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SPECTROELECTROCHEMICAL DETERMINATION OF THE HETEROGENEOUS ELECTRON TRANSFER KINETICS OF SOLUBLE SPINACH FERREDOXIN

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SUMMARY: Heterogeneous electron transfer rate parameters for soluble spinach ferredoxin are reported using a recently developed single potential step spectroelectrochemical technique. The reductive kinetics were measured by monitoring the decrease in absorbance as a function of time for several overpotential steps at methyl viologen modified optically transparent gold minigrid electrodes. These measurements yielded an average formal heterogeneous electron transfer rate constant ($k^{o'}_{f,h}$ = 6.5 (±1.3) x 10^{-5} cm/s) and electrochemical transfer coefficient (α = 0.60 ±(0.16)) at pH 7.5. These results are the first heterogeneous electron transfer rate parameters reported for this species.

Soluble spinach ferredoxin is a 2Fe-2S iron-sulfur protein of molecular weight 11,640 daltons (1) which exhibits a low formal reduction potential, $E^{o'}$ = -0.423 V vs. NHE (2). In the oxidized form it exhibits an absorbance maximum characteristic of the active site at 420 nm. Reduction results in a decrease in absorbance at this wavelength with a difference molar absorbtivity, $\Delta\varepsilon$, of 5130 $M^{-1}cm^{-1}$ (1).

Ferredoxin functions as a one electron transfer agent in the green plant chloroplast and is believed to undergo direct heterogeneous reduction by membrane bound iron-sulfur protein(s) of Photosystem I. Ferredoxin is also believed to be the most negative solution soluble species in the photosynthetic apparatus and is known to be an essential catalyst involved in the direct conversion of radiant energy into chemical energy (3). The low redox potential and inherent instability to oxygen of soluble ferredoxin has led to few reports of its homogeneous electron transfer kinetics (4) and no reports of its heterogeneous electron transfer kinetics.

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Spinach ferredoxin cannot be completely reduced by chemical reductants in solution except at high pH which can cause protein denaturation. The attainable negative solution potential for chemical reduction is defined by the Nernst relation; E = -0.059 pH. In order to chemically reduce 99% of a sample of ferredoxin in aqueous solution the pH must exceed 9.25 (5). More negative solution potentials can be achieved at native protein pH conditions with the use of electrodes having sufficiently large hydrogen overpotentials. Both gold and antimony-doped tin oxide electrodes satisfy this criterion and exhaustive mediated reductions of spinach ferredoxin have been reported (5).

In order to measure heterogeneous electron transfer kinetic parameters of an electroactive species, it must be possible to drive the redox reaction at a mass transfer controlled rate. Biological molecules do not typically exhibit mass transfer controlled rates of electron transfer at electrodes. Landrum et al. (6) reported a gold minigrid electrode surface, electrochemically modified with methyl viologen, which exhaustively reduced and oxidized soluble spinach ferredoxin at pH 7.1. Bowden et al. (7) used this electrode to measure the reductive heterogeneous electron transfer kinetics of myoglobin using a recently developed single potential step spectroelectrochemical technique (8). This same approach has now been applied to the measurement of the reductive heterogeneous electron transfer kinetic parameters for soluble ferredoxin.

EXPERIMENTAL

Type III spinach ferredoxin solution was purchased from SIGMA and was used directly from stock in 0.15 M Trizma buffer, pH 7.5, 0.2 M NaCl. The concentration was determined spectroscopically using $\varepsilon(420)$ nm = 9680 $M^{-1}cm^{-1}$ (1). Methyl viologen was obtained from K & K Laboratories and was used without further purification. Trizma buffer was from SIGMA and a 0.15 M, pH 7.5 solution was prepared when sample dilution was required. All other chemicals were reagent grade and all solutions were prepared in doubly distilled water. The gold minigrid electrodes were obtained from Buckbee-Mears, St. Paul, Minnesota, and had an optical transparency of 67% with 200 lines per inch.

Optical measurements were made with a Beckman Acta MVII spectrophotometer operating in time drive at a fixed wavelength of 420 nm. Potentials were applied using a conventional potentiostat constructed with

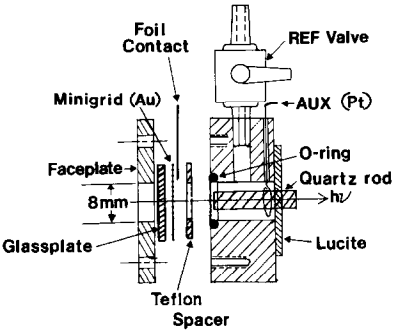

Figure 1. Quartz rod optically transparent electrochemical (OTE) cell.

operational amplifiers, Philbrick-Nexus 1026, and were monitored with a Keithley 202 digital voltmeter. These potentials were referenced to a Ag/AgCl (1.0 M KCl) reference electrode which was calibrated against a saturated quinhydrone solution, pH 7.0 to be 0.226 ±0.005 V vs. NHE.

The spectroelectrochemical cell, electrode mounting and modification procedures have been previously described (7). This cell was modified by inserting a quartz rod into the lightpath similar to that reported by Shu and Wilson (9) as shown in Figure 1. For reductive kinetic measurements of ferredoxin a decrease in absorbance must be monitored. Conventional potential step transmission spectroelectrochemistry requires the monitoring of an increase in absorbance due to the small dimensions of the electrochemical diffusion layer relative to normal cell pathlengths. The small optical pathlengths of the cell employed, ca. 0.06 cm, served to minimize the background absorbance and to increase the signal to noise ratio of the optical signal. This latter point was important when considering the small changes in absorbance which were measured in this work.

RESULTS AND DISCUSSION

The reaction scheme for ferredoxin reduction is as indicated below:

$$Fd_{ox} + e^- \xrightarrow{k_{f,h}} Fd_{red}$$

where Fd_{ox} and Fd_{red} represent the oxidized and reduced forms, respectively, and $k_{f,h}$ is the heterogeneous electron transfer rate constant for a given potential step. The absorbance-time transients for three different overpotentials are depicted in Figure 2. Because of the noise at this level of sensitivity, the solid lines were drawn to approximate averaged transients. Kinetic analysis was performed with data taken from the solid lines. Signal averaging was not possible due to the 20 minute equilibration period

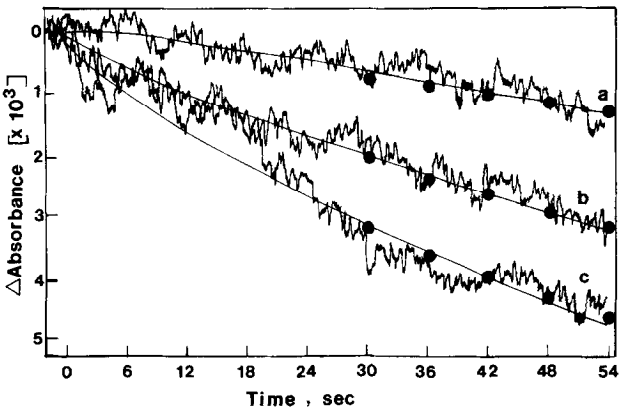

Figure 2. Theoretical and experimental absorbance versus time behavior of spinach ferredoxin at a modified gold minigrid electrode. Solution conditions: 117.8 μM ferredoxin in 0.15 M tris buffer, pH 7.5, 0.2 M NaCl. Trace, potential step (mV vs. NHE) and overpotential, η, (mV vs. NHE).
(a) -439 mV, 16 mV; (b) -489 mV, 66 mV, (c) -524 mV, 101 mV.

between potential steps which allows the system to return to initial bulk concentration conditions.

Solid circles represent the predicted chronoabsorptometric behavior for a 117.8 μM ferredoxin sample as calculated from the theoretically derived time dependent optical response and the average $k_{f,h}$ for each transient (8). Because of the "hole filling" process, which is characteristic of the minigrid electrode (10), only data taken after 25 seconds were evaluated (7).

A detailed description of the data analysis for a simple one-electron transfer has been presented (8). The rate constant, $k_{f,h}$, at a given overpotential was obtained from the chronoabsorptometric results through use of the normalized absorbance vs. log ($k_{f,h}\, t^{\frac{1}{2}}/ D^{\frac{1}{2}}$) working curve where $D = 1.19 \times 10^{-6}$ cm/sec (11) for spinach ferredoxin. Figure 3 is a diagram of the working curve with experimental results at various overpotentials represented by symbols. Representative $k_{f,h}$ values for a series of overpotentials are given in Table 1. The formal heterogeneous rate constant, $k^{o'}_{f,h}$, is determined from the intercept of the linear $k_{f,h}$ vs. η plot and

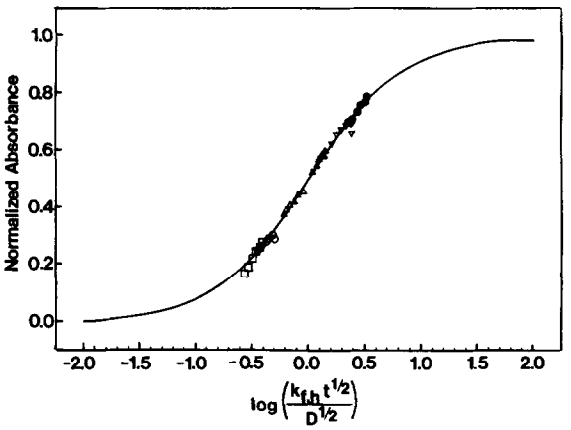

Figure 3. Normalized absorbance versus log ($k_{f,h}\, t^{\frac{1}{2}} / D^{\frac{1}{2}}$) working curve for the reduction of spinach ferredoxin at a modified gold minigrid electrode.
Same solution conditions as in Figure 2. Calculated log $k_{f,h}$ and transient number given in parentheses;
●, η=86 (-3.34,20); △, η=101 (-3.49,2); ▲, η=66 (-3.68,32); ▽, η=51 (-3.82,25); ○, η=26 (-4.17,35); □, η=16 (-4.29,9).

the transfer coefficient, α, is evaluated from the slope (8). A typical result is shown in Figure 4.

Table 1. Heterogeneous electron transfer rate constants for the reduction of spinach ferredoxin at a methyl viologen electrochemically modified gold minigrid electrode.

η (mV)	$k_{f,h}$ (cm/s)
16	7.8 (±0.1) x 10^{-5}
26	1.1 (±0.2) x 10^{-4}
41	1.4 (±0.1) x 10^{-4}
51	1.7 (±0.1) x 10^{-4}
66	2.0 (±0.3) x 10^{-4}
76	3.9 (±0.3) x 10^{-4}
101	4.7 (±0.2) x 10^{-4}

Solution contained 117.6 μM ferredoxin, pH 7.5 tris buffer, 0.2 M NaCl. Rate constants are mean values of at least 3 transients at each overpotential. Rate constants for individual transients are mean values of six observations over the 30 to 60 second time domain. Parentheses contain one standard deviation.

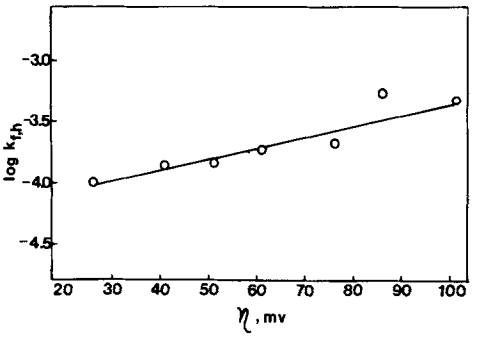

Figure 4. Dependence of $k_{f,h}$ on overpotential. Linear regression slope $=7.8 \pm(0.1) \times 10^{-3}$ mV^{-1}; intercept = $-4.20 \pm(0.06)$ cm/sec; Correlation coefficient = 0.945.

A summary of the overall kinetic results is presented in Table 2 for experiments conducted with different ferredoxin samples at four modified gold grid electrodes. Numbers in parentheses represent one standard deviation in the intercept. This study demonstrates the viability of applying the potential step spectroelectrochemical kinetic method to the study of a low potential biological redox molecule. Access to heterogeneous electron transfer kinetic parameters for ferredoxin may aid in the elucidation of its electron transfer mechanism.

Table 2. Formal heterogeneous electron transfer rate constants and electrochemical transfer coefficients for spinach ferredoxin at four modified minigrid electrodes.

[FERREDOXIN] μM	$k^{o'}_{f,h}$ cm/s	α
78.5	$4.9 (\pm0.2) \times 10^{-5}$	$0.78 \pm(0.18)$
117.8	$6.3 (\pm0.1) \times 10^{-5}$	$0.46 \pm(0.09)$
123.9	$6.8 (\pm0.1) \times 10^{-5}$	$0.47 \pm(0.07)$
117.8	$8.1 (\pm0.1) \times 10^{-5}$	$0.67 \pm(0.06)$
Average values	$6.5 (\pm1.3) \times 10^{-5}$	$0.60 \pm(0.16)$

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