas per molecule of 16-AP in the monolayers of horizontal and vertical states at the corresponding F value and are estimated on the extension of the line Pa and the line Qbc, respectively, in Figure 2. ϕ_{16AP} is the volume fraction of hydrophobic moiety of 16-AP in the liquid hydrocarbon layer of the duplex film. The $\Delta\delta$ value of the 16-AP–DMPC mixture is 0, as seen in Table I; therefore, f_{16AP} = 1. The $F - X_{16AP}$ relation calculated from eq 5 with f_{16AP} = 1 agreed well with line af in Figure 4, as obtained experimentally. This calculation indicates that the $\Delta\delta$ value of the 16-AP–DMPC mixed monolayer, M, is actually 0 as expected by eq 3.

With the vertically oriented 16-AP molecule, CH, DMPC, PA, and SA are immiscible in monolayer, as a result of steric hindrance in the close packing of the rigid aromatic group of 16-AP with the hydrocarbon chain or steroid ring and also from the probable increase in δ value of 16-AP with contribution of the 9-anthroyloxy moiety located in the uppermost layer of monolayer to the molecular interaction.

Cholesterol in Biological Membrane. The preferential interactions of lipid with membrane proteins have been investigated by using myelin proteolipid apoprotein (Boggs et al., 1977; Brophy et al., 1984) and Ca^{2+}-ATPase (Silvius et al., 1984; Simmonds et al., 1982, 1984). Lower affinities of the rigid steroid structure of CH to the integral proteins were demonstrated. The effect of lipid composition on the activity of membrane protein cannot be attributed to the effect on overall fluidity of the membrane but rather to the local environment around the protein as a result of interaction of lipid components with the protein (Warren et al., 1974; Johannsson et al., 1981; East & Lee, 1982).

We found that the rigid lipid, cholesterol, does not readily accommodate the horizontally oriented 16-AP and cannot constitute the mixed duplex monolayer. Similar results were also observed in the mixed monolayer of CH and DMPC in the case of valinomycin (unpublished data). The latter compound also orientates horizontally in the monolayer. In biological and reconstituted membranes, the flexible hydrocarbon chains of phospholipids stabilize the structure of integral protein in the membrane while the rigid molecule of cholesterol is probably excluded from close proximity to the membrane protein. The effects of cholesterol on the enhancement of membrane protein activity are evident in transport systems (Saito & Silbert, 1979) and enzymatic systems (Sinha et al., 1977). In these unique cases, cholesterol seems to play a rather specific role in activation of membrane protein. It must, therefore, be noted that the results obtained in the present work suggest only nonspecific effects in the preferential interaction of flexible lipid with membrane protein.

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Kinetic Analysis of Fusion of Hemagglutinating Virus of Japan with Erythrocyte Membrane Using Spin-Labeled Phosphatidylcholine

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ABSTRACT: HVJ* (hemagglutinating virus of Japan containing spin-labeled phosphatidylcholine in its envelope around 10 mol %) was adsorbed onto erythrocytes or erythrocyte ghosts at various doses, and the ESR spectrum of the virus–cell system was measured at 37 °C. The peak-height increase for the HVJ*–ghost system was satisfactorily analyzed on the basis of envelope fusion by a first-order kinetic equation with two different rate constants. The rate constant was obtained as $k_1 = 0.84$ min^{-1} and $k_2 = 0.011$ min^{-1}, independent of the virus dose. The fraction of virus fused at the rate constant k_1 decreased with the dose. However, the average number of fast-fusing viruses per cell was nearly independent of the dose, and the value was one to two. The peak-height increase in the HVJ*–erythrocyte system was caused by both envelope fusion and phospholipid exchange catalyzed by the virus-induced hemolyzate. At lower doses, where the virus-induced hemolysis was small and, therefore, the rate of phospholipid exchange was small, the peak-height increase could be analyzed by the same kinetic equation with nearly the same rate constant value for k_1 as that for HVJ*–ghosts. However, the k_2 was larger than that for HVJ*–ghost, owing to the additional transfer by phospholipid exchange.

Fusion of virus envelope with target cell membranes is an essential step in virus infection [see White et al. (1983) and Ohnishi (1985)]. We have been studying envelope fusion using viruses having spin-labeled phosphatidylcholine (PC)*[1] in-

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