Photophysical Probes of a Protein/Semiconductor Electrode Interface

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Immobilization of redox molecules on solid electrodes offers a way of investigating the electron transfer of a donor/acceptor interface without the presence of diffusion processes.1 Such interactions also have important implications for analytical and clinical biochemistry. Direct electrochemistry of proteins and enzymes is therefore an active research field.2 One example of direct protein electrochemistry relevant to this work is cytochrome c (Cyt c) adsorbed on a tin oxide (SnO2) surface.3 This protein is rich in lysine amino acid residues and thus carries positive charges at neutral pH. It has been found that the native protein, Fe-Cyt c, absorbs strongly on SnO2, presumably through the electrostatic interaction with the deprotonated hydroxyl groups on SnO2 surface (Scheme 1A). The electron transfer rate constant between the two at zero free energy has been measured by cyclic voltammetry and is found to be relatively slow (3–8 s−1). In terms of modern theoretical understanding of electron transfer,3 the parameters (electronic coupling, V, and reorganization energy, Λ) which govern this rate remain uncharacterized. We report here a novel approach to obtain such parameters by combining thermal and photochemical interfacial electron transfer measurements within the context of a novel theoretical formalism. We used time-resolved fluorescence spectroscopy to measure the interfacial electron transfer rate constant from zinc-substituted cyt c (Zn-Cyt c) to SnO2 and estimated electronic coupling between the protein and semiconductor and the reaction reorganization energy. The lowest photoexcited state of Zn-Cyt c has a much higher energy level4 than the conduction band edge5 of SnO2 (Scheme 1B). Electron injection to SnO2 (route a in Scheme 1B) is therefore a thermodynamically favorable process that competes with fluorescence decay emission (route b). Zn-Cyt c was prepared6 and adsorbed7 onto a fluorine-doped SnO2 substrate according to the published procedures. The adsorption was confirmed by the UV-vis spectrum of Zn-Cyt c adsorbed on a tin oxide (SnO2) surface.8

Integration of either the anodic or the cathodic wave in the interface without the presence of diffusion processes.9 Such waves, which are substantially less than the calculated full monolayer constant from zinc-substituted cyt c, are thermodynamically favorable process that competes with fluorescence (FL) emission.

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Cyt c as 30 Å. Protein-adsorbed SnO2 samples were removed from solution and used in the fluorescence experiments. When excited with 582 nm light, Zn-Cyt c gave a broad emission spectrum with a maximum at 640 nm, where SnO2 alone did not emit. Time-resolved fluorescence emissions were collected by the single photon counting technique and are shown in Figure 1. The emission curves of Zn-Cyt c both on SnO2 and on glass (on which there is no interfacial ET) can be fitted reasonably well with double-exponential decay. The lifetime of the major component is in the range of 10−9 s and is shorter on SnO2 than on glass by 30%, resulting in an average radiationless rate constant of 2 × 109 s−1. Energy transfer between protein molecules is unlikely since the surface coverage is submonolayer.

We also ruled out the possibility of energy transfer between the protein and SnO2 due to the fact that this semiconductor does not absorb light at the excitation wavelength. Therefore, apart from fluorescence, electron injection from the excited Zn-Cyt c to SnO2 seems to be the only route for de-excitation. Photoinduced interfacial electron transfer is confirmed by the observation of anodic photocurrents of Zn-Cyt c/SnO2 sample immersed in an electrochemical cell (Figure 2a). The photocurrent excitation spectrum (Figure 2b) corresponds to the UV-vis spectrum of Zn-Cyt c in aqueous solution. Bare SnO2 did not show such behavior.

With these data available, information on two key parameters can be obtained, as follows. First, since the high density of acceptor states ensures an optimal Franck–Condon factor, the electronic coupling can be assessed directly from the electron transfer rate constant. The observed rate is relatively slow in comparison with (similar) photoexcited dye molecules directly adsorbed to a semiconductor surface, which exhibited subpicosecond electron injection.3 Thus the rate of electron injection from bound Cyt c is nominally 106 slower than that for directly adsorbed dyes. This suggests an interfacial interaction in which the reactive zinc porphyrin is relatively sequestered from the semiconductor surface by the intervening protein matrix. If one uses the broadest assumption of an exponential decay of rate with distance1, k = exp(−βR), with β ≈ 1.4 Å−1, the porphyrin...
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We then have for the Fermi golden rule:

\[ K_1 = \frac{2\pi|V|^2}{\hbar} \]  

(2)

In eqs 1 and 2, \( K_1 \) and \( K_2 \) are the interfacial electron transfer rate constants, \( V \) and \( V' \) the electronic coupling, and \( \rho \) the density of final electronic states. The following points should be made: (a) \( V \) is different from \( V' \); \( V' \) is the coupling per state, whereas \( V \) is the total integrated coupling. We can write \( N|V'|^2 = |V|^2 \), where \( N \) is the effective number of strongly coupled electronic states. (b) \( K_1 \) is not generally proportional to the density of states. Equation 2 is somewhat misleading. In practice, when \( \rho \) is large, then \( |V'|^2 \sim 1/\rho \), so that we reach a thermodynamic limit where the rate does not depend on the density of states.

We can estimate \( N \) by recognizing that the energy uncertainty (line width) associated with the initial state due to the Heisenberg principle is \( \Delta E = \hbar K_1 \). The number of states in this energy range is \( N = \Delta E/\hbar \). Putting all of these together, we get

\[ K_1 = \frac{2\pi|V|^2}{\hbar} \frac{N}{\hbar K_1} \]

so that

\[ K_1^2 = \frac{2\pi|V|^2}{\hbar} \frac{N}{\hbar K_1} \frac{K_1}{2\sqrt{\pi kT}} = \frac{\sqrt{\pi x}}{\exp(\pi x/4)} \]

(3)

Dividing eqs 3 and 1, we finally obtain

\[ \frac{\hbar K_1}{2\sqrt{\pi kT}K_2} = \frac{\sqrt{\pi x}}{\exp(\pi x/4)} = 54 \]

solving for \( x \) we obtain

\[ \lambda = 0.28 \text{ eV} \]

The reorganization energy of Cyt c has been measured or estimated before by many research groups\(^9\) using different physical methods, with values ranging from 0.14 to 1.2 eV. Our value of 0.28 eV falls within the range.\(^10\)

In summary, when photophysical luminescence techniques can be coupled with photoelectrochemical techniques, they together can provide unique insights into the nature of the protein/electrode interaction in direct protein electrochemistry. This approach, when applicable, can also provide a direct estimate of the fundamental parameters governing interfacial electron transfer, i.e., the electronic coupling and the reorganization energy.

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(9) The estimated \( \lambda \) has some uncertainty due to the variation of the fluorescence lifetimes measured by SPC. However, the uncertainty is very small. For example, variation of \( \pm 10\% \) in lifetimes would result in an uncertainty of only \( \pm 0.01 \) eV for \( \lambda \).


(11) Note that this analysis provides the value of \( \lambda \) for Fe-Cyt c only. It need not assume an equivalent value of \( \lambda \) for Zn-Cyt c, since the value of \( \lambda \) for Zn does not control the photochemical rate. This rate constant depends on \( |V'| \), which depends only on the structure of the Cyt c/SnO\(_2\) interface and on the electronic density of states of the semiconductor.