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Miscibility of dipalmitoylphosphatidylcholine, oleic acid and cholesterol measured by DSC and compression isotherms of monolayers

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Abstract

The miscibility of dipalmitoylphosphatidylcholine and oleic acid (DPPC/OA) and of dipalmitoylphosphatidylcholine/oleic acid/cholesterol (DPPC/OA/Chol) in mixed monolayers was determined. According to the thermodynamic calculations, the energy involved in the process is very low, suggesting the lack of strong interactions. Moreover, the presence of oleic acid or cholesterol has little influence on the transition temperature of DPPC.

INTRODUCTION

It is well known that cholesterol has a rigidifying effect on native phosphatidylcholine [1]. In contrast, the presence of unsaturation in the alkyl chain has been associated with less effective packing and lower thermal stability. Oleic acid is known to be a penetration enhancer for a great variety of molecules; the mechanism proposed to explain this effect has been related to a lipid phase separation [2]. Oleic acid lowers the transition temperature of stratum corneum lipids, increasing the flexibility of lipid alkyl chains. Cholesterol has been added to phospholipids in liposomal formulations in order to obtain stable preparations. The addition of a small percentage of oleic acid to these vesicles could combine both beneficial effects, i.e. penetration enhancement and liposomal stability. The present research was designed to determine the physicochemical behaviour of DPPC/OA/Chol mixtures when ordered in mono- and bilayers.

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EXPERIMENTAL

Chemicals

DPPC, cholesterol and oleic acid were purchased from Sigma and used without further purification. Chloroform (Merck, pro analysis) was used as the spreading solvent.

Water employed for the surface studies was distilled twice over permanganate and passed through a Milli Q filtration system. Its resistivity was always greater than 18 $M\Omega\,cm^{-1}$ and the pH was 5.5–6; it was always freshly prepared.

Methods

Compression isotherms

Compression isotherms were recorded on a Langimur film balance equipped with a Wilhelmy platinum plate, as described in ref. 3. The output of the pressure pickup (Beckmann LM 600 microbalance) was calibrated by recording the well-known isotherm of stearic acid. This isotherm is characterized by a sharp phase transition at 25 $mN\,m^{-1}$ on pure water at 20°C. The Teflon trough (surface area, 495 cm^2; volume, 309.73 ml) was regularly cleaned with hot chromic acid; in addition, before each experiment it was washed with ethanol and rinsed with double-distilled water. Before each run, the platnium plate was cleaned with chromic acid and rinsed with double-distilled water.

Films were spread on the aqueous surface with a Hamilton microsyringe, and at least 10 min was allowed for solvent evaporation. Films were compressed continuously at a rate of 4.2 $cm\,min^{-1}$; changes in the compression rate did not alter the shape of the isotherms. All the isotherms were run at least 3 times in the direction of increasing pressure with freshly prepared films. The accuracy of the system under the conditions in which the bulk of the reported measurements were made was 0.5 $mN\,m^{-1}$ for surface pressure.

Preparation of liposomes

From a standard solution of DPPC in chloroform of 6 $mg\,ml^{-1}$, samples were prepared containing 6 mg of phospholipid and different volumes of oleic acid. The system was freeze dried and samples were rehydrated by adding 150 μl of distilled water. The system was gently shaken and heated at 60°C for 1 h.

A similar protocol was followed for the three-component mixtures. A mother solution of DPPC/Chol (10:1) in chloroform was prepared and different volumes of oleic acid solution in chloroform were added to attain

the following ratios: DPPC/Chol/OA = 1:0.5:0.26 and 1:0.5:0.6. Mixtures containing higher amounts of OA were not homogeneous after hydration.

Calorimetric analysis

Calorimetric analyses were performed with a differential scanning calorimeter (Perkin-Elmer DSC-2 with intracooler). Weighed amounts of the liposomal samples were sealed in stainless steel pans. For each sample several scans were performed, in both heating and cooling modes between 0 and 50°C with heating rates of 5°C min^{-1}.

Indium was used as the calibration standard.

RESULTS

Because of the different surface characteristics of DPPC and oleic acid, it was necessary to pre-select the optimal amount of both components to achieve adequate compression isotherms: 10 mM solutions were chosen for both components and their mixtures.

Under these conditions, oleic acid gives isotherms in a liquid-expanded state that changes to a liquid-condensed state, without a real phase transition, at around 8 mN m^{-1}. Collapse is reached at 31.68 mN m^{-1}. When spreading an equimolecular amount of DPPC under the same conditions, there is an initial surface pressure increase of 1.44 mN m^{-1}. Nevertheless, the isotherms have the same shape as described in the literature, and the phase transition appears at 8.88 mN m^{-1}. Although working under other conditions, we found that isotherms of DPPC collapse at around 58.7 mN m^{-1} [4]; in this case, due to the higher amount of phospholipid spread, the system behaved anomalously and no clear collapse point could be detected.

Compression isotherms of mixed monolayers show that the presence of oleic acid does not greatly modify the ordered state of DPPC molecules in the monolayers. The phase transition at around 8 mN m^{-1}, characteristic of DPPC monolayers, appears in the isotherms irrespective of the OA content. Moreover, collapse pressures increase steeply from 31.6 mN m^{-1} for pure OA monolayers to 54.7 with a DPPC molar fraction of 0.7 (Table 1).

In order to determine the miscibility and the interaction energies, we used the equation based on the surface rule [5], developed initially to establish miscibility in multicomponent monolayers.

The surface area occupied by immiscible or totally miscible components is the sum of the areas of the individual components. Deviations from this ideal behaviour indicate miscibility as well as some sort of molecular interaction. Figure 1 shows these values for the different molar compositions of the monolayers. Apparently, although the mixtures are not ideal, deviations from ideality are very small. To evaluate the energy associated

TABLE 1

Collapse and phase transition pressures of mixed monolayers of DPPC/OA, given as a function of the oleic acid molar fraction

Collapse/mN m^{-1}	X Oleic	Transition/mN m^{-1}
31.68	1.0	–
31.68	0.9	8.16
33.12	0.8	8.16
34.08	0.7	8.16
42.72	0.6	8.16
51.84	0.5	8.16
51.84	0.4	8.58
54.72	0.3	8.64
–	0.2	8.64
–	0.1	8.64
–	0.0	8.88

with these interactions, the thermodynamic parameters were calculated [6]. The value of the excess free energy of mixing can be calculated from the difference between areas under the isotherms of the experimental and ideal films for a specified surface pressure.

The sign gives information about the stability of these mixtures. In the present case, a negative excess free energy of mixing indicates that the interactions are more favored than in the ideal state. The values of ΔG_M^{EX} were calculated by applying eqn. (1) which was obtained by following the Goodrich, Pagano and Gershfeld approaches. Numerical values were calculated according to the mathematical method of Simpson. The region below the lowest reproducible pressure was assumed to go to zero at the

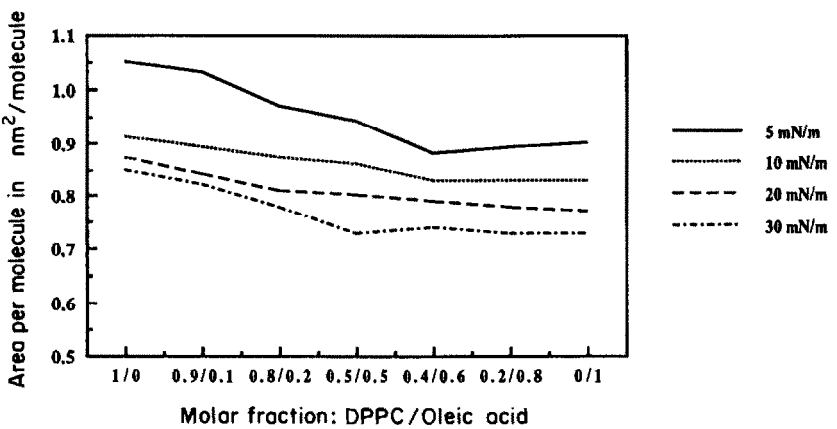

Fig. 1. Mean area per molecule values in mixed monolayers of DPPC/OA measured at different surface pressures.

TABLE 2

Excess energy of mixing, interaction parameters and energies in DPPC/OA mixed monolayers measured at 10 and 20 mN m^{-1}

Molar fraction (DPPC/oleic acid)	10 mN m^{-1}			20 mN m^{-1}		
	ΔG_m^{ex}/ (J mol^{-1})	α	ΔH/ (J mol^{-1})	ΔG_m^{ex}/ (J mol^{-1})	α	ΔH/ (J mol^{-1})
0.9/0.1	85.48	0.39	–	18.05	0.08	–
0.8/0.2	+178.15	−0.46	–	−397.26	−99.0	–
0.5/0.5	−126.40	−0.21	−282.80	−198.15	−0.32	−396.3
0.4/0.6	−34.90	−0.06	−72.73	−18.04	−0.03	−37.5
0.2/0.8	−213.09	−0.54	−665.87	−282.22	−0.72	−811.9

lift-off print. For this reason the two-phase liquid–gas and gaseous phase regions are not included in the integration

$$\Delta G_M^{Ex} = \int_0^{\pi} A_{12}\,d\pi - N_1 \int_0^{\pi} A_1\,d\pi - N_2 \int_0^{\pi} A_2\,d\pi \quad (1)$$

where A_{12} is the mean molar area in the mixed film, A_1 and A_2 are the molar areas of the two pure components, and N_1 and N_2 are the molar fractions of the monolayer components 1 and 2.

Values for ΔG_M^{EX}, α and ΔH are given in Table 2. These calculations confirm the low energy involved in the interaction, as was observed qualitatively from the area/molecule values represented in Fig. 1.

Mixed monolayers of three components

A new set of experiments was carried out starting from a DPPC/OA mixture (1:0.5), adding different amounts of cholesterol. These experiments were designed to determine the influence of increasing amounts of Chol on the ordered state of DPPC/OA monolayers. Compression isotherms of the pure components are given in Fig. 2. The monolayers of DPPC/OA are more expanded than those of cholesterol.

The monolayers containing different molar fractions of the three components gave isotherms with area/molecule values intermediate between those of the pure components. To better evaluate the differences between the monolayers, the compressibility was calculated by applying the equation

$$C = -\frac{1}{A}\left(\frac{\delta A}{\delta \pi}\right)_T \quad (2)$$

The values of compressibility calculated at 5 mN m^{-1} of surface pressure were 4.05×10^{-2} m mN^{-1} and 2.80×10^{-2} m mN^{-1} for DPPC/OA and

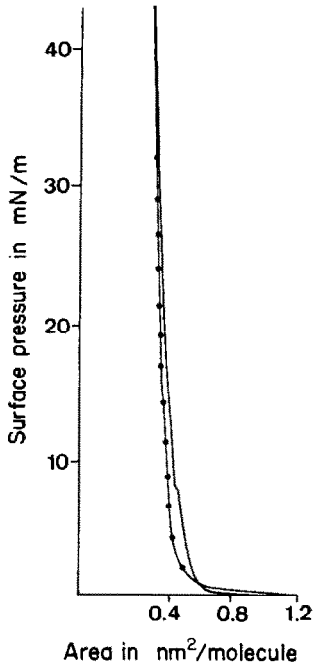

Fig. 2. Compression isotherms of Chol and DPPC/OA spread on pure water.

cholesterol, respectively. The calculations were made at these low surface pressures because here the shape of the isotherms show the highest differences.

The area/molecule values calculated at 5, 10 and 20 mN m^{-1} for the different mixed monolayers are given in Fig. 3. It is clear that deviations from ideality are very small, thus suggesting an ideal miscibility. The

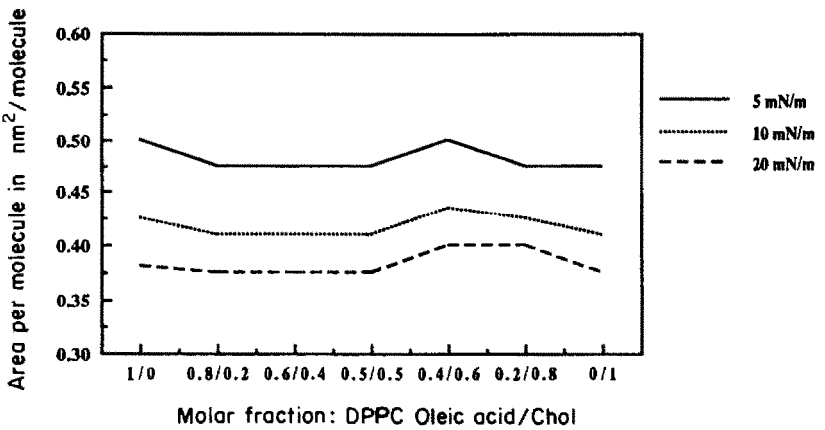

Fig. 3. Mean area per molecule values in mixed monolayers of DPPC-OA-Chol measured at different surface pressures.

TABLE 3

Excess energy of mixing, interaction parameters and energies in DPPC/OA/Chol mixed monolayers measured at 10 and 20 mN m^{-1}

Molar fraction (DPPC–Oleic Acid/Chol)	10 mN m^{-1}			20 mN m^{-1}		
	ΔG_m^{ex}/ (J mol^{-1})	α	ΔH/ (J mol^{-1})	ΔG_m^{ex}/ (J mol^{-1})	α	ΔH/ (J mol^{-1})
0.8/0.2	−109.55	−0.20	−33.94	−113.40	−0.29	−117.2
0.6/0.4	−89.70	−0.15	−94.05	−117.87	−0.20	−122.7
0.5/0.5	−78.24	−0.13	−78.24	−103.24	−0.17	−68.8
0.4/0.6	59.60	0.10	62.07	191.27	0.33	199.2
0.2/0.8	1.08	0.00	1.67	26.25	−0.07	−41.0

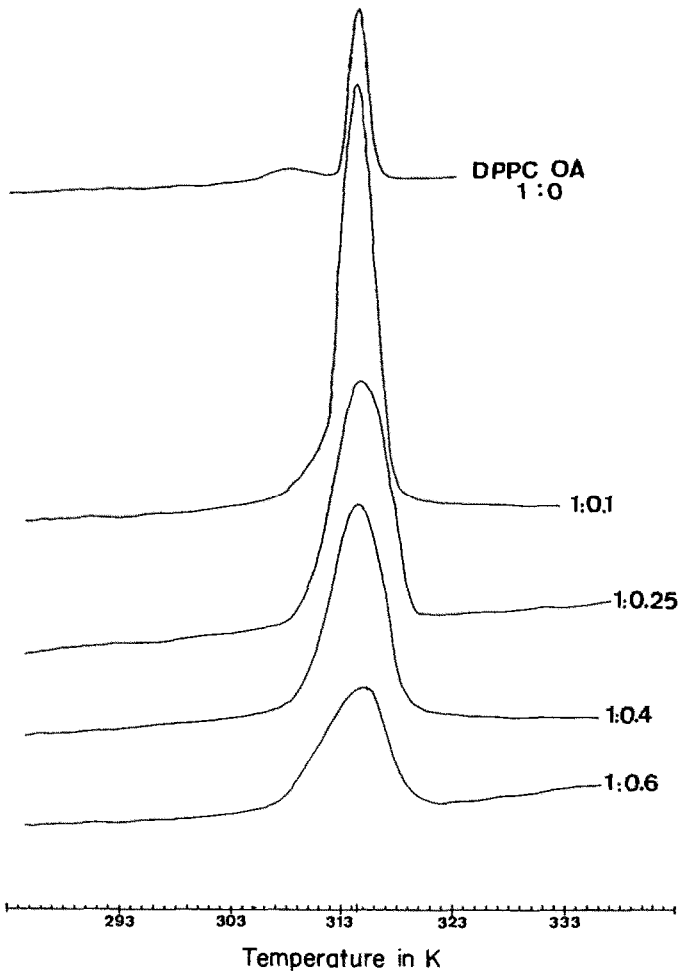

Fig. 4. DSC scans of DPPC liposomes containing varying amounts of OA. Molar fractions are indicated on the curves.

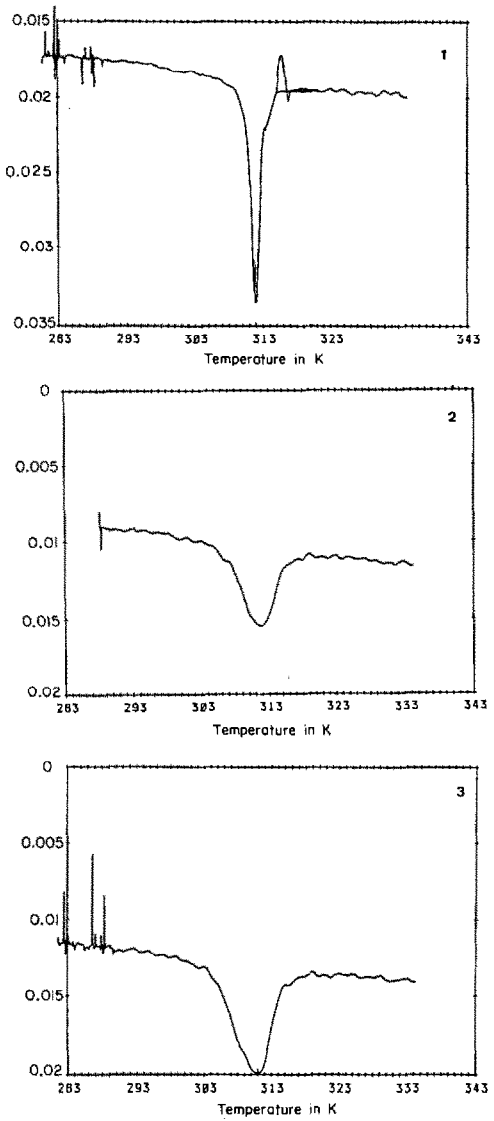

Fig. 5. DSC scans of DPPC/Chol liposomes containing varying amounts of OA. Molar fractions of DPPC/Chol/OA: (1) 1:0.5:0; (2) 1:0.5:0.26; (3) 1:0.5:0.6.

collapse pressures for all the monolayers of different molar compositions show a gradation in their values according to an ideal miscibility behaviour.

As described above, the interaction parameters were calculated from the area/molecule values and are given in Table 3. It can be seen that the energies associated with the mixing process are nearly zero which confirms the ideality of the mixing behaviour.

Calorimetric studies

The miscibility of DPPC/OA was also studied in liposomes using differential scanning calorimetry. Samples were prepared as described above but because of the low solubility of OA in water, the maximum molar fraction of this molecule in the mixture was 0.4. Samples with a higher OA content gave non-stable emulsions.

The thermograms are given in Fig. 4. The presence of increasing amounts of OA in liposomes containing DPPC increases the transition temperature slightly and broadens the peaks significantly. This broadening of the transition profile suggests that there is a reduction in the cooperativity of the transition. Nevertheless, the lack of significant changes in T_c corroborates the low interactions detected in the miscibility parameters of the monolayers.

The influence of oleic acid and cholesterol on the transition phase of DPPC was also studied in mixtures of these three lipids. Mixtures of DPPC/Chol (1:0.5) show a T_c of 39°C, see Fig. 5. The presence of oleic acid at equimolar concentrations of cholesterol results in a lower T_c of 38°C. Comparing these data with those of OA/DPPC, it seems that the presence of cholesterol reverses the effect of oleic acid. This is consistent with the dual effect already described for cholesterol in the sense that it fluidizes rigid bilayers and rigidifies fluid bilayers, and is also in agreement with the results of Ongpipattanakul et al. [2], who found that oleic acid reduces the T_c of a stratum corneum mixture of lipids.

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