Scanning Electrochemical Microscopy. 38. Application of SECM to the Study of Charge Transfer through Bilayer Lipid Membranes

Michael Tsionsky,[†] Junfeng Zhou, Shigeru Amemiya, Fu-Ren F. Fan, and Allen J. Bard*

Department of Chemistry and Biochemistry, The University of Texas at Austin, Austin, Texas 78712

Robert A. W. Dryfe

Department of Chemistry, UMIST, P.O. Box 88, Manchester M60 1QD, U.K.

The use of the scanning electrochemical microscope (SECM) to probe the kinetics of charge-transfer processes at bilayer lipid membranes (BLM) is presented. Analysis of the SECM tip response demonstrates that an unmodified BLM behaves as an insulator, whereas a BLM doped with iodine shows some positive feedback. The SECM technique thus allows one to probe processes at a BLM and determine the kinetics of the charge-transfer process. The SECM can also be used to determine the shape of the BLM.

The self-assembly of amphiphilic molecules, such as phospholipids, to form bilayers has been widely studied because of the similar physical properties of such artificial membranes and membranes found in biological cells.[1] Recent interest in the bilayer lipid membrane (BLM) has also focused on its potential in analytical devices using the BLM as the basis to form biosensors.[2] The resistance and capacitance values of an unmodified BLM and cell membranes are very similar.[3,4] However, the conductivity of both types of membrane can be increased by the inclusion of certain lipophilic molecules that can form channels or act as carriers and facilitate the transfer of selected ions.[5] Certain lipophilic molecules in the BLM can also transfer electrons by acting as redox "shuttles". Tien has shown how a BLM modified with lipophilic electron donors (such as ferrocene and tetrathiafulvalene) or lipophilic electron acceptors (e.g., 7,7,8,8,-tetracyanoquinodimethane) can effectively act as a working electrode.[6,7] The dynamics of such charge-transfer processes have hitherto been probed by patch-clamp experiments, where a portion of the membrane under study is trapped at the orifice of a micropipet.[8]

Previous work has demonstrated how the scanning electrochemical microscope (SECM) can be used to probe dynamic processes at interfaces,[9] including the metal/solution interface,[10] the semiconductor/solution interface,[11] the air/liquid interface,[12] and the liquid/liquid interface.[13] In SECM, an ultramicroelectrode (UME) is used as the tip to probe interfacial charge-transfer reactions by studying the change of the steady-state current that flows at the tip. More recently, microelectrode techniques have been applied to extract kinetic information about ion transport across the BLM; such transport is often complicated by the presence of unstirred layers near the membrane. Antonenko and co-workers have used ion-selective microelectrodes to probe the steady-state concentration gradients of the solute ions that were induced within the unstirred layers adjacent to the BLMs.[14–16] With this technique, an amperometric-positioning electrode is usually required to determine the tip-to-sample distance.[17–19] In other studies, Matsue and co-workers have used amperometric microelectrode techniques to probe ion fluxes in biological membranes.[20–22] (In most of these studies, a microelectrode tip was positioned near a BLM ($\sim$10–70 μm) and the ionic permeability of the membrane found from comparisons with those calculated by digital simulation. The shape of the SECM approach curves or the question of electronic conductivity of the membrane was addressed only for simple permeation of relatively hydro-

† Current address: IGEN, 16020 Industrial Drive, Gaithersburg, MD 20877.

(1) Tien, H. T. *Bilayer Lipid Membranes (BLM): Theory and Practice*; Marcel Dekker: New York, 1978.

(2) Tien, H. T.; Barish, R. H.; Gu, L.; Ottova, A. L. *Anal. Sci.* **1998**, *14*, 3.

(3) Hanai, T.; Haydon, D. A.; Taylor, J. *Proc. R. Soc. London, Ser. A* **1964**, *281*, 377.

(4) Montal, M.; Mueller, P. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 3561.

(5) Gennis, R. B. *Biomembranes: Molecular Structure and Function*; Springer-Verlag: New York, 1989.

(6) Tien, H. T. *J. Phys. Chem.* **1984**, *88*, 3172.

(7) Tien, H. T. *Bioelectrochem. Bioenerg.* **1984**, *13*, 299.

(8) Brown, K. T.; Flaming, D. G. In *Advanced Micropipet Techniques for Cell Physiology*; Wiley: New York, 1986; Chapter 17.

(9) Bard, A. J.; Fan, F.-R. F.; Mirkin, M. V. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Marcel Dekker: New York, 1993; Vol. 18; p 243.

(10) Mandler, D.; Bard, A. J. *J. Electrochem. Soc.* **1989**, *136*, 3143.

(11) Mandler, D.; Bard, A. J. *J. Electrochem. Soc.* **1990**, *137*, 2468.

(12) Slevin, C. J.; Ryley, S.; Walton, D. J.; Unwin, P. R. *Langmuir* **1998**, *14*, 5331.

(13) Tsionsky, M.; Bard, A. J.; Mirkin, M. V. *J. Am. Chem. Soc.* **1997**, *119*, 10785.

(14) Antonenko, Y. N.; Bulychev, A. A. *Biochim. Biophys. Acta* **1991**, *1070*, 474.

(15) Antonenko, Y. N.; Pohl, P.; Denisov, G. A. *Biophys. J.* **1997**, *72*, 2187.

(16) Pohl, P.; Saparov, S. M.; Antonenko, Y. N. *Biophys. J.* **1998**, *75*, 1403.

(17) Horrocks, B. R.; Mirkin, M. V.; Pierce, D. T.; Bard, A. J.; Nagy, G.; Tóth, K. *Anal. Chem.* **1993**, *65*, 1213.

(18) Wei, C.; Bard, A. J.; Nagy, G.; Tóth, K. *Anal. Chem.* **1995**, *67*, 1346.

(19) Wei, C.; Bard, A. J.; Kapui, I.; Nagy, G.; Tóth, K. *Anal. Chem.* **1996**, *68*, 2651.

(20) Yamada, H.; Matsue, T.; Uchida, I. *Biochem. Biophys. Res. Commun.* **1991**, *180*, 1330.

(21) Matsue, T.; Shiku, H.; Yamada, H.; Uchida, I. *J. Phys. Chem.* **1994**, *98*, 11001.

(22) Yasukawa, T.; Uchida, I.; Matsue, T. *Biochim. Biophys. Acta* **1998**, *1369*, 152.

A.

B.
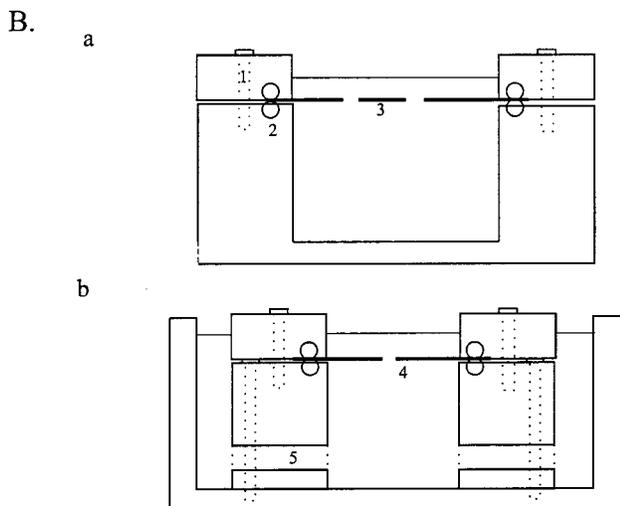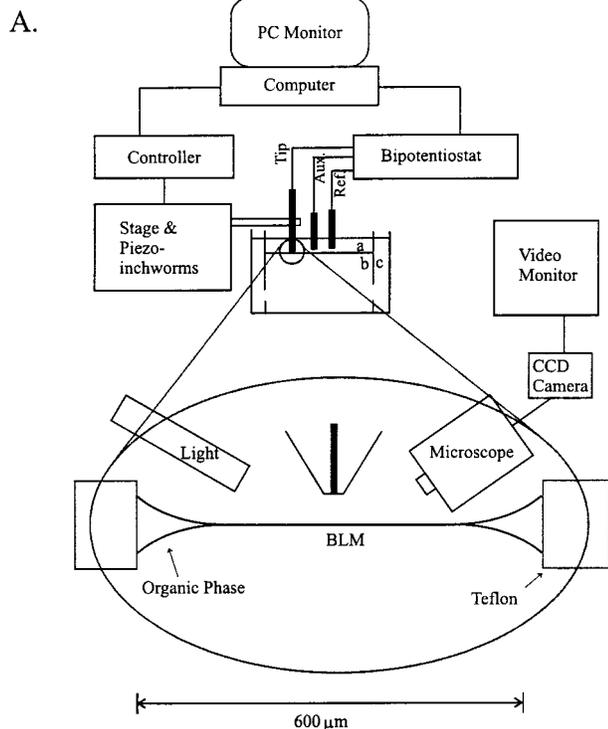
Figure 1. (A) Schematic diagram of SECM apparatus and a cell for the preparation of horizontally oriented BLM. The solutions in the different parts of the cell are represented by a, b, and c. (B) Schematic diagram of the two different cells used: (1) screw, (2) O-ring, (3) Teflon sheet with two holes, (4) Teflon sheet with one hole, (5) holes in the inner wall of the Teflon barrier.

phobic ions.[22]) In this report, we described the utilization of a horizontally oriented BLM for SECM measurements. The examination of a BLM of lecithin, which behaves as an insulator in Ru-$(NH_3)_6^{3+}$ and $Fe(CN)_6^{4-}$ systems, is described. The SECM was also used to measure the effective flux of iodide through the BLM when it was modified by addition of iodine.

EXPERIMENTAL SECTION

As opposed to conventional BLM apparatus, where the membrane is oriented vertically, in the experiments described

here a horizontally oriented membrane that separated upper and lower compartments containing the same solution was employed (Figure 1A) to make the cell compatible with conventional SECM apparatus. To prevent differential pressures across the membrane, two different cells were used (parts A and B(a) of Figure 1). In the type of cell shown in Figure 1A, the upper and lower solutions (a and b, respectively) were separated by an inner Teflon cell that contained several holes (7 mm diameter) between solutions b and c so that the pressures in the upper and lower compartments were the same (for details of the cell, see Figure 1B(b)). In the cell shown in Figure 1B(a), a second pinhole, spaced about 5 mm from the membrane pinhole (600 μm diameter) was made in the Teflon partition (250 μm thickness). Unless provision was made to equalize the pressures above and below the membrane, stable membranes could not be obtained. A difficulty with the cell in Figure 1B(a) is that both compartments are in ionic contact through the hole, so that one cannot apply a differential potential across the membrane or easily maintain different solution compositions in the two compartments. Thus, the cell in Figure 1B-(b) is advantageous for a wider range of BLM studies, e.g., where the upper solution composition differs from that of the lower solution. In this study, it was used for the topographical imaging experiments. All cells were made of Teflon, with all electrodes in the upper compartment. BLMs were prepared from solutions of lecithin in *n*-heptane or *n*-decane by the standard brush-technique.[1,23] Aliquots of 3.5 μL of lipid solution were added to the cell with a micropipet. Formation of the BLM was monitored with a color CCD camera (model TK-C1380, JVC, Yokohama, Japan), mounted on a microscope (Katoptaron LDM-1/s, META Geraeteteknik, Koln, Germany) to detect the interference fringes caused by the thinning of the organic layer. Lecithin was supplied as a chloroform solution (Avanti Polar Lipids, Alabaster, AL). The chloroform was evaporated with a stream of N_2, and the lipid was redissolved in *n*-heptane or *n*-decane. A solution concentration of 1.25 mg lipid/mL was used for all experiments except in topographical imaging experiments where 20 mg lipid/mL solutions were used to obtain more stable BLMs. Iodine was added to the lipid solution before producing the BLM in some experiments. All solutions were prepared from deionized water (Milli-Q, Millipore Corp.). The BLM formed was approached with a platinum SECM tip (25 μm diameter),[24] which was polished, rinsed with ethanol and then with water, and dried before each experimental run. The three-electrode cell was completed by a Pt wire counter electrode and an Ag/AgCl reference electrode. The potential and positioning of the tip were controlled via a PC using an SECM (Model CHI 900, CH Instruments, Austin, TX).

RESULTS AND DISCUSSION

Insulating BLM-Approach Curves. The insulating properties of an unmodified BLM were investigated by approaching the UME tip toward the BLM (*z*-direction) or by scanning the tip horizontally (*x*- or *y*-direction) in close proximity to the BLM. Previous studies by Matsue and co-workers have indicated that the permeability of an unmodified BLM by a hydrophilic ion should be very low.[20] Therefore, $Ru(NH_3)_6^{3+}$ and $Fe(CN)_6^{4-}$ were used as hydrophilic

(23) Hanke, W.; Schlue, W. *Planar Lipid Bilayers: Methods and Applications*; Academic Press: London, New York, 1993.
(24) Bard, A. J.; Fan, F.-R. F.; Kwak, J.; Lev, O. *Anal. Chem.* **1989**, *61*, 132.
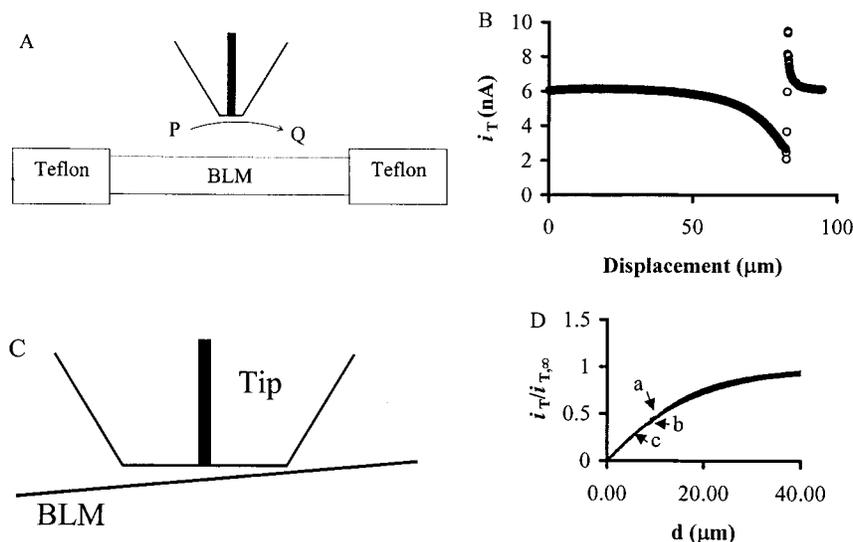
Figure 2. (A) Processes at BLMs for mediator (e.g., $Ru(NH_3)_6^{3+}$ or $Fe(CN)_6^{4-}$) at the tip. (B) Current approach curve obtained at the 25 μm diameter Pt SECM tip as the tip−BLM separation was reduced. The BLM was formed from a solution of lecithin (1.25 mg/mL) in *n*-heptane. The aqueous solution contained $Ru(NH_3)_6^{3+}$ (1 mM) as the electroactive species, with 0.1 M KCl as supporting electrolyte. The tip potential was −0.35 V vs the Ag/AgCl reference electrode. (C) Schematic representation of the tilted alignment between the tip and bilayer. (D) Analysis of the experimental approach curve given in A. The current is normalized by its steady-state bulk solution value ($i_{T,\infty}$). (a) Approach to the BLM, (b) approach to the Teflon cell, and (c) theoretical curve for an insulating interface.

redox mediators (species P in Figure 2A). Figure 2B shows a typical experimental (current−displacement) approach curve obtained for the diffusion-limited reduction of $Ru(NH_3)_6^{3+}$ at the SECM tip. As the BLM is approached in the *z*-direction, the tip current decreases from its transport-limited value, $i_{T,\infty}$, at large *d*, the tip-to-substrate distance, described by[25]

$$i_{T,\infty} = 4nFDca \qquad (1)$$

where *n* is the number of electrons transferred per molecule, *F* is Faraday's constant, *D* is the diffusion coefficient of the electroactive molecule, *c* is its bulk concentration, and *a* is the radius of the tip. The current decrease is due to the hindered diffusion of $Ru(NH_3)_6^{3+}$ toward the tip, caused by the presence of the BLM. The tip current tends toward zero as *d* approaches zero, indicating that the BLM is acting as an insulator. However, when the tip makes contact with the BLM, the bilayer breaks and the current returns to the $i_{T,\infty}$ value once more. The initial higher current and transient decay when the tip first penetrates the membrane represent the tip contacting fresh solution and reforming a steady-state concentration profile. Note that the position where the BLM film breaks represents the point where the glass sheath touches the membrane, and because the tip plane is not precisely in the plane of the BLM (Figure 2C), the conducting electrode is 5−10 μm from the BLM at this point. The declining portion of the approach curve shown in Figure 2B was compared with the theoretical decay curve assuming purely negative feedback (i.e., no regeneration of the electroactive material occurring at the interface being approached). The theoretical curve is described by[26]

(25) Saito, Y. *Rev. Polarogr.* **1968**, *15*, 177.
(26) Mirkin, M. V.; Fan, F.-R. F.; Bard, A. J. *J. Electroanal. Chem.* **1992**, *328*, 47.
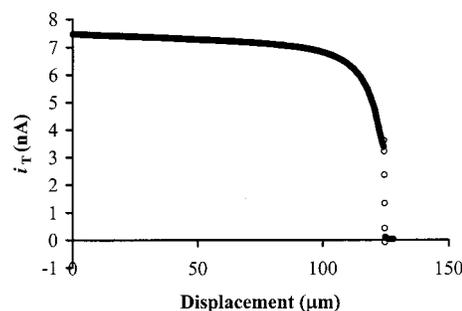
Figure 3. Approach curve obtained at a "multilayer" formed from lecithin solution. The current decay again corresponds to the negative feedback case. The aqueous solution contained 1 mM $Fe(CN)_6^{4-}$ and 0.1 M KCl. Tip potential, 0.5 V vs Ag/AgCl.

$$i_{ins}/i_{T,\infty} = 1/\{0.15 + (1.5358/L) + [0.58 \exp(-1.14/L)] + 0.0908 \exp[(L - 6.3)/(1.017L)]\} \quad (2)$$

where i_{ins} represents the current (as a function of *d*) and *L* is the normalized tip−BLM separation ($L = d/a$). Figure 2D shows the experimental BLM approach curve, the theoretical approach curve (eq 2), and an approach curve obtained using the Teflon wall of the cell partition as an inert interface. As can be seen, all three curves essentially superimpose, when the position corresponding to $d = 0$ is adjusted for the experimental data, indicating that the BLM was completely insulating under these conditions. In particular, the BLM is effectively impermeable with respect to $Ru(NH_3)_6^{3+}$ ions, in agreement with the findings of Matsue and co-workers.[20] A different behavior was obtained when larger amounts of stock lipid solution were used to form a layer over the pinhole in the cell. Under these conditions, the lipid did not thin to form a bilayer (no interference colors were seen); instead, a gray multilayer appeared to form that was also insulating with respect to ion transfer. A typical approach curve obtained from such a multilayer is given in Figure 3. The decay in this case was also characteristic of the negative feedback expected for an insulating

Figure 4. Top (a) and side (b) views of schematic diagram for the measurement of the topographical images of the BLM by SECM. The tip approached to close proximity of the surface at point a and was then scanned toward point d (a → b → c → d) at a constant height. Teflon membrane thickness, 150 μm.

Figure 5. Transport-limited current image (upper) and the corresponding topographical image (lower) of a bilayer obtained by scanning 25 μm diameter Pt SECM tip along one axis parallel to the surface in a solution containing 1 mM Fe(CN)$_6^{4-}$ and 0.1 M NaCl. Tip potential, 0.4 V vs Ag/AgCl; scan rate, 3 μm/s. The indicated points, a−d, correspond to the positions in Figure 4, as observed by optical microscopy.

interface. However, in this case, the organic phase did not break when contacted by the tip. Instead, an insulating layer of lipid remained on the electrode surface as it moved into the lower compartment solution, so that the tip current remained at zero. This result is consistent with previous SECM experiments at liquid−liquid interfaces, where the tip carries and maintains a thin layer of upper layer liquid on its surface as it penetrates the interface and moves into the lower layer.

SECM Scans Across an Insulating BLM. When a BLM is formed by painting the lipid solution across a hole, only the middle portion represents the BLM. A thicker annulus of the lipid solution is formed near the edges of the hole (in contact with the Teflon support (150 μm in this experiment), which influences the bilayer behavior (Figure 4).[27] We employed Fe(CN)$_6^{4-}$ as a mediator ion which shows negative feedback at both the BLM and the annulus material to obtain a surface topographic image of the bilayer film surrounded by the annulus. The resulting topography, which indicates the side view of the BLMs, could be compared with their top view, which was simultaneously observed with an optical microscope. The UME tip was held at a potential for the diffusion-limited oxidation of ferrocyanide and was moved into close proximity toward the annulus surface (point a in Figure 4) until the current flowing at the electrode was about $0.80i_{T,\infty}$. Then, the UME tip was kept at a constant height and scanned (at 3 μm/s) toward the opposite side of the annulus over the bilayer (a → b → c → d in Figure 4). The position of the tip relative to the surface was simultaneously observed with an optical microscope. A typical two-dimensional SECM image is shown in Figure 5a. At all tip

positions the current detected, i_T, was smaller than $i_{T,\infty}$ ($i_T/i_{T,\infty} <$ 1), demonstrating that the tip was always in close proximity to the surface so that the transport of the mediator to the tip was hindered by the bilayer and the annulus. Because both the bilayer and the annulus are impermeable to ferrocyanide, i_T depended only on the tip/surface distance, enabling the diffusion-limited current image in Figure 5a to be converted to the topographical image on the basis of eq 2.[26] Figure 5b shows the SECM topographical image of a bilayer surrounded by the annulus. The small asymmetry noted in the figure probably results from the fact that the tip scan in the $x-y$ plane was not exactly parallel to the plane of the BLM. A gradual change of the tip-to-surface distance was observed when the tip was moving above the annulus (a → b and c → d), corresponding to the shape of the annulus around the bilayer.[27] On the other hand, the tip-to-surface distance did not change significantly when the tip was scanned over the bilayer (b → c). Because the tip was scanned at constant height, the small change in d over the bilayer indicates that it is planar but tilted with respect to the tip scan plane. The diameter of the bilayer that was estimated from Figure 5b (~350 μm) agrees well with that observed by optical microscopy. The region of the membrane is thicker (~20 μm at point a) and thickens even more as it gets near the edge of the hole. This region could not be scanned in the constant height mode without either contacting the layer or losing resolution for the BLM portion. It should also be noted that a higher scan rate of the tip (15 μm/s) resulted in a less planar topographical image of the bilayer. This result suggests the disturbance of the bilayer by the fast movement of the tip. Indeed, BLM shaking by vigorous stirring of the solutions have recently been reported.[15]

Charge-Transfer Mediation by Iodine. Analogous experiments with iodide as the aqueous phase electroactive species, led to a different tip response as the BLM was approached. Oxidation of I$^-$ at a large BLM−tip separation gave $i_{T,\infty}$, which yielded a D of 2.62×10^{-5} cm^2 s^{-1} (eq 1). As the BLM was approached, the

(27) White, H. S. In *Ion Channel Reconstitution*; Miller, C., Ed.; Plenum Press: New York, 1986; p 3.
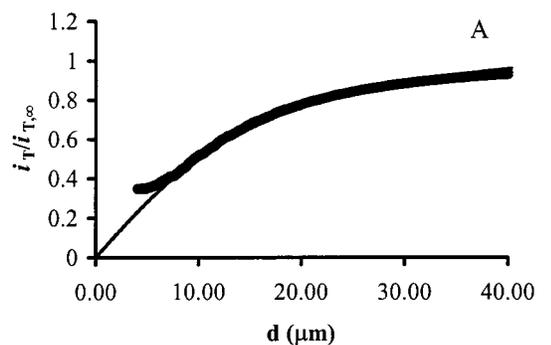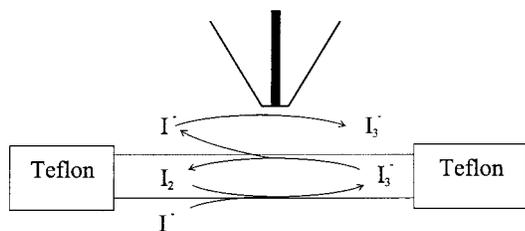
Figure 6. (A) Analysis of an approach curve for the oxidation of I⁻ (2.53 mM) in 0.1 M KCl. The solid line represents SECM theory for an insulating substrate (eq 2). Tip potential, 0.83 V vs the Ag/AgCl. Other conditions as in Figure 2A. (B) Processes at BLMs for I⁻/I₂ mediator oxidized at the tip.

tip current did not decrease with d as quickly as in Figure 2A, indicating that the membrane was not acting as a pure insulator. Comparison of the experimental data with eq 2 confirmed that there was a slight increase in the tip current at small d values (Figure 6A), implying that the depletion of I⁻ by oxidation at the SECM induced a net transfer of ions across the BLM. BLM permeability toward iodide has previously been shown to increase in the presence of iodine,[28] where the main pathway is likely to be a facilitated transfer of I⁻ by I₂, with I₃⁻ thus being the active lipid-bound species (Figure 6B).[29–31] Indeed, without reducing reagents, e.g., Na₂S₂O₃, in the solutions, I₂ generated by air oxidation of I⁻ produces an enhanced flux of the I⁻.[28] In our case, oxidation of I⁻ at the tip surface generates I₂, which diffuses into the bilayer and mediates the transport process.[21] When the lipid solution for BLM preparation was made with I₂-saturated *n*-heptane, the experimental data showed a dramatic flattening of the SECM approach curve (Figure 7A), indicating that the net flux of I⁻ to the tip had been markedly increased by the added I₂. A comparison of the data with eq 2 is given in Figure 7B. Further experiments were carried out where the concentration of I₂ in the membrane-forming solution was varied over the millimolar concentration range; a comparison of the data with eq 2 is presented in Figure 7C. It is clearly shown in Figure 7B,C that, as expected, a higher concentration of iodine in the BLMs results in large positive feedback, indicating faster transfer of I⁻ across the BLM. Note that the electroneutrality in the top and bottom phases is maintained during the tip-induced transfer of I⁻ from the bottom to the top phases because of the ionic contact through

(28) Läuger, P.; Richter, J.; Lesslauer, W. *Ber. Bunsen-Ges. Phys. Chem.* **1967**, *71*, 906.
(29) Bender, C. J.; Tien, H. T. *Anal. Chim. Acta* **1987**, *201*, 51.
(30) Klotz, K.-H.; Benz, R. *Biophys. J.* **1993**, *65*, 2661.
(31) Klotz, H.-K.; Benz, R. *Biochim. Biophys. Acta* **1995**, *1235*, 378.
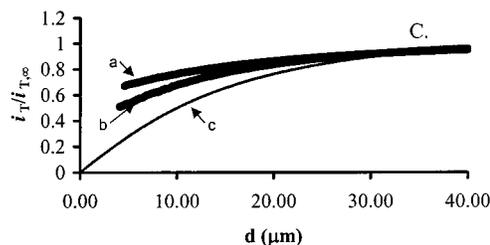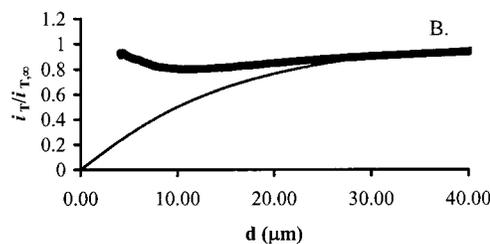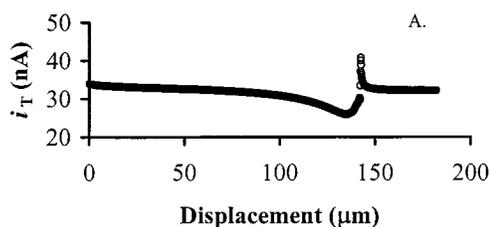
Figure 7. (A) Approach curve for the oxidation of I⁻ (2.53 mM) in 0.1 M KCl, when the bilayer was prepared with a lipid solution saturated with I₂ in *n*-heptane. Conditions otherwise correspond to those of Figure 2A. (B) Analysis of the data in A in terms of eq 2. A strong enhancement of the current at small separations (*d*) is seen, compared to that predicted by eq 2 and found in Figure 6. (C) Analysis of approach curves for the oxidation of I⁻ (2.53 mM) in 0.1 M KCl with bilayer prepared with lipid from (a) 18 mM and (b) 9 mM solutions of I₂ in *n*-heptane. The solid line (c) represents SECM theory for an insulating substrate (eq 2). Conditions otherwise correspond to those of Figure 2A.

the second hole in the cell (Figure 1B(a)) and because of the charge compensating transfer of I⁻ across the entire area of the BLMs. These processes are not expected to be rate-limiting.

On the SECM scale, the sub-nanometer thickness of the BLM can be approximated as being infinitesimally thin; thus, mass-transport to the tip can be treated as a two-phase diffusion process with an interfacial barrier. Such a situation has recently been numerically characterized at the SECM by Unwin and co-workers and used to determine partitioning kinetics of electroactive solutes at the interface between two immiscible phases.[32] Recent in-depth studies of I₂-mediated I⁻ transport using the charge-pulse[30] and voltage-clamp[31] techniques has shown that the model proposed by Läuger and Stark[33] describes well the transport process across BLMs. This model assumes that I⁻ from the aqueous phase is transported as I₃⁻ through the BLM. Note an alternative, that could not be distinguished, would be effective transport by electron transfer to reduce tip-generated I₂ in the upper phase with oxidation of I⁻ in the lower phase, mediated by I₂ in the BLM. We assume for simplicity that the transfer of I⁻ between the two aqueous phases can be represented as follows

(32) Barker, A. L.; Macpherson, J. V.; Slevin, C. J.; Unwin, P. R. *J. Phys. Chem. B* **1998**, *102*, 1586.
(33) Läuger, P.; Stark, G. *Biochim. Biophys. Acta* **1970**, *211*, 458.

$$I^-(\text{lower phase}) \overset{k}{\rightleftharpoons} I^-(\text{upper phase}) \qquad (3)$$

where k is the first-order interfacial rate constant for the transfer of I^- between the two aqueous phases. This simplified assumption allows for the rough estimation of the rate constant on the basis of the recently developed model.[32] Because the solutions on either side of the BLM are identical, the case studied here corresponds to a partition coefficient (K_e) and diffusion coefficient ratio (γ) of 1, which corresponds to the parameters that were used for the calculation of the approach curves in Figure 12 of ref 32. Comparison of the calculated approach curves with, for example, the experimental data for the BLM with 18 mM I_2 shows that the approach curve calculated with the dimensionless interfacial rate constant, K, where

$$K = ka/D \qquad (4)$$

of 0.5 fits well to the experimental data. Substitution of the experimental a and D values (12.5 μm and 2.62×10^{-5} cm^2 s^{-1}, respectively) gives a value of about 0.01 cm s^{-1} for k. The interfacial parameter k derived from the treatment above is effectively equivalent to the permeability coefficient, P, which characterizes transport across a BLM[5]

$$J_{\text{BLM}} = P(c_b - c_a) \qquad (5)$$

where J_{BLM} is the flux through the bilayer and c_a and c_b are the concentrations of the transported species in the upper and lower phases, respectively. In one report on this system,[28] Läuger et al. pointed out that the thickness of the diffusion layers on either side of the BLM prevented the extraction of large values of P. However, in our case, measurement of the ion fluxes in close proximity to the bilayer surface by SECM and the theoretical treatment taking into account the diffusional and depletion effects of I^- in both aqueous phases[32] enables extraction of the fast kinetic parameter. Although the approach used here, where the BLM is treated as infinitesimally thin, does not allow us to identify the rate-determining step.[30,31]

CONCLUSION

The SECM has been used to probe transfer kinetics in BLM systems. The technique thus presents a nonspectroscopic alternative to the voltage-clamp method for the extraction of in situ kinetic data from a BLM. We could extract the kinetic data without the limitation by the unstirred diffusion layers near the bilayer membrane.[28] The extended dynamic range of the SECM results from the diffusion layer thickness being reduced to the order of micrometers.