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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