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Lateral Electrical Conductivity of Mica-Supported Lipid Bilayer Membranes Measured by Scanning Tunneling Microscopy

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ABSTRACT Lateral electric conductivity of mica-supported lipid monolayers and of the corresponding lipid bilayers has been studied by means of scanning tunneling microscopy (STM). The surface of freshly cleaved mica itself was found to be conductive when exposed to humid air. Lipid monolayers were transferred onto such a surface by means of the Langmuir-Blodgett technique, which makes the mica surface hydrophobic and suppresses the electric current along the surface in the experimentally accessible humidity (5–80%) and applied voltage (0–10 V) range. This is true for dipalmitoylphosphatidylethanolamine (DPPE) as well as dipalmitoylphosphatidylcholine (DPPC) monolayers. Repeated deposition of DPPC layers by means of the Langmuir-Blodgett LB technique does not lead to the formation of a stable surface-supported bilayer because of the high hydrophilicity of the phosphatidylcholine headgroups that causes DPPC/DPPC bilayers to peel off the supporting surface during the sample preparation. In contrast to this, a DPPE or a DPPC monolayer on top of a DPPE monolayer gives rise to a rather stable mica-supported bilayer that can be studied by STM. Electric currents between 10 and 100 fA, depending on the ambient humidity, flow along the DPPE bilayer surface, in the humidity range between 35 and 60%. The DPPC surface, which is more hydrophilic, is up to 100 times more conductive under comparable conditions. Anomalous high lateral conductivity thus depends on, and probably proceeds via, the surface-adsorbed water layers. The prominence of ambient humidity and surface hydrophilicity on the measured lateral currents suggests this. The combination of our STM data and previously published water adsorption isotherms as a function of the relative humidity indicate that one layer or less of adsorbed water suffices for mediating the measurable lateral currents. The fact that similar observations are also made for other hydrophilic substrates supports the conclusion that lateral conductivity via surface-adsorbed water is a rather general phenomenon.

INTRODUCTION

Electrostatic phenomena are of paramount importance in nature. They are not only responsible for the redistribution of any ions in the vicinity of charged surfaces but, moreover, are also at the very roots of bioenergetics and bioelectrochemistry. Charge transport plays a crucial role in these respects. This is especially true when the electric current is confined to, or affected by, the presence of a biological membrane. Indeed, proton transport along the surfaces of proteolipid membranes is essential for the energy conversion in the mitochondria as well as in the thylakoids of light-harvesting plants (Boyer et al., 1977). Ion or other charge transport in, and often along, the surface of the conductive proteins or protein complexes, moreover, provides the basis for signal transfer in all living systems (Kell, 1979).

Many attempts have therefore been made to investigate the mechanism of charge transport and the electrostatic characteristics of biologically relevant hydrophilic surfaces in detail. Owing to their simplicity and similarity to the biological membranes, lipid bilayers turned out to be particularly useful in this respect (for reviews see, e.g., Läuger 1987; Teissié and Tsong, 1981; Cevc, 1990).

The first detailed investigations of the electric current through the model bilayers were done with black lipid membranes (Müller et al., 1962), which show that lipid bilayers are, per se, very good insulators. They must therefore be enriched with carriers, pores, or defects to become conductive. The advent of patch-clamp technology (Hamill et al., 1981) has improved the sensitivity as well as the spatial resolution of the transbilayer current measurements, which can now be done with a bilayer spanning the tip of a micropipette and permit monomolecular resolution when the transbilayer channels are investigated.

In contrast, the high resolution studies of charge transport along the lipid bilayer surface were not possible to date. Perhaps the main reason for this is the very strong background signal from the bulk fluid surrounding the membrane surface, which is typically many orders of magnitude higher than that of the surface itself (short-circuit problem). Early experiments with the lateral conductivity of lipid membranes were, therefore, done with incompletely solvated membrane stacks (Jendrasiak and Mendible, 1976). One of the problems with such systems was their inhomogeneity and poor reproducibility. This has precluded reliable conclusions on the basis of such experiments to be drawn at the molecular scale. To overcome the underlying problems, single lipid layers, which form spontaneously at the air-water interface, have thus been employed.

Several studies of the lateral conductivity of a monolayer surface have been published in the past. The results of these studies seemed to support the idea of appreciable electric

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currents flowing along the polar surface of the lipid monolayer (Jendrasiak and Mendible, 1976; Prats et al., 1986, 1987; Teissié et al., 1985; Sakurai and Kagamura, 1987; Morgan et al., 1991). Such a conclusion was based on the results of macroscopic fluorescence intensity changes during an alleged diffusion of the surface-associated protons along the monolayer surface. This was typically monitored after introducing an excess of H^+ at one end of a Langmuir trough. The validity of such a deduction was questioned, however (Kasianowicz et al., 1987 a,b), in principle as well as in experimental terms. Indeed, the use of fluorescent labels and macroscopic system dimensions may hamper the reliability of such studies too much to permit unambiguous conclusions.

At first glance, the other experimental system used to date for measuring the lateral surface conductivity is more robust and simple. In such an experimental set-up, the lateral conductivity of an air-water interface is directly monitored by means of macroscopic electrodes immersed into the bulk subphase. The lipid layer is then gradually deposited onto the water surface. Alternatively, lipids can be compressed in a Langmuir trough. Simultaneously, the electric current between two electrodes is measured. Any significant change in this current is ultimately taken to be diagnostic of the contribution from the lateral surface conductivity. Unfortunately, such an inference is only true if the surface tension of the investigated system does not affect the solvated electrode area significantly. However, this is rarely true, if ever. In our very numerous tests of such surface conductivity measurements we have always seen an abrupt increase in the electric current when lipid molecules have formed a sufficiently dense monolayer at the air-water interface. Meticulous tests have revealed, however, that the detected current change is always accountable to the increased contribution from the bulk water conductivity; the lipid-dependent decrease of the surface tension of water results in an increase of the wetted electrode area. This in turn gives rise to an extra current contribution that can, erroneously, be attributed to the surface conductivity itself. Our calculations even suggest that such a bulk contribution normally creates signals much bigger than those stemming from the surface conductivity for all realistic measuring conditions.

To get unambiguous information about the electrical conductivity along the surface of a polar lipid layer, and also to improve the signal-to-noise ratio and thus the experimental resolution, it is thus essential to get rid of the bulk signal. Scanning tunneling microscopy (STM) provides the most elegant solution to this problem (for a review on STM in biology, see Guckenberger et al., 1992).

To date, STM has been applied exclusively to studies of objects on conductive substrates. The electric current was therefore made to pass directly from the tip to the substrate, not allowing any conclusions about the conductivity along the sample surface. STM measurements to date have been done with dimyristoylphosphatidic acid, dipalmitoylphosphatidylcholine (DPPC) and arachidic acid monolayers deposited onto a highly oriented pyrolytic graphite (Lang et al., 1988; Fang et al., 1992; Fuchs, 1988). The electrical conductivity of such surface-supported lipid Langmuir-Blodgett (LB) films was then found to be up to 10 orders of magnitude higher than expected on the basis of the bulk lipid data (Guckenberger et al., 1992). This was interpreted in terms of the two-dimensional conductivity along the lipid headgroup layer (Mizutani et al., 1988), as lipid chains are practically nonconducting (Cevc and Marsh, 1987).

To derive more direct information from the STM measurements pertaining to the lateral conductivity of lipid layers we have chosen mica, an insulator, as a substrate. Such an experimental set-up should prevent current flowing from the tip over short vertical distances (~2 nm) to the substrate. A current measured on the mica-supported samples should thus be forced to advance over a longer distance (1–3 mm) laterally along the sample surface, which should deliver direct information on the surface conductivity.

MATERIALS AND METHODS

General considerations about sample configuration

To be suitable for the STM measurements, lipids must be well oriented and mechanically stable. We have achieved this goal by depositing lipids onto freshly cleaved mica by means of the LB technique (Blodgett and Langmuir, 1937). The advantage of mica as a substrate is that it offers a perfectly planar, atomically smooth, and nonconducting surface. If the substrate was conductive, electric current would flow directly from the tip through the layer into the substrate; no conclusions would then be possible about the lateral surface conductivity of the deposited film. An adequate sample configuration for surface conductivity examinations is illustrated in Fig. 1.

A typical sample consists of a lipid-coated mica surface pressed to the sample holder (a stainless steel ring of 6 mm diameter) by a spring. Any measured current must thus flow between the probing tip and the steel ring along the investigated surface. To get the most reliable lipid-specific conductivity data we have investigated lipid bilayers in which the polar lipid headgroups were pointing toward the air and thus toward the STM tip. In this configuration, the conductive surface, consisting of a lipid headgroup layer, is contacted directly by tip and sample holder. A bilayer configuration also minimizes the interference between mica and the studied lipid headgroups. All of these requirements would also be met by a lipid

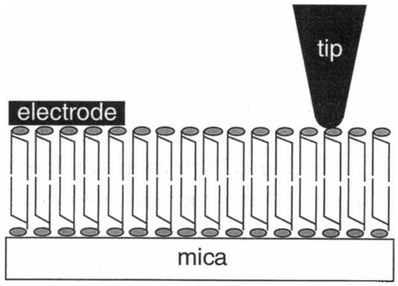

FIGURE 1 Schematic representation of an adequate sample configuration for measurements of the lateral conductivity of lipid bilayers. A lipid bilayer is deposited on mica. The tunneling tip and the conductive sample holder are both in mechanical contact with the lipid headgroups. Any current measured with this conformation must flow from the tip along the lipid headgroup layer to the sample holder (distance between tip and sample holder is ~3 mm). Such current provides evidence for the lateral electrical conductivity of the membrane surface. For STM imaging of the sample topography, the tip moves at a finite distance above the surface.

monolayer deposited onto a nonconductive and hydrophobic substrate. Because of the lack of a hydrophobic, nonconductive, and sufficiently smooth substrate, mica was chosen as a substrate after having been hydrophobized by a downward facing lipid monolayer.

Chemicals

1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) were purchased from Sygena (Liestal, Switzerland). NaCl and $MgCl_2$ were used in p.a. quality of Merck (Darmstadt, Germany). Filtered water (milli Q) with a resistance higher than 18 MΩcm was used as the film balance subphase. The relative humidity in the sample chamber was adjusted by a controlled mixing of pure N_2 with the water-saturated N_2 that was bubbled through a milli Q water-filled bottle.

Sample preparation

Lipids were deposited at the air-water interface of a film balance by the solvent method (1 mg lipid/1 ml $CHCl_3$). The lipids were compressed into the crystalline phase with a lateral pressure between 35 and 45 mN/m at 20 °C. Before each transfer onto the solid substrate, each lipid film was maintained at the air-water interface in the compressed state for 45 min, which promoted annealing of defects in the lipid monolayer and thus eliminated weak spots at which the film could detach (Heim, 1994). The first lipid layer was transferred onto mica by pulling the substrate out of the LB trough; the second layer was deposited by dipping the sample into the film balance. The transfer rates for all lipid layers were close to one, when the lift speed was 0.1 mm/s.

After the successful bilayer depositions, the coated mica was in a water-filled container. To become amenable to STM, samples first had to be exposed to the air. (Many researchers believe that lipid bilayers on hydrophilic substrates are stable in water only. This belief is not always justified, however. DPPE, for example, has a very low affinity for water. The chance of exposing a DPPE bilayer to the air without damage is therefore high.) To expose the sample to air, the sample was lifted out of the trough in a glass filled with water. The remaining lipid monolayer at the surface of the container was removed by addition of enough water to cause overflow.

We have tested different drying methods (Heim, 1994), mostly with devastating results. The only convenient method in which the samples could be exposed to air without bilayer destruction was rapid dehydration; the samples were exposed to air by quickly pulling them out of water by using a forceps. This was done vertically, with the sample surface parallel to the air-water interface. Residual water in some surface-attached drops was blown away quickly with compressed air.

Ions often increase the attraction between biological molecules (Donald et al., 1992a,b). We have therefore tested the influence of $MgCl_2$ and NaCl (in concentrations of 10 and 100 mM) on the lipid-mica adhesion. The presence of ions did not improve the final lipid film stability, however (Heim, 1994). All results presented in this article therefore pertain to samples prepared with pure water in the subphase.

Conductivity measurements

The following four basic lipid film configurations were prepared for the conductivity measurements: (1) DPPE and (2) DPPC monolayers with air-exposed chains, (3) DPPE bilayer with PE headgroups facing the air, and (4) DPPE/DPPC bilayer with PC headgroups facing the air. For comparison, the surface conductivity of freshly cleaved mica was also examined. The instability of DPPC/DPPC bilayers prevented experiments with such samples.

All samples were first brought into mechanical contact with the tunneling tip. (The mechanical contact between tip and sample can be determined by the appearance of interference phenomena observed with a light microscope.) The current between the tip and the conductive sample holder was measured at different ambient humidities. The tip position was kept constant during the conductivity measurements (STM scan and feedback was switched off). Currents were determined with a tip-sample bias of -10 V (sample being the negative electrode). DC-etched tungsten tips were used for the conductivity measurements as well as for the STM imaging.

STM imaging of the sample topography

STM imaging was done mainly for two reasons: (1) to show that it is possible to do stable STM based only on the conductivity along the sample surface and (2) to characterize the structure of the lipid bilayer samples, which is necessary to know on which surfaces the different conductivity measurements were done.

To get topographic information, the feedback regulation was switched on. In this mode the current is kept constant by adjusting the distance between tip and sample. The tip then follows the sample surface at an approximately constant distance. For stable STM imaging with our instrument, the current must be permanently >0.05 pA. Therefore the surface conductivity on the sample must be sufficiently high to transport at least this current value. We chose the scan speed to be 1 μm/s. To get an image as shown in Fig. 3 *c* (2 × 2 μm), it takes, therefore, ~20 min. (For a description of our STM set-up see Guckenberger et al., 1988.)

Atomic force microscopy (AFM) imaging of the sample topography

To image the surface of nonconducting samples as well as to confirm the STM images of the well conducting samples, corresponding AFM pictures were taken. This was done in air in the constant force mode while simultaneously taking elasticity maps (for detailed description of our home-built AFM, Wiegräbe et al., to be published). Areas in the order of 500 × 500 μm^2 that had previously been tested for conductivity in the STM (in each case at more than 10 different positions) were repositioned by light microscopy for the AFM. (Scan speed in the AFM was 3 μm/s; therefore it took ~10 min to scan the AFM images in Fig. 3.)

RESULTS

Conductivity measurements

Freshly cleaved mica exhibits a strongly humidity-dependent electrical conductivity along its surface. Currents of >500 pA are measured at a relative humidity of 80% (see Fig. 2 *a* and Guckenberger et al., 1994). All currents were measured at -10 V tip-sample bias.

DPPE monolayers transferred onto freshly cleaved mica support no current when exposed to air with a relative humidity between 10 and 80% (see Fig. 2 *a*). The same is true for DPPC monolayers studied at humidities <80%. (The detection limit of our instrument is ~10 fA, because of noise that increases slightly with increasing humidity.)

DPPE bilayers with the lipid headgroups facing the tip are conductive and transport an electric current of ~0.1 pA at a relative humidity of 60% (Fig. 2 *b*). Because the DPPE monolayer is nonconductive one can conclude that this current is transported along the DPPE headgroups in the top layer.

DPPE/DPPC bilayers, with the latter type of lipids facing the tip, are the most conductive of all lipid films studied in this work. They support a current starting from several femtoamperes at low humidities (~5%) up to 25 pA at 68% relative humidity (Fig. 2 *b*). For the same reasons as in the case of DPPE bilayers we conclude that such current is

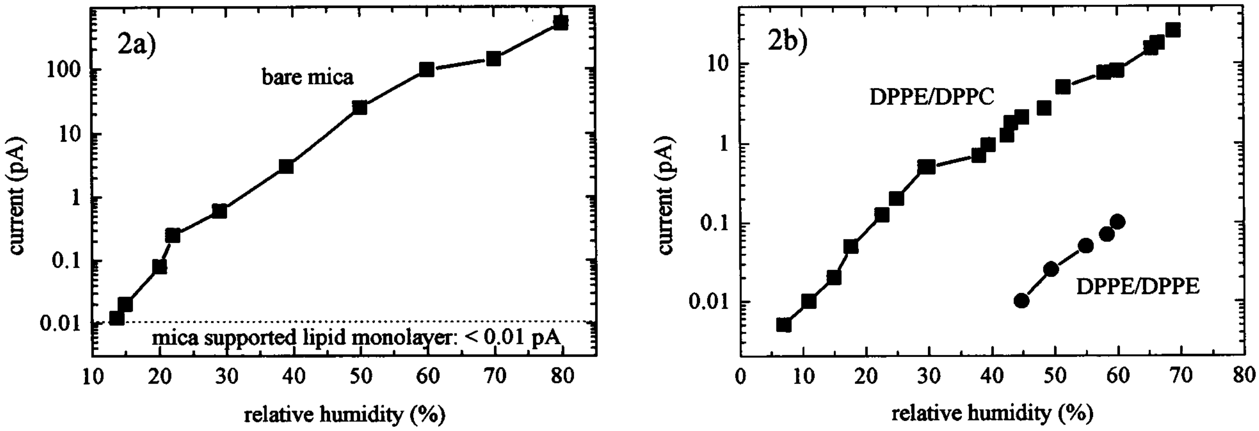

FIGURE 2 (*a*) Lateral current versus relative humidity for plain mica and the surface of DPPE and DPPC monolayers. The surface of freshly cleaved mica is conductive. Dependent on the humidity of the ambient air, currents between 10 fA and ~500 pA are measured for an applied voltage of 10 V. When the mica surface is covered with lipid monolayers of DPPE or DPPC, the surface is hydrophobic and the conductivity is suppressed. (*b*) Mica covered with a lipid bilayer is conductive dependent on the ambient humidity and the hydrophilicity of the lipid headgroups. The hydrophilic surface of DPPC is much more conductive than the less hydrophilic DPPE.

transported along the DPPC headgroup layer. (The supporting DPPE monolayer is nonconductive). Lateral currents vary by a factor of two (occasionally more) for different tips and samples; different contact areas between tip and sample, and various electrochemical effects, may be responsible for this. Bilayer samples, moreover, often have only a few conductive areas, which indicates that the second, and the only conductive, lipid layer is not intact.

Imaging the topography of mica-supported lipid bilayers by STM and AFM

Fig. 3 *a* shows the AFM image of a mica-supported DPPE monolayer. Its featurelessness documents the homogeneity of the hydrophobized mica surface after the lipid monolayer adsorption. Two small rectangular areas in Fig. 3 *a* appear deeper. These areas were scanned before with higher force that locally destroyed the lipid monolayer. (The square in the center was scanned four times with 100 nN. The square left of it was scanned twice with 100 nN.) The deepest areas of the holes are ~1.6 nm deep. The imaging force used to get Fig. 3 *a* was 15 nN. The meandering vertical stripes in Fig. 3, *a* and *f*, are artifacts.

During imaging of the sample topography our STM needs to detect a tunneling current of at least ~0.05 pA. This means that the samples with an exposed DPPC layer, as well as freshly cleaved mica, are both conductive enough to be imaged easily in the STM as long as the ambient air is humid enough (Fig. 3 *c*). DPPE bilayers, with a maximum current of 0.1 pA, are more difficult to study, but still amenable to STM imaging (Fig. 3 *b*). On the latter type of sample, structures of very small height (less than 1 nm) are seen. On plain mica no structures can be found except for sporadic steps (data not shown).

Fig. 3 *c* gives the STM image of a lipid bilayer consisting of DPPE (bottom) and DPPC (top) layers. It is noteworthy that the taking of this image (as well as Fig. 3 *b*) was based on lateral electric current only. The STM image clearly shows ~5.0-nm-deep holes, corresponding to the expected lipid bilayer thickness. The density and the size of holes observed in the phospholipid bilayer vary strongly between different places on the sample, but each hole bottom is conductive. Images can be taken at voltages as low as 0.4 V and up to 10 V (upper value is limited by the electronics). This kind of sample sometimes shows many protrusions. We did not find any regularity in their height or in the propensity to their formation, however, between the preparations.

When the ambient humidity is below a certain threshold (40–50%), the measured hole depth increases dramatically (20 nm and more). The same happens when the voltage falls under some threshold value that is on the order of 2 V at 50% relative humidity. This is because of insufficient current in such holes, which induces strong regulation of the vertical tip position toward the sample. An overestimation of the hole depths results from this.

Fig. 3, *d* and *e*, shows the structure of a DPPE/DPPC bilayer imaged by AFM. Before taking the latter picture, a 1-μm square was scanned with a force of 100 nN. In this area the lipid bilayer was destroyed by the tip, leaving a 6-nm-deep hole, corresponding to the bilayer thickness. In Fig. 3 *f* the topography of the DPPE/DPPC bilayer structure was imaged by AFM in an area where the sample was nonconductive (as determined by previous STM measurements). The bilayer was found to be incomplete here. The same was true for the DPPE bilayers in nonconductive areas (Fig. 3 *g*).

DISCUSSION

Conductivity measurements

The surfaces of mica-supported lipid monolayers are nonconductive. In contrast to this, a humidity-dependent electric cur-

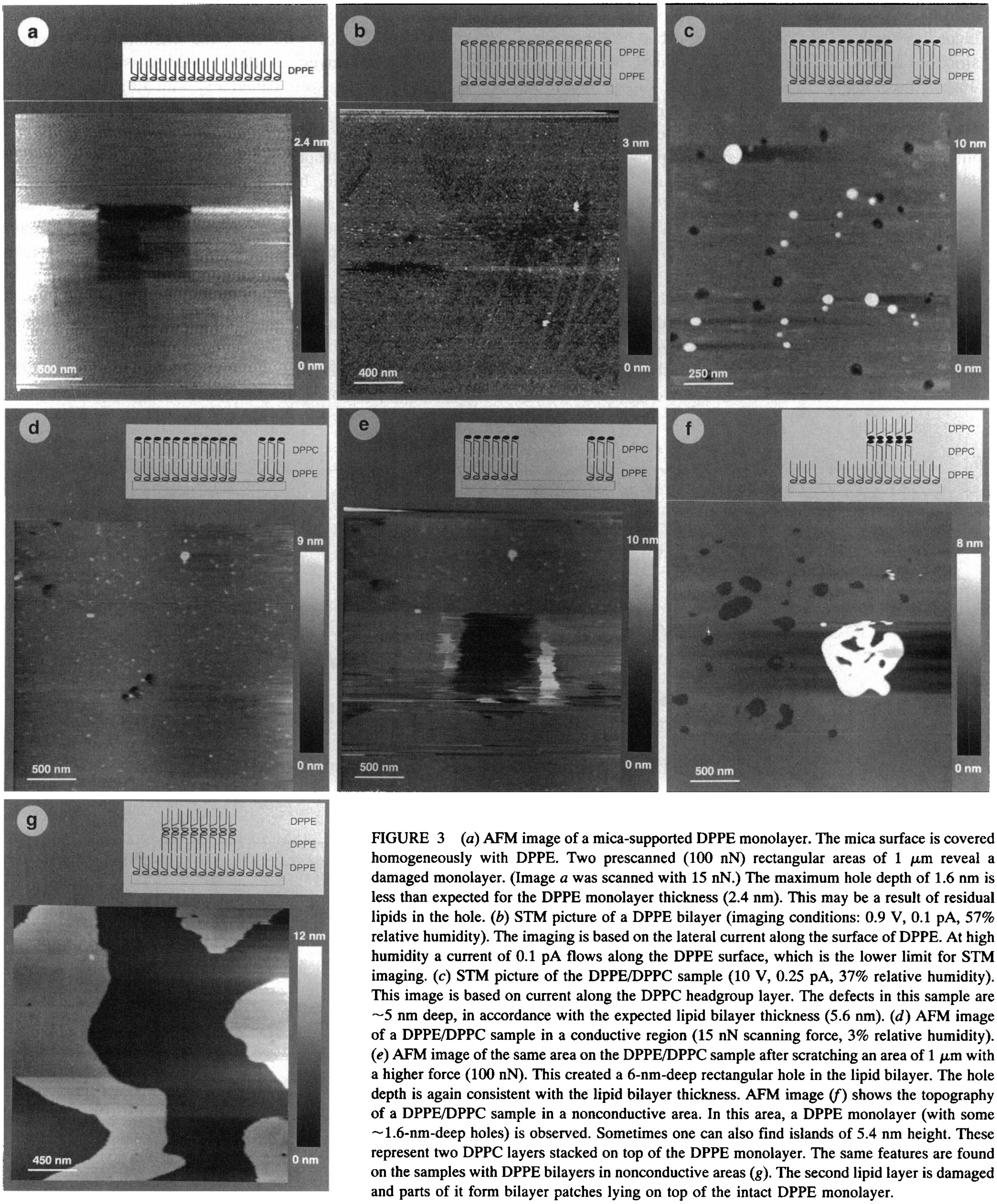

FIGURE 3 (*a*) AFM image of a mica-supported DPPE monolayer. The mica surface is covered homogeneously with DPPE. Two prescanned (100 nN) rectangular areas of 1 μm reveal a damaged monolayer. (Image *a* was scanned with 15 nN.) The maximum hole depth of 1.6 nm is less than expected for the DPPE monolayer thickness (2.4 nm). This may be a result of residual lipids in the hole. (*b*) STM picture of a DPPE bilayer (imaging conditions: 0.9 V, 0.1 pA, 57% relative humidity). The imaging is based on the lateral current along the surface of DPPE. At high humidity a current of 0.1 pA flows along the DPPE surface, which is the lower limit for STM imaging. (*c*) STM picture of the DPPE/DPPC sample (10 V, 0.25 pA, 37% relative humidity). This image is based on current along the DPPC headgroup layer. The defects in this sample are ~5 nm deep, in accordance with the expected lipid bilayer thickness (5.6 nm). (*d*) AFM image of a DPPE/DPPC sample in a conductive region (15 nN scanning force, 3% relative humidity). (*e*) AFM image of the same area on the DPPE/DPPC sample after scratching an area of 1 μm with a higher force (100 nN). This created a 6-nm-deep rectangular hole in the lipid bilayer. The hole depth is again consistent with the lipid bilayer thickness. AFM image (*f*) shows the topography of a DPPE/DPPC sample in a nonconductive area. In this area, a DPPE monolayer (with some ~1.6-nm-deep holes) is observed. Sometimes one can also find islands of 5.4 nm height. These represent two DPPC layers stacked on top of the DPPE monolayer. The same features are found on the samples with DPPE bilayers in nonconductive areas (*g*). The second lipid layer is damaged and parts of it form bilayer patches lying on top of the intact DPPE monolayer.

rent between tip and sample holder is measured on the lipid bilayer samples. This indicates that any measured current advances from the tip along the surface of the second lipid layer to the sample holder at the periphery of each sample.

The measured current values strongly depend on the lipid headgroup hydrophilicity as well as on the relative humidity of the ambient air. The hydrophilic surface of freshly cleaved mica is conductive also, dependent on the ambient humidity. This indicates that for both systems (on lipid bilayers as well as on mica) the adsorbed water plays a major role in the process of lateral conductivity.

Fig. 4 illustrates this in some detail. It shows how much water is adsorbed to lipid multilayers containing PE or PC headgroups (Cevc and Marsh, 1987). The water adsorption isotherm for DPPE in the crystalline phase is probably below the one given in Fig. 4, which relates to this lipid in a gel phase. (Adsorption isotherms of DPPE in the crystalline phase are not available.) Humidity-dependent water layer thickness on mica, as measured by ellipsometry, is given in Beaglehole et al., 1991.

Fig. 4 *a* suggests that the DPPC headgroup lattice adsorbs two to three water molecules per lipid headgroup at a humidity of 40% in addition to the two strongly bound solute molecules that are not shown in the adsorption isotherm.

Inasmuch as every DPPC molecule under such conditions occupies an area of ~0.5 nm^2, the thickness of the entire adsorbed water layer[5] at this humidity is ~0.1 nm, on average. (The data were calculated with an area of 0.5 nm^2 per PC headgroup and a space requirement of 0.063 nm^2 for each water molecule.) Despite its minute thickness, this layer is able to transport an electric current of 1 pA. DPPE is less hydrophilic, binds less water molecules at every given humidity, and, therefore, is less conductive than DPPC. Much higher ambient humidity is necessary to make the DPPE layer conductive enough for STM (at 60% relative humidity, the current is 0.1 pA).

Ellipsometric data (Beaglehole et al., 1991) for mica-adsorbed water, as a function of the ambient humidity, shows that even plain, freshly cleaved mica surfaces support a current of 1 pA in a water layer not much thicker than 0.1 nm. This represents less than one layer of adsorbed water

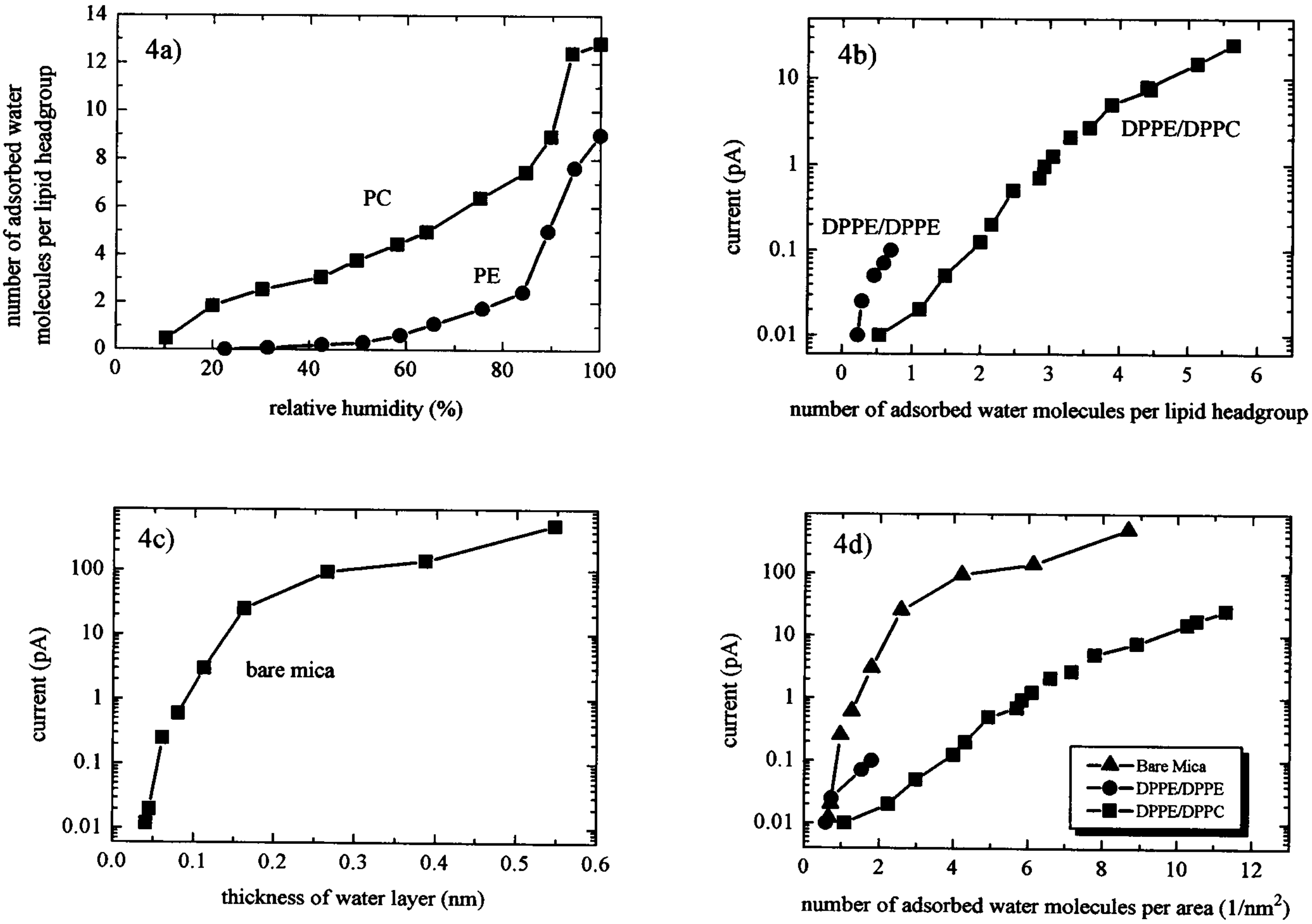

FIGURE 4 With the adsorption isotherms for water on PE and PC headgroups (*a*) one can relate the current to the number of adsorbed water molecules (*b*). Ellipsometric data gives the relationship between the water layer thickness and the relative humidity on mica (Beaglehole et al., 1991). With such data one can calculate the dependence of the lateral current on the thickness of the adsorbed water layer (*c*). The current increases strongly with the adsorbed water layer thickness up to a thickness of ~0.25 nm. The change of the slope in *c* indicates that the charge transport mechanism might change when the water layer thickness reaches 0.25 nm, at which value the first water monolayer is complete. Additional water forming a second water layer is more similar to bulk water and thus does not contribute so much to the lateral conductivity. (*d*) Knowing the area per water molecule and the size of the lipid headgroups, one can compare the conductivities of water layers on different surfaces. (The data were calculated assuming a water diameter of 0.27 nm, which is the average distance between two water molecules in the bulk.) Indicated in *d* is that comparably thick water layers adsorbed on different surfaces may mediate different currents. This suggests that water as well as the surface properties both influence the surface conductivity.

molecules. In the bulk water, a layer thickness of ~40 nm would be needed to transport the same current (calculated with a tip radius of 10 nm and water resistance higher than 18 MΩcm). This means that the water adsorbed on lipids, as well as on mica, exhibits an electrical conductivity two to three orders of magnitude higher than bulk water.

Fig. 4 *d* suggests that the density of water molecules at the investigated surface is probably not the only parameter affecting the lateral surface conductivity. Various polar surfaces may have different lateral conductivity even when their water coverage is comparable.

Sample structure

In the conductive areas of lipid bilayers, defects (holes) are present. (Such holes are not created by the tip as neither their size nor their density in an individual image is a function of the scanning process.) The comparison of the STM and the AFM images (Fig. 3, *c*, *d*, and *e*) shows that the surface of the DPPE/DPPC samples is conductive and contains holes as deep (5.0 nm) as expected for the bilayer conformation. Furthermore, the mica-supported lipid bilayer is destroyed by the AFM tip when scanned with a force of 100 nN. This leaves a 6-nm-deep rectangular area in Fig. 3 *e*. The hole depth is in accordance with the expected lipid bilayer thickness.

It is sometimes difficult to find a conductive area on the mica-supported lipid bilayer sample. This may be because parts of such a bilayer are often destroyed by exposure to the air. Fragments of the second layer then detach and form more stable oligolayer segments (with lipid chains facing the air) that come to lie on top of the monolayer. A complete monolayer and fragmented double-layer islands of DPPE or DPPC on top of the monolayer are, therefore, left after exposure to air (see Fig. 3, *f* and *g*). The DPPC or DPPE islands are not connected electrically to each other, as the surrounding monolayer is nonconductive. Nevertheless, the surface conductivity of such triple-layer islands, probably supported by the water that is sandwiched between the two polar headgroup surfaces, is observed indirectly by charging or discharging one fragment according to its electrical capacitance. The charging current is high enough to trigger the electronics to stop the mechanical approach of the sample toward the tip. Permanent current between tip and sample holder cannot be detected in such a case, as the DPPC islands are not connected to the sample holder.

This interpretation of the sample configuration is supported by additional AFM measurements. Fig. 3 *f* shows a nearly complete DPPE monolayer with one DPPC double-layer island on top of the monolayer. Several holes are also seen in the monolayer. By scanning with higher forces it is possible to remove the deposited lipid layers step by step. At first, the lipid layer on top of the sample detaches. Then, the next lipid layer is removed until only the smooth surface of the supporting DPPE monolayer, with some defects, can be seen (Knapp et al., 1995). During such an abrasion, the friction of the tip on the sample surface changes according to the changing surface properties.

It can be assumed that bare mica is seen at the hole bottom for several reasons. (1) The hole depth measured under suitable experimental conditions (voltage and humidity above the threshold values) is constant and of the expected magnitude (~5.0 nm). (2) If the hole bottom would not be bare it could contain only a lipid monolayer; such a monolayer is nonconductive and thus would not be imageable by means of STM. (3) AFM elasticity scans show the highest rigidity in the holes, which is indicative of the presence of pure mica (Knapp et al., to be published).

For STM images the mica surface at the bottom of the holes has to be connected electrically to the DPPC surface as this is the only medium that can transport electric current laterally to the sample holder. We hypothesize that the bottom of each hole is connected to the polar lipid bilayer surface by defect lines at the rims of such holes. Such defect lines form resistive barriers between the DPPC surface and the bottom of the holes. Another possibility is that hydration rings are formed along the exposed lipid chains at the hole edges, thus acting as proton wires. Such wires have been postulated previously to explain the anomalous high trans-bilayer conductivity of protons as compared with other ions (Gutknecht, 1987; Cevc, 1993).

This suggestion is not borne out by the lack of lateral current along the hydrophobic surface. The electrical field, which drives the current, and the absolute resistance value, which determines the current magnitude for a given applied potential, differ strongly when going along and through the hydrophobic layer. In the former case, the magnitude of the electrical field is low whereas the resistance value is relatively high. In the latter case, the opposite is true. It is therefore possible that an electric current flows through the proton wire along an individual lipid chain but not along the extended surface consisting of the ends of many such chains.

Conduction mechanism

Not much is known about the conduction mechanism in ultrathin water films. The mechanism of standard ionic conductivity is irrelevant in this respect as the layers are less than one molecule thick. Such a thin layer simply does not have enough space to accommodate ions that wish to retain their hydration and thus prefer bulk water. Ion movement on top of the surface is also unlikely. Electrostatic forces between lipid headgroups and ions should suppress lateral motion of the ions (Pethig, 1979). From the experimental point of view the contamination with ions is extremely unlikely owing to the design of our experiment, which has maintained the CO_2, and thus the CO_3^- concentration very low.

The emerging picture is thus as follows. Lateral surface conductivity might be based on proton transport along the network of the surface-adsorbed water molecules. Under certain binding conditions, a proton in such a network is shared between two or more water molecules. This can be described in the simplest approximation by a double symmetrical potential (Vinogradov, 1971). Thermal and/or electric excitations force such protons to move from one potential minimum to the other. This facilitates proton movement between the molecules. If voltage is applied between two edges of the hydrated surface, the proton propensity to move along the potential gradient results in a directed proton flow, detectable as an electric current. As water molecules are known to form a network along a lipid layer surface (Marrink et al., 1993), such protons then travel easily along the surface over long distances in the external lateral electric field gradient. The extraordinary high water conductivity arises from the fact that the network structure of the adsorbed water layer prevents dissipation into the third dimension. Lateral charge transport thus takes place microscopically, with a low activation energy, resulting in an effective current transport over long distances.

This presumptive model of the conduction mechanism is supported by the data given in Fig. 4 *d*. The latter suggests that the lateral conductivity is probably not determined by the thickness of the surface-adsorbed water layer alone. Equally thick water layers mediate different electrical currents, depending on the adsorbing surface. The physical properties of the water-adsorbing surface, therefore, influence the measured current values. This could arise from the fact that the density or the strength of the hydrogen bonds in the surface-adsorbed water is influenced by the adsorbing surface. Such an implication is also supported by the results of recent computer simulations of lipid hydration (Marrink et al., 1993).

CONCLUDING REMARKS

We have shown for the first time directly that bilayers made of DPPE or DPPE and DPPC are able to support lateral electric currents along the layers of their headgroups. This was demonstrated in situ by STM measurements. The detected currents were found to depend strongly on the ambient humidity as well as on the hydrophilicity of the investigated surface. Both dependencies suggest that the measured current proceeds along the surface through the adsorbed water layer. This solvent layer is very thin. Sometimes a single water molecule bound per lipid headgroup is enough to transport a current of 0.1 pA in the lateral direction. Two to three water molecules bound to each lipid headgroup mediate an electric current of up to 1 pA.

The less hydrophilic DPPE adsorbs less water and therefore depends on high humidity (~60%) to get sufficiently hydrated to transport a lateral current of 0.1 pA. For the DPPC surface, a similar amount of water is adsorbed when the relative humidity is ~15%; this latter lipid is, therefore, laterally much more conductive than DPPE. Ellipsometric data indicate that the water layer on mica, which mediates a current of ~1 pA, is also on average less than one water molecule thick (~0.1 nm) (Beaglehole et al., 1991).

We have thus shown for two unrelated systems (lipids as a biological system and mica as an insulator) that they can support an anomalous high electric current along their hydrated surface (two to three orders of magnitude higher than in bulk water).

The surface-adsorbed water layers thus act as an ultrathin conductive coating suitable for STM of a wide variety of biological samples (most of which are hydrophilic). With such a water layer, high resolution data can be obtained because the conductive cover is much thinner (0.2 nm) than any currently known metal coating (at least 1 nm). Recently, we were able to image DNA, tobacco mosaic virus, and collagen on mica in humid air (Guckenberger et al., 1994).

We infer that the conduction mechanism on such biological surfaces will probably be the same as on mica and the lipid bilayers. The anomalous high conductivity of ultrathin surface-adsorbed water films thus seems to be a rather general phenomenon. The well defined physicochemical properties of lipid bilayer surfaces allow systematic studies of the conduction mechanism of such thin water films.

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