# Lateral proton conduction in mixed monolayers of phosphatidylethanolamine and cetyltrimethylammonium bromide

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# Lateral Proton Conduction in Mixed Monolayers of Phosphatidylethanolamine and Cetyltrimethylammonium Bromide

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ABSTRACT: Proton conduction is known to be facilitated along phospholipid monolayers spread on aqueous phases. This property was monitored with mixed cetyltrimethylammonium bromide/phosphatidylethanolamine monolayers. The film was shown to be metastable by surface pressure and fluorescence measurements. The detergent was leaving the interface for the bulk phase. Nevertheless, a fraction of the detergent remained in the lipid matrix, as shown by the binding of the fluorescent probe 8-anilino-1-naphthalenesulfonate. Its dissociation constant decreased, and the nature of its binding site was affected, as shown by a shift of its emission spectrum. Apart from film expansion, the properties of the film were affected only at the water/membrane interface. Proton conduction was prevented only when the surface concentration of the detergent was larger than a critical value. Such an effect could be due either to the disruption in the continuity of the conducting hydrogen-bond network or to an electrostatic repulsion of the protons by the interface.

Lateral conduction of protons along lipid monolayers has been observed by using various methodologies (Teissié & Tocanne, 1990). They can be either direct ones through the observations of the local concentrations of protons at the interface by fluorescence (Teissié et al., 1985), or surface potential (Prats et al., 1986) measurements, or more indirectly through the associated changes in surface pressure (Prats et al., 1987b) and the increase in surface conductance (Sakurai & Kawamura, 1987; Morgan et al., 1988). The process is not at the present time well-characterized on bilayered systems, and conflicting conclusions have been reported by one group where a rather complicated set of equations was solved on a computer in order to explain the experimental fact (Nachliel & Gutman, 1988).

All previous studies were performed on pure lipid systems with only one class of compounds at a time. The main conclusions were that the phenomenon was a diffusion along the interface, presumably through a hydrogen-bond network involving the polar head groups and the hydration layer (Prats et al., 1987a, 1989; Morgan et al., 1988; Teissié et al., 1990). The chemical nature of the polar group of the phospholipid did not play a role, but the state of the monolayer must be liquid (Prats et al., 1987a). In order for diffusion to occur, a decisive property of the monolayer was the need for continuity in the hydrogen-bond network. This was indicated by the collapse of the conduction for very expanded films where

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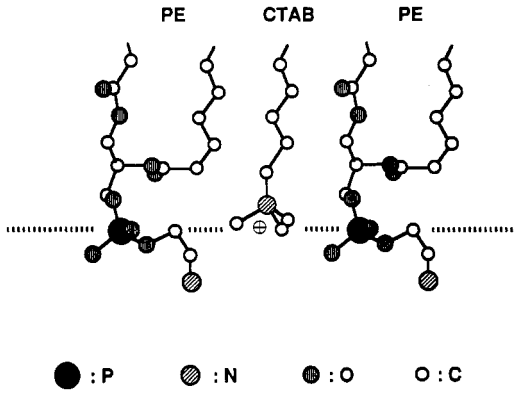


FIGURE 1: Molecular structure of CTAB and of the polar region of PE.

the surface potential was nil (Teissié et al., 1985) or when a structural configurational change was triggered upon compression (Teissié et al., 1990).

The phenomenon was supposed to occur in three steps: adsorption of protons from the bulk phase to the interface, diffusion along the interface, and leakage back to the bulk phase. The last process must be of minor amplitude in order to allow long-range diffusion to be observed but was responsible for the occurrence of a very steep pH gradient perpendicular to the interface (Prats et al., 1986).

In order to get more information on the phenomenon and on its relevance to more biological events, the use of more complicated systems than a pure single lipid was needed. Surface charges should modulate such conduction by changing the electrical properties of the interface and, as shown by recent NMR studies, the local conformations of the polar head regions (Seelig & MacDonald, 1987). This can be obtained through the insertion of charged amphiphilic molecules such as fatty amines in the lipid monolayers. Such mixed films can be obtained by the cospreading of the two molecules at the air/water interface.

This was the purpose of the present study. As it was previously observed that negatively charged phospholipids supported proton conduction, we have focused our study on the influence of a positively charged amine, cetyltrimethylammonium bromide (CTAB), on the conducting properties of phosphatidylethanolamine (PE) monolayers (Figure 1). This lipid was chosen because it does not show a phase transition, being a natural product purified from *Escherichia coli* (Gottschalk, 1986). The mixed monolayer was first characterized in reference to the pure lipid system. The presence of the amine was shown by the change of the interfacial properties of the film but was proved not to affect the organization and the dynamics of the lipid matrix too much. Proton conduction was observed to be prevented when a high level of amines was present in the film, confirming per se the interfacial character of the conductance. A change in the chemical composition of the monolayer while keeping its structural and dynamic properties unchanged was enough to prevent the lateral proton conduction.

## Materials and Methods

*Chemicals.* Phosphatidylethanolamine (PE) from *E. coli*, 12-(9-anthroyloxy)stearic acid (12-9-AS) and cetyltrimethylammonium bromide (CTAB) were obtained from Sigma. 8-Anilino-1-naphthalenesulfonate (magnesium salt) (ANS) was purchased from Kodak.

Synthesis of the pH fluorescent indicator probe fluorescein phosphatidylethanolaminothiocarbamide (FPE) was described previously (Soucaille et al., 1988). Salts were analytical grade. Ultrapure water, free from surfactant, was prepared with a Milli-Q system (Millipore, France).

*Monolayer Preparation.* Buffered saline solutions were prepared with ultrapure water. Lipid/detergent mixtures were spread from solution in chloroform/methanol (5:1, v/v) and a 5–15 min period was observed to allow for solvent evaporation. The film surface pressure was monitored by means of a platinum plate (Prolabo, France) connected to a force transducer of our own construction. The sensitivity of the surface pressure determination exceeded 0.2 mN/m. The temperature was 20 °C (±0.5 °C).

*Fluorescence Measurements.* An interface fluorimeter constructed in the laboratory was used, in which front-face fluorescence was monitored (Teissié et al., 1976; Teissié, 1979a). Emission from a small illuminated area (about 2 mm in radius) was measured for differing compression states of the monolayer. The trough was milled in Plexiglas in order to maintain a degree of low light scattering. Compression was obtained by moving a teflon barrier in order to change the total surface area of the monolayer. Excitation wavelengths were selected by means of optical filters. The fluorescence intensity was measured by means of a photomultiplier tube (EMI 9558, England) connected to a data acquisition unit.

Photobleaching experiments were carried out as follows (Teissié et al., 1978). The background signal, due to the light scattered by the subphase, was set at zero fluorescence electronically. Then a mixture of probe [12-(9-anthroyloxy)stearic acid] and phospholipid/detergent in a chloroform/methanol mixture (5:1 v/v) was spread at the air/water interface in the dark to avoid photodegradation. The probe-to-PE molar ratio was 2:98. After an equilibration period of 5–15 min to allow for complete solvent evaporation, the film was then compressed to a given pressure and allowed to settle for an additional 5 min. The optical shutter was then opened, and the decrease in fluorescence intensity resulting from the dimerization reaction was recorded. After a 30-s bleaching period, the shutter was closed, and recovery was detected by observing the fluorescence signal associated to the irradiation of the monolayer for 1 s at differing time lags following the bleaching reactions. The film was then compressed to a new value of the surface pressure, and the bleaching procedure was repeated.

As the surface concentration of the probe increased with compression of the monolayer, the intensity was expressed as the "reduced intensity" $I_f$, which is proportional to the fluorescence emitted by each probe molecule. We have previously shown that the reduced intensity is obtained as the product of the observed intensity multiplied by the molecular area of the lipid matrix (for a given probe to lipid molar ratio) (Teissié et al., 1976). $I_f$ is a linear function of the quantum yield of the chromophore and is dependent on the nature of the environment (Franck Codon effect).

The dimerization reaction of the 12-(9-anthroyloxy)stearic acid is second order in respect to the probe. As the dimer is nonfluorescent and as the fluorescence intensity of the monomer is linearly related to its concentration, the extent of the reaction is obtained from the decay in fluorescence emission during illumination. The rate constant of dimerization $K_d$ is obtained by plotting the reciprocal of the fluorescence intensity versus the time of illumination during the early steps of the photoreaction. In control experiments, no deviation from linearity, reflecting occurrence of a recovery process during bleaching, was detected. $K_d$ was shown to relate to spectroscopic and structural contributions (Theretz et al., 1984). The former are direct functions of the reduced fluorescence intensity $I_f$, and the latter $K_{DS}$ reflect the influence of both the

structure and the dynamics of the probe environment.

After photoreaction, the local concentration of monomers in the previously illuminated area is lower than in the nonbleached surface. This concentration gradient then drives the diffusion of fluorescent monomers into the bleached zone. The extent of recovery of fluorescent monomers relates directly to the lateral diffusion coefficient $D$ of the probe, which is under the control of the order parameter of the lipid matrix (Teissié et al., 1978). Recovery experiments were analyzed by use of a mathematical approach adapted for uniform disk illumination (Teissié et al., 1978) after statistical analysis taking the nonlinear relationship between the extent of recovery and its duration into account (Denicourt et al., 1987).

*Proton Diffusion Experiments.* Proton lateral diffusion experiments were run with the proton "window" jump technique using a trough and an experimental procedure previously described (Teissié et al., 1985). Monolayers were obtained by spreading a mixture of phospholipid, detergent, and FPE (PE/FPE molar ratio 98:2) in solution in $CHCl_3$/MeOH (5:1, v/v) onto an aqueous subphase (1 mM phosphate buffer at a well-defined pH). The movement of protons from the injection compartment to the fluorescence observation area was observed by a change in fluorescence emission in the pH-sensitive fluorescent chromophore (FPE at the lipid/water interface). This proton lateral diffusion is described by two parameters: $T_{H^+}$, the time between the acid injection and the beginning of the decrease in fluorescence, and $\Delta F$, the amplitude of this decrease (Teissié et al., 1985).

*Determination of the Apparent pK ($pK_{app}$).* $pK_{app}$ was taken as the subphase pH at which the fluorescence $F$ emitted by the film for a given surface pressure $\Pi$ obeyed the relationship

$$\frac{F(pK_{app}, \Pi) - F(\text{pH } 4, \Pi)}{F(\text{pH } 7.5, \Pi) - F(pK_{app}, \Pi)} = 1$$

This operational definition was based on the observation that the fluorescence was not related in any obvious way to the subphase pH for values of pH outside the range 4.5–7.2. It was obtained by compressing films on subphases with differing pH values and recording the fluorescence intensity and the surface pressure in relation to the molecular area (Soucaille et al., 1988).

*ANS Binding Isotherms.* 8-Anilino-1-naphthalenesulfonate is known to display a strong increase in fluorescence yield and a large blue-shift in the emission spectrum when bound to a structured and/or hydrophobic environment (Slavik, 1982). The monitoring of its interaction is thus easy. The binding is mainly due to hydrophobic forces but is modulated by electrostatic forces (McLaughlin & Harary, 1976; Teissié, 1979, 1987). When the dye was present in the subphase, the detected signal was the sum of the scattered light, the fluorescence from the subphase, and the fluorescence of the bound amphiphile. The scattered light and the fluorescence of the aqueous dispersed probe were first measured before spreading the film. This signal was very reproducible and was the baseline in the evaluation of the binding isotherms. It was observed to increase linearly with the probe concentration up to 20 $\mu$M. From the changes in the emission in the bound probe upon compression, which were evaluated for different concentrations, the dissociation constant $K_{diss}$ and the saturating emission $I_\infty$ were computed. As the number of binding sites was always small, the concentration of dye in the subphase was not really affected. $K_{diss}$ and $I_\infty$ were then obtained from the reciprocal plotting of the fluorescence of the bound probe versus the ANS concentration. Emission shifts were evaluated from the ratio of the emission at 476 nm ($\Delta\lambda$ = 10 nm) to the emission at 498 nm ($\Delta\lambda$ = 10 nm). An increase was indicative of a blue-shift (Teissié et al., 1976).

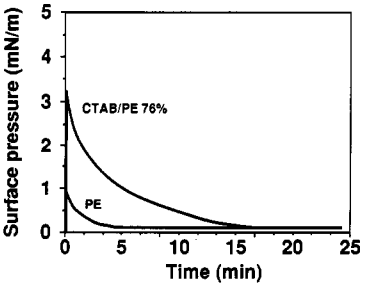


FIGURE 2: Changes in the surface pressure of the film after spreading the solution. (Lower curve) Pure PE film. (Upper curve) CTAB/PE mixture (spreading molar ratio 76%).

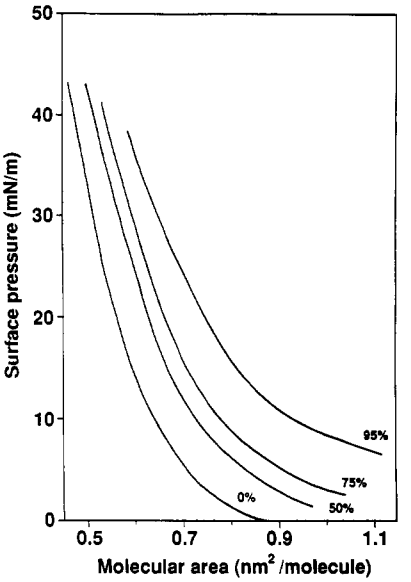


FIGURE 3: Compression isotherms of mixed films. Detergent/phospholipid mixtures were spread at the indicated relative content in CTAB. Each compression isotherm has been reproduced three times.

## Results

*Surface Pressure Changes of the Film.* Spreading a mixture of the detergent and of the lipid gives the formation of a metastable film. The surface pressure of the monolayer was observed to strongly decrease during the stabilization period, which was observed in order to obtain good evaporation of the solvent (Figure 2). This decrease was many times larger than that classically observed with pure lipid films. Its amplitude was larger when films with a higher content in CTAB were spread, suggesting that the presence of the detergent was clearly involved in this metastability. After waiting for a period that depended on the detergent-to-lipid ratio, almost constant surface pressure was obtained.

If a film spread at an initially low surface pressure was compressed, the compression isotherms were observed to be dependent on the detergent-to-lipid spreading molar ratio (Figure 3). The higher the molar ratio in CTAB was, the more the film expanded. Nevertheless, at high packing densities, the surface pressures were closer to each other whatever the initial composition of the film.

All this experimental evidence strongly suggested that CTAB was present in the film, but the stability of the complex was clearly dependent on the packing density of the film.

*Surface Fluorescence of FPE.* Being a phospholipid, the pH reporter probe was assumed to remain at the air/water

Table I: Spectroscopic and Dynamic Parameters of Different Mixtures of PE and CTAB Spread as Monolayers Probed by 12-9-AS[a]

| | $I_{f_{AS}}$ ($\times 10^{13}$ mV·cm$^2$·mol$^{-1}$) | $D$ diffusion coefficient (cm$^2$·s$^{-1}$) | $K_D$ dimerization constant ($\times 10^{14}$ cm$^2$·mol$^{-1}$·min$^{-1}$) | $K_{DS}$ structural dimerization constant (mV$^{-1}$·min$^{-1}$) |
|---|---|---|---|---|
| PE | 4.87 ± 0.12 | $1.5 \pm 0.2 \times 10^{-4}$ | 7.32 ± 0.47 | 16.7 ± 1.4 |
| CTAB/PE (50%) | 6.53 ± 0.14 | $5.2 \pm 1 \times 10^{-5}$ | 10.5 ± 0.5 | 15.0 ± 0.6 |
| CTAB/PE (83%) | 6.69 ± 0.96 | $2.7 \pm 1.6 \times 10^{-5}$ | 21.1 ● 3.1 | 37.7 ± 7.8 |

[a] The film pressure was always set at 5 mN/m. The different parameters were obtained by photodimerization and FRAP experiments. Each value is the mean of three different determinations (±SD).

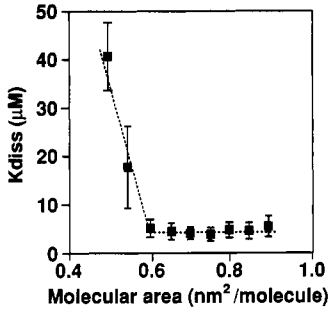


FIGURE 4: Dissociation constant of ANS binding to a pure PE film in relation to the film packing. The subphase was 10 mM NaCl and 1 mM phosphate buffer, pH 6.8. Each point is the mean value of three different determinations (± SD).

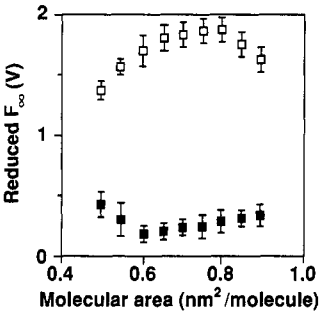


FIGURE 5: Saturating emission of bound ANS to a mixed film in relation to its packing. The CTAB/PE mixed film was spread at a CTAB relative content of 83% (□). A 3 μM concentration in ANS is observed to be saturating. The saturating emission is obtained by extrapolation of the binding isotherms to infinite ANS concentration for the pure PE monolayer (■). It is indicative of the number of ANS-binding sites in the mixed film. The subphase was 10 mM NaCl and 1 mM phosphate buffer, pH 6.8.

interface. Its density can be easily monitored from the fluorescence intensity changes for the differing conditions of spreading or of compression. The observed behavior of the emission was similar to what we previously got when dealing with pure PE host monolayers (Soucaille et al., 1988). This is indicative that there are not major alterations of the lipids such as a detergent-induced solubilization. $pK_{app}$ values were not strongly affected by the insertion of the detergent in the lipid matrix and were close to 5.6.

*ANS Binding.* The binding of the fluorescent amphiphile was strongly affected by the presence of the detergent in the lipid monolayer. The dissociation constant $K_{diss}$ greatly decreased down to less than 3 μM and kept this value whatever the packing of the film. The constant for the pure PE film was observed to increase dramatically for tightly packed films (Figure 4). The behavior of the pure PE film is relevent to our previous analysis of the interaction of another fluorescent amphiphilic probe, *N*-phenylnaphthylamine (NPN) (Teissié, 1990). The dissociation constant was observed to be constant for loosely packed films but to increase strongly when the film pressure was larger than 15–20 mN/m. It was suggested that it might be associated with a reorganization of the matrix, changing its entropy. Such a change is either not present in the CTAB-PE film or, if present, would not affect the structure of the ANS-binding site enough to be detected. This site is apparently different from what it is in a pure PE monolayer. This conclusion is supported by the red-shift in the emission of bound ANS when CTAB is present (data not shown) and by the change in the total number of binding sites (Figure 5).

The most important conclusion of this binding assay is the evidence that CTAB is present in the lipid monolayer and strongly affects its interfacial properties. This is valid whatever the packing density of the film. CTAB might be partly expelled from the matrix upon compression, but some molecules remain present nevertheless.

*Structural and Dynamic Investigations of the Films.* Lateral proton conduction along phospholipid monolayers is known to be abolished when the film is brought to the solid condensed state (Prats et al., 1978a). Such an effect was observed with conductance by another research group, but, in their conclusions, the authors missed that point (Menger et al., 1989). It is then of major importance when dealing with such processes to check if the modifications of the film do not bring it to a gel-like state. Photochemistry of 12-(9-anthroyloxy)stearic acid is a powerful tool in the analysis of such phase transitions in monolayers (Teissié et al., 1978; Denicourt et al., 1987). 12-9-AS is dimerized under UV illumination. The reduced fluorescence $I_{f_{AS}}$ is indicative of the environment of the probe. The structural dimerization constant $K_{DS}$ is controlled by the organization of the matrix. The diffusion coefficient $D$ (obtained through FRAP experiments) is directly related to the order parameter of the matrix, i.e., the so-called fluidity. This information is, of course, relevent to the localization of the fluorescent moiety, i.e., at the level of $C_{12}$.

Results are shown in Table I. Increases, both in $I_{f_{AS}}$ and in $K_{DS}$, are detected when CTAB is present in the matrix. The lateral diffusion coefficient $D$ is decreased by the presence of detergent. As $K_{DS}$ increased and $D$ decreases with an increase in the detergent-to-lipid ratio, this is indicative of an increase in the matrix order when CTAB is inserted, order increases when more detergent molecules are present. Nevertheless, the mobility of the probe is still very high, showing that in any case the matrix remains fluid.

The noticeable modifications of the structure and dynamics of the film are further pieces of experimental evidence that CTAB is indeed present in the interfacial film. PE from *E. coli* is known to be highly unsaturated (Gottschalk, 1986), and the free volume between the chains is going to be very high as shown by the amazingly high value of $D$. The insertion of CTAB between the phospholipid molecules would either decrease this free volume if freely dispersed or hinder the diffusion by an archipelago phenomena if a molecular aggregation is present (Saxton, 1987).

*Lateral Proton Conduction Along the Monolayer.* Proton conduction along the mixed monolayer at a 5 mN/m surface pressure was observed when the spreading molar ratio in

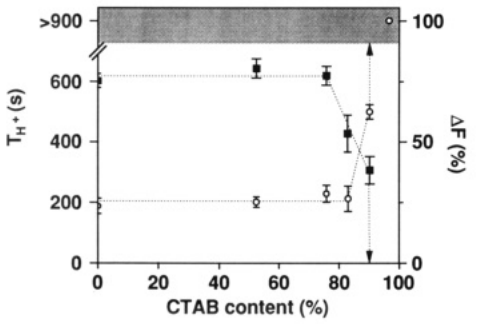


FIGURE 6: Proton-facilitated conduction along a mixed film in relation to the CTAB relative content. The subphase was 1 mM phosphate buffer, pH 6.8. The surface pressure was set at 5 mN/m. The time lag $T_{H^+}$ (○) and the relative amplitude of the fluorescence change (■) are shown. The gray area at the top of the graph represents a time domain where it is considered that no facilitated conduction along the interface takes place. Each point is the mean value of three to six different determinations (±SD).

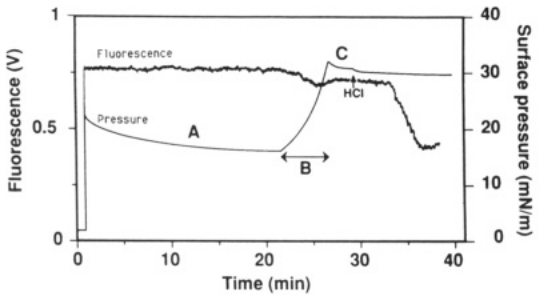


FIGURE 7: Change in surface pressure and in proton conduction of a mixed film. The spreading molar ratio was 96%. After surface pressure stabilization, the film was not in a state able to support proton conduction (surface pressure of 15 mN/m) (A). The film was then compressed up to 30 mN/m (B). Film relaxation was then observed with a small surface pressure relaxation (C). In this new state, proton-facilitated conduction was observed.

CTAB was kept low, as shown in Figure 6. $T_{H^+}$ was always of the same range as observed with pure PE monolayers, and $\Delta F$ kept a constant value. This is similar to all the previous observations with other phospholipids. A dramatic change was observed when the molar ratio was larger than 80%. $T_{H^+}$ was then observed to increase, and concomitantly $\Delta F$ was observed to decrease (Figure 6). Furthermore, when working with a film that was spread at a 96% molar ratio, no facilitated conduction was observed, i.e., $T_{H^+}$ was larger than 900 s. Proton conduction is then controlled by the composition of the film.

A striking feature of the behavior of the mixed film was that it was possible to reinduce the proton conduction in a nonconducting film (i.e., spread at a 96% molar ratio as described just above) by compression (Figure 7). But this was obtained only when the film was compressed up to a surface pressure larger than 25 mN/m, the detergent-to-lipid ratio during spreading being 96%. This recovery in conduction was obtained whatever the initial packing of the film (if less than 25 mN/m of course). Conduction is observed with a slight increase in $T_{H^+}$ but with a large decrease in $\Delta F$ as compared to the plateau value present in Figure 6 (data not shown).

## Discussion

A basic feature of the detergent/phospholipid mixed film was its metastability after spreading and upon compression. The simplest interpretation is to assume that the detergent leaves the interface by a desorption mechanism, a process that does not affect the phospholipids, PE as well as FPE. This can be described in a reaction

$$(\text{Det, PL})_{\text{spread}} \rightarrow (\text{Det, PL})_{\text{film}} + \text{Det}_{\text{bulk}} \quad (1)$$

where Det is CTAB and PL the PE/FPE mixture.

The evidence that PL always stays at the interface is our observation of the change in fluorescence intensity of FPE. This is supported by the observation that phospholipids, when pure and when spread under similar conditions, remain at the air/water interface. From our experiments (Figure 2), we should conclude that the reaction in eq 1 is slow (the time scale in the order of several minutes). It can be monitored by the change in the surface pressure of the film, but one should take into account that it is not quantitatively reliable due to the positive charges of the detergents, which are prone to alter the wetting angle of the plate.

Equation 1 would depend on the relative ratio between spread lipid and detergent molecules such as

$$(m\text{PL } p\text{Det})_{\text{spread}} \rightarrow (m\text{PL, } n\text{Det})_{\text{film}} + (p - n)\text{Det}_{\text{bulk}} \quad (2)$$

where $m$ is the number of phospholipid molecules, $p$ is the number of spread CTAB molecules, and $n$ is the number of those remaining at the interface. From our observations, we can conclude that, for a given film total area (spreading conditions), for a given $m$, $n$ will depend on $p$, the derivative of $n$ versus $p$ being positive. If $m$ is increased, $p$ being kept constant, $n$ will decrease. If the film is compressed, $n$ will decrease. In other words, the results in Figure 3 indicate that the detergent is partly expelled from the film under compression. Nevertheless, the compression isotherms as well as the results on the binding isotherms of ANS to the monolayers all show that, even when the film is tightly compressed, a fraction of the spread CTAB molecules is always present at the interface.

A rough estimate of the number of CTAB molecules present at the interface can be obtained from the film expansion and from eq 2. The major drawback of this approach is, as written above, the reliability of the plate to measure the pressure with a positively charged film. We make the assumption that CTAB molecular area is that of a single fatty acid chain, i.e., 0.2 nm$^2$. We get

$$\text{Area}(\text{PL},n\text{Det})\pi - \text{Area}(\text{PL})\pi = 0.2n \quad (3)$$

$\pi$ being the film pressure.

Using equation 3 and the differing compression isotherms in Figure 3, this approach shows that for a surface pressure of 5 mN/m, when the molar ratio of spread molecules is 75%, $n$ should be equal to 1. For a ratio of 95%, $n$ must be between 2 and 3. This means that under these conditions the hexagonal matrix of phospholipid molecules is strongly altered (Figure 8C).

From the data on the photodimerization of 12-9 AS, it is clear that the presence of CTAB in the lipid matrix does not bring many changes in the hydrocarbon layer, except the expansion of the film. The increase in $K_{DS}$ and the decrease in $D$ are indicative of an increase in matrix order, but no drastic change as in the case of a phase transition is present. The binding of ANS indicates that a dramatic alteration of the interface has been induced by the insertion of CTAB. The binding affinity is strongly affected as was expected due to the change in the electrical surface charge. A dramatic modification of the properties of the binding sites is observed through a red-shift of the emission of the bound probe. As a conclusion, the main effect of the insertion of CTAB in the lipid matrix is a modification of the interfacial properties. Such a change is detected even under conditions where only 1 CTAB molecule is present per phospholipid (i.e., with a spreading ratio of 75%) and where the lateral conduction is not affected by the insertion of the detergent (Figure 6).

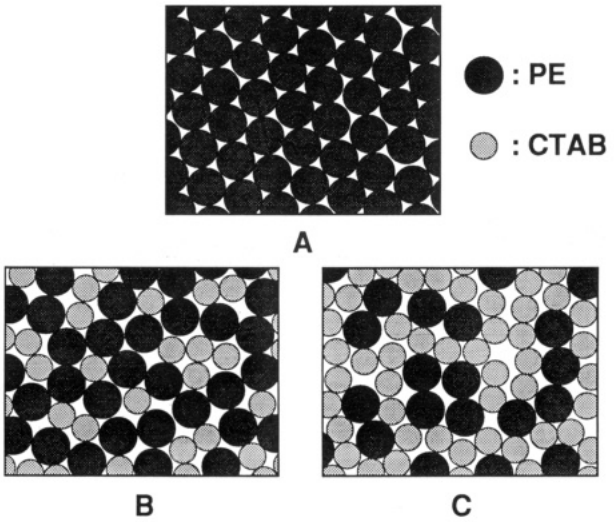


FIGURE 8: Change in the structure of the hexagonal matrix of lipids with an increase in the CTAB relative content. PE molecules are supposed to build a hexagonal matrix in a monolayer (A). A continuity between PE molecules is present at a low CTAB content (B) but disappears when three detergent molecules are present per PE molecules (C).

The proton lateral conduction is prevented for critical values of $n/m$. At a surface pressure of 5 mN/m, we observed that this critical value is between 2 and 3. At the present state of knowledge, this may be due to two processes. First, insertion of detergent molecules in the lipid matrix is shown to affect its interfacial properties and as such is prone to disrupt the hydrogen-bond network associating polar head groups and interfacial water molecules. We proposed that a decisive role was played by the network in supporting the "hop and turn" process carrying the movement of protons. Destruction of the network or only of its continuity will prevent the conduction. Second, insertion of positively charged CTAB molecules will definitively give a change in the electric charge density of the interface. Electrostatic repulsion will then modulate the access of protons to the interface in the injection compartment of the trough as we already observed under other conditions in a previous study (Prats et al., 1985). This electrical repulsion will of course increase with an increase in $n$, and a critical value will prevent the feeding of the interface with protons. The observation that conduction can be reobtained by a small compression of the matrix is indicative that a very subtle change in packing is enough to bring the film back to the conducting state. As shown in Figure 3, the film expansion for a pressure larger than 25 mN/m between a 96% mixed film and a pure PE monolayer is less than 0.2 $nm^2$, i.e., less than the molecular area of a CTAB molecule. Conduction is thus present when there is less than one detergent molecule per lipid molecule in a packed film, as predicted in Figure 8. But for a film of similar composition but with looser packing (surface pressure between 7 and 25 mN/m), we observed in Figure 3 that between 1 and 2 CTAB molecules are present per PE molecule. No conduction is detected. From Figure 8B, we must conclude that as the $n/m$ ratio is less than 2, continuity between the PE molecules is present. The conclusion of these observations is that the stopping of the facilitated lateral proton conduction is associated with an electrostatic repulsion due to an interfacial CTAB density larger than a critical value.

A final conclusion of this work is to demonstrate that changing the nature of the interface is enough to prevent the facilitated proton conduction along it. All other parameters are unchanged. This definitely proves the interfacial character of the proton movement and rules out the comment that it might be due to a bulk phase convection (Kasianowicz et al., 1987).

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