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Long-range electron-transfer reaction rates to cytochrome *c* across long- and short-chain alkanethiol self-assembled monolayers: Electroreflectance studies†

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The kinetics of electron transfer (ET) between cytochrome *c* and a gold (111) electrode through self-assembled monolayers of alkanethiols with terminal carboxylic acid groups, $COOH(CH_2)_nSH$, have been studied for $n = 2$–11 using an ac potential-modulated UV–VIS reflectance spectroscopic technique (electroreflectance spectroscopy, ER). For $9 \leqslant n \leqslant 11$, the standard ET rate constant, k_{app}, depends exponentially on the chain lengths and the exponential decay factor is 1.09 per methylene group; for $n < 9$, however, k_{app} deviates from the exponential plot. The ET reaction through short-chain alkanethiol monolayers is controlled by the preceding chemical reaction. The rate-controlling step is very likely to be the reorganization of cytochrome *c* to the favourable conformation for the ET reaction. The ET reaction rate constant from cytochrome *c* in the favourable conformation to the electrode surface obeys Marcus theory for long-range ET. The ET reaction through long-chain alkanethiol monolayers is controlled by the ET rate through alkanethiols.

The interfacial electron transfer (ET) between gold electrodes and an electrochemically active species through a self-assembled monolayer (SAM) has been extensively studied.[1–7] In recent years, alkanethiol monolayers have been widely used to study the effects of distance, ordering, and chemical environment on heterogeneous ET processes. According to Marcus theory,[8] the long-range ET reaction is non-adiabatic and the reaction rates are expected to decay exponentially with the distance between the redox sites when the distance becomes greater than the van der Waals contact distance. Li and Weaver[1] studied the electrode reaction of cobalt(II) complexes attached to gold and mercury surfaces by thioalkylcarboxylate ligands. They were the first to find that the rate of this reaction decreased exponentially with alkyl chain length, with an exponential decay coefficient (the electronic coupling constant), β, of 1.0 A^{-1}. Finklea and co-workers[2,5,6] studied the long-range ET between a metal electrode and redox-active pentammine(pyridine)ruthenium complexes covalently attached to alkanethiol monolayers, and interpreted log k_{app} *vs.* overpotential plots in terms of the Marcus free energy-rate relation.[8] They revealed that the standard ET reaction-rate constant k_1 varies exponentially with the number of methylene groups (n) in the alkyl chain:

$$k_1 = k_1^0 \exp(-\beta n) \quad (1)$$

where k_1^0 is the extrapolated value of the rate constant for $n = 0$; in this case, the value of β was found to be approximately 1.02–1.10 per CH_2. The reorganizational energy barriers of the ET reaction were also evaluated. Their symmetrical Tafel plots supported a through-bond tunnelling mechanism.

A number of other groups have also investigated the temperature[7] and distance dependence of heterogeneous ET reactions of thiol self-assembled monolayers, with terminal ferrocene groups on gold and silver electrodes.[9,10] The experimental values of β reported by these groups are approximately 1.10 per methylene unit, and are independent of the redox moieties covalently bonded to alkanethiols when $n > 10$. Smalley *et al.*[11] recently studied a rapid ET reaction between a gold electrode and ferrocene attached to the alkanethiol monolayers by using the indirect laser-induced temperature jump method (ILIT). They reported that the relation of the electronic coupling factor between the ferrocene moiety and the surface-bound sulfur of the thiol layer is not strictly exponential when $n < 8$. Bowden and co-workers[12–14] and our group[15] have studied the ET reaction rate between a gold electrode and cytochrome *c* attached to alkanethiols with terminal COOH groups, which formed an SAM on a well defined gold (111) surface. Bowden and co-workers demonstrated that the standard ET reaction-rate constant of cytochrome *c* through alkanethiol monolayers with $10 \leqslant n \leqslant 17$ varies exponentially with n, using traditional electrochemical techniques (ac impedance and cyclic voltammetry).[12–14] However, no electron-transfer reaction rates through short-chain alkanethiol layers have been reported, because the rate is too rapid to be measured by traditional electrochemical techniques.

We have previously proposed a new spectroelectrochemical technique, ac potential modulated UV–VIS electroreflectance spectroscopy (electroreflectance, ER), which is suitable for the determination of rapid ET reaction rates.[15,16] Using this ER method, we have studied dye adsorbed on glassy carbon electrodes[16] and cytochrome *c* immobilized on gold electrodes modified with two kinds of alkanethiol monolayers with terminal carboxylic acid groups: $HS(CH_2)_2COOH$ and $HS(CH_2)_{10}COOH$.[15]

Of particular interest in the present work is the characterization of the distance dependence of the ET rate constant of cytochrome *c* through short-chain alkanethiols ($n < 10$). We have used the ER method to investigate the dependence of the standard rate constant of the ET reaction of cytochrome *c* on the chain length of self-assembled monolayers through a homologous series of alkanethiols ($2 \leqslant n \leqslant 11$).

Experimental

Chemicals

Horse heart cytochrome *c* (type VI from Sigma) was purified chromatographically as described elsewhere.[17] β-Mercaptopropionic acid $HS(CH_2)_2COOH$ (Dojin Laboratories, Japan),

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was of reagent grade. The long-chain alkanethiols, $HS(CH_2)_nCOOH$ ($n = 5$, 6, 9, 10 or 11), were synthesized and purified in our laboratory according to standard literature procedures[18] and the structures were verified by NMR and mass-spectrometric analysis. Water was purified with a Milli-Q system (Millipore Co.) and its resistivity was greater than 18 MΩ cm. All other chemicals were of reagent grade and used as received.

Instrumentation

The instrumentation for cyclic voltammetric, ac impedance, and ER measurements has been described previously.[19,20] The gold (111)–mica substrates were prepared by vapour deposition for the working electrodes instead of the gold disc electrodes described previously.[15] An open-bottomed glass electrochemical cell was employed for the ER measurements. The electrode area (3.1 cm^2) was defined by a sealing rubber o-ring positioned between the electrode and the glass cell. The potentials were measured against a silver/silver chloride reference electrode in saturated potassium chloride solution, with a platinum wire counter electrode.

Procedures

A well defined gold (111) electrode was prepared by vapour deposition of Au (99.99%) on a fresh mica surface at 580 °C using an ULVAC VPC-260 compact vacuum coater equipped with an ULVAC UTM-150 turbomolecular pump. Prior to deposition, mica sheets were heated at 580 °C for *ca.* 5 h. The substrate was maintained at 580 °C and the pressure in the vacuum chamber was $< 2 \times 10^{-6}$ Torr throughout the deposition process. Afterwards, the vapour-deposited Au–mica sheets were annealed at 550 °C for at least 5 h and quenched in purified water immediately prior to use as an electrode. An alkanethiol SAM on the gold surface was formed by immersing the Au–mica sheet into an ethanolic 1 mM alkanethiol solution for 2 h at room temperature. After rinsing with ethanol to remove the excess of alkanethiol from the surface, the electrode was soaked in a 50 μM cytochrome *c* solution (pH 7.0) for *ca.* 30 min in order to immobilize cytochrome *c* on the modified electrode. Excess of cytochrome *c* was removed by rinsing the electrode with the base solution (10 mM phosphate buffer at pH 7.0). The ER spectroscopic technique reported previously[15,16] was used to evaluate the heterogeneous ET reaction rate constants. The ER response was measured at the formal potential of cytochrome *c* and the peak wavelength at which the maximum response is obtained. Cyclic voltammetric and ac impedance techniques were also used to characterize the electrode reaction. All measurements were carried at 25 ± 1 °C under an argon atmosphere.

Results and Discussion

Cyclic voltammograms of cytochrome *c* immobilized on alkanethiol monolayers at the well defined gold (111) electrode were found to be quite stable and reproducible. Fig. 1 shows the dc cyclic voltammograms of the cytochrome *c* –$HOOC(CH_2)_9SH$ electrode system acquired at sweep rates between 20 and 200 mV s^{-1}. The formal potential was +50 mV, which suggests that cytochrome *c* immobilized on the monolayers contain terminal carboxylic acid groups is in its native state, as reported previously.[15,21] The amount of cytochrome *c* immobilized on the SAM modified electrode, Γ_t, was 13.6×10^{-12} mol cm^{-2} from the area of the dc voltammetric peak. As the sweep rate increases, the peak potentials (E_{pa} and E_{pc}) shift symmetrically in positive and negative directions, respectively, from the formal potential, as would be expected for a system undergoing a transition from reversible to quasi-reversible electron transfer. However, the heterogeneous rate constant of cytochrome *c* immobilized on shorter chain alkanethiol monolayers with terminal carboxylic acid groups is so rapid that the peak potential separation remains the same and the ET rate cannot be measured by traditional voltammetric techniques.

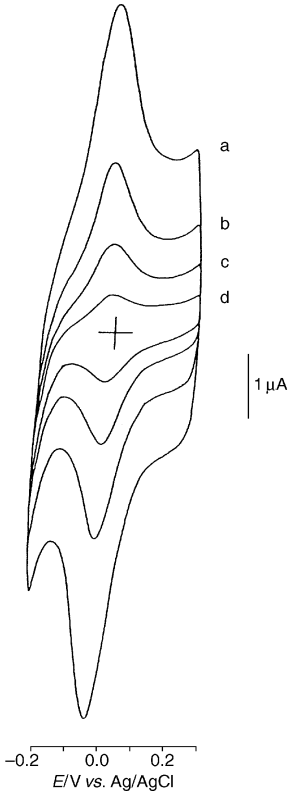

Fig. 1 Cyclic voltammogram of the adsorbed cytochrome *c* –$HOOC(CH_2)_9S$–Au (111) composite electrode. 10 mM phosphate buffer solution, pH 7.0. Scan rates in mV s^{-1}: (a) 200, (b) 100, (c) 50, (d) 20.

In a previous paper,[16] we reported that the frequency dependence of the ER responses in the frequency domain is represented by eqn. (2)

$$-\omega \cot \phi = (1 - \omega^2 R_s R_{ct} C_a C_d)/(R_{ct} C_a + R_s C_a + R_s C_d) \quad (2)$$

where cot ϕ is the ratio of the magnitudes of the real and imaginary components of the ER signal; ω is the angular frequency of the potential modulation; R_{ct} and C_a are the charge-transfer resistance and capacitance, respectively; and R_s and C_d are the solution resistance and double-layer capacitance, respectively, which are evaluated by the ac impedance technique. According to eqn. (2), R_{ct} and C_a due to the redox reaction of cytochrome *c* can be calculated from the slope and intercept in the plot of the ER responses with respect to $-\omega^2$. Fig. 2(A) shows a plot of $-\omega \cot \phi$ against $-\omega^2$ for the ER response of the cytochrome *c*–$HOOC(CH_2)_9SH$ electrode system. The values of R_{ct} and C_a from the slope and intercept of the linear plot at $\omega^2 = 0$ were calculated to be 129 Ω cm^2 and 12.1 μF cm^{-2}. The heterogeneous rate constant, k_{app}, was subsequently calculated to be 320 s^{-1} by using eqn. (3).[16]

$$k_{app} = (2R_{ct} C_a)^{-1} \quad (3)$$

The kinetic parameters of cytochrome *c* immobilized on a homologous series of alkanethiol monolayers $HOOC(CH_2)_nSH$ ($n = 2$–11) were determined from ER measurements. Fig. 2(B) shows a plot of $-\omega \cot \phi$ against $-\omega^2$ for the ER response of cytochrome *c* immobilized on a short-chain alkanethiol SAM ($n = 2$). The slope and intercept of the linear plot at $\omega^2 = 0$ yielded R_{ct} and C_a values of 41 Ω cm^2

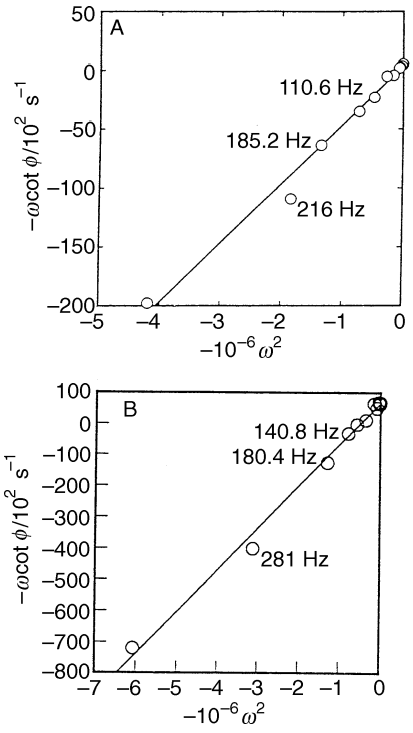

Fig. 2 Plot of $-\omega\cot\phi$ against $-\omega^2$ for the ER response of (A) the cytochrome *c*–HOOC$(CH_2)_9$S–Au (111) and (B) the cytochrome *c*–HOOC$(CH_2)_2$S–Au (111) composite electrode in the same solution as in Fig. 1, at $E_{dc} = +50$ mV, $\Delta E_{ac} = 60$ mV and $\lambda = 420$ nm. Open circles are experimental values; the solid line is a simulated least-squares fit.

and 9.4 μF cm^{-2}, respectively. The standard rate constants for the ET reaction of cytochrome *c* immobilized on the HOOC$(CH_2)_n$SH electrode systems ($n = 2$, 5, 6, 9, 10 or 11) and other kinetic parameters are summarized in Table 1.

By using the value of C_a determined from the ER measurement and $n_a = 0.95$ (n_a is the apparent number of electrons involved in the electrode reaction evaluated from the full width at half-maximum of the cyclic voltammogram in Fig. 1), the surface coverage of the electrode by cytochrome *c* for the HOOC$(CH_2)_9$SH monolayer, Γ_t, was calculated to be 1.35×10^{-11} mol cm^{-2} from eqn. (4),[16]

$$C_a = nn_aF^2\Gamma_t/4RT \quad (4)$$

where n is the number of electrons involved in the electrode reaction. This value agrees well with that evaluated from the peak area of the cyclic voltammogram and that reported as a monolayer by Bowden and co-workers.[12]

The logarithm of apparent ET standard rate constants of cytochrome *c* are plotted as a function of the number of methylene groups in the alkanethiol chain in Fig. 3. The ET reaction rate, k_{app}, of cytochrome *c* through long-chain alkanethiol monolayers ($n = 9$, 10 or 11) shows a linear relationship between $\log k_{app}$ *vs.* n. Bowden and co-workers[14] measured the ET reaction rate of the same system with

Table 1 Kinetic parameters for the cytochrome *c* –HOOC$(CH_2)_n$S–Au (111) systems

n	k_{obs}/s^{-1}	Γ_t/pmol cm^{-2}	C_a/μF cm^{-2}	R_{ct}/Ω cm^2
2	1.3×10^3	10.5	9.4	41
5	1.1×10^3	12.3	11.0	42
6	8.3×10^2	11.3	10.1	60
9	3.2×10^2	13.5	12.1	129
10	98	13.0	11.6	440
11	36	12.8	11.4	1220

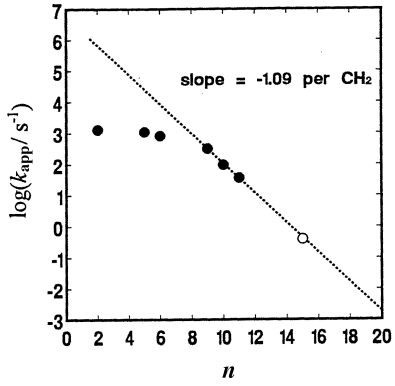

Fig. 3 Logarithmic plots of the apparent standard rate constant of cytochrome *c* as a function of the number of methylene groups of the alkanethiol self-assembled monolayers: (●) obtained by the ER technique in this work; (○) obtained by ac impedance measurements[12]

longer-chain alkanethiols ($n = 10$–17) by ac impedance and cyclic voltammetric techniques. Their results fall on the line determined by our data and confirm the validity of both methods. The slope of the dotted line in Fig. 3 yields the exponential decay parameter β to be 1.09 per methylene unit, which agrees well with the values determined for both the ruthenium complex[2] and the self-assembled monolayers containing terminal ferrocene groups.[10,11] With the assumption that the ET takes place through the molecular channel formed by the alkane backbone, with a C—C bond distance of 0.127 nm,[2] the tunnelling parameter, β, is evaluated to be 8.8 nm^{-1}.

For $n < 9$, on the other hand, the ET rate constant does not increase as rapidly as expected from eqn. (1), as shown in Fig. 3. The rate constant through shorter-chain alkanethiols reaches a limiting value of the order of 10^3 s^{-1}. We have reported that the upper limit of the measurable ET rate constant by the ER method can be estimated from eqn. (5).[16]

$$k_{app} < (\Delta\varepsilon/nF)^2C_a^2/8\tau I_h^2 \quad (5)$$

where $\Delta\varepsilon = \varepsilon_{red} - \varepsilon_{ox}$, and ε_{red}, and ε_{ox} are the apparent absorption coefficients of the reduced and oxidized forms of the adsorbed redox active species, respectively; $\tau = R_sC_d$, the time constant of the cell, and I_h is the detection limit of the ER signal. In the present electrochemical system, the time constant of the cell, τ, is *ca.* 1 ms and the detection limit of the ER signal is 5×10^{-4} V^{-1}. The upper limit of the measurable k_{app} by ER is expected to be 5×10^4 s^{-1} *i.e.*, rate constants of the order of 10^3 s^{-1} can easily be measured.

The intramolecular ET rates of cytochrome *c* derivatives have been widely investigated.[22] The typical model of the ET pathway between donor and acceptor was envisaged as being a combination of covalent bonding, hydrogen bonding, and through-space jumping, and each of these has a different decay factor. In the case of cytochrome *c* immobilized on alkanethiol monolayers with terminal carboxylic acid groups, the ET reaction may take place through the following scheme. There are at least two states of cytochrome *c* [states (I) and (II)] on the modified surface and there is a reorganization equilibrium between cytochrome *c* (I) and cytochrome *c* (II). The orientation of the cytochrome *c* (I) form is unfavourable for the ET reaction and it is necessary to form cytochrome *c* (II) prior to the ET reaction. Cytochrome c (II) has a specific conformation to form a strong electronic coupling between the binding sites (cytochrome *c* and carboxylic acid groups) and a non-adiabatic ET takes place from the haem edge to the electrode surface. The reorganization equilibrium between two states can be written by eqn. (6) and (7).

$$\text{cytochrome } c \text{ (I)} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{cytochrome } c \text{ (II)}$$

$$\text{cytochrome } c \text{ (II)} + \text{e} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} \text{cytochrome } c \text{ (red)} \quad (6)$$

$$K = \Gamma_{ox}(\text{II})/\Gamma_{ox}(\text{I}) = k_1/k_2 \quad (7)$$

where K is the equilibrium constant between the cytochrome c (I) and cytochrome c (II), and Γ_{ox}(I) and Γ_{ox}(II) are the surface concentrations of state (I) and state (II), k_1 and k_2 are the transformation rate constants from state (I) to state (II) and *vice versa*, k_f and k_b are the ET rate constants for forward and reverse ET reactions, respectively.

The ET reaction rate constant between the haem edge of cytochrome c (II) to the electrode can be represented by Marcus theory.

$$k_{et} = k^0 \exp(-\beta_1 d - \beta_2 n - \beta_3 l) \quad (8)$$

where d is the distance between the haem edge to the binding site (intramolecular ET distance), n is the number of methylene groups in the alkanethiol monolayers with terminal carboxylic acid groups, l is the ET distance of the alkanethiols without a methylene group, and β_i ($i = 1$, 2, and 3) are the exponential decay factors of the ET rates through the respective pathways.

The ET reaction rate is given by

$$v_{et} = k_f \Gamma_{ox}(\text{II}) - k_b \Gamma_{red} \quad (9)$$

where Γ_{red} is the surface concentration of the reduced form of cytochrome c. With the small perturbation of the electrode potential ($\eta = E - E^{0'}$) around the formal potential, $E^{0'}$, of electrode reaction, eqn. (9) can be written as

$$v_{et} = k_{et} \Gamma_{ox}(\text{II}) \quad (10)$$

The total concentration of cytochrome c at the surface can be derived from eqn. (11) and (12)

$$d\Gamma_{ox}(\text{II})/dt = k_1 \Gamma_{ox}(\text{I}) - (k_2 + k_{et})\Gamma_{ox}(\text{II}) = 0 \quad (11)$$

$$\Gamma_t = \Gamma_{ox}(\text{I}) + \Gamma_{ox}(\text{II}) + \Gamma_{red} = [(2k_1 + k_2 + k_{et})/k_1]\Gamma_{ox}(\text{II}) \quad (12)$$

The reaction rate equation is obtained by substituting eqn. (12) into eqn. (10)

$$v_{et} = k_{et} \Gamma_{ox}(\text{II}) = [k_1 k_{et}/(2k_1 + k_2 + k_{et})]\Gamma_t \quad (13)$$

The apparent ET rate constant, k_{app}, is given by eqn. (14).

$$k_{app} = k_1 k_{et}/(2k_1 + k_2 + k_{et}) \quad (14)$$

In the case of the ET through long-chain alkanethiols, k_1, $k_2 \gg k_{et}$. The apparent ET rate constant becomes

$$\ln k_{app} = \ln k_{et} + \ln[k_1/(2k_1 + k_2)] \quad (15)$$

The slope of $\ln k_{app}$ *vs.* n is β_2 ($= -1.09$ per CH_2 as shown in Fig. 3) and the extrapolated value to $n = 0$ is given by

$$\ln k_{app} = \ln k^0 + (-\beta_1 d - \beta_3 l) + \ln[k_1/(2k_1 + k_2)] \quad (16)$$

The value of $k^0 \exp(-\beta_1 d)$ corresponds to the intramolecular ET rate constant of cytochrome c from the haem edge to the binding site, which is greater than 10^6 s^{-1} (see Fig. 3).

In the case of short-chain alkanethiols, on the other hand, the ET rate through alkanethiol is much larger than the transformation reaction rate from state (I) to state (II), *i.e.*, $k_{et} \gg k_1$, k_2, and the ET rate constant becomes constant.

$$k_{app} = k_1 \quad (17)$$

Fig. 4 displays the simulated results (solid line) from eqn. (14), from which k_1 was estimated to be 3×10^3 s^{-1}.

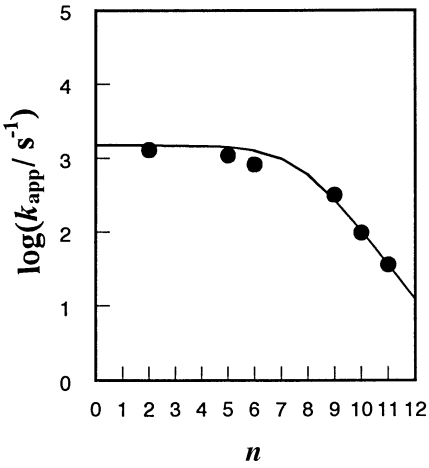

Fig. 4 Simulated plot (solid line) of K_{app} *vs.* n for data calculated from eqn. (14)

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