Electron-Transfer Dynamics of Cytochrome C: A Change in the Reaction Mechanism with Distance

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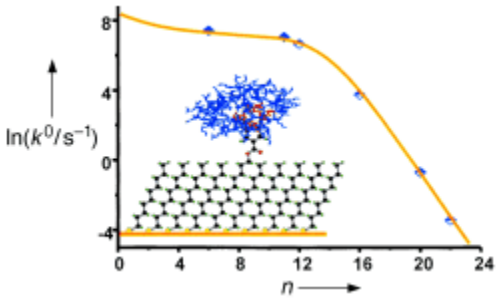
Abstract:



Directly linking a gold electrode coated with pyridine-terminated self-assembled monolayers (SAMs) to the redox-active heme unit of cytochrome c has enabled the electron-transfer mechanism to be studied as a function of distance. Comparison of the kinetic data for this system (see graph) with earlier data on COOH-terminated SAMs requires a change in the electron-transfer mechanism with distance from the electrode surface that does not involve large-amplitude conformational rearrangement.

Keywords: Redox processes | electron transfer | cytochrome *c* | protein environment | metal electrode

Article:

Redox processes are ubiquitous in nature, and the understanding of electron transfer in complex systems, for example, biological structures such as proteins, membranes, and the photosynthetic reaction center, is an outstanding challenge. Here we provide new results on the electron-transfer dynamics of the protein cytochrome c as a function of distance from a metal electrode. Comparison of this distance-dependence with previous studies indicates that a conformationally gated mechanism involving a large amplitude protein motion is not operative, but a change in the electron-transfer mechanism occurs and is linked to the protein environment.

The redox protein cytochrome c is very well characterized and numerous studies of its electron transfer have been performed, both under homogeneous and heterogeneous conditions.[1] A number of research groups have immobilized cytochrome c on gold electrodes that are coated with a self-assembled monolayer (SAM) of -S-$(CH_2)_{n-1}$-COOH, presumably by binding to the protein's lysine groups.[2] The electronic coupling strength between the electrode and the protein can be varied by changing the length of the alkane chain. At large SAM thicknesses the electron-transfer rate constant declines exponentially with distance (electron tunneling mechanism), but it is distance-independent at lower thicknesses, hence there is a change in the rate-limiting step and the mechanism of reaction. More recently, mixed monolayer films of pyridine-terminated alkanethiols embedded in an alkanethiol diluent have been used to directly tether the heme to the surface.[3] This strategy for immobilization (Figure 1) should eliminate large-amplitude conformational motion of the protein on the surface of the SAM as a gating mechanism for the electron transfer, because the heme is directly linked to the alkanethiol tunneling barrier.

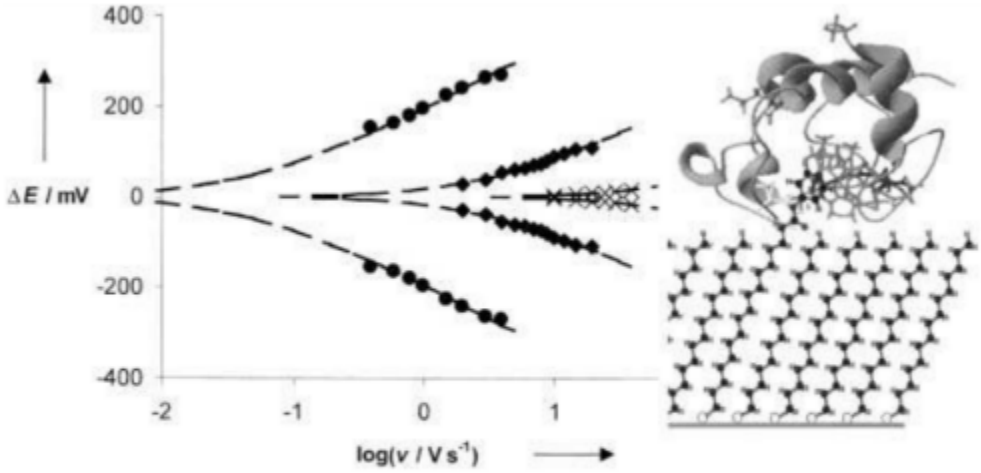


Figure 1. The dependence of the peak separation ΔE on the voltage scan rate ρ is shown for pyridine-terminated chains having lengths of 20 methylene groups (circles), 16 methylene groups (diamonds), and 6 methylene groups (×). In each case the data is fit to the Marcus model with a reorganization energy of 0.8 eV. A schematic diagram of the cytochrome immobilization strategy is shown on the right.

The immobilization of the cytochrome on the film has been demonstrated through electrochemical control experiments and by direct imaging by STM.[3b] The primary evidence for binding near the heme is the negative shift of the redox potential, relative to that in solution, and the differential adsorption strength of different functional end groups (nitriles, imidazole, and pyridine).[3b] The rate constants for electron-transfer between the Au electrode and the cytochrome

c were determined by cyclic voltammetry.[3b] The composition of the mixed films (Table 1) consists of 3–4 % pyridine-terminated chains in a diluent of alkane-terminated chains, and the coverage of cytochrome corresponds to about 10 % of the pyridine sites, which is less than 1 % overall. The nearly ideal quality of the voltammograms stands in strong contrast to that reported with pure layers of pyridine-terminated alkanes,[3a] for which the voltammetry studies showed there to be severe asymmetry in the redox rates and significant inhomogeneity. The homogeneous behavior of the voltammetry that is observed for the mixed films indicates that the protein does not denature.[4] Spectroscopic studies are underway to characterize the adsorbed cytochrome and will be reported elsewhere.

Table 1. Rate-constant data for cytochrome c immobilized on pyridinylalkanethiols.[8]

System	k^0 [Hz]	No. trials
C6py/C5	1670 ± 60	2
C11py/C10	1150 ± 80	5
C12py/C11	783 ± 36	3
C16py/C15	43 ± 10	7
C20py/C19	0.50 ± 0.06	3
C22py/C21	0.032 ± 0.026	2

Figure 1 shows the dependence of the voltammetric peak positions on the voltage scan rate for three different systems. The shift of the peak position with voltage scan rate is used to quantify the standard rate constant k^0 for electron transfer.[5] The eicosanethiol (C20) chain has the slowest k^0 value: its peaks move apart at lower scan rates than the peaks for shorter methylene chain lengths (C16 and C6). The dashed curves in this figure show the best fit to the Marcus theory model with a reorganization energy λ_o of 0.8 eV for each of the systems. The fits are not very sensitive to the value of the reorganization energy because the data do not extend to high overpotentials, for example, a λ_o value of 0.5 eV gives similar quality fits and a k^0 value that only differs by a few percent from those in Table 1.[5]

The thickness dependence of the k^0 value is summarized in Table 1 and plotted in Figure 2, together with earlier data for cytochrome c adsorbed on COOH-terminated SAMs. Both data sets obtained at large thicknesses display an exponential dependence on distance. The value of k^0 is proportional to the electronic coupling squared for nonadiabatic electron transfer [Eq. (1)]. $|V^0|^2$ is the electronic coupling matrix element at the minimum donor–acceptor separation distance and β is a characteristic decay factor.[6,7]

$$k^0_{NA} \propto |V|^2 = |V^0|^2 \exp(-\beta R) \qquad (1)$$

The two data sets (COOH and pyridinyl SAMs) should have the same distance dependence in the nonadiabatic ("tunneling") regime since the distance in both cases is being changed by the number of methylene units in the tether. A best fit to the rate data at long distance gives a β value of 1.22 per CH_2 group for the COOH-terminated SAMs and 1.19 per CH_2 group for the pyridine-terminated SAMs. Although the slopes are similar, the absolute value of the rate constant is significantly larger (at a given methylene number) for the pyridine-terminated tethers, which indicates a larger tunneling probability (electronic coupling).

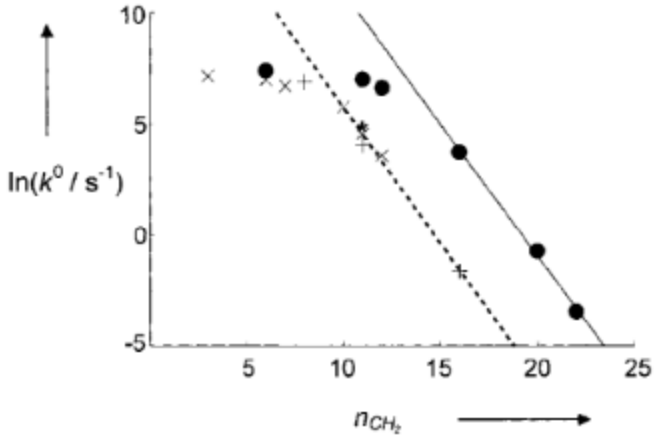


Figure 2. Plot of k^0 versus number of methylene groups for cytochrome c on SAM-coated gold electrodes (× from ref. [2c,d], + from ref. [2a,b], and ● from this work for COOH and · for pyridine-terminated layers). The lines are fits to Equation (1).

Both data sets show a plateau region at short donor–acceptor separations, however, the plateau region for the pyridine-terminated SAMs extends to larger film thicknesses (about 12 methylene groups). The maximum rate constants for both film types are similar (the hexyl chains have a rate constant of about 1100 Hz for the COOH-terminated SAMs and about 1700 Hz for the pyridine-terminated SAMs) and display plateau behavior. Previous research groups[2] explained the plateau behavior as resulting from a change in the rate-determining step from electron tunneling at large distances to conformational rearrangement of the protein–SAM system to a redox-active state at short thickness; this situation is analogous to the conformationally gated mechanism used to describe protein–protein electron transfer. Since the pyridine ring binds near the heme, the conformationally gated mechanism would need to involve local changes near the redox center, rather than large-amplitude motion of the protein. In addition, the alternating current impedance and cyclic voltammetry data indicate a typical charge-transfer step[2, 5] and do not support a more complex mechanism involving a conformational rearrangement step. Direct spectroscopic detection of redox species immobilized on the SAMs terminated with carboxylic acids[9] support the view that the conformational changes are small. In summary, the mode of binding restricts the type of conformational change that can be linked to the electron-transfer process at short distances, which implies that large-amplitude motion of the protein is not involved.

Murgida and Hildebrandt[9] observed a significant deuterium isotope effect for the electron-transfer rate constant on thin (C2 and C3) films and suggested that proton transfer may be coupled to the electron transfer or that rearrangement of the hydrogen-bonded network in the protein may constitute a rate-limiting step. In addition, they observed a thickness dependence for the isotope effect and postulated that the change in mechanism is modulated by the applied electric field. Thus, the influence of a D_2O buffer on the electron-transfer rate constant was evaluated for the C16 and C11 pyridinyl systems. The rate constant for the C16-pyridinal tether was 50 Hz, which is very similar to that observed in the H_2O buffer. The rate constant for the C11-pyridinyl tether was 900 Hz, which is a factor of 0.78 smaller than that found in the H_2O buffer. These findings are consistent with those of Murgida and Hildebrandt.

The large difference in the extent of the plateau region and the higher electron-transfer rates for the pyridine system (see Figure 2) is consistent with a larger electronic coupling for the pyridine-immobilized cytochrome c than for the carboxylate-immobilized cytochrome c. The enhanced electronic coupling suggests that the change in the electron-transfer mechanism may be linked to the change in electronic coupling with distance from the electrode. In the adiabatic, or strong coupling, regime the rate constant k_A^0 does not display an exponential distance dependence, but does depend on the polarization relaxation in the medium.[10] The simple limit is given by Equation (2),

$$k_A^0 = \frac{1}{\tau_{eff}}\sqrt{\frac{\lambda_o}{\pi^3 RT}}\exp\left(-\frac{\Delta G_a^*}{RT}\right) \qquad (2)$$

where the activation free energy is given by Equation (3)

$$\Delta G_a^* = \frac{\lambda_o}{4} - |V| \qquad (3)$$

when the free energy of the reaction is zero. The reorganization energy λ_o is difficult to calculate since it depends in a detailed manner on the protein structure,[11a] the SAM-coated electrode,[11b] and the solvent. The characteristic polarization relaxation time τ_{eff} gives a measure of the time-scale for the response of the surrounding medium (the solvent molecules, protein interior, etc.[10]) to the change in the charge distribution associated with the electron transfer, and will depend on detailed properties of the SAM-associated protein. A simple approximation treats this relaxation time as the longitudinal dielectric relaxation, which in a Debye dielectric continuum model is given by Equation (4).

$$\tau_{eff} \approx \tau_L = \left(\frac{\varepsilon_\infty}{\varepsilon_s}\right)\frac{3\eta V_m}{RT} \qquad (4)$$

τ_L is the longitudinal relaxation time of the solvent polarization, η is the solvent shear viscosity, V_m is the molar volume, ε_s is the static dielectric constant, and ε_∞ is the high-frequency dielectric constant. Hence, the experimental characteristic of electron transfer in the adiabatic limit is a friction-dependent rate constant,[12] and the rate constant for the cytochrome c in the plateau region displays a viscosity dependence.[2d, 13] This model is also consistent with the results obtained in D_2O, since the D_2O-"hydrated" protein would have a different relaxation time than the H_2O-hydrated protein and D_2O has a slower dielectric relaxation time than H_2O. Although the pyridinyl system has a larger rate constant in the plateau region than that of the COOH system, their similarity suggests that the free energies of activation in the two cases are similar, despite the different manner of protein immobilization. This observation requires that any significant changes in the reorganization energy between the two systems must be compensated for by changes in the polarization relaxation time and the electronic coupling magnitude, which also modifies the relaxation time.[10, 12] A more detailed study of this correspondence is being pursued since the current method does not provide a precise measurement of λ_o.

This work demonstrates how a new strategy for immobilizing cytochrome c on electrode surfaces which directly tethers the redox-active site to the metal electrode can be used to explore the change in the mechanism of electron transfer with distance between the protein and the electrode. The distance was changed through the variation in the number of methylene groups in the tether, but differs from earlier studies by the nature of the SAM–cytochrome interaction. The difference in binding modes provides a stronger electronic coupling for the pyridinyl systems than for the COOH system and changes the SAM thickness at which the onset of a plateau (nonexponential dependence on distance) is observed. This circumstance also causes different rate constants in the tunneling regime for the two different binding modes (but same number of methylene groups in the chain). These findings indicate that electron transfer at short distances need not be linked to a large-amplitude conformational change of the protein with respect to the electrode surface. A change in the electron-transfer mechanism that arises from the enhanced electronic coupling at short distance is also consistent with the observations.

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[8] The reported error is two standard deviations. In each case the SAM is a pyridine-terminated alkanethiol (for example, C6py has six CH_2groups) immersed in an alkanethiol diluent. See ref. [3b] for details offilm composition and characterization.

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