Micellar Accelerated Reduction of Ethylenediaminetetraacetatocobaltate(III) by 1-Benzyl-1,4-dihydronicotinamide

Kohichi YAMASHITA, Masahiro CHIBA, Hitoshi ISHIDA, and Katsutoshi OHKUBO*
Department of Applied Chemistry, Faculty of Engineering, Kumamoto University, Kumamoto 860
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The electrostatic and hydrophobic effects of ionic micelles on the reduction of ethylenediaminetetraacetato cobaltate(III) ([Co(edta)]$^-$) by 1-benzyl-1,4-dihydronicotinamide (BNAH) were investigated with dodecyltrimethylammonium chloride (DTAC) and/or sodium dodecyl sulfate (SDS). Cationic DTAC accelerated the reduction of [Co(edta)]$^-$, while anionic SDS retarded the reaction. The micellar effects of DTAC on the present reaction at 30 °C were kinetically analyzed by using the ion-exchange model and the Berezin's approach: the evaluated rate constant, $(1.0\pm0.1—1.1\pm0.4)\times10^{-4}$ mol^{-1} dm^3 s^{-1}, for the micellar-phase reaction was ten times smaller than that, 1.46×10^{-3} mol^{-1} dm^3 s^{-1}, for the aqueous-phase one. This fact was well-explained by the electron-transfer rate constant calculated with the experimentally determined redox potentials of BNAH and [Co(edta)]$^-$. The cationic DTAC micelles, however, resulted in considerably larger value (3.58×10^{-3} mol^{-1} dm^3 s^{-1}) of overall reaction rate constant than that (1.46×10^{-3} mol^{-1} dm^3 s^{-1}) of nonmicellar one, because the apparent concentrations of the reactants in the micellar phase increased remarkably by condensation of BNAH and [Co(edta)]$^-$ to the micelles through the hydrophobic interaction and the electrostatic interaction, respectively.

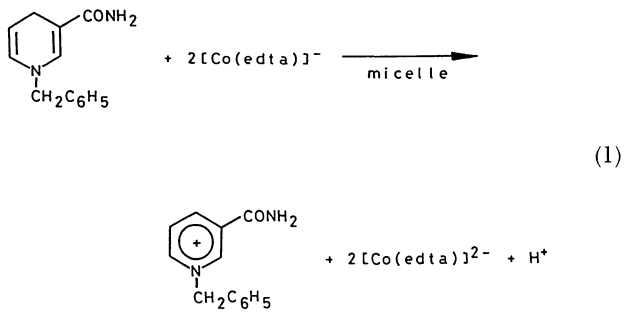
The hydride-transfer reactions from a reduced nicotinamideadenine dinucleotide (NAD(P)H) and its model compounds have been shown to proceed through the sequential electron-proton-electron transfer.[1,2] Since the first electron-transfer step plays a significant role in the reaction of NADH model compounds,[1,2] the reduction of one-electron oxidants such as transition metal complexes by NADH model compounds is useful to evaluate the reactivity of NADH model compounds.[3-9]

Although biochemical processes involving NAD(P)H proceed in a microheterogeneous system composed of an aqueous phase and a lipophilic phase, less attention has been paid to the study of microenvironmental effects on the reaction of NADH model compounds. Since the micellar systems represent a convenient simple model of enzymes to provide important information about the microenvironmental effects on the reaction,[10,11] it is of interest to study the micellar effects on the redox reactions of NADH model compounds.

In a previous paper,[12] we investigated the effects of ionic surfactants on the reduction of hydrophobic and nonionic tris(acetylacetonato)cobalt(III) by 1-benzyl-1,4-dihydronicotinamide (BNAH). The micelles retarded the reaction rates by incorporation of the reactants through the hydrophobic interaction because low polar environments in the micellar phase destabilized the transition states of the reaction.

The reduction of hydrophilic and ionic substrates like ethylenediaminetetraacetatocobaltate(III) ([Co(edta)$^-$] by BNAH might give further information about the micellar effects on the reaction of NADH model compounds, since the ionic micelles can concentrate the ionic substrate by electrostatic interaction.

In this paper, we describe the micellar promoted reduction of [Co(edta)]$^-$ by BNAH in the presence of cationic dodecyltrimethylammonium chloride (DTAC) and anionic sodium dodecyl sulfate (SDS).



(1)

Experimental

The materials and the procedure of reaction were the same as used in the previous work,[12] except that potassium ethylenediaminetetraacetatocobaltate(III) (K[Co(edta)]·2H$_2$O) were prepared according to the literature.[13] The cyclic voltammetry measurements were performed on a Hokuto Denko HA-301 potentiostat/galvanostat by using a glassy carbon working electrode and a saturated calomel reference electrode. The ion-exchange constant for the equilibrium between [Co(edta)]$^-$ and the surfactant counter ion (Cl$^-$) was determined by employing a molecular sieve filtration on Sephadex G-15.[14] A column (diameter=3 cm and bed-height=30 cm) was maintained at 30±0.1 °C with an outer jacket by circulating water. The void volume (V_o) of the packed column was estimated to be 29.0 cm^3 by using Blue Dextran 2000. Before run the column was equilibrated with 1000 cm^3 of 4%(v/v)methanol-borate buffer (pH 9.0, μ=0.02 mol dm^{-3}) containing the surfactants (0—9.0×10^{-2} mol dm^{-3}). The each run was started by the addition of 1.0 cm^3 of 2×10^{-3}mol dm^{-3} K[Co(edta)]·2H$_2$O. Elution with the appropriate eluent was followed at a rate of 1.0 cm^3 min^{-1}. Fractions (0.5—1.0 cm^3) were collected employing an automatic fraction collector and were monitored at 275

nm spectrophotometrically. The elution volume was given by the total volume of the eluent until the fraction gave the maximum absorbance peak of [Co(edta)]$^-$.

Results and Discussion

When BNAH (5.0×10^{-4} mol dm^{-3}) and [Co(edta)]$^-$ (1.0×10^{-3} mol dm^{-3}) were thermally reacted in 4%(v/v)methanol-0.02 mol dm^{-3} H_3BO_3-NaOH buffer (pH 9.0, μ=0.02 mol dm^{-3} with KCl) containing the surfactants of DTAC or SDS (0—6.0×10^{-2} mol dm^{-3}), both absorptions of BNAH (λ_{max}=360 nm) and [Co(edta)]$^-$ (λ_{max}=540 nm) decreased with time. The spectral changes indicated that 2 moles of [Co(edta)]$^-$ were consumed by 1 mole of BNAH. All the present reactions followed by monitoring the absorbance change of [Co(edta)]$^-$ at 540 nm were found to obey the second-order rate law (Eq. 2).

$$-\frac{d[[Co(edta)]^-]}{dt}=2k_{obsd}[BNAH][[Co(edta)]^-]. \quad (2)$$

Therefore, the following mechanism (Eqs. 3a—c) seems appropriate for the reduction of [Co(edta)]$^-$ by BNAH as reported in other systems.[3,12]

$$BNAH+[Co(edta)]^- \longrightarrow BNAH^{\cdot+}+[Co(edta)]^{2-}, \quad (3a)$$

$$BNAH^{\cdot+} \longrightarrow BNA^{\cdot}+H^+, \quad (3b)$$

$$BNA^{\cdot}+[Co(edta)]^- \longrightarrow BNA^+ +[Co(edta)]^{2-}. \quad (3c)$$

Since the reduction potential (E_{red}^0) of [Co(edta)]$^-$ and the oxidation potential (E_{ox}^0) of BNAH in 4%(v/v)methanol-borate buffer were estimated to be -0.53 ± 0.1 and -0.01 ± 0.1 V vs. SCE, respectively, the first electron-transfer reaction (Eq. 3a) is endothermic. The subsequent deprotonation (Eq. 3b) and the second electron-transfer reaction (Eq. 3c) might proceed very quickly as can be seen from the pK_a value of BNAH$^{\cdot+}$ (3.6)[2] and the oxidation potential of BNA$^{\cdot}$ (-1.08 V vs. SCE).[2] Therefore, the first-electron-transfer step (Eq. 3a) may be a rate-determining step, which might suffer micellar effects.

Figure 1 depicts the micellar effects of SDS and DTAC on the second-order rate constant (k_{obsd}) observed for the reduction of [Co(edta)]$^-$ by BNAH under [surfactant]>cmc. The reaction rate was enhanced remarkably by cationic DTAC and was maximized at [DTAC]=4.0×10^{-2} mol dm^{-3}, while anionic SDS retarded the reaction rate. (The broken line in Fig. 1 will be discussed later.)

Since these micellar effects could result from distribution of the reactants between a micellar phase and an aqueous phase,[10,11] the incorporation of BNAH and [Co(edta)]$^-$ into the micelles were evaluated by using the binding constants and the ion-exchange constants, respectively.[15] The binding constants (K_{BNAH}) of BNAH to SDS and DTAC in 4%(v/v)methanol-borate buffer (pH 9.0, μ=0.02 mol dm^{-3}) at 30 °C were determined to be 236±15 and 102±13 mol^{-1} dm^3, respectively, in our previous work.[12]

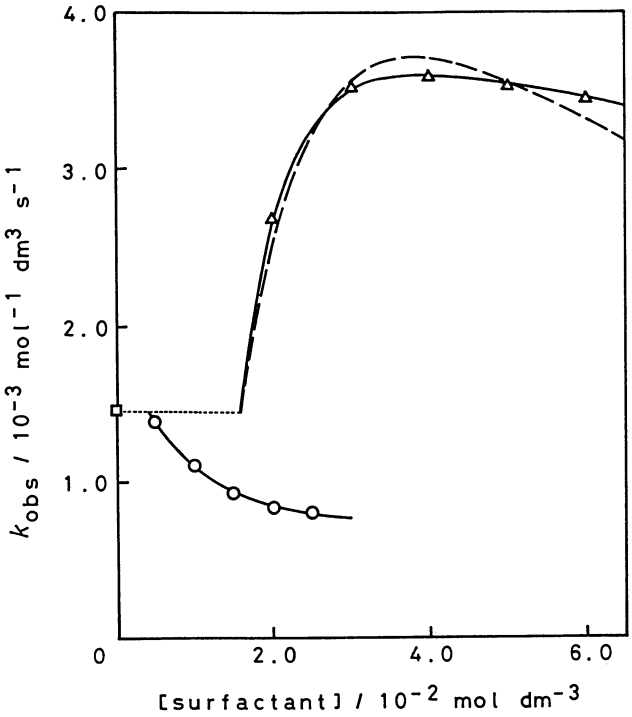


Fig. 1. Micellar effects on second-order rate constants obtained for thermal reduction of [Co(edta)]$^-$ by BNAH with SDS (○) or DTAC (△). The broken line represents the rate constants calculated by Eq. 10.

The ion-exchange constant for the equilibrium between [Co(edta)]$^-$ and the surfactant counter ion was estimated by using a gel filtration chromatography.[14,15] The elution volume (V_e) and the ion-exchange constant (K_1) are related as follows;

$$\frac{1}{V_e-V_o}=\frac{1}{V_i k' K_D}\left(1+K_1\frac{[\overline{Cl^-}]_m}{[\overline{Cl^-}]_w}\right)(1-C_m v), \quad (4)$$

$$k'=(kV_g+V_i)/V_i$$

where V_o and V_i are the void volume and the imbibed (stationary) volume, respectively, V_g is the total volume of a gel matrix, v is the partial molar volume of a surfactant molecule in the micelles, K_D is the molecular sieving constant, C_m, (C_m=[surfactant]—cmc) is the concentration of micelles, k is the constant of proportionality between [Co(edta)]$^-$ absorbed per unit volume of a gel matrix and the equilibrium concentration of [Co(edta)]$^-$ in a liquid phase, [$\overline{Cl^-}$] is the stoichiometric (bulk) concentration of Cl$^-$, and subscripts w and m refer to an aqueous phase and a micellar phase, respectively.

The concentrations of Cl$^-$ in the micellar phase and in the aqueous phase are calculated by the following equations.[15]

$$[\overline{Cl^-}]_m=(1-\alpha)C_m-[\overline{[Co(edta)]^-}]_m-[\overline{H_2BO_3^-}]_m, \quad (5)$$

$$[\overline{Cl^-}]_w=\alpha C_m+\text{cmc}+[\overline{[Co(edta)]^-}]_m+[\overline{H_2BO_3^-}]_m+[\overline{Cl^-}]_{add}, \quad (6)$$

$$K_1=\frac{[\overline{[Co(edta)]^-}]_m\,[\overline{Cl^-}]_w}{[\overline{[Co(edta)]^-}]_w\,[\overline{Cl^-}]_m}, \tag{7}$$

$$K_2=\frac{[\overline{[Co(edta)]^-}]_m\,[\overline{H_2BO_3^-}]_w}{[\overline{[Co(edta)]^-}]_w\,[\overline{H_2BO_3^-}]_m}, \tag{8}$$

$$A\,[\overline{[Co(edta)]^-}]_m^3+B\,[\overline{[Co(edta)]^-}]_m^2+C\,[\overline{[Co(edta)]^-}]_m+D=0, \tag{9}$$

$$A=(1-K_1)(1-K_2),$$

$$B=E(1-K_2)+(K_1[[Co(edta)]^-]_t+[H_2BO_3^-]_t)(1-K_1),$$

$$C=[EK_1+K_1\{[[Co(edta)]^-]_t-(1-\alpha)C_m(1-K_1)\}][[Co(edta)]^-]_t,$$

$$D=-(1-\alpha)C_mK_1K_2[[Co(edta)]^-]_t^2,$$

$$E=\alpha C_m+\mathrm{cmc}+[\overline{Cl^-}]_{add}+K_1[[Co(edta)]^-]_t+(1-\alpha)C_mK_1,$$

where α is the degree of ionization of the micelle, $[\overline{[Co(edta)]^-}]$ and $[\overline{H_2BO_3^-}]$ are the stoichiometric (bulk) concentrations of $[Co(edta)]^-$ and $H_2BO_3^-$, $[\overline{Cl^-}]_{add}$ is the concentration of Cl^- added as KCl, and the subscript t refer to the total concentration. Thus, the $(V_e-V_o)^{-1}$ value is calculated at a given C_m value by using Eqs. 4—9.

When the values of α=0.2 and v=0.256 $mol^{-1}dm^3$ were used,[15,16] the data experimentally obtained for the gel filtration chromatography with DTAC were quite adequately simulated by Eq. 4 with values of K_1=1.2±0.1 and K_2=0.2±0.1 as shown by the broken line in Fig. 2. On the other hand, the $(V_e-V_o)^{-1}$ values decreased with increasing the SDS micellar concentration. The fact indicates that $[Co(edta)]^-$ is not distributed to the SDS micellar phase due to the electrostatic repulsion between $[Co(edta)]^-$ and anionic SDS. Thus, the anionic SDS micelles retarded the reaction rates by suppressing the reaction of BNAH in the micellar phase with $[Co(edta)]^-$ in the aqueous phase. The K_{BNAH}, K_1, and K_2 values are summarized in Table 1.

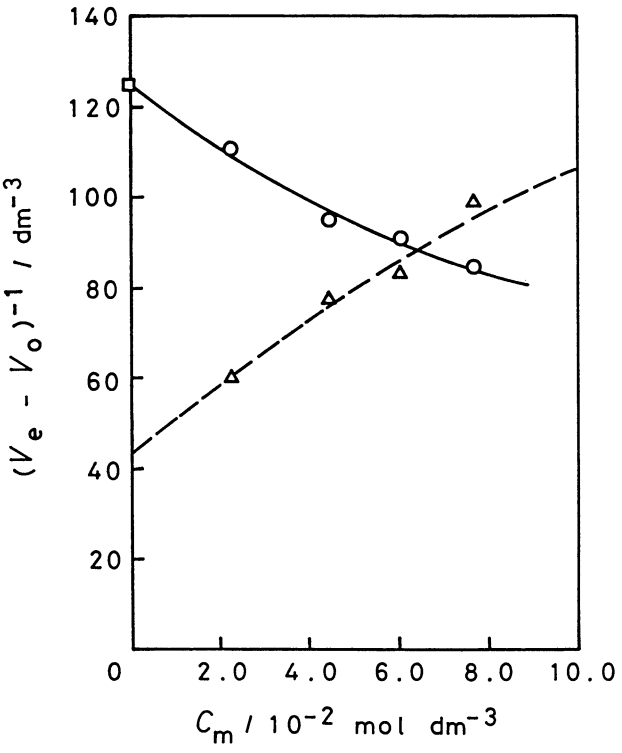


Fig. 2. Plots of $(V_e-V_o)^{-1}$ vs. C_m for elution of $[Co(edta)]^-$ on column of Sephadex G-15 in the presence of SDS (○) or DTAC (△) at 30 °C. The broken line represents the $(V_e-V_o)^{-1}$ values calculated by Eq. 4.

The following reaction scheme can be depicted for the reduction of $[Co(edta)]^-$ by BNAH in the presence of DTAC micelles.[15]

$$\begin{array}{ccc} BNAH_m+[Co(edta)]^-_m & \longrightarrow & \text{products} \\ \uparrow\downarrow K_{BNAH} \qquad \uparrow\downarrow K_1 & & \\ BNAH_w+[Co(edta)]^-_w & \longrightarrow & \text{products} \end{array}$$

According to the ion-exchange model, the observed second-order rate constants (k_{obsd}) can be given by Eq. 10.[15]

$$k_{obsd}=\frac{(k^m/v)K_{BNAH}K_1\dfrac{[\overline{Cl^-}]_m}{[\overline{Cl^-}]_w}+k^w}{(1+K_{BNAH}C_m)\left(1+K_1\dfrac{[\overline{Cl^-}]_m}{[\overline{Cl^-}]_w}\right)}, \tag{10}$$

where k^m and k^w are the rate constants of reactions in the micellar phase and the aqueous phase, respectively. The broken line depicted in Fig. 1 shows the second-order rate constants calculated from Eqs. 5—10 by using the above mentioned values of K_1=1.2±0.1 and K_2=0.2±0.1, and α=0.2. Thus, the rate constants (k^m) of the reaction in the DTAC micellar phase was evaluated to be $(1.0\pm0.1)\times10^{-4}$ $mol^{-1}dm^3s^{-1}$ as listed in Table 1. The k^m value obtained by the ion-exchange model was confirmed by comparing with that ($k^m=(1.1\pm0.4)\times10^{-4}$ $mol^{-1}dm^3s^{-1}$) estimated by using the following equations of Berezin's approach in stead of Eqs. 4 and 10.[17]

Table 1. Binding Constants of BNAH to DTAC and SDS (K_{BNAH}), Ion-Exchange Constants for $[Co(edta)]^-$ (K_1 and K_2), and Second-Order Rate Constants (k^w for the Aqueous Phase and k^m for the DTAC Micellar Phase) in 4%(v/v)Methanol-Borate Buffer (pH 9.0) at 30 °C

Surfactant	K_{BNAH}[a] / $mol^{-1}dm^3$	K_1	K_2	k^w or k^m / 10^{-4} $mol^{-1}dm^3s^{-1}$
None				14.6±0.6
DTAC	102±13	1.2±0.1	0.2±0.1	1.0±0.1
SDS	236±15			

a) Ref. 12.

$$\frac{1}{V_e-V_o}=\frac{K_{Co}}{V_i k' K_D}C_m+\frac{1}{V_i k' K_D}, \quad (11)$$

$$k_{obsd}=\frac{(k^m/v)K_{BNAH}K_{Co}C_m+k^w}{(1+K_{BNAH}C_m)(1+K_{Co}C_m)}, \quad (12)$$

where K_{Co} (=15.3±2.8 mol^{-1}dm^3) is the binding constant of [Co(edta)]$^-$ to the micelles.

The second-order rate constant (k^m) in the DTAC micellar phase was ten times as small as that (k^w=(1.46±0.06)×10^{-3} mol^{-1}dm^{3}s^{-1}) in the aqueous phase. This fact suggests that the reactivities of BNAH and [Co(edta)]$^-$ were affected by the microenvironments in the micellar phase. In this regard, the present micellar system including BNAH was not so effective for the formation of substrate-micelle complex and the activation of the substrate as compared with the native enzymatic system including NADH, because the former excludes the functional parts such as histidine, cysteine, and metal ions.

To elucidate the microenvironmental effects of the micelles on the reactivity of BNAH, the oxidation potential (E_{ox}^0) of BNAH and the reduction potential (E_{red}^0) of [Co(edta)]$^-$ were determined with or without DTAC by cyclic voltammetry measurements.[18] The oxidation and reduction peak potentials (E^p) of BNAH and [Co(edta)]$^-$ are related to the transfer coefficient (β) as Eqs. 13—15 based on Rehm-Weller, Marcus, and Marcus-Levine free energy relationships, respectively.[18]

$$E^p=E^0+\frac{1-2\beta}{[\beta(1-\beta)]^{1/2}}\Delta G_0^{\neq}, \quad (13)$$

$$E^p=E^0+4(1-2\beta)\Delta G_0^{\neq}, \quad (14)$$

$$E^p=E^0+\frac{\ln(\beta^{-1}-1)}{\ln 2}\Delta G_0^{\neq}. \quad (15)$$

The transfer coefficient (β) is calculated from Eq. 16 by using the width of the wave ($|E^p-E^{p/2}|$).

$$\beta=\frac{1.857RT}{F|E^p-E^{p/2}|}. \quad (16)$$

Figure 3 depicted the plots according to Eqs. 13—15 for BNAH oxidation with or without 5.0×10^{-2} mol dm^{-3} DTAC in 4%(v/v)methanol-borate buffer. The E_{ox}^0 values of BNAH in the aqueous solution and micellar solution were estimated to be −0.01±0.1 and 0.14±0.1 V vs. SCE, respectively, from the intercepts of the linear plots.[19] On the other hand, the cyclic voltammetry for [Co(edta)]$^-$ in the presence of DTAC was complicated probably due to the electrostatic interaction between [Co(edta)]$^-$ and DTAC. Therefore, the reduction potentials of [Co(edta)]$^-$ were determined in 4%(v/v) and 30%(v/v)methanol-borate buffer (Fig. 4) because the reaction circumstances in the micellar phase have been reported to be akin to those in solution of 30—50%(v/v)methanol-borate buffer.[12] The E_{red}^0 value (−0.44±0.1 V vs. SCE) obtained for [Co(edta)]$^-$ in 30%(v/v)methanol-borate

Table 2. Oxidation Potentials (E_{ox}^0) of BNAH and Reduction Potentials (E_{red}^0) of [Co(edta)]$^-$ with or without 5.0×10^{-2} mol dm^{-3} DTAC in 4%(v/v)Methanol-Borate Buffer (pH 9.0) at 30 °C

[DTAC]	E_{ox}^0	E_{red}^0
10^{-2} mol dm^{-3}	V vs. SCE	V vs. SCE
0	−0.01±0.1	−0.53±0.1
5.0	0.14±0.1	−0.44±0.1[a]

a) Determined in 30%(v/v)methanol-borate buffer without DTAC.

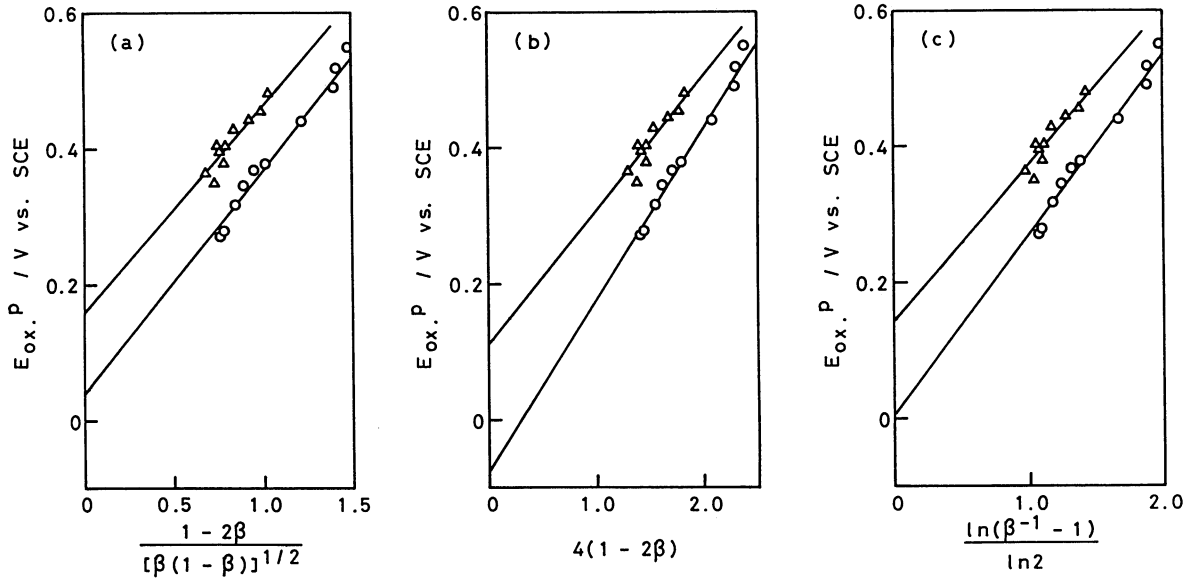


Fig. 3. Plots of E_{ox}^p vs. (a) $(1-2\beta)/[\beta(1-\beta)]^{1/2}$, (b) $4(1-2\beta)$, and (c) $\ln(\beta^{-1}-1)/\ln 2$ for BNAH without surfactants (○) or with 5.0×10^{-2} mol dm^{-3} DTAC (△) in 4% (v/v) methanol-borate buffer.

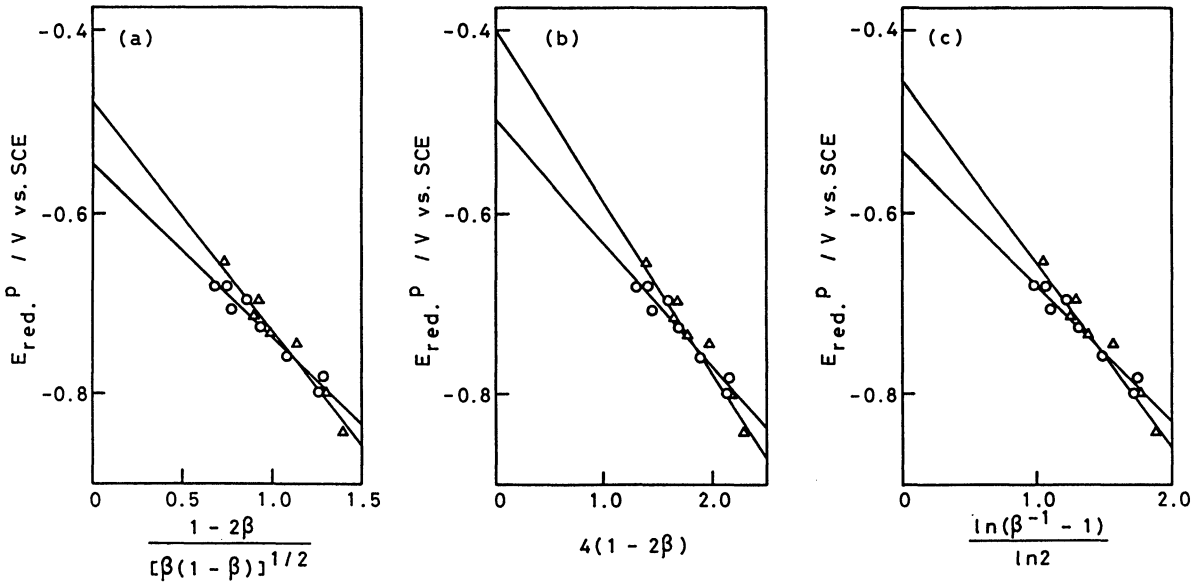


Fig. 4. Plots of E_{red}^{p} vs. (a) $(1-2\beta)/[\beta(1-\beta)]^{1/2}$, (b) $4(1-2\beta)$, and (c) $\ln(\beta^{-1}-1)/\ln 2$ for $[Co(edta)]^-$ in 4% (v/v)- (○) and 30% (v/v) methanol-borate buffer (△).

buffer was slightly positive than that (−0.53±0.1 V) obtained in 4%(v/v)methanol-borate buffer. The E_{ox}^{0} and E_{red}^{0} values were summarized in Table 2.

According to Marcus theory, the rate constant (k_e) of the electron transfer from BNAH to $[Co(edta)]^-$ is related to the E_{red}^{0} and E_{ox}^{0} values by Eq. 17.[21,22]

$$RT \ln k_e = RT \ln K_e k_d + (E_{red}^{0} - E_{ox}^{0}) - (w_p - w_r), \quad (17)$$

where K_e is the equilibrium constant for the formation and the dissociation of the encounter complex ([BNAH $Co(edta)^-$]), k_d is the rate constant for the dissociation of the successor complex ([$BNAH^+$ $Co(edta)^{2-}$]) to $BNAH^{\cdot+}$ and $[Co(edta)]^{2-}$, and w_r and w_p are the work terms required to bring together the reactants and the products, respectively. When the $K_e k_d$ and $(w_p - w_r)$ values were assumed to be constant both in the micellar phase and in the aqueous phase, the k_e value in the micellar phase was calculated to be $k_e = 1.5\times10^{-4}$ mol^{-1}dm^{3}s^{-1} from Eq. 17 by using the above-mentioned E_{red}^{0}, E_{ox}^{0}, and k^w values. Since the rate constant (k_e) estimated for the reaction between BNAH and $[Co(edta)]^-$ in the micellar phase were very close to that ($k^m = (1.0\pm0.1 - 1.1\pm0.4)\times10^{-4}$ mol^{-1}dm^3 s^{-1}) obtained experimentally, the small k^m value in the micellar phase compared with the k^w value in the aqueous phase was attributable to weak reducing ability of BNAH in the micellar phase. That is, low polar microenvironments in the micellar phase are unfavorable for production of ionic species such as $BNAH^{\cdot+}$ and BNA^+. Furthermore, the cationic charge of the DTAC micellar surface suppresses the formation of a positive charge on the nicotinamide ring of BNAH (or BNA·) and the charge separation of produced $[Co(edta)]^{2-}$. Thus, the electron-transfer reaction from BNAH to $[Co(edta)]^-$ was retarded in the DTAC micellar phase. However, the total reaction rate was accelerated by DTAC micelles because $[Co(edta)]^-$ and BNAH were considerably concentrated to the DTAC micellar phase through the electrostatic force and the hydrophobic force, respectively.

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