Effects of Anionic Surfactants on the Hydride-Transfer Reaction of NADH with Methylene Blue: In Premicellar and Micellar Regions

Takeshi Matsumoto, Takamasa Otsuki, Yoshimi Sueishi, and Shunzo Yamamoto*

The Graduate School of Natural Science and Technology, Okayama University,
3-1-1 Tsushimanaka, Okayama 700-8530

Received September 13, 2007; E-mail: yamashun@cc.okayama-u.ac.jp

The kinetics of the hydride-transfer reaction between methylene blue (MB) and reduced nicotinamide–adenine dinucleotide (NADH) were studied in basic buffer solutions containing anionic surfactants (sodium dodecyl sulfate (SDS), sodium decyl sulfate (SDES), and sodium tetradecyl sulfate (STS)) under oxygen-saturated conditions. Initially, the absorbance of MB at 666 nm decreased with SDES concentration, but increased gradually near and above the cmc. The observed first-order rate constant (k_{obsd}) decreased gradually at first, more steeply with increasing SDES concentration and then reached to a small value and became constant. The changes in the absorbance of MB and the observed rate constant were explained by changes in the form of MB in the solution with increasing SDES concentration. The concentration of SDES can be divided into four regions, in connection with the changes in the absorbance and the observed rate constant. The profiles of the changes in the absorbance of MB and the apparent rate constant for SDS and STS with surfactant concentrations were very similar to those for SDES.

Extensive research has been carried out to investigate the effects of surfactants on the electron absorption spectra of many dyes.[1–15] Generally, as the anionic surfactant concentration gradually increases, the absorbance of cationic dyes initially decreases and then gradually increases. At very low surfactant concentrations, far below their cmc, formation of a dye–surfactant aggregate occurs, beginning with the ion pairs ($D^+{\cdot}S^-$) and continuing to a dye–surfactant aggregate represented by $(D^+{\cdot}S^-)_n$. Near and just below the cmc, $(D^+{\cdot}S^-)_n$ aggregates reorganize into premicelles and micelles with a monomeric D^+ content, resulting in an increase in the absorbances of the dyes in this premicellar and micellar region. With a further increase in surfactant concentration, the absorbance reaches its limiting value, and all dye molecules are attached on the surface of normal micelles as monomeric molecules. Recently, we have also observed that the absorbance of methylene blue (MB) at 665 nm at first decreases rapidly with increasing sodium dodecyl sulfate (SDS) concentration but then gradually increases near and above the cmc.[16]

Micellar effects on bimolecular reaction rates are due mainly to the increase or decrease of reactant concentrations in the micellar pseudophase, and the changes in the reaction rate with surfactant concentration can often be explained in these terms.[17] Generally, it is easier to evaluate the partition of hydrophobic reactants between the aqueous phase and micellar pseudophase. In the case of ionic reactants, the Columbic interaction between the ions and the charge of the micellar surface takes on an important role. Micelles can cause acceleration or inhibition of a given reaction relative to the equivalent reaction in aqueous solutions. The influence of micellar systems on chemical reactivity is often analyzed in terms of the pseudophase model.[18–23]

The biological importance of NADH (reduced nicotinamide–adenine dinucleotide) as an electron source has stimulated extensive studies on electron-transfer reactions from NADH and its analogues to various oxidants.[24–32]

Sevcik and Dunford studied the kinetics of the oxidation of NADH by methylene blue under anaerobic and aerobic conditions in closed systems.[33] They obtained a saturation curve by plotting the observed rate constant against $[\mathrm{NADH}]_0$ and proposed mechanisms which included the formation of a 1:1 complex between NADH and MB^+.

In a previous paper,[34] the effects of SDS on the rate of the hydride-transfer reaction between MB and 1-benzyl-1,4-dihydronicotinamide (BNAH) were examined. Since BNAH is insoluble in water, the reaction was studied in 10 vol % ethanol aqueous buffer solution. In 10 vol % ethanol aqueous solution, the absorbance of MB changed only slightly with increasing SDS concentration. This indicates that the formation of a 1:1 MB–SDS ion pair is not important in 10 vol % ethanol aqueous buffer solution. It was found that binding of MB and BNAH to SDS micelles played an important role in the effects of SDS on the reaction rate. On the other hand, NADH is soluble in water and does not associate to the micelles of anionic surfactants. We can examine the effect of the change in the form of MB on the reaction rate in the reaction of MB with NADH in aqueous solution.

In this manner, the spectral changes of the absorption spectra of dyes with increasing surfactant concentration have been well studied, but the change in the reactivity of the dye molecules with the surfactant concentration seems not to be well investigated. In this paper, we study the effects of anionic surfactants on the reaction of MB with NADH. More detailed insight into the origin of the effects of surfactants can be obtained by simultaneous measuring the changes in the absorbance of MB and the apparent first-order rate constant.

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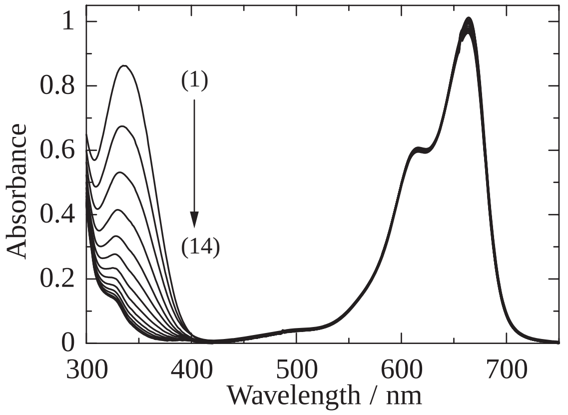


Fig. 1. Repetitive scan of absorption spectrum for the hydride-transfer reaction of MB with NADH in 0.20 mol dm^{-3} NaOH–sodium tetraborate buffer at pH 9.4 and 25 °C in the presence of O_2. $[NADH]_0 = 5.74 \times 10^{-4}$ mol dm^{-3}, $[MB]_0 = 1.25 \times 10^{-5}$ mol dm^{-3}. (1) Taken immediately after mixing, (2)–(14) taken at subsequent 15 min intervals.

Experimental

Materials. MB, surfactants (sodium dodecyl sulfate (SDS), sodium decyl sulfate (SDES), and sodium tetradecyl sulfate (STS)) were of the highest commercially available purity (Wako Pure Chemical Industries) and were used without further purification. NADH was purchased from Wako Pure Chemical Industries, LTD and was used as supplied. In order to reduce the spontaneous decomposition of NADH, basic buffer solutions (0.2 mol dm^{-3} NaOH–sodium tetraborate buffer; pH 9.4) were used for kinetic measurements.[33,35]

Measurements. In this study, the reactions of MB with NADH were followed by recording the decrease in the absorbance of NADH at 336 nm in the presence of O_2 using a Hitachi spectrophotometer (model 2001) at 40.0 °C, because the absorption spectra of MB significantly depends on the concentrations of surfactants. In the presence of O_2 (oxygen-saturated condition), the absorbance of NADH at 336 nm decreased smoothly, while the absorbance of MB at 666 nm decreased a little at first, then became almost constant, even under conditions of an excess of NADH over MB (Fig. 1). Figure 1 shows that the leuco methylene blue (MBH) produced by reduction with NADH was reoxidized by dissolved oxygen and that the steady-state was attained for MB. This suggests that the reaction obeys pseudo-first-order kinetics after the steady-state was reached and that the absorbance at 336 nm could be used to obtain the apparent first-order rate constants.[33,34] All runs gave good first-order plots over two half-lives. The steady-state concentration of MB was assumed to be equal to the initial concentration.

Absorption spectra were recorded on a Shimadzu MultiSpec-1500 photodiode-array spectrophotometer at 25.0 ± 0.1 °C.

Surface tensions of the surfactant solutions in aqueous buffer solutions were measured at 25 and 40 °C by the drop-weight method.[34,36,37] The cmcs of the surfactant solutions were determined by plots of surface tension versus surfactant concentration.[37] To ensure that the instruments worked properly and that the method of determination of the cmc was appropriate, the cmc of SDS in aqueous solutions was determined. The result was 8.2×10^{-3} mol dm^{-3} for SDS, which is in good agreement with a value (8.26×10^{-3} mol dm^{-3} for SDS) reported in the literature.[37] The values of the cmc obtained at 25 °C were 0.65 and 7.2×10^{-3} mol dm^{-3} for SDS and SDES, respectively, and those at 40 °C were 0.25, 1.1, and 10.0×10^{-3} mol dm^{-3} for STS, SDS, and SDES, respectively.

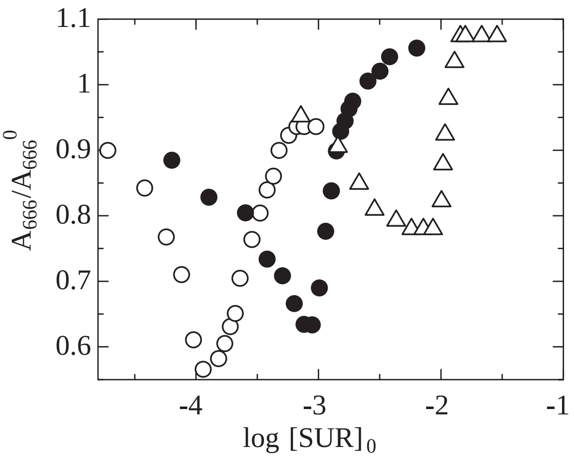
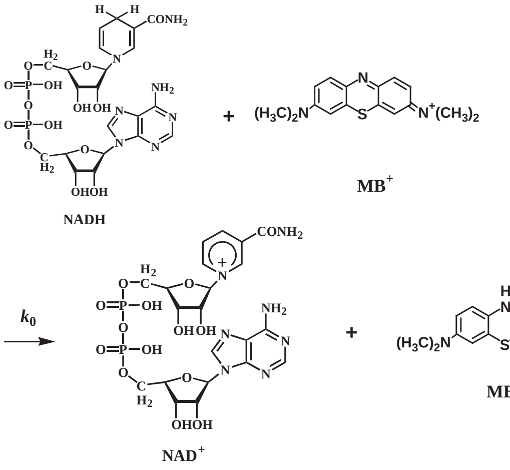


Fig. 2. Effects of STS (○), SDS (●), and SDES (△) on the absorbance of MB at 666 nm.

Results and Discussion

Effects of Surfactants on the Absorption Spectrum of MB. Figure 2 shows the change in the absorbance of MB at 666 nm with the additions of STS, SDS, and SDES. As shown in Fig. 2, the absorbance of MB demonstrated an initial decrease with increasing surfactant concentrations and a gradual increase above 0.16, 0.95, and 7.0×10^{-3} mol dm^{-3} for STS, SDS, and SDES, respectively. The tendencies shown in Fig. 2 are consistent with previous observations for many dye–surfactant systems. As mentioned above, these findings were explained in terms of the formation of ion pairs between MB and an anionic surfactant molecule, the aggregation of the ion pair and the accommodation of monomeric MB molecules into the micelles.

Kinetics of the Oxidation of BNAH by MB in the Presence of O_2. The reaction of NADH with MB was studied in aqueous buffer solutions in the presence of O_2.



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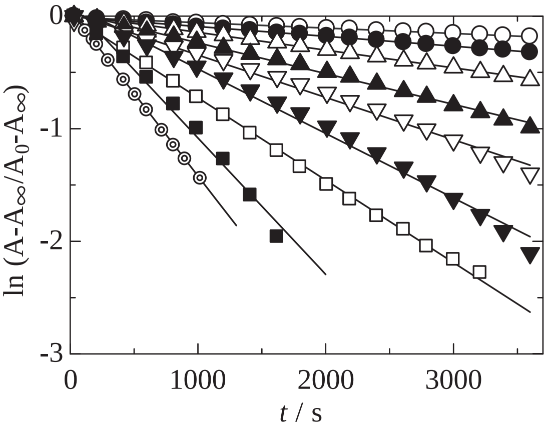

Fig. 3. Pseudo-first-order plots of the reaction of MB with NADH at pH 9.4 in the presence of O_2. $[NADH]_0 = 5.74 \times 10^{-4}\,mol\,dm^{-3}$; $[MB]_0 =$ (○) 0.21, (●) 0.43, (△) 0.86, (▲) 1.49, (▽) 1.89, (▼) 2.67, (□) 5.35, (■) 7.29, and (◎) $10.6 \times 10^{-5}\,mol\,dm^{-3}$.

Figure 1 shows the time dependence of the absorption spectrum for the reaction of NADH with MB in the presence of O_2 (oxygen-saturated condition). The absorption of NADH at 336 nm gradually decreased after mixing a NADH solution with an MB solution ($[NADH]_0 = 5.74 \times 10^{-4}\,mol\,dm^{-3}$, $[MB]_0 = 1.25 \times 10^{-5}\,mol\,dm^{-3}$), while that of MB at 666 nm showed only a slight decrease. Under the conditions that the initial concentrations of MB were less than that of NADH, the absorbance of MB was kept nearly constant while the absorbance of NADH decreased. As mentioned above, the leuco methylene blue (MBH) produced by the reaction with NADH was rapidly re-oxidized by dissolved O_2 and the steady-state for MB was attained. All the runs gave good first-order plots (Fig. 3). The apparent first-order rate constants (k_{obsd}) were obtained at some initial concentrations of MB. A saturation curve was obtained by plotting the k_{obsd}-values against $[MB]_0$. As mentioned above, Sevcik and Dunford also obtained a saturation curve between k_{obsd} and $[NADH]_0$ under the conditions of an excess of NADH. The saturation curve shows the existence of a 1:1 complex between reactants in this reaction. However, the plots between k_{obsd} and $[MB]_0$ at concentrations below $2.50 \times 10^{-5}\,mol\,dm^{-3}$ approximately gave a straight line with a small intercept. This intercept showed the rate constant for the spontaneous decomposition of NADH, but it can be neglected in the present study. The equation for k_{obsd} in these regions of MB concentration is given as

$$k_{obsd} = k_0[MB]_0, \tag{2}$$

where k_0 is the rate constant for the reaction (4) shown later ($k_0 = 11.7\,dm^3\,mol^{-1}\,s^{-1}$) and the rate constant of the autodecomposition of NADH was small and can be neglected at pH 9.4. This indicates that at the low concentrations of MB and NADH, the concentration of the complex could be neglected. All measurements of the effects of surfactants on the reaction rate were done in the low-concentration regions.

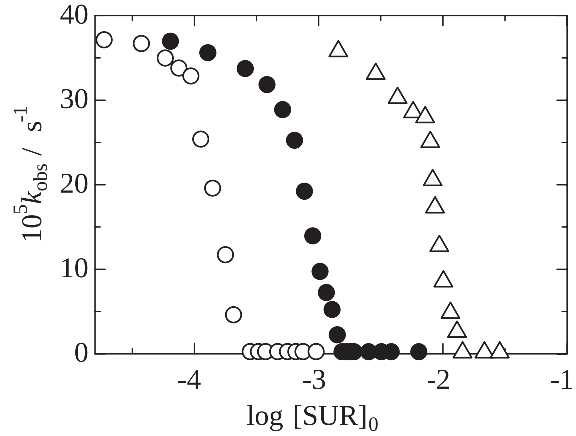

Fig. 4. Effects of STS (○), SDS (●), and SDES (△) on the apparent first-order rate constant for the hydride-transfer reaction of MB with NADH.

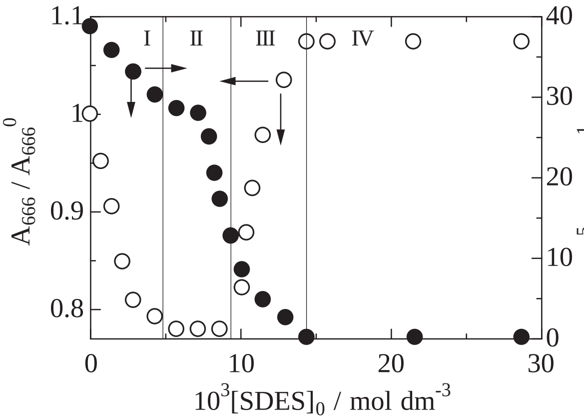

Fig. 5. Effects of SDES on the absorbance of MB (○) and the apparent first-order rate constant for the hydride-transfer reaction of MB with NADH (●).

Figure 4 shows the changes in the observed first-order rate constant (k_{obsd}) for the reaction of NADH with MB^+ on the addition of SDES. As shown in Fig. 4, the rate constant decreased gradually at first, and then more steeply, and finally the rate constant reached its limiting value.

Figure 5 shows the changes in the absorbance of MB and the observed rate constant with the addition of SDES. The concentrations of SDES can be divided into four regions as shown in Fig. 5. In regions I and II (the premicellar region), the absorbance of MB decreases (it seems to be constant in region II) and the k_{obsd}-value also decreases (k_{obsd} decreases more steeply in region II) with increasing SDES concentration. In a previous paper, the effects of SDS on the absorbance of MB were examined, and it was reported that in these regions, ion pairs between MB and SDS and aggregates of the ion pairs are formed with increasing anionic surfactant concentration. In region III (near and above the cmc), MB molecules are accommodated at the surface of the micelles as monomeric molecules. In this region, the absorbance of MB increases with increasing SDES concentration. In region IV, the absorbance of MB is constant, and only monomeric MB molecules attached to the micelles are present.

As mentioned above, the change in the absorbance of MB at low surfactant concentrations must be attributed to the formation of the 1:1 ion pairs (MB–S). Therefore, in this region the following reactions are considered.

$$\mathrm{MB} + \mathrm{S} \rightleftharpoons \mathrm{MB{-}S} \qquad K_1 \qquad (3)$$

$$\mathrm{MB} + \mathrm{NADH} \rightarrow \mathrm{MBH} + \mathrm{NAD} \qquad k_0 \qquad (4)$$

$$\mathrm{MB{-}S} + \mathrm{NADH} \rightarrow \mathrm{MBH} + \mathrm{NAD} + \mathrm{S} \qquad k_1 \qquad (5)$$

where K_1 is the equilibrium constant for the formation of the 1:1 MB–S ion pair and k_0 and k_1 are the rate constants for the reactions of free MB and MB in the ion pair with NADH, respectively.

The absorbance, A, of MB and the observed rate constant ($k_{\rm obsd}$) for the reaction of MB with NADH are given by

$$A = \mathcal{E}_0[\mathrm{MB}] + \mathcal{E}[\mathrm{MB{-}S}] \qquad (6)$$

$$k_{\rm obsd} = k_0[\mathrm{MB}] + k_1[\mathrm{MB{-}S}] \qquad (7)$$

where $\mathcal{E}_0$ and $\mathcal{E}$ are the molar absorption coefficients of free MB and the MB–S ion pair at 666 nm, respectively, and [MB] and [MB–S] are the concentrations of free MB and the ion pair, respectively. Since $[\mathrm{S}]_0 \gg [\mathrm{MB}]_0$, the following equations are obtained

$$K_1 = \frac{[\mathrm{MB{-}S}]}{[\mathrm{MB}][\mathrm{S}]} \qquad (8)$$

$$\frac{[\mathrm{MB{-}S}]}{[\mathrm{MB}]_0} = \frac{K_1[\mathrm{S}]_0}{1 + K_1[\mathrm{S}]_0} \qquad (9)$$

where $[\mathrm{MB}]_0$ and $[\mathrm{S}]_0$ are the initial concentrations of MB and S. From Eqs. 6, 7, and 9, the following equations are derived:

$$\frac{A}{A_0} = 1 - \frac{\mathcal{E}_0 - \mathcal{E}_1}{\mathcal{E}_0}\frac{K_1[\mathrm{S}]_0}{1 + K_1[\mathrm{S}]_0} \qquad (10)$$

$$\frac{k_{\rm obsd}}{k^0_{\rm obsd}} = 1 - \frac{k_0 - k_1}{k_0}\frac{K_1[\mathrm{S}]_0}{1 + K_1[\mathrm{S}]_0} \qquad (11)$$

where A_0 and $k^0_{\rm obsd}$ are the absorbance of MB and the observed rate constant in the absence of surfactants, respevtively.

Figure 6 shows the changes in A/A_0 and $k_{\rm obsd}/k^0_{\rm obsd}$ with increasing SDES concentration. As shown in Fig. 6, A/A_0 and $k_{\rm obsd}/k^0_{\rm obsd}$ decreased with increasing SDES concentration. The solid lines in Fig. 6 show the value calculated by Eqs. 10 and 11 using $K_1 = 95.0\,\mathrm{dm^3\,mol^{-1}}$, $\mathcal{E}/\mathcal{E}_0 = 0.16$, and $k_1/k_0 = 0.26$. As shown in Fig. 6, the agreements between the observed and calculated values for A and $k_{\rm obsd}$ are good below $3.0 \times 10^{-3}\,\mathrm{mol\,dm^{-3}}$. This indicates that the decrease in A and $k_{\rm obsd}$ below $3.0 \times 10^{-3}\,\mathrm{mol\,dm^{-3}}$ are attributed to the formation of 1:1 MB–S ion pairs.

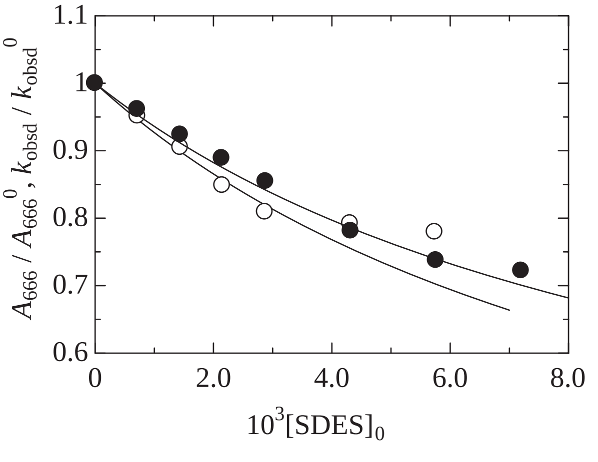

Fig. 6. Plots of $A/A^{\rm o}$ (○) and $k_{\rm obsd}/k^{\rm o}_{\rm obsd}$ (●) against $[\mathrm{SDES}]_0$.

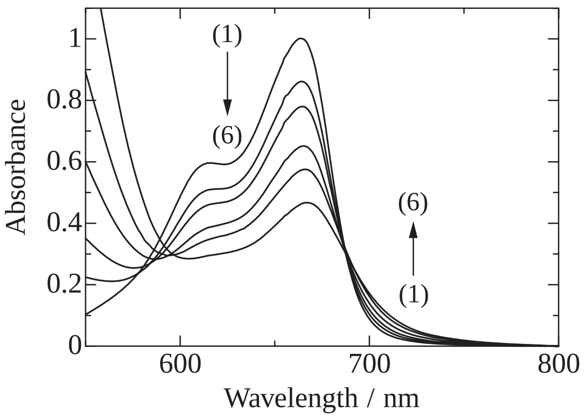

Fig. 7. Absorption spectra of MB ($1.25 \times 10^{-5}\,\mathrm{mol\,dm^{-3}}$) in aqueous buffer solutions containing various concentrations of NpO. $[\mathrm{NpO}]_0$ = (1) 0, (2) 0.85, (3) 1.61, (4) 3.22, (5) 4.83, and (6) $8.05 \times 10^{-5}\,\mathrm{mol\,dm^{-3}}$.

Hamai and Satou[38] reported on the spectral changes of MB with the addition of naphthol orange (NpO) and pointed out that at low NpO concentrations a 1:1 MB–NpO complex is formed, while at high NpO concentrations a 1:2 MB–NpO complex is also formed.

$$\mathrm{MB} + \mathrm{NpO} \rightleftharpoons \mathrm{MB{-}NpO} \qquad K_{\rm m} \qquad (12)$$

$$\mathrm{Mb{-}NpO} + \mathrm{NpO} \rightleftharpoons \mathrm{Mb{-}(NpO)_2} \qquad K_{\rm d} \qquad (13)$$

where $K_{\rm m}$ and $K_{\rm d}$ are the equilibrium constants for the formation of the 1:1 and 1:2 complexes. At low NpO concentrations, the formation of a 1:2 complex can be neglected. Since the 1:1 MB–NpO complex corresponds to the 1:1 MB–S ion pair, we quantitatively examined the effects of the formation of the 1:1 complex on the absorption spectrum of MB and the rate of the reaction of MB with NADH.

Figure 7 shows the absorption spectra of MB solutions containing various concentrations of NpO. The addition of NpO resulted in a slight shift in the absorption peak of MB, accompanied by a reduction in the MB absorption intensity with an isosbestic point, indicating the formation of a complex between MB and NpO. These spectral changes are similar to those reported by Hamai and Satou.

The absorbance, A, of MB in the MB–NpO system is given by

$$\begin{aligned} A &= \mathcal{E}_0[\mathrm{MB}] + \mathcal{E}_{\rm m}[\mathrm{MB{-}NpO}] \\ &= \mathcal{E}_0[\mathrm{MB}]_0 - (\mathcal{E}_0 - \mathcal{E}_{\rm m})[\mathrm{MB{-}NpO}] \end{aligned} \qquad (14)$$

where $\mathcal{E}_0$ and $\mathcal{E}_{\rm m}$ are the molar absorption coefficients of free MB and the MB–NpO complex, respectively, and [MB] and [MB–NpO] are the concentrations of free MB and the complex, respectively.

Figure 8 shows the change in the observed rate constant ($k_{\rm obsd}$) on the addition of NpO. As Figure 8 shows, the rate constant decreases with increasing NpO concentration. In the system of MB–NpO, the following reactions are considered

$$\mathrm{MB} + \mathrm{NADH} \rightarrow \mathrm{MBH} + \mathrm{NAD} \qquad (1)$$

$$\mathrm{MB{-}NpO} + \mathrm{NADH} \rightarrow \mathrm{MBH} + \mathrm{NAD} + \mathrm{NpO} \qquad (15)$$

and the observed rate constant ($k_{\rm obsd}$) is given by

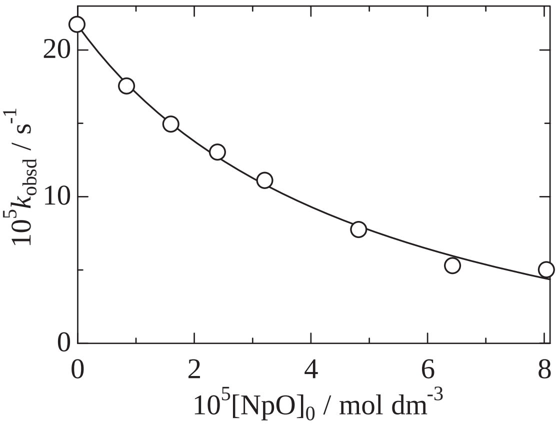

Fig. 8. Effects of NpO on the apparent first-order rate constant for the hydride-transfer reaction of MB with NADH.

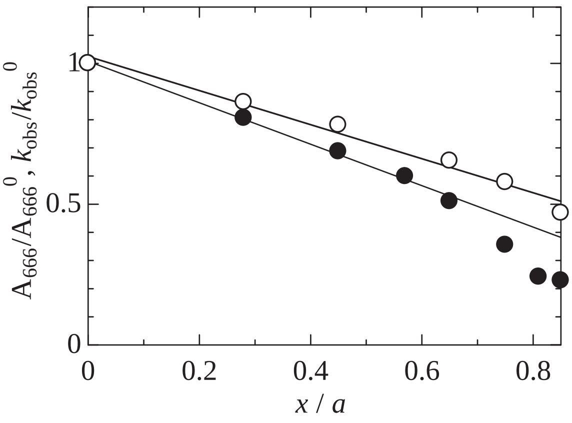

Fig. 9. Plots of $A/A^{\rm o}$ (○) and $k_{\rm obsd}/k^{\rm o}_{\rm obsd}$ (●) against $[{\rm NpO}]_0$.

$$\begin{aligned} k_{\rm obsd} &= k_0[{\rm MB}] + k_{\rm m}[{\rm MB{-}NpO}] \\ &= k_0[{\rm MB}]_0 - (k_0 - k_{\rm m})[{\rm MB{-}NpO}] \end{aligned} \qquad (16)$$

where $k_{\rm m}$ is the rate constant for the reaction of $\rm MB^+$ in the complex with NADH. From Eqs. 14 and 16, the following relations are derived

$$\frac{A}{A_0} = 1 - \frac{\varepsilon_0 - \varepsilon_{\rm m}}{\varepsilon_{\rm m}}\frac{[{\rm MB{-}NpO}]}{[{\rm MB}]_0} \qquad (17)$$

$$\frac{k_{\rm obsd}}{k^0_{\rm obsd}} = 1 - \frac{k_0 - k_{\rm m}}{k_0}\frac{[{\rm MB{-}NpO}]}{[{\rm MB}]_0} \qquad (18)$$

where A_0 and $k^0_{\rm obsd}$ are the absorbance of MB and the observed rate constant in the absence of NpO.

From reaction (12), $K_{\rm m}$ is expressed as follows:

$$K_{\rm m} = \frac{[{\rm MB{-}NpO}]}{([{\rm MB}]_0 - [{\rm MB{-}NpO}])([{\rm NpO}]_0 - [{\rm MB{-}NpO}])} \qquad (19)$$

where $[{\rm MB}]_0$ and $[{\rm NpO}]_0$ are the initial concentrations of MB and NpO, respectively. By using the value of $K_{\rm m}$ ($K_{\rm m} = 79700\,{\rm mol^{-1}}$) reported by Hamai and Satou[38] and the initial concentrations of MB and NpO, the concentration of the complex can be calculated.

Figure 9 shows the plots of A/A_0 and $k_{\rm obsd}/k^0_{\rm obsd}$ against $[{\rm MB{-}NpO}]/[{\rm MB}]_0$ (x/a). As shown in Fig. 9, straight lines are obtained below $[{\rm MB{-}NpO}]/[{\rm MB}]_0 = 0.65$. This indicates that relations (17) and (18) hold in the region of low NpO concentration. The values of $\varepsilon_{\rm m}/\varepsilon_0 = 0.64$ and $k_{\rm m}/k_0 = 0.58$ were obtained.

In this manner, the decreases in the absorbance of MB and the rate constant for the reaction of MB with NADH observed in the low-concentration regions in the MB–SDES and MB–NpO systems can be explained by the formation of the 1:1 ion-pair between $\rm MB^+$ and SDES or the 1:1 complex between MB and NpO.

As mentioned above, the concentration of SDES can be divided into four regions as shown in Fig. 7. In region I shown in Fig. 7, the absorbance of MB decreases and the $k_{\rm obsd}$ value also decreases with increasing SDES concentration. As mentioned above, in this region the 1:1 MB–S ion pair is formed. In region II, the absorbance of MB is almost constant and the $k_{\rm obsd}$ value further decreases. In this region, the aggregates of the ion pair are formed. The absorbance of the aggregate is similar to that of the ion pair. However, the formation of the aggregates further reduces the reactivity of MB. In region III, the absorbance of MB increases, while the $k_{\rm obsd}$ value decreases successively with increasing SDES concentration. In this region, MB molecules are accommodated in the micelles of SDES as monomeric molecules. MB molecules accommodated in the micelles looses almost completely their reactivity toward NADH. Therefore, the $k_{\rm obsd}$ value decreases to the limiting value with increasing the fraction of MB accommodated in the micelles. On the other hand, the absorbance of MB increases and becomes somewhat larger than the original value. In region IV, the absorbance of MB becomes constant and the $k_{\rm obsd}$ value becomes very low (similar to that of the auto-decomposition of NADH). In this region, only monomeric molecules of MB accommodated in the SDES micelles are present.

The reason for the inhibition effects on the hydride-transfer reaction of the formation of the complex with NpO and the ion-pair with SDES may be (1) the decrease in the diffusion of the complex and the ion-pair compared with free MB, and (2) the protection of the complexed methylene blue against the reaction with NADH. These two factors are larger for the association of MB to SDES micelles owing to the bulkiness of the micelle than the formation of the complex and the ion-pair. In region I, the large change in MB absorption spectrum was observed. This shows that the electronic property of MB changes. It is possible that the ability of MB as an electron acceptor decreases and the reactivity of MB toward NADH decreases. In region IV, however, the absorption spectrum of MB is similar to that of a free monomeric MB. This indicates that ability of MB as an electron acceptor does not decrease in this region. Therefore, the decrease in the reactivity of MB in region IV (and perhaps also in region I) is based on the decrease in the diffusion and the steric hindrance.

Figures 10 and 11 show the changes in the absorbance of MB and the observed rate constant with the additions of STS and SDS, respectively. The profiles of the changes shown in Figs. 10 and 11 are very similar to those for SDES shown in Fig. 7. The changes are shifted to lower surfactant concentration in the order STS, SDS, and SDES, corresponding to the order of decrease in the cmc.

As mentioned above, the effects of SDS on the rate of the hydride-transfer reaction between MB and BNAH were stud-

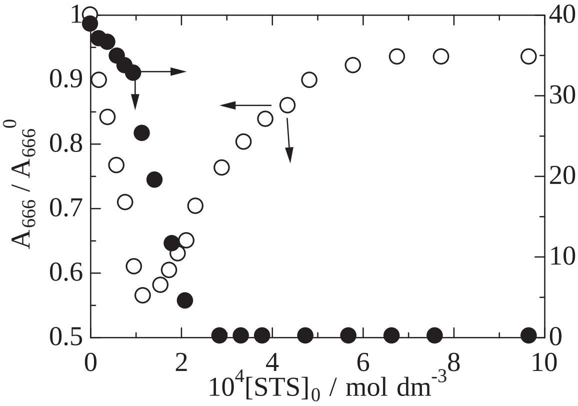

Fig. 10. Effects of STS on the absorbance of MB (○) and the apparent first-order rate constant for the hydride-transfer reaction of MB with NADH (●).

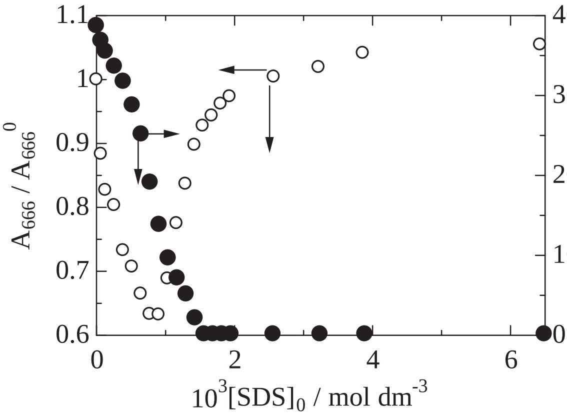

Fig. 11. Effects of SDS on the absorbance of MB (○) and the apparent first-order rate constant for the hydride-transfer reaction of MB with NADH (●).

ied in 10 vol % ethanol aqueous buffer solution. The k_{obsd} value was constant in the low [SDS] concentration (below the cmc), sharply increased at higher [SDS] concentration than the cmc, passed through a maximum value, then decreased at higher surfactant concentrations. In 10 vol % ethanol aqueous solution, the formation of a 1:1 MB–SDS ion pair was not important and the rate did not change in the premicellar region. In the micellar region (above the cmc), binding of BNAH and MB to SDS micelles began at the cmc, hence the concentration of both reactants in the small volume of the micelles explained the sharp increase in k_{obsd}, but the continuous dilution of reactants within the micellar interface with the increase in [SDS] caused a decrease in k_{obsd} (the fraction of BNAH incorporated into the micelles which were not associated with MB at their surface increased with increasing micelle concentration).[34]

In this manner, the incorporation of BNAH into the micelles played an important role in the effect of SDS on the rate for the reaction between MB and BNAH. On the other hand, NADH can not be incorporated into the micelles because of its bulkiness and hydrophilic nature. In the reaction of MB with NADH, the effects of anionic surfactants on the k_{obsd} value were attributed only to the change in the form of MB with increasing surfactant concentration.

Conclusion

The absorbance of MB and the apparent first-order rate constant for the reaction of MB and NADH changed with the addition of anionic surfactants. The absorbance of MB at first decreased rapidly with increasing surfactant concentration but then gradually increased above the cmc. The rate constant decreased gradually at first and then more steeply with increasing surfactant concentration until it reached to a small value and became constant. By simultaneously measuring the changes in the absorbance of MB and the rate constant, the effects of anionic surfactants on the rate of the reaction of MB with NADH can be explained in more detail.

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