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THE MONOLAYER BEHAVIOR AND TRANSFER CHARACTERISTICS OF PHOSPHOLIPIDS AT THE AIR/WATER INTERFACE

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Abstract—The monolayer behavior of phospholipids at the air/water interface and their transfer characteristics on the solid substrates have been investigated with a constant-perimeter type Langmuir trough. From the surface pressure-area (Π-A) isotherm, evaluated were the cross-sectional area of an oriented molecule, phase transition, and the miscibility of mixed monolayers. The monolayer state depends on the chain length and its mobility, and by adding proper salts, the monolayers were stabilized. The miscibility of mixed monolayers was also discussed with collapse pressure and excess area. The monolayers of all lipids were transferred into only one layer on hydrophobic substrates, and up to two layers on hydrophobic substrates. The multilayer formation of dipalmitoylphosphatidic acid (DPPA) was significantly affected by the subphase pH and the addition of multivalent salts. As a transfer promoter, DPPA or octadecylamine (ODA) was used as a component in a mixture with lipid materials, and their mixed monolayers resulted in good transfer characteristics. The transferred mass and film thickness of DPPA, determined by using a quartz crystal microbalance (QCM) and by an ellipsometry respectively, were proportional to the number of transfer. The lattice spacing of DPPA was 20.3 Å per layer by ellipsometry, and 22.78 Å per layer by X-ray diffraction. These indicated that the transferred multilayers had well-defined layered structures of Y-type.

Key words: Phospholipid Monolayer, Π-A Isotherm, Mixed Monolayer, Langmuir-Blodgett (LB) Film, Transfer Characteristics

INTRODUCTION

The monolayers of phospholipids from cell membranes have been studied as a model of biological membranes [Singer and Nicolson, 1972]. During last 10 years, the challenge of understanding complex natural systems has been a driving force in this area and therefore, leads to development of the biomimetic systems of synthetic membranes [Ringsdorf et al., 1988]. Also, as a precursor of Langmuir-Blodgett (LB) films, the phospholipid monolayer spread on the air/water interface has been investigated in a well-defined way [Möhwald, 1990]. The organized and planar array of a LB film can provide a new method of arranging lipid membranes, suitable for probing the cooperative interactions between its constituents, and the properties of surface as a whole.

The understanding of composition, structure, and dynamics of monolayers can not only account for its functions *in vivo* [Swart, 1990], but also can artificially realize the biological characteristics of biosensors [Roberts, 1990]. The phospholipid monolayers are characterized by the planarity over macroscopic dimensions and the ordered two-dimensional structures, and their surface properties are controlled by the molecular density of monolayers, the ionic conditions of subphase, temperature, and composition. The surface pressure-area (Π-A) isotherms of various phospholipids provide useful information on monolayer states [Phillips and Chapman, 1968], including the phase transition of the liquid expanded (LE)/liquid condensed (LC) phases [Mitchell and Dluhy, 1988]. Film deposition of floating monolayer can practically be analyzed by the transfer ratio [Langmuir et al., 1937]. The monolayers of pure phospholipids can be hardly transferred into multilayers under normal conditions [Taylor and Mahboubian-Jones, 1982], but the monolayers of carboxylic acids, having ionizable head group, can be transferred in the existence of divalent metal ions [Berg and Claesson, 1989]. In fact, it is not well understood whether there are operational windows for transferring phospholipids on solid substrates.

In this work, we studied the monolayer behavior of phospholipids at the air/water interface using a Π-A isotherm. Thereafter, the transfer properties of pure phospholipid and mixed monolayers were examined under various conditions of transfer. The transfer of monolayer was observed by the transfer ratio and transferred mass on the quartz crystal microbalance, and the transferred multilayers were also characterized with X-ray diffraction and ellipsometry.

MATERIALS AND METHODS

1. Materials

The lipid materials used in this work were cholesterol, phosphatidylcholine (PC), phosphatidic acid (PA), phosphatidylethanolamine (PE), and phosphtydylserine (PS). The detailed nomenclature of phospholipids may vary with the lengths and saturation degrees of hydrocarbon chains. In our experiments, the hydrocarbon chains of phospholipids were fixed to dipalmitoyl group, and phospholipids of having different chain lengths or unsaturated linkages were also used. All lipids were purchased from Sigma Co. and were used without further purification.

The subphase was deionized water obtained from a Milli-Q system. The pH of subphase was adjusted by the addition of HCl

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or NaOH. The subphase ions were varied with $CaCl_2$, $BaCl_2$, $CdCl_2$, $AlCl_3$, $La(NO_3)_3$, and poly(sodium 4-styrenesulfonate) (PSSS). Mixed films of phospholipids were made of stearic acid (SA), dipalmitoylphosphatidic acid (DPPA) and octadecylamine (ODA). The lipids dissolved in a chloroform/methanol (4 : 1) solution were spread to form monolayer, and all experiments were conducted at room temperature.

2. Monolayers and Multilayer Transfer

A constant-perimeter type Langmuir trough was constructed and reported else where [Kim et al., 1992]. The trough was equipped with a Wilhelmy microbalance (NL-004-PS, Nippon Laser Co.) and a motor-driven compression barrier controlled by an electronic feedback system. The barrier was a glass fiber tape coated with polytetrafluoroethylene. Also, an automated vertical dipping unit was attached so that a LB multilayer could be obtained by repeatedly lowering and raising the solid substrate through the monolayer.

The monolayers were transferred onto the solid substrates by a conventional dipping technique, varying the dipping speed from 5 to 200 mm/min. The substrates used were glass microscope slides. The hydrophobicity of the glass surface was modified by treating with long-chain silane, if necessary. The glass was immersed into a toluene solution of 5%(v/v) octadecyltrichlorosilane for 1 hr and then rinsed with pure toluene. The contact angle of water on glass was originally 69° but after silanization, it increased to 96°.

3. Characterization of LB Film

The characteristics of transferred multilayer were also determined by measuring the transferred mass, ellipsometric thickness, and lattice spacing. For DPPA monolayer, the strong interaction between divalent metal salts and the ionizable head groups gives good transferability and layer structure measurable by X-ray diffraction. The transferred mass was determined by a 9 MHz AT-cut quartz crystal microbalance (QCM) with silver electrodes on both sides (Ilshin Co.). The QCM was connected to a TTL-type oscillation circuit driven at 5 volt DC, and the frequency signal output was measured by a frequency counter (SC-7201, Iwatsu Co.) on-lined to the personal computer. The film thickness was measured by Rudolph Research/Auto E(II) with the specific wave length of $\lambda = 638$ nm, and the substrate as a reference material was a silicon wafer. In the X-ray analysis, a thin film X-ray diffractor (Model No. D/MAX-RC, Rigaku Co.) with a Cu, Kα source and $\lambda = 1.54$ Å was applied. The scanning rate was 4°/min.

RESULTS AND DISCUSSION

1. Monolayer Behavior

1-1. Π-A Isotherms of Phospholipids

Fig. 1 shows the Π-A isotherms of various phospholipids at room temperature, and the trends of isotherms were consistent with the result of Phillips and Chapman [1968]. Out of the five lipids studied, i.e., cholesterol, DPPA, dipalmitoylphosphatidylcholine (DPPC), dipalmitoyl-phosphatidylethanolamine (DPPE), and dipalmitoylphosphatidylserine (DPPS), DPPC showed a plateau region, which indicates the transition of LE/LC phases, but the other four lipids had only the condensed phase. As the temperature increases, the slope of the plateau increases and finally the plateau disappears [Mitchell and Dluhy, 1988].

The monolayer state can be also described by the compressibility of a phase. The modulus (C_S^{-1}) of compressibility of a monolayer, defined as [Davies and Rideal, 1963]

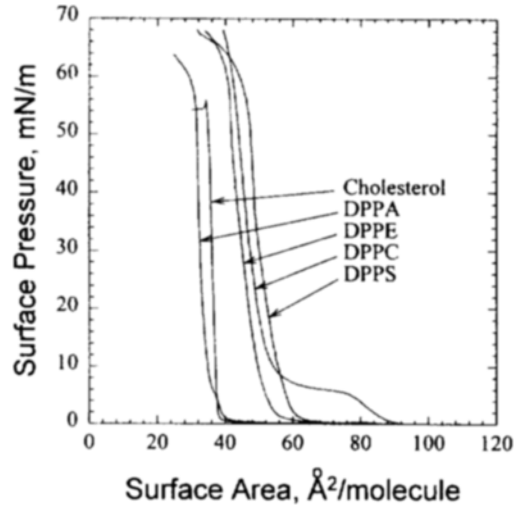

Fig. 1. Π-A isotherms of lipids; cholesterol, DPPA, DPPC, DPPE and DPPS.

Table 1. The compressional moduli of lipid monolayers

Material	Π, mN/m	C_S^{-1}, mN/m	Monolayer state
Cholesterol	50	709	condensed
DPPA	50	704	condensed
DPPS	50	419	condensed
DPPE	50	407	condensed
DOPE	50	124	liquid condensed
DPPC	5	29	liquid expanded
	20	187	liquid condensed
	50	276	condensed

Table 2. Limiting areas of lipid monolayers before the collapse

Material	Limiting area, Å²/molecule
Cholesterol	37.65
DPPA	33.57
DPPC	51.90
DPPE	50.14
DPPS	53.16

$$C_S^{-1} = -A(\partial \Pi/\partial A)_T = -(\partial \Pi/\partial \ln A)_T \qquad (1)$$

can be calculated directly from the slope of a Π-A isotherm. In general, C_S^{-1} depends on the state of the monolayer, such that a condensed monolayer has a greater modulus than an expanded one. Table 1 gives the moduli of phospholipid monolayers and indicates the LE/LC transition of DPPC. The monolayer states of all lipids just below the collapse pressure were in the range of condensed states. However, DPPC has three different moduli of monolayers corresponding to three different conditions of the states before and after phase transition, and just before the collapse.

Table 2, obtained from Fig. 1, shows the limiting areas of five lipid monolayers. DPPA with a small acid group shows smaller limiting area than the other three dipalmitoylphospholipids do. Also, cholesterol occupies with small area because it orients vertically at the interface and tightly pack in the condensed state [Ries et al., 1988; Yamauchi et al., 1993].

1-2. Effects of Hydrocarbon Chain Length

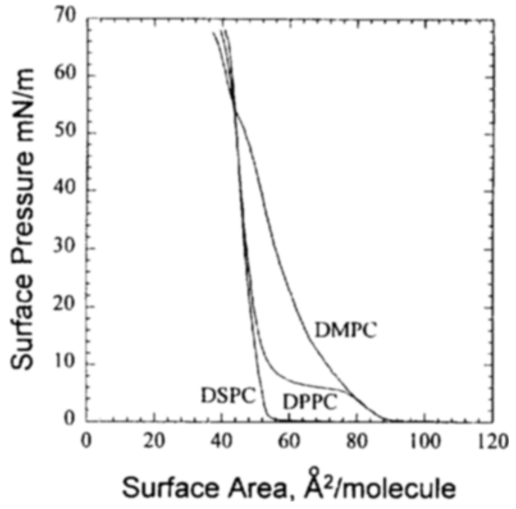

Fig. 2. Π-A isotherms of DMPC, DPPC and DSPC; These materials have the saturated hydrocarbon chains with different chain lengths.

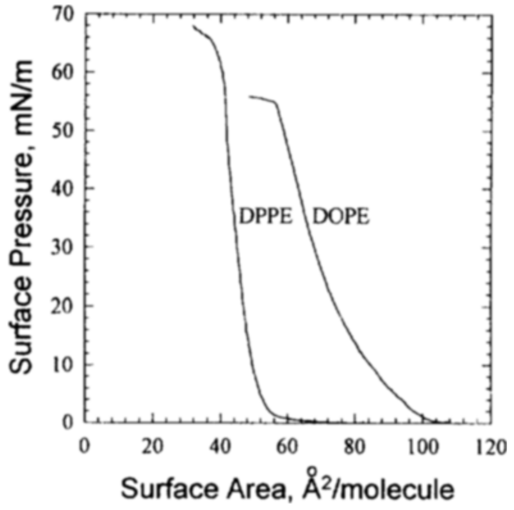

Fig. 3. Π-A isotherms of DPPE and DOPE; DPPE has a saturated hydrocarbon chain with 16 carbon chains, and DOPE has an unsaturated hydrocarbon chain with 18 carbon chains with one double bond of the cis-form at the 9th position.

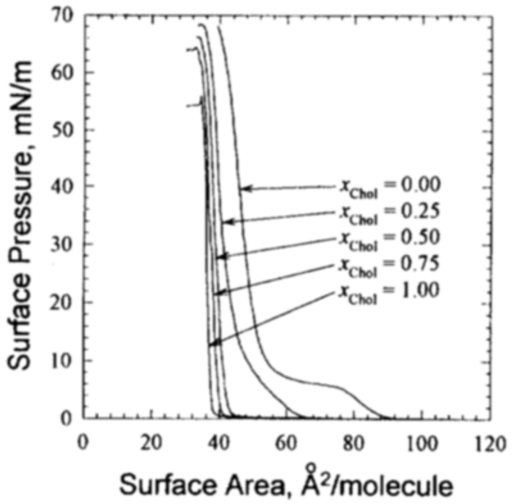

Fig. 4. Π-A isotherms of DPPC/cholesterol mixtures; x_{Chol} represents the mole fraction of cholesterol.

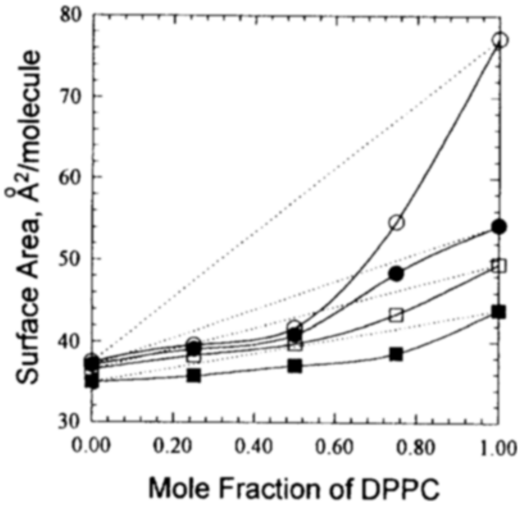

Fig. 5. Average molecular area vs. mole fraction of DPPC for monolayers of the DPPC/ cholesterol mixture.

The surface pressure for each plot are 5 (○), 10 (●), 20 (□) and 50 mN/m (■); Dotted lines represent the values calculated by the additivity rule.

The Π-A isotherms of dimyristoylphosphatidylcholine (DMPC), DPPC, and distearoylphosphatidylcholine (DSPC) are shown in Fig. 2. DPPC shows the distinctive LE/LC phase transition around 5 mN/m, and DMPC has the expanded phase and a slight tendency of phase transition around 50 mN/m, while DSPC shows the condensed phase without distinguishable LE/LC phase transition feature. Similar trends of chain contribution to the phase behavior could be found in fatty acid monolayers and bilayers [Adam, 1968]. That is, the phase of homologous series of fatty acids varies smoothly from a LC state to a LE state with a decrease in chain length. Also, the phase transition of Fig. 2 can be explained by the endothermic chain-melting [Tscharner and McConnell, 1981] at the Chapman transition temperature (T_C), where T_C depends on the chain length [Bell et al., 1981; Tredgold, 1977]. Therefore, the phase transition of lipid monolayer is attributed from the chain interaction just like the case of the fatty acid monolayer and bilayer, and it is concluded that the phase transition is significantly influenced by the mobility associated with the variation in hydrocarbon chain length.

1-3. Effects of Double Bond

Fig. 3 shows the Π-A isotherms of DPPE and dioleoylphosphatidylethanolamine (DOPE). DPPE has a saturated hydrocarbon chain with 16 carbons and DOPE has an unsaturated one with 18 carbons and a double bond. The isotherm of DPPE is more condensed than that of DOPE. If only the effect of chain length is considered, it would be opposite from the observations in Fig. 2, where the long chain phospholipid formed the condensed monolayer. For DOPE, however, the *cis*-conformation of a double bond could significantly increase the total volume occupied by the hydrocarbon chain [Israelachvili, 1992]. Further, the compressional modulus (Table 1) indicates that DOPE forms more flexible monolayer than DPPE does.

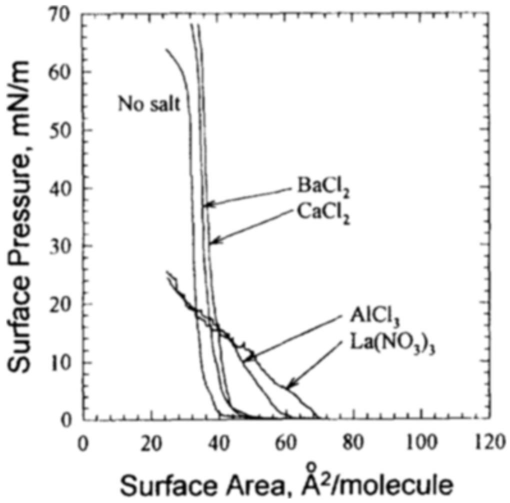

Fig. 6. The salt effects on the monolayer behavior of DPPA; The salt concentrations are 10^{-4} M, and the subphase was maintained at pH 8.

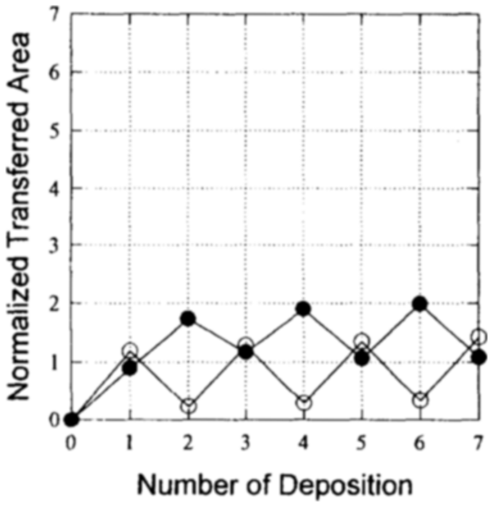

Fig. 7. Transfer characteristics of DPPE with the hydrophilicity of substrate.

The slide glass as a hydrophilic substrate (○) and the silanized glass as a hydrophobic substrate (●) are used. The dipping speeds are 5 mm/min and the deposition pressure is 40 mN/m.

1-4. Mixed Monolayer

Fig. 4 shows the Π-A isotherms of DPPC/cholesterol mixtures of different compositions. As the amount of cholesterol in the monolayer increases, the region of the LE/LC phase transition decreases and then disappears. Such a transition would depend on the miscibility of the two components. In a monolayer, however, the miscibility of two components can hardly be determined, but in the case of two materials showing distinguishable collapse pressures, the surface phase rule is often used [Gaines, 1966]. If the equilibrium spreading pressure (Π_{eq}) of a mixed film is independent of composition, two components are immiscible at Π_{eq}. However, it is difficult to determine Π_{eq}, and thus collapse pressure is used more conveniently. Therefore, if two components are immiscible, the isotherm of the mixture shows two distinguishable collapse pressure, each value corresponding to the collapse pressure of a single pure component. Fig. 4 shows that the collapse pressure clearly varies with the monolayer composition, and it gives a satisfactory result for homogeneous mixing.

Another method is to use the excess area, in which the average molecular area at a fixed pressure is plotted against the composition [Gaines, 1966]. If components are immiscible, one component will exist in the form of patches. In this case, each component acts independently in the monolayer, and the average area per molecule in a mixed film will follow the additivity rule.

$$A_{12}=x_1A_1+x_2A_2 \tag{2}$$

where, A_{12} is the average molecular area in the mixed film, x_i the mole fraction of the i component, and A_i the molecular area in a pure film at the same surface pressure. In Fig. 5 obtained from Fig. 4, the average molecular areas were given with negative deviations from the additivity rule, indicating that there exist some attractive interactions between DPPC and cholesterol. These attractive interactions sustained up to the collapse pressure. From the observations of collapse pressure and attractive interactions, it is tentatively concluded that DPPC and cholesterol would form a mixed monolayer homogeneously.

1-5. Effects of Salts

Fig. 6 shows the effects of salts on the monolayers of DPPA. All the salt concentrations were 10^{-4} M, and the subphase was maintained at pH 8 by the addition of NaOH. Divalent ions (Ba^{2+}, Ca^{2+}) formed more stable monolayers and higher collapse pressures than trivalent ions (Al^{3+}, La^{3+}). The trivalent ions made very unstable and dissociable monolayers. The ionization of carboxylic or phosphatic acids can be controlled by changing the pH and salts. Such divalent ions are often necessary for monolayers to be transferred into multilayers [Kuhn and Möbius, 1971].

2. LB Multilayer Formation

Film deposition of floating monolayer can practically be characterized by the transfer ratio [Langmuir et al., 1937], and during the transfer it can be generalized by using the normalized transferred area, N_A, given by

$$N_A=\Delta A_{L,t}/A_S \tag{3}$$

where, $\Delta A_{L,t}$ is the cumulative decrease in the area of a monolayer by a film transfer, and A_S is the area of solid substrate.

2-1. Pure Lipid Film

For all pure lipids, only one layer was transferred at upward stroking. In the first up trip, molecules deposited on the hydrophilic surface of glass anchoring their polar head groups. However, when the substrate was immersed, the whole preceding layer peeled off from the substrate, mostly because of the weak interactions between the polar head groups and the hydrophilic surface of the glass substrate. In general, it was reported that pure phospholipids can hardly form LB multilayers [Taylor and Mahboubian-Jones, 1982].

2-2. Surface Treatment of the Substrate

The slide glass was silanized with octadecyltrichlorosilane. In Fig. 7, the silanized substrate allowed only two layers of LB film of DPPE. Here, as the surface was hydrophobic, dipping started downward first. On the first down- and second up-trips, the molecules of two layers formed a head-to-head configuration. However, if the substrate traveled down again, the whole preceding layer peeled off from the surface. The interactions between polar heads in the multilayers are too weak to form a stable multilayer [Hasmonay et al., 1979], and therefore the film can be successfully transferred by increasing the head-to-head interaction of phos-

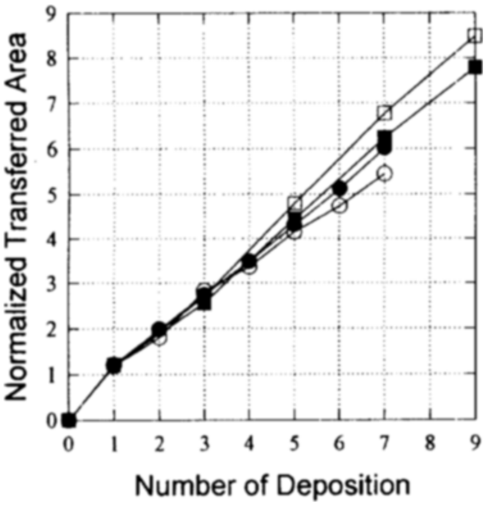

Fig. 8. Transfer characteristics of DPPA with divalent metal ions. The concentrations of $CaCl_2$ (○) and $BaCl_2$ (●) are 10^{-4} M at pH 8. The dipping speeds are 5 mm/min, and the deposition pressure is 40 mN/m. The effect of $BaCl_2$ can be observed by the comparison between N_A by glass (●, $T_R=0.80$) and calculated $N_{A,QCM}$ (□: for DPPA only, $T_R=0.93$ and ■: for Ba^{2+} and $DPPA^-$ interaction, $T_R=0.84$).

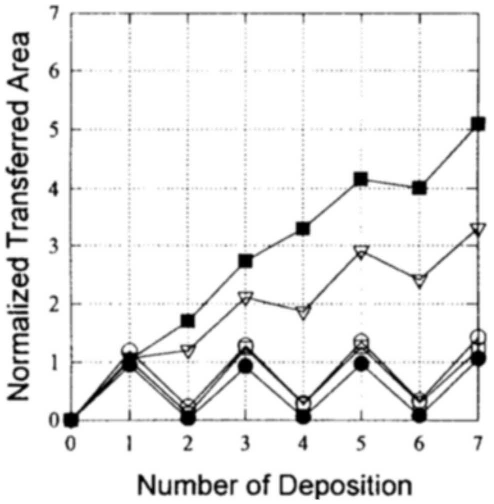

Fig. 9. Transfer characteristics of DPPE with dipping speeds and salts. Speed for the up-trip is 5 mm/min, and for the down-trip, 5 (○) and 200 (others). The deposition pressure is 40 mN/m, and the subphase conditions are as follows-○ & ●: no salts at pH 5.5, □: 10^{-3} M PSSS at pH 3, ■: 10^{-4} M $CaCl_2$ at pH 10 and ▽: 10^{-3} M PSSS & 10^{-4} M $CaCl_2$ at pH 10.

pholipids.

2-3. Effects of Salts

Fig. 8 shows the transfer characteristics of a DPPA monolayer in the existence of divalent metal ions. The DPPA monolayer was transferred in the presence of divalent ions because of the increased interaction between head groups.

The transfer ratio of a LB film was also measured by a QCM, of which the oscillation frequency shift is proportional to the mass loaded on the crystal surface. The operating principle of a QCM can be described by the following idealized equation [Sauerbrey, 1959, 1964],

$$\Delta f = -2.3\times10^{6}\ f_o^{2}\cdot\Delta m/A \qquad (4)$$

where, Δf is the frequency change by mass loading [Hz], f_o the oscillation frequency [MHz], Δm the mass loaded on a quartz surface [g], and A the active area of QCM [cm^2]. Therefore, the 9 MHz QCM has a mass sensitivity of 5.37 ng/cm^2 for 1 Hz change.

Following Eqs. (3) and (4), N_A can be also deduced from the mass change by the QCM, and the normalized transfer ratio by the QCM, $N_{A,QCM}$ is defined as

$$N_{A,QCM} = \Delta m/\Delta m_{Ideal} \qquad (5)$$

Assuming that the monolayer density can be determined from the Π-A isotherm, the ideal mass change by film deposition (Δm_{Ideal}) becomes,

$$\Delta m_{Ideal} = 2A\cdot M_W/A_\Pi\cdot N_{Avg} \qquad (6)$$

where M_W is the molecular weight of the film molecule, A_Π the surface area per molecule at the deposition pressure [Å²/molecule], and N_{Avg} the Avogadro number. The mass change by film deposition, Δm, can be determined from Eq. (4). Therefore, $N_{A,QCM}$ can be given by the following equation,

$$N_{A,QCM} = -13.1\Delta f\cdot A_\Pi/f_o^{2}\cdot M_W \qquad (7)$$

Fig. 8 shows the normalized transferred areas of a DPPA LB film obtained from Eqs. (4) and (7). As the interaction between DPPA and Ba^{2+} affects M_W and A_Π in Eq. (7), $N_{A,QCM}$ is calculated for two cases; one is for pure DPPA and the other is for a case where two negatively charged DPPA molecules bind to one Ba^{2+} ion. For DPPA with salts, the values of calculated normalized areas $N_{A,QCM}$ are the same as the values given by Eq. (3), but those of pure DPPA deviate.

2-4. Control of Dipping Speed

The dipping speed of substrates may control the peel-off of the transferred film at down trip. It has been reported that neither deposition occurs in the rapid down trip, nor the transferred layer is lost [Albrecht et al., 1982]. Therefore normal deposition takes place only in the up-trip. Fig. 9 shows that the downward dipping speed had no effects on the transfer but shows an improvement in the existence of appropriate salts. The negative charge of PSSS had not improved the transferability significantly, but we have some progress in the complex salt system.

2-5. Mixed Film

The mixed films of nontransferable amphiphiles with SA has been already transferred in various laboratories [Lee et al., 1992; Hann, 1990]. In the transfer of mixed phospholipid system, however, lipid monolayers mixed with SA did not give any distinguishable progress above 80% of SA, and even if salts were added, it did not show the satisfactory transferability at rapid downward dipping. Therefore, we used the other amphiphiles for achieving good quality of multilayer even at a high concentration of phospholipid. Fig. 10 shows that DPPA and ODA also promoted the film transfer and that the transfer characteristics of mixed DPPE could be improved under normal condition. For most lipids, the films were transferred above 70% of DPPA or ODA, but the mixtures of DPPC was not transferred until 80%.

3. Layer Structure

The structure of DPPA multilayers was determined by ellipsometric analysis and X-ray diffraction analysis. In Fig. 11, the film

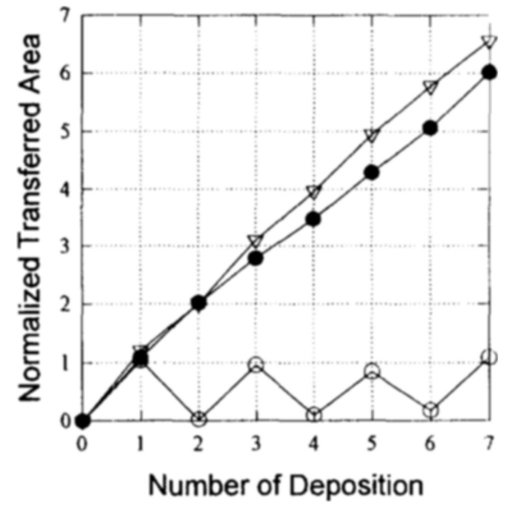

Fig. 10. Transfer characteristics of a DPPE/DPPA mixture.
(○: pure DPPE, ●: 3/7 mixture, ▽: pure DPPA); The deposition pressure is 40 mN/m, and the dipping speeds are 5 mm/min. The subphase is maintained at 10^{-4} M $BaCl_2$ and pH 9.

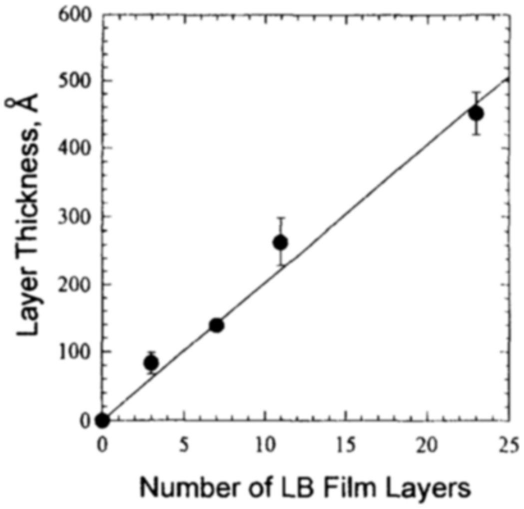

Fig. 11. The layer thickness of DPPA LB films measured by the ellipsometry as the number of layers increased.
The subphase is maintained at 10^{-4} M $BaCl_2$ and pH 9. The substrate is a Si wafer. The dipping speed is 5 mm/min, and the deposition pressure is 40 mN/m. From the slope, the film thickness of one layer is about 20.3 Å.

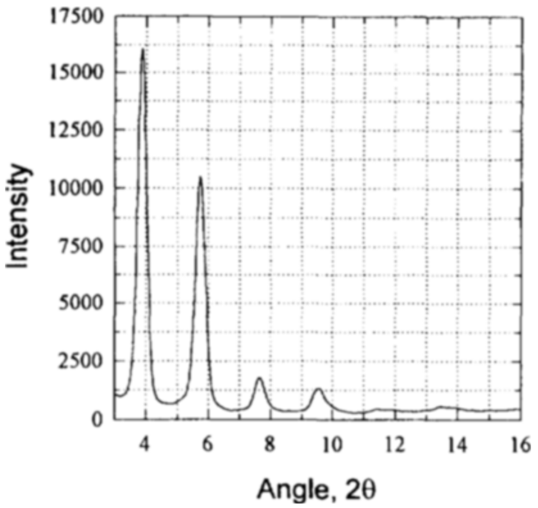

Fig. 12. The X-ray diffraction pattern of a 40-layered DPPA LB film.
The subphase is maintained at 10^{-4} M $CdCl_2$ and pH 9. The dipping speed is 5 mm/min, and the deposition pressure is 40 mN/m. From Bragg's equation, the film thickness of one layer is 22.78 Å.

thickness measured by ellipsometry increases linearly along the number of transferred layers. The slope shows that the film thickness of one layer was evaluated to be about 20.3 Å.

The lattice spacing has also been determined by X-ray analysis. Fig. 12 shows the thin film X-ray diffraction patterns of a 40-layered DPPA LB film. $CdCl_2$, which enhances the X-ray intensity, also promotes the transferability of the monolayers. The addition of Cd^{2+} to the subphase condenses the negatively charged DPPA monolayer by binding of Cd^{2+} and by screening of repulsive forces [Lösche et al., 1985]. After the transfer, the Cd^{2+} ions exist between the head and head groups in the Y-type film structure. The thickness of one layer is about 22.78 Å a half of the lattice spacing of the Bragg's equation.

CONCLUSIONS

The monolayer behavior of phospholipids was characterized by the Π-A isotherm. The limiting area and compressional modulus indicated most of lipids to be in the condensed phase, but DPPC showed a LE/LC phase transition behavior at room temperature. The mixed monolayer of DPPC/cholesterol mixture showed the concentration-dependency of collapse pressure and the negative deviation of excess area, ant it indicated the homogeneous mixing of two components. For DPPA, having an ionizable head group, divalent ions made more stable monolayers, but trivalent ions formed unstable and dissociable monolayers.

The monolayer of all pure phospholipids were transferred into only one layer on the hydrophilic substrate and up to two layers on the hydrophobic substrate. It is because the interaction between the hydrophilic substrate and the head groups or the head-head interaction is week. The metal salt promoted the formation of DPPA multilayer with good qualities. Some possibilities of multilayer formation were observed by controlling the dipping speed and the subphase conditions. Especially, the lipid monolayers mixed with DPPA or ODA gave good transfer characteristics at slow dipping.

The DPPA multilayer was analyzed by measuring the transferred mass with a QCM, and its film thickness and lattice spacing were determined through ellipsometry and X-ray diffraction, respectively. These analyses indicate the well-defined layered structures of Y-type with 22.78 Å/layer.

ACKNOWLEDGMENT

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NOMENCLATURE

A : surface area per molecule [Å^2/molecule]
A : cross-sectional area of quartz crystal [cm^2]
A_{12} : average molecular area in the mixed film of species 1 and 2 [Å^2/molecule]
A_i : molecular area in the pure film of species i [Å^2/molecule]
$\Delta A_{L,t}$: cumulative decrease in the area of monolayer by film transfer [cm^2]
A_S : area of the solid substrate for film transfer [cm^2]
A_Π : surface area per molecule at deposition surface pressure [Å^2/molecule]
C_S^{-1} : modulus of compressibility of monolayer [mN/m]
DMPC : dimyristoylphosphatidylcholine
DOPE : dioleoylphosphatidylethanolamine
DPPA : dipalmitoylphosphatidic acid
DPPC : dipalmitoylphosphatidylcholine
DPPE : dipalmitoylphosphatidylethanolamine
DPPS : dipalmitoylphosphatidylserine
DSPC : distearoylphosphatidylcholine
Δf : frequency change by mass loading on QCM [Hz]
f_o : intrinsic oscillation frequency of QCM [MHz]
LB : Langmuir-Blodgett
LC : liquid condensed
LE : liquid expanded
Δm : mass loading of foreign species on QCM [g]
M_W : molecular weight of film molecule
N_A : normalized transferred area of monolayer calculated by area change
$N_{A,QCM}$: normalized transferred area of monolayer calculated by QCM
N_{Avg} : Avogadro number [6.02×10^{23} molecules/mole]
ODA : octadecylamine
PA : phosphatidic acid
PC : phosphatidylcholine
PE : phosphatidylethanolamine
PS : phosphtydylserine
PSSS : poly(sodium 4-styrenesulfonate)
QCM : quartz crystal microbalance
SA : stearic acid
T_C : Chapman transition temperature [℃]
x_i : mole fraction of the i component
Π : surface pressure [mN/m]
Π_{eq} : equilibrium spreading pressure [mN/m]

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