MISCIBILITY, CHAIN PACKING, AND HYDRATION OF 1-PALMITOYL-2-OLEOYL PHOSPHATIDYLCHOLINE AND OTHER LIPIDS IN SURFACE PHASES

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ABSTRACT The miscibility of 1-palmitoyl-2-oleoyl phosphatidylcholine with triolein, 1,2-diolein, 1,3-diolein, 1(3)-monoolein, oleyl alcohol, methyl oleate, oleic acid, and oleyl cyanide (18:1 lipids) was studied at the argon-water interface. The isothermal phase diagrams for the mixtures at 24° were characterized by two compositional regions. At the limit of miscibility with lower mol fractions of 18:1 lipid, the surface pressure was composition-independent, but above a mixture-specific stoichiometry, surface pressure at the limit of miscibility was composition-dependent. From the two-dimensional phase rule, it was determined that at low mol fractions of 18:1 lipids, the surface consisted of phospholipid and a preferred packing array or complex of phospholipid and 18:1 lipid, whereas, above the stoichiometry of the complex, the surface phase consisted of complex and excess 18:1 lipids. In both regions of the phase diagram, mixing along the phase boundary was apparently ideal allowing application of an equation of state described earlier (J. M. Smaby and H. L. Brockman, 1984, *Biochemistry*, 23:3312–3316). From such analysis, apparent partial molecular areas and hydrations for phospholipid, complex, and 18:1 lipid were obtained. Comparison of these calculated parameters for the complexed and uncomplexed states shows that the aliphatic moieties behave independently of polar head group. The transition of each 18:1 chain to the complexed state involves the loss of about one interfacial water molecule and its corresponding area. For 18:1 lipids with more than one chain another two water molecules per additional chain are present in both states but contribute little to molecular area. In contrast to 18:1 lipids, the phospholipid area and hydration change little upon complexation. The uniformity of chain packing and hydration behavior among 18:1 lipid species contrasts with complex stoichiometries that vary from 0.04 to 0.65. This suggests that the stoichiometry of the preferred packing array is determined by interactions involving the more polar moieties of the 18:1 lipids and the phospholipid.

INTRODUCTION

It is well established that the physical properties of the cores of lipoproteins reflect closely the physical behavior of their constituent lipids (Small and Shipley, 1974). In contrast, the behavior of lipids in surfaces of lipoproteins and other naturally occurring emulsions is less clearly understood. Particularly difficult to define have been the availability and state of apolar core lipids at the lipoprotein surface. This has occurred because their mass in the surface monolayer is very small relative to the particle as a whole (Miller and Small, 1983*a*; Miller and Small, 1983*b*) and because apolar molecules in the surface phase are probably in rapid equilibrium with those in the core. As a consequence, the contribution to measurable physical parameters of apolar lipids in the surface phase may be very small relative to that from the core or may be lost through signal averaging. Knowledge of the concentration and state of apolar lipids in surface phases is important because lipolytic enzymes, such as hepatic and lipoprotein lipases, and presumably transfer proteins act at the surface of the substrate particle, not its core (Brockman, 1984).

One approach to elucidating surface structure has been to study the incorporation of apolar lipids into phospholipid bilayers (Hamilton et al., 1983) although the relationship between the bilayer state and lipoprotein surfaces is not well defined. Alternatively, the behavior of apolar lipids, particularly cholesteryl esters (Smaby and Brockman, 1981*a*, Smaby and Brockman, 1981*b*), has been studied in lipid films at the air/water interface. As with bilayers, however, the relation between monolayer state and that at the emulsion surface is not understood. One way to strengthen the relation between model systems and natural emulsions is to study the properties of the models at or beyond the solubility limit of the apolar lipid in the more polar surface lipids. This fixes the activity of the apolar lipid to that in the bulk phase, as in natural emulsions. With bilayers this approach has enabled the determination of phase diagrams but has not established what regulates the solubilities of apolar lipids in surfaces. As an alternative, miscibilities of cholesteryl esters have been determined in lipid films at the air/water interface. Analysis of such data led to the development of a thermodynamic equation of state that predicts that along phase boundaries each cholesteryl ester or solubilizing lipid has associated

with it a fixed number of water molecules and behaves as a discrete unit. Moreover, mixing of these units is apparently ideal (Smaby and Brockman, 1984).

Because of the potential of this approach to provide a rational, thermodynamic basis for understanding the surfaces of lipoproteins and model emulsions in general, we have studied its applicability to glycerides and other oleoyl- or oleyl-containing lipids in mixtures with a representative phosphatidylcholine. The results reveal the existence of two packing densities and hydration states for 18:1 chains and suggest that the thermodynamic model is not limited to cholesteryl ester-containing surface phases, but may have more general applicability.

MATERIALS AND METHODS

Lipids

Triolein, oleyl alcohol, methyl oleate, oleic acid, 1(3)-monoolein, 1,2-diolein, 1,3-diolein, and oleyl cyanide (10-monodecene nitrile) were purchased from NuCheck Prep, Inc., Elysian, MN. The purity of each of these 18:1 lipids was checked by thin-layer chromatography and from measured detection limits, after spraying with chromic-sulfuric acid and charring, purity was shown to be greater than 99%. 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine was purchased from Avanti Biochemicals, Birmingham, AL. Purity of the phospholipid was also greater than 99% when analyzed by thin-layer chromatography. Phospholipid concentration in stock solutions was determined by assaying aliquots for organic phosphorus (Bartlett, 1959).

Solvents

Petroleum ether was purified as previously described (Smaby and Brockman, 1981*a*). Ethanol was distilled from KOH and zinc. The absence of surface active impurities in each solvent was determined as described earlier (Smaby et al., 1983). Water was purified by reverse osmosis, mixed-bed deionization, adsorption on activated charcoal, and filtration through a 0.2-μm polycarbonate membrane (Nuclepore, Pleasanton, CA).

Surface Pressure-Molecular Area Isotherms

In all cases the lipids were spread in a humidified argon atmosphere onto a 10 mM potassium phosphate-0.1 M sodium chloride subphase, pH 6.6 at 24°. Surface pressure was measured as a function of area using a fully automated Langmuir film balance system (Brockman et al., 1980; Brockman et al., 1984). All mixtures were spread in 51.67 μl of petroleum ether or petroleum ether/ethanol (95:5). After standing at a large molecular area for four minutes, the films were compressed at ≤ 5 Å^2/min/molecule. Phase transitions were identified using second and third derivatives, as previously described (Brockman et al., 1980). Area calibration was accomplished by plotting estimated geometric area vs. number of molecules of a test lipid at several surface pressures. The responses were linear at each pressure with a common nonzero intercept on the area axis. From this, an area correction term was obtained that was used to achieve true proportionality. The magnitude of the correction showed that previously reported molecular areas could have been 5–10% larger than those reported herein, when measured at small absolute areas.

RESULTS

To help define the determinants of lipid miscibility and packing in surface phases, a family of lipids containing identical aliphatic moieties (18:1 lipids) was studied at the Ar/buffer interface in binary mixtures with 1-palmitoyl-2-oleoyl phosphatidylcholine. All of these lipids gave expanded type surface pressure-area isotherms in pure form and did not exhibit expanded-condensed phase transitions at 24°. The surface pressure-area isotherms for each set of mixtures exhibited a common type of behavior, which is exemplified in Fig. 1. When the surface pressure is plotted as a function of the apparent molecular area of one species, here 1-palmitoyl-2-oleoyl phosphatidylcholine, a pattern of behavior is apparent. As the mol fraction of 1,3-diolein is increased, from left to right in Fig. 1, an expansion of the apparent molecular area of the phospholipid occurs. At mol fractions of diolein up to 0.25, a single discontinuity in the curves is observed at a surface pressure of 45.6 mN/m, the collapse pressure of pure 1-palmitoyl-2-oleoyl phosphatidylcholine monolayers. At higher mol

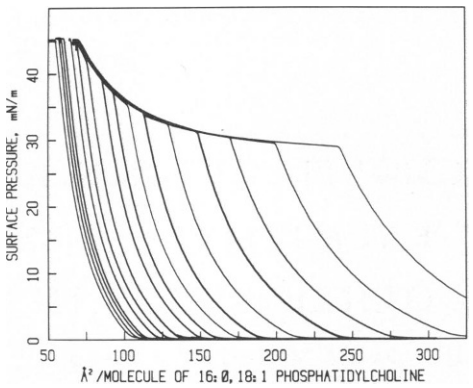

FIGURE 1 Surface pressure-molecular area isotherms for mixtures of 1,3-diolein and 1-palmitoyl-2-oleoyl phosphatidylcholine. The subphase was 10 mM potassium phosphate and 0.1 M NaCl, pH 6.6, 24°C. The mol fractions of 1,3-diolein from left to right were 0.0, 0.049, 0.109, 0.150, 0.199, 0.250, 0.291, 0.360, 0.399, 0.466, 0.499, 0.552, 0.603, 0.649, 0.703, and 0.752.

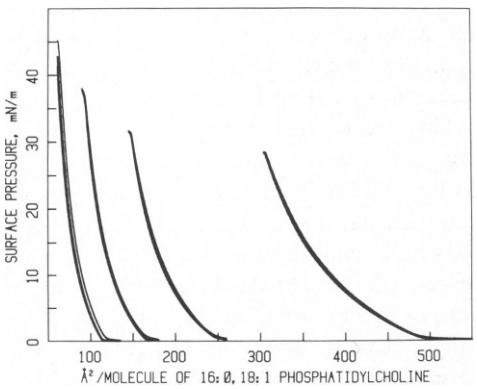

FIGURE 2 Surface pressure-molecular area isotherms for mixtures of 1,3-diolein and 1-palmitoyl-2-oleoyl phosphatidylcholine. The subphase was 10 mM potassium phosphate, 0.1 M NaCl, pH 6.6, 24°C. The lipid films were compressed to just beyond the point of collapse, expanded and recompressed. The mol fractions of 1,3-diolein from left to right were 0.150, 0.399, 0.603, and 0.799.

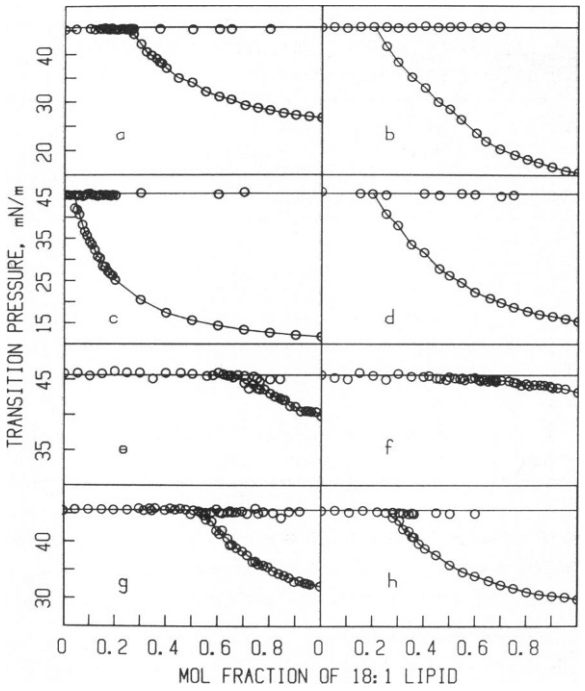

FIGURE 3 Isothermal phase diagrams for mixtures of 18:1 lipids with 1-palmitoyl-2-oleoyl phosphatidylcholine. Phase transitions were identified as described in the text. 18:1 lipids (*a*) 1,3-diolein; (*b*) oleyl cyanide; (*c*) triolein; (*d*) methyl oleate; (*e*) oleic acid; (*f*) monoolein; (*g*) oleyl alcohol; and (*h*) 1,2-diolein.

fractions of 1,3-diolein, a second transition is observed, which approaches the collapse pressure of pure 1,3-diolein. Note that the lower transitions in this range define an envelope curve on which the isotherms coincide above their transition pressure. The qualitative interpretation of such data follows from the two-dimensional phase rule as elaborated by Crisp (1949*a* and *b*). The absence of any transitions at compositions below 0.25 and pressures below 45.6 mN/m indicates that 1-palmitoyl-2-oleoyl phosphatidylcholine and 1,3-diolein are not only miscible in this composition range, but form a complex or preferred packing array that collapses at a surface pressure of 45.6 mN/m. The continuous variation of transition pressure with composition above 0.25 indicates miscibility between the complex and 1,3-diolein.

To determine if the transitions shown are reversible on our time scale, i.e., equilibrium is being achieved, films at selected compositions were compressed to just beyond the point of collapse, expanded, and recompressed. As shown in Fig. 2, the near identity of the three curves in each set suggests strongly that the transition pressures and areas are near equilibrium values. This is also supported by Fig. 1, which shows that the curves break directly onto the envelope curve without significant overshoot, as well as by the observation that at several compositions and over a tenfold range of film compression rate, transition areas and pressures were unchanged (data not shown).

From data of the type shown in Fig. 1, phase transition pressure-composition phase diagrams can be constructed. For 1,3-diolein-phospholipid mixtures, the phase diagram (Fig. 3 *a*) shows the region of monolayer formation that consists of the entire area from mol fractions between zero and 1.0 at surface pressure below the lower transitions. The region of monolayer-bulk phase coexistence lies between the two sets of transitions at mol fractions greater than 0.25. Above 45.6 mN/m no monolayer is present. For the other phospholipid-18:1 lipid systems studied, similar phase diagrams were obtained (Figs. 3 *b–h*). These indicate that the formation of preferred packing arrays or complexes is not specific to diolein, but is a general property of such lipids.

The point of intersection of the curves in each phase diagram is the mol fraction of 18:1 lipid at which only complex is present, X_c. For most of the mixtures this value can be determined accurately by inspection of the phase diagrams. For systems like monoolein-1-palmitoyl-2-oleoyl phosphatidylcholine (Fig. 3 *f*), however, the collapse pressures of the pure components are similar. To better estimate X_c and to minimize human bias, data were plotted as transition pressure vs. $-\log$ (mol fraction of 18:1 lipid). Complex stoichiometry was defined as the mol fraction at which a linear extrapolation of the data near the complex stoichiometry intersects 45.6 mN/m. This method gave values of X_c that agree within ± 0.02 with those determined by inspection, and was used to obtain X_c for the phase digrams shown in Fig. 3. These values are summarized in Table I.

In a previous study involving cholesteryl esters mixed with more amphipathic lipids (Smaby and Brockman, 1984) surface phase behavior similar to that described here was observed. Importantly, however, because $X_c \approx 0$, the formation of preferred packing arrays or complexes at the limits of miscibility was not explicitly recognized. In the prior study it was shown that data obtained along the phase boundary, delineating the solubility of cholesteryl esters in the monolayer phase, obeys a simple relationship (Smaby and Brockman, 1984),

$$\bar{A} = \sum_{i=2}^{n} X_i \omega_i, \qquad (1)$$

where $\bar{A}$ is the average molecular area of the lipid components 2 to n, X_i is the mol fraction of the i'th component relative to components 2 to n, and ω_i is its apparent partial molecular area. In the present study, this phase boundary corresponds to the line describing the miscibility of 18:1 lipid with complex, e.g., the lower set of transitions to the right of the complex stoichiometry in Figs. 3 *a–h*. To test this model for lipid miscibility where values of X_c are not zero, Eq. 1 must be modified. Specifically, the equation must reflect that above X_c, $X_c/(1 - X_c)$ mol of 18:1 lipid are complexed, i.e., above X_c the surface is composed of complex and excess 18:1 lipid. Thus, as the mol fraction of 18:1 lipid changes from X_c to 1.0, the mol fraction of uncomplexed 18:1 lipid changes from zero to 1.0. More-

TABLE I

MISCIBILITY, AREA AND HYDRATION PARAMETERS FOR 18:1 LIPIDS WITH 1-PALMITOYL-2-OLEOYL PHOSPHATIDYLCHOLINE AT THE MONOLAYER PHASE BOUNDARY

18:1 lipid	Complex stoichiometry	Apparent partial molecular area, Å²			Collapse pressure	Associated water, mol/mol		Activity coefficient
		Uncomplexed phospholipid	Complex	Uncomplexed 18:1 lipid		Complex	18:1 lipid	
	X_c	ω_2	ω_c	ω_3	π_{3c}	a_c	a_3	f_1
Triolein	0.04	52.6	58.7	101.2	11.7	0.653	6.612	0.880
1,3-Diolein	0.25	53.5	70.8	63.5	26.8	0.882	2.852	0.724
1,2-Diolein	0.26	53.8	69.6	59.2	29.8	1.081	3.949	0.623
Methyl Oleate	0.20	54.3	63.9	36.1	15.3	0.386	1.534	1.161
Oleic Acid	0.65	53.1	96.2	25.8	39.7	0.271	0.355	1.485
Oleyl Alcohol	0.52	53.6	78.4	29.5	32.0	0.529	1.086	0.926
Monoolein	0.58	53.1	92.0	29.2	43.1	0.326	0.343	1.398
Oleyl Cyanide	0.21	52.4	60.9	31.0	15.5	0.267	0.808	1.574

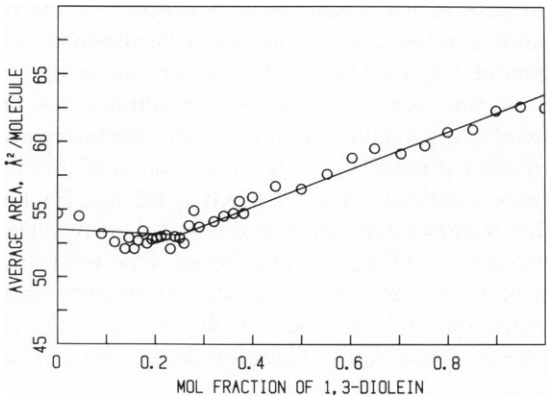

FIGURE 4 Average molecular area at the phase boundary as a function of composition for mixtures of 1,3-diolein and 1-palmitoyl-2-oleoyl phosphatidylcholine.

over, since the choice of ordinate is arbitrary the surface at mol fractions of 18:1 lipid less than X_c should also obey Eq. 1, modified to reflect that as the mol fraction of 18:1 lipid changes from 0.0 to X_c, the mol fraction of complex changes from 0.0 to 1.0. In this range, the other species present is uncomplexed phosphatidylcholine. If X_3 is the mol fraction of 18:1 lipid in the two component lipid mixture, Eq. 1 becomes

$$\bar{A} = \omega_2 + X_3\left[\frac{(\omega_c - \omega_2)\,(1 - X_c)}{X_c} - \omega_2\right] \qquad (2)$$

for $0 < X_3 < X_c$ and

$$\bar{A} = \omega_c - \omega_3\frac{(X_c)}{1 - X_c} + X_3\left[\frac{\omega_3}{1 - X_c} - \omega_c\right] \qquad (3)$$

for $X_c < X_3 < 1$ where ω_2, ω_3, and ω_c are the apparent partial molecular areas of uncomplexed phospholipid, uncomplexed 18:1 lipid and complex, the latter being expressed per mol of phospholipid. Because both equations are linear with respect to X_3, a biphasic plot of $\bar{A}$ vs. X_3 should be obtained. For the 1,3-diolein-phospholipid data of Fig. 3 *a*, this plot is shown in Fig. 4. For each of the mixtures studied, reasonable agreement with the biphasic model of $\bar{A}$ vs. X_3 was observed and the values of ω_2, ω_c, and ω_3 calculated from the slopes and intercepts using Eqs. 2 and 3 are given in Table I.

For $X_3 < X_c$ the phase diagrams for the mixtures are characterized by a constant surface pressure at the limit of miscibility (Figs. 3 *a–h*), the value of which is the collapse pressure of the phospholipid. Because each pure 18:1 lipid has a lower collapse pressure (Table I), the formation of the complexes must involve a dehydration process. This follows from the thermodynamic relationship (Gaines, 1978).

$$\pi = \frac{-kT}{\omega_1}\ln f_1 X_1, \qquad (4)$$

where π is the surface pressure, ω_1 is the partial molecular area of water, f_1 is the activity coefficient of interfacial water, and X_1 is its mol fraction in the three-component surface phase. The amount of water associated with the complex and with uncomplexed 18:1 lipid can be estimated by a further application of the thermodynamic model used to describe cholesteryl ester miscibility in surfaces (Smaby and Brockman, 1984). For cholesteryl ester containing mixtures, it was shown that not only is the apparent partial molecular area of each lipid species constant at the limits of miscibility, but also that each molecule of each lipid species has associated with it a stoichiometric number of water molecules. These are defined as the interfacial water molecules which determine the surface pressure at the limit of miscibility. By combining Eqs. 3 and 4, it can be shown that at $X_3 > X_c$ along the phase boundary, where $\pi = \pi_c$, i.e., at the limits of miscibility of complex and 18:1-lipid,

$$\pi_c = \frac{-kT}{\omega_1}\ln \cdot \left\{\frac{f_1\,[a_c + (a_3 - a_c)\,X_3]}{a_c + 1 - \dfrac{X_c}{1 - X_c} + \left(a_3 - a_c + \dfrac{X_c}{1 - X_c}\right)X_3}\right\}. \qquad (5)$$

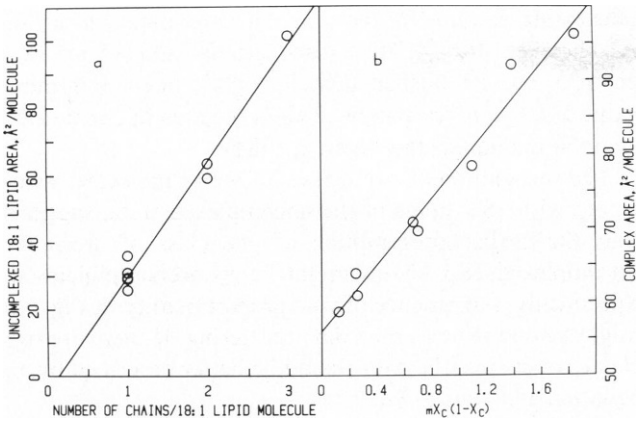

FIGURE 5 (*a*) Apparent partial molecular area of uncomplexed 18:1 lipid as a function of the number of aliphatic chains per molecule, (*b*) apparent partial molecular area of the complex as a function of the mol of 18:1 chains per mol of complex. Complex stoichiometries (X_c) and areas (ω_c) are from Table I.

This general equation degenerates to a form equivalent to the previously reported relationship (Smaby and Brockman, 1984, Eq. 3) for the case that $X_c = 0$, i.e., no complexation occurs.

For the eight binary systems studied, values of f_1, a_c and a_3 were determined using the fitting procedure previously described (Smaby and Brockman, 1984). These are reported in Table I. Note that, as would be predicted by the collapse pressures of pure 18:1 lipids (Table I) and Eq. 2, the hydration (a_3) of uncomplexed triglyceride is the greatest, the dioleins are less and the single chain 18:1 lipids are least hydrated in the surface phase.

DISCUSSION

In a previous study, an equation of state was developed to describe cholesteryl ester miscibility in surface phases (Smaby and Brockman, 1984). The basis for the model is

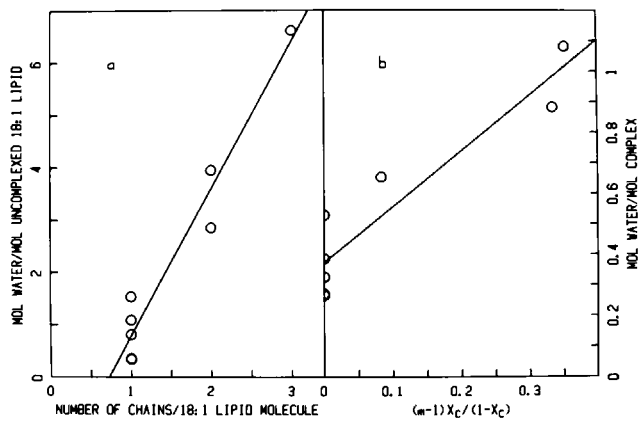

FIGURE 6 (*a*) Mol of water per mol of uncomplexed 18:1 lipid as a function of number of chains per molecule, (*b*) Mol of water per mol of complex as a function of the mol of chains (less one per 18:1 lipid molecule) per mol of complex. Stoichiometrics (X_c) and hydration parameters (a_c) are from Table I.

that at the surface pressure that defines the miscibility limit for a particular composition, each lipid species occupies a state that is characterized by an apparent partial molecular area and degree of hydration. Moreover, these parameters are independent of composition and surface pressure, i.e., are characteristic of the lipid species itself, and mixing of species is apparently ideal. In the present study, this model was tested with mixtures of 18:1 lipids with 1-palmitoyl-2-oleoyl phosphatidylcholine. When allowance is made for the formation of preferred packing arrays or complexes between the species, the present data indicate that the model is well obeyed for each binary system studied. That each species behaves in a mixture-independent manner is suggested by the constancy of the apparent partial molecular area for phospholipid in mixtures with 18:1 lipid-phospholipid complexes, ω_2. Averaging the values in Table I gives 53.3 ± 0.63 Å^2.

For the 18:1 lipids, there is a considerable variation in the head group structure. If polar head group is the primary determinant of packing, uniformity of behavior among 18:1 lipid species would not be expected. In contrast, if the aliphatic chains predominate, a simple proportionality should exist between the apparent partial molecular areas of uncomplexed 18:1 lipids and the number of aliphatic chains in each species, m. Fig. 5 *a* shows that such a relation is approximately obeyed ($r = 0.991$) for the eight systems studied. Assuming proportionality, the chains would each occupy 34.3 ± 1.9 Å^2.

Inspection of the apparent partial molecular areas of the complexes, ω_c in Table I, shows no obvious pattern. If in the complexes 18:1 chain packing and phospholipid packing were uniform, as in the uncomplexed state, then

$$\omega_c = \omega_{2c} + \omega_{3c}\, m\left[\frac{X_c}{1 - X_c}\right], \qquad (6)$$

where the subscripts 2c and 3c refer to the apparent partial molecular areas of phospholipid and 18:1-chains in the complexed state. Fig. 5 *b* shows that a plot of ω_c vs. m $X_c/(1 - X_c)$ is linear ($r = 0.985$) and from the intercept and slope ω_{2c} is 56.0 ± 1.3 and ω_{3c} is 22.4 ± 1.6 Å^2. Thus, although complexed phospholipid occupies nearly the same area as uncomplexed phospholipid, the 18:1 chain area has decreased to 22.4 Å^2. This suggests that it is primarily the conformation or hydration of 18:1 lipid, not phospholipid, which changes when it mixes with 1-palmitoyl-2-oleoyl phosphatidylcholine in the preferred array or complex. Moreover, the uniformity of chain packing together with the diverse complex stoichiometries observed (Table I) suggest that complex stoichiometry is determined largely by specific interactions between the polar moieties of the lipids.

Changes in lipid hydration can be examined by an analysis of hydration parameters (Table I) analagous to that described above for apparent partial molecular areas. If the hydration of 18:1 lipids was proportional to the number of chains per molecule, a plot of a_3 vs. m should be

linear with an intercept of zero. As shown in Fig. 6 *a*, such a plot is reasonably linear ($r = 0.974$) but does not show proportionality. Such behavior can be explained by postulating that two classes of hydration sites exist. It can be assumed that each 18:1 chain has a_{3a} water molecules and that for 18:1 lipids with more than one chain each additional chain has an additional a_{3b} water molecules. This yields

$$a_3 = a_{3a}m + a_{3b}(m - 1), \tag{7}$$

which predicts linearity as is observed for a plot a_3 vs. m (Fig. 6 *a*). From the slope and intercept a_{3a} is 0.78 ± 0.67 and a_{3b} is 2.03 ± 0.40. Physically, this analysis suggests that the addition of the second and third 18:1 chains to the glycerol backbone in the series monoolein-diolein-triolein considerably increases lipid hydration at interfaces.

Because even monochain 18:1 lipids lose area upon complexation, it is reasonable to propose that the water molecules represented by a_{3a} are lost from each chain. It is also reasonable, given the similarity of areas occupied by complexed and uncomplexed 1-palmitoyl-2-oleoyl phosphatidylcholine, that its hydration is constant in the complexes. If phospholipid hydration is denoted by a_{2c}, it is readily shown by analogy to the derivation of Eq. 6 that

$$a_c = a_{2c} + a_{3b}\left[\frac{X_c}{1 - X_c}\right](m - 1). \tag{8}$$

Fig. 6 *b* shows that, as predicted by the equation, a plot of $(m - 1)\,X_c/(1 - X_c)$ is approximately linear ($r = 0.936$). The alternative model, loss of only the waters greater than a_{3a} per chain, was also tested but gave extremely poor agreement with the data ($r = 0.275$). The intercept from Fig. 6 *b* is 0.37 ± 0.03. This value for the hydration of 1-palmitoyl-2-oleoyl phosphatidylcholine, is similar to those obtained in an earlier study of this phospholipid mixed with cholesteryl esters (Smaby and Brockman, 1984). The slope, 1.82 ± 0.28, is the number of water molecules associated with each of the second and third 18:1 chains. Note that, as predicted from the derivation of the equation, the value of this parameter is approximately equal to that obtained in the uncomplexed state, 2.03 ± 0.40. Thus, the hydration data are consistent overall with about one molecule of water per chain being lost from each 18:1 chain upon complexation, leaving two waters per 18:1 chain for those 18:1 lipids having more than one chain.

It should be noted that the parameters calculated above and listed in Table I are thermodynamic quantities and are not based on any particular lipid structural or conformational model. In particular, the consideration of area or hydration parameters on a per-chain basis for 18:1 lipids does not necessarily indicate that water moelcules must be associated with particular chains. However, consideration of these parameters in view of the cross-sectional areas of the molecules or particular groups can suggest or eliminate locations for the water molecules. For such comparisons, a reasonable estimate for the area of a water molecule is 9.65 Å^2 (Fowkes, 1962). The cross-sectional area of an 18:1 chain is not established (Small, 1984), but a minimum value of 23 Å^2 is reasonable. This is the area of a saturated chain in the liquid state (Small, 1984).

The recognition of two classes of water molecules associated with 18:1 lipids in the uncomplexed state, suggests that the earlier presumption of one class of areas for uncomplexed 18:1 chains might be an oversimplification. Specifically, the absence of true proportionality in Fig. 5 *a* might be due to more than data scattering. If there are two classes of chains, the data should be better described by an equation analgous to Eq. 7

$$\omega_3 = \omega_{3a}\,m + \omega_{3b}(m - 1), \tag{9}$$

where ω_{3a} is the area of each chain and its 0.78 water molecules and ω_{3b} is the additional area of the second and third chains, when present.

From the intercept of Fig. 5 *a*, ω_{3b} is 4.6 Å^2. Thus, the additional 2.03 water molecules associated with each of the second and third chains increase the area of those chains by no more than 4.6 Å^2. On the other hand, the calculated area of two water molecules is $2 \times 9.65 = 19.3$ Å^2. Clearly, these water molecules cannot be coplanar with the lipid chains in the surface phase. More likely, they are aligned with the lipid perpendicular to the interface. The notion of alignment is also supported by the analysis of 18:1 chains in complexes. Fig. 5 *b* shows excellent agreement with a model in which all chains are considered equivalent.

From the slope and intercept of Fig. 5 *a*, Eq. 9, yields 29.7 Å^2 for the area of each 18:1 chain and its 0.78 water molecules. Upon complex formation, the 18:1 chain area is reduced to 22.4 Å (Fig. 5 *b*). The change in area is $29.7 - 22.4 = 7.3$ Å^2 and could be due to changes in hydration, conformation or both. The maximum contribution, which dehydration alone could make to complexation of an 18:1 chain, is $0.78 \times 9.65 = 7.6$ Å^2. The similarity between this and the difference calculated above, strongly suggests that the 0.78 water molecules per chain are coplanar with the lipid and that the lipid does not undergo major conformational change upon complexation.

The hydration of the phospholipid in the uncomplexed state was not determined. However, the apparent partial molecular areas for phospholipid in the complexed (56.0 Å^2) and uncomplexed state (53.3 Å^2) are similar. This suggests that complexation with 18:1 lipid involves only small absolute changes in other phospholipid hydration or conformation. Thus, overall complex formation appears to be mostly a dehydration process.

The predicted locations of water molecules has been made without any direct consideration of molecular conformation. However, their plausibility can be examined by construction of molecular models. As a basis for this, a predicted conformation of triglyceride at interfaces (Brockman, 1984) was used. If water is hydrogen bonded

to each of the acyl carbonyls, it will be coplanar with the lipid when the lipid is oriented with its long axis perpendicular to the interface. Secondly, the additional four water molecules can be aligned with the glycerol portion of the molecule such that they would not contribute significantly to the molecular area. While such model building is speculative, it supports the predictions that arise from comparison of hydration and area parameters.

It is easy to visualize from molecular models how removal of the carbonyl-associated water molecules will allow the closer packing of the chains. Not so obvious is what determines complex stoichiometry. Because of the uniformity of 18:1 chain packing in the complexed state, the arrangement of molecules is likely determined by interactions involving the more polar portions of the lipids. Also of interest, are the effects of the two packing modes, complexed and uncomplexed, on the availability of 18:1 lipids and phospholipids as substrates for enzymatic reactions. An intriguing observation is that the activation of lipoprotein lipase by apolipoprotein C-II was greatest at mol fractions of triolein <4 mol% in egg phosphatidylcholine monolayers (Demel et al., 1982). As shown in Table I, this is the range in which triolein is complexed.

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