Scanning Electrochemical Microscopy Studies of Electron Transfer through Monolayers Containing Conjugated Species at the Liquid−Liquid Interface

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The rates of electron transfer (ET) between two redox species through a monolayer of saturated dipalmytoyl phosphocholine and polyconjugated 2(3-(diphenylhexatrienyl)propanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine phospholipids adsorbed at the interface of two immiscible electrolyte solutions (ITIES) were measured by scanning electrochemical microscopy. Comparison of the ET rates shows that addition of phospholipids with conjugated hydrocarbon chains increases the ET rate by at least a factor of 2 compared to films with only saturated hydrocarbon chains. This difference was sufficiently high to obtain information about distribution of lipid molecules in monolayers formed by mixing two lipids. Lateral scanning of an ultramicroelectrode tip across the lipid monolayer comprised of two different phospholipids showed that each lipid forms microsize domains. The rate of ET across a monolayer of lipid adsorbed at the ITIES was also probed as a function of temperature. A sharp decrease in ET rate with temperature suggests a phase transition of the hydrocarbon chains of the lipid molecules. The phase transition increases the ET distance between the two redox centers with a resultant decrease in ET rate.

Introduction

The introduction of a phospholipid monolayer at the interface between two immiscible liquids decreases the rate of the electron transfer (ET) reaction between redox species confined to the different liquid phases. We show here that addition of a phospholipid with conjugated hydrocarbon chains increases the rate of ET, suggesting that these molecules either behave as electron shunts (or molecular wires) through the monolayer or disrupt the monolayer structure allowing closer approach of the reactants. Phospholipids comprise the major components of biological membranes and are responsible for their structural integrity. Because they represent simplified models of the biological membrane, phospholipid monolayers and bilayers have been intensely studied.[1] The amphiphilic nature and rich two-dimensional phase properties of phospholipids allow convenient assembly of well-ordered monomolecular films at interfaces. There is currently much interest in understanding the molecular interaction in organized, oriented membrane model systems.[2] In particular, the formation of domain structures within phospholipid monolayers has been an area of intense study,[1b] because domain formation implies lateral phase separation and partitioning of membrane function within microdomains of a defined structure. The molecular order and organization within phospholipid monolayers have been characterized by a variety of spectroscopic techniques including internal and external reflection infrared spectroscopy,[3a,b] scanning tunneling microscopy,[1c] atomic force microscopy,[4] glancing incident angle X-ray techniques,[5] ^{2}H NMR,[6] and fluorescence microscopy.[7] These techniques have been applied with great success to the study of monolayers at the air−water interface. Fluorescence microscopy has provided the most direct evidence for the formation of shaped, phase-separated regions as the films are compressed in phospholipid monolayers at the air−water interface.[7] The extension of these studies to the oil−water interface, however, is generally difficult.[8] The thickness of one of the two solutions has to be taken into account, and a beam probing the interface has to penetrate this thick liquid film. This creates a large background in X-ray or fluorescent studies, as well as interference and attenuation in IR microscopy, for example.

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The electrochemistry at the interface between two immiscible electrolyte solutions (ITIES) has been extensively studied over the past 2 decades,[9] and phospholipid monolayers at water/organic interfaces have been employed in studies of charge and ion transfer.[10,11] In the course of our work on the applications of scanning electrochemical microscopy (SECM),[12] we have demonstrated that SECM allows characterization of charge-transfer processes at liquid/liquid interfaces.[13] SECM was also used to investigate ET occurring at the ITIES via a bimolecular reaction between redox species confined to the two different solvents.[14] These studies showed that the ET rate was a function of the potential drop across the interface and that conventional ET theories[15] are applicable to the liquid/liquid interface. SECM has also been used to probe the kinetics of heterogeneous ET through a liquid/liquid interface covered with a monolayer of phospholipid.[16] The rate constant for electron transfer between two redox couples (one in each phase) through the monolayer was smaller than that at the unfilmed interface and depended on (i) the driving force given by the difference between the standard potentials of the two couples, (ii) the potential drop across the two ITIES, and (iii) the length of hydrocarbon chain of phospholipids adsorbed at the liquid/liquid interface. In this paper, we use the SECM at the ITIES to obtain more information about the effect of adding a second conjugated lipid to this monolayer.

Experimental Section

Chemicals. NaCl, $NaClO_4$, and $Na_4Fe(CN)_6$ from Johnson Matthey (Ward Hill, MA), 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine Zn (ZnPor) from Aldrich (Milwaukee, WI), and benzene (J. T. Baker Inc., Phillisburg, NJ) were used as received. Tetrahexylammonium perchlorate ($THAClO_4$; Fluka Chemika, Switzerland) was recrystallized twice from an ethyl acetate/ether (7:3) mixture and was dried under vacuum overnight at room temperature. Before measurements, the benzene solution containing 0.25 M $THAClO_4$ and 0.5 mM ZnPor was treated with at least twice its volume of pure water by vigorous shaking to make a water/benzene emulsion. This emulsion was centrifuged to separate the benzene solution from the aqueous layer. This procedure was repeated twice to remove any trace amounts of surfactants from the organic phase that might adsorb on the benzene/water interface. Chloroform solutions of symmetric saturated synthetic lipids (Figure 1A) (1,2-diacyl-*sn*-glycero-3-phosphocholine) with different numbers of methylene groups in the hydrocarbon chains (n = 10, dicaproyl phosphocholine (DCPC); n = 16, dipalmytoyl phosphocholine (DPPC), from Avanti Polar Lipids, Inc. (Alabaster, AL), were stored at −20 °C. The conjugated phospholipid 2(3-(diphenylhexatrienyl)propanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (DPHC) (Figure 1B) was obtained from Molecular Probes (Eugene, OR) and was dissolved in chloroform to form a stock solution of known concentration which was stored at −20 °C. Before the experiments, a flow of nitrogen was passed above a small volume (25 μL) of the phospholipid solution in $CHCl_3$ to evaporate the solvent completely. The lipid was then redissolved in 500 μL of a benzene solution containing 0.25 M $THAClO_4$ and 0.5 mM ZnPor. This new solution constituted the stock solution to be added in definite amounts to the benzene solution containing 0.25 M $THAClO_4$ and 0.5 mM ZnPor, which served as the organic phase in our experiments. All aqueous solutions were prepared from deionized water (Milli-Q, Millipore Corp.)

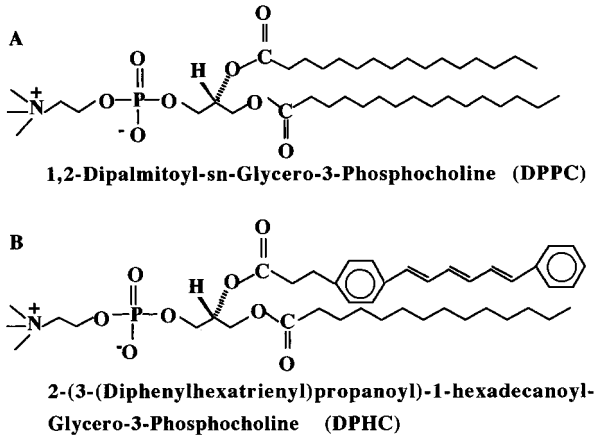

Figure 1. (A) Synthetic saturated phosphatidylcholine lipid DPPC. (B) Synthetic conjugated phosphatidylcholine lipid DPHC.

Electrodes and Electrochemical Cells. Pt wires (25 μm diameter) (Goodfellow, Cambridge, U.K.) were heat-sealed in glass capillaries to prepare SECM tips as described elsewhere.[17] The tip electrode was rinsed with ethanol and water and then polished and dried before each measurement. A three-electrode configuration was used in all experiments with all three electrodes (tip, auxiliary, and reference) placed in the top (organic) phase. The cell for the SECM/ITIES experiments was described previously.[14] An ionic bridge containing a NaCl + $NaClO_4$ solution served as a junction between the Ag/AgCl reference electrode and the benzene solution. In the measurements of the ET rates, the upper phase consisted of a 0.5 mM solution of ZnPor in benzene with 0.25 M $THAClO_4$ and 0−150 μM of the lipid or mixture of lipids. The bottom phase was a solution of 0.1 M NaCl, 0.1 $NaClO_4$, and 7 mM $Na_4Fe(CN)_6$ in water.

SECM Apparatus and Procedure. The SECM instrument has been described in detail previously.[18] Before SECM measurements, the 25-μm tip was positioned in the top phase and cyclic voltammetry was recorded. ZnPor shows two well-defined one-electron waves corresponding to the oxidation of ZnPor to $ZnPor^{\bullet+}$ and $ZnPor^{2+}$. The shape of the CV was not perturbed by the presence of lipids.[16]

Results and Discussion

As in previous SECM studies[14,16] of electron transfer through the ITIES, two redox couples, each dissolved in

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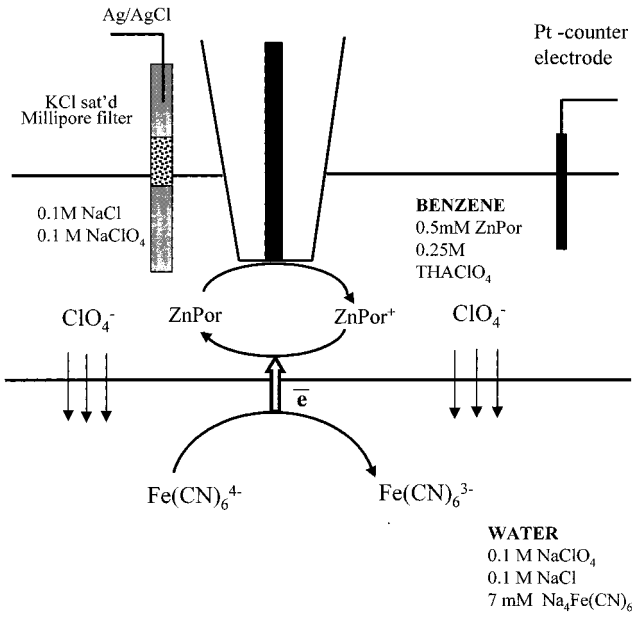

Figure 2. Schematic diagram of SECM measurements in the feedback mode of the kinetics of ET between $ZnPor^{\bullet+}$ in benzene and $Fe(CN)_6^{4-}$ in water. Electroneutrality was maintained by the transfer of perchlorate ions across the interface. The ITIES is modified by a monolayer of phospholipids which is represented here as a thick line.

one phase, were used. ZnPor/$ZnPor^{\bullet+}$ was dissolved in benzene in the upper, organic phase (o), and $Fe(CN)_6^{4-}$/$Fe(CN)_6^{3-}$ was dissolved in water in the lower, aqueous phase (w). The evidence from previous studies of the system is that there is no appreciable solubility of $Fe(CN)_6^{4-/3-}$ in benzene, nor of $ZnPor^{0/+}$ in the water phase. In all these experiments, a tip ultramicroelectrode (UME) with radius a was placed in the upper phase and held at the potential for the first oxidation of ZnPor to produce the cation radical $ZnPor^{\bullet+}$ (Figure 2):

$$ZnPor - e \rightarrow ZnPor^{\bullet+} \qquad E^\circ = 0.9 \text{ V vs Ag/AgCl (tip)}$$

$$ZnPor^{\bullet+}(o) + Fe(CN)_6^{4-}(w) \rightarrow ZnPor(o) + Fe(CN)_6^{3-}(w) \text{ (ITIES)}$$

In the absence of any reaction at the ITIES to regenerate ZnPor, the current decreases as the tip approaches the interface, which serves to block diffusion of ZnPor to the tip. This yields an approach curve of i_T vs d, which is the same as that for a tip approach to an insulating interface.[12] In the presence of an ET reaction at the ITIES, ZnPor is generated at the interface and diffuses back to the tip to produce larger currents (positive feedback). Thus, in the presence of $Fe(CN)_6^{4-}$ in the aqueous solution, $ZnPor^{\bullet+}$ diffuses to the ITIES where it is reduced back to ZnPor. The approach curves were obtained by moving the tip toward the liquid/liquid interface and recording the UME tip current, i_T, as a function of d with $d = 0$ taken as the position of the sharp increase in tip current when the tip touched the ITIES, showing direct oxidation of $Fe(CN)_6^{4-}$ at the tip.[14]

In previous experiments,[16] it was shown that a monolayer of phospholipids adsorbed at the ITIES significantly decreased the ET between the two separated redox centers. The most reasonable interpretation of this behavior was that the monolayer, in the presence of excess lipid in the benzene, acts as an insulating layer and increases the distance over which ET occurs between $Fe(CN)_6^{4-}$ and

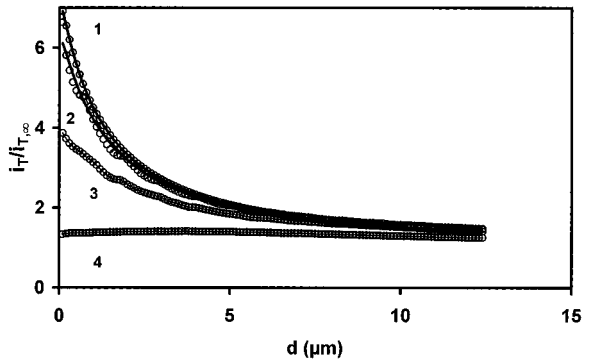

Figure 3. Effect of lipid concentration on the shape of the normalized SECM current−distance curves. Aqueous solution was 0.1 M NaCl + 0.1 M $NaClO_4$ + 7 mM $Na_4Fe(CN)_6$. The concentration of DPPC lipid in benzene was (1) 0, (2) 2, (3) 15, and (4) 100 μM. Circles are experimental points. Solid lines represent the theory for k values of (1) 0.033, (2) 0.030, (3) 0.018, and (4) 0.005 cm/s. The tip approached at 1 μm/s. $i_{T,\infty}$ is the current for a tip far from interface.

$ZnPor^+$. In this paper, we studied the enhancement of ET through the lipid monolayer by the introduction of conjugated molecules into this lipid monolayer and made a subsequent step toward a system with bioactive molecules embedded into lipid bilayer of membrane cells. The polyconjugated phospholipid DPHC was chosen as a simple model of such conductive molecules. This phospholipid presents a *trans,trans,trans*-1,6-diphenyl-1,3,5-hexatriene chain, which is the prototype of polyene systems and has previously been used as a fluorescent probe[19] because it has an intense relatively long-lived emission that is sensitive to its environment. It is relatively compatible with membranes because of its nonpolar hydrocarbon structure. It could also serve as an electron relay through the blocking monolayer via electron delocalization through the conjugated chain. In addition to its conjugated chain, DPHC also contains one saturated C16 alkyl chain which interacts well with the DPPC molecules of the blocking monolayer (Figure 1B). However, the DPHC could also disrupt the monolayer of DPPC, and this could also affect the ET rate.

ET through a Pure DPPC Monolayer. The amphiphilic phospholipid molecules dissolved in the organic phase spontaneously form a monolayer film at the water/benzene interface. The orientation of the monolayer is such that the hydrophilic head is immersed in water solution and the hydrophobic tails are directed toward the organic phase. They progressively induce a blocking effect of the interface as their concentration increases in solution; the area of the interfacial film grows[16] and a dramatic decrease in the rate of interfacial ET between the two redox couples, signaled by a decrease in the feedback current, occurs as shown in Figure 3. Although the electron-transfer rate decreases markedly with increasing concentration of lipid, it does not vanish completely and reaches a limiting value higher than that seen at an insulating interface; beyond a given point, on the order of 50−100 μm, the rate does not change further for higher lipid concentrations. This point is taken to signal the formation of a complete DPPC monolayer.[16] The $i_T/i_{T,\infty}$ vs d curve under these conditions can be used to determine the rate constant for electron transfer, k.

ET through a Pure DPHC Monolayer. The same kind of experiment, with a progressive increase of lipid

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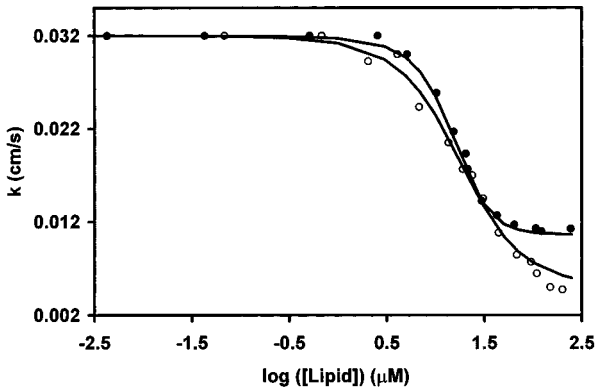

Figure 4. Heterogeneous electron-transfer rate constant, k, as a function of the concentration of the phospholipids (●) DPHC and (○) DPPC. Lines drawn through the points are for clarity.

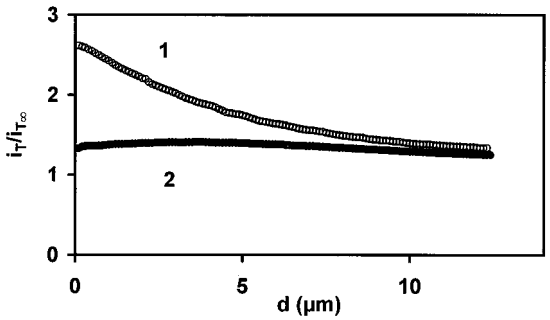

Figure 5. Approach curves for a full coverage (100 μM) of DPHC (curve 1) and DPPC (curve 2). A good theoretical[14] fit to the experimental points was produced with k = (1) 0.012 and (2) 0.005 cm/s. The tip approached at 1 μm/s.

concentration in benzene solution, was carried out with DPHC (Figure 4). The electron-transfer rate decreased with concentration of DPHC, but to a lesser extent than in the DPPC case. The limiting ET rate was 2.4 times larger than that obtained for DPPC, showing a better ET through this lipid monolayer (Figure 5). One can account for this result in several ways. One possibility is that ET occurs more readily through the delocalized conjugated chain of the DPHC. If this is so, the ET process would be quite efficient, given that only half of the chains consist of this conductive conjugated assembly. Alternatively, the structure of the DPHC layer might allow closer approach of the reactants, for example, greater penetration of $ZnPor^+$ into the phenyl-rich regions of the DPHC chains. This DPHC lipid allows about the same electron-transfer rate as that previously observed for the much shorter DCPC. The difference in the values of ET rates for saturated and conjugated hydrocarbon chains of adsorbed phospholipids provides a method to probe the distribution of lipids in a monolayer made with a mixture of phospholipids.

ET through Mixed Monolayers (DPPC and DPHC). Saturation of DPPC with Addition of DPHC. In a second series of experiments, the ET rates were measured at a monolayer of one phospholipid adsorbed at the ITIES while another phospholipid was added to the benzene solution. This can provide information about (i) the ability of a phospholipid to embed into a preexisting monolayer of the other lipid at the ITIES and (ii) the effect of such modifications of the layer on the rate of electron transfer.

At full coverage of the interface with DPPC, electron transfer through the interface is relatively slow (k = 0.005 cm/s). The progressive increase of the concentration of the conjugated phospholipid (DPHC) in the organic phase induced an increase in the magnitude of the rate constant

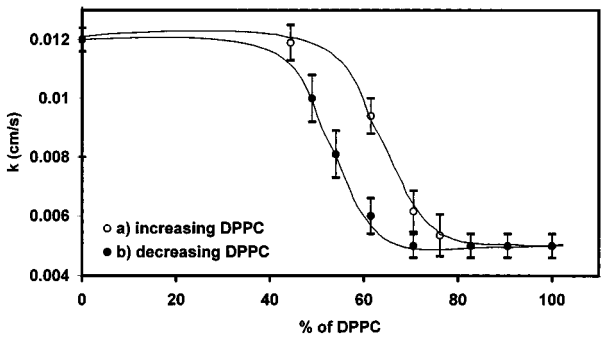

Figure 6. Variation of k at the ITIES through a monolayer made of a mixture of phospholipids. (a) After full coverage with DPHC, increasing amounts of DPPC were added to the solution. (b) After full coverage with DPPC, increasing amounts of DPHC were added to the solution. Other experimental details as in Figure 2. Standard deviations are measured at each ratio for at least four different approach curves. Lines drawn through the points are for clarity.

(Figure 6, curve b). The increase in k was not continuous with increasing DPHC concentration. No change in k was noted until a threshold amount of 30% DPHC had been added, then k increased until it reached a constant value at 55% DPHC.

Saturation of DPHC with Addition of DPPC. Essentially the same behavior was observed when increasing amounts of DPPC were added to a solution in the presence of a monolayer of DPHC (Figure 6, curve a). At full coverage with DPHC (the more conductive phospholid) k = 0.012 cm/s, and at 65% DPPC, we observe a strong decrease in k. The values of k are different at a given fractional amount of DPHC on the rising (or falling) portion of the curves. This hysteresis behavior suggests that the monolayer film does not attain a true equilibrium composition in these experiments during the 15 min allowed following each concentration change. This is consistent with a rather slow process for lipid exchange in an interfacial layer.[10,16,20]

ET through a Monolayer Prepared from a Mixture of DPPC and DPHC. In another series of experiments, the monolayer was prepared from mixtures of DPPC and DPHC at different total lipid concentrations. In these experiments, the stock solution of ZnPor in benzene (0.25 M $NaClO_4$) also contained the two lipids in different proportions. Mixtures of phospholipids at ratios of 50/50 and 70/30 DPPC/DPHC were prepared and added in small amounts to increase progressively the total concentration of lipids in the organic phase. The results are shown in Figure 7 along with the results obtained for pure DPPC and DPHC. The trend for the variation of k as a function of increasing concentrations of the mixtures of phospholipids was generally the same as that in Figure 6. The inset in Figure 7 is an expanded view for concentrations of added lipids greater than 40 μM. Each k value is the average obtained of at least four different approach curves to the interface for each concentration and uncertainties are based on at least four different approach curves (the results for the 50/50 mixture have been omitted from the inset for clarity). An equilibration time of ~15 min was used between each new addition. The general trend, whether the phospholipids were pure or in mixtures, was that the electron-transfer rate decreased at a total lipid concentration above ~4 μM and reached a limiting value at a total concentration above ~0.10 mM. The rates for

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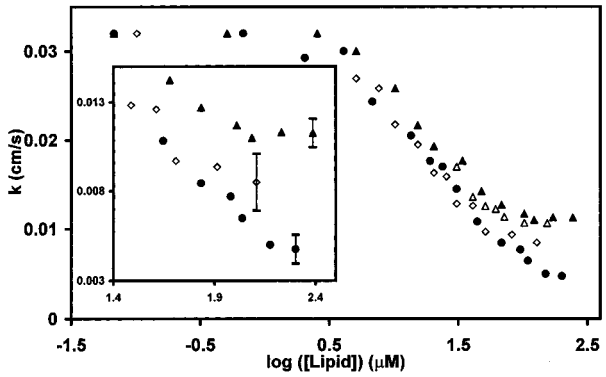

Figure 7. Change of k (cm/s) with increasing concentration of phospholipid in solution (●) DPPC only, (▲) DPHC only, (◇) 70/30 DPPC/DPHC, and (△) 50/50 DPPC/DPHC. Experimental conditions as in Figure 3. Inset shows an expanded view of the concentration range close to full coverage of the interface. The 50/50 curve was omitted from the inset for clarity. Standard deviations shown were obtained at each ratio from at least four different approach curves and three different preparations of monolayer.

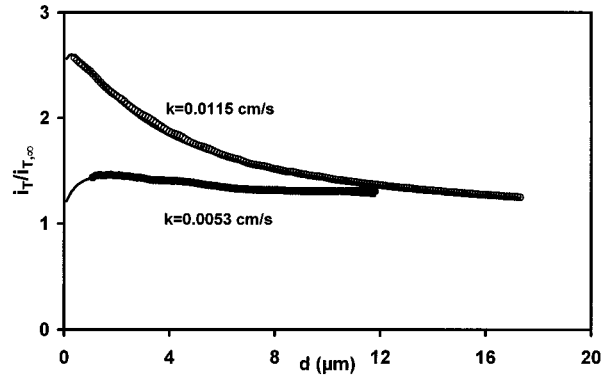

Figure 8. Two different approach curves obtained on the same ITIES formed from a mixture of 70 mol % DPPC and 30 mol % DPHC at a total lipid concentration of 62 μM. The two curves were taken ~10 min apart.

mixtures were intermediate between those of pure samples. We interpret these results to indicate that below 4 μM the interfacial lipid molecules do not form a sufficiently organized monolayer to block ET, and the fractional surface coverage is low. Above 0.15 mM, a complete and relatively leak-free monolayer exists. As described in the next section, the values for k deduced with mixtures varied significantly and those shown in Figure 7 are averages over a number of approach curves.

Effect of Lateral Tip Position for Lipid Mixtures. While the approach curves for monolayers of pure DPPC or pure DPHC were reproducible for repeated approaches and yielded k values that agreed within a standard deviation of ±0.0008 cm/s, those for monolayers prepared from mixtures of DPPC and DPHC showed larger variations. For example, Figure 8 shows two of the different approach curves recorded on a monolayer formed with a 62 μM mixture of 70% DPPC and 30% DPHC that was prepared for imaging (vide infra). Care was taken before this set of measurements to wait until the equilibrium conditions were attained. For this concentration ratio, the set of curves showed a wide spread of k over a number of measurements. Indeed, the individual values of k shown for the curves presented in Figure 8 are very close to those of pure DPPC or pure DPHC. An explanation of this behavior is that the two phospholipids are segregated into separate DPPC and DPHC domains and the observed response depends on which type of domain the UME tip

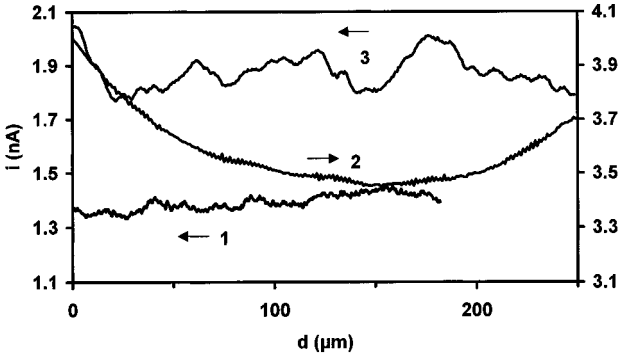

Figure 9. Images of the ITIES obtained by scanning the UME tip along one axis parallel to the interface: (1) current stability far from the interface; (2) bare interface with pronounced meniscus; (3) ITIES covered with 70/30 DPPC/DPHC at a total lipid concentration of 62 μM.

was positioned above as it approached the interface. To see this type of behavior, the domain sizes had to be larger than the tip diameter of 25 μm.

SECM Imaging of Domains in DPPC/DPHC Monolayers. The nature of the approach curves in the mixed monolayers suggests the existence of segregated domains of DPPC and DPHC at the ITIES. To obtain direct evidence of the presence of such domains, the SECM tip was scanned across the monolayer (X or Y axes) parallel to the interface. The current response for the oxidation of ZnPor (1.5 mM) at +0.95 V vs Ag/AgCl in the bulk benzene (far from the interface) was stable and constant with X and Y positions (curve 1, Figure 9). The UME was moved close to the interface (within a distance of 3−5 μm, as estimated from the approach curve and the nature of the domain located below the tip) and was scanned laterally above the interface at 5 μm/s over a distance of 250 μm. A bare interface and interfaces covered with monolayers prepared from solutions of 60 μM of each phospholipid alone were recorded as controls for the response for monolayers formed from a mixture of lipids. The current fluctuations with the controls were within the range 5−10% (similar to those shown in curve 1, Figure 9). The response with a bare interface was smooth, showed no large variations, and followed the meniscus (curve 2, Figure 9). The interfaces covered with a monolayer of a single phospholipid (DPPC or DPHC) were also flat and smooth. Monolayers of a mixture of lipids (50/50 or 70/30 DPPC/DPHC (curve 3, Figure 9)) showed much stronger fluctuations of the current. Care was taken in these scans to make sure that the UME tip did not touch the interface during tip movement. The scan of the tip along the interface allowed observation of zones corresponding to a higher current response and thus a larger k value as compared to other zones. We take the former to correspond to domains rich in DPHC, where there is a higher ET rate through the interface, and the latter to correspond to domains rich in DPPC. The sizes of the domains estimated from curve 3 of Figure 9 are in the range of 10−30 μm. This size is in good agreement with domain sizes found with fluorescent probe microscopy for DPPC adsorbed at the air/water interface.[8] The observed gradual transitions at the zone boundaries are caused by the size of the electrode (12.5 μm radius, which is comparable with domain size), the scan rate used for the experiment, and the distance that separates the tip and the interface. There is also the possibility of some lateral motion of the domains at the interface. To address this point, the interface was imaged using forward (Figure 10, curve 1) and reverse (Figure 10, curve 2) scans at the same spot of the interface. The interface showed reproducible behavior within the

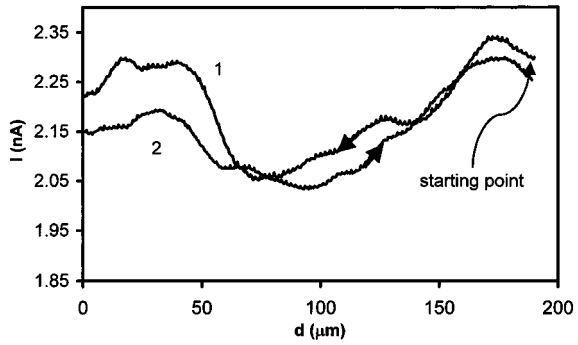

Figure 10. Images of the ITIES obtained by scanning the UME tip along one axis parallel to the interface covered with 70/30 DPPC/DPHC: forward scan (curve 1) and backward scan (curve 2) showing the reproducibility of the method within a recording time of 2 min.

time scale of the experiment, so we conclude that only small changes and movement of the monolayer occur during experiment times of less than 2 min.

Effect of Temperature on Interfacial ET Rate. To study whether the method is capable of detecting phase transitions in a phospholipid monolayer at the interface, k was measured as a function of temperature. It is well-known that lipid monolayers adsorbed at the air/water interface can undergo a phase transition as a function of surface pressure or temperature.[10,21] Different types of phases have been proposed for lipid molecules adsorbed at the air/water interfaces: gas, liquid-flexible, liquid-extended, solid-tilted, and solid-vertical.[10a] Each of these phases is characterized by its own area per lipid molecule, conformation, and tilt angle of hydrocarbon chain. Transformation of a lipid monolayer from one phase to another should change the separation distance between two redox species dissolved in the contacting solutions and hence the ET rate. The temperature dependences of the ET rate for monolayers of DCPC and DPPC are shown in Figure 11 over the temperature range of 5−25 °C. Lowering the temperature below 5 °C caused solidification of the benzene solution. Over the range 15−25 °C, the value of the ET rate for DCPC decreased only slightly (Figure 11, curve a). However, there was a sharp drop in k when the temperature was decreased to 10 °C. The decrease on cooling to 5 °C was small and the slope in the range of 5−10 °C was about the same as that in the 15−25 °C range. The expected behavior of ln k as a function of $1/T$ would be a straight line with a slope corresponding to the activation energy of the ET process (eq 1) as long as the properties of the monolayer remain constant.:

$$k = A \exp(-\Delta G^{\ddagger}/RT) \qquad (1)$$

where A is a preexponential factor and $\Delta G^{\ddagger}$ is the free energy of activation. The sharp decrease of the ET rate between 15 and 10 °C may indicate a phase transition of the DCPC lipid monolayer at the ITIES. The observed temperature range for this drop in the ET rate is in good agreement with the data obtained for phase transition from impedance measurements.[21a] In comparison, the curve for a monolayer of DPPC at the interface (Figure 11, curve b) shows no transition of this kind over the temperature range of 25−5 °C, although the slight decrease in the value of k in the temperature range of

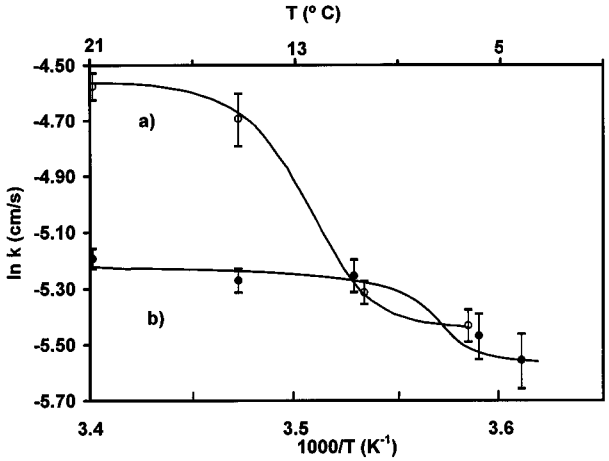

Figure 11. Change of the rate constant of electron transfer between $ZnPor^{\bullet+}$ and $Fe(CN)_6^{4-}$ through an ITIES completely covered with (a) 60 μM DCPC and (b) 110 μM DPPC as a function of the temperature. Temperatures were regulated within ±0.1 °C. Standard deviations are calculated from at least four different approach curves. Lines drawn through the points are for clarity.

10−6 °C might indicate some greater organization of the DPPC alkyl tails. However, the effect is much smaller than that for DCPC, confirming, as suggested previously,[8] the greater induced disorder for the long alkyl chain of DPPC adsorbed at the ITIES and the organic phase screening effect of intermolecular interactions.

Conclusions

The SECM can be used to investigate ET occurring at a monolayer of phospholipids adsorbed at the ITIES via a bimolecular reaction between two redox centers confined to different solutions.[16] When two different types of lipids (e.g., phospholipids with saturated and polyconjugated hydrocarbon chains) were used to form a monolayer, the observed ET rate was at least two times faster through films with conjugated chains compared to those with saturated chains. Whether this involves better ET through the conjugated chains or a different structure of the monolayer allowing clear approach of the reactants will require further experiments with a wider range of phospholipids. These studies suggest that ET between redox centers of biological molecules and with layers containing enzymes, DNA, or pore-forming molecules embedded into a lipid monolayer at the ITIES can be probed by SECM. SECM studies of ET across an ITIES should allow one to probe the factors, such as separation distance and potential drop between redox centers, that affect ET.

UME lateral scanning across monolayers formed from different lipids clearly shows that mixed monolayers contain domains with sizes in the tens of micrometer range at the ITIES. To our knowledge, this is the first experimental evidence of such domain formation at the liquid/liquid interface, although domain formation at the air/water interface has been observed by the fluorescent probe technique.[8]

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