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Ion-Induced Interfacial Dynamics of Phospholipid Monolayers

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Ion-induced interfacial dynamics of phospholipid monolayers were studied by various electrochemical techniques. The lipid monolayers were constructed by using the mercapto derivatives of natural lipids that were self-assembled directly onto gold electrode surfaces in a tails-down fashion. The supported lipid assemblies appeared to act as rather effective electron-tunneling barriers with $K_3Fe(CN)_6$ as the redox probe, despite a relatively low surface coverage and/or a disordered surface structure. Upon the stimulation by alkaline-earth ions, the lipid layers appeared to undergo surface reorganization, exposing part of the electrode surface which resulted in the formation of microscopic mass-transfer lipid channels. The dimensions and/or the number of these channels increased with increasing ion concentrations, and this ion-gate effect appeared to be quite selective, with the most pronounced effects observed among the series of alkaline-earth ions with Ca^{2+}.

Biological cell membranes serve as the functional interface between the intracellular and the extracellular domains. They play a fundamental role in numerous biological processes by, for instance, providing a physical and biochemical compartmentalization, providing regions for organization of the energy transduction mechanism, and acting as sites for enzymatic reactions.[1] Investigations of the biomembrane interfacial dynamics are crucial in understanding the molecular origin and mechanism of these biological events, such as membrane fusion, adhesion, and cell−cell recognition, which are generally initiated at the membrane "defects".[1]

There has been extensive interest in employing simple chemical systems to model biological cell membranes, including, for instance, liposomes/vesicles, micelles, and monomolecular films.[1,2] Among these, due to the relative ease of fabrication and the requirement of only a small quantity of materials, monomolecular films, in particular, of natural phospholipid molecules, have been used quite extensively as biomembrane models.[1−8] Lipid monolayers are generally formed at the air/water interface using the Langmuir−Blodgett (LB) method and then are transferred onto a *planar* substrate surface, typically with a preformed organic thin-film interlayer, forming a surface composite structure.[2−6] However, previous works were mainly focused on lipid assemblies that were fabricated by depositing the lipid layers onto the supporting substrates (with the organic adhesion layer) at a relatively high surface pressure, where the resulting lipid layers are consequently very compact and ordered with few defects; whereas at low surface pressures, the conformation of the deposited films might differ from that on the air/water interface depending on the specific substrates.[5] Additionally, the interactions between the lipids and supporting underlayers are relatively weak, involving mainly hydrophobic and/or electrostatic interactions, where the structural integrity and mechanical stability of the lipid assemblies could sometimes be compromised. This sometimes renders the study of lipid interfacial dynamics rather difficult.

Direct studies on lipid monolayers at the air/water or oil/water interface (again, by the LB method) do offer the benefits of easy variation of surface pressure and hence lipid conformations, such as the coexistence of binary phases.[2−6] However, detection and measurements of transmembrane mass-transfer in these systems are technically challenging.

Self-assembling provides an efficient alternative to the construction of phospholipid monolayers directly onto an electrode surface, with membrane defects formed "naturally" during the self-organization process. Additionally, the lipid molecules are attached to the substrates via relatively strong linkages, e.g., covalent bonding or sorptive interactions, which help stabilize the lipid

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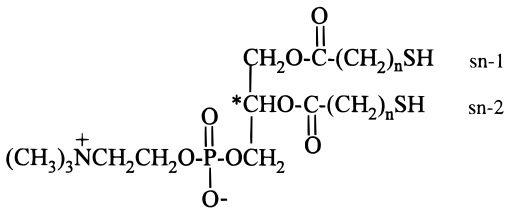
assemblies significantly.[7,8] Also, by bringing the lipids in direct contact with an electrode surface (without the dielectric insulating layer), one can achieve better potential control and signal detection. This is particularly important in electrochemical investigations of membrane interfacial structural transitions, which can be amplified as detectable electrochemical responses (e.g., current, impedance, etc). As phospholipid molecules are amphiphilic with a charged/dipolar headgroup, and the structure and properties of the lipid assemblies are largely dependent upon their charged states, electrochemistry, among the many techniques available,[1−8] has proven to be an effective tool in investigating the dynamics in structural transitions of supported phospholipid assemblies.[7]

Experimentally, by taking advantage of the strong affinity of thiols on noble transition metal (e.g., gold, silver) surfaces, thiol-derivatives of natural phospholipids can be custom-made and these can then be self-assembled onto electrode surfaces to form mechanically and macroscopically stable surface-adsorbed layers, akin to monomeric alkanethiols that have been well-studied.[9] Various electrochemical and surface-sensitive techniques can then be employed to elucidate the interfacial structure and dynamics of these lipid assemblies.[1−8]

In this paper, we describe electrochemical studies of and the construction of lipid monolayers, the latter of which is done by the spontaneous self-assembling of thiols onto gold surfaces. Specifically, the lipid molecules are custom-tailored with thiol groups attached to the apolar tail termini. When a cleaned gold electrode surface is exposed to the lipid solution, a lipid monolayer can be readily formed in a tails-down fashion. As the lipid molecules are immobilized on the substrate surface by chemisorption, the mechanical stability of the adlayers is significantly strengthened, which is one of the striking differences from other lipid monolayer systems.[2−7,8b] In addition, thanks to the strong affinity of thiols to a variety of transition metal surfaces,[9] the lipid assemblies can, in principle, be easily constructed on different electrodes, where chemical sensing might be achieved by the specific electrode materials. To the best of our knowledge, there has been no prior electrochemical investigation of these relatively novel lipid assemblies.

Upon the stimulation by inorganic divalent ions (e.g., alkaline-earth ions), the lipid assemblies appear to undergo interfacial reorganizations, leading to the formation of ion-gated lipid channels. Previous works on transmembrane mass transfer have been mainly focused on membrane ion-channels which consist of special proteins, polypeptides or their synthetic analogues that are embedded in the lipid membranes.[10−12] The channel opening/closing can be controlled either internally (involving chemical messengers) or externally (such as lights, electrical potentials). However, ion-induced formation of lipid channels has not received much attention and results on this end are somewhat inconclusive.[13] It has been speculated that the formation of mass-transfer channels might be one of the intrinsic properties of lipid membranes, whose biological significance has yet to be defined.[13] This paper represents our continuing efforts geared at gaining a better understanding of the relationship between the lipid structure and the interfacial dynamics involved.

Scheme 1. Chemical Structure of ω-Thiolated Phospholipids



EXPERIMENTAL SECTION

Materials. Tris(hydroxymethyl)aminomethane (Tris, Aldrich, 99%), 11-mercaptoundecanoic acid (MUA, Aldrich, 95%), 4-(dimethylamino)pyridine (Aldrich, 99%), dithiothreitol (ACROS, 99%), Aldrithiol (Aldrich, 98%), *N*,*N*′-dicyclohexylcarbodiimide (ACROS, 99%), ethylene-diaminetetraacetic acid (EDTA, disodium salt dihydrate, 99+%, Aldrich), potassium ferricyanide ($K_3Fe(CN)_6$), and $CaCl_2$ (both from ACROS) were used as received. L-α-glycerophosphorylcholine/$CdCl_2$ (Sigma, 99%) was dried overnight at 50 °C in vacuo. NaCl (ACROS) was recrystalized twice prior to use. All solvents were purchased from typical sources and used as received, except for chloroform which was freshly distilled over phosphorus pentoxide prior to use for lipid synthesis. Water was supplied from a Barnstead Nanopure system (18.3 MΩ cm).

Lipid Synthesis and Monolayer Formation. The mercapto derivatives of phospholipids were synthesized by following a literature procedure,[8a] where two ω-thiolated fatty acids were attached to the *sn-1* and *sn-2* positions of the glycerol backbone via ester linkages, resulting in two thiol groups at both termini of the apolar tails (Scheme 1). The final products were characterized by proton NMR spectroscopy, which showed consistent spectra with literature results.[8a] The self-assembled monolayers (SAMs) of the thiol-terminated phospholipids were constructed by exposing a cleaned gold disk electrode into a 1 mM chloroform solution of the desired phospholipid for ~5 days. The gold electrode (area 0.85 mm^2), constructed by sealing a gold wire in a soft glass tubing, was first polished with 0.05-μm Al_2O_3 slurry (from Bruehler) then rinsed thoroughly with dilute H_2SO_4, Nanopure water, and ethanol, subsequently, followed by electrochemical etching in 0.5 M H_2SO_4 by cycling electrode potentials within −0.4 to +1.2 V until a reproducible voltammetric response was

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obtained. The time for the formation of a stable lipid adlayer is somewhat longer than that for formation of monomeric alkanethiols/arenethiols,[9] possibly due to the more complicated molecular structures of the lipid adsorbates where two anchoring sites were involved; however, further extended deposition time did not appear to improve the film quality very much. In the case that divalent ions were introduced into the lipid solution during self-assembling, the lipid was dissolved in methanol (again, at 1 mM), whereas the deposition time was kept the same (5 days). In either case, the lipid-modified electrodes were then rinsed with copious solvents (chloroform or methanol), to remove loosely bound ligands, and dried in a N_2 stream before being transferred to a cell for electrochemical measurements. In the absence of the divalent ions, the voltammetric responses of the electrode-supported lipid assemblies prepared from the chloroform or from the methanol solution were virtually indistinguishable.

In the study of ion-assisted surface tailoring, the lipid-modified electrode was first treated in a 100 mM solution of Ca^{2+} and subsequently incubated into a 1 mM solution of *N*-methyl-*N*′-(10-mercaptodecyl)viologen dihexafluorophosphate ($MMDV^{2+}(PF_6^-)_2$, synthesized by following a literature procedure[14]) in acetone for varied periods of time. Again, the electrode was then rinsed with copious acetone and water before being transferred into an electrolyte solution for electrochemical measurements.

Electrochemistry. Electrochemical measurements were carried out with a BAS Electrochemical Workstation 100B/W, with Ag/AgCl (3 M NaCl, from BAS) as the reference electrode and a platinum coil as the counter electrode. The solution was degassed with N_2 for ~20 min prior to data acquisition and was blanketed with a N_2 atmosphere during the entire experimental period. Electrode double-layer capacitance was evaluated by ac impedance measurements using an EG&G 1025 Frequency Response Analyzer and an EG&G potentiostat/galvanostat (model 283). Impedance spectra were collected in the frequency range from 1 Hz to 100 kHz with a 10 mV ac amplitude and varied dc voltage bias, and the spectra were fitted to an equivalent circuit by using a commercial program from EG&G.

RESULTS AND DISCUSSION

In this section, we begin with a description of the electrochemical characterization of the lipid self-assembled monolayers on gold electrode surfaces, and follow that with a description of investigations of effects of inorganic divalent ions on the transmembrane mass transfer using redox-active $K_3Fe(CN)_6$ as the probe molecule. Discussions of a possible reorganization (conformational change) mechanism of the dynamic transitions will also be presented.

Self-Assembled Phospholipid Monolayers. We take 1,2-bis-(11-mercaptoundecanoyl)-*sn*-glycero-3-phosphocholine (abbr. PC-2C11SH) as the illustrating example. Figure 1 shows the cyclic voltammograms of a gold electrode with and without the lipid adlayer in 0.1 M NaCl (pH 7.93, buffered by 0.010 M Tris-HCl). One can see that (i) the double-layer charging current at the lipid-modified electrode decreases quite significantly, compared with that for the same bare electrode, consistent with the formation of a dielectric lipid monolayer on the electrode surface and (ii) the

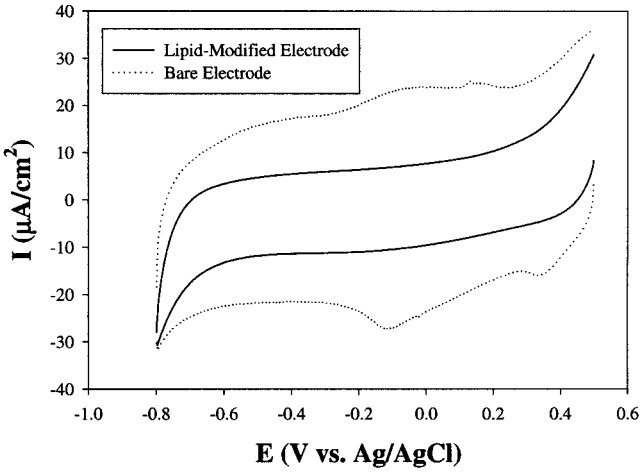

Figure 1. Cyclic voltammograms of a gold electrode with and without a lipid (PC-2C11SH) monolayer in 0.10 M NaCl (pH = 7.93 buffered by 0.010 M Tris-HCl). Potential sweep rate 100 mV/s. Electrode area 0.85 mm^2.

structure of the lipid SAM was relatively insensitive to electrode potentials within the potential range of −1.0 to +0.6 V (vs Ag/AgCl) (see Supporting Information), demonstrating mainly featureless double-layer charging responses. Previous studies[7] on lipid layers that were *physisorbed* onto a mercury electrode surface showed that the lipids underwent two rapid and reversible potential-induced structural reorientations, manifested by two voltammetric current waves at very negative potentials (around −1.0 V). These were interpreted on the basis of the postulation that some of the "active" lipid molecules, when induced by electrode potentials, flipped from a tails-down fashion to a head-down fashion on the electrode surface, followed by desorption from the electrode surface and formation of a partial bilayer structure, exposing part of the electrode surface.[7] However, it should be noted that in these studies the structure of the phospholipid layers supported on the mercury electrode surface was somewhat ambiguous. Nonetheless, this observed discrepancy of electrode-potential dependence might be, at least in part, ascribed to the strong *chemisorptive* interactions of thiolated phospholipids on gold, which hinder the "vertical" structural transitions[7] of the surface-immobilized lipid molecules.

The lipid SAMs obtained above did not appear to be fully packed, as revealed by ac impedance and voltammetric measurements (more details below) which showed an effective electrode double-layer capacitance of about 10 μF/cm^2, much greater than that expected for an ordered and compact lipid monolayer or an alkanethiolate self-assembled monolayer (SAM) of similar chain-length (~2 μF/cm^2).[9a] However, abundant existence of monolayer defects (pinholes) seemed to be ruled out, as shown below by measuring the electrochemistry of a redox probe (vide infra). Therefore, this seems to suggest that the electrode-bound lipid molecules might be very disordered or tilted (i.e., low surface coverage), which could be partly attributed to the less than optimal conformations of the two apolar tails (as both tails are active anchoring sites; however, self-assembled monolayers of *sn-2 mono*thiolated phospholipids showed rather reproducible electrochemical responses). However, simply prolonging SAM deposition time did not result in substantial improvement of the film quality.

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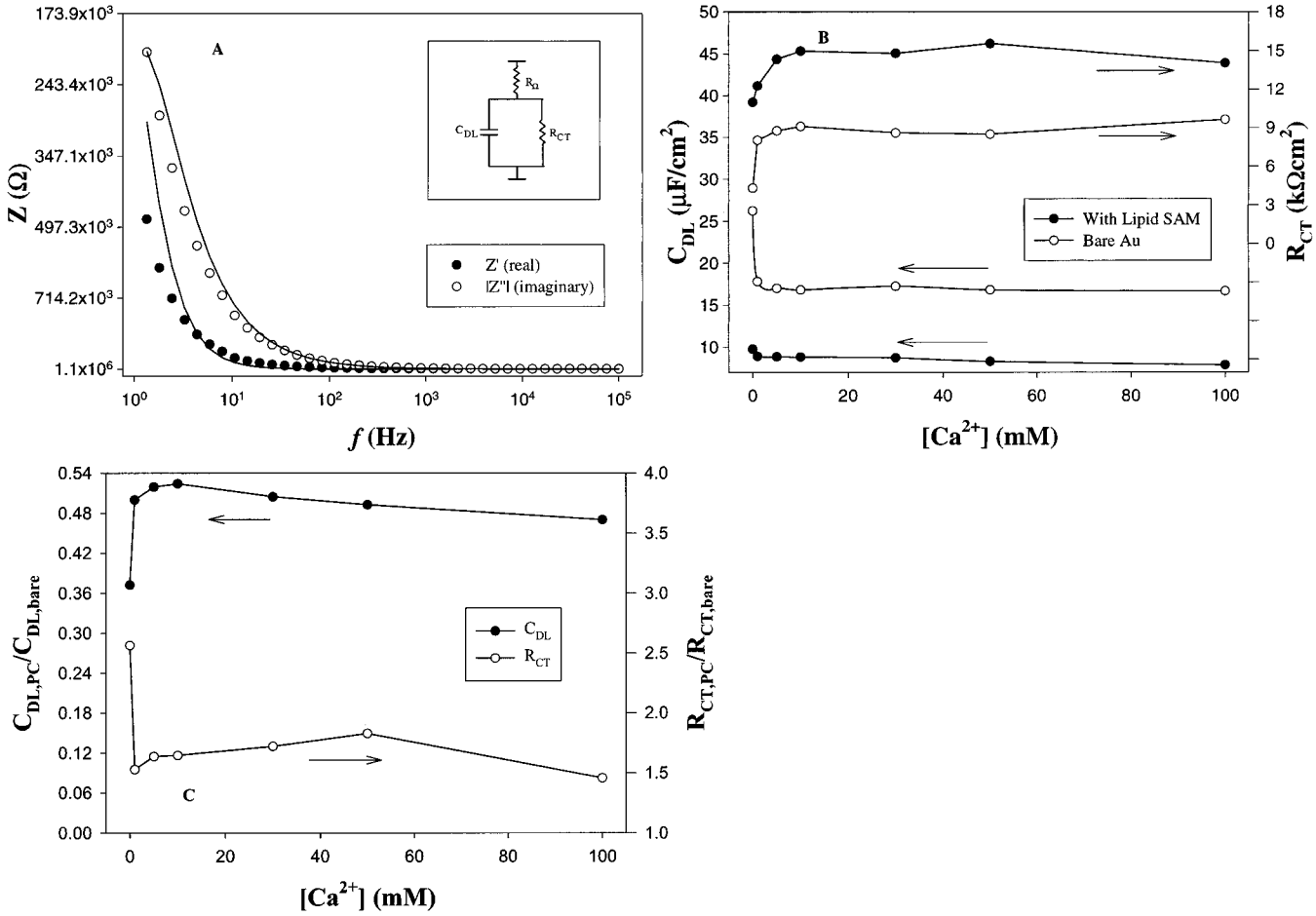


Figure 2. (A) Representative impedance spectra (symbols) of a lipid-modified electrode (conditions same as in Figure 1) at $[Ca^{2+}] = 5$ mM. Frequency range from 1 to 100 kHz, ac amplitude 10 mV, dc potential bias −0.2 V. The spectra were fitted (solid lines) by the equivalent circuit shown in the inset. (B) Variation of electrode interfacial capacitance (C_{DL}) and charge-transfer resistance (R_{CT}) with Ca^{2+} concentration which were determined from (A). (C) Variations of the ratios of C_{DL} and R_{CT} in the presence and absence of lipid adlayers with Ca^{2+} concentration.

It turns out that it is this unique lipid conformation that gives rise to the interesting interfacial dynamic behaviors, as detailed below.

Ion-Induced Interfacial Dynamics. It has been found that the structures of phospholipid assemblies are very sensitive to certain inorganic (divalent) ions such as alkaline-earth ions, the so-called condensing effect,[1] where the divalent ions specifically bind to the phosphate moieties of the polar heads of two neighboring lipid molecules. The resulting conformational (structural) change within the lipid adsorbed layers can then be readily monitored by electrochemical and impedance measurements. Using Ca^{2+} as the representative example, we carried out a series of impedance spectroscopic experiments to investigate the variation of electrode double-layer capacitance (C_{DL}) with the ion concentration at a lipid-modified and naked electrode, respectively. Figure 2A shows the representative impedance spectra (symbols) of a lipid-modified Au electrode in 0.1 M NaCl containing 5 mM Ca^{2+} that were fitted (solid lines) by the equivalent circuit shown in the inset. Here the lipid adlayers were modeled as a simple RC circuit with R_Ω denoting the solution uncompensated resistance and R_{CT} and C_{DL} the *effective* charge-transfer resistance and double-layer capacitance of the electrode-supported lipid monolayers, respectively. The fitting results (R_{CT} and C_{DL}) were then depicted in Figure 2B where the Ca^{2+} concentration was varied within the range of 0−100 mM. Several observations warrant attention here. First, upon the formation of lipid self-assembled monolayers on the electrode surface, C_{DL} decreased quite drastically (∼50%), and correspondingly, R_{CT} increased about 2-fold, compared with that at the bare electrode, consistent with the above voltammetric measurements (Figure 1). Second, with the introduction of Ca^{2+} into the electrolyte solution, the *effective* C_{DL} decreased, and concomitantly, R_{CT} increased, with increasing Ca^{2+} concentration both with lipid-modified and naked electrodes; and the ion effects were most significant when 1 mM Ca^{2+} was initially added into the solution, and further increase of Ca^{2+} concentration appeared to have only subtle effect. Third, the ratios of C_{DL} (R_{CT}) (Figure 2C) at the lipid-modified electrode and that at the naked electrode increased (decreased) markedly with the introduction of 1 mM Ca^{2+}; and further addition of Ca^{2+} actually led to a slight decrease.

As suggested by these measurements (Figures 1 and 2), the lipid surface coverage appeared to be much less than that of a fully packed monolayer. Thus, it is speculated that the condensing effects induced by the divalent ions might lead to the rearrangements of surface lipid molecules that result in the formation of surface "defects" embedded within (somewhat) ordered lipid domains. This is, in essence, akin to the formation of transmembrane channels which are gated by solution chemical messengers (inorganic divalent ions). The above observations are consistent

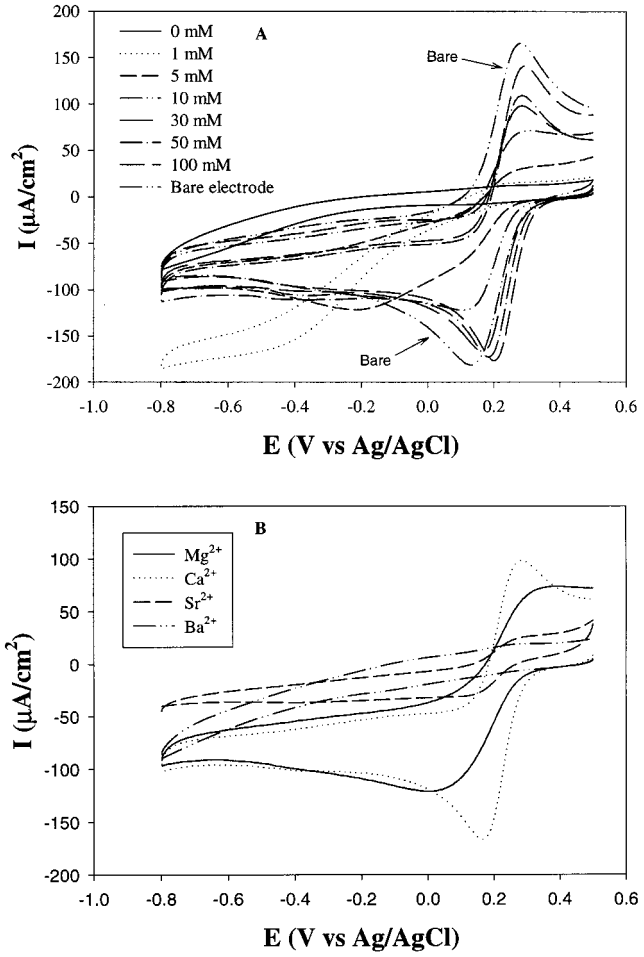
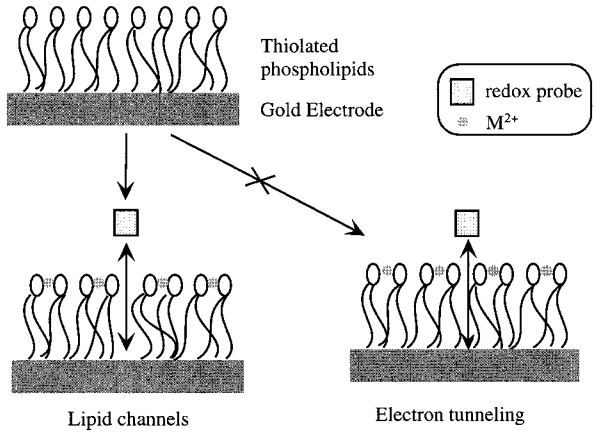


Figure 3. (A) Cyclic voltammograms of a PC-2C11SH SAM-modified gold electrode in 0.1 M NaCl (pH = 7.93 buffered by 0.01 M Tris-HCl) with 1 mM $K_3Fe(CN)_6$ and varied concentration of Ca^{2+} (no Ca^{2+} was added with the bare electrode). (B) Cyclic voltammograms of the lipid-modified electrodes in the same electrolyte solutions containing varied divalent ions. The divalent ion concentrations were all 30 mM. Potential sweep rates in (A) and (B) all at 100 mV/s.

with this speculation. For instance, in the absence of Ca^{2+}, $C_{DL,PC}/C_{DL,bare} = 0.37$, and $R_{CT,PC}/R_{CT,bare} = 2.56$; whereas at $[Ca^{2+}] = 1$ mM, $C_{DL,PC}/C_{DL,bare} = 0.50$, and $R_{CT,PC}/R_{CT,bare} = 1.53$ (Figure 2C). These variations (i.e., *relative* increase in interfacial capacitance and decrease in charge-transfer resistance) might be ascribed to the ion-induced creation of "defect" sites within the lipid assemblies. Higher Ca^{2+} concentrations did not appear to have substantial impact on the C_{DL} and R_{CT} measurements, suggesting that surface saturated binding might have been reached or the variation of interfacial structure was not significant enough to be detectable by this technique.

Using a redox probe molecule, the above ion-gated dynamic transitions can be observed more visibly by monitoring the corresponding voltammetric responses. We take $K_3Fe(CN)_6$ as the illustrating example. It was found that, in the absence of divalent ions, the electrochemical response of $K_3Fe(CN)_6$ was virtually invisible (Figure 3A, solid line), despite the relatively low surface coverage or disordering of the lipid adlayers, as speculated above. This appears to suggest that there were no substantial "pinholes" within the lipid monolayer, and the lipid adlayers served as a rather effective barrier against electron tunneling (Scheme 2). However, upon the addition of divalent alkaline-earth ions into the electrolyte solution, the reversible voltammetric features of $K_3Fe(CN)_6$ emerged with a pair of peaks showing up at $E^{\circ\prime} = +0.24$ V which became better-defined with increasing ion concentrations. Figure 3A shows the representative voltammetric responses of the lipid-modified electrode with varied concentrations of Ca^{2+} in the electrolyte solution. Similar effects were also observed for other alkaline-earth ions but to a less significant extent (Figure 3B), where the ion-gating effect increased in the order of $Ba^{2+} < Sr^{2+} < Mg^{2+} < Ca^{2+}$.

Scheme 2. Schematic Illustration of the Lipid Self-Assembled Monolayer on a Gold Electrode Surface and the Ion-Induced Interfacial Dynamic Transitions



These observed variations of voltammetric responses are, again, likely related to the condensing effects of divalent ions on lipid adlayers, as one of the immediate consequences of the specific interactions between divalent ions and lipid phosphate groups is conformational reorganizations within the lipid assemblies, resulting in the formation of more ordered (and hence more compact) surface domain structures. However, there could be other contributing factors as well. For instance, the binding of cationic alkaline-earth ions to the lipid polar headgroups rendered the lipid surface (partially) positively charged, which might then facilitate the transport of anionic $Fe(CN)_6^{3-}$ to the electrode surface. On the other hand, the lipid charge state might affect the electron-tunneling kinetics of anionic $Fe(CN)_6^{3-}$, due to the electrostatic interactions.

As within the potential range of +0.6 to −1.0 V, no desorption of lipid molecules from the electrode surface occurs (i.e., no vertical transitions; Figure 1), the experimental observations appeared to be consistent with the postulation of ion-induced (lateral) conformational reorganization of lipid adlayers, which resulted in the formation of microscopic transmembrane mass-transfer channels (Scheme 2). In addition, the channel dimensions appeared to increase with increasing ion concentrations, which is further supported by the observations that, at high Ca^{2+} concentrations (e.g., ≥30 mM), the voltammetric peak current of $Fe(CN)_6^{3-}$ increases linearly with the square root of potential sweep rates, indicating a linear-diffusion-controlled redox process (Figure 4A), corresponding to the case at a macrosized electrode. In contrast, at low Ca^{2+} concentrations (e.g., <30 mM), the peak current appears to be virtually invariant at low sweep rates (Figure 4B), consistent with *microelectrode* voltammetry where the redox

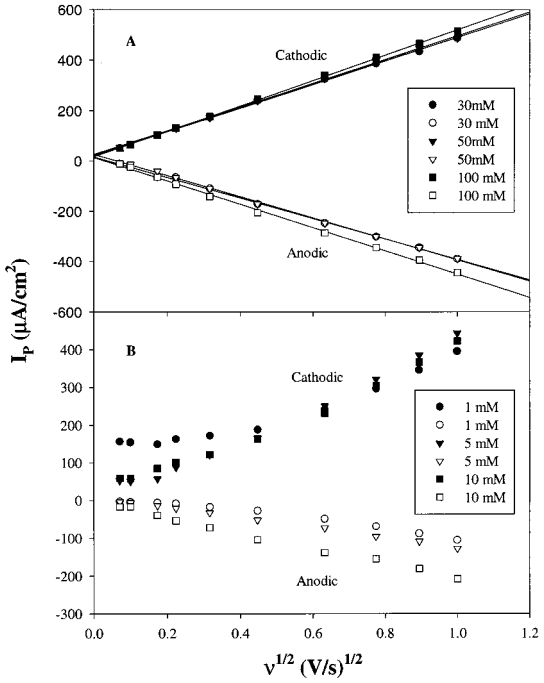


Figure 4. Variation of $K_3Fe(CN)_6$ anodic and cathodic peak currents with potential sweep rate at various Ca^{2+} concentrations: (A) 30, 50, and 100 mM; (B) 1, 5, and 10 mM. Experimental conditions the same as in Figure 3. Lines shown in (A) are linear regressions.

process is radial-diffusion-controlled, corresponding to plateau current responses as shown in Figure 3A.[15] Another equally plausible interpretation for these observations is that the number of channels increases, whereas the channel size remains microscale, and at high ion concentrations, the close vicinity of the microchannels renders the overall voltammetric currents to behave like that from a macrosized electrode, as the diffusion profiles overlap between neighboring channels.[16] At the moment, however, it is difficult to distinguish the contributions from these two varied mechanisms. It is more likely that both play a role here in governing the voltammetric responses measured.

Additional experimental observations appear to further support the lipid channel mechanism. For instance, after the treatment with a high concentration of Ca^{2+} (e.g., 50 mM), the lipid-modified electrode was re-immersed into the lipid solution (in chloroform) for additional deposition. The resulting lipid assemblies became much less sensitive to the stimulation by divalent ions where the voltammetric responses of $Fe(CN)_6^{3-}$ were significantly depressed even at $[Ca^{2+}] = 50$ mM (Figure 5). Additional cycles of treatment resulted in even better blocking of the $Fe(CN)_6^{3-}$ electrochemistry. Correspondingly, from impedance spectroscopic measurements in a 0.10 M NaCl electrolyte solution with no divalent ions or $K_3Fe(CN)_6$, the lipid adlayer capacitance (C_{DL}) was found to decrease from 13.5 to 8.0 μF/cm^2, whereas the charge-transfer resistance (R_{CT}) increased from 39.5 to 63.2 kΩ cm^2. As mentioned earlier, in the initial monolayer formation process, simply prolonging deposition time did not result in a compact/ordered lipid monolayer. The above experimental observations are consistent with

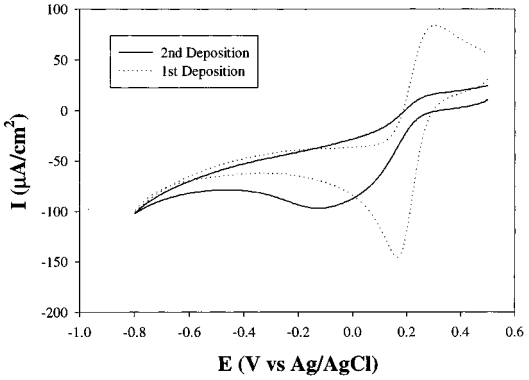


Figure 5. Cyclic voltammograms of a PC-2C11SH SAM-modified gold electrode in 0.1 M NaCl (pH = 7.93 buffered by 0.01 M Tris-HCl) with 1 mM $K_3Fe(CN)_6$ and 50 mM Ca^{2+}. The electrode was pretreated in 50 mM Ca^{2+} followed by immersion into the lipid solution for additional deposition (—); also shown is the CV with the first lipid assembly (···, from Figure 3). Potential sweep rate 50 mV/s.

the lipid-channel mechanism, where the exposed electrode area (lipid channels) was coated with additional lipid molecules during the second deposition period and hence helped block the electron-tunneling of $K_3Fe(CN)_6$.

In a second control experiment where the lipids were initially self-assembled onto the gold electrode surface in the presence of Ca^{2+} ions (in a methanolic solution), the resulted lipid SAMs were found to be rather insensitive to divalent ions as well (not shown), with no well-defined electrochemical features of $Fe(CN)_6^{3-}$. The interfacial capacitance and charge-transfer resistance also exhibited similar variations, as described above.

In these control experiments, the absence of well-defined electrochemical features of $K_3Fe(CN)_6$ also appeared to discount the speculation of electrostatic effects where the binding of divalent cations (e.g., Ca^{2+}) to the lipid (zwitterionic) polar head region might facilitate the transport of anionic $Fe(CN)_6^{3-}$. In addition, the electron-tunneling mechanism seemed to be disfavored as well, as prior to the electrochemical measurements in $K_3Fe(CN)_6$ solutions, the resulting lipid monolayers presumably had already had saturated binding of Ca^{2+}.

Therefore, these observations seem to further support the ion-induced channel-forming hypothesis (Scheme 2), implying a rather high degree of lateral mobility of these surface-anchored lipid molecules despite the relatively strong *chemisorptive* interactions with the electrode surface. Similar observations have been reported previously. For instance, Ulman et al.[17] found that, under ambient conditions, alkanethiol monolayers containing mixtures of OH and CH_3 groups at their air/monolayer interface appeared to undergo gradual (lateral) surface reorganization, manifested by contact angle, ellipsometry, and *x*-ray photoelectron spectroscopy (XPS) measurements. This surface reorganization was ascribed to the result of trans-gauche isomerization at chain termini, which might start from surface defects.[17] Additionally, ion-gate responses have also been observed with self-assembled monolayers of glutathione (and its fragment dipeptides) on gold electrode surfaces in the presence of alkaline-earth ions.[18] In the lipid adsorbed monolayers under study here, the surface transi-

(15) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; John Wiley & Sons: New York, 1980.
(16) (a) Amatore, C.; Savéant, J. M.; Tessier, D. *J. Electroanal. Chem.* **1983**, *147*, 39. (b) Sabatani, E.; Rubinstein, I. *J. Phys. Chem.* **1987**, *91*, 6663.
(17) Evans, S. D.; Sharma, R.; Ulman, A. *Langmuir* **1991**, *7*, 156.
(18) Takehara, K.; Aihara, M.; Miura, Y.; Tanaka, F. *Bioelectrochem. Bioenerg.* **1996**, *39*, 135.

tions appear to be triggered by the binding of divalent ions to the lipid polar head regions (receptors). They also strongly suggest that the dynamic transitions are contingent upon the packing/ordering of the lipid assemblies, consistent with early observations that it is energetically favorable that membrane dynamics initiate at the defect/active sites.[1]

However, it should be noted that in these measurements (Figures 3 and 5), only the first cycles of voltammetric responses were collected after the electrode was introduced into the electrolyte solutions at each ion concentration, to minimize possible effects of electrode potentials. As mentioned earlier, in the absence of divalent ions (e.g., see Figure 3), the lipid adsorbed layers appeared to be very insensitive to repeated potential cyclings, showing basically featureless double-layer charging current. However, with the binding of divalent ions to the lipid polar-head region, the headgroup charge state changes and the experimental data shown here cannot completely rule out the contributions from the headgroup interactions with electrode potentials, although effects of electrode potentials do not appear to be as significant.

It should be noted that the binding constants of these ions to the polar headgroups (phosphate moieties) of phosphatidylcholine increase in the order of $Ba^{2+} < Sr^{2+} < Mg^{2+} < Ca^{2+}$.[1b] One can see that the selective effect of ion-induced lipid dynamic transitions observed above is quite consistent with this binding sequence, where Ca^{2+} appears to be the most effective gating ion with the strongest binding whereas Ba^{2+} the least effective with the weakest binding. This chemoselectivity might be exploited for chemical sensing in transmembrane mass transfer.

Ion-Assisted Surface Grafting. One might note that these ion-induced interfacial dynamic transitions can be exploited for surface tailoring in an increasing level of definition. For instance, when a new thiol ligand is present in the second deposition solution, more complicated surface assemblies can be constructed. The resulting surface composition will be governed by the initially divalent ion concentration, which controls the overall lipid channel dimensions (and hence the exposed electrode area, vide ante) and can be determined by various surface-sensitive techniques.[15] Here we used the thiol derivatives of viologens as the illustrating examples, in part because viologens have been used rather extensively as efficient electron-transfer mediators in a number of biological systems (such as the NAD^+/NADH couple).[19] By incorporating viologen moieties into the lipid membrane layers, we aimed at providing a mechanistic basis toward the manipulation of lipid surface structures and reactivities.

Figure 6 shows the cyclic voltammograms (CVs) of lipid-modified electrodes that have been pretreated in 100 mM Ca^{2+} and following which have been immersed into a viologen thiol derivative ($MMDV^{2+}(PF_6^-)_2$, see the Experimental Section) solution for (A) 3 h and (B) 46 h; also shown are the CV responses of the electrodes without Ca^{2+} treatment prior to viologen deposition (Figure 6, left panels). The latter case constitutes the control experiments where only surface exchange reaction takes place. In all cases, a pair of voltammetric peaks can be found at $E^{o\prime} = 0.45$ V, which are ascribed to the first electron-transfer reaction of the viologen moieties, namely, $V^{2+} \Leftrightarrow V^{+\bullet}$, and the peak currents

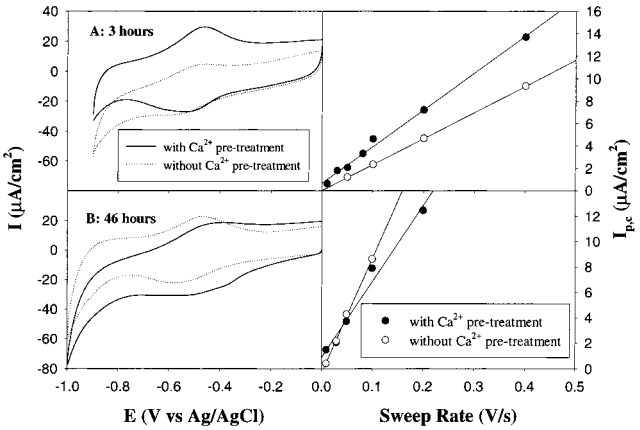


Figure 6. Cyclic voltammograms of the lipid-modified electrodes that were incubated into viologen thiol-derivative solution for a second deposition for (A) 3 h and (B) 46 h, with and without a pretreatment of 100 mM Ca^{2+} (left panels). Sweep rate 200 mV/s. The right panels show the corresponding variation of peak current with sweep rate (symbols) fitted with the linear regressions (solid lines).

show a linear increase with potential sweep rates, consistent with surface confined species (Figure 6 right panels). From these, the surface concentration (coverage) of viologens can be evaluated. For instance, when the incubation time was short, e.g., 3 h (A), the surface coverage of viologen increased from 2.45×10^{-11} mol/cm^2 to 3.45×10^{-11} mol/cm^2 when the electrode was pretreated with 100 mM Ca^{2+} before viologen deposition, compared with the case where no pretreatment of Ca^{2+} was initiated. This clearly demonstrates the facilitation of ion binding on the lipid surface reorganization, resulting in the ready access of electrode surface by solution molecules, consistent with the lipid channel mechanism described earlier. In contrast, when the deposition time was very long, e.g., 46 h (B), although the overall viologen surface concentration increased, the pretreatment of the electrode-supported lipid assemblies with 100 mM Ca^{2+} actually resulted in a decrease of viologen surface coverage, 6.33×10^{-11} mol/cm^2, as compared with that without any Ca^{2+} pretreatment, 9.62×10^{-11} mol/cm^2. This might be accounted for, again, by the ion-induced lipid conformational transitions. At longer incubation times, the anchoring of viologen ligands onto the electrode surface was most likely limited by surface−ligand exchange reactions and therefore it was much slower than the initial adsorption process onto exposed electrode surface. It is therefore anticipated that the exchange reaction will be impeded by the condensing effects of Ca^{2+} during the pretreatment process and hence an eventual lower surface coverage will result.

It is anticipated that this approach can be extended for other thiol ligands as well, which might then be exploited for more complicated surface functionalization.

Transition Reversibility. The reversibility of the dynamic processes was investigated by purging the cationic stimulants with strong chelating ligands such as EDTA, which is of great importance in the study of the regulation of transmembrane mass transport by chemical messengers.[13a] In the preliminary study, we found that upon the removal of ions from the lipid headgroups by EDTA, the lipid molecules appeared to be (at least partially) relaxed back to the initial conformation and hence channels closed, as manifested voltammetrically (not shown). An understanding of the molecular mechanism of channel opening and

(19) (a) Maeda, H.; Kajiwara, S. *Biotechnol. Bioeng.* **1985**, *27*, 596. (b) Kim, S.; Yun, S.-E.; Kang, C. *J. Electroanal. Chem.* **1999**, *465*, 153.

closing will be critical in the design of ion-triggered transmembrane mass-transfer pathways.

CONCLUDING REMARKS

Self-assembled monolayers of phospholipids were constructed on gold electrode surfaces by the self-assembling of ω-mercapto-derivatized lipid molecules. Despite the relatively low surface coverage and/or disordering of the lipid adlayers, they behaved as very effective electron-tunneling barriers. As the lipid molecules were anchored to the electrode surface by a relatively strong linkage, there was little effect of electrode potential on the interfacial structure of the lipid assemblies in the absence of inorganic divalent ions. However, upon the stimulation by alkaline-earth ions, the lipid layers appeared to undergo (lateral) surface reorganization, forming microscopic lipid channels. These observations suggest that the ionophoretic capabilities of lipid assemblies might be one of the intrinsic properties of phospholipid membranes.[13]

Further and more detailed studies will focus on the characterizations of the lipid surface structures by using other complementary surface-sensitive techniques (such as ellipsometry, surface plasmon resonance spectroscopies, scanning probe microscopies) and the effects of lipid chemical structure including polar headgroups and apolar tails, due to their influence on the neighboring lipid−lipid interactions. For instance, when lipid monolayers were constructed with 1,2-bis(16-mercaptohexadecanoyl)-*sn*-glycero-3-phosphocholine (abbr. PC-2C16SH), the surface-supported lipid assemblies were found to be much less sensitive to the stimulation by these alkaline-earth ions. Also, solution parameters (e.g., temperature, pH) are anticipated to influence the lipid layer structure and conformation (viz., fluidity), and hence, the interfacial dynamics. On the other hand, other redox probe molecules of varied charge states (e.g., cationic, neutral, or anionic) will be used to study the lipid interfacial dynamics where effects of the electrostatic interactions between lipid polar headgroups and the redox probes can be differentiated. These aspects are currently being pursued and results will be reported in due course.

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SUPPORTING INFORMATION AVAILABLE

Supporting Information Available. Cyclic voltammogram of a PC-2C11SH-modified gold electrode in 0.1 M NaCl (Tris, pH 7.93) in the potential range of 0 to −1.2 V (1 page). This material is available free of charge via the Internet at http://pubs.acs.org.

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