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Intermolecular biological electron transfer: an electrochemical approach

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Abstract

We investigated the electron transfer (ET) rates between a well-defined gold electrode and cytochrome *c* immobilized at the carboxylic acid terminus of alkanethiol self-assembled monolayers (SAMs) by using the potential modulated electroreflectance technique. A logarithmic plot of ET rates against the chain length of the alkanethiol is linear with long chain alkanethiols. The ET rates become independent of the chain length with short alkanethiols. It is proposed that the rate-limiting ET step through short alkyl chains results from a configurational rearrangement process preceding the ET event. This "gating" process arises from a rearrangement of the cytochrome *c* from a thermodynamically stable binding form on the carboxylic acid terminus to a configuration, which facilitates the most efficient ET pathways (surface diffusion process). We propose that the lysine-13 of mammalian cytochrome *c* facilitates the most efficient ET pathway to the carboxylate terminus and this proposal is supported by the ET reaction rate of a rat cytochrome *c* mutant (RC9–K13A) [Elektrokhimiya (2001) in press], in which lysine-13 is replaced by alanine. The ET rate of K13A is more than six orders of magnitude smaller than that of the native protein.

Keywords: Self-assembled monolayer; Alkanethiolate; Carboxylic acid-terminated; Electroreflectance; Electron transfer kinetics; Cytochrome *c*; Cytochrome *c* mutant

1. Introduction

It is well known that redox active sites attached to ω-derivatized alkanethiol SAMs on a gold electrode undergo a redox reaction through the alkanethiol SAMs. Electrostatic interactions between the positively charged lysine amino groups immediately surrounding the heme crevice of cytochrome *c* and the negatively charged carboxylate termini of the SAMs stabilize the binding of cytochrome *c*, analogous to its complex with negatively charged physiological redox partners such as the cyt. *c*/cyt. b_5 complex (see Ref. [1] and references therein). Electrochemical approaches that utilize such SAMs for investigating biological ET mechanisms are advantageous in that the driving force of the ET reaction can be regulated through control of the electrode potential, and the ET path length can be varied by using alkanethiols of varying chain lengths. In this regard, the electrode reaction of cyt. *c* through carboxylic acid-terminated alkanethiol SAMs is considered to be a simplified model system for biological ET processes.

It has been shown that the long-range ET rate between metal electrodes and electroactive species through alkanethiol SAMs decreases with the chain length of the SAMs. For these systems, the ET reaction rate constants of the electrochemical active sites depends on the number of methylene groups *n* in the alkyl chain:

$$k_s = k_{(n=0)}\exp(-\beta n) \quad (1)$$

where $k_{(n=0)}$ is the apparent ET rate constant extrapolated to chain length of zero methylene group ($n=0$). The exponential decay factor β has been found to be 1.09 ± 0.02 per methylene group (0.71 ± 0.01 Å^{-1}) regardless of the type of redox species at the terminus of the alkanethiol SAMs (see Refs. [2,3] and references therein). In the tunneling-pathway mode, the total coupling (the matrix element) of a single ET pathway, which consists of different intervening

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bridges, is given as a product of the couplings for the individual bridges.

$$H_{AB}^2 \propto \Pi\kappa(\text{covalent})\Pi\kappa(\text{hydrogen})\kappa(\text{SAM}) \quad (2)$$

where H_{AB} is the electronic matrix element describing the electronic coupling of the reactants A and B, and κ is the transmission coefficient (averaged transition probability for electron transfer per passage of the system through the intersection region of the potential energy surface). In the case of cyt. *c* immobilized on the carboxylic acid-terminated alkanethiol SAMs, Π κ(covalent) and Π κ(hydrogen) (see Ref. [4] and references therein), and κ(SAM) (see Refs. [2,3] and references therein) have been extensively investigated.

Intermolecular ET processes between the positively charged and negatively charged sites of electron transfer protein molecules such as cyt. *c*/cyt. b_2, cyt. *c*/cyt. *c* peroxidase, cyt. *c*/plastocyanine, and cyt. *c*/cyt. b_5 couples have been investigated in solutions having different ionic strength, pH, and viscosity (see Ref. [1] and references therein). Effects of solution viscosity on the configurational rearrangement reaction rate between ET protein complexes have been studied theoretically and experimentally (see Refs. [5–7]). The non-uniform charge distributions on the surface of protein molecules often lead to multiple configurational states in the protein coupling. The initial step in the formation of the protein complex is a non-specific electrostatic association between the two proteins, followed by rotational diffusion on the molecular surface to reach the proper configuration for the ET event. When the protein couple forms multiple configurations, the intermolecular ET rate is limited by the rotational diffusion of the molecules. This process can be viewed as directional ET regulated by a "gating mechanism" [8]. Intermolecular ET rates depend strongly on the ionic strength, pH, and temperature of the solution and are reported to be in the range of 10^3–10^5 s^{-1}.

2. Experimental

Non-*N*-terminally acetylated cytochrome *c* (fraction II) was prepared by expression in yeast, as described by Koshy et al. [10]. The mutant cytochrome *c*, RC9–K13A, in which lysine-13 is replaced by alanine, was prepared by expression in the corresponding recombinant yeast by the procedure of Wang and Margoliash [11]. Electroreflectance set-up and experimental procedures were reported elsewhere [12,13].

3. Results and discussion

In electrochemical studies of the ET mechanism of cyt. *c* immobilized on the carboxylic acid-terminated alkanethiol SAMs, one can deconvolute the intermolecular ET event at the interface from the intramolecular ET event, provided that one can measure ET rate constants up to 10^5 s^{-1}. The charging current of the electrical double layer at the electrode interface limits the measurable ET rate when traditional electrochemical techniques (cyclic voltammetry and ac impedance) are used. At well-defined surfaces having geometrical areas of approximately 1 cm^2, the fastest reliable ET rates that have been reported are around 100 s^{-1}, which are much slower than intermolecular ET rates of protein complexes. That is, it would be difficult to measure the intermolecular ET event by traditional electrochemical techniques. With the UV–VIS potential-modulated reflectance spectroscopic technique, on the other hand, the effect of double-layer charging on the optical response can be minimized and enables one to measure ET rates up to approximately 10^4 s^{-1} [14].

The logarithmic plot of the ET rate constant with respect to the chain length of carboxylic acid-terminated alkanethiols is shown in Fig. 1. The ET reaction rate constant decreases exponentially with the chain length when $n > 6$ and, thus, the ET rates through the long-chain alkanethiol SAMs are controlled by the electron transfer through the alkanethiol bonds. The ET reaction rates through the short-chain alkanethiol monolayers, on the other hand, are nearly independent of the chain length. It is assumed that there is a configurational rearrangement of cytochrome *c* on the SAM prior to the ET reaction given by reaction (3): the thermodynamically stable adsorbed structure of cyt. *c* (ox)(I), which is formed upon the adsorption of oxidized cytochrome *c* from the solution to the carboxylate termini, undergoes a configurational rearrangement to cyt. *c* (ox)(II),

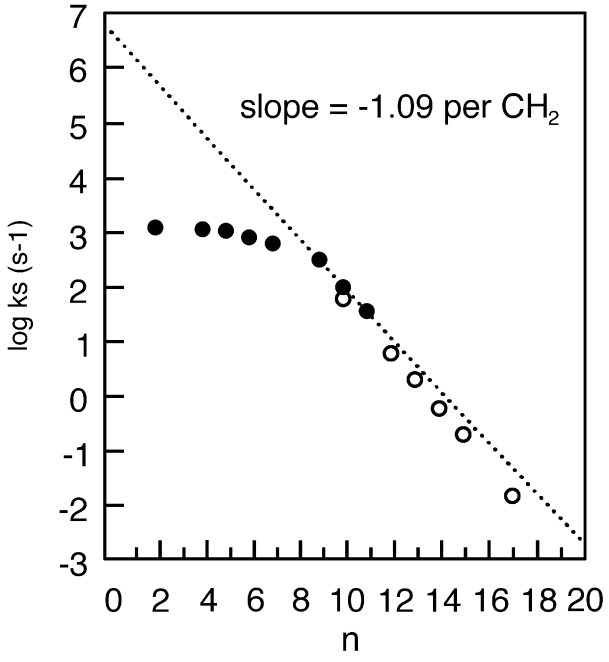

Fig. 1. Logarithmic plot of the ET rate constant k_s of cytochrome *c* immobilized on $HOOC(CH_2)_n$S-/Au electrodes vs. the number of methylene groups *n* in the alkyl chain. The filled circles are our results obtained by the electroreflectance technique [3a, 3b] and the open circles are obtained by the ac impedance technique [9].

from which the most efficient ET reaction takes place as given by reaction (4). The ET reaction is followed by a second configurational rearrangement given by reaction (5) to form a thermodynamically stable binding state, cyt. *c* (red)(I). The rate-controlling step of the ET reaction through a short alkanethiol chain is very likely to be the conformational rearrangement of cytochrome *c* on the SAM surface given by reaction (3) and the transformation rate constant k_1 of the forward reaction is estimated to be 2.6×10^3 s^{-1} [3].

$$\text{cyt. } c \text{ (ox)(I)} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{ Cyt. } c \text{ (ox)(II)} \quad (3)$$

$$\text{cyt. } c \text{ (ox)(II)} + \text{e} - \underset{k_b}{\overset{k_f}{\rightleftharpoons}} \text{ Cyt. } c \text{ (red)(II)} \quad (4)$$

$$\text{cyt. } c \text{ (red)(II)} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \text{ Cyt. } c \text{ (red)(I)} \quad (5)$$

It is possible to elucidate the binding site of cyt. *c* (ox)(II), which facilitates the most efficient ET pathway, from the extrapolated value of k_s to $n=0$ at low ionic strength, the value of which is about 5×10^6 s^{-1}. The ET rates from the heme edge to various lysine residues on the surface of cytochrome *c* can be estimated from the results of Gray and Winkler (see Ref. [4] and references therein) and the intramolecular ET rate from the heme edge to lysine-13 is estimated to be 2×10^5 s^{-1}. The intramolecular ET rates from the heme center to other lysine residues are much smaller than 2×10^5 s^{-1}. It is very probable that Lys-13 forms the hydrogen bonding to the carboxylate terminus of the alkanethiol SAMs and is very unlikely that the lysine residues other that Lys-13 could be the binding site to form an efficient ET pathway to the carboxylic acid terminus.

We then studied the ET kinetics of a cytochrome *c* mutant (RC9–K13A) immobilized on the carboxylic acid-terminated alkanethiol SAMs to confirm our proposal about the binding site of cytochrome *c* to the carboxylate terminus. The ET rate constant of RC9–K13A immobilized on 3-mercaptopropionic acid SAM on gold electrode was measured to be 0.2 ± 0.05 s^{-1}. The logarithmic plot of the apparent ET rate constant vs. the number of methylene groups *n* in the carboxylic acid terminated alkanethiol SAM is given by Eq. (6) (see Ref. [3] and references therein).

$$k_{et}(\text{app}) = 7.6 \times 10^6 \exp(-1.1\ n) \quad (6)$$

The ET rate constant of the native cytochrome *c* through 3-mercaptopropionate SAM ($n=2$) is estimated to be 8.4×10^5 s^{-1}. One can calculate the difference in the effective number of covalent bonds in the ET path Δn_{eff} between the native cytochrome *c* and RC9–K13A cytochrome *c* by using the value of k_{et} (app) = 0.2 for RC9–K13A cytochrome *c*.

$$0.2 = 8.4 \times 10^5 \exp(-0.99 \Delta n_{eff})$$

$$\Delta n_{eff} = 15.4\ C \quad (7)$$

where *C* represents a covalent bonding defined by Gray and Winkler (see Ref. [4] and references therein).

A potential candidate of the binding site of RC9–K13A could be the Lys-8 because the difference in the effective number of covalent bonds from the heme edge to Lys-13 and that Lys-8 is estimated to be 15 (see [4,15,16] and references therein). Other lysine residues such as Lys-25 ($\Delta n_{eff} = 13C$), Lys-72 ($\Delta n_{eff} = 13.6C$), Lys-86 ($\Delta n_{eff} = 16C$), which are highly conserved lysine residues surrounding the heme crevice, could form multiple ET pathways in RC9–K13A.

It is also interesting to analyze the data for the ET rate constants of yeast iso-1 cytochrome *c* through carboxylic acid-terminated alkanethiol SAM reported by Kasmi et al. [17] by using our prediction. For the ET rate constants of cytochrome *c* through -S-$(CH_2)_7$COOH and -S-$(CH_2)_{10}$ COOH are calculated to be 2430 and 92 s^{-1}. In the present calculation, we prefer to use our data measured by a potential modulated electroreflectance technique [2,3,14] because the measurements of rapid ET rate constants by an ac impedance are erroneous. Indeed, a measurable upper limit of the ET rate by a traditional electrochemical technique is around 100 s^{-1}. The ratios of the ET rate constants for horse cytochrome *c* [2,3] and yeast iso-1 cytochrome *c* are given by $18/2430 = 7.4 \times 10^{-3}$ and $0.21/92 = 2 \times 10^{-3}$. If we assume that the ET reaction from electrode to yeast iso-1 cyt. *c* takes place through the SAM in the same manner as that of horse heart cyt. *c*, the ET reaction site of iso-1 cytochrome *c* could be calculated by using Eq. (7). The differences in the effective number of covalent bonds in the ET path Δn_{eff} between the horse heat cytochrome *c* and yeast iso-1 cytochrome *c* are $5.0C$ (for $n=7$ alkanethiol) and $6.3C$ (for $n=10$ alkanethiol), respectively. That is, the ET pathway from the carboxylate terminus to the heme edge of yeast iso-1 cytochrome *c* comprises five to six more effective covalent bonds than that of horse heart cytochrome *c*. In the case of yeast iso-1 cytochrome *c*, it is very likely that Lys-11 could be the binding site to facilitate the ET reaction. The Arg-13 in yeast iso-1 cyt. *c* is very unlikely to form a strong hydrogen bonding to the carboxylate terminus of the SAM because the arginine structure is stabilized by the resonance structure [18].

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