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Phospholipid Monolayers at the Triolein–Saline Interface: Production of Microemulsion Particles and Conversion of Monolayers to Bilayers

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ABSTRACT: Interfacial tensions of phospholipid monolayer at the triolein (TO)–saline interface were measured. The adsorption isotherms and the interfacial pressure–molecular area curves were evaluated on the basis of the measurements. Phosphatidylcholine (PC) forms a highly condensed monolayer, with a large lateral attractive interaction; phosphatidylethanolamine (PE) and phosphatidylserine (PS) form expanded monolayers with smaller lateral interaction energies. At the lowest interfacial tension (the highest interfacial pressure), the mole fractions of PC, PE, and PS in the monolayers are estimated as 0.95, 0.73, and 0.88, respectively. Therefore, PC forms the most stable monolayer at the interface. These results are consistent with the finding that the stable TO particles in aqueous solution were produced by using PC as an emulsifier, and PE and PS did not stabilize the particles. The phase diagram of TO and PC mixtures in saline obtained from theoretical considerations predicts the equilibrium conversion of the monolayers on TO particles to bilayers. This process may be closely related to the transformations of very low density lipoproteins and chylomicrons to high-density lipoproteins in plasma. The particle sizes of the emulsion are calculated theoretically as a function of PC mole fracton in the TO–PC mixture and compared with the experimental values obtained from quasi-elastic light scattering (QLS) measurements.

Lipoproteins may be viewed as emulsion particles whose component lipids are in dynamic equilibrium with lipids in each class of lipoproteins as well as with those in the membranes of various tissues. Both chylomicrons and nascent very low density lipoproteins (VLDLs),[1] formed in the small intestine and the liver cells, respectively, are large (100–1000-nm) emulsion particles composed of triglyceride-rich cores stabilized by surface phospholipid (mainly phosphatidylcholine) monolayers containing a very small amount of several different apoproteins (Patton et al., 1984). In plasma, these particles attach to capillary endothelium, where lipoprotein lipase promotes triglyceride hydrolysis (Mjos et al., 1975; Vigne & Havel, 1981; Redgrave & Small, 1979). The digestion of triglyceride shrinks the core to produce the core remnant particle (VLDL or chylomicron remnants) (Atkinson & Small, 1986; Miller & Small, 1983a): As the shrinkage proceeds, the redundant surface monolayers change into bilayers and finally separate from the particles as surface remnants (Tall & Small, 1980). The surface remnants, mainly composed of phospholipids, fuse with some elements of the high-density lipoprotein (HDL) system (Tall et al., 1982). Thus, the surface monolayers play important roles in the particle stabilization and in the conversion of emulsion particles into bilayers.

Emulsion models for triglyceride-rich lipoproteins, prepared by vortexing egg phosphatidylcholine, cholesterol, and triolein (TO) in excess water, have been characterized: Phosphatidylcholine is found exclusively in the surface monolayers separated from the neutral lipid cores. The surface composition of TO is ca. 3 mol % (Miller & Small, 1982, 1983a,b). The β-carbonyl group of TO is less hydrated than the α groups (Hamilton & Small, 1981). Also, addition of cholesterol increases the size of emulsion particles (Miller & Small, 1983b).

In this connection, we have measured the interfacial tension of phospholipid films at the TO–saline interface to evaluate monolayer compositions, lateral interaction parameters, and phase behaviors of TO–phospholipid mixtures in water by using thermodynamic approaches. On the basis of our results,

[1] Abbreviations: VLDL, low-density lipoprotein; HDL, high-density lipoprotein; TO, triolein; PC, egg yolk phosphatidylcholine; PE, egg yolk phosphatidylethanolamine; PS, bovine brain phosphatidylserine; QLS, quasi-elastic light scattering.

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we discuss the stability of the TO–phospholipid microemulsion, the transformation of the monolayers on emulsion particles into bilayers, and the size of the particles. The behavior of the monolayers at the TO–saline interface is quite different from that at the air–saline interface, and the experimental results obtained at the air–saline interface seem not to be correlated directly to the emulsion properties.

Experimental Procedures

Materials. Egg yolk phosphatidylcholine (PC) was kindly provided by Asahi Kasei Co. The purity (over 99.5%) was determined by thin-layer chromatography. The small amount of impurity (0.5%) was identified as sphingomyelin by HPLC. The acyl chain composition of PC was 0.2% 14:0, 34.9% 16:0, 0.3% 16:1, 11.9% 18:0, 30.4% 18:1, 15.0% 18:2, 0.2% 20:2, 0.3% 20:3, 3.2% 20:4, 3.3% 22:6, and 0.3% others. Egg yolk phosphatidylethanolamine (PE) and bovine brain phosphatidylserine (PS) were purchased from Sigma Chemical Co. The purities of both PE and PS were over 97% as checked by thin-layer chromatography. The fatty acid compositions of PE and PS were 14.9% 16:0, 0.3% 16:1, 27.6% 18:0, 17.4% 18:1, 10.5% 18:2, 16.9% 20:4, 3.8% 22:4, 7.5% 22:6, and 1.1% others and 41.3% 18:0, 31.2% 18:1, 4.5% 18:2, 1.0% 20:0, 1.1% 20:1, 1.4% 20:4, 6.1% 22:4 and 22:5, 6.4% 22:6, and 7.0% 34 small peaks possibly due to some other unidentified polyene fatty acids, respectively. TO obtained from Taiyo Chemical Co. was purified by silicate (Wakogel C-200, Wako Pure Chemicals) column chromatography to remove fatty acid, diglyceride, and monoglyceride by using chloroform/methanol (99/1) as an eluent. The purity of TO thus obtained was over 99%. Water was doubly distilled with a quartz still.

Solubility Determination. Each phospholipid was dissolved in chloroform (150 mM). The solvent of an aliquot of the solution was evaporated, and the residue was dried in vacuo for 15 h. TO (5 mL) was added, and the phospholipid was dispersed at 45 °C by vortexing. After cooling to 25 °C, the optical density at 550 nm of the mixture was measured. An inflection in the optical density–concentration curve was regarded as the solubility at 25 °C. The solubilities of PC and PS, thus obtained, agreed with those from the inflections of the interfacial tension–concentration curves. The solubility of PE was much higher than the inflectional concentration of the interfacial tension–concentration curve.

Interfacial Tension Measurement. The interfacial tension of the triolein–saline (10 mM Tris-HCl/150 mM NaCl, pH 7.0) interface was measured by the drop weight method as a function of the phospholipid concentration in the triolein phase. The inverted TO drops (TO was less dense than the surrounding aqueous solution) were formed by using an all-glass micrometer syringe in a double-walled jacket equipped with a Teflon stopper. The temperature was maintained at 25 °C by circulating water through the outer jacket. Harkins and Brown correction factors were used for estimating interfacial tension values (Harkins & Brown, 1919). The density of TO was determined by using a 5-mL pycnometer. The interfacial tension values were reproducible within ±0.2 mN/m. The particulars in interfacial tension measurement have been described elsewhere (Handa & Mukerjee, 1981; Mukerjee & Handa, 1981).

Preparation of Microemulsion. Each phospholipid and triolein were dissolved in chloroform at 150 and 75 mM, respectively. After evaporation of the solvent the mixture was dried in vacuo for 15 h. The saline (10 mL) was added to the dried lipid mixture in a cylindrical tube (diameter 2.5 cm, height 10 cm) at 60 °C. The total concentration of each phospholipid and TO was kept at 1 mM. The suspension was briefly vortexed, degassed by nitrogen purging (5 min), and then sonicated for 30 min under a stream of nitrogen gas at 60 °C. The top of the tube was covered by a pin-holded Teflon film. The probe type sonicator used was a UD-200 from Tomy Seiko Co. Ltd. (power setting 100 W). The lipid-dispersed solution (microemulsion) was centrifuged for 10 min to remove the titanium dust. At 0–5 °C, even 2-h sonication did not lead to a stable optical density. On the other hand, at 60 °C, a lower stable optical density was attained within 20 min. No free fatty acids resulting from the hydrolysis of the lipids during sonication were detected by thin-layer chromatography. Klein's (1970) oxidation index of lipids extracted from the sonicated dispersion showed no lipid oxidation.

Particle Size Measurement. Quasi-elastic light scattering (QLS) measurements of the autocorrelation functions of light scattered from microemulsion particles were performed at 25 °C, on a Photal LPA-3000/3100 equipped with a 5-mW He–Ne laser and a correlator of 1000 channels. The correlation functions were analyzed by the histogram method (Gulari et al., 1979), and the weight-averaged particle sizes of the whole dispersion were evaluated.

Results

Interfacial Tension. Figure 1 shows the interfacial tension at the TO–saline interface, γ, as a function of the phospholipid concentration in the TO phase, C. The γ value at $C = 0$ (γ_0) was 31.08 mN/m. The solubilities of PC and PS in TO at 25 °C were 8×10^{-4} and 1.5×10^{-3} M (mol/L), respectively. The interfacial tensions of the TO saturated with PC and PS, γ_c, were 17.60 and 10.00 mN/m, respectively. The solubility of PE in TO was higher than 5×10^{-3} M. The interfacial tension of the PE solution, however, became stationary (γ_c = 21.2 mN/m) in the concentration region above 7×10^{-4} M. Therefore, the association of PE would occur in the TO phase. Figure 1 demonstrates that the γ value of the PC solution is stable over the concentration range of $(1–3) \times 10^{-4}$ M but falls abruptly in the higher concentration region; the slope of the curve is not as steep for PE and PS as for PC. These features of interfacial tension lead to particularly important results in the adsorption isotherms.

Adsorption Isotherms and Interfacial Pressure–Molecular Area Curves. The adsorption amount at interface, Γ (mol/cm^2), is correlated with the change of interfacial tension, $(\partial\gamma/\partial \ln C)_{T,P}$, by the Gibbs adsorption equation:

$$\Gamma = -\frac{1}{RT}\left(\frac{\partial\gamma}{\partial \ln C}\right)_{T,P} \quad (1)$$

Here, R is the gas constant. Figure 2 shows the adsorption amounts, Γ, calculated by use of eq 1 as a function of the phospholipid concentration, C. The experimental uncertainties, resulting from the differential procedures in the application of eq 1, are shown by the error bars. A remarkable cooperativity is observed in the adsorption of PC at the TO–saline interface.

The interfacial pressure of the phospholipid monolayer, F, is calculated as

$$F = \gamma_0 - \gamma \quad (2)$$

Surface area per lipid molecule (molecular area), A, is evaluated as $A = 1/(N\Gamma)$. Here, N is the Avogadro number. Figure 3 shows the interfacial pressure–molecular area curves (F–A curves) for PC, PE, and PS. In the F value range 5–20 mN/m the PC monolayer is condensed, whereas the PE and PS monolayers expanded. It is noteworthy that a very small A value of 40×10^{-2} nm^2/molecule indicates the strong con-

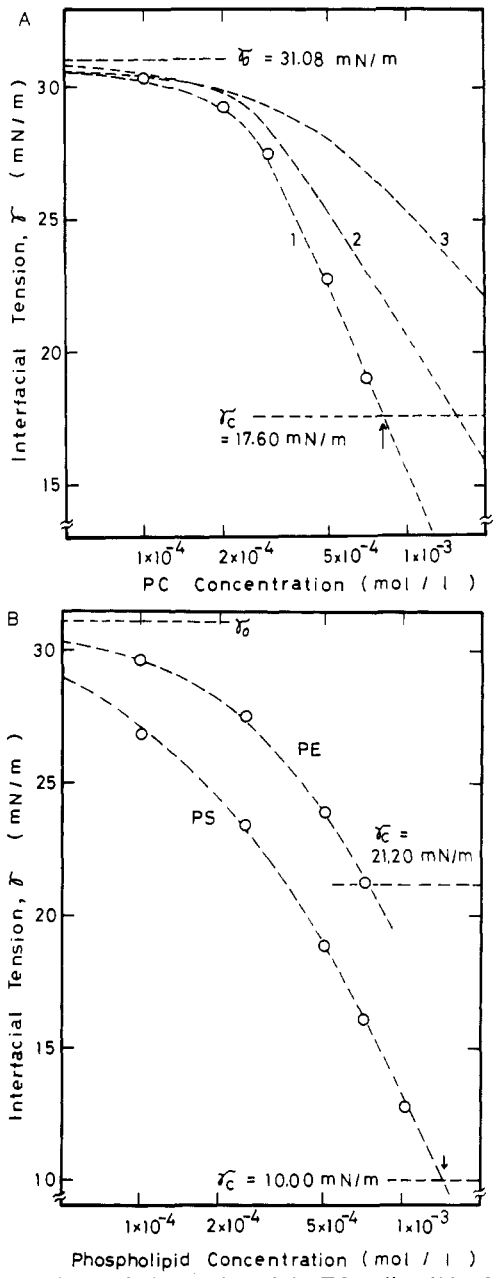

FIGURE 1: (A) Interfacial tension of the TO–saline (10 mM Tris-HCl/150 mM NaCl, pH 7.0) interface as a function of PC concentration in TO solution. γ_0 the interfacial tension without PC; γ_c = the lowest interfacial tension for the TO solution saturated with PC. The arrow indicates the solubility of PC in TO. Theoretical curves (broken lines) 1–3 are calculated by eqs 3 and 4 (see Results in the text). (1) $\Gamma_s = 4.1 \times 10^{-10}$ mol/cm^2, $K = 7 \times 10^2$ L/mol, $\omega = 1.8$; (2) $\Gamma_s = 2.77 \times 10^{-10}$ mol/cm^2 ($a = 60.5 \times 10^{-2}$ nm^2/molecule), $K = 7 \times 10^2$ L/mol, $\omega = 1.8$; (3) $\Gamma_s = 4.1 \times 10^{-10}$ mol/cm^2, $K = 7 \times 10^2$ L/mol, $\omega = 0$. (B) Interfacial tension of the TO–saline interface as a function of phospholipid concentration in TO solution. γ_c: for PE, 21.20 mN/m; for PS, 10.00 mN/m. The arrow indicates the solubility of PS in TO. Theoretical curves (broken lines) are calculated by eqs 3 and 4 (parameters: see Table I).

densation of the PC monolayer. A similar A value has been reported at the hexane–saline interface (Beatrice et al., 1976). The F–A curve of PC at the air–water interface (Shah & Schulman, 1967; Phillips & Chapman, 1968) is considerably expanded, different from that at the oil–water interface.

Mixed Monolayer of Phospholipid and Triolein. Triolein forms an insoluble monolayer at the air–water interface (Nakagaki & Funasaki, 1974) and also at the TO–water interface. For the ideal interface, Antonoff's relationship gives a rough value of interfacial tension as the difference between

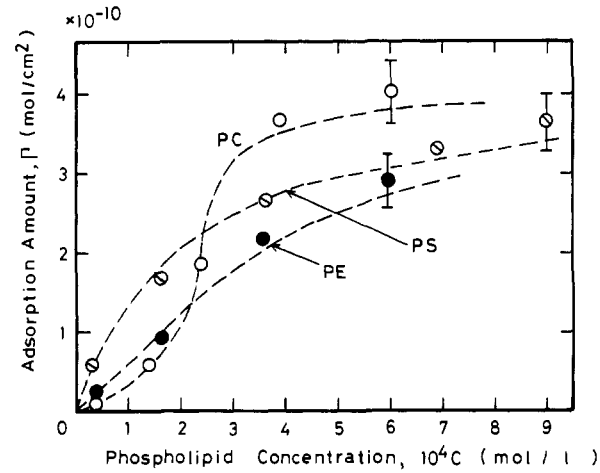

FIGURE 2: Adsorption isotherms of phospholipids at the TO–saline interface. The experimental uncertainties in their evaluation with eq 1 are shown by error bars (±10%). Theoretical curves (broken lines) are calculated by eq 3 (parameters: see Table I).

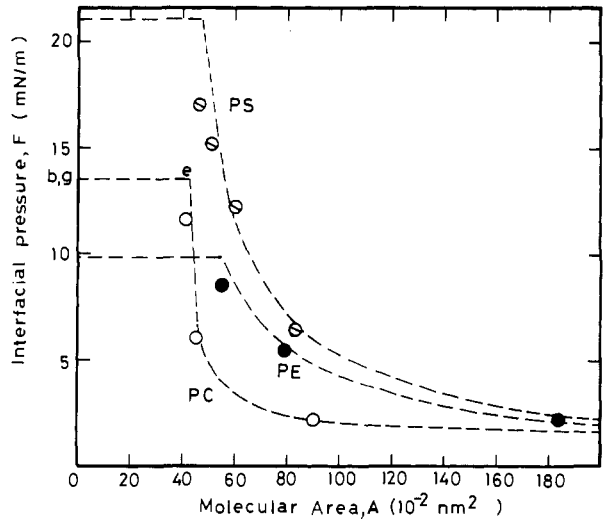

FIGURE 3: Interfacial pressure–molecular area curves of phospholipids at the TO–saline interface. Theoretical curves (broken lines) are calculated by eq 5 (parameters: see Table I). The F values of the horizontal line, F_c, are evaluated as $F_c = \gamma_0 - \gamma_c$. For points b, e, and g, see Figure 4A.

the surface tensions of the two liquids (Davies & Rideal, 1961). For example, the interfacial tension at the heptane–water interface is 50.56 mN/m, which is close tothe surface tension difference, 52.18 (=71.96–19.78) mN/m (Handa & Mukerjee, 1981). For the TO–saline interface, the calculated value 37.66 (=71.96–34.30) mN/m, deviates considerably from the observed value (γ_0 = 31.08 mN/m), suggesting the presence of a TO monolayer at the interface. The TO monolayer at the interface is in equilibrium with the liquid phase of TO, and the spreading (or collapse) pressure of TO is considered to be 6.58 (=37.66 – 31.08) mN/m (Nakagaki et al., 1985; Handa et al., 1985).

When a phospholipid is dissolved in the TO phase, the phospholipid molecules adsorb and form a mixed monolayer with TO at the interface. The cross-sectional molecular area of a phospholipid was (40–41) × 10^{-2} nm^2/molecule. The molecular area of TO is not known experimentally, because TO exhibits only an expanded monolayer at the air–water interface (Adam, 1968). We estimate (65–75) × 10^{-2} nm^2/molecule as the cross-sectional area of TO (Appendix I).

Defay et al. (1966) have described chemical potential in a mixed monolayer constituted of lipids of similar size based on

Table I: Experimental Values of Saturated Adsorption Amount, Γ_s, Cross-Sectional Molecular Area, a, Magnitude of Adsorption, K, and Lateral Interaction Parameter, ω

	Γ_s (mol/cm^2)	a (nm^2/molecule)	K (L/mol)	ω
PC–TO	4.10×10^{-10}	40.5×10^{-2}	7.0×10^2	1.8
PE–TO	4.10×10^{-10}	40.5×10^{-2}	1.5×10^3	0.6
PS–TO	4.00×10^{-10}	41.5×10^{-2}	5.0×10^3	0

Butler's equation. A theory for the equilibrium between the monolayer and the solution has been developed by Lucassen-Reynders (1966), by using the chemical potential. The adsorption iostherms of the phospholipids were evaluated by the equation (see Appendix I):

$$\Gamma/\Gamma_s = X_m = \frac{K[\exp(2\omega X_m)]C}{1 + K[\exp(2\omega X_m)]C} \quad (3)$$

where Γ_s is the saturated adsorption amount of phospholipid and is related to the cross-sectional molecular area, a, as $\Gamma_s = (Na)^{-1}$. The mole fraction of phospholipid in the monolayer, X_m, is equal to $\Gamma/\Gamma_s = a/A$. K and ω are parameters showing the magnitude of adsorption and the lateral interaction in the monolayer, respectively. The lateral attractive interaction between PC molecules leads to a positive value of ω, and the repulsive interaction results in a negative value. When the ω value exceeds 2, a phase separation occurs in the monolayer. Interfacial tension, γ, is described as a function of X_m:

$$\gamma = \gamma_0 + \Gamma_s RT[\ln(1 - X_m) + \omega X_m^2] \quad (4)$$

$$\gamma = \gamma_0 + \Gamma_s RT[\ln(1 - a/A) + \omega(a/A)^2] \quad (5)$$

The γ–C relations were simulated by eqs 3 and 4. The best fittings were obtained with the Γ_s, K, and ω values summarized in Table I. The theoretical relations are in good agreement with the experimental ones as Figure 1 shows.

The Γ–C and F–A relations were also obtained with eqs 3 and 5, respectively. Figures 2 and 3 demonstrate satisfactory agreements between the experimental and the theoretical values. The use of a Γ_s value of 2.77×10^{-10} mol/cm^2 (corresponding to a value of 60×10^{-2} nm^2/molecule observed at the air–water interface) in eqs 3–5 did not give any calculated values of γ, Γ, and A consistent with the experimental results (see Figure 1A).

The stabilization of TO particles by a phospholipid in saline involves the formation of a monolayer at the TO particle–saline interface (the monolayer–TO liquid equilibrium). The relation between the interfacial pressure, F, and the composition of the monolayer, X_m, can be evaluated on the basis of the experimental results of the interfacial tension (Figure 1) and the adsorption isotherm (Figure 2) at the interface. The experimental results were represented well by the theoretical equations (eqs 3–5). The coexistence of the TO emulsion particles and liposomes (shown in the next section), which implies the equilibrium among the TO liquid, the monolayer, and the bilayer (excess phospholipid), leads to a stationary value of the interfacial tension, γ_c, as seen in Figure 1. These equilibria among the TO liquid, the monolayer, and the bilayer constitute the phase diagram of the phospholipid–TO mixture in saline. Figure 4A shows the phase diagram of the TO–PC mixture in saline. Line ce, representing the equilibrium between the monolayer and the TO liquid phase, was calculated by using eq 4 with the parameters listed in Table I. The F value of line beg, 13.48 mN/m, was obtained as $\gamma_0 - \gamma_c$. On this line, the monolayer ($X_m = 0.95$), the TO liquid (the mole fraction of PC, X_c, is 8×10^{-4}), and the bilayer (the mole fraction of PC, X_b, is 0.97; Miller & Small, 1982) coexist in equilibrium. Coexisting in the region bce are the mixed monolayer and the TO liquid phase. The TO particles can be stabilized as microemulsion in the upper part of the region. Figure 4B shows the diagrams for PE and PS evaluated in similar ways. The maximum attainable mole fractions of PE and PS in the monolayer at the interface are 0.73 and 0.88, respectively.

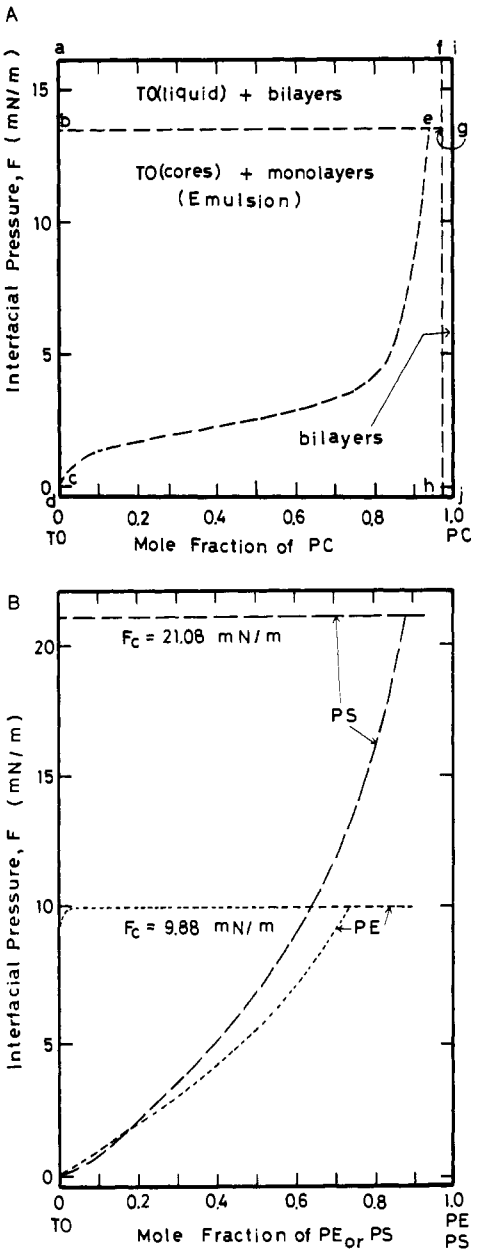

FIGURE 4: (A) Phase diagram of PC–TO mixture in saline. The line ce is calculated from eq 4 (parameters: see Table I). The F value is highest at the line beg, and $F_c = \gamma_0 - \gamma_c = 13.48$ mN/m. The TO–PC–saline emulsion is produced in the region bce. The bilayer is formed in the region fhji. On the line beg, the emulsion (monolayer, e, + core, b) is in equilibrium with the bilayer, g. $X_m = 0.95$ at e, and $X_b = 0.97$ at g. Theoretically, a mixed monolayer of PC and TO exist in the region ecdhg, but this region is unrealizable at the TO–saline interface. (B) Relations between interfacial pressure and mole fraction of phospholipid in monolayer calculated by eq 4 (parameters: see Table I). The diagrams show that remarkable amounts of TO remain in the monolayer of PE and PS even at the highest interfacial pressure, F_c.

Formation of Microemulsion. The procedures described under Experimental Procedures produced stable microemulsions of TO–PC–saline mixtures. PE or PS as an emulsifier did not stabilize TO particles in saline: rapid separation of TO and saline was observed within 30 s after sonication. A

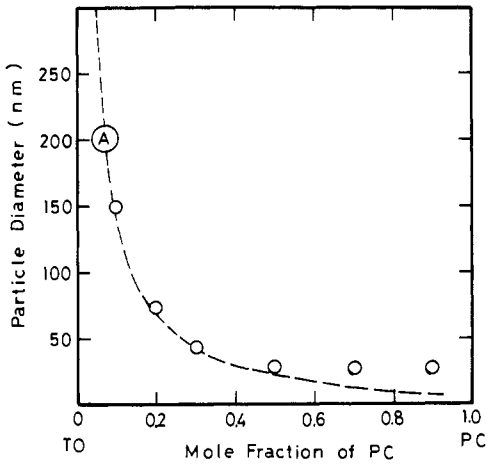

FIGURE 5: Diameter of emulsion particle as a function of PC mole fraction, ξ. A shows lymphatic chylomicrons (PC:triglyceride = 0.7:9.3) (Atkinson & Small, 1986). The broken line is calculated by eq 7 (δ = 2.1 nm, X_c = 0, and X_m = 0.95).

phosphatidylethanolamine sample of a fatty acid composition similar to PC (34.6% 16:0, 1.4% 16:1, 12.1% 18:0, 32.2% 18:1, 14.5% 18:2, 2.3% 20:4, and 2.9% 22:6), prepared by the choline–ethanolamine exchange of egg PC at Research Laboratory, Q.P. Co. (Tokyo), also failed to stabilize the TO particles in saline (unpublished data). Figure 5 depicts the weight-averaged diameter of the emulsion particles measured by the QLS method as a function of the mole fraction of PC in the TO–PC mixture, ξ. The increase of ξ decreases the particle size. Coexistence of bilayers (liposomes) with the emulsion particles was observed on electron micrographs of the negatively stained microemulsion at ξ = 0.5 (data not shown). The presence of liposomes in the microemulsion was also confirmed from a differential quenching of *N*-dansyl-PE incorporated in the outer and the inner PC monolayers of liposomes by Cu^{2+}. The fractions of PC participating in the liposome formation were ca. 10 and 2 mol % for the mixtures of ξ = 0.5 and 0.3, respectively (data not shown).

Discussion

Phospholipid Monolayer and Emulsion Stability. PC molecules form a condensed monolayer at the TO–saline interface with the lateral interaction parameter, ω, as large as 1.8, indicating the lateral attractive interaction of $1.8RT$ between the PC molecules. PE and PS form more expanded monolayers. The ω values of phospholipid–TO mixed monolayers were evaluated also at the air–saline interface: ca. −2 for PC–TO and ca. 0 for PE–TO and PS–TO mixtures (unpublished data). These results suggest that the type of interface remarkably affects the molecular interaction between lipids in monolayers. The maximum interfacial concentration of phospholipid at the TO–saline interface is attained at the minimum interfacial tension, γ_c, where the mixed monolayer, the TO liquid, and the phospholipid bilayer phases are in equilibrium. The Gibbs phase rule shows that the degree of freedom of the system is zero; thus γ, X_m, X_c, and X_b are fixed (Nakagaki et al., 1985). The X_m values for PC, PE, and PS are 0.95, 0.73, and 0.88, respectively. Therefore, an appreciable area of the interface is occupied by TO in the PE or PS monolayer; that is, the exposure of the less hydrophilic monolayer to saline leads to unstable interface.

TO droplets dispersed in aqueous solution are in constant motion, and there are frequent collisions between them. If, on the collision, the interfacial films surrounding the two colliding droplets rupture, the two droplets will coalesce into a larger one. The mechanical strength of the interfacial monolayer is the prime factor determining the emulsion stability (Sherman, 1968; Adamson, 1967; Rosen, 1978). For the stabilization of the emulsion, the phospholipid monolayer at the TO droplet surface should be condensed with strong lateral intermolecular forces, exhibiting a high film elasticity: When the collision and the subsequent deformation of the emulsion particles expand the surface area (the decrease of X_m), the resultant decrease of F is quickly compensated by the transfer of PC molecules from the adjacent region of the monolayer and/or from the core solution. Induction of a large restoring force for the expansion requires the high interfacial elasticity, $-A(dF/dA)$, which is calculated from eqs 4 and 5:

$$-A(dF/dA) = X_m(dF/dX_m) = \Gamma_s RT\left(\frac{X_m}{1-X_m} - 2\omega X_m^2\right) \quad (6)$$

At the maximum surface pressure, F_c, the restoring coefficients, $X_m(dF/dX_m)$, are 160, 70, and 20 mN/m for PC, PS, and PE, respectively (see Figure 4). These investigations predict that the PC monolayer can stabilize most effectively the TO droplets in saline, consistent with the experimental observations. This may be related to the fact that PC is by far the most abundant phospholipid in human lipoproteins (Nichols et al., 1986; Chapman, 1986).

Conversion of Monolayers to Bilayers. As mentioned before, electron micrography and differential quenching experiments show the presence of liposomes (bilayers) in the emulsion. Similar results have been reported for TO–cholesteryl oleate–cholesterol–PC emulsions (Miller & Small, 1983a,b). The coexistence of liposomes and emulsion particles is explained on the basis of the equilibrium line beg in Figure 4A. Irrespective of the PC/TO ratio of the emulsion, the composition of the monolayer at the emulsion particle surface is fixed at X_m = 0.95, when the bilayers (liposomes) coexist with the emulsion particles. The points b, e, and g represent the TO liquid (i.e., the core of the emulsion particles), the PC monolayer containing 5 mol % of TO (i.e., the monolayers on the particles) and the PC bilayers (liposomes). Miller and Small (1982) have separated the PC bilayers from the emulsion particles by ultracentrifugation and analyzed their lipid composition as X_b = 0.97. The composition of the bilayer, X_b (g in Figure 4A) is not necessarily equal to that of the monolayer on the emulsion particle, X_m (e in the same figure). The difference between X_m and X_b in this case is small.

If the amount of TO in the core is reduced, the surface monolayer becomes redundant and finally the bilayer separates from the particle through the process b + e → g in Figure 4A (collapse of the monolayer). This equilibrium process presumably plays an important role in the conversion of the surface monolayers of chylomicrons or VLDLs to HDLs in plasma (Tall et al., 1982). The process is schematically illustrated in Figure 6.

Size of Microemulsion Particles. PC and TO substantially separate each other in a microemulsion particle and constitute the surface monolayer and the core, respectively. The particle radius, r, is controlled geometrically by the volume ratio of the monolayer to the core. If the fraction of PC participating in the bilayer formation is very small, the r value is related to the PC composition of the PC–TO mixture, ξ (see Appendix II):

$$\frac{(r-\delta)^3}{r^3-(r-\delta)^3} = \left[\frac{X_c V_{PC} + (1-X_c)V_{TO}}{X_m V_{PC} + (1-X_m)V_{TO}}\right]\left(\frac{X_m - \xi}{\xi - X_c}\right) \quad (7)$$

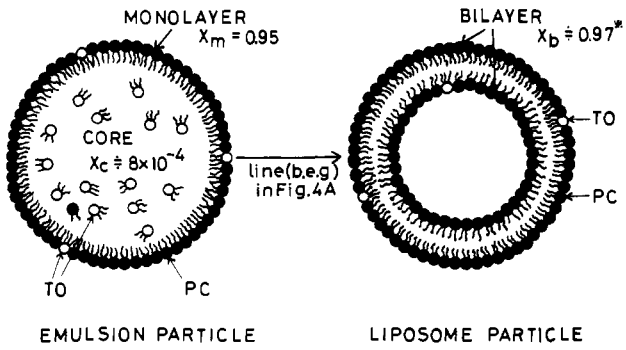

FIGURE 6: Schematic representation for conversion of emulsion particles to bilayers (liposomes).

Here, δ is the monolayer thickness, which is 2.1 nm (Huang & Mason, 1978). The molar volumes V_{PC} and V_{TO} are 760 and 967 cm^3/mol, respectively. The values of X_m and X_c are 0.95 and 0.8 × 10^{-4}, respectively. The broken line in Figure 5 shows the particle diameter, $2r$, calculated from eq 7, as a function of ξ. When $\xi < 0.4$, the experimental value of the diameter is in good agreement with the calculated one. The diameter of lymph chylomicrons (A) (Atkinson & Small, 1986) is shown in this figure. When $\xi > 0.4$, appreciable deviations are observed and the experimental values are nearly constant ($2r$ = 27–29 nm). The equilibrium between the monolayers and the bilayers (i.e., coexistence of the emulsion and the liposome particles) is partly responsible for the deviations. This effect, however, could be minimized by effective sonication (energy supply) in the preparation. On the other hand, the geometrical packing of the PC molecules, which constitute the monolayer on the emulsion particles, restricts the formation of very small emulsion particles ($2r$ < ca. 25 nm) (Huang & Mason, 1978; Israelachvili et al., 1976; Ruckenstein & Nagarajan, 1977). At the higher value of ξ, a part of the PC molecules participate in the formation of the emulsion particles of the minimum size (ca. 25 nm), and the excess (of PC molecules) constitutes the bilayers.

Further work on the coexistence of the emulsion and the liposome particles and the effects of apoprotein on the monolayer and the particle properties is in progress and will be published later.

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Appendix I

Defay et al. (1966) introduced a lateral interaction parameter to Butler's chemical potentials of lipids in a monolayer. For a mixed monolayer of lipids of similar size, TO and a phospholipid, PL, their chemical potentials are expressed as

$$\mu_{PL}^{m} = \mu_{PL}^{mo} + RT \ln (f_{PL}^{m} X_m) - N a_{PL} \gamma \quad \text{(A1)}$$

$$\mu_{TO}^{m} = \mu_{TO}^{mo} + RT \ln [f_{TO}^{m}(1 - X_m)] - N a_{TO} \gamma \quad \text{(A2)}$$

Here, μ^{mo} is a standard potential for a pure lipid and is constant under a constant temperature and a pressure. f^m is an activity coefficient based on the symmetrical choice of standard state. X_m is the mole fraction of PL in the monolayer. N, a, and γ are the Avogadro number, the cross-sectional area of the lipid molecule, and the interfacial tension, respectively.

For a dilute solution of PL in TO, the chemical potentials are described in a usual way:

$$\mu_{PL}^{b} = \mu_{PL}^{b*} + RT \ln X_c \quad \text{(A3)}$$

$$\mu_{TO}^{b} = \mu_{TO}^{bo} + RT \ln (1 - X_c) \quad \text{(A4)}$$

Here, X_c is the mole fraction of PL in the solution and very small as $1 - X_c \doteq 1$ and μ_{PL}^{b*} is a standard chemical potential based on the asymmetric choice.

In the equilibrium of TO between the monolayer and the solution, eqs A2 and A4 are equated with each other to give the relation:

$$F = \gamma_0 - \gamma = -[RT/(N a_{TO})] \ln [f_{TO}^{m}(1 - X_m)] \quad \text{(A5)}$$

Here γ_0 is the interfacial tension at the TO–saline interface without the PL monolayer (i.e., $X_m = X_c = 0$), and $N a_{TO} \gamma_0 = \mu_{TO}^{mo} - \mu_{TO}^{bo}$. In the equilibrium of PL between the monolayer and the solution, eqs A1 and A3 similarly give the relation:

$$F = \gamma_0 - \gamma = -[RT/(N a_{PL})][\ln (f_{PL}^{m} X_m) - \ln (f_{PL}^{m*} K) - \ln C] \quad \text{(A6)}$$

Here, f_{PL}^{m*} is the limiting value of f_{PL}^{m}, when $X_c \to 0$ ($X_m \to 0$), and

$$K = \left(\frac{v_{TO} M_{TO}}{1000}\right) \lim_{X_c \to 0} \left(\frac{X_m}{X_c}\right) = \text{constant} \quad \text{(A7)}$$

v_{TO} (=1/density of TO) and M_{TO} are the specific volume and molecular weight of TO, respectively. At 25 °C, $v_{TO} M_{TO}/1000$ = 0.967 M^{-1}. From eqs A5 and A6, one can obtain

$$(f_{PL}^{m} X_m)/[f_{TO}^{m}(1 - X_m)] = f_{PL}^{m*} K C \quad \text{(A8)}$$

The Bragg–Williams theory (Hill, 1960) is applied for the lateral interaction of lipids in the monolayer as

$$f_{PL}^{m} = \exp[\omega(1 - X_m)^2] \qquad f_{TO}^{m} = \exp[\omega(X_m)^2] \quad \text{(A9)}$$

and $f_{PL}^{m*} = \exp(\omega)$. ω is the interaction parameter. Similar treatments have been employed by Lucassen-Reynders (1966).

The a value of PL is ca. 40 × 10^{-2} nm^2. Marsden and Rideal (1938) showed an area per molecule of 25 × 10^{-2} nm^2 at F = 28 mN/m for a cis fatty acid, erucic acid, at the air–water (pH 7.2) interface at 21 °C. We estimated a similar value as the cross-sectional area of oleic acid on the basis of a molecular model, which leads to 75 × 10^{-2} nm^2/molecule as the value of a_{TO}. At the TO–water interface, a more closely packed conformation is possible, if the α-carbonyl groups protrude into the water phase and the β group is buried in the TO phase (Hamilton & Small, 1981). Therefore, we assumed (65–75) (70 ± 5) × 10^{-2} nm^2/molecule for a_{TO}. The a value for triglyceride of saturated fatty acid is about 60 × 10^{-2} nm^2/molecule (Small, 1986). We introduce the approximation: $a_{PL} = a_{TO} = a$ = ca. 40 × 10^{-2} nm^2. Although, this approximation results in a 40% higher value of ω in the simulation, the selected values of ω, K, and a shown in Table I gave theoretical values of γ (F) and Γ in good agreement with the experimental values.

The adsorption amount, Γ, and the molecular area, A, are related to X_m:

$$\Gamma = X_m/(N a) = \Gamma_s X_m \quad \text{(A10)}$$

and

$$A = 1/(N\Gamma) = a/X_m \quad \text{(A11)}$$

Eqs A8 and A5 are rewritten with eqs A9–A11 to lead to eqs 3 and 5, respectively.

Appendix II

The volumes of the cores and the surface monolayers of emulsion particles are

$$n[V_{PC}X_c + V_{TO}(1 - X_c)] = N_e(4\pi/3)(r - \delta)^3 \quad \text{(A12)}$$

$$m[V_{PC}X_m + V_{TO}(1 - X_m)] = N_e(4\pi/3)[r^3 - (r - \delta)^3] \quad \text{(A13)}$$

n and m are the sums of moles of PC and TO constituting the cores and the monolayers, respectively. V represents the partial molar volume of each lipid. N_e is the number of the emulsion particles. The overall mole fraction of PC, ξ, is related with n/m:

$$n/m = (X_m - \xi)/(\xi - X_c) \quad \text{(A14)}$$

From eqs A12–A14, eq 7 is obtained.

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