April, 1980] © *1980 The Chemical Society of Japan* *Bull. Chem. Soc. Jpn.*, **53**, *947—950 (1980)* 947

Electrochemical Oxidation and Reduction of Ferredoxin Adsorbed on Mercury Electrode Surface. Phase-selective A. c. Polarography at DME†

Tadaaki KAKUTANI, Kazunobu TORIYAMA, Tokuji IKEDA, and Mitsugi SENDA*
Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, Kyoto 606

(Received November 5, 1979)

The electrochemical behavior of *Clostridium pasteurianum* ferredoxin (Cl.Fd) was studied by a.c. polarography with the dropping mercury electrode. The a.c. polarograms observed in acidic media (pH$<$7) were assigned to RSH/RSHg redox reaction of the cysteine residues of apoferredoxin, whereas those observed in basic media (pH$>$8) were assigned to a two-step one-electron transfer (EE mechanism) to the iron-sulfur clusters of Cl.Fd adsorbed on the mercury electrode surface ($(\mathrm{Cl.Fd})_{\mathrm{ad}}$). The a.c. polarographic behavior of $(\mathrm{Cl.Fd})_{\mathrm{ad}}$ observed in basic media was interpreted by theory of a.c. polarography of a two-step surface redox reaction. The formal standard oxidation-reduction potential of $(\mathrm{Cl.Fd})_{\mathrm{ad}}$ was determined to be −0.33 V *vs.* NHE at pH 7.0, and its pH dependence was −30 mV/pH between pH 8 and 10.

In a previous paper[1] we have shown that ferredoxin is adsorbed irreversibly on the surface of mercury electrode and decomposes gradually to apoferredoxin (cluster-free ferredoxin) in the adsorption layer. D.c. and a.c. polarographic studies on ferreoxins have been reported by several workers.[2-7] However, these authors did not fully consider the adsorption nature of ferredoxins on the electrode surface. Accordingly further examination is needed to find the exact relationship between the measured potentials being characteristic of the polarograms of ferredoxins and their oxidation-reduction potentials. In this study we have investigated the a.c. polarographic behavior of *Clostridium pasteurianum* ferredoxin (Cl.Fd) at the dropping mercury electrode (DME) with exact reference to the adsorption of ferredoxin on the electrode surface. The results show that the a.c. polarographic study of Cl.Fd with DME in basic media enables us to determine the electrochemical oxidation-reduction potential of the iron-sulfur clusters of the ferredoxin adsorbed on the electrode surface.

Experimental

Preparations of *Clostridium pasteurianum* ferredoxin and apoferredoxin as well as apparatus and methods of electrochemical measurements have been described previously.[1] The dropping mercury electrode was of a common type having a mercury flow rate of 1.01 mg s^{-1} and a drop time of 9.5 s at open circuit in distilled water at 45 cm height of mercury reservoir. Real and imaginary components of the alternating currents were recorded against d.c. potential applied to the DME. The a.c. current intensities were taken at the moment of the maximum growth of each mercury drop. Potentials were measured against a saturated calomel electrode (SCE). All measurements were carried out at 25 °C.

Results and Discussion

Figure 1 shows the real and imaginary components of the a.c. waves of 1.7 μM Cl.Fd at pH 4.4 (Fig. 1A) and at pH 9.2 (Fig. 1B). The wave heights grew up to a certain limit with increasing concentration of Cl.Fd, as seen in Fig. 2, where the peak heights of the imaginary a.c. wave, $\delta_1 I_{\mathrm{p}}^{\mathrm{imag}}$, are plotted against the Cl.Fd concentration, $C_{\mathrm{Cl.Fd}}$, at pH 6.9. This indicates that Cl.Fd is accumulated on the DME surface and that the a.c. polarograms are due to the redox reaction of Cl.Fd adsorbed on the electrode surface. Upon assuming that the adsorption is a diffusion-controlled process,[1,8] $\delta_1 I_{\mathrm{p}}^{\mathrm{imag}}$ is given by $\delta_1 I_{\mathrm{p}}^{\mathrm{imag}} = kA\Gamma = kA(6/7)(7D_{\mathrm{Cl.Fd}}\tau/3\pi)^{1/2} C_{\mathrm{Cl.Fd}}$ at the initial linear portion of $\delta_1 I_{\mathrm{p}}^{\mathrm{imag}}$ *versus* $C_{\mathrm{Cl.Fd}}$ plot in Fig. 2, where k is the proportionality constant, Γ the surface concentration in mole per cm^2

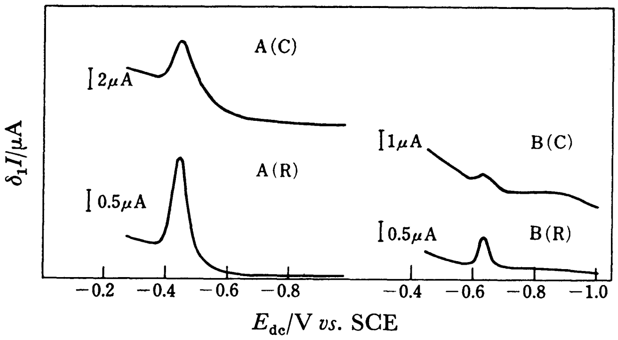

Fig. 1. A.c. polarograms of 1.7 μM Cl.Fd at 200 Hz. A(C) and A(R): Imaginary and real components at pH 4.4, B(C) and B(R): imaginary and real components at pH 9.2.

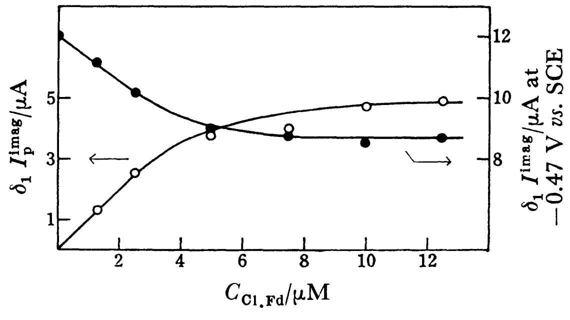

Fig. 2. Plots of $\delta_1 I_{\mathrm{p}}^{\mathrm{imag}}$ and $\delta_1 I^{\mathrm{imag}}$ at −0.47 V against $C_{\mathrm{Cl.Fd}}$ at pH 6.9 (frequency=200 Hz).
○: $\delta_1 I_{\mathrm{p}}^{\mathrm{imag}}$ (at −0.575 V *vs.* SCE), ●: $\delta_1 I^{\mathrm{imag}}$ at −0.47 V *vs.* SCE.

---

† A main part of the experimental results of this work was presented at the 23rd Annual Meeting on Polarography, Nov. 4—5, 1977, Osaka: abstract; T. Ikeda, K. Toriyama, and M. Senda, *Rev. Polarogr. (Kyoto)*, **23**, 26 (1977).

of the adsorbed Cl.Fd, $D_{\mathrm{Cl.Fd}}$ the diffusion coefficient of Cl.Fd, and A and τ are the surface area and the drop time of the DME, respectively. At high Cl.Fd concentrations a maximum current, $\delta_1 I_{\mathrm{p}}^{\mathrm{max}}$, is attained, which is given by $\delta_1 I_{\mathrm{p}}^{\mathrm{max}} = kA\Gamma^{\mathrm{max}}$, where Γ^{max} is the maximum surface concentration of adsorbed Cl.Fd. Applying these two equations to the plot in Fig. 2, we estimated the Γ^{max} value as 1.4×10^{-11} mol/cm^2, $D_{\mathrm{Cl.Fd}} = 1.4 \times 10^{-6}$ cm^2 s^{-1} being assumed.[1] Adsorption of Cl.Fd also caused a decrease of the imaginary a.c. current in the potential region where the faradaic current does not appear, indicating that the differential double layer capacity of the mercury-solution interface decreases due to the adsorption of Cl.Fd on the electrode surface (see Fig. 2). Upon assuming that the relative decrease of the double layer capacitance, C_{dl}, is proportional to the surface coverage, θ, due to adsorption of Cl.Fd, that is, $\theta = \Gamma/\Gamma^{\mathrm{max}} = (C_{\mathrm{dl}}^{\circ} - C_{\mathrm{dl}})/(C_{\mathrm{dl}}^{\circ} - C_{\mathrm{dl}}^{\mathrm{max}})$, where C_{dl}° and $C_{\mathrm{dl}}^{\mathrm{max}}$ are the double layer capacitances in the absence of Cl.Fd and at the maximum adsorption of Cl.Fd, respectively, Γ^{max} can be estimated by $\Gamma^{\mathrm{max}} = (6/7)(7D_{\mathrm{Cl,Fd}}/3\pi)^{1/2}C_{\mathrm{Cl.Fd}}/((C_{\mathrm{dl}}^{\circ} - C_{\mathrm{dl}})/(C_{\mathrm{dl}}^{\circ} - C_{\mathrm{dl}}^{\mathrm{max}}))$, where a diffusion-controlled adsorption of Cl.Fd is assumed. Application of this equation to the relative decrease of the imaginary a.c. current at -0.45 V at pH 6.9 leads to the Γ^{max} value of 1.3×10^{-11} mol/cm^2. These two Γ^{max} values agree well with each other and with the Γ^{max} value of 1.3×10^{-11} mol/cm^2 obtained for apoferredoxin (cluster-free ferredoxin) with the hanging mercury drop electrode (HMDE).[1]

In all a.c. polarograms of Cl.Fd recorded in this study, the peak potentials of the real and imaginary components, $E_{\mathrm{p}}^{\mathrm{real}}$ and $E_{\mathrm{p}}^{\mathrm{imag}}$, coincided with each other and were independent of a.c. frequency between 50 and 500 Hz (see Table 1). As shown in Fig. 3 the peak potentials shifted by -60 mV/pH at pH lower than 7 and by -30 mV/pH at pH higher than 8. In the previous paper[1] we have shown that Cl.Fd adsorbed on the HMDE surface decomposes to the corresponding apoferredoxin and that the apoferredoxin gives d.c. and a.c. voltammograms due to the RSH/RSHg redox reaction of the cysteine residues of the apoferredoxin. At pH lower than 7 the peak potentials of the a.c. polarograms obtained with the DME agreed with the peak potentials of the RSH/RSHg redox couple observed with the HMDE.[1] Furthermore we found that at pH lower than 7 apo-Cl.Fd gives practically the same a.c. polarograms as Cl.Fd with the DME. These results indicate that at pH lower than 7 Cl.Fd adsorbed on the DME surface decomposes rapidly to apo-Cl.Fd and that the a.c. polarograms of Cl.Fd obtained here at pH lower than 7 should be assigned to the RSH/RSHg redox reaction of the cysteine residues of apo-Cl.Fd.

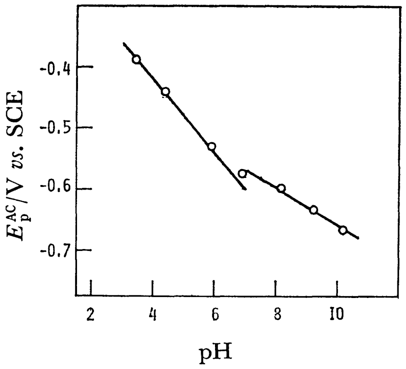

Fig. 3. Dependence of the peak potential, $E_{\mathrm{p}}^{\mathrm{AC}}$, on pH.

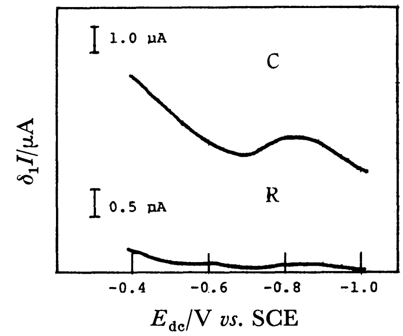

Fig. 4. A.c. polarograms of 1.5 μM apo-Cl.Fd at pH 9.2. R: Real component, C: imaginary component.

On the other hand at pH higher than 8 the a.c. polarograms of Cl.Fd with the DME (Fig. 1B) were obviously different from those of apo-Cl.Fd with the DME (Fig. 4). Furthermore the peak potentials of the a.c. polarograms of Cl.Fd with the DME in basic media (pH>8) were much more positive than the peak potentials of the steady-state cyclic a.c. or d.c. voltammograms at the HMDE which were assigned to the RSH/RSHg redox reaction of cysteine residues of apoferredoxin.[1] These results indicate that in basic media Cl.Fd adsorbed on the DME surface may remain undecomposed at least for a drop life of the DME, usually less than 9 s, and that the a.c. polarograms observed with DME in basic media should be assigned to the redox reaction of the iron-sulfur clusters of Cl.Fd adsorbed on the electrode surface. Figure 5 shows

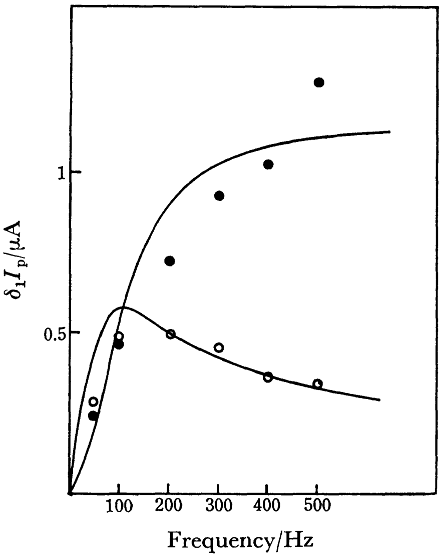

Fig. 5. Dependence of the peak heights of a.c. polarograms of 1.7 μM Cl.Fd on ac frequency at pH 9.2. ○: Real component, ●: imaginary component.

Table 1. Peak potential and half-peak width of a.c. polarograms of Cl.Fd adsorbed on DME surface at pH 9.2

Frequency/ Hz	E_p^{real}/V *vs.* SCE	E_p^{imag}/V *vs.* SCE	$\Delta E_{p/2}^{real}$/mV		$\Delta E_{p/2}^{imag}$/mV	
			Obsd	Calcd[a)]	Obsd	Calcd[a)]
50	−0.64	−0.64	25	24	35	35
100	−0.64	−0.64	30	29	40	45
200	−0.64	−0.64	35	37	—	64
300	−0.64	−0.64	40	43	—	77
400	−0.64	−0.64	45	48	—	87
500	−0.64	−0.64	50	51	—	96

a) Calculated by using $K=1$, $k_{sap}=3.9\cdot10^3\ s^{-1}$, and $G\theta_t=3.2$.

dependences of the peak heights of the two a.c. components on a.c. frequency at pH 9.2. The peak potentials, E_p^{real} and E_p^{imag}, and the half-peak widths, $\Delta E_{p/2}^{real}$ and $\Delta E_{p/2}^{imag}$ of the two components at pH 9.2 are gievn in Table 1.

The redox reaction of the iron-sulfur clusters of Cl.Fd in bulk solution has been studied by several authors using equilibrium techniques.[9–13)] These studies have revealed that the stoichiometry of electron equivalents, n, is 2, whereas the potentiometric data fit the Nernst equation for which $n=1$.[10–13)] Eisenstein and Wang[13)] interpreted these results by assuming that Cl.Fd, which has two iron-sulfur clusters, is reduced in two reduction steps, each step involving a one-electron transfer to each cluster. More direct evidence was provided by Orme-Johnson and Beinert[14)] and Mathews *et al.*,[15)] who observed two different ESR signals arising sequentially during a reductive titration of Cl.Fd. Therefore, it is reasonable to suppose that the adsorbed Cl.Fd also is electrochemically reduced in two reduction steps at the mercury electrode surface.

According to theory of a.c. polarography[16)] for a two-step surface redox reaction,

$$\begin{array}{l} O_{ad} + n_1e \rightleftarrows S_{ad} \\ S_{ad} + n_2e \rightleftarrows R_{ad} \end{array} \qquad \text{(I)}$$

in which the interaction between the adsorbed reactants is taken into account, the real and imaginary components of the a.c. wave, $\delta_1 I_F^{real}$ and $\delta_1 I_F^{imag}$, for d.c. reversible process and $n_1=n_2=1$ may be, under appropriate simplifications,[17)] expressed as

$$\delta_1 I_F^{real} = \frac{(r_1r_2-y_1y_2-x_1x_2)(r_1+r_2)+(r_1y_2+r_2y_1)(y_1+y_2+x_1-x_2)}{(r_1r_2-y_1y_2-x_1x_2)^2+(r_1y_2+r_2y_1)^2}\delta_1 E \qquad \text{(1a)}$$

$$\delta_1 I_F^{imag} = \frac{(r_1y_1+r_2y_1)(r_1+r_2)-(r_1r_2-y_1y_2-x_1x_2)(y_1+y_2+x_1-x_2)}{(r_1r_2-y_1y_2-x_1x_2)^2+(r_1y_2+r_2y_1)^2}\delta_1 E \qquad \text{(1b)}$$

with

$$r_1 = [(F^2/RT)\Gamma_t k_{sap}\bar{f}_1^{1/2}\bar{f}_2^{1/2}]^{-1}$$

$$r_2 = [(F^2/RT)\Gamma_t k_{sap}\bar{f}_2^{1/2}(1-\bar{f}_1-\bar{f}_2)^{1/2}]^{-1}$$

$$y_1 = [(F^2/RT)\Gamma_t\omega]^{-1}[(1/\bar{f}_1)+(1/\bar{f}_2)-G\theta_t]$$

$$y_2 = [(F^2/RT)\Gamma_t\omega]^{-1}[(1/\bar{f}_2)+(1/(1-\bar{f}_1-\bar{f}_2))-G\theta_t]$$

$$x_1 = -x_2 = [(F^2/RT)\Gamma_t\omega]^{-1}[(1/\bar{f}_2)-(1/2)G\theta_t]$$

In these equations $\delta_1 E$ is the amplitude of applied ac voltage, ω the angular frequency, Γ_t the total surface concentration defined by $\Gamma_t=\Gamma_O+\Gamma_S+\Gamma_R$, Γ_i(i=O, S, or R) being the surface concentration of species i, θ_t the total surface coverage defined by $\theta_t=\Gamma_t/\Gamma^{max}$, Γ^{max} being the maximum value of Γ_t, $\bar{f}_1$ and $\bar{f}_2$ are the mean fractions of the surface coverages defined by $\bar{f}_1=\bar{\Gamma}_O/\Gamma_t$ and $\bar{f}_2=\bar{\Gamma}_S/\Gamma_t$, respectively, $\bar{\Gamma}_i$ is the mean or "d.c." value of Γ_i, k_{sap} the apparent rate constant of the charge transfer which depends on the adsorption parameters and on the total surface coverage θ_t, and G the parameter representing the interaction between the adsorbed reactants. The mean fractions $\bar{f}_1$ and $\bar{f}_2$ are related to the d.c. potential, E_{dc}, by

$$E_{dc} = E_o' + (RT/F)[\ln\sqrt{K}+\ln(\bar{f}_1/\bar{f}_2) - (1/2)(1-2\bar{f}_2)G\theta_t] \qquad \text{(2a)}$$

$$E_{dc} = E_o' + (RT/F)[-\ln\sqrt{K}+\ln(\bar{f}_2/(1-\bar{f}_1-\bar{f}_2)) + (1/2)(1-2\bar{f}_2)G\theta_t] \qquad \text{(2b)}$$

with

$$E_o' = (E_{o1}'+E_{o2}')/2 \qquad \text{(3)}$$

$$K = \exp[(F/RT)(E_{o1}'-E_{o2}')] \qquad \text{(4)}$$

where E_{o1}' and E_{o2}' are the formal standard potentials of the first and second surface redox steps, respectively. E_o' corresponds to the formal standard potential of the adsorbed redox couple O_{ad}/R_{ad}. The parameter K corresponds to the formation constant of the intermediate S_{ad}. These equations predict that both components of the a.c. wave are symmetrical with respect to the E_o'-axis and that within limited values of the parameters K, $G\theta_t$, and (ω/k_{sap}) the peak potentials of both components are independent of a.c. frequency and coincide with each other and with E_o'. These predictions are consistent with the present results. In Table 1 are also given values of the half-peak widths as calculated from these equations by assuming $K=1$, $G\theta_t=3.2$, and $k_{sap}=3.9\times10^3\ s^{-1}$. Solid lines in Fig. 5 are drawn by these equations using the same values of the parameters as above. Apparent agreement between the theory and experimental results seems reasonably good. However, it must be noted that the data can equally well fit the simplified equations (1) and (2) with other sets of k_{sap}, $G\theta_t$, and K for $0\leqq K\lesssim3$: for example, $k_{sap}=4.3\times10^3\ s^{-1}$, $G\theta_t=4$ and $K=3$, or $k_{sap}=5.8\times10^3\ s^{-1}$, $G\theta_t=2.5$ and $K=0.1$, or even $k_{sap}=5.0\times10^2\ s^{-1}$, $G\theta_t=0.95$ and $K=0$. The last set with $K=0$ corresponds to a single two-electron redox reaction, $O_{ad}+2e\leftrightarrows R_{ad}$[18)] (II). In conclusion we may state that the a.c. polarographic behavior of Cl.Fd at DME in basic solution is explained by the redox reaction mechanism (I) with $0\leqq K\lesssim3$ (note that $K=0$ corresponds to the mechanism (II)).

As described above, the peak potentials of the a.c. polarograms, E_p^{AC}, in basic media should correspond to the formal standard redox potential of the iron-sulfur clusters of Cl.Fd adsorbed on the electrode surface. Table 2 summarizes our E_p^{AC} values and the values (extra- or interpolated to pH 7.0) of the standard redox potential, E_o', of Cl.Fd determined by various equilibrium techniques.[9–13)] The E_o' value cited from

TABLE 2. FORMAL STANDARD OXIDATION-REDUCTION POTENTIAL OF Cl.Fd

pH	E'_o/V *vs.* NHE	Method	Ref.
7.0	−0.33[a]	A.c. polarography	This work
8.2	−0.355		
9.2	−0.390		
10.2	−0.420		
7.0	−0.33[a]	Potentiometric titration in the presence of mediators.	9
7.0	−0.39[a]	Equilibrium with H_2/H^+, hydrogenase.	10
7.0	−0.405		11
7.0	−0.403		12
7.4	−0.383	Equilibrium with methyl viologen (reduced)/(oxidized)	13

a) Value extra- or interpolated to pH 7.0.

Ref. 9 was determined by the direct potentiometric titration in the presence of mediators using a Pt electrode as an indicator electrode [direct method]. On the other hand, the E'_o values cited from Refs. 10 to 13 were measured by equilibrating a ferredoxin with another reducing redox couple such as H_2/H^+[10–12] or methyl viologen[13] using spectrophotometry [indirect method]. As seen from Table 2, our E_p^AC value extrapolated to pH 7 (= −0.33 V *versus* NHE) is *ca.* 60 to 70 mV more positive than the E'_o values determined by the "indirect" method.[10–13] This difference may be due to the difference in the free energy between the adsorbed redox couple and the redox couple in bulk of solution. On the contray the E_p^AC value agreed well with the E'_o value determined by the "direct" method (the value interpolated to pH 7.0 is −0.33 V *versus* NHE).[9] In addition the E'_o determined by the "direct" method shifted by −30 mV/pH, which is also in agreement with the pH dependence of the E_p^AC observed here in basic media. In the "direct" method a Pt electrode was used as an indicator electrode. Adsorption of plant ferredoxins on a Pt electrode was also reported.[5] Therefore it is likely that the E'_o determined by the "direct" method corresponds to the formal standard redox potential of the iron-sulfur clusters of Cl.Fd adsorbed on the Pt electrode surface.

This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education.

References

1) T. Ikeda, K. Toriyama, and M. Senda, *Bull. Chem. Soc. Jpn.*, **52**, 1937 (1979).

2) P. D. J. Weitzman, I. R. Kennedy, and R. A. Caldwell, *FEBS Lett.*, **17**, 241 (1971).

3) H. Dalton and J. Zubieta, *Biochim. Biophys. Acta*, **322**, 133 (1973).

4) Y. W. Chien, *J. Pharm. Sci.*, **65**, 1471 (1976).

5) B. A. Kiselev, A. A. Kazakova, V. B. Evstigneev, V. K. Gins, and E. N. Mukhin, *Biofizika*, **21**, 35 (1976).

6) C. L. Hill, J. Renaud, R. H. Holms, and L. E. Mortenson, *J. Am. Chem. Soc.*, **99**, 2549 (1977).

7) P. Bianco and J. Haladjian, *Biochem. Biophys. Res Commun.*, **78**, 323 (1977).

8) J. Koryta, *Collect. Czech. Chem. Commun.*, **18**, 206 (1953).

9) B. Ke, W. A. Bulen, E. R. Shaw, and R. H. Breeze, *Arch. Biochem. Biophys.* **162**, 301 (1974).

10) K. Tagawa and D. I. Arnon, *Biochim. Biophys. Acta*, **153**, 602 (1968).

11) E. T. Lode, C. L. Murray, and J. C. Rabinowitz, *J. Biol. Chem.*, **251**, 1683 (1976).

12) N. A. Strombaugh, J. E. Sundquist, R. H. Burris, and W. H. Orme-Johnson, *Biochemistry*, **15**, 2633 (1976).

13) K. K. Eisenstein and J. H. Wang, *J. Biol. Chem.*, **244**, 1720 (1969).

14) W. H. Orme-Johnson and H. Beinert, *Biochem. Biophys. Res. Commun.*, **36**, 337 (1969).

15) R. Mathews, S. Charlton, R. H. Sands, and G. Palmer, *J. Biol. Chem.*, **249**, 4326 (1974).

16) T. Kakutani, K. Toriyama, T. Ikeda, and M. Senda, Paper presented at the 25th annual meeting on polarography, Oct. 5—6, 1979; abstract, *Rev. Polarogr. (Kyoto)*, **25**, 17 (1979).

17) These simplifications involve the following assumptions; $\alpha_1=\alpha_2=\beta_1=\beta_2=0.5$, $k_\mathrm{s}(1)=k_\mathrm{s}(2)$, $B_\mathrm{O}(1)B_\mathrm{S}(1)=B_\mathrm{S}(2)B_\mathrm{R}(2)$, $a_\mathrm{OO}+a_\mathrm{SO}=a_\mathrm{OS}+a_\mathrm{SS}=a_\mathrm{OR}+a_\mathrm{SR}=a_\mathrm{SO}+a_\mathrm{RO}=a_\mathrm{SS}+a_\mathrm{RS}=a_\mathrm{SR}+a_\mathrm{RR}$, $a_\mathrm{ij}=a_\mathrm{ji}$ (i, j=O, S, or R) where α_i and β_i are the cathodic and anodic transfer coefficients of the *i*-th charge transfer step, respectively, $k_s(\mathrm{i})$ is the standard rate constant of the *i*-th charge transfer step, $B_\mathrm{i}(1)$ and $B_\mathrm{i}(2)$ are the constants representing the adsorption free energy of species i on the electrode surface at $E=E_\mathrm{o1}$ and $E=E_\mathrm{o2}$, respectively, E_o1 and E_o2 the standard redox potentials of the redox couples O/S and S/R supposedly in the bulk of solution, respectively, and a_ij (i, j=O, S, or R) is the Frumkin's *a*-parameter of the interaction between the adsorbed reactions i and j.

18) T. Kakutani and M. Senda, *Bull. Chem. Soc. Jpn.*, **52**, 3236 (1979).