CAPSULES notes and applications from BASi

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Investigation of the Reaction Between Cytochrome b_5 and Cytochrome c Using Cyclic Voltammetry

Purpose

Electron transfer reactions (e.g., from NADH to oxygen) are important components of many physiological processes (e.g., respiration), and the mechanism(s) by which electrons are transferred between electron transfer proteins have been the focus of many studies. In this study, the electron transfer reaction between cytochrome *c* and cytochrome b_5 was studied using cyclic voltammetry.

Reference

Electrochemical measurement of Second-Order Electron Transfer Rate Constants for the Reaction between Cytochrome b_5 and Cytochrome c, R. Seetharaman, S.P. White, and M. Rivera, Biochemistry 35 (1996) 12455-12463.

Method

Cyclic voltammetry is a very useful method for qualitative characterization of chemical reactions coupled to electron transfer reactions. Quantitative data (e.g., rate constants) can also be extracted from cyclic voltammograms, but this typically requires the use of simulation software (e.g., DigiSim®).

Results

The aim of this experiment was to reduce ferricytochrome b_5 (iron(III)) to ferrocytochrome b_5 (iron(II)), which then reduced ferricytochrome *c* via an (second-order) homogeneous electron transfer reaction. These reactions can be represented by the following mechanism:

ferricytochrome $b_5 + e^- =$ ferrocytochrome *b5*
ferrocytochrome -b_5 + ferricytochrome *c* =
ferricytochrome b_5 + ferrocytochrome *c*

This is a catalytic mechanism, since the species reduced at the electrode surface is regenerated by the homogeneous electron transfer reaction. Since the redox potential of cytochrome b_5 is more negative than that of cytochrome *c*, the homogeneous electron transfer reaction is thermodynamically favorable. This mechanism also requires that ferricytochrome *c* is not reduced at the electrode surface; that is, cytochrome b_5 must be preferentially reduced in the presence of cytochrome *c*. This can be achieved using electrostatic discrimination, since cytochrome b_5 is negatively charged, and cytochrome *c* is positively charged.

It has been previously shown that cytochrome b_5 can be reduced using a gold disk electrode modified with b-mercaptopropionate in the presence of a polycation such as polylysine (1). The proposed mechanism for electron transfer requires a ternary complex between the carboxylate groups of the propionate ligands, the polylysine, and the cytochrome b_5 (***F1***). Cytochrome *c* cannot be reduced at this electrode, due to repulsive interactions with the positively charged polylysine.

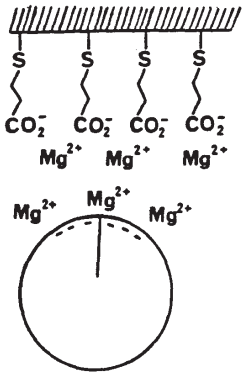

Figure 1. *Model of ternary complex between* b-*mercaptopropionate adsorbed to the surface of a gold electrode, multivalent cations (e.g., polylysine), and negatively charge cyctochrome* b_5. *Reprinted with permission from reference 1. Copyright 1994 American Chemical Society.*

The cyclic voltammogram of a solution containing only cytochrome b_5 using the above modified gold electrode is shown in ***F2a***, and the voltammogram of a solution containing both cytochrome b_5 and cytochrome *c* is shown in ***F2b***. Both voltammograms show a pair of coupled reduction-oxidation peaks that can be attributed to cytochrome b_5. The other

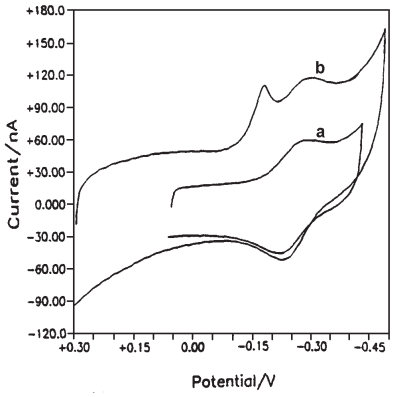

Figure 2. *Cyclic voltammograms of (a) cytochrome b_5 (0.1 mM) and (b) cytochrome c (0.1 mM) and cytochrome b_5 (0.1 mM) in a 100 mM MOPs solution (pH 7) containing polylysine (0.025 mM). Scan rate = 50 mV s^{-1}, all potentials measured with reference to Ag/AgCl. Reprinted with permission from primary Reference. Copyright 1996 American Chemical Society.*

reduction peak in ***F2b*** (the pre-peak) is related to the homogeneous electron transfer reaction between cytochrome b_5 and cytochrome *c*, and is present only if all the components required for this reaction are present (i.e., ferricytochrome *c*, cytochrome b_5, poly-L-lysine, and the b-mercaptopropionate modified electrode). A value of 3.6 x 10^8 $M^{-1}s^{-1}$ for the rate constant for the homogeneous electron transfer reaction (k_f) was extracted using DigiSim, which is comparable the value obtained using flash photolysis (2).

The effect of the homogeneous electron transfer reaction on the current response can be illustrated using DigiSim. All simulation parameters are taken from the primary Reference (***F3***); in addition, the rate

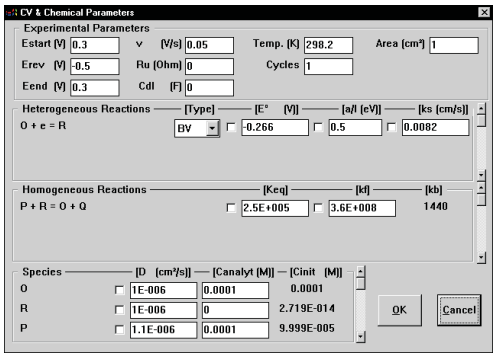

Figure 3. *Parameters used for simulation of proposed catalytic mechanism.*

constant for the homogeneous reaction (k_f) is varied to illustrate the effect of this reaction on the current response (***F4***). At intermediate values of k_f, only one

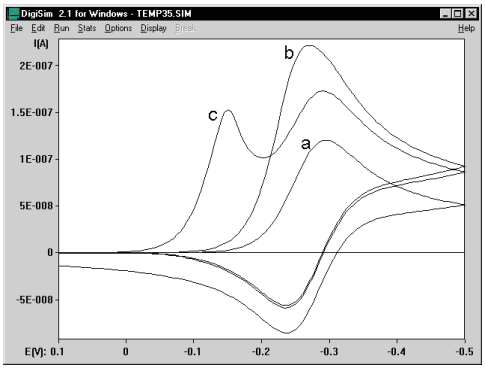

Figure 4. *Simulated cyclic voltammograms for proposed catalytic mechanism using the parameters in Table 1. k_f = 0 (a), 3.6 x 10^5 (b), and 3.6 x 10^8 (c) $M^{-1}s^{-1}$.*

reduction peak is seen, although the faradaic current is higher than it is when there is no reaction. Peak splitting is only seen at higher values of k_f. One way to rationalize these effects is to consider that there are two contributions to the faradaic current; direct reduction of cytochrome b_5 at the electrode surface, and mediated reduction of cytochrome *c* using cytochrome b_5 as the mediator. The rate of the direct reduction reaction (and hence its contribution to the current) is determined by the heterogeneous electron transfer rate constant, whereas the rate of the mediated reduction is determined by both the heterogeneous electron transfer rate constant and k_f. It should also be noted that, since the overall mechanism is catalytic, only a small amount of the reduced mediator (ferrocytochrome b_5) is required to obtain a measurable mediated current (current amplification). Therefore, if the homogeneous electron transfer reaction is fast enough, the mediated current can be observed at potentials significantly positive of the redox potential of the mediator; that is, the mediated current can be observed at potentials at which the current due to direct electron transfer is negligible. This can be illustrated by examining the concentration profiles (3) for k_f = 3.6 x 10^8 $M^{-1}s^{-1}$ (the value reported in the primary Reference). The current for the pre-peak is due only to the mediated electron transfer, since the only (net) changes are in the concentrations of oxidized and reduced cytochrome *c* (c(III) and c(II), respectively) (***F5a***). The peak can be attributed to depletion of cytochrome *c* at the electrode surface. In contrast, the main peak is due to depletion of oxidized cytochrome b_5 at the electrode surface due to the heterogeneous electron transfer reaction (***F5b***).

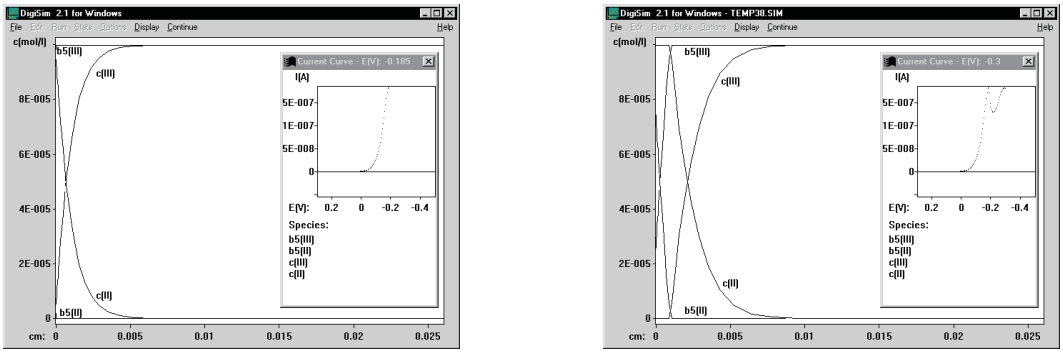

Figure 5. *Concentration profiles generated using CV - the Movie for $k_f = 3.6 \times 10^8\ M^{-1}s^{-1}$.*

DigiSim is a registered trademark and CV - the Movie is a trademark of Bioanalytical Systems, Inc.

References:
1. *M. Rivera, M.A. Wells, and F.A. Walker, Biochemistry 33 (1994) 2161-2170.*
2. *T.E. Meyer, M. Rivera, F.A. Walker, M.R. Mauk, A.G. Mauk, M.A. Cusanovich, and G. Tollin, Biochemistry 32 (1993) 622-627.*
3. *Concentration profiles generated using CV - the MovieÔ.*