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The effect of pH on the phase transition temperature of dipalmitoylphosphatidylcholine-palmitic acid liposomes

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The shift in the gel-liquid crystal phase transition temperature (t_m) of dipalmitoylphosphatidylcholine liposomes induced by incorporation of 10 mol% palmitic acid, was measured by 90° light scattering at different bulk pH values. It has been found that the t_m shift decreases sigmoidally from 4.7 to −0.3°C as the bulk pH is raised from 5 to 11. Since it is in this range that the carboxyl group of a membrane-bound fatty acid should ionize, our results can be interpreted to mean that there is relationship between the t_m shift and the degree of dissociation of palmitic acid, the uncharged fatty acid increasing t_m and its conjugate, anionic form, slightly decreasing the transition temperature of dipalmitoylphosphatidylcholine liposomes. The experimental results are fitted by a modified form of the Henderson-Hasselbach equilibrium expression which takes into account the effect of the anionic fatty acid on the surface potential and hence, on the surface pH of liposomes, according to Gouy-Chapman and Boltzmann equations, respectively. Best fit between theory and experiments is found when the intrinsic interfacial pK of palmitic acid is set equal to 7.7. This high pK value can be explained as due to the effect of the lower dielectric constant of the interfacial region, as compared to bulk water, on the acid-base dissociation of the carboxyl group. The results presented here show that upon incorporation of palmitic acid, the phase transition of dipalmitoylphosphatidylcholine bilayers becomes extremely sensitive to changes of pH in the vicinity of the physiological range. This property is not shown by the pure phospholipid bilayers in the same pH range.

Introduction

It has been demonstrated that free fatty acids modulate a large number of membrane phenomena, ranging from transport of nucleotides and ions [1] to activities of many enzymes immersed into the lipid matrix [2,3]. As a consequence, the question has been raised of whether the observed changes in such different functions are related to modifications induced by the long-chain carboxylic acids in the physical properties of membranes [2]. In this regard, many investigations have been conducted to gather information on the perturbations that free fatty acids can induce in lamellar systems [4–8].

Calorimetric and optical studies have shown that the temperature t_m, which defines the transition from the gel to the liquid-crystalline state of phosphatidylcholine lamellae, is strongly shifted by incorporation of free fatty acids into the bilayers [4–7]. Palmitic acid, in particular, has been shown to induce a concentration-dependent in-

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Abbreviation: DPPC, dipalmitoylphosphatidylcholine.

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crease in the t_m of dipalmitoylphosphatidylcholine bilayers at pH 7.0 [4]. Although it has been found that sodium palmitate in unbuffered media has a different effect which consists in slightly lowering the t_m of the phospholipid bilayers [7], in subsequent investigations on the interactions of dipalmitoylphosphatidylcholine and palmitic acid [8], no attention has been paid to the possible role of pH as modulator of the t_m shifts induced by the fatty acid.

Because of their amphipathic character, long-chain carboxylic acids can be expected to induce perturbations not only in the hydrophobic moiety of membranes, but also in the polar region. In fact, the ionization of the carboxyl group affects the membrane/water interface by generating negative surface charge as the pH is raised [9]. In view of the electrostatic effects on lipid phase transitions demonstrated by several authors [10–14], we decided to perform a detailed investigation of the influence of bulk pH on the phase transition temperature of dipalmitoylphosphatidylcholine/palmitic acid mixtures, to test the hypothesis that the t_m shift induced by the fatty acid, should be dependent on the ionization of its carboxyl group. We used sonicated liposomes and measured the phase transition temperature by the change in the 90° light scattering intensity of the lipid dispersions [15–17]. The study was carried out from pH 5 to 11, since it is in this range that the carboxyl group of a fatty acid incorporated into membranes has been shown to ionize [9,18–20].

The experimental data are fitted by an equation which relates the t_m shift induced by palmitic acid to the bulk pH, on the basis of the Henderson-Hasselbach equilibrium expression as modified by introducing the surface potential which, according to the Gouy-Chapman theory [21–23], will be generated by the unprotonated fatty acid [9]. The equation also includes the prediction of the Boltzmann's law on the difference between surface and bulk pH that should be induced by the surface potential [24].

Materials and Methods

Synthetic DL-α-dipalmitoylphosphatidylcholine and palmitic acid were purchased from Sigma Chemical Company. The reported purity of the lipids was higher than 99%. The purity of the phosphatidylcholine was periodically verified by thin-layer chromatography. All other reagents were analytical grade. Ultrapure water obtained through a Milli RO – Milli Q Purification System of Millipore, was used throughout.

Depending on the required pH, lipids were dispersed in one of the following aqueous buffers: 3 mM sodium acetate/acetic acid (pH 5.0–5.5), 2.5 mM imidazole-HCl (pH 6.0–7.5) or 2.5 mM Tris-HCl (pH 8.0–8.9). For higher pH values, NaOH solutions were used. In all cases, NaCl was added to bring the final electrolyte concentration to 0.010 M.

For the preparation of the lipid vesicles, a chloroform/methanol (9:1, v/v) solution containing the appropriate proportion of phosphatidylcholine and palmitic acid was dried with nitrogen. Thereafter, to help remove the last traces of retained chloroform, benzene was added and evaporated with nitrogen. After addition of the required amount of buffer solution to give a lipid concentration of 0.3 mM, the mixture was sonicated for 10 min under nitrogen using a Branson Sonifier (Model B12) equipped with a titanium microtip, at a power output of 60 watts [25,26]. During sonication the sample was kept at 60°C, i.e. above the phase transition temperature [27], by means of a Lauda K-4/RD circulating bath. The vesicles were annealed at 60°C for 1 h, before performing the phase transition experiments. No titanium particles were observed after centrifugation at 2500 rpm for 7 min.

Phase transitions of the lipid dispersions were followed by the change in the 90° light scattering intensity [15–17] at 400 nm using an Aminco Bowman Spectrofluorometer. The lipid dispersions were cooled from 60°C to 30°C in approx. 20 min by circulating water through the sample compartment. Temperature was measured directly in the sample using a microprobe thermocouple type IT-18 (time constant = 0.1 s) connected to a BAT 8 Digital Thermometer from Bailey Instruments.

The pH of the samples was measured before and after running the phase transition experiments using a Radiometer PHM84 Research pH meter equipped with a combination electrode.

Results

In Fig. 1 are shown some examples of the phase transition determinations for DPPC/palmitic acid (9:1) vesicles, at different bulk pH values. The transition temperature was taken at the midpoint of the normalized curves representing the change in the 90° light scattering intensity vs. temperature. Similar experiments were carried out with the mixed liposomes as well as with the pure DPPC vesicles, at approx. 0.5 pH unit intervals, to investigate in detail the pH dependence of t_m in the range from 5 to 11. The results are shown in Fig. 2. It can be observed that at pH 5 the t_m of the mixed vesicles is about 5°C higher than that of DPPC vesicles. However, as the pH is raised, the difference decreases until above pH 9, the t_m of DPPC-PA dispersions first becomes similar and then even slightly smaller than the transition temperature of pure DPPC vesicles.

The difference between the transition temperatures of DPPC/palmitic acid and pure DPPC vesicles at the same pH, denoted by Δt_m and plotted as a function of bulk pH, is shown in Fig. 3, where the circles represent the experimental values and the lines the fitting of an equation which is derived in the following. (It should be mentioned that ionic activity coefficients will be taken to be unity throughout [11]).

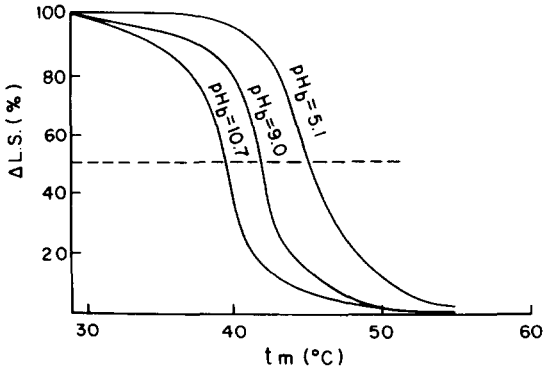

Fig. 1. Determination of the gel-liquid crystal transition temperature (t_m) of DPPC/palmitic acid (9:1) liposomes at different bulk pH alues. The normalized change in the 90° light scattering intensity (Δ L.S.) of the lipid dispersions at 400 nm, is plotted as a function of temperature. The phase transition teperature (t_m) is taken as the temperature corresponding to 50% of the total light scattering change. Lipid concentration: 0.3 mM; temperature sweep rate: approx. −1.5°C/min.

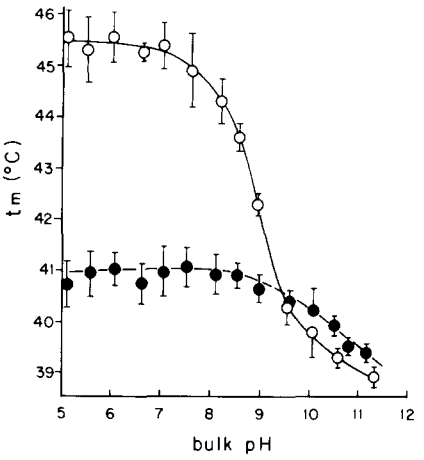

Fig. 2. Phase transition temperature (t_m) of DPPC/palmitic acid (9:1) (○) and DPPC (●) liposomes as a function of bulk pH. The phase transition temperatures were determined from curves similar to those in Fig. 1. The values plotted are the means ± S.D. of at least five experiments.

As shown in Fig. 3, the experimental Δt_m decreases sigmoidally from 4.7 to −0.3°C as the bulk pH is increased from 5 to 11. Since in this range, a long-chain fatty acid incorporated to membranes should dissociate [9,18–20], our results could be interpreted to mean that there is a linear relationship between Δt_m and the degree of dissociation (α) of palmitic acid, the uncharged fatty acid (AH) increasing t_m and its conjugate, anionic form (A^-), slightly decreasing the transition temperature of DPPC liposomes. Thus, at any pH, the combined influence of both fatty acid species on the t_m shift, should be reflected

$$\Delta t_m = a \cdot [AH] + b \cdot [A^-] \quad (1)$$

where a and b are different proportionality constants.

If [PA] is the total fatty acid concentration, then

$$\Delta t_m = [PA] \cdot (a - \alpha \cdot (a - b)) \quad (2)$$

The existence of a linear relationship between Δt_m and the fatty acid content of the vesicles, at least up to 10 mol% palmitic acid, which is the concentration employed in our experiments, is shown in Fig. 4, where Δt_m vs. [PA] is plotted at five different pH values. It can be observed that deviations from linearity only occur at concentra-

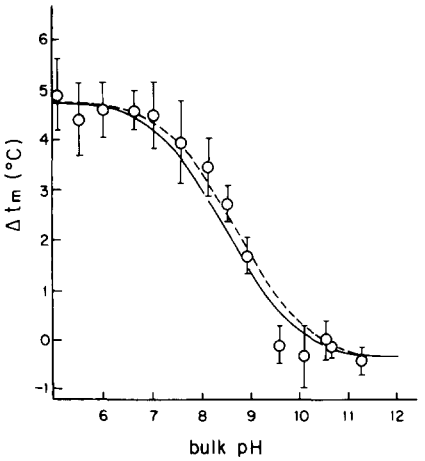

Fig. 3. The shift in the transition temperature of DPPC liposomes (Δt_m) induced by incorporation of 10 mol% palmitic acid, as a function of bulk pH. The circles are the experimental results (±S.D.) obtained from Fig. 2 as the difference between the t_m values corresponding to DPPC/palmitic acid and DPPC vesicles, at the same bulk pH; the square root of the sum of the squares of the standard deviations of those two t_m values, gives the S.D. for each Δt_m. The lines correspond to the predictions of Eqn. 6, with the a and b constants equal to 0.47 and $-0.03°C/(mol\%)$, respectively, according to the limiting slopes obtained from Fig. 4. Lipid mean molecular areas of 45 Å^2 (———) and 65 Å^2 (— — —) were assumed as corresponding to the gel and liquid-crystalline states, respectively; $c = 0.01$ M. The intrinsic interfacial pK of palmitic acid, pK_I, was set equal to 7.7. No significant differences in the predicted Δt_m values are found if the calculations are performed for 312.15 or 323.15 K, these two temperatures limiting a range which includes all the experimental t_m values, expressed in the Kelvin scale.

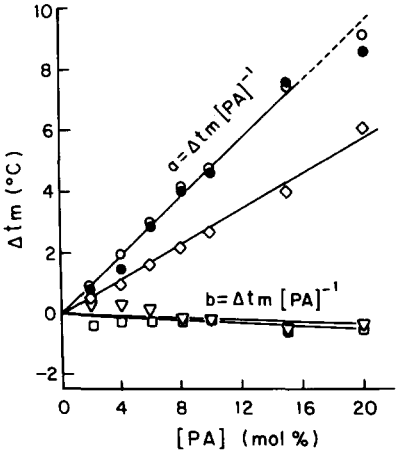

Fig. 4. Δt_m as a function of the molar percentage of palmitic acid in DPPC/palmitic acid vesicles, at different bulk pH values: 5.1 (●), 5.6 (○), 8.5 (◇), 10.5 (□) and 11.2 (▽), the slopes of these curves being 0.47, 0.28, 0.02, -0.03 and $-0.03°C/(mol\%)$, respectively. The limiting slopes are 0.47 and $-0.03°C/(mol\%)$ corresponding to the constants a and b, respectively, of Eqns. 1–4 and 6. As explained in the text, Δt_m is the difference between the transition temperatures of DPPC/palmitic acid and pure DPPC liposomes at the same pH_b. [PA], total fatty acid concentration.

tions much higher than 10 mol% fatty acid. These results validate the use of Eqn. 2 to analyze the data of Fig. 3.

Likewise, in Fig. 4 is observed that, although the slope of the Δt_m vs. [PA] plot changes dramatically with pH, limiting slopes at the acidic and alkaline sides of the pH range studied, can be considered to represent the separate effects on the t_m shift of the fully protonated or fully ionized fatty acid, respectively. If the behavior at the acidic side represents the approach of α to zero, Eqn. 2 reduces to

$$\Delta t_m = [PA] \cdot a \tag{3}$$

At the alkaline extreme, on the other hand, α should approach unity. Then, Eqn. 2 becomes

$$\Delta t_m = [PA] \cdot b \tag{4}$$

In this way, the parameters a and b representing the distinct effects on Δt_m of the uncharged and charged forms of palmitic acid, were determined to be 0.47 and $-0.03°C/(mol\%)$, respectively (Fig. 4).

The ionized fatty acid will generate a surface potential ψ [9] which, according to the Boltzmann's law, will shift the surface pH (pH_s) with respect to the bulk pH (pH_b) [24]. Thus, the relationship between α and pH_b is given by a modified form of the Henderson-Hasselbach equilibrium expression in which the term $(pH_b + (F\psi/2.3RT))$ derived from the Boltzmann equation, represents the surface pH.

$$\alpha = \frac{1}{1 + 10^{\left(pK_I - \left(\frac{F\psi}{2.3RT} + pH_b\right)\right)}} \tag{5}$$

where pK_I is the intrinsic interfacial pK of palmitic acid in the absence of surface electrostatic effects, F and R are the Faraday and gas

constants, respectively, and T is the Kelvin temperature.

By inserting Eqn. 5 into Eqn. 2, the final expression relating Δt_m to bulk pH is obtained

$$\Delta t_m = [PA] \cdot \left(a - \frac{(a-b)}{1 + 10^{\left(pK_I - \left(\frac{F\psi}{2.3RT} + pH_b \right) \right)}} \right) \quad (6)$$

The use of this equation to calculate Δt_m, requires a previous evaluation of the surface potential of the DPPC/palmitic acid vesicles. In a previous paper from this laboratory [23] it has been ascertained that the surface potential of liposomes can be accurately estimated using the Gouy-Chapman theory

$$\psi = \frac{2kT}{e} \sinh^{-1} \left(\frac{\sigma^2}{8\varepsilon_0 \varepsilon_r N c k T} \right) \quad (7)$$

k being the Boltzmann constant, e the electron charge, N the Avogadro's number, ε_r the aqueous dielectric constant, ε_0 the permittivity of free space, c the univalent salt concentration (0.010 M, i.e. 10 $mol \cdot m^{-3}$ in the SI system) and σ the electronic surface charge which will be given by

$$\sigma = \frac{-[PA] \alpha e}{100 A_m} \quad (8)$$

where [PA] is the molar percentage of fatty acid and A_m the lipid mean molecular area. In order to obtain ψ for a certain pH from Eqns. 5, 7 and 8, an iterative procedure, as suggested by Von Tscharner and Radda [9], was followed. First, the surface potential in Eqn. 5 is taken as zero; the value of α thus obtained is substituted in Eqn. 8 and with this σ, the surface potential is calculated according to Eqn. 7. This new value of ψ is inserted again in Eqn. 5 and the routine is repeated until no significant variation is observed between consecutive values of ψ. For the purpose of calculations, all quantities were expressed in SI units.

As shown in Fig. 3 the experimental results are fitted fairly well by Eqn. 6, assuming a value of 7.7 for the intrinsic interfacial pK (pK_I) of palmitic acid and either 45 or 65 Å^2 (i.e. 4.5 or $6.5 \cdot 10^{-19}$ m^2 in the SI system) for the mean molecular area of the lipids (Eqn. 8). These areas were chosen according to the following considerations. The lipid mean molecular area of dipalmitoylphosphatidylcholine has been determined to be 48 Å^2 in the gel phase and 70 Å^2 in the liquid-crystalline state [10]. If the area of a palmitic acid molecule is taken as 20 Å^2 [28] and if ideal behavior concerning the additivity of areas is assumed, lipid mean molecular areas of 45 and 65 Å^2, in the gel and liquid-crystalline state, respectively, can be calculated for the mixed lecithin vesicles containing 10 mol% fatty acid. Surface charge densities, and therefore, ψ, α and Δt_m, were computed for both extreme values of molecular areas, instead of taking an intermediate estimate for the area at the phase transition temperature. The theoretical curves obtained in both cases are very close (Fig. 3).

It can be noted that Eqn. 6 is an implicit expression for temperature and that the calculation of ψ according to Eqn. 7 requires not only the insertion of a certain value of T, but also of the aqueous dielectric constant corresponding to the selected temperature. However, no significant differences in the calculated Δt_m values are found even if T in Eqns. 5, 6 and 7 is varied from 312.15 to 323.15 K and ε_r in Eqn. 7 is taken for any of these temperatures. The range limited by the above mentioned temperatures includes all the experimental t_m values expressed in the Kelvin scale and is much braoder than the maximal experimental Δt_m. It should be mentioned that the calculated surface potential (Eqn. 7) does show a variation if T is modified from 312.15 to 323.15 K, the maximal change within the pH range studied being from -98.6 to -102.5 mV at pH 11.5 and for a lipid mean molecular area of 45 Å^2. This small change, however, is not significantly reflected in the Δt_m (Eqn. 6) which varies less than 0.01°C.

Discussion

A simple analysis of the bulk pH dependence of the t_m shift induced in dipalmitoylphosphatidylcholine liposomes by incorporation of palmitic acid, can be done by drawing a smooth line (not shown) through the experimental points of Fig. 3 and taking the pH_b at the midpoint of the curve, as the apparent pK of palmitic acid at the liposome/water interface. The apparent pK obtained

in this way is about 8.7, very similar to the value of 8.4 reported by Kantor and Prestegrad [18] from NMR titrations of egg phosphatidylcholine vesicles containing 16 mol% fatty acid. Ptak et al. [20] found an apparent pK between 7.2 and 7.4 when titrating, also by NMR, 3.3 mol% fatty acid incorporated to egg phosphatidylcholine liposomes. In this same study, a strong effect of the ionic strenth is described, such that for 5 mol% fatty acid in phosphatidylcholine liposomes, the apparent pK increases from 7.4 to about 8 when the phosphate buffer concentration is reduced from 100 to 1 mM. Such variation was to be expected since a more rigorous analysis [9] shows that the large shift of the apparent interfacial pK of fatty acids with respect to the pK of *n*-alkyl carboxylic acids in water (4.7–5.0) [29] includes a surface potential component. This electrostatic component has been taken into account in the theoretical analysis of our data. Best fit between theory (Eqn. 6) and experiments, is obtained when the intrinsic interfacial pK, pK_I, of palmitic acid, i.e. the interfacial pK in the absence of surface electrostatic effects, is set equal to 7.7, a value which is still very high as compared to the aqueous pK. On the basis of previous studies on the effect of the dielectric constant on the pK of cationic and molecular acids [24,30] one can assign this shift to the lower polarity of the interface as compared to bulk water. A similar point of view has been taken, on qualitative grounds, by other authors [9,20].

Depending on the medium in which the reaction proceeds, the dissociation of an acid into base and proton(s), is characterized by different pK values which have been thermodynamically analyzed by Fernández and Fromherz, to include interfacial dissociation processes [24]. Most of the following discussion on pK values is based on the theoretical considerations of that study. An acid-base dissociation occurring in water is characterized by pK_w, whereas for a reaction taking place in non-aqueous media or in mixtures of water and an organic solvent such as dioxane, the pK is denoted by pK_m. By increasing the proportion of organic solvent in those mixtures, the dielectric constant is reduced and the dissociation process is affected in different ways according to the nature of the acid: at low polarities the pK_m of cationic acids shows only a small decrement with respect to pK_w whereas for molecular acids pK_m is much higher than pK_w. A decrease in the dielectric constant is unfavorable for the dissociation of a molecular acid such as a carboxylic acid, since upon ionization ,two charges are generated. To illustrate this effect, we have plotted in Fig. 5, data taken from Harned and Owen [29] for the dissociation of propionic acid in mixtures of dioxane-water of different polarity. The continuous line representing the pK_m of propionic acid vs. the reciprocal of the dielectric constant ε of the solvent, shows that pK_m increases dramatically as ε is lowered.

As for the intrinsic interfacial pK, pK_I, of palmitic acid (Eqn. 6), it can be interpreted as corresponding to the dissociation of a membrane-bound acid into a membrane-bound base and a proton, in the water near the interface, i.e. in equilibrium with the surface concentration of protons [24]. Therefore, the dissociation can be considered as a two-phase reaction. This pK_I is different, not only from the pK_w characterizing a dissociation process taking place in water, but also from the pK_m of a reaction proceeding in homogeneous non-aqueous or partially non-aqueous solutions of low polarity.

In order to make a comparison of the high intrinsic pK_I of palmitic acid at the liposome surface, with the pK_m of a carboxylic acid such as propionic acid dissociating in homogeneous media of low polarity, the pK_m has to be transformed into pK_I. As described elsewhere [24] the transformation can be achieved by taking into account the work involved in the transfer of a proton from the low-polarity medium into water, i.e. by considering the degenerate activity coefficient of the proton ($f^0_{H^+}$) [31] in each dioxane-water mixture, according to the following equation

$$pK_I = pK_m - \log f^0_{H^+} \qquad (9)$$

To perform the calculations, the degenerate activity coefficient of the proton is approximated by the mean degenerate activity coefficient of HCl in the corresponding dioxane/water mixture [29]. In this way, we have obtained the dashed line of Fig. 5, representing the effect of the polarity on the hypothetical pK_I of propionic acid. If the pK_I of

palmitic acid at the liposome/water interface (7.7) is interpolated in this curve, a dielectric constant of 13 is obtained. Such interpolation procedure implies that the possible influence of the longer alkyl chain of palmitic acid as compared to propionic acid, on the pK_I of the carboxyl group, is disregarded. This assumption is justified by the findings of several authors indicating that the pK of *n*-alkyl carboxylic acids in water [32,33] or in organic solvents of low polarity such as ethoxyethanol [34] does not depend significantly on the chain length.

The effective polarity affecting the dissociation of palmitic acid at the liposome/water interface, obtained by interpolation from Fig. 5, would correspond to location of the free fatty acid carboxyl groups near the oxygen-rich carboxyl ester portions of the phospholipid bilayer, a region for which a dielectric constant between 10 and 30 has been estimated from capacitance and conductance measurements [35,36].

The effect of palmitic acid on the phase transition temperature of dipalmitoylphosphatidylcholine bilayers, has been attributed to steric factors. It has been proposed that the large trimethylammonium group of phosphatidylcholine makes it difficult for the acyl chains to pack as closely as the chains of the analogous phospholipid phosphatidylethanolamine, the amino group of which is devoid of methyl substituents [37]. This would explain why the t_m is 20°C higher for dipalmitoylphosphatidylethanolamine than for dipalmitoylphosphatidylcholine. The insertion of palmitic acid into the phosphatidylcholine bilayers, by relieving the crowding of the phospholipid polar groups, would allow a closer interaction of the acyl chains. As a consequence, the mixture shows an increased t_m as compared with the pure lecithin [4]. In view of our results demonstrating that the increase in the t_m of dipalmitoylphosphatidylcholine induced by palmitic acid, depends on the ionization degree of its carboxyl group, the above interpretation should be valid only for the fully protonated fatty acid. Our present work with DPPC/palmitic acid (9 : 1) dispersions, shows that the Δt_m of about 5°C measured at pH 5, decreases gradually as the pH is raised, until above pH 10, i.e. when the palmitic acid carboxyl group is almost fully ionized, a negative Δt_m of small magnitude (0.3°C) is obtained. It can be presumed that the closer packing of acyl chains promoted by the fatty acid through spacing of the voluminous phosphatidylcholine polar groups, is impaired by the repulsive effect resulting from the negative charge density generated in the bilayer surface, by ionization of the carboxyl group of palmitic acid.

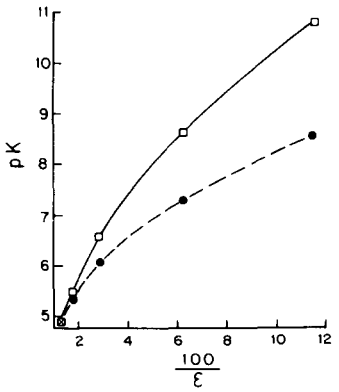

Fig. 5. pK of an *n*-alkyl carboxylic acid as a function of the reciprocal of the dielectric constant of the solvent. The experimental data, taken from Harned and Owen [29], correspond to dissociation of propionic acid at 40°C in dioxane/water mixtures containing 0, 20, 45, 70 and 82 wt% dioxane, giving dielectric constants of 73.28, 56.26, 35.35, 16.26 and 8.84, respectively. □, pK_m; ●, pK_I calculated from pK_m according to Eqn. 9; ⊠, pK_w.

It is interesting to mention that incorporation of very high concentrations of fatty acid, induces instability in the phospholipid bilayers [4]. In this regard, a thermotropic transition which takes place directly from the gel to the inverted hexagonal phase, has been described for mixtures of DPPC and 67 mol% palmitic acid [38]. Such concentration, however, is very far from the low fatty acid concentrations found in biological membranes [7,9].

Most studies on phase transitions induced by electrostatic effects [10–12], have been conducted using aqueous dispersions of a single, pure phospholipid, such that the modifications in pH or electrolyte concentration affect ionic groups which belong to the same molecule undergoing the phase change. In the present work, a mixture of lipids has been studied. The phase transition temperature of one of them, dipalmitoylphosphatidylcholine, is relatively insensitive to changes of

pH in the range from 5 to 11. The incorporation into the bilayers of a second lipid, palmitic acid, generates a system showing a strong pH dependence of its phase transition temperature, the pH sensitive element being the carboxyl group of the fatty acid (Fig. 2). Several years ago, Fromherz [39] defined a 'lipid assembly' as a mixture of lipids showing properties not found with the pure lipid components. The DPPC/palmitic acid lamellar system could be considered as a 'lipid assembly', at least concerning the sensitivity of its phase transition temperature to pH changes, which is developed upon mixing the pure lipids (Fig. 2).

It has been suggested that fatty acids can work like membrane surface buffers in the physiological range because of their ability to generate changes in surface charge upon modification of the pH [9]. This capacity, coupled to the ability of palmitic acid of inducing pH-dependent phase transitions in phosphatidylcholine bilayers, could reveal this fatty acid as an exquisite modulator of phase changes in biomembranes.

Triggering of lipid phase transitions at constant temperature by changes in pH or salt concentration, can be considered of possible relevance of homeothermal organisms [11]. We have shown that by appropriate combination of lipids, a system transducing the variation of a pH signal into phase changes, can be generated (Fig. 2). Whether similar transduction mechanisms play any role in physiological phenomena, remains to be explored.

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