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# Interaction of free fatty acids with phospholipid bilayers

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**Abstract**

The partition of free fatty acids (FFA) to egg-phosphatidylcholine (egg-PC) and egg-phosphatidylethanolamine (egg-PE) vesicles was studied. Upon the addition of FFA to the suspension of vesicles, the pH of the aqueous phase changed depending on the length and saturation of the FFA hydrocarbon chain, as well as on the vesicle composition. The medium pH decreased faster if FFA was added to egg-PE as compared to egg-PC vesicles. The fluorescent free fatty acid indicator (ADIFAB) was used to measure the amount of FFA remaining in the aqueous phase. Most of the FFA added to the suspension of egg-PE vesicles remained in the aqueous phase, whereas in the presence of egg-PC vesicles the FFA partitioned preferentially into the lipid phase. The amount of FFA incorporated into the lipid bilayers was estimated by measuring the changes of pH at the lipid bilayer surface, using fluorescein-PE. At high surface concentrations of FFA, decreasing pH at the bilayer surface caused the protonation of FFA, and raised the p$K$ of FFA at the bilayer surface from 5 to about 7. The partition of FFA in egg-PE vesicles was an order of magnitude lower than that in egg-PC vesicles. The incorporation amount was determined more by the molecular packing than by the nature of lipid headgroups, because steroylcaprioyl-PE, which preferred the bilayer structure, behaved more like egg-PC than egg-PE. Understanding FFA partition characteristics would help to interpret the hydrolysis measurements of phospholipids, and to explain many biological activities of FFA.

*Keywords:* Phospholipid; Bilayer; Free fatty acid; Partition

## 1. Introduction

Exogenous free fatty acids (FFA) are known to affect a variety of cellular functions. The mechanism by which FFA affects cellular functions is still unknown. From reports so far, the activity of exogenous FFA seems to depend on their hydrocarbon chain length and saturation. In general, unsaturated FFA are more active, whereas saturated FFA are usually more inert [1–6]. In most of these experiments, the effect of FFA occurs immediately or shortly after exposure, and in some cases the effect is reversible. Therefore, the involvement of FFA metabolism in these cases is unlikely. On the other hand, the partition of FFA into the membrane may alter the physical properties of membranes. It is known that exogenous FFA perturbs lipid phases of plasma membranes [7–9]. However, the presence of a large quantity of FFA is necessary to cause any measurable effects such as fluidity.

Exogenous FFA must enter the cell or the plasma membrane to cause an effect, therefore the partition into a lipid bilayer may be the first criterion of their biological activity. The partition of a variety of FFA into lipid bilayers has been studied previously. It has been shown that FFA partition depends on their hydrocarbon chain lengths and saturation. The partition of FFA may also depend on the type and the composition of the host lipid bilayer (acceptor). However, available data do not seem to indicate such dependence. In this study we intend to examine the effect of the host bilayer on the partition of FFA.

The partition of FFA has been measured by a variety of techniques [10–13]. The most commonly used methods are based on fluorescence labeled FFA [14–16]. However, the fluorophore, usually attached to the hydrocarbon chain of FFA, often causes significant perturbation in the membrane and modifies the FFA properties. Since properties of FFA are sensitive to their structure, such as saturation, data obtained with labeled FFA have to be interpreted with caution. Anel et al. [17] developed a technique that allows measurement of FFA partition into lipid bilayers. This technique uses a fluorescent, water soluble FFA-binding protein. The fluorescence of the dye attached to the protein

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is modified upon the binding of FFA in the aqueous phase. In this paper we propose a new method to measure the relative partition of FFA into the lipid phase using fluorescein-PE. The pH sensitive fluorescein attached to the headgroup of phospholipid molecules detects changes of the surface pH caused by the partition of FFA. We applied these techniques in the study to determine the partition of FFA into different host or acceptor bilayers. The good agreement among these techniques gives confidence to our measurements.

## 2. Materials and methods

### 2.1. Materials

Egg phosphatidylcholine (egg-PC) and egg phosphatidylethanolamine (egg-PE) were obtained from Avanti Polar Lipids (Alabaster, AL). *N*-(5-Fluoresceinthiocarbamoyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (fluorescein-PE) and acrylodated intestinal fatty acid binding protein (ADIFAB) were purchased from Molecular Probes (Eugene, OR). *cis*-9-Octadecenoic (oleic), dodecanoic (lauric) acids and oleic acid methyl ester were obtained from Sigma (St. Louis, MO). 1-Palmitoyl-2-caprioyl-*sn*-glycero-3-phosphatidylethanolamine (SCPE) was obtained from Dr. Ching-Shien Huang, University of Virginia.

### 2.2. Vesicle preparation

Lipid vesicles were prepared with or without fluorescence dye. The fluorescence dye was mixed with lipids in a chloroform suspension. Samples were evaporated under a vacuum, and dispersed in water or buffer. In order to obtain unilamellar vesicles, samples were extruded using 0.2 $\mu$m filter (Nuclepore, Pleasanton, CA) according to procedure described by Hope et al. [18]. Vesicles were formed at pH 9.0 and kept on ice to prevent hexagonal phase formation in egg-PE vesicles. Size of vesicles was determined using the dynamic light scattering method (Submicron Particle Sizer Model 370, NICOMP, Santa Barbara, CA). The average sizes were 190 nm and 175 nm for egg-PC and egg-PE vesicles, respectively. Both egg-PE and egg-PC remained in the lamellar phase after extrusion when kept at room temperature. X-rays diffraction shows that the lamellar-hexagonal phase transition for the suspension of egg-PE vesicles is about 60° C (data not shown).

FFAs were added to the suspension of vesicles from 1 mM ethanol stock solutions, except in bulk pH measurements where FFA stock solution was 10 mM. We assumed that FFA equilibrates instantaneously within the lipid bilayer [19,20]. All figures show the amount of FFA as a total concentration in the sample and as a molar fraction of lipids and FFA combined (mol%). X-ray diffraction measurements did not indicate the presence of the hexagonal phase in the egg-PE suspension at room temperature when 20 mol% of oleic acid was added (data not shown).

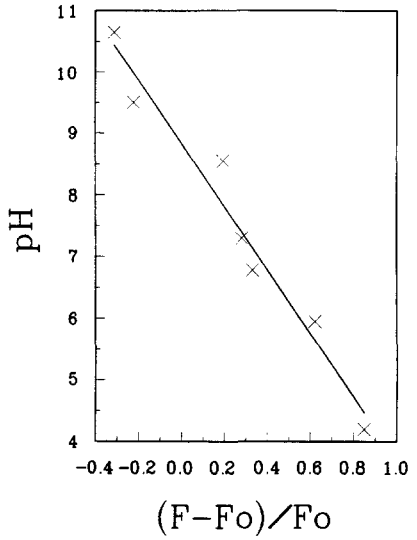


Fig. 1. The normalized fluorescence of fluorescein-PE incorporated into egg-PC vesicles as a function of bulk pH. The fluorescence intensity ($F$) was normalized to that at pH 9.0 ($F_0$). The straight line was calculated with the least square method.

### 2.3. Measurements of pH at the membrane surface

We applied the fluorescein-PE as a probe of pH at the lipid-water interface. It is known that the fluorescence intensity of fluorescein derivatives is sensitive to changes of pH [21–23]. We measured the fluorescence of fluorescein-PE in egg-PC vesicles as a function of bulk pH. We have shown that the relative decrease of fluorescence is proportional to pH in the aqueous phase (Fig. 1). This result is similar to that obtained by Thelen and co-workers [23]. This dependence can be well approximated with linear function, as shown in Fig. 1. In all subsequent experiments, the fluorescence intensity was normalized to that at pH 9. In all surface pH measurements, the lipid content of samples was 0.1 mg/ml and the fluorescence labeled PE never exceeded 0.5 mol% of the total lipid. Lipid vesicles were suspended in 5 mM NaCl solution with pH adjusted to 9.0.

### 2.4. Partition of FFA into lipid bilayers measured with ADIFAB

Lipid vesicles were suspended in 10 mM Tris, 150 mM NaCl, 1 mM EGTA buffer at pH 8.0, and incubated for a few minutes with FFA and 0.1 $\mu$M of ADIFAB. The emission spectra of ADIFAB were taken after each addition of FFA. The probe was excited at 390 nm. As previously described by Anel et al. [17], the spectral shift of ADIFAB emission allows the determination of FFA concentration. The ratio of the fluorescence intensities at 505 nm and 423 nm is a measure of FFA concentration in

the aqueous phase. All fluorescence measurements were performed on the SLM 8000 fluorimeter with a thermoregulated cuvette holder.

The FFA-ADIFAB dissociation constants ($K_d$) and partition coefficients ($K_p$) were calculated as described by Anel et al. [17]. The concentration of FFA in the aqueous phase was calculated according to this equation:

$$[\mathrm{FFA}] = K_d Q(R - R_o)/(R_{max} - R) \quad (1)$$

where $R$ is the measured ratio of fluorescence intensities at 505 nm and 432 nm, $R_o$ is this ratio without FFA, $R_{max}$ is the value when ADIFAB is saturated and $Q$ is an empirical constant. Values of $Q$, $R_{max}$ and $R_o$ are 19.5, 11.5 and 0.25, respectively ($Q$ and $R_{max}$ were taken from Anel et al. [17], $R_o$ was calculated from the ADIFAB fluorescence spectrum).

The FFA-lipid partition constants were calculated using the following equation:

$$K_p = (([\mathrm{FFA}]_o - [\mathrm{FFA}])/[\mathrm{FFA}])V_a/V_m \quad (2)$$

where $[\mathrm{FFA}]_o$ is the total FFA concentration in the sample, [FFA] is the aqueous concentration of FFA calculated from Eq. (1), and $V_a$ and $V_m$ are the volumes of the aqueous and lipid phases, respectively. The value of $V_m/V_a$ for lipid vesicles is $10^{-3}$ for each millimolar of phospholipid [17]. In all experiments with ADIFAB lipid concentration was 20 $\mu$M.

### 2.5. Estimation of FFA partition into lipid bilayers using bulk pH measurement

The measurement of FFA in the suspension of vesicles was estimated using a method based on the principle that the presence of FFA in the aqueous phase causes a drop of bulk pH. This method is used extensively to monitor the activity of phospholipase $A_2$ [24–26]. In our experiments, a concentrated solution of FFA was added in small doses (30 nmol) to the vesicles suspended in water. The change of pH was registered after each addition of FFA. The initial pH in vesicle suspensions was adjusted to 9.0. The total amount of lipid in a sample was 5 $\mu$mol in 3 ml water. pH measurements were done on a Beckman ∅40 pH meter with an attached plotter.

## 3. Results

### 3.1. Measurements of bulk pH as a function of FFA concentration

The pH of the bulk aqueous phase of a vesicle suspension containing FFA is determined by the number of ionized FFA molecules. When lauric or oleic acid was added to suspension media (without vesicles), the bulk pH decreased rapidly and similarly (crosses Fig. 2). Open symbols in Fig. 2 show the change of aqueous pH when

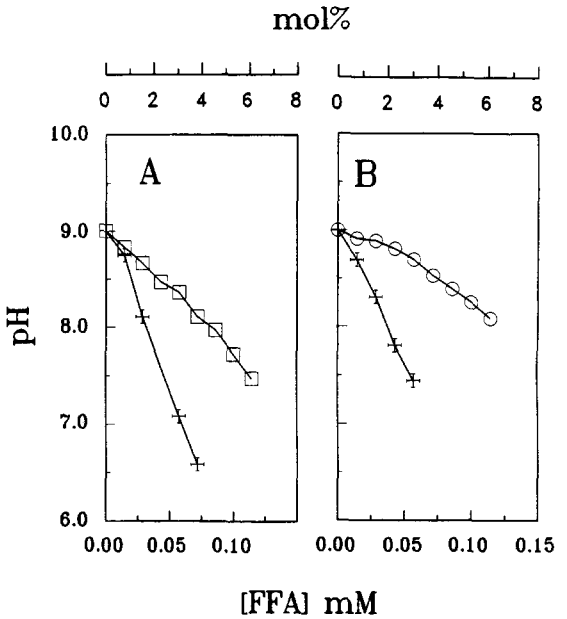


Fig. 2. The bulk pH as a function of FFA bulk concentration in the presence (open symbols) and absence (crosses) of egg-PC vesicles suspension. Fig. 2A represents data with lauric acid whereas Fig. 2B with oleic acid. The total lipid concentration was 5 $\mu$molar for each sample. The molar fraction of FFA is also indicated.

lauric or oleic acids were added to the suspension of egg-PC vesicles. In both cases, the drop of pH was reduced. The decrease of bulk pH was more pronounced when lauric acid was added to vesicles. This observation confirms data reported earlier [17,20,27], that long chain fatty acids partition more readily into lipid bilayers, consequently the buffering effect is more pronounced.

When lauric acid was introduced to bilayers of different host lipids, the dependence of aqueous phase pH on FFA concentration was strikingly different. Fig. 3 shows that in the presence of egg-PE vesicles, changes of aqueous pH were quite close to that in suspension medium (water) alone. When egg-PC vesicles were present, changes of aqueous pH were reduced. Similar results were obtained with oleic acid (not shown).

### 3.2. The concentration of FFA in the aqueous phase

In order to measure the amount of FFA in the aqueous phase, we used the FFA binding protein with an attached fluorescence indicator (ADIFAB). The amount of FFA bound to proteins is proportional to the ratio of fluorescence intensities at 505 nm and 430 nm [17,28]. The ratio is higher for increasing amounts of FFA in the aqueous phase. The amount of FFA in the aqueous phase was estimated according to Eq. (1). When FFAs were added to the ADIFAB suspension in the absence of lipid vesicles the resulting fluorescence depends only on the FFA dissociation constant. The dissociation constants calculated for oleic and lauric acids are $(0.29 \pm 0.005) \cdot 10^{-6}$ $M^{-1}$ and

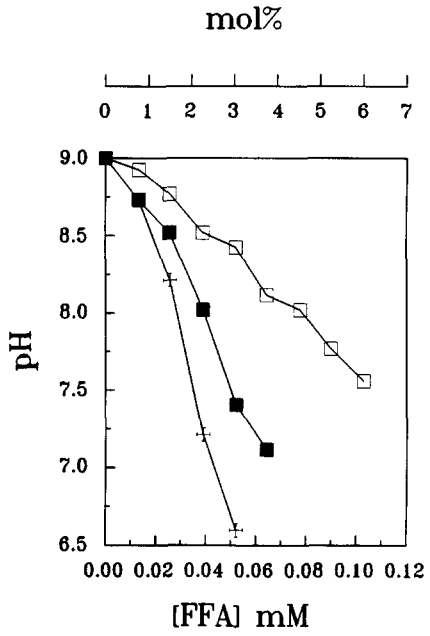


Fig. 3. The bulk pH of samples when lauric acid was added to aqueous media (crosses), egg-PE (filled symbols) and egg-PC (open symbols) vesicles suspensions.

$(0.71 \pm 0.16) \cdot 10^{-6}$ $M^{-1}$, respectively. The value calculated for oleic acids is identical to that obtained by Anel et al. [17].

Fig. 4 shows fluorescence ratios calculated from ADIFAB spectra. The fluorescence ratios obtained in the presence of vesicles formed from egg-PE do not differ much from that without vesicles when the partition of oleic acid was measured. Only in high concentrations of oleic acid did the ratio in PE suspension start to depart from those in the medium (Fig. 4B). The partition of lauric acid into egg-PE bilayer was significant but still smaller than that into egg-PC vesicles (Fig. 4A). FFA-egg-PE partition coefficients calculated for lauric and oleic acids were $(4.4 \pm 2.2) \cdot 10^4$ and $(2.4 \pm 1.8) \cdot 10^4$, respectively.

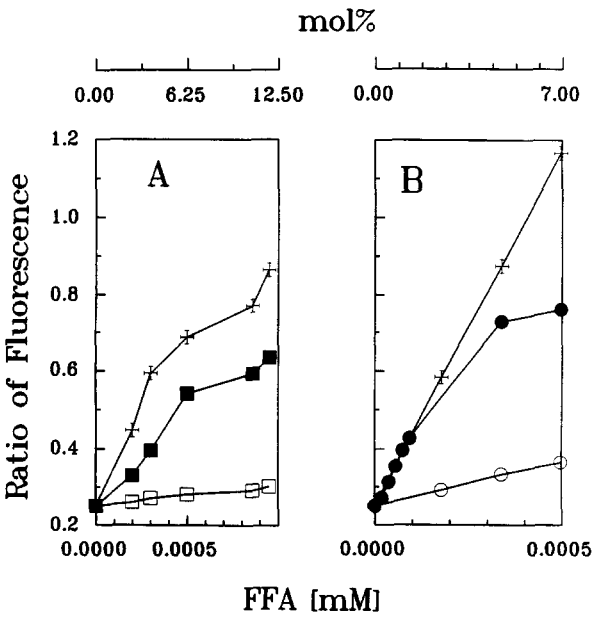


Fig. 4. The amount of fatty acids in the aqueous phase measured with ADIFAB in the presence of egg-PE (filled symbols) and egg-PC (open symbols) vesicles. Crosses represent free fatty acids in the solution without vesicles. Plot A and B represent data obtained with lauric and oleic acids, respectively.

When oleic and lauric acids were added to egg-PC vesicles, the aqueous concentration of FFA remained low and almost unchanged with an increasing amount of FFA in the sample. Fluorescence ratios obtained from spectra of ADIFAB in the presence of egg-PC vesicles with oleic and lauric acids are shown on Fig. 4A and B. This experiment shows that in the presence of egg-PC vesicles the majority of FFA are in the lipid phase. Calculated partition coefficients are now $(74 \pm 37) \cdot 10^4$ for lauric acid and $(31 \pm 3) \cdot 10^4$ for oleic acid. The partition coefficient of oleic acid measured in the presence of egg-PC vesicles agrees with that presented by Anel et al. $(36 \cdot 10^4$ [17]). The measurements of FFA concentrations in water support the conclusion obtained with bulk pH measurements. FFA enters more readily into lipid bilayers formed from egg-PC than those formed from egg-PE. Errors estimated for the partition coefficients of lauric acid were in the range of 50%. These large uncertainties were caused by the low accuracy in the determination of the dissociation constant (23% error). The estimation of the partition coefficient of oleic acids was more accurate since the dissociation constant was estimated with an error smaller than 3%.

### 3.3. Measurements of the surface pH

The partition of the ionized FFA into lipid vesicles increases the surface charge of the membrane and the surface pH. The fluorescein moiety attached to phosphatidylethanolamine headgroup detects changes in the surface pH caused by the presence of charged carboxyl groups of FFA at the membrane surface. Since the aqueous pH (9.0) is much higher than the bulk p$K$ of FFA (5.0), free fatty acids in the aqueous phase are predominantly in the deprotonated form. Consequently, the partition of FFA into the lipid bilayer might be estimated from the amount of charge present at the membrane surface. This assumption is valid only when the surface pH is sufficiently far from the p$K$ of FFA. The flattering out of the surface pH above 0.01 mM of oleic acid (Fig. 5B) is likely due to the increased protonation rather then aggregation of FFA in the aqueous phase. The aqueous concentration of FFA is below its solubility limit (6 $\mu$M; [29]). The p$K$ of FFA in the lipid bilayer was measured to be around 7.6 [13,29]. In addition, changes of the fluorescence should be sensitive only to the changes of local pH. When the methyl ester of oleic acid (up to 20 mol%) was added to the egg-PC vesicles the fluorescence of fluorescein was not effected. Addition of ethanol alone, within appropriate range, did

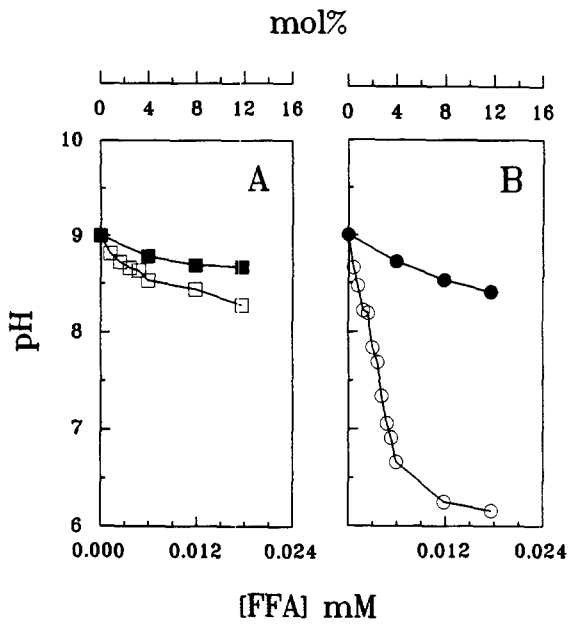


Fig. 5. Surface pH measured with fluorescein-PE in vesicles formed from egg-PE (filled symbols) and from egg-PC (open symbols). Plot A and B represent data obtained by adding lauric and oleic acids, respectively.

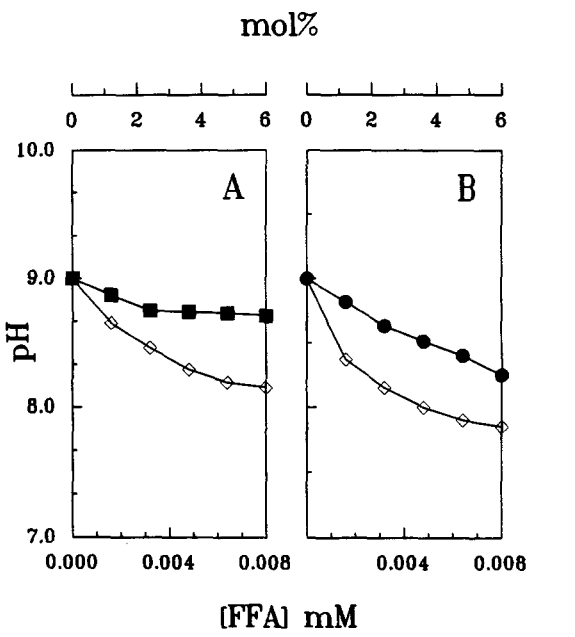


Fig. 6. Surface pH measured with fluorescein-PE when FFA was added to egg-PE vesicles (filled squares and circles) and to the mixture of 5 mol% egg-PC in egg-PE (open diamonds). A and B represent data obtained with lauric and oleic acids, respectively.

not effect the fluorescence of fluorescein-PE of membranes. All measurements of surface pH were performed at a bulk pH of 9.0.

Again, pronounced differences in surface pH was observed when both FFAs were added to egg-PE or egg-PC vesicles. Fig. 5A and B shows the dependence of the surface pH on the concentrations of lauric and oleic acids. When oleic acid was added to egg-PC vesicles, the surface pH dropped sharply. The drop of pH was much smaller when FFA was added to vesicles formed from egg-PE. These results confirm similar observations using two other methods, that FFA partition more into egg-PC than into egg-PE bilayers.

In addition, we were able to increase FFA partition into egg-PE bilayer by adding small amounts of egg-PC. Fig. 6 shows pH at the surface of vesicles formed from egg-PE mixed with 5 mol% egg-PC. The intake of FFA by membrane is substantially enhanced by egg-PC. When the amount of egg-PC increases to 20 mol%, the intake of FFA is practically indistinguishable from that of egg-PC alone.

In order to determine whether the partition coefficient of FFA into the lipid bilayer depends on the structure of lipid headgroups or the propensity of the membrane to undergo lamellar-non-lamellar phase transition we compared the surface pH of egg-PE and 1-stearyl-2-caprioyl-PE (SCPE), which does not experience lamellar-non-lamellar phase transition at laboratory temperatures. The result is shown in Fig. 7. The decrease of surface pH when FFA are added to SCPE vesicles is very similar to that of egg-PC vesicles. This experiment shows that the hydrocarbon packing in lipid bilayers, but not the headgroup, determines the efficiency of FFA partition.

We calculated the partition coefficient of FFA into lipid bilayers from surface pH measurements using the Gouy-Chapman-Stern theory. The concentration of protons at the membrane surface depends on the surface charge density. The charge density equals to the amount of FFA in the

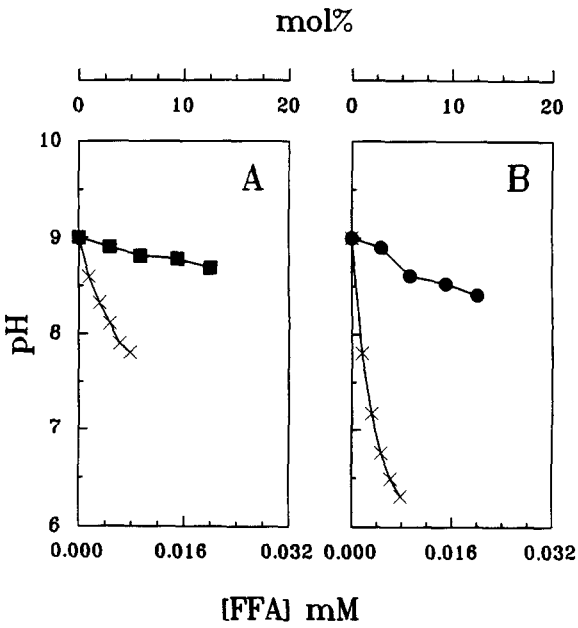


Fig. 7. Surface pH when lauric (A) and oleic (B) acids were added to the vesicles formed from egg-PE (filled squares and circles) and from 1-stearyl-2-caprioyl-PE (SCPE) (crosses).

lipid bilayer when the surface pH is sufficiently far from p$K$ of FFA. The surface concentration of protons $[H^+]$ is related the bulk concentration $[H^+]_o$ by the Boltzman equation [30,31]:

$$[H^+] = [H^+]_o \exp(-F\psi_o/RT)$$

where $\psi_o$ is the surface potential, $F$ is Faraday constant, $R$ is the gas constant, and $T$ is the absolute temperature. The electrostatic potential at the bilayer surface ($\psi_o$) may be calculated from the surface charge density according to the equation:

$$\psi_o = \sigma/\epsilon\epsilon_o\kappa$$

where $\kappa = (2e^2z^2NC/\epsilon\epsilon_o kT)^{1/2}$, $\epsilon$ is a dielectric constant of the medium, $\epsilon_o$ is the permittivity of free space, $z$ is the valance of the ion, $C$ is the salt concentration, $N$ is the Avogadro number, and $k$ is the Boltzman constant. The charge density at the surface of the lipid bilayer was calculated assuming that each FFA molecule carries the elementary charge and the surface area of lipid molecule is 65 Å$^2$. We assumed that at low concentrations of FFA the total surface area of membrane did not increase. The partition coefficients of oleic and lauric acid into egg-PC membrane, when calculated from the surface pH data, are $(8.9 \pm 3.2) \cdot 10^4$ and $(2.5 \pm 1.0) \cdot 10^3$, respectively. Partition coefficients in the presence of egg-PE vesicles were much lower, $(1.2 \pm 0.7) \cdot 10^3$ for oleic acid and $(4.0 \pm 1.2) \cdot 10^2$ for lauric acid.

## 4. Discussion

The partition of FFA into lipid bilayers is an important process that affects cell and membrane functions, as well as membrane biogenesis and FFA cytotoxicity. There are virtually no reports concerned with the effect of the composition of host (acceptor) lipid vesicles on FFA partition. Only recently has it been shown that FFA partition differently into vesicles in the fluid phase than in the gel phase [17]. The physical factors determining the partition coefficients are largely unknown.

There are a number of approaches to measure the partition of FFA into lipid bilayers. The bulk pH method has been used to measure the FFA generated from the activity of phospholipase $A_2$ [24,32]. ADIFAB was designed for the purpose of monitoring FFA left in the aqueous phase as a result of partition [4]. Another approach was applied by Kamp and Hamilton [20,21], who encapsulated pH sensitive dye to report the amount of FFA present in the inner layer of vesicles. In this study, we applied a surface pH measurement method to provide additional information about factors affecting FFA partition into bilayers. All three methods give complementary results, that lend confidence to the reliability of these methods.

First, measurements of pH in the bulk medium show that there is difference between egg-PE and egg-PC vesicles in the respect of the modifying changes of bulk pH caused by FFA. The only reasonable explanation is that the partition coefficients for these membranes are different. To test these hypotheses we measured the concentration of FFA in the aqueous phase applying the FFA sensitive protein, ADIFAB. Partition coefficients derived from these experiments for lauric and oleic acids were an order of magnitude higher for egg-PC bilayers than that for egg-PE bilayers.

This finding raises the question: why do FFA in lipid membrane contribute so little to the bulk pH?

When the surface concentration of FFA is very low, and the carboxyl groups are charged, the proton concentration in the vicinity of the membrane surface varies with the FFA concentration at the vesicle surface [30]. When FFA is added to a vesicle suspension, a portion of FFA molecules goes to the bilayer [17]. When the bulk pH is above the p$K$ of FFA (5.9; [13]), and the concentration of FFA is low, the majority of FFA carboxyl groups are charged regardless of their location. Subsequent additions of FFA progressively lower the bulk pH of the vesicle suspension. With an increasing amount of FFA that favorably partition in the membrane, the charge density on the membrane surface increases. As a result, in the vicinity of the membrane surface, proton concentration is raised due to electrostatic interaction [30,31], and carboxyl groups are protonated. This effect is manifested as the fact that the p$K$ of FFA in lipid bilayers is shifted from about 5.0 in the bulk phase, to about 7.0 at the membrane surface [13,14]. Because of to FFA protonation at the membrane surface, further addition of FFA does not change the proton concentration in the bulk aqueous phase. Consequently, changes of bulk pH are buffered when additional FFA partition preferably into the lipid phase.

To measure the effect of FFA on the proton concentration at the membrane surface, we used a pH sensitive dye, fluorescein, covalently linked to the PE headgroup [21]. Since the fluorophore is attached to the headgroup, it is sensitive to the environment at the vicinity of the membrane surface [33]. The p$K$ for fluorescein-PE was measured previously and was estimated to be approx. 6.0 [21]. Our calibration experiment shows that the fluorescence intensity of the fluorescein-PE increases proportionally with the bulk pH within a wide range (Fig. 1). During the calibration, we assumed that the proton concentration at the surface of the phosphatidylcholine bilayer is the same as that in the bulk. Since the majority of FFA is in the membrane, the aqueous concentration of FFA is low. This is indeed the case; changes of pH at the membrane surface are more pronounced then that in the bulk (Figs. 3 and 5). To further verify this assumption, we measured the effect of these lower concentrations of FFA on the bulk pH, using a water soluble, pH sensitive dye, carboxyl-fluorescein. When FFA was added to the vesicle suspension, the decrease of fluorescence of carboxyl-fluorescein did

not exceed 5% of the initial intensity, while the fluorescence of fluorescein-PE changed significantly. Therefore, it is justified to assume that most changes in the fluorescence of fluorescein-PE are caused by the variation of the proton concentration at the bilayer surface, in spite of the absence of surface pH calibration.

During the partition experiments, FFA in the aqueous phase was in the monomer form even when the total FFA concentrations exceeded CMC and/or solubility limits (12 $\mu$M for lauric acid [34] and 6 $\mu$M for oleic acid [28,35]). FFAs partition preferentially into the lipid phase therefore their concentrations in the aqueous phase are below solubility limits. When partition coefficients were calculated from data obtained with fluorescein-PE we assumed that majority of FFA in the aqueous phase is ionized. The lack of any significant differences in the fluorescence measurements when the pH varies from 8.5 to 9.5 (data not shown) seems to confirm that assumption. Similar observations for myristic acid were made by Peitzsch and McLaughlin [27]. In addition, the amount of FFA applied in ADIFAB and surface pH experiments did not change the bulk pH, as measured with carboxyl-fluorescein (data not shown). Therefore, we do not expect any changes in ionization of FFA when the partition was measured by ADIFAB and surface pH measurements.

The values of the partition coefficients of oleic acid calculated from data obtained from ADIFAB and surface pH experiments are consistently different. The value of the partition coefficient for oleic acid obtained with ADIFAB is similar to that obtained by Anel et al. ($36 \cdot 10^4$; [17]) but the surface pH measurements yield values about 1/3 of that measured by ADIFAB. Peitzsch and McLaughlin [27] using radiolabeled FFA and zeta potential measurements estimated the partition coefficient of myristic acid to egg-PC to be $(0.8–7.4) \cdot 10^4$, whereas Pjura et al. [10] estimated the partition coefficients of oleic acid to egg-PC to be in order of $(1 \text{ and } 7) \cdot 10^4$ by two different procedures. It seems that measurements by ADIFAB consistently give higher values of partition coefficients than those by other methods. Our measurements by ADIFAB and surface pH support this tendency. The difference for lauric acid is even more pronounced. This is perhaps due to the fact that lauric acid, with a 12 carbon chain, is more soluble in water and therefore associates less with the bilayer and ADIFAB. The uncertainty of measurements is thus much higher.

Despite the discrepancies in calculated values of partition coefficients, a main conclusion can be derived from our experiments, that properties of the host lipid bilayer have a significant effect. We selected two different host lipid systems: egg-PC and egg-PE as our model. In the bilayer form, the area per molecule of PE is smaller than that of PC, therefore the molecules of PE of similar acyl chains are packed tighter [36]. The differences in the molecular packing between egg-PE and egg-PC are expected to result in a reduction of FFA partition into tightly packed PE membrane. Because of the different tightness of hydrocarbon chain packing, phosphatidylcholines form stable bilayers, whereas most unsaturated phosphatidylethanolamines may transform to the inverted hexagonal phase at elevated temperatures [37–40]. The fact that the bilayer-preferred SCPE behaves more like PC than PE signifies the importance of the tightness of hydrocarbon chain packing or equivalently the curvature energy, rather then the headgroup preference, on the partition of FFA. The difference in FFA partition is responsible for the different buffering effect of these two phospholipids. The buffering effect should be taken into consideration when bulk pH is used as a reaction assay.

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