Directional Electron Transfer from Ubiquinone-10 to Cytochrome c at a Biomimetic Self-Assembled Monolayer Modified Electrode

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ABSTRACT

The redox behavior of cytochrome c (Cyt c) at a ubiquinone-10 (UQ) incorporated self-assembled monolayer (SAM)-modified electrode was studied by cyclic voltammetry. A well-defined catalytic wave due to the reduction of Cyt c by UQ was observed at around −0.4 V vs. Ag/AgCl (saturated KCl). However, the re-oxidation peak of UQ at around +0.3 V was small, suggesting no significant catalytic ability of UQ for the re-oxidation of Cyt c. These voltammetric behaviors could be well simulated by digital simulation with a simple reaction model in which UQ and Cyt c coexist homogeneously in a reaction layer on the base gold electrode. The parameters obtained by curve fitting of cyclic voltammograms showed that the re-oxidation of Cyt c by UQ is somewhat thermodynamically unfavorable and, importantly, kinetically slow. This slow process is probably originated from spatial separation between the redox species. Such a directional or one-way electron transfer may be occurring in the mitochondrial respiratory chain system to achieve efficient energy production.

Keywords: SAM, Biomembrane Model, Digital Simulation

1. Introduction

It is well known that biological energy production in the mitochondrial respiratory chain system is realized by electron transfer (ET) occurring at biomembranes.1 In this ET pathway, electrons are directionally shuttled between specific components incorporated in the biomembrane. Such directional or one-way electron transfer would be preferable for efficient energy production, however its authenticity or origin has not yet been established.

So far, several biomembrane models, including planar or supported bilayer lipid membranes2–5 (BLMs), polarized oil/water interfaces,6–9 and self-assembled monolayer (SAM) modified electrodes,10–12 have been used to clarify the mechanism of potential-dependent ET reactions. In a previous study,11 Takehara et al. successfully incorporated ubiquinone-10 (UQ; also known as coenzyme Q10) into alkanethiol SAMs formed on gold, and then used cyclic voltammetry (CV) to observe a pair of separated cathodic and anodic peaks due to the redox reactions of UQ. In our recent study,12 it was found that when a longer alkanethiol (1-dodecylmercaptan; DM or 1-octadecylmercaptan; OM) was used to form a SAM, the redox reaction of UQ was significantly slowed down not only by the separation of UQ from the Au|SAM interface but also by the inhibition of the ET-coupled proton transfer across the SAM|solution interface. However, when a strongly hydrophobic supporting-electrolyte cation such as tetratramethylammonium ion was used, the adsorption of the cation on the SAM|solution interface induced a possible structural disorder of the monolayer and then facilitated the supply of protons from the solution to UQ in the monolayer and thus the ET between UQ and the base electrode.

In this study, we studied a biomimetic ET process between UQ and cytochrome c (Cyt c) at the DM SAM-modified gold electrode. In the mitochondrial respiratory chain, the reduced form of UQ (ubiquinol; H2UQ) transfers electrons to Cyt c via complex III (ubiquinol–Cyt c reductase).1 Thus, the in vivo ET between H2UQ and Cyt c occurs via enzymatic reaction. Nevertheless, it would be fruitful to study the ET reaction in a simple non-enzymatic system such as the SAM modified electrode, as it may provide a basic principle for understanding ET processes occurring at biomembranes. In this study, we found that a reduction peak of UQ at the DM SAM-modified electrode was increased by adsorption of the oxidized form of Cyt c (Cyt (ox)) to the SAM|solution interface, showing a catalytic reduction of Cyt (ox) by UQ. Interestingly enough, however, the reoxidation peak of UQ was not increased by the addition of Cyt (ox). This suggested a one-way ET from H2UQ to Cyt (ox) at the biomimetic SAM modified electrode. This would show a possible directional ET in biological systems. Here, we analyzed cyclic voltammograms obtained for the SAM modified electrode by means of digital simulation with a simple reaction model, and then discussed the possible origin of the directional ET.
5 min under ultrasonic field. In reference to Chen et al.’s paper, Cyt c was immobilized on the SAM electrode by exposure to a solution of x (20–160) μM Cyt c in 5 mM KH2PO4–K2HPO4 buffer (pH 6.8) for 15 min at room temperature. The resultant Cyt c-immobilized SAM electrode was washed with pure water before CV measurements.

2.3 CV measurements

CV was performed as reported previously. In short, the above-described SAM electrode, a platinum wire electrode, and a Ag/AgCl (saturated KCl) electrode were immersed in a buffered solution (5.0 mM NaH2PO4–Na2HPO4; pH 7.2), being used as the working, counter, and reference electrodes, respectively. In a similar manner to that reported previously, no supporting electrolyte other than the phosphate buffer was added to the electrolyte solution in order to avoid desorption of Cyt c from the SAM electrode. The voltage sweep rate (v) was changed in the range from 0.01 to 0.2 V s⁻¹. The electrolytic solution was thoroughly deaerated by purging with nitrogen gas for at least 15 min. The electrolytic cell was placed in an air-conditioned room (at 25°C).

2.4 Digital simulation

Cyclic voltammograms recorded were analyzed with the help of a digital-simulation technique. A spread-sheet program for the analysis was written in Microsoft Excel 2007 and used for manual curve-fitting analyses.

3. Results and Discussion

3.1 Voltammetric behavior of Cyt c at the SAM electrode

First, we examined the possibility of direct electron transfer (DET) for Cyt c at the SAM-modified gold electrode. Figure 1 shows the cyclic voltammograms obtained with the DM SAM-modified electrode in the absence (a) and presence (b) of Cyt c immobilized on the SAM with x = 20 μM (vide supra); note that UQ was not incorporated therein. As shown in the figure, Cyt c gave an ill-defined voltammetric wave at around −0.4 V. The cathodic peak was relatively clear, but the anodic peak was very weak and not very reproducible (sometimes, appeared as two separate small peaks).

On the other hand, a better-defined voltammetric wave was reported for Cyt c adsorbed on a similar DM SAM-modified electrode. The previous authors claimed the large negative shift (about 0.4 V) of the redox potential of Cyt c due to its denaturation on the hydrophobic surface of the DM SAM. However, our digital simulation analysis of cyclic voltammograms (vide infra) has shown that such a large negative shift of the redox potential is inadequate for reproducing the voltammograms of UQ in the presence of Cyt c.

The observed small wave for Cyt c may be related to the presence of pinholes or defects of the DM SAM. Though data are not shown, such a wave was never observed for a longer CH3-terminated alkanethiol SAM (i.e., OM SAM).

Thus, no well-defined DET was achieved between Cyt c and the gold electrode through a relatively long CH3-terminated alkanethiol (DM or OM) SAM. This is quite different from those observed for carboxylic acid-terminated alkanethiol SAMs, in which a strong electrostatic interaction between the carboxylate termini and Cyt c induces a possible structural disorder of the monolayer to realize relatively fast DET between Cyt c and the base gold electrode. For the CH3-terminated longer alkanethiol SAMs, however, the adsorption of Cyt c on the SAM surface seems not to break significantly the neat structure of the monolayer, and hence the DET between Cyt c and the electrode may be rather strongly inhibited by the alkyl-chain monolayer.

3.2 Voltammetric behavior of UQ at the DM-SAM electrode

Figure 2 shows cyclic voltammograms obtained for the UQ-incorporated DM-SAM electrode in the absence of Cyt c. As reported previously, a pair of well-defined cathodic and anodic peaks was obtained. These peaks were much widely separated by more than 0.8 V, suggesting a very strong inhibition effect of the SAM on the redox reaction of UQ. As described previously, this effect is increased with increasing the carbon number of the alkanethiols, being induced not only by the separation of UQ from the gold/SAM interface but also by the inhibition of the ET-coupled proton transfer across the SAM/solution interface. The cyclic voltammogram shown in Fig. 2 could be well reproduced by digital simulation with a simple reaction model (vide infra).

With the increase of v (in V s⁻¹) from 0.01 to 0.2, the cathodic and anodic peak potentials (Epc and Ep a) were shifted to more negative and positive values, respectively, however the midpoint potential, Em id ≡ (Epc + Ep a)/2, was slightly dependent on v as Em id/V = −0.024 + 0.230v: From this result, the formal potential of UQ at pH 6.8 (E°CQ) was estimated to be −0.024 V vs. Ag/AgCl (saturated KCl). This value is +0.17 V vs. SHE, being close to the formal potential of UQ adsorbed at a mercury electrode in water, i.e., +0.1 V vs. SHE at pH 7. This may suggest that the redox moiety of UQ incorporated in the SAM is in a hydrophilic environment, due to possible preferential hydration of the redox moiety even in the hydrophobic membrane.

A pair of cathodic and anodic peaks shown in Fig. 2 is a typical adsorption wave having no diffusion tail. The cathodic peak area (marked by diagonal lines) was almost identical with the anodic peak area; the amount of electricity calculated from the cathodic peak area was almost identical with that reported previously.

![Figure 1](image1.png)

Figure 1. Cyclic voltammograms obtained with the DM SAM-modified gold electrode in the absence (a) and presence (b) of Cyt c immobilized on the SAM with x = 20 μM; note that UQ was not incorporated therein. pH = 6.8; v = 0.1 V s⁻¹.

![Figure 2](image2.png)

Figure 2. Cyclic voltammograms obtained with the DM SAM-modified gold electrode in the absence (a) and presence (b) of UQ in the SAM; note that Cyt c was not immobilized on the SAM. pH = 6.8; v = 0.1 V s⁻¹.
peak area was not very dependent on \( v \) in the range of 0.01–0.2 \( \text{V s}^{-1} \). By considering that the observed peaks are due to the two-electron redox reaction of UQ (i.e., \( \text{UQ} + 2e^- + 2H^+ \rightleftharpoons \text{H}_2\text{UQ} \)), we then estimated the total surface concentration of the UQ species on the electrode, e.g., \( I_\text{f} = 7.17 \times 10^{-12} \text{mol cm}^{-2} \) for the electrode used in Fig. 2; however, the reproducibility of \( I_\text{f} \) was not very good, the relative standard deviation being \( \approx 25\% \).

3.3 Catalytic reduction of Cyt \( c \) by UQ

It has been found that UQ incorporated in the DM SAM can catalyze the reduction of Cyt \( c \) immobilized on the SAM. As shown above, Cyt \( c \) undergoes DET at the DM SAM electrode even in the absence of UQ, however the voltammetric signal was weak and ill-defined. In contrast, Cyt \( c \) gave a larger and well-defined catalytic wave at the UQ-incorporated SAM electrode, as shown in Fig. 3. The cathodic peak current at around \(-0.4 \text{ V}\) was not very dependent on \( x \) (i.e., \(-0.19, -0.22, \) and \(-0.24 \mu \text{A} \text{ for } x = 20, 80, \) and 160 \( \mu \text{M} \), respectively). This may suggest a possible saturation of Cyt \( c \) on the SAM surface under the concentration conditions used. Owing to this possible saturation, the observed catalytic wave did not show a typical sigmoidal curve, but was definitely influenced by a catalytic effect of UQ on both the cathodic and anodic scans, as shown by diagonal lines in Fig. 3.

Thus, as expected, we observed a catalytic ability of UQ for the reduction of Cyt \( c \) on the DM-SAM electrode as a biomembrane model. However, it is noteworthy that the anodic peak for the oxidation of UQ was almost unchanged in amplitude, though the peak potential was somewhat shifted to a more negative value; this negative shift is probably because of the facilitation of proton transfer induced by a structural disorder of the SAM due to the hydrophobic adsorption of Cyt \( c \); it was reported that hydrophobic ions such as tetrapentylammonium ions caused a possible structural disorder of SAM to facilitate the redox reaction of UQ. Anyway, the result shown in Fig. 3 clearly demonstrates that the re-oxidation of Cyt \( c \) is not catalyzed by the oxidized form of UQ incorporated in the DM SAM.

3.4 Reaction model

To understand the above voltammetric behaviors of UQ/Cyt \( c \) at the SAM electrode, we employed a digital-simulation technique for reproduction of the cyclic voltammograms. For the simulation, we assumed a simple reaction model shown in Fig. 4. This figure has been drawn by imagining that UQ is buried in the monolayer and Cyt \( c \) is placed at the SAM/solution interface, however the location or position of these redox species has nothing to do with the results of digital simulation (vide infra). Nevertheless, in the real electrode system, the location or orientation of UQ and Cyt \( c \) on the electrode is important; their certain portions cannot participate in the electrode reactions. In the present model, we have taken into account only electrochemically “active” species to explain the voltammetric behaviors.

In the reaction model, it is assumed that the two-electron redox reaction of UQ incorporated in the SAM proceeds consecutively:

\[
\text{UQ} + e^- + H^+ \rightleftharpoons \text{H}_2\text{UQ}
\]

(1)

\[
\text{HUQ} + e^- + H^+ \rightleftharpoons \text{H}_2\text{UQ}
\]

(2)

The formal potentials of these one-electron reactions are denoted by \( E_{1}^\text{c} \) and \( E_{2}^\text{c} \), respectively. As observed in the voltammetric measurement (Fig. 2), reactions (1) and (2) occur in one step as

\[
\text{UQ} + 2e^- + 2H^+ \rightleftharpoons \text{H}_2\text{UQ}
\]

(3)

The formal potential of this two-electron reaction (\( E_{12}^\text{c} \)) could be estimated experimentally (vide supra), and should be related to \( E_{1}^\text{c} \) and \( E_{2}^\text{c} \) as

\[
E_{12}^\text{c} = \frac{E_{1}^\text{c} + E_{2}^\text{c}}{2}
\]

(4)

In the present simulation analysis, we set \( E_{12}^\text{c} \) to be an adjusting parameter, from which the value of \( E_{12}^\text{c} \) was obtained by using Eq. (4).

The faradaic current (\( I \)) should be given by the sum of the contributions from reactions (1) and (2):

\[
I = I_1 + I_2
\]

(5)

with

\[
I_1 = -FA(k_{1i}c_{\text{UQ}} - k_{b1i}c_{\text{HUQ}})
\]

(6)

\[
I_2 = -FA(k_{2i}c_{\text{HUQ}} - k_{b2i}c_{\text{H}_2\text{UQ}})
\]

(7)

where \( F \) is the Faraday constant, \( A \) is the surface area of the gold electrode (here, 0.0707 \text{ cm}^2), \( c \) is the concentration in the SAM for the active UQ species indicated by the subscript (i.e., UQ, HUQ, or H2UQ), and \( k_1 \) and \( k_b \) are the forward and backward rate constants, respectively, for reactions (1) and (2) (indicated by subscript 1 or 2). The rate constants are then given by the function of the electrode potential (\( E \)), i.e., Butler–Volmer equations.

\[
k_{1i} = k_i^\text{e} \exp \left[ -\frac{\alpha_1 F}{RT} (E - E_1^\text{c}) \right]
\]

(8)

\[
k_{b1} = k_i^\text{e} \exp \left[ \frac{(1 - \alpha_1) F}{RT} (E - E_1^\text{c}) \right]
\]

(9)
where $k$ and $\alpha$ are the standard rate constant or the transfer coefficient for reaction (1) or (2), and $R$ and $T$ have usual meanings.

In the model shown in Fig. 4, it is assumed for simplicity that Cyt $c$ adsorbed on the SAM does not undergo DET via the SAM. Though the DET process cannot be entirely excluded, the above experimental results suggest that Cyt $c$ reacts more efficiently with the UQ species in the monolayer:

\[
\text{H}_{2}\text{UQ} + \text{Cyt} (\text{ox}) \rightleftharpoons \text{UQ} + \text{Cyt} (\text{red}) \quad (12)
\]

The reaction rates for these chemical reactions are given respectively by

\[
\frac{dc_{\text{UQ}}}{dt} = -\frac{dc_{\text{H}_{2}\text{UQ}}}{dt} = \frac{k_{\text{EC}}}{C_{\text{Cyt}}C_{\text{H}_{2}\text{UQ}}} - k_{b\text{EC}} \text{Cyt} \text{C}_{\text{H}_{2}\text{UQ}} \text{(red)} \quad (14)
\]

\[
\frac{dc_{\text{Cyt}}}{dt} = \frac{dc_{\text{H}_{2}\text{UQ}}}{dt} = \frac{k_{\text{EC}}}{C_{\text{Cyt}}C_{\text{H}_{2}\text{UQ}}} - k_{b\text{EC}} \text{Cyt} \text{C}_{\text{H}_{2}\text{UQ}} \text{(red)} \quad (15)
\]

In the present simulation analysis, $k_{\text{EC}}$ was used as an adjusting parameter; therefore, $k_{b\text{EC}}$ was evaluated from $E_{\text{Cyt}}^\text{red}$ with $E_{\text{Cyt}}^\text{ox}$ or $E_{\text{Cyt}}^\text{ox}$ and the formal potential of Cyt $c$ ($E_{\text{Cyt}}^\text{red}$):

\[
K_{\text{EC}} = \frac{k_{\text{EC}}}{k_{b\text{EC}}} = \exp\left[\frac{F}{RT}(E_{\text{Cyt}}^\text{red} - E_{\text{Cyt}}^\text{ox})\right] \quad (16)
\]

\[
K_{\text{EC}} = \frac{k_{\text{EC}}}{k_{b\text{EC}}} = \exp\left[\frac{F}{RT}(E_{\text{Cyt}}^\text{red} - E_{\text{Cyt}}^\text{ox})\right] \quad (17)
\]

In the reaction model shown in Fig. 4, the redox species responsible for the electrochemical reactions, (1) and (2), and the chemical reactions, (12) and (13), are assumed to exist in a reaction layer with the thickness of $d$. Here, the value of $d$ is arbitrary, but was chosen to be the same as the DM SAM, i.e., $d = 1.3 \times 10^{-7} \text{cm}$, which has been estimated from the molecular length (1.5 nm) and the tilt angle (~30°). Thus, the reaction layer has the thickness of $d$, however, it is assumed for simplicity that the concentration of any redox species is “homogeneous” in the reaction layer. This assumption means that the location of the transport of UQ/Cyt $c$ in the SAM were not taken into consideration. The concentration ($c$) of a redox species in the reaction layer was then obtained simply by dividing the surface concentration ($f$) by the above $d$-value: $c = f/d$. We would like to add that the diameter of a Cyt $c$ molecule is 3.1 nm, which is about twice as large as the SAM thickness (note that the relative sizes of Cyt $c$, UQ, and SAM in Fig. 4 are not to scale).

### 3.5 Digital simulation

In digital simulation of cyclic voltamograms, calculation was repeated at a time interval of $\Delta t = 1/(1000 \text{ s}) = 0.01 \text{ s}$ at $v = 0.1 \text{ V s}^{-1}$. The electrochemical rate constants at an arbitrary time $t = m\Delta t$ ($m = 0, 1, 2, \ldots$) are given by Eqs. (8)–(11) as

\[
k_{11}^m = k_1^\text{f} \exp\left[\frac{-\alpha F}{RT}(E_m - E_1^\text{f})\right] \quad (18)
\]

\[
k_{12}^m = k_2^\text{f} \exp\left[\frac{-(1 - \alpha)F}{RT}(E_m - E_2^\text{f})\right] \quad (19)
\]

\[
k_{12}^m = k_2^\text{f} \exp\left[\frac{-(1 - \alpha)F}{RT}(E_m - E_2^\text{f})\right] \quad (20)
\]

\[
k_{12}^m = k_2^\text{f} \exp\left[\frac{-(1 - \alpha)F}{RT}(E_m - E_2^\text{f})\right] \quad (21)
\]

where $E_m$ is the electrode potential at $t = m\Delta t$.

The concentrations of the UQ species at $t = m\Delta t$ ($m = 1, 2, \ldots$) can be expressed by the following equations (for derivation, see the Supporting Information):

\[
c_{\text{UQ}}^m = \frac{d(\Delta t k_{11}^m + d)\epsilon_{\text{UQ}}^{\text{ox}} - \Delta t k_{12}^m (c_1 - \epsilon_{\text{Cyt}}^{\text{ox}})}{\Delta t k_{12}^m + d - (\Delta t)^2 k_{11}^m k_{12}^m - \Delta t k_{11}^m k_{12}^m} \quad (22)
\]

\[
c_{\text{H}_{2}\text{UQ}}^m = \frac{d\epsilon_{\text{H}_{2}\text{UQ}}^{\text{ox}} + \Delta t k_{12}^m (c_1 - \epsilon_{\text{Cyt}}^{\text{ox}})}{\Delta t k_{12}^m + d} \quad (23)
\]

where $k_{11}^m = k_{11}^0 + k_{11}^m$, $k_{12}^m = k_{12}^0 + k_{12}^m$, and $c_1 = c_{\text{UQ}} + c_{\text{H}_{2}\text{UQ}} + c_{\text{Cyt}}^{\text{ox}}$ is the total concentration of the UQ species. The initial concentrations for the species at $t = 0$ (i.e., $m = 0$) are: $c_0^{\text{UQ}} = c_1$, $c_0^{\text{H}_{2}\text{UQ}} = 0$, and $c_0^{\text{Cyt}} = 0$.

The correlation of concentrations for chemical reactions (12) and (13) was made by using the following equations:

\[
c_{\text{UQ,cont}}^m = c_{\text{UQ}}^m + \Delta c_{\text{UQ}}^m \quad (25)
\]

\[
c_{\text{H}_{2}\text{UQ,cont}}^m = c_{\text{H}_{2}\text{UQ}}^m + \Delta c_{\text{H}_{2}\text{UQ}}^m \quad (26)
\]

\[
c_{\text{Cyt}}^{\text{ox,cont}}^m = c_{\text{Cyt}}^{\text{ox,cont}}^m + \Delta c_{\text{Cyt}}^{\text{ox,cont}} \quad (27)
\]

where $\Delta c_{\text{UQ}}^m$ and $\Delta c_{\text{Cyt}}^{\text{ox,cont}}$ represent the concentration changes by chemical reactions (12) and (13), respectively, and are given by (cf. Eqs. (14) and (15))

\[
\Delta c_{\text{UQ}}^m = \Delta t(k_{\text{EC}} C_{\text{Cyt}} C_{\text{H}_{2}\text{UQ}}^{\text{ox}} - k_{b\text{EC}} C_{\text{Cyt}}^{\text{ox,cont}} \text{C}_{\text{H}_{2}\text{UQ}}^{\text{ox}}) \quad (28)
\]

\[
\Delta c_{\text{Cyt}}^{\text{ox,cont}}^m = \Delta t(k_{\text{EC}} C_{\text{Cyt}}^{\text{ox,cont}} C_{\text{H}_{2}\text{UQ}}^{\text{ox}} - k_{b\text{EC}} C_{\text{Cyt}}^{\text{ox,cont}} C_{\text{H}_{2}\text{UQ}}^{\text{ox}}) \quad (29)
\]

The thus corrected concentrations were employed for calculation of the concentrations of the UQ and Cyt $c$ species at $t = (m + 1)\Delta t$ ($m = 0, 1, 2, \ldots$) as

In Eq. (34), $c_{\text{Cyt}}^{\text{ox,cont}}$ is the total concentration of “active” Cyt $c$ molecules being responsible for the catalytic wave shown in Fig. 3. For the digital simulation analysis, $c_{\text{Cyt}}^{\text{ox,cont}}$ ($= c_{\text{Cyt}}^{\text{ox,cont}}$; note that $c_{\text{Cyt}}^{\text{ox,cont}}$ was estimated in advance from the amount of electricity for the catalytic wave, which is marked by diagonal lines in Fig. 3. As seen in the figure, the catalytic current was also
absence of Cyt c on the SAM. pH = 6.8; v = 0.1 V s⁻¹. The solid line shows the experimental voltammogram, whereas open circles represent the “theoretical” voltammogram that is shown by adding the experimental base current to the digital simulation curve.

**Table 1.** Parameters used or obtained in the curve fitting of cyclic voltammograms (Figs. 5 and 6).

| Parameter      | Unit       | 0              | 20             | 80             | 160            |
|----------------|------------|----------------|----------------|----------------|----------------|
| $c_1$          | M          | $5.51 \times 10^{-5}$ | $7.19 \times 10^{-5}$ | $5.51 \times 10^{-5}$ | $7.00 \times 10^{-5}$ |
| $\Gamma_1$     | mol cm⁻²   | $7.17 \times 10^{-12}$ | $1.29 \times 10^{-11}$ | $7.17 \times 10^{-12}$ | $9.10 \times 10^{-12}$ |
| $E_{\text{Cyt}}$ | V          | $-0.024$       | $-0.024$       | $-0.024$       | $-0.024$       |
| $E_{\text{c}}$ | V          | $+0.0016$      | $+0.0016$      | $+0.0016$      | $+0.0016$      |
| $k_1$          | cm s⁻¹     | $2.0 \times 10^{-10}$ | $8.0 \times 10^{-10}$ | $3.0 \times 10^{-10}$ | $3.0 \times 10^{-10}$ |
| $k_2$          | cm s⁻¹     | $3.0 \times 10^{-10}$ | $2.0 \times 10^{-10}$ | $3.0 \times 10^{-10}$ | $1.0 \times 10^{-10}$ |
| $a_1$          |           | 0.52           | 0.40           | 0.55           | 0.50           |
| $a_2$          |           | 0.66           | 0.70           | 0.50           | 0.50           |
| $c_i^*$        | M          | 0              | $9.28 \times 10^{-4}$ | $1.42 \times 10^{-3}$ | $1.05 \times 10^{-3}$ |
| $\Gamma_i$     | mol cm⁻²   | 0              | $1.26 \times 10^{-10}$ | $1.84 \times 10^{-10}$ | $1.36 \times 10^{-10}$ |
| $E_{\text{c}}$ | V          | +0.030         | +0.030         | +0.030         | +0.030         |
| $K_{\text{Cyt}}$ |           | 23             | 23             | 23             | 23             |
| $k_{\text{Cyt}}$ | M⁻¹ s⁻¹   | $1.0 \times 10^2$ | $1.0 \times 10^2$ | $1.0 \times 10^2$ | $1.0 \times 10^2$ |
| $k_{\text{C}}$ | M⁻¹ s⁻¹   | 4.4            | 4.4            | 4.4            | 4.4            |
| $K_C$          |           | 3.0            | 3.0            | 3.0            | 3.0            |
| $k_{\text{C}2}$ | M⁻¹ s⁻¹   | $1.8 \times 10^3$ | $2.8 \times 10^3$ | $2.8 \times 10^3$ | $2.8 \times 10^3$ |
| $k_{\text{C}2}$ | M⁻¹ s⁻¹   | $5.9 \times 10^2$ | $9.3 \times 10^2$ | $9.3 \times 10^2$ | $9.3 \times 10^2$ |

*Adjusting parameters.

observed on the anodic reverse scan following the cathodic forward scan; therefore, this current was also counted to estimate the total amount of electricity. In the curve fitting of cyclic voltammograms, we have referred to the thus estimated $c_i^*$ but have obtained its more appropriate value as an “adjusting” parameter.

Finally, using the above parameters, we can calculate the current at $t = m\Delta t$ ($m = 0, 1, 2, \ldots$) (cf. Eqs. (5)-(7)):

$$I^* = -F \Delta \left[ \kappa_{\text{Cyt}} \left( \kappa_{\text{Cyt}} \delta_{\text{Cyt}}^* \nu \right) - \left( \kappa_1 \delta_1 - \kappa_2 \delta_{\text{Cyt}}^* - \kappa_2 \delta_{\text{Cyt}}^* \right) \right]$$

### 3.6 Curve fitting of cyclic voltammograms

The above mentioned simulation method enabled us to perform manual curve fitting of cyclic voltammograms.

First, as shown in Fig. 5, the cyclic voltammogram of UQ in the absence of Cyt c could be well reproduced by digital simulation. In the simulation, the values of $E_{\text{c}}$ and $c_i^*$ were estimated in advance by the analysis of cyclic voltammograms (vide supra). On the other hand, the values of $E_{\text{Cyt}}$ and $k_1$, $k_2$, $a_1$, and $a_2$ were used as adjusting parameters. The value of $E_{\text{Cyt}}^*$ was obtained from Eq. (6) with the values of $E_{\text{c}}$ and $E_{\text{Cyt}}$. The experimentally observed large separation of the cathodic and anodic peaks could be reproduced by smaller values of $k_1$ and $k_2$ (see Table 1 shown below).

As shown in Fig. 6, the cyclic voltammograms in the presence of Cyt c could also be well reproduced by digital simulation with the reaction model of Fig. 4. Here, the values of $E_{\text{c}}$ and $c_i^*$ were
Figure 7. Simulated cyclic voltammograms for the catalytic redox reactions of Cyt c by UQ incorporated in a SAM-modified electrode (v = 0.1 V s⁻¹). $X = (k_{b,C1}/4.4 \text{ M}^{-1} \text{s}^{-1}) = 1$ (a), 10 (b), 20 (c), 50 (d), 100 (e), 200 (f), and 500 (g). The other parameters are the same as those shown for $x = 80 \mu$M in Table 1.

3.7 Origin of the directional ET

Table 1 shows the parameters used or obtained in the curve fitting of cyclic voltammograms. First, we focus on the value of $E_{c,C1}$ ($= +0.030 \text{ V vs. Ag/AgCl (saturated KCl)}$) obtained as an adjusting parameter. This value is close to the reported half-wave potential of $+0.255 \text{ V vs. SHE}$ (i.e., $+0.058 \text{ V vs. Ag/AgCl (saturated KCl)}$), which was determined at the gold electrode in the presence of an effector, 4,4’-bipyridyl (pH 7). This suggests that the solvation environment of the redox center of Cyt c on the SAM surface should be similar to that in aqueous solution.

Combination of the value of $E_{c,C1}^\prime$, with those of $E_{c,C1}^*$ and $E_{c,C1}^\prime$, yield the values of $K_{C1}$ and $K_{C2}$, respectively (cf. Eqs. (16) and (17)). As shown in Table 1, $K_{C1}$ and $K_{C2}$ are larger than unity, i.e., 23 and 3.0, respectively. Such equilibrium constants suggest that the reduction of Cyt c with the UQ species, i.e., the forward processes in reactions (12) and (13), are somewhat thermodynamically unfavorable, but the re-oxidation reactions of Cyt c, i.e., the corresponding backward processes, are unfavorable. In the voltammetric measurement, however, a sufficiently large overpotential ($\eta > 0.2 \text{ V}$) was imposed on the electrode at around the anodic peak potential (about $+0.3 \text{ V}$). Accordingly, there may be a high degree of probability that the large overpotential facilitates re-oxidation of Cyt c by UQ to increase the anodic peak current. Figure 7 shows the cyclic voltammograms reproduced by digital simulation, in which we can find a considerable increase of the anodic wave as well as the cathodic one on multiplying the rate constant $k_{b,C1}$ (experimentally observed as $4.4 \text{ M}^{-1} \text{s}^{-1}$) by the factor of $X$ ranging from 1 to 500. In this simulation, $k_{b,C1}$ has been increased synchronously with $k_{b,C1}$ so that the equilibrium constant, $K_{C1}$, is unchanged (cf. Eq. (16)). Thus, theoretical simulation demonstrates that the catalytic current of Cyt c by UQ is obtained not only for the cathodic reduction of Cyt c but also for the anodic oxidation. Actually, however, the re-oxidation of Cyt c hardly occurs on the UQ-incorporated SAM as shown in Fig. 3(b). This is probably because UQ and Cyt c are spatially separated by the presence of the SAM, suggesting one of the most significant roles of biomembranes. It may be imagined that phospholipids prevent redox components from getting too close to each other.

4. Conclusions

A catalytic reduction of Cyt c by UQ could be observed at the DM SAM-modified electrode. The cyclic voltammogram shows a well-defined catalytic wave at around $-0.4 V$ vs. Ag/AgCl (saturated KCl), however no clear catalytic wave was observed for the re-oxidation of Cyt c by UQ, though the re-oxidation peak of UQ was observed at around $+0.3 V$. A digital simulation analysis with a simple reaction model (Fig. 4) has successfully been performed to show that the re-oxidation of Cyt c is somewhat thermodynamically unfavorable and, importantly, that this process is kinetically slow. Such a slow process is probably due to spatial separation of the redox species located in the SAM. Thus, it has been suggested that a similar situation may be occurring in real biomembranes so that an efficient energy production system can be achieved. However, we should mention that the biomimetic ET system proposed in this study is not necessarily the same as the real respiratory chain system, in which UQ reacts with Cyt c not directly but via a redox protein (ubiquinol–Cyt c reductase). Furthermore, the CH1-terminated alkanethiol SAM might show rather different characteristics from those in phospholipid bilayer membranes. Further study should be needed to establish a model system much closer to biomembranes.

Supporting Information

The Supporting Information is available on the website at DOI: https://doi.org/10.5796/electrochemistry.18-00059.

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