Single Molecule Electrochemistry

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Abstract: By using specially constructed nanometer tips of sharpened Pt-Ir wire in a wax sheath, small numbers of molecules (1–10) can be trapped between the tip and a substrate. Repeated electron transfers of an electroactive molecule as it shuttles by diffusion between tip and substrate produce a current (~0.6 pA/molecule) that can be used to detect the trapped molecules. The tip electrode size and shape can be found from the electrode approach curves (current vs tip-to-substrate distance) based on approximate equations and digital simulations. Analysis of the observed fluctuating currents by autocorrelation, spectral density, and probability density functions is also described.

Introduction

We report here electrochemical studies at the level of single molecules and small numbers of molecules. The detection of single molecules, their characterization, and their chemical and physical manipulation have come within current scientific capability and have become topics of significant interest. Electrochemical measurements on single molecules should allow determination of standard half-reaction potentials and free energies, diffusion coefficients, and kinetic parameters. To our knowledge the work reported here represents the first example of controlled redox chemistry at the single molecule level. Electroanalytical chemistry at this level represents the ultimate sensitivity in trace chemical analysis, and such studies are being undertaken with the expectation that investigations of properties and reactions at the single molecule level will allow one to discover effects, e.g., of molecular environment, that cannot be observed because they are averaged out when one makes measurements on a large number of molecules. A number of techniques have been used in recent years to detect single molecules or ions in different environments. These include the detection of ions that are confined in a vacuum in electromagnetic traps, single-molecule spectroscopy of molecules in solid matrices, the detection of individual dye molecules in solution by far-field confocal fluorescence microscopy, and the detection of molecules on surfaces with high spatial resolution by near-field scanning optical microscopy. Single-molecule detection (SMD) has also been achieved for molecules spatially restricted in microdroplets and in a thin flow cell. Recently, Collinson and Wightman observed individual chemical reactions in solution by detecting temporally resolved electrogenerated chemiluminescence at an ultramicroelectrode with a multichannel scaler.

In a recent communication, we reported SMD for an electroactive molecule in solution as it repeatedly undergoes electron-transfer reactions at an electrode held at a small distance from a substrate in a scanning electrochemical microscope (SECM). Briefly, the current at a small Pt disk electrode (radius ~10–20 nm) encased and slightly recessed in a wax sheath is measured as it is moved toward a conductive substrate in a solution containing an electroactive species and excess inert supporting electrolyte. The tip contacts the substrate and traps one or more molecules in the gap between disk and substrate. The electroactive molecules diffuse back and forth between the disk electrode and substrate undergoing redox processes at each, thus producing a current flow (Figure 1A). In this report we describe further electrochemical SMD experiments focussing on the SECM characterization of the cell geometry, the effect of substrate conductance on the tip current fluctuation at various distances, and statistical analyses of the response based on the time correlation, power spectral density, and probability density functions.

Experimental Section

Materials. Unless otherwise mentioned, in all of the experiments, (trimethylammonio)methylferrocene (CP-FeTMA) was used as the electroactive molecule. This species was selected because both it and its oxidized product are stable in aqueous media and undergo rapid heterogeneous electron-transfer reactions at electrodes in a convenient range of potentials. Both indium tin oxide (ITO) on glass (Delta Technologies, Inc., Stillwater, MN) and a 1-mm-thick, (001)-oriented n-TiO2 single-crystal sample (Fuji Titan, ca. 5 mm × 5 mm) were used as the substrates. ITO was degreased in trichloroethylene (Aldrich, Milwaukee, WI) and was subsequently washed with ethanol and dried in air before use. The n-TiO2 (001) single-crystal sample was polished, finished, cleaned, and reduced according to previously reported

† University of Texas.
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1) Sec, for example, Itano, W. M.; Bergquist, J. C.; Wineland, D. J. Science 1987, 237, 612.
5) Betzig, E; Chichester, R. J. Science 1993, 262, 1422.
Figure 1. (A) Idealized diagram of the trapping of molecule A that is oxidized at the tip electrode. The product A⁺ is reduced at the substrate. (B) Schematic diagram of the effect of compressing wax tip coating against substrate.

procedures. A layer of In-Ga alloy was then plated onto the back-side of the sample to serve as an ohmic contact, which was connected to a thin platinum wire. The entire crystal (except its front surface) including the ohmic contact and part of the connecting wire was then electrically shielded with (Micro Super XP2000) stop-off lacquer (Tolber Division, Michigan Chrome and Chemical Company, Hope, AR). A 1 M NaNO₃ solution was used as the supporting electrolyte. All other chemicals were reagent grade and were used without further purification. Millipore reagent water (18 MΩ) was used for the preparation of aqueous solutions. Pt-Ir (80%-20%) wire (0.250-mm diameter), used to construct tips, was obtained from FHC Co. (Brunswick, ME).

Tip Preparation and Apparatus. The ultramicrotips used in this experiment were prepared by the procedures described previously. Insulation of the tip was done with Apiezon wax or polyethylene glue following the procedure reported by Nagahara et al. The insulated tip was then mounted on the SECM in a cell containing a redox electrolyte (e.g., 2 mM Cp₂FeTMA⁺ in 1 M NaNO₃). The success of the tip insulation procedure was checked by cyclic voltammetry in this solution. For a well-insulated tip, the tip current during an initial scan, irt, was less than 50 fA. The very end of the tip was then exposed in the SECM by the following procedure. The potentials of the tip and a conductive substrate; (e.g., 0.60 V vs SCE) were biased at suitable values. When it is reverse-biased or biased at a potential negative of its flat-band potential (V_{fb}), the n-TiO₂ surface becomes positive of V_{fb} (e.g., 0 V vs SCE), curve 2, in a solution containing 2 mM Cp₂FeTMA⁺ and 1.0 M NaNO₃. The tip was biased at 0.60 V vs SCE. The tip moved to the substrate surface at a rate of 38 Å/s. Solid curves are experimental data and symbols are theory. Simulated data for conducting substrate; (+) calculated data based on eq 3; (Δ) simulated data for insulating substrate. Fitting parameters: a = 45 nm, a₀ = 67 nm, l = 28 nm, and D = 5.0 × 10⁻⁹ cm²/s. Experimental irt, is equal to 9.0 pA.

is capable of both STM and SECM measurements with a current sensitivity as low as 50 fA. The electrochemical cell contained a Pt counter electrode and either an SCE or a Pt quasi-reference electrode (PtQRE) as a reference electrode.

Results and Discussion

The results described below are representative of the large number of experiments that were carried out. A chronology of the experiments and additional results and figures (Figures S1–S9) showing related experiments are given in the supporting information.

Tip Current vs Distance. The SMD experiment requires a tip of small diameter that is slightly recessed within the wax sheath. While it is not possible to remove the tip after preparation from the SECM cell and examine it by electron microscopy, information about the exposed area of the tip and the shapes of the tip and insulating sheath can be obtained from electrochemical measurements, that is, from SECM approach curves where the tip current, irt, is measured as a function of tip-substrate spacing, d, as the tip is moved toward the substrate. Tips with the correct configuration show approach curves like those in Figure 2; these were obtained with a solution containing 2 mM Cp₂FeTMA⁺ and 1.0 M NaNO₃, with the tip biased at 0.6 V vs SCE (where Cp₂FeTMA⁺ oxidation is diffusion-controlled). In the experiment in Figure 2 (curve 1), the n-TiO₂ substrate was biased at −0.7 V vs SCE, so that Cp₂FeTMA⁺ generated at the tip was rapidly reduced back to Cp₂FeTMA⁺ at the n-TiO₂ surface. A similar approach curve has previously been reported for the same solution and ITO substrate. Since n-TiO₂ is a semiconductor, when it is biased at a potential negative of its flat-band potential (V_{fb} ≈ −0.25 V vs SCE in this solution), it is forward-biased and is electronically conductive. When it is reverse-biased or biased at a potential positive of V_{fb} (e.g., 0 V vs SCE), the n-TiO₂ surface becomes insulating. Hence, experiments can be carried out with the

References

same tip and solution without making any changes, while the conductance of an n-TiO$_2$ substrate is controlled by adjusting its bias potential. Additional experiments of this type are shown in Figures S2, S3, and S7.

The exposed radius $a$ of a normal disk-shaped electrode can be determined from the steady-state current, $i_{T,\infty}$, when it is far away from the substrate from the equation:

$$i_{T,\infty}^0 = 4nFDC^b a$$  \hspace{1cm} (1)

where $n$ is the number of electrons involved in the redox reaction (1 for Cp$_2$FeTMA$^+$), $F$ is the Faraday constant, $D$ is the diffusion coefficient, and $C^b$ is the bulk concentration of Cp$_2$FeTMA$^+$. However, as discussed below, the tip produced by the procedure described here is recessed slightly within the insulating wax sheath. Such a recessed disk electrode produces a different response.$^{21}$ For a recessed disk, the SECM positive-

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<th>normalized tip current for a recessed disk electrode</th>
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different from a normal disk electrode (i.e., one with $l = 0$), which shows a monotonically increasing tip current, with tips constructed as described above, the tip current increases to reach a maximum and then decreases as the tip comes even closer before showing a large increase (not shown) presumably because of the occurrence of electron tunneling between tip and substrate. Although this current decrease might be attributed to heterogeneous kinetic effects on the electron-transfer reactions,$^{22}$ this behavior was found at even more extreme tip or substrate potentials for various substrates and other redox couples. We thus believe that this region of current behavior is related to the deformation of the insulating sheath as it is pressed against the substrate at very small distances (as shown in Figure 1B). Consider an idealized thin-layer cell as shown in Scheme 2, where we assume that the insulating wax flows inward and outward by equal amounts as it is compressed against the substrate. When this occurs, the steady-state current of the thin-layer cell confined by the disk, substrate, and insulating sheath is given by the following equation (see Appendix C).

$$i_{T,\infty}^0 = \frac{\pi[2la^2 - \Deltaa(a_0^2 + a^2)]/[8a(l - \Deltaa)^2]}{l_{T,\infty}}$$  \hspace{1cm} (3)

where $i_{T,\infty}^0$ is given by eq 1, $a$ is the initial disk radius, $a_0$ is the initial outer radius of the insulating sheath before the compression occurs, and $l$ is the change of the recessed depth of the disk electrode during compression. As implied in eq 3, if $a_0 < \sqrt{3a}$, the tip current will increase to a maximum at $\Deltaa$ equal to $(3a^2 - a_0^2)/(a_0^2 + a^2)$ and then decrease as the tip comes even closer to the surface of the substrate; otherwise, it will reach the maximum at $\Deltaa = 0$. As shown in Figure 2, curve 1, there is some discrepancy between the experimental curve and the theoretically calculated data based on eq 3, perhaps because $\Deltaa$ is not exactly equal to the nominal tip displacement due to the finite compressibility of the insulating wax and deviations from the assumed idealized model. With a diffusion coefficient of Cp$_2$FeTMA$^+$ of $5 \times 10^{-5} \text{ cm}^2/\text{s}$, the theoretical fit of the experimental approach curve yields $a = 45 \text{ nm}$, $a_0 = 67 \text{ nm}$, and $l = 28 \text{ nm}$.

When the n-TiO$_2$ substrate is in its insulating state (e.g., $E_S = 0 \text{ V vs SCE}$), the approach curve is that shown in Figure 2, curve 2. Here the behavior of the recessed tip is similar to that of a usual disk electrode and decreases monotonically with decreasing distance.

**Fluctuation of the Tip Current at Small Distances. The Effect of Substrate Conductance. **The previous study of tip current fluctuations at small distances where a small number of electroactive molecules were trapped between tip and substrate focussed on a conductive substrate.$^{12}$ Here we compare the fluctuation behavior of tip current on both

conductive and insulating substrates. As found in the previous
studies, the fluctuation intensity was strongly dependent on the
distance. To ensure that we were measuring two different
fluctuation behaviors at the same distance, we used the same
substrate, n-TiO$_2$, held at a fixed distance and changed its
conductance simply by varying its bias potential. As shown in
Figure 3A, when the tip was far away from the substrate (e.g.,
$d/d > 2$), only a very small fluctuation in the tip current was
observed when the substrate was either conductive ($E_S = -0.7$ V vs SCE) or insulating ($E_S = 0.0$ V vs SCE). As expected at
such a distance, the current was slightly higher when the
substrate was conductive than when it was insulating. When
the tip approached to within the recessed depth of the disk
electrode (i.e., $d < 1$ nm), considerable fluctuation in the tip current
was observed when the substrate was conductive, while
$i_T$ was
essentially zero and its fluctuation was negligibly small when
the substrate was insulating (see Figure 3B). This suggests that
the positive feedback of the SEC is responsible for the high
amplification of the tip current, which allows for very high
detection sensitivity of only a few molecules of electroactive
species. When the tip was moved even closer to the surface of
the substrate, the tip current decreased because of the decrease
of the active area of the electrode (as discussed in the previous
section), but the relative fluctuation of $i_T$ intensified (see Figure
3C).

Data Analysis. Exact mathematical descriptions of the data
shown in Figure 3C are not possible in the absence of a better
understanding of the processes causing the larger current
fluctuations and the microscopically detailed cell geometry.
However, we can analyze the results by taking the data to be
nondeterministic or random. In cases where no explicit

mathematical equation can be written for the time histories
produced by a random phenomenon, statistical procedures
are usually used to define the descriptive properties of the
data.$^{23,24}$ Three of the basic statistical properties for describing
random data, i.e., the autocorrelation function, the spectral
density function, and the probability density function, for the
time series in Figure 3C are shown in Figure 4. The autocor-
relation function for a time history record $x(t)$, or the time
correlation function (TCF), is a measure of time-related proper-
ties in the data that are separated by fixed time delays and is
defined as

$$TCF(\tau) \equiv \lim_{T \to \infty} \frac{1}{T} \int_{0}^{T} x(t)x(t + \tau)dt$$

in which $\tau$ is the time delay and $T$ is the available record length or
some desired portion of the record length. The power spectral
density function, PSDF, of $x(t)$ is related to the TCF through
Fourier transformation and is given by

$$PSDF(f) = 4 \int_{0}^{\infty} TCF(\tau) \cos(2\pi f \tau)d\tau$$

in which $f$ is the frequency. Both TCF and PSDF provide information on the rate of fluctuation in a time history record.
As shown in Figure 4A, the coherence of the data is high, and
TCF indicates that several fluctuation processes occur at
frequencies on the order of a few tenths of a hertz. Parallel to
the TCF, the PSDF of the time series is fairly broad and contains
several peaks at frequencies on the order of fractions of hertz
(Figure 4B). From the autocorrelation of the time series at zero
and infinite time displacements, a mean square tip current of
37.3 (pA)$^2$ and a mean tip current of 6.1 pA can be obtained,

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*(23) Bendat, J. S.; Piersol, A. G. Random Data Analysis and Measure-

(24) Van Kampen, N. G. Stochastic Processes in Physics and Chemistry;
North Holland: Amsterdam, 1992.*
The time series observed on ITO that we reported previously. This current time series observed on a different conductive substrate, pA apart, with a standard deviation of the peaks of Gaussian peaks. The most probable tip currents are spaced 0.5 pA. A more accurate analysis is based on the probability density function of the time series of Figure 3C contains several probability density plot shown in Figure 5C again is character-

\[ i_{\text{TLC}} = nFDCb/d = nF\pi a^2 DCb/d \]  

For the cylindrical geometry,

\[ i_{\text{TLC}} = nFDN/d^2 N_{AV} \]  

where \( N_{AV} \) is Avogadro’s number. From eq 8 with \( n = 1 \) and the conditions given above, \( N \) is about 10. Thus, each molecule contributes 0.6 pA of current. Note that the TLC in eq 8 is equivalent to the expression derived from the average transit time between tip and substrate, \( t_d \).

\[ t_d = d^2/2D \]  

\[ i = nNe/2t_d = nNeD/d^2 \]

where \( e \) is the electronic charge \((e = F/N_{AV})\). The factor of 2 in eq 10 accounts for the fact that it takes twice the time, \( t_d \), for one electron-transfer event to occur at the tip. Taking \( N = 1 \), \( D = 5 \times 10^{-6} \text{ cm}^2/\text{s} \), and \( d = 11 \text{ nm} \), a tip current of \(-0.6 \text{ pA} \) is obtained. Note that the fluctuation amplitude of the tip current around the mean value as shown in Figure 3C is 0.6 pA.

An exact mathematical description of the entire time series of the tip current based on a deterministic model is not possible. Not only does it require sufficient knowledge of the basic mechanisms of the fluctuation phenomena, which are still not clear, but also it involves inherently stochastic processes involving movement of molecules into and out of the tip/substrate chamber, thermal fluctuations, and other random processes. However, a limited description of the general shape of the current fluctuation is of interest. Assuming that the redox molecules diffuse inside the TLC with a constant \( D \), as suggested by eq 10, there are at least two possibilities which might cause the tip current to change with time: (1) changes in \( d \) and the electrode area, for example, because of temperature fluctuations and (2) fluctuations in the number of molecules, \( N \). A continuous change in \( d \) without changing \( N \) cannot cause a stepwise fluctuation in the tip current; however, it might account for a slow drift of the TLC current. The probability of large step or quantized changes in \( d \) without changing \( N \) is small. We cannot rule out the possibility that the electric field in the gap may also affect the observed behavior, especially at very small supporting electrolyte concentrations.25,26 In the work described here, however, a high concentration of supporting electrolyte was employed. Further work will be required to explore possible electric field effects.

The experimental shapes of the rise and fall of the current can be fit very approximately (Figure 6) by an exponential rise and fall with time constants of the order of \( 2-10 \text{ s} \) (attributed to thermal variations in \( d \)), a residence time (time for the current to rise and fall) of \( 5-15 \text{ s} \), and a time between events of the
order of 10 s, for the conditions of this experiment. These results are consistent with the TCF and PSDF analyses shown in Figure 4.

Conclusions

This study has shown that the positive feedback of the SECM is responsible for the observation of significant fluctuations in the tip current. Both TCF and PSDF analyses indicate that multiple fluctuation processes with the frequencies of fluctuation on the order of a few tenths of a hertz. The PDF contains several bell-shaped Gaussian peaks. The most probable tip currents at the given cell parameters (base radius = 15 nm and height = 11.2 nm) and \( C^b \) equal to 2 mM are spaced 0.5 (±0.1) pA apart. These fluctuation amplitudes of the current time series correspond quite well with the contribution current expected for a single molecule in a TLC of that geometry. Very similar fluctuation behavior of the tip currents was observed for both ITO and n-TiO₂ in the conductive state.

Appendix A

Approximate Equation for Recessed Electrode. An equation for the recessed ultramicrodisk can be obtained by assuming that the small recessed layer behaves as a thin layer cell (TLC) bounded by the disk and the bulk solution. The concentration gradient at steady state at the surface of the usual disk electrode coplanar with its insulating sheath with the following boundary conditions

\[
\begin{align*}
C &= C^l \quad z = l \quad r \leq a \\
\partial C / \partial z &= 0 \quad z = 0 \quad r > a \\
C &= C^b \quad z = \infty \quad r \geq 0 \\
C &= C^b \quad z \geq 0 \quad r = \infty
\end{align*}
\]

has been solved by several authors\(^{21}\) in different ways and is given by\(^{27}\)

\[
(\partial C / \partial z)_{z=0} = 2C^b / \pi [1/((a^2 - r^2)^{1/2})], \quad 0 \leq r \leq a
\]

For the recessed disk-shaped electrode shown in Scheme 1, a simplified model with the following boundary conditions is proposed:

\[
\begin{align*}
C &= C^l \quad z = l \quad r \leq a \\
\partial C / \partial z &= 0 \quad z = l \quad r > a \\
C &= C^b \quad z = \infty \quad r \geq 0 \\
C &= C^b \quad z \geq 0 \quad r = \infty
\end{align*}
\]

The concentration gradient at \( z = l \) can be solved in a similar way and is given by

\[
(\partial C / \partial z)_{z=l} = [2(C^b - C^l) / \pi] [1/(a^2 - r^2)^{1/2}] \quad (14)
\]

For simplicity, we have assumed that the concentration at \( z = l \), \( C^l \), is constant, independent of \( r \). Thus, eq 2 derived here is only approximate. Equation 1 can be obtained by the following integration

\[
i^b_{T,\infty} = \int_0^\infty nFDC \frac{\partial C}{\partial z} / \partial z = 0 \quad 2\pi r dr
\]

where the concentration gradient is given by eq 12.

Similarly, the steady-state current on a recessed disk electrode is obtained from the following integration with the concentration gradient given by eq 14.

\[
i^b_{T,\infty} = \int_0^\infty nFDC \frac{\partial C}{\partial z} / \partial z = 0 \quad 2\pi r dr = 4nFDC(C^b - C^l)a
\]

For a cylindrical TLC with a base radius of \( a \) and a height equal to \( l \), the steady-state diffusion current is given by

\[
i_{TLC} = \pi a^2 nFDC^l / l
\]

Here, we have assumed boundary conditions: \( C = 0 \) at \( z = 0 \) and \( C = C^l \) at \( z = l \).

From the requirement of current continuity, we have \( i_{TLC} = i^b_{T,\infty} \). Thus,

\[
C^l = 4lC^b / (4l + \pi a)
\]

Substituting \( C^l \) of eq 18 into eq 17, we obtain

\[
i_{TLC} = i^b_{T,\infty} = [\pi a(4l + \pi a)](4nFDC^b a)
\]

Thus,

\[
i^b_{T,\infty} = [\pi a(4l + \pi a)]^b_{T,\infty}
\]

Appendix B

Digital Simulation of Current at a Recessed Tip Approaching a Conductive Substrate. A finite difference method\(^{28,29}\) was employed to simulate the observed response


for cylindrical coordinates.

\[
\frac{\partial C_i(r,z,t)}{\partial t} = D_i \left[ \frac{\partial^2 C_i(r,z,t)}{\partial r^2} + \frac{1}{r} \frac{\partial C_i(r,z,t)}{\partial r} + \frac{\partial^2 C_i(r,z,t)}{\partial z^2} \right]
\]

where \( C_i \) is the concentration of species \( i \) as a function of time, \( t \), and coordinates \( r \) (radial) and \( z \) (normal). An exponentially increasing space grid\(^{30,31} \) and the alternate-direction implicit method\(^{31,32} \) was employed. Details of the simulation are given in supporting information.

Appendix C

Approximate Equation for Compression of Recessed Electrode against Substrate. We take the idealized geometry shown in Scheme 2 for the wax-sheathed metal tip and assume that the insulating wax flows inward and outward by equal amounts as it is compressed against the substrate. Thus,

\[
(i_{\text{TLC}}) = \frac{\pi (a - \Delta a)^2 \Delta l}{2} \left( \frac{a_0^2 + a^2}{8a} \right) nFDC^b (l - \Delta l) \]  \( i_{\text{TLC}} = \{ \pi [2la^2 - \Delta l(a_0^2 + a^2)]/[8a(l - \Delta l)] \} i_{\text{T},\infty}^0 \)  \( (3) \)

By defining several dimensionless variables \( I \equiv i_{\text{TLC}}/i_{\text{T},\infty}, L \equiv (l - \Delta l)/a, A_0 \equiv a_0/a, \Delta L \equiv \Delta l/a, \) and \( L^0 \equiv l_{\text{T},0}/l_{\text{T},\infty}, \) we can write eq 3 in a normalized form

\[
I = \{ \pi [2L + \Delta L(1 - A_0^2)]/8L^2 \} L^0 \]  \( (20) \)

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Supporting Information Available: Chronology of the single molecule electrochemistry experiments and details of the digital simulation of current at a recessed tip approaching a conductive substrate (15 pages). See any current masthead page for ordering and Internet access instructions.

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