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Partitioning of lysolipids, fatty acids and their mixtures in aqueous lipid bilayers: Solute concentration/composition effects

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ABSTRACT

Distributions of lysopalmitoylphosphatidylcholine (LPPC), palmitic acid (PA) and their 1:1 mixtures between water and dipalmitoylphosphatidylcholine (DPPC) bilayer were determined using a fluorescence probe that selectively detects only the solutes in water. Water solute concentrations were obtained at each of several lipid concentrations. Dynamic Light Scattering experiments confirmed that the lipid/solute aggregates were vesicles in the concentration range investigated. Lipid concentration dependence of the solute component in water was fit to a thermodynamic model of solute distribution between two coexisting solvents. Water/bilayer partition coefficient and the free energy of transfer, for each of these solutes were determined from the fit. Main findings are: (1) Water/bilayer partition coefficient of solute is greater for 2 to 10% solute mole fraction than for 0 to 2%, signaling solute induced bilayer perturbation that increases bilayer solubility, beginning at 2% solute mole fraction. (2) Partition coefficients are in the order LPPC < PA < LPPC + PA at 37 °C and LPPC + PA ≤ LPPC < PA at 50 °C. This signifies synergism toward increased solute solubility in the bilayer-gel phase and lack of it in the bilayer-liquid phase when LPPC and PA are present together. Implications of the solute concentration/composition and bilayer phase dependences of the partition coefficients to the reported solute induced enhancements in transmembrane permeability are discussed.

1. Introduction

Lysophosphatidylcholines (LPC) and fatty acids (FA) are either synthesized directly or formed as a result of enzymatic breakdown of membrane phospholipids by Phospholipase A_2 [1–4]. LPC and FA being amphiphilic are present in water as well as in the membrane. Within the membrane they contribute to its structure and phase [5,6]. They are responsible for several physiological functions and therefore their concentrations in water and in the membrane are of biological significance [7–10]. The lipid/solute systems studied in this work were: lysopalmitoylphosphatidylcholine (LPPC), palmitic acid (PA), and 1:1 LPPC/PA in dipalmitoylphosphatidylcholine (DPPC) aqueous bilayer solutions. The mixtures are of relevance to Phospholipase A_2 mediated enzymatic hydrolysis of phospholipids at lipid membrane interfaces that results in equal amounts of the products: LPC and FA, which then distribute between the membrane and water. The LPC/FA mixtures realize the actual situation present in phospholipid membrane hydrolysis. Partition coefficients and knowledge of solute concentration in water are essential for measurements of enzymatic activity as shown in previous work [11]. Their effects on bilayer properties are responsible for various phenomena like lag time in hydrolysis, and changes in enzymatic activity with product accumulation [12]. The present work is motivated by its significance to elucidating the underlying physicochemical basis of such phenomena, its need in PLA_2 activity measurements, and also to the phenomenon of solute-induced enhancement of transmembrane permeability.

The solute concentration in water was measured using a fluorescence assay in which the fluorescent probe ADIFAB (Acrylodan-labeled rat-intestinal fatty acid binding protein) responds to the presence of only the solute component in water. In this assay, the bilayer is not perturbed because ADIFAB is present only in water. ADIFAB, when first introduced was used only to study FA binding [13]. In recent work ADIFAB was shown to bind LPC as well [11]. This fact permits investigation of partitioning of LPC in addition to that of FA.

Distribution of LPC and FA may be treated as a thermodynamic problem of solute partitioning between two coexisting solvents: the lipid bilayer and water. The observed lipid concentration dependence of the solute component in water was fit to the basic thermodynamic model of solute distribution between two coexisting solvents. The water/bilayer partition coefficient, and hence the free energy of transfer, for each of these solutes were determined from the fit.

In the present case of water/bilayer partitioning, the partition coefficient was found to depend on solute concentration. Two solute concentration ranges could be identified. Solute concentrations in the range of 0 to 2% of the lipid have lower water/bilayer partition coefficient than in the range of 2 to 10%. When LPPC and PA were present together the

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measured water/bilayer partition coefficient at 37 °C was higher than when they were present individually. This represents synergism toward solubilization in the bilayer. Such solute concentration/composition dependent behavior was absent at 50 °C where the DPPC bilayer is in the fluid phase. A similar reported phenomenon is the synergistic enhancement in the transmembrane permeability of drug molecules upon simultaneous incorporation of LPPC and PA in membranes in the gel phase and the lack of such an effect in the fluid phase [14]. The emphasis of this work is on the effect of solute concentration and composition on the behavior of the lipid bilayer as a solvent as revealed by the water/bilayer partition coefficient of the solutes.

The hydrodynamic radii of the phospholipid/LPPC/PA vesicles, at various compositions, were determined by Dynamic Light Scattering measurements to determine the composition at which bilayer dissolution into mixed micelles occurred. The main purpose was to ensure that the partition coefficient experiments are conducted in the composition range where the structures are vesicles.

2. Materials and methods

The partitioning of LPPC, PA, and a 1:1 mixture of LPPC and PA between small unilamellar vesicles (SUV) of DPPC and water was measured by a fluorescence assay that employed ADIFAB as the fluorescence probe. Fluorescence measurements of ADIFAB in aqueous solutions of DPPC + solute (LPPC or PA, or 1:1 LPPC + PA mixtures) were conducted for various concentrations of DPPC between 10 and 400 μM. At each of the phospholipid concentration, the solute concentration was varied from 0 to 10% of phospholipid concentration.

2.1. Materials

Dipalmitoylphosphatidylcholine (DPPC) and lysopalmitoylphosphatidylcholine (LPPC) were obtained from Avanti Polar Lipids as lyophilized powders. Sodium salt of palmitic acid (PA) was obtained from TCI America. The fluorescence probe, ADIFAB, was obtained from FFA Sciences (San Diego, CA).

2.2. Methods

The partition coefficient K_W defined by the ratio of the bilayer to water solute molar fractions is related to the free energy of transfer from water to bilayer, $(G^0_{SB} - G^0_{SW})$, as;

$$K_W = \frac{[solute]_B / [DPPC]}{[solute]_W / C_W} = \exp \frac{-\left(G^0_{SB} - G^0_{SW}\right)}{RT}, \quad (1)$$

where $[solute]_{B,W}$ are the solute concentrations in bilayer or water, $C_W = 55.6$ is the molar concentration of water, G^0_{SB} and G^0_{SW} are the molar Gibbs free energies of the solute in the lipid bilayer and water respectively, R is the gas constant and T is the temperature. The fraction x_s of the total $[solute] = [solute]_B + [solute]_W$ present in water is obtained by rearranging Eq. (1) as

$$x_s = \frac{[solute]_W}{[solute]} = \frac{C_W}{C_W + [DPPC]K_W}. \quad (2)$$

The experimental quantity measured in the fluorescence assay described below is proportional to x_s.

2.2.1. Fluorescence assay

ADIFAB fluorescence spectra were measured with a Horiba Scientific Fluoromax-4 spectrometer. The excitation wavelength was 386 nm. Fluorescence emission peak was at 432 nm. The ADIFAB concentration in all samples was 0.4 μM. ADIFAB binds only to the LPPC and PA present in the water [11]. The emission peak shifts to 505 nm upon binding. The fluorescence response to the presence of binding entities represented by the Generalized Polarization defined by [11,15,16],

$$GP = \frac{I_{505} - I_{432}}{I_{505} + I_{432}}, \quad (3)$$

was calculated, where I_{505} and I_{432} are the fluorescence emission intensities at 505 nm and 432 nm respectively.

The GP was observed to be linearly proportional to the total solute concentration in water [11]. The GP data of LPPC and PA in buffer could be fit to a line for concentrations up to 0.9 μM. The GP data of ADIFAB for solutes added to DPPC solutions also showed linear variation with total [solute]. In the presence of DPPC, assuming no direct exchange between membrane and ADIFAB [17], the relations between GP and solute concentrations are,

$$GP - GP_0 = c_1[solute]_W = c[solute], \quad (4)$$

where c and c_1 are proportionality constants, $[solute]_W$ is the solute concentration that partitions into water (including solute bound to ADIFAB and free solute in water) and GP_0 is the GP value at zero solute concentration. The quantity c is the slope of GP vs. [solute], given by;

$$c = \frac{c_1[solute]_W}{[solute]} = c_1 x_s, \quad (5)$$

where x_s is the solute fraction in water, given by Eq. (2). Thus, the slope of GP vs. the total solute concentration in units of ΔGP/M, where M is the concentration unit of moles per liter, represents the solute fraction, x_s (Eq. (2)), in the aqueous phase. In the present experiments the slope is determined for various [DPPC].

Fluorescence emission spectra were measured for several solute concentrations at each of eight different DPPC concentrations between 10 and 400 μM. For every DPPC concentration, the GP vs. total [solute] was plotted and the slope of the GP variation with solute concentration was obtained as the experimental quantity proportional to x_s.

2.2.2. Samples

Hepes buffer (pH = 7.4, 20 mM) was the aqueous medium for all samples. Small unilamellar vesicles of DPPC or DPPC + solute were prepared by vortexing a thin film of DPPC with added Hepes followed by sonication in an ultrasonic bath (model no. G112SP1G from Laboratory Supplies Inc., NY) for 5 min. The ultrasound power output was 80 W. No change in the pH of Hepes was observed after the addition of DPPC or the solutes. ADIFAB was added to the sample solution just before measurements to a concentration of 0.4 μM. The samples were prepared and studied as follows:

(i) LPPC and DPPC: For each of the eight DPPC concentrations, a stock solution of LPPC (in Hepes buffer; pH = 7.4) at the same concentration was prepared. LPPC from the stock solution was added in steps to the DPPC solution in the fluorescence cuvette to achieve various solute concentrations of up to 10% of DPPC. The fluorescence emission spectra were measured at the end of each addition and stirring for 2 to 5 min.

(ii) PA and DPPC: Due to low aqueous solubility of PA, its stock solution at concentration 2 mM was first prepared at pH = 10. This stock solution was then diluted with Hepes buffer to obtain PA stock solutions of the same concentrations as DPPC. No significant change in the pH of Hepes buffer due to the addition of PA solution at pH 10 was observed. Measurements were conducted with PA from these latter stock solutions added to DPPC just as for LPPC and DPPC.

(iii) LPPC + PA (1:1) and DPPC. Samples were prepared by two methods: (1) In the first method, each of the mixtures was individually prepared. PA was dissolved in ethanol in a glass vial and a thin film of PA was prepared by subsequent evaporation of

ethanol under N_2 flux. Equimolar stock solution of concentration 400 μM of LPPC + PA was prepared by adding required amounts of LPPC solution to the PA film. Appropriate amounts of LPPC + PA solution were then added to the DPPC vesicle solution to yield different mixtures with LPPC + PA concentration from 0 to 10% of DPPC. Each LPPC + PA + DPPC mixture was sonicated for 10 min. Fluorescence emission spectrum of ADIFAB in every individually prepared mixture was measured. (2). In the second method, DPPC vesicles were formed first and aliquots from stock of equimolar mixture of LPPC and PA were added to the preformed vesicles and fluorescence measurements were conducted after each addition and stirring for 5 min.

A two to five minute stirring time was found to be sufficient for equilibration at the end of each addition from stock. There was no change observed in the results for various periods of waiting/stirring up to 2 h. In the present experiments, adding LPPC and/or PA to preformed vesicles showed a less than 0.25% change in GP over a period of 2 h. Fatty acid equilibration times were reported to be about 5 min and FA translocation rates are in the millisecond time scales [17–19].

NMR experiments have shown that LPPC translocation in egg-PC vesicles is a very slow process, with a $t_{1/2}$ for translocation of about 12 h [20]. In cosonicated vesicles of LPPC + egg-PC, LPPC was found to be present in the outer and inner monolayers with the outer to inner ratio of 3:1. But the addition of LPPC into preformed egg-PC vesicles showed the presence of LPPC only in the outer monolayer with no change in the signal even after 24 h. Incorporation of lysolipids into the membrane outer leaflet has been reported to be a fast process with a rate constant of about 0.2 s^{-1} [21]. In the LPPC + DPPC samples, the LPPC most likely partitions into the outer monolayer.

The slopes of ADIFAB fluorescence GP vs. [solute] (LPPC or PA or 1:1 LPPC to PA), denoted by ΔGP/M, were determined for each of the DPPC concentrations, from the observed linear variations. This slope is proportional to x_s (Eq. (5)). Its dependence on the lipid concentration is given by Eq. (2), and may be written as,

$$\Delta GP/M = \frac{A}{C_W + [DPPC]K_W}, \quad (6)$$

where, A is directly proportional to C_W. The slope data vs. [DPPC] were fit to Eq. (6). The fit yields K_W.

2.2.3. Dynamic Light Scattering

DLS measurements were carried out on DynaPro Nanostar Model WDPN06 (Wyatt Technologies), equipped with a GaAs laser (120 mW) operating at a nominal wavelength of 658 nm. The scattered light was collected at 90° by a solid state Single Photon Counting Module (SPCM) detector. The sampling time was set to an optimum value, typically 10 s, to obtain a fully decaying intensity correlation function (ICF). The ICFs were single exponential decays with baselines that were unity within the precision of the measurements. The exponential fit to the ICF yielded the translational diffusion coefficient (D_t) of the particles in the sample. The hydrodynamic radius (R_h) of the sample was then derived from D_t using the Stokes–Einstein equation [22]. All sample solutions were filtered through 0.2 μm Whatman nylon syringe filters. The temperature of the sample solutions was controlled by an internal Peltier effect heat pump with an accuracy of ± 0.01 °C.

3. Results

Measurements were conducted at 37 °C and 50 °C. The spectra in Fig. 1 illustrate the response of ADIFAB to LPPC, PA, and a 1:1 mixture of LPPC and PA in aqueous DPPC solutions at 37 °C for various solute concentrations. The GP of the fluorescence was calculated for each of the spectra. Its variation with solute concentration, shown in Fig. 2 for the case of [DPPC] = 50 μM, was then examined. Data as in Fig. 2 were obtained for several DPPC concentrations.

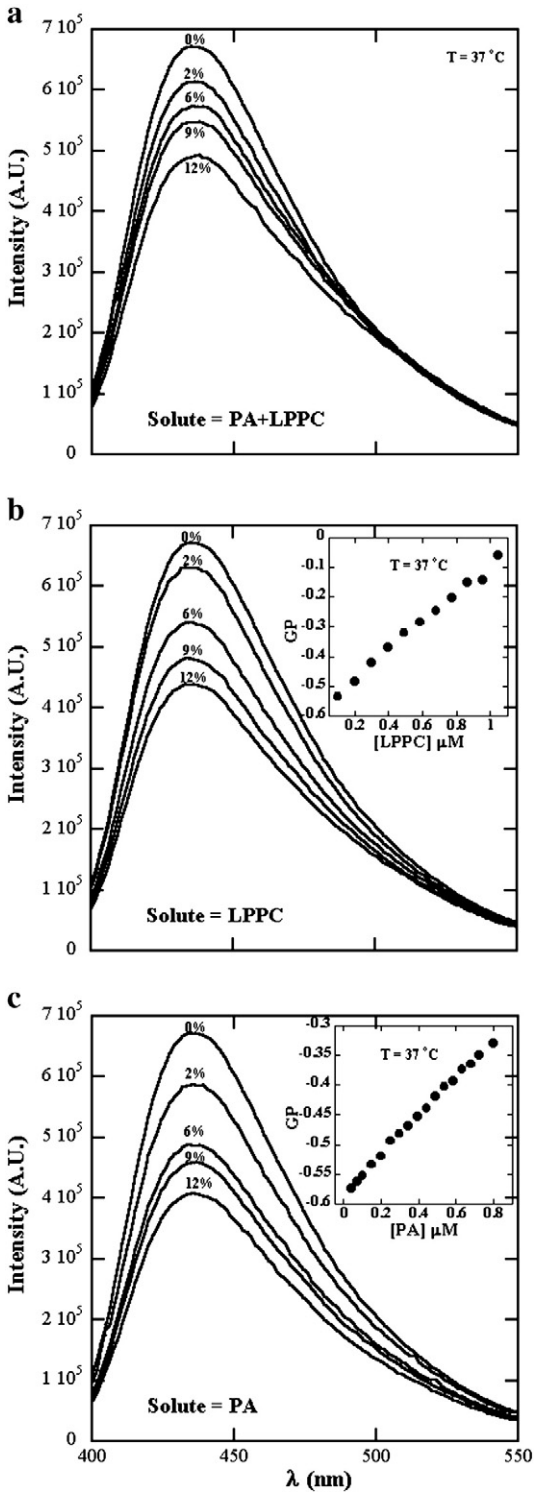

Fig. 1. Fluorescence spectra of ADIFAB, excited at 386 nm, in aqueous solutions of small unilamellar vesicles of DPPC at 37 °C, for various solute concentrations. Solutes were (a) 1:1 mixtures of LPPC and PA, (b) LPPC, and (c) PA. [DPPC] + [solute] = 50 μM. Insets in b and c show the linear variation of GP with the solutes, LPPC and PA respectively, in buffer.

Two linear regions, differing in slopes by more than 10%, in the GP vs. solute concentration behavior were identified at each of the DPPC concentrations for LPPC and PA at 37 °C. The 1:1 LPPC/PA mixed solutes did not exhibit two linear regions at 37 °C. GP vs. [solute] at 50 °C was

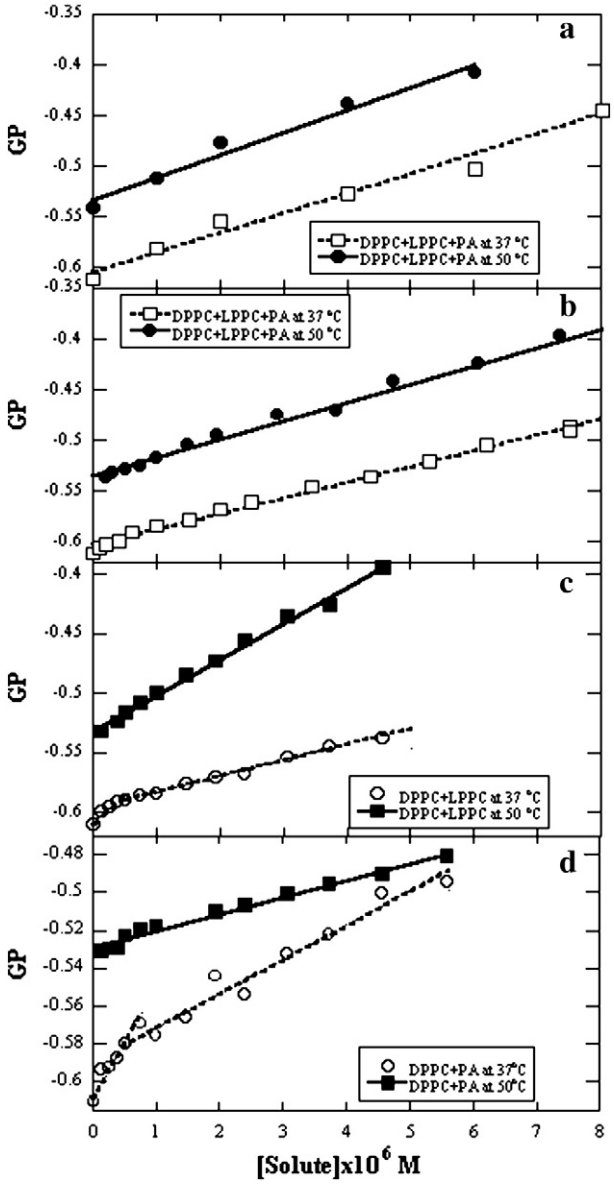

Fig. 2. Variation of GP of ADIFAB fluorescence with solute concentration. [DPPC] + [solute] = 50 μM. The solutes were (a) 1:1 mixtures of LPPC and PA where each of the solute concentration was prepared by cosonication of DPPC + LPPC + PA (Method 1), (b) 1:1 mixtures of LPPC and PA where each of the solute concentration was obtained by titration into preformed DPPC vesicles from a stock of LPPC + PA (Method 2); (c) LPPC, and (d) PA.

characterized by a single linear variation for all of the three solutes; LPPC, PA, and LPPC + PA.

The break in the behavior of GP vs. [solute] at 37 °C, in the solute concentration range studied in this work, denotes a transition from one linear variation to another. The slope of each of the regions represents the solute fraction x_s in water as described in Subsection 2.2.1. The change in the slope means a change in the partition coefficient and is an expression of change in the bilayer property. That is, each of these lipid/solute concentration regions is a solvent differing from the other, with the difference being brought about by the compositional change in the bilayer. The slope of the lower solute concentration region represents partitioning between lipid bilayer and water, while the slope of the higher solute concentration region represents partitioning between mixed lipid/solute bilayer and water.

The dependence of the GP slope, of each of these regions, on [DPPC] was then examined. The observed concentration dependence is shown in Fig. 3a and b for LPPC and PA respectively. The data were fit to the form in Eq. (6) rather than Eq. (2), because the GP slope is only proportional to the solute fraction, x_s, in the aqueous phase. The standard deviations in the GP slopes, from repeated measurements on three different samples at [DPPC] = 100 μM for LPPC and two for PA were 10%. The fit values for K_W and the uncertainty from the fit are given in Table 1. The solutes have a lower free energy in the bilayer with the change for PA being higher in magnitude. Fatty acids have been reported to be less soluble in water and more soluble in bilayers than LPC [6].

Results for the 1:1 mixtures of LPPC and PA in DPPC are shown in Fig. 4. The solid symbols in Fig. 4 are the slopes of GP variation with the mixed solute concentration and represent the mixed x_s. Accordingly the data were fit to Eq. (6). Experiments on the mixed solutes were conducted four times for [DPPC] = 100 μM, twice for [DPPC] = 20 μM, and five times for [DPPC] = 10 μM. These repetitions are independent measurements; on different samples. The standard deviations in the data, from these repeated measurements, were 10%. The partition coefficient and uncertainty determined from the fit and the calculated free energy of transfer are given in Table 1. The mixed solute solutions exhibited only a single slope in the GP vs. mixed solute concentrations of 0 to 10%. The partition coefficient of the combined LPPC and PA is higher than that of the individual solutes at 37 °C, demonstrating synergistic enhancement of the solubility of mixed solutes in the bilayer gel phase.

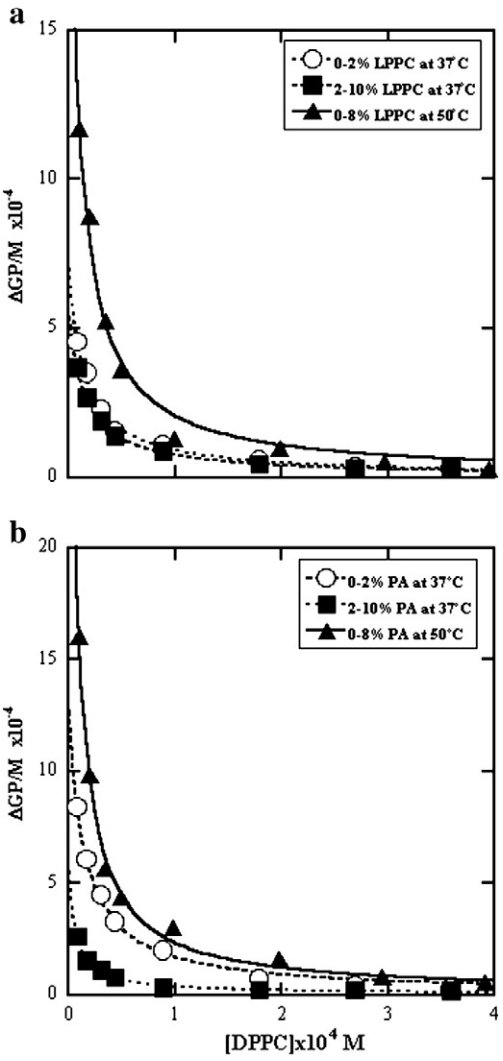

Fig. 3. Dependence of the solute mole fraction x_s, defined by Eq. (2) and experimentally represented by the slope of GP vs. [solute] (Fig. 2) on lipid concentration. Solutes were LPPC or PA. Lines are fits to Eq. (6).

Table 1
Partition coefficient (K_W) and the free energy of transfer (ΔG) of solute from aqueous phase to bilayer.

T (°C)	Solute (mol%)	$K_W \times 10^{-6}$			$G^0_{SB} - G^0_{SW}$ (kJ mol^{-1})		
		LPPC	PA	LPPC + PA	LPPC	PA	LPPC + PA
30[a]	–	0.63[a]	4.17[a]	–	–	–	–
37	0–2%	3.17 ± 0.53	3.78 ± 0.46	–	−38.6	−39.0	–
37	2–10%	3.69 ± 0.19	8.63 ± 1.57	–	−39.0	−41.2	–
37	0–10%	–	–	14.1 ± 1.93	–	–	−42.4
37[b]	0–10%	–	–	15.8 ± 6.2[b]	–	–	−42.7[b]
50	0–8%	6.32 ± 1.78	9.89 ± 2.61	5.98 ± 0.98	−42.1	−43.3	−41.9
50[b]	0–8%	–	–	7.16 ± 2.56[b]	–	–	−42.4[b]
50[a]	–	5.17[a]	2.92[a]	–	–	–	–
60[a]	–	6.07[a]	8.95[a]	–	–	–	–

[a] K_W values from Høyrup et al. [21]. These values are given by the product of the reported K values and C_w (= 55.6 M).
[b] For samples prepared by method 2 where solute concentrations were obtained by adding equimolar solution of PA + LPPC to preformed DPPC vesicles.

The partition coefficient for the mixed solutes when added to preformed vesicles is about the same as for cosonication with DPPC. In samples prepared by cosonication, the solutes have enough time to distribute between the layers. In the cosonicated vesicles therefore, LPPC is expected to be present in both outer and inner monolayers (but more dominantly in the outer monolayer), whereas LPPC partitions into the outer monolayer when added to preformed vesicles [20]. Observation of similar K_W values for these proposed distributions implies that the time of 5 min employed for equilibration when adding solutes to preformed vesicles is sufficient and also that LPPC partitioning into the inner monolayer does not appear to affect K_W perhaps because the majority of it is in the outer monolayer.

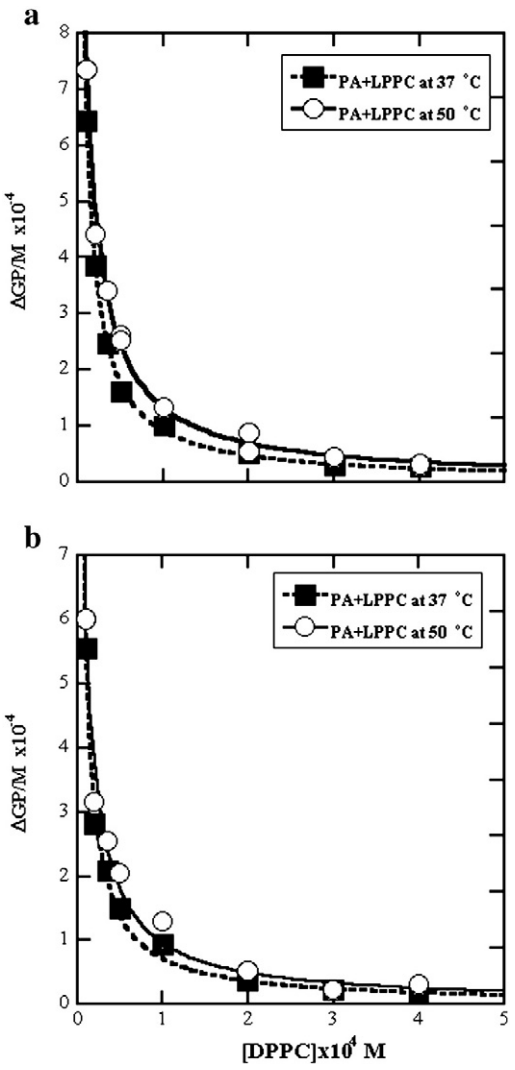

Fig. 4. Dependence of the solute mole fraction x_s, defined by Eq. (2) and experimentally represented by the slope of GP vs. [solute] (Fig. 2) on lipid concentration. Solutes were 1:1 mixtures of LPPC and PA and solute concentrations were obtained (a) individually by cosonication of DPPC + LPPC + PA (Method 1) and (b) by titration into preformed DPPC vesicles from a stock of LPPC + PA (Method 2). Lines are fits to Eq. (6).

Results of fitting of the data at 50 °C where the DPPC bilayer is in the liquid phase are also presented in Table 1. There is no dependence of K_W on the solute concentration. The partition coefficient of LPPC increases as temperature increases and that of PA shows a smaller change. The behavior of the partition coefficients, with respect to temperature, namely that LPPC distribution is sensitive to temperature and phase change of the bilayer and PA is not, is in agreement with reported observations by Calorimetry [21]. The comparison is for the higher solute concentration range, because the Calorimetry data are at high solute mole fractions. Interestingly, synergistic effects in partitioning into the bilayer in the fluid phase (50 °C) were not observed.

DLS data of the hydrodynamic radii of LPPC/DPPC and PA/DPPC aggregates of various compositions are shown in Fig. 5a and b respectively. Radius values of about 50 nm are typical of small unilamellar vesicles. The results show that the aggregates are vesicles at the solute/lipid concentrations and compositions on which the partition coefficient measurements were carried out. In the case of LPPC/DPPC, at a total concentration of 200 μM, no major size change was detected even up to about an LPPC mole fraction of 0.8. Data at a total LPPC + DPPC concentration of 20 mM are presented in Fig. 6. The radius remains at about 50 nm until about an LPPC mole fraction of 0.75, but changes to small aggregates of radius 6 nm at about 0.85. Pure LPPC aggregates form micelles and the measured radius was 4.3 nm. These results show the occurrence of vesicles to small mixed micelle transformation at about an LPPC mole fraction of 0.85. DLS measurements also show that the lipid/solute mixtures are vesicles. Neither bilayer dissolution; initiated by formation of detergent micelles in water, nor structural transformation of mixed lipid/solute aggregates occurs for the concentration/composition range studied.

4. Discussion

Partitioning of fatty acids into lipid membranes is primarily driven by the hydrophobic effect [17,21]. The present experiments show that solute presence itself and the physical state of the membrane also play a role. The partition coefficient of the solute LPPC or PA into DPPC bilayers increases from one value to another at about a solute concentration of 2 mol%. Lower solute concentrations, in general, are closer to representing intrinsic bilayer properties. Given that a small but clear change was observed at 2% solute mole fraction, the experimental values of K_W above 2% may be considered to represent a lipid-solute mixed bilayer property and the values below 2% at least approximate those of the lipid bilayer.

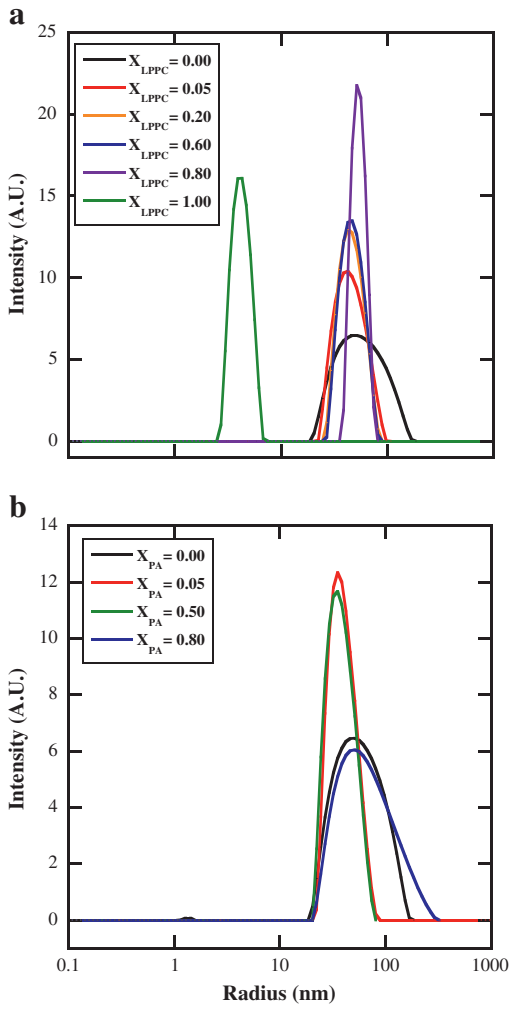

Fig. 5. (a) Dynamic Light Scattering results for the hydrodynamic radii of (a) DPPC + LPPC aggregates at various mole fractions, X_{LPPC}, of LPPC. (b) DPPC + PA aggregates at various mole fractions, X_{PA}, of PA. [DPPC + solute] concentration was 200 μM.

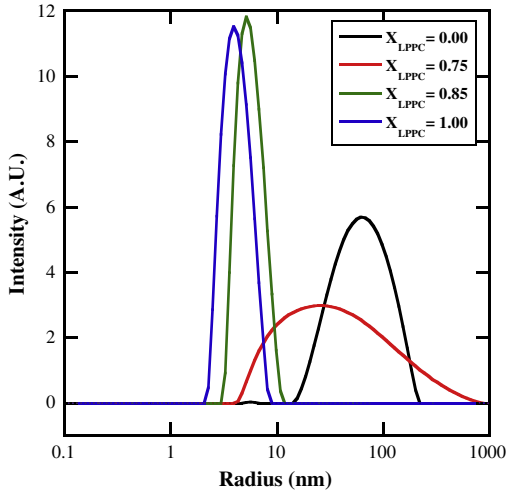

Fig. 6. Dynamic Light Scattering results for the hydrodynamic radii of (a) DPPC + LPPC aggregates at various mole fractions, X_{LPPC}, of LPPC. [DPPC + solute] concentration was 20 mM.

Synergistic enhancement in the partition coefficient was observed when both LPPC and PA were present. These effects were observed only in the gel phase. LPPC or PA induced enhancement and synergistic enhancement under the combined presence of LPPC and PA in membrane permeability have been observed in experiments on the release of encapsulated calcein from lipid membranes [14]. Leakage of calcein may be ascribed to solute induced defects in the membrane that facilitate movement of encapsulated molecules across the membrane. Partition coefficients give the amounts of LPPC and PA in the membrane and the amounts of LPPC and PA in the membrane affect permeability. The possibility of the ability of fatty acids to perturb the dense head group region of the gel-phase membrane, thereby promoting incorporation of lysolipids and the possible connection between partition coefficient and permeability have been presented before [14,21]. While the mechanisms of K_W and permeability enhancement are not fully understood, the present results provide experimental evidence for solute induced and synergistic enhancement of K_W, and thus support the suggestion that solute induced membrane perturbation leads to increase in K_W and thereby to permeability. K_W and permeability enhancements thus appear to be related effects.

It is interesting that the enhancement effects are not present in the liquid phase in both permeability and partition coefficient. Differences between solute induced perturbation of ordered and disordered membrane states are to be expected. The physical state of the membrane in the liquid phase is quite different from that in the gel phase. The absence of the enhancement effects in permeability as well as partition coefficient in the liquid phase could be because LPPC and PA do not perturb the membrane in the liquid phase as in the gel phase, that is, solute induced effects are mitigated by the fluidity of the liquid phase. The physical state of the membrane, and hence K_W, in the gel phase are affected by the presence of PA and LPPC, due perhaps to lateral phase separation, and induced defects [23,24]. The DPPC bilayer expands by about 5% upon transition from gel to liquid [25,26]. At 50 °C, it is in the more relaxed liquid phase to begin with. The effect of bilayer bending stress due to solute would also be lessened in the liquid phase. Lack of solute induced enhancement in K_W and in permeability in the liquid phase could possibly be ascribed to the increased fluidity that precludes significant membrane perturbation in the liquid phase [14,21].

5. Conclusions

Membrane (DPPC) partition coefficients of lysophosphatidylcholine, palmitic acid, and their mixtures were measured using a fluorescence assay. While partitions into lipid membrane of long chain hydrocarbon molecules are mainly due to hydrophobic forces; solute induced membrane perturbation and lipid phase also contribute to the behavior of the partition coefficient with respect to solute concentration and temperature. Solute-induced perturbation of the bilayer structure manifested as an increase in the water/bilayer partition coefficient (increased affinity to the bilayer) in the gel phase. The effect of change in the bilayer structure was first observed at 2% solute mole fraction in the case of LPPC and PA in the bilayer gel phase. The presence of LPPC and PA together enhances their partition coefficient over that of the individual values at 37 °C, where the DPPC bilayer is in the gel phase. Solute induced enhancement effects on the partition coefficient are not present in the bilayer liquid phase. Similarity in character of the observed enhancement in both of membrane partition coefficient and transmembrane permeability in the gel phase and absence in the liquid phase supports the idea that solute presence in the bilayer gel phase perturbs the membrane toward further partitioning and increasing permeability; whereas solute concentration and composition in the membrane liquid phase do not perturb the membrane enough to cause changes in permeability.

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References

[1] O.G. Berg, M.H. Gelb, M.-D. Tsai, M.K. Jain, Interfacial enzymology: the secreted phospholipase A2-paradigm, Chem. Rev. 101 (9) (2001) 2613–2653.
[2] D.A. Six, E.A. Dennis, The expanding superfamily of phospholipase A2 enzymes: classification and characterization, Biochim. Biophys. Acta 1488 (2000) 1–19.
[3] D. Mehta, Lysophosphatidylcholines: an enigmatic lysolipid, Am. J. Physiol. Lung Cell. Mol. Physiol. 289 (2005) L174–L175.
[4] D.L. Ingle, D.E. Bauman, U.S. Garrigus, Lipogenesis in the ruminant: in vivo site of fatty acid synthesis in sheep, J. Nutr. 102 (1971) 617–624.
[5] J.B. Henshaw, C.A. Olsen, A.R. Farnbach, K.H. Nielson, J.D. John, D. Bell, Definition of the specific roles of lysolecithin and palmitic acid in altering the susceptibility of dipalmitoylphosphatidylcholine bilayers to phospholipase A_2, Biochemistry 37 (1998) 10709–10721.
[6] A. Arouri, O.G. Mouritsen, Membrane perturbing effects of fatty acids and lysolipids, Prog. Lipid Res. 52 (2013) 130–140.
[7] N. Abumrad, C. Harmon, A. Ibrahimi, Membrane transport of long-chain fatty acids: evidence for a facilitated process, J. Lipid Res. 39 (1998) 2309–2318.
[8] R.T. de Jongh, E.H. Serné, R.G. IJzerman, G. de Vries, C.D.A. Stehouwer, Free fatty acid levels modulate microvascular function: relevance for obesity-associated insulin resistance, hypertension, and microangiopathy, Diabetes 53 (2004) 2873–2882.
[9] X. Ye, Lysophospholipid signaling in the function and pathology of the reproductive system, Hum. Reprod. Update 14 (2008) 519–536.
[10] S.E. Gardell, A.E. Dubin, J. Chun, Emerging medicinal roles for lysophospholipid signaling, Trends Mol. Med. 12 (2006) 65–75.
[11] J. Singh, R. Ranganathan, Quantitation of lysolipids, fattyacids, phospholipase A2 activity and correlation with membrane polarity, J. Lipid Res. 53 (2012) 1993–2001.
[12] S.A. Tatulian, Toward understanding interfacial activation of secretory phospholipase A_2: membrane surface properties and membrane-induced structural changes in the enzyme contribute synergistically to PLA_2 activation, Biophys. J. 80 (2001) 789–800.
[13] G.V. Richieri, A. Anel, A.M. Kleinfeld, Interactions of long-chain fatty acids and albumin: determination of free fatty acid levels using the fluorescent probe ADIFAB, Biochemistry 32 (1993) 7574–7580.
[14] J. Davidsen, O.G. Mouritsen, K. Jørgensen, Synergistic permeability enhancing effect of lysophospholipids and fatty acids on lipid membranes, Biochim. Biophys. Acta 1564 (2002) 256–262.
[15] E.D. Olson, J. Nelson, K. Griffith, T. Nguyen, M. Streeter, H.A. Wilson-Ashworth, M.H. Gelb, A.M. Judd, J.D. Bell, Kinetic evaluation of cell membrane hydrolysis during apoptosis by human isoforms of secretory phospholipase A2, J Biol. Chem. 285 (2010) 10993–11002.
[16] F.M. Harris, S.K. Smith, J.D. Bell, Physical properties of erythrocyte that determine susceptibility to secretory phospholipase A2, J. Biol. Chem. 276 (2001) 22722–22731.
[17] A. Anel, G.V. Richieri, A.M. Kleinfeld, Membrane partition of fatty acids and inhibition of T cell function, Biochemistry 32 (2) (1993) 530–536.
[18] F. Kamp, J.A. Hamilton, Movement of fatty acids, fatty acid analogs, and bile acids across phospholipid bilayers, Biochemistry 32 (41) (1993) 11074–11085.
[19] F. Kamp, D. Zakim, F. Zhang, N. Noy, J.A. Hamilton, Fatty acid flip-flop in phospholipid bilayers is extremely fast, Biochemistry 34 (37) (1995) 11928–11937.
[20] S.P. Bhamidipati, J.A. Hamilton, Interactions of lyso 1-palmitoylphosphatidylcholine with phospholipids: a 13C and 31P NMR study, Biochemistry 34 (16) (1995) 5666–5677.
[21] P. Høyrup, J. Jesper Davidsen, K. Jørgensen, Lipid membrane partitioning of lysolipids and fatty acids: effects of membrane phase structure and detergent chain length, J. Phys. Chem. B 105 (2001) 2649–2657.
[22] P.J. Wyatt, Light scattering and the absolute characterization of macromolecules, Anal. Chim. Acta. 272 (1993) 1–40.
[23] W.R. Burack, Q. Yuan, R.L. Biltonen, Role of lateral phase separation in the modulation of phospholipase A2 activity, Biochemistry 32 (1993).
[24] J.D. Bell, J.M. Burnside, J.A. Owen, M.L. Royall, M.L. Marilyn, L. Baker, Relationships between bilayer structure and phospholipase A2 activity: interactions among temperature, diacylglycerol, lysolecithin, palmitic acid, and dipalmitoylphosphatidylcholine, Biochemistry 35 (15) (1996) 4945–4955.
[25] J.F. Nagle, Theory of biomembrane phase transitions, J. Chem. Phys. 58 (1973) 252–264.
[26] K.M. Hallinen, S. Tristram-Nagle, J.F. Nagle, Volumetric stability of lipid bilayers, phys. chem. chem. phys. 14 (2012) 15452–15457.