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Proton transport at the monolayer-water interface

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It is shown that when monolayers of stearic acid, palmitic acid, DPPC, or DPPS are compressed above some critical area A_c a lateral conduction mechanism is initiated at the monolayer/water interface. The interfacial conductance increases on further increasing the molecular packing density in the monolayer. All compounds also show major changes in surface potential at A_c the potential becoming more positive in all cases. It is argued that this is a consequence of structural reorganisation at the headgroup/water interface causing a significant reduction in the local permittivity. The critical area, A_c, is approximately double the molecular areas estimated from the pressure-area isotherm, and experiments with stearic acid monolayers show that A_c decreases significantly when the chaotropic ion SCN^-, which is known to disrupt the molecular structure of water, is added to the subphase. It is likely, therefore, that the structural changes occurring at A_c involve the formation of a hydrogen bonded network between monolayer headgroups and adjacent water molecules at the monolayer/water interface. It is suggested that the conduction mechanism initiated at A_c arises from proton hopping along this hydrogen-bond network.

1. Introduction

Phospholipid monolayers serve as useful, easy-to-study models of cell membranes [1], and a variety of monolayer properties relevant to biological processes have been investigated, e.g., lateral diffusion of proteins [2] and binding of anaesthetics [3].

Of relevance to the present work are the attempts to demonstrate experimentally that proton transport can occur at a phospholipid monolayer/water interface, thus supporting the hypothesis of localised chemiosmosis in which it is assumed that membrane-bound proton pumps are linked by proton fluxes localised at or near the membrane surface [4]. In this respect, the fluorescence experiments reported by Teissié's group in Toulouse [5–8] are of great interest. These were performed by injecting acid into the aqueous subphase below a monolayer and following the diffusion of the injected protons through the subphase and along the monolayer/water interface using pH sensitive fluorescent probes. Experiments with a number of different phospholipid and glycolipid monolayers appeared to confirm that proton transport readily occurred along the monolayer/water interface. Furthermore, the measurements indicated that proton diffusion rates were about 20 times faster along the interface of a condensed monolayer than through the bulk subphase. It was suggested that this rapid proton transport occurred via a 'hop and turn' mechanism along a bidimensional hydrogen-bond network formed between the lipid headgroups and neighbouring water molecules.

In a recent paper, Kasianowicz et al. [9] questioned the experimental technique of Teissié's group and concluded that the fast interfacial proton diffusion was an experimental artefact. They argued [10] that because the subphase was stirred during the addition of acid, a convective flow of lipid molecules was produced in the monolayer which in turn caused a drag effect on the unstirred aqueous layer at the interface. Since these layers also contained a high proportion of the injected acid, they concluded that the observation of rapid proton transport was inevitable.

Teissié's group [11] has refuted this criticism but was unable to convince Kasianowicz et al. who continue to believe [10] that experimental artefacts were responsible for the effects observed. Although their arguments seem plausible, Kasianowicz et al. appear to neglect two important experimental facts.

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(a) Rapid proton transport is observed only above a critical packing density. At low packing densities no effect was observed. However, when the monolayer was compressed to a surface pressure of 25 mN/m interfacial proton transport was clearly present. At this elevated surface pressure the greater viscosity of the condensed monolayer makes it less likely that stirring of the aqueous subphase will give rise to convective flow in the monolayer and the adjacent unstirred layer.

(b) Fluorescent probes attached to the monolayer detected the arrival of injected protons far earlier than probes in the aqueous subphase. This is clear evidence for enhanced proton diffusion at the interface and is further supported by the observation that calcium ions strongly influence the interfacial diffusion process.

In an independent study [12,13], we have measured directly the electrical current flow along the interface between a condensed monolayer and the aqueous subphase. The evidence from our work support the findings of Teissie's group, the electrical measurements showing clearly that an interfacial charge transport mechanism is induced when a monolayer is compressed below a critical packing density. Increasing the packing density above the critical value causes the measured conductance to increase steadily.

Although the induced conduction was highest for negatively charged DPPA and stearic acid monolayers, nevertheless, we were able to eliminate the excess ion concentration associated with the Gouy-Chapman double-layer as the source of this enhanced conductivity [14]. The degree of dissociation, α, of a fatty acid monolayer on ultrapure water is so low ($\alpha = 2 \cdot 10^{-3}$) that the total charge in the double-layer would be insufficient to support the extra current flow observed. Furthermore, the zwitterionic lipid dipalmitoylphosphatidylethanolamine (DPPE) investigated in the earlier work was electrically neutral at the pH of our experiments so that a conduction mechanism based on diffusion through the double-layer is implausible for this lipid.

We have now extended our studies to palmitic acid and to two other phospholipids viz. the dipalmitoyl homologues of phosphatidylcholine (DPPC) and phosphatidylserine (DPPS). In these latest experiments we confirm that the interfacial conductance is common to a range of different monolayers. Further, we show that a strong correlation exists between the conductance behaviour and the surface potential of monolayers. Finally, we show that chaotropic ions, which are believed to disrupt the molecular organisation in bulk water [15,16], have a significant effect on the surface potential-area (ΔV-A) curves of stearic acid monolayers.

2. Materials and Methods

DPPC and DPPS were obtained from Koch Light Ltd. Palmitic acid and stearic acid were obtained from Sigma Chemical Co. Ltd. All samples were quoted as better than 99% pure and were used without further purification. Palmitic and stearic acids were dissolved in chloroform (approx. 1 mg/ml) and the lipids in a 1:4 (v/v) mixture of chloroform and methanol (approx. 1 mg/ml). All solvents used were of HPLC grade.

Monolayer experiments were performed on a PTFE trough ($60 \times 26 \times 2$ cm) located on a thermostatically controlled metal base plate placed on an antivibration table and housed in a Class 2 Semiconductor Clean Room. Prior to use, the trough was cleaned by rinsing in hot chloroform, followed by repeated rinses in hot and then room temperature ultrapure water. The ultrapure water for cleaning the apparatus and for the subphase was obtained from a Millipore R060 reverse osmosis system, coupled to Super-Q polishing filters comprising ion exchange, organex and 0.2 μm ultra filtration cartridges. When freshly drawn, the pH of this water was 7.0, but fell to 5.6 after standing in the trough owing to the absorption of atmospheric CO_2. At this pH DPPC is electrically neutral but zwitterionic, stearic and palmitic acids are negatively charged and DPPS is negatively charged but also zwitterionic. Solutions of NaCl (Gold Label) and NaSCN (AnalaR) were prepared with ultrapure water as required.

Using an automated data acquisition system, surface pressure and either the surface potential or lateral conductance were recorded simultaneously as continuous functions of area per molecule. Monolayer surface pressure was measured with a Wilhelmy plate and electrobalance to an accuracy of 0.1 mN/m, whilst surface potential measurements made with a Kelvin probe were accurate to ± 10 mV. The experimental arrangement for measuring lateral conductance has been described previously [12,13] so only a brief description is required here.

Two measuring electrodes made from 25-mm wide bright platinum plates were held parallel to each other at a separation of 20 mm and their bottom edges immersed to a depth of 2–4 mm in the subphase. A constant voltage of 1.5 V was applied across the electrodes and the resulting current flow measured with a Keithley electrometer, model 616. The electrometer output was passed to a 12 bit ADC. This enabled the small currents associated with the monolayer to be identified above the larger background current which flows through the bulk aqueous phase. Although only the first ten bits were reliable, nevertheless, interface currents less than 0.1% of the bulk subphase currents could easily be detected and measured.

In a typical experimental sequence, the trough was filled with ultrapure water and the surface cleaned by aspiration. The electrodes, previously washed by sonication in hot chloroform followed by boiling in ultrapure water, were placed in position and the voltage applied. After the initial transient had decayed and the current through the subphase had stabilised (this takes about 10

min or so), a 50–100-μl aliquot of the spreading solution was carefully introduced onto the water surface and 2 min allowed for the solvent to evaporate before the monolayer was compressed.

3. Results

Figs. 1–4 show surface pressure, π, surface potential, ΔV, and lateral conductance, G, plotted as functions of the area per molecule for stearic acid, palmitic acid, DPPC and DPPS, respectively. The curves are representative plots taken from approximately 10 experiments per compound. The molecular area, A_0, obtained by extrapolating the high pressure portion of the π-A isotherm back to zero pressure, is listed for each compound in Table I. Also listed for comparison are the values obtained for DPPA and DPPE from our earlier work [13].

In the expanded phase, the surface potentials of the monolayers in Figs. 1–4 are almost constant until a critical area is reached, below which ΔV increases significantly. The changes in ΔV occur at much larger areas in the compression cycle than the increases in surface pressure, suggesting that surface potential is a more sensitive detector of surface activity than surface pressure. In Table I the area, A_c at which ΔV begins to rise is listed for the compounds investigated here as well as for those investigated earlier [13]. (In an earlier paper [12] we reported larger values for A_c than those quoted in Table I for stearic acid, DPPE and DPPC. These higher values were found to be associated with trace impurities in the water used in those experiments, a problem which has been discussed fully elsewhere [17]). Also listed in the table are the surface potentials of the fully compressed monolayers i.e., the values achieved just prior to film collapse. These results have been discussed already [18] and are given here only for completeness.

Significantly, the area at which ΔV begins to rise coincides also with an increase in the measured conductance (see Figs. 1–4). At large areas, G is independent of area and has a value corresponding to the volume conductance of the water subphase enclosed by the electrodes. However, as the film is compressed, G rises rapidly, passes through a maximum and decreases again in the fully condensed monolayer. The maximum in conductance and the subsequent decrease appear to coincide with the area per molecule at which the surface pressure of the monolayer begins to increase.

Since the conductance changes during monolayer compression were small, about 1–5% of the total for the compounds investigated here, a series of control experiments were carried out in order to confirm that they were indeed associated with the monolayer and not with experimental artefacts. The control experiments and the results obtained are described below.

(a) When the compression barriers were closed in the absence of a spread monolayer the measured current remained constant at a value determined by the conductivity of the subphase.

(b) When pure solvent was spread onto the subphase and allowed to evaporate, again no change was noted in

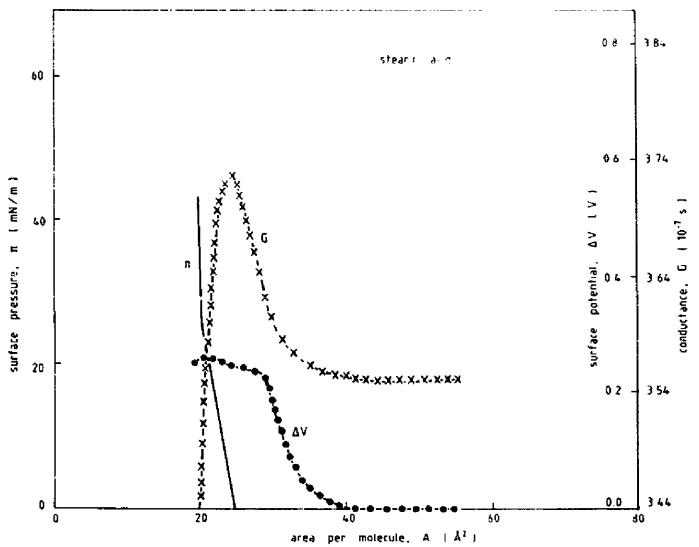

Fig. 1. Surface pressure (———), conductance (× ——— ×) and surface potential (● ——— ●) plotted as functions of the area per molecule for a stearic acid monolayer. In all measurements shown in Figs. 1 to 4 the subphase was ultrapure water at $T = 20\,^{\circ}C$.

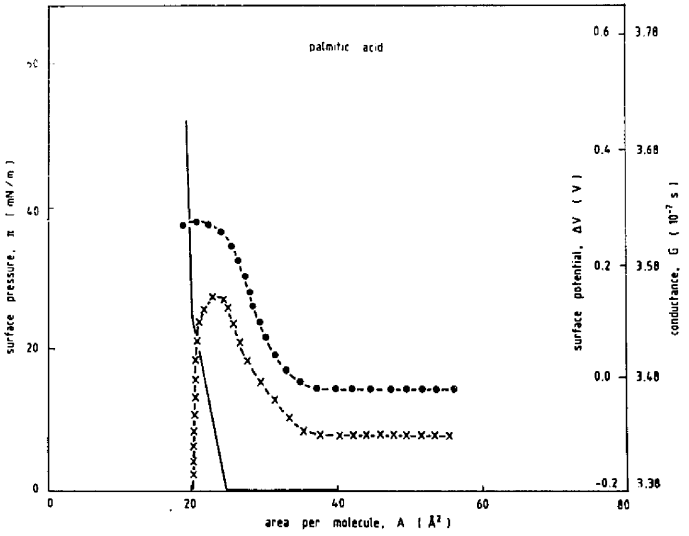

Fig. 2. Surface pressure (———), conductance (× ——— ×) and surface potential (● ——— ●) plotted as functions of the area per molecule for a palmitic acid monolayer.

the measured conductance on closing the compression barriers.

(c) The critical area per molecule, A_c for the onset of the conductance increase was found to be independent of both the volume (between 50 and 100 μl) and concentration (between 0.6 and 1.2 mg/ml) of the spreading solution, and independent also of the compression rate (between 0.2 and 0.4 Å^2/molecule per s).

(d) Values of G and ΔV remained constant when the monolayer area was held constant.

(e) The conductance increase was independent of the depth to which the electrodes were immersed in the

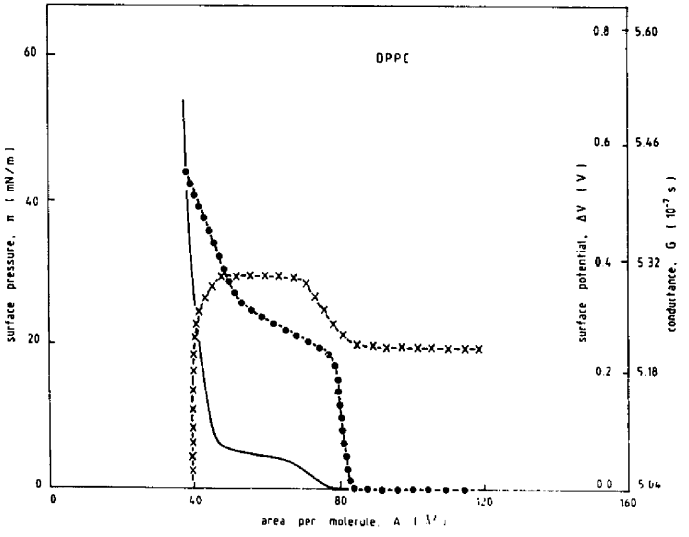

Fig. 3. Surface pressure (———), conductance (× ——— ×) and surface potential (● ——— ●) plotted as functions of the area per molecule for a DPPC monolayer.

TABLE I

Surface potential and lateral conductance data

Summary of surface potential and lateral conductance data obtained on all compounds investigated to date. A_0 is the area per molecule obtained by extrapolating the high pressure portion of the π-A isotherm to zero pressure. A_c is the critical area at which both surface potential and conductance begin to rise. ΔV is the surface potential at the maximum compression and ΔG is the maximum increase in conductance. In all cases the subphase was ultrapure water at $T = 20\,^{\circ}C$.

Compound	A_0 (Å^2)	A_c (Å^2)	ΔV (mV)	ΔG (10^{-8}S)
DPPE [a]	41±2	85±5	520±10	0.8±0.2
DPPC	44±2	84±5	544±10	0.9±0.2
DPPA [a]	42±2	96±5	242±10	3.4±0.3
DPPS	42±2	80±5	226±10	0.6±0.2
Stearic acid	20±1	39±3	260±10	1.8±0.3
Palmitic acid	20±1	38±3	263±10	1.2±0.3

[a] See Ref. 13.

subphase. However, the background current measured when the monolayer was fully expanded was directly proportional to the immersed area. This experiment confirms that the conductance increase must take place at or near the monolayer/water interface and that the background current flows through the subphase water.

A rise in the surface pressure of the monolayer, i.e., a decrease in surface tension of the subphase, is expected to reduce the height of the meniscus present at the electrodes, thereby decreasing the effective area of the electrode in contact with the subphase and reducing the background current.

In an effort to estimate the magnitude of the meniscus effect, subsidiary experiments were carried out in which the behaviour of the meniscus during monolayer compression was examined in detail using a microscope and videorecording equipment. The height of the meniscus was seen to decrease as soon as the surface pressure started to increase, and when a monolayer of stearic acid was compressed to a surface pressure of 50 mN/m, a total decrease of approx. 0.55 mm was observed [13]. Generally, the electrodes were immersed to a depth of 2 to 4 mm, so the consequent reduction in the effective area of the electrodes immersed in the subphase could give rise to a decrease of approx. 14–28% in the background current through the subphase. This is more than sufficient to account for the decreases observed experimentally. Within the accuracy of our measurement (approx. 0.01 mm) no change was seen in the height of the meniscus during the first stages of monolayer compression, i.e., when $\pi \cong 0$, so the *increase* in conductance observed in this range was not caused by the movement of the meniscus. (An *increase* of 0.2 mm in the meniscus height would have been required to account for the conductance increase observed).

It is concluded from the above experiments that the conductance increase is a real effect associated with the monolayer/water interface but that the decrease in conductance which occurs when the surface pressure begins to increase is an experimental artefact caused by a decrease in the height of the meniscus at the electrodes.

The maximum increases in conductance, ΔG, are given in Table I and are seen to depend on the nature of

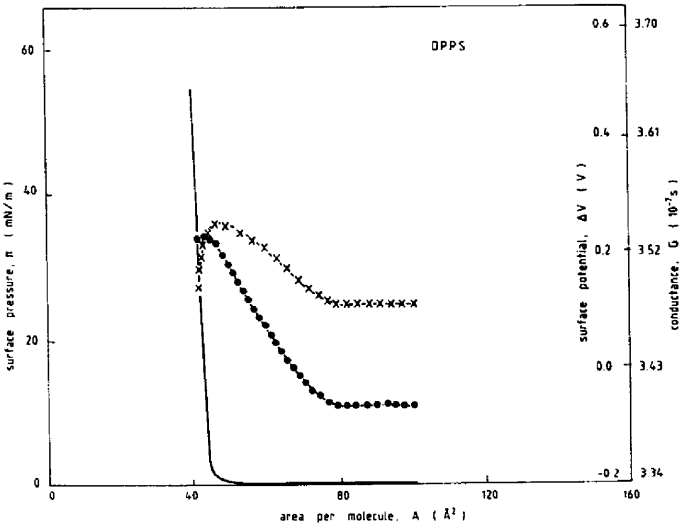

Fig. 4. Surface pressure (———), conductance (× ——— ×) and surface potential (● ——— ●) plotted as functions of the area per molecule for a DPPS monolayer.

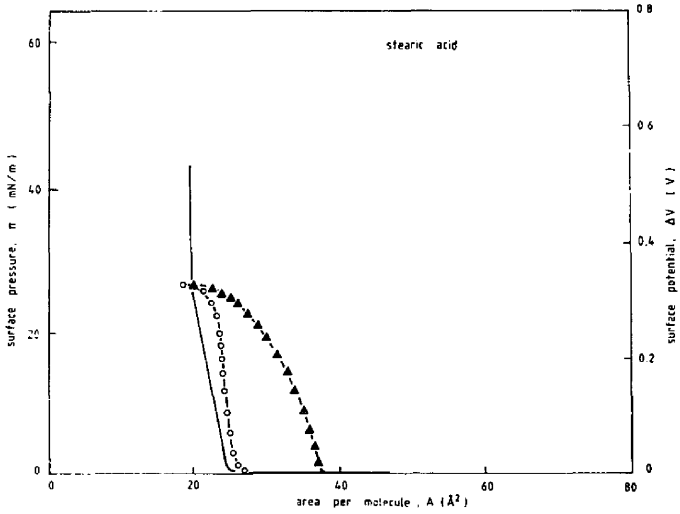

Fig. 5. Surface potential vs. area per molecule (ΔV-A) plots for stearic acid monolayers formed on two aqueous subphases: (▲) 0.1 M NaCl and (○) 0.1 M NaSCN. The two subphases gave identical π-A isotherms (———).

the monolayer. In general, the negatively charged monolayers show the largest conductance increases while the neutral lipids show the smallest increases. DPPS, which is charged but also zwitterionic at the pH of the experiment, appears to be an exception, showing the smallest effect of all the compounds studied.

Fig. 5 shows the effect on the ΔV-A curve of a stearic acid monolayer of adding the chaotropic ion, SCN^-, to the subphase water. It is clear that a reduction has occurred in the critical area, A_c from 39 Å^2 on a 0.1 M NaCl subphase (the same result as on pure water) to 27 Å^2 on a 0.1 M NaSCN subphase. The addition of such high concentrations of salt to the aqueous subphase increased the background current to such an extent that the interfacial current could not be measured with the present arrangement.

4. Discussion

The control experiments show clearly that the conductance increase observed during monolayer compression must arise from a charge transport mechanism located at or very near the interface between the monolayer and the subphase. We have already eliminated the excess ions in the diffuse part of the double-layer as the source of the conductance increase [14]. The degree of dissociation of the charged monolayers is insufficient to account for the magnitude of the conductance change observed, while for the neutral lipids no double-layer is present to make such a contribution.

Similar changes in lateral conductance were observed by Richard et al. [19] in their measurements on monolayers of a mixed valence conducting TCNQ salt spread on a glycerol subphase. In their case the increase was attributed to the onset of electronic conduction through the charge transfer complex when intermolecular distances were sufficiently small. The rapid decrease in conductance which occurred when the monolayer became close packed was attributed to film collapse. In our experiments, the effect of film collapse is hidden by the decrease in current which is already occurring as a result of the meniscus effect.

Jendrasiak and co-workers [20,21] have discounted electronic conduction as the dominant mode of conduction in phospholipids on the grounds that the conductivity of compressed powder samples was found to depend on the degree of hydration of the samples and almost independent of the length and the degree of saturation of the alkyl chains. On the otherhand, protonic conduction in phospholipids is widely reported. For example, the evolution of hydrogen gas at the cathode during the passage of electric current through hydrated DPPE samples was taken by Murase et al. [22] as definitive evidence for protonic conduction. Proton conduction has also been reported in condensed lipid monolayers [23] and in deposited monolayers and bilayers of dimyristoylphosphatidylcholine [24]. A positive thermovoltage coupled to the evolution of hydrogen in DPPC was taken by Szundi [25] as strong evidence for proton conduction in this lipid.

A large body of literature (Ref. 26 provides an excellent review) is now available which points to the ex-

istence of hydrogen bonding in lipids. Infrared spectroscopy has revealed [27] that phospholipid headgroups are linked by hydrogen bonds with large proton polarisabilities suggesting that they are capable of forming an extensive two-dimensional hydrogen-bonded structure. Hydrogen bonding has been studied in phospholipid crystals [28], in hydrated phases [29] and in organic solvents [30]. Hydrogen bonding has also been shown to occur [31] in fatty acids where the alkyl chain is thought to stabilise the R-COO^- $\cdots$ HOOC-R hydrogen bonds.

It is likely, therefore, that during compression on the surface of water in a Langmuir trough, alkanoic acid and lipid monolayers will, at some critical packing density, form a hydrogen-bond network between the headgroups and the adjacent water molecules. Indeed, an increase in the ellipsometric angle at a critical area per molecule in PC, PE and PS is strongly suggestive of molecular structuring during compression [32]. Of greater significance, though, is that the changes in the optical properties of the monolayer occur at precisely the same area per molecule as the increase in ΔV [32]. Our observation of an enhanced conductance occurring simultaneously with a change in ΔV is consistent with the work of Teissié et al. [6] who showed that enhanced proton transport along the monolayer occurred only for areas per molecule smaller than that at which the surface potential of phospholipids became non-zero.

The formation of a hydrogen-bond network at the monolayer/water interface would be expected to reduce significantly the local permittivity at the interface. This is because the previously free water molecules adjacent to the monolayer would now be bound into the network. This is expected to increase the contribution of the headgroup dipole moment to the overall surface potential of the monolayer [18,33–35]. Since the headgroup moments of the compounds investigated here are expected to be positive [18], the observed changes in ΔV are consistent with a large reduction in local permittivity having occurred.

Evidence that the decrease in permittivity is associated with the formation of a hydrogen-bond network is given in Fig. 5, where the addition of the chaotropic ion, SCN^-, to the subphase water is seen to reduce the critical area, A_c at which ΔV increases. In the absence of the chaotropic ion, A_c is approximately double the extrapolated area, A_0, suggesting that a sheath of water molecules around each headgroup participates in the hydrogen bonding. The reduction in A_c in the presence of chaotropic ions, implies that the structure in this sheath is disrupted and that closer packing of molecules in the monolayer is necessary if the hydrogen-bond network is to form.

All the evidence presented above suggests, therefore, that the enhanced monolayer conductance observed in Figs. 1–4, arises from the transport of protons along a two-dimensional hydrogen-bond network formed between the monolayer headgroups and adjacent water molecules.

Proton transport might be expected to occur within such a network provided each water molecule is both a donor and an acceptor of hydrogen bonds, thus allowing intermolecular hopping of the proton via L and D type defects followed by rotation of the defect to a favourable orientation to receive the next proton [36].

The data in Table I indicate that the measured conductance is consistently larger for the charged monolayers (DPPA, stearic acid and palmitic acid), than for the zwitterionic ones. The only exception is ΣPPS which is both charged and zwitterionic and shows a very small conductance. A possible explanation for the larger conductance of the charged monolayers is given by Haines [37], who suggests that protonic conduction may be further enhanced in ionised monolayers because of the availability of negatively charged sites to which the protons responsible for the conduction can hop.

Some hysteresis is found in the conductance-area plots of all the compounds studied here. On expanding the monolayer, the conductance remains higher than the background, returning to the initial value only after a lapse of some 10–15 min. This is easily explained since on expansion, the monolayer held between the two electrodes is likely to disperse only slowly thus maintaining a proton network between the electrodes even when the remainder of the monolayer is in the gaseous phase.

5. Conclusions

It has been shown that when phospholipid or alkanoic acid monolayers are compressed, a critical molecular area is reached at which a lateral conduction process is initiated at the monolayer/water interface. The critical area, which depends on the specific compound investigated, coincides with a positive change in the surface potential of the monolayer and is reduced significantly by adding the chaotropic ion, SCN^- to the aqueous subphase. Based on these findings and supporting evidence from the literature, it is concluded that the conduction process arises from the transport of protons along a two-dimensionai hydrogen-bond network formed between the monolayer headgroups and adjacent water molecules. Proton transport along the network is probably by means of a 'hop and turn' mechanism as suggested by Teissie et al. [6]. The formation of this network causes a sharp decrease in the local permittivity at the water/headgroup interface which in turn produces an increase in the surface potential of the monolayer.

The higher conductance associated with the negatively charged monolayers is attributed to the fact that a

larger number of sites are available for protons to undergo the 'hop and turn' mechanism.

The results reported here corroborate the work of Teissie and co-workers reported in Refs. 5–8. In this context it should be stressed that our results are not subject to the criticism by Kasianowicz et al. [9,10] that the high interfacial diffusion coefficients for protons measured by Teissié et al. [6] and Prats et al. [7] was caused by convective currents in the subphase.

Our work supports the concept that localised proton transport can occur along the surface of biomembranes.

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