Reduction of Methylene Blue with L-Ascorbic Acid or L-Cysteine in Micellar Systems

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The reduction of Methylene Blue (MB) with L-ascorbic acid to the corresponding leuco compound has been studied in aqueous surfactant solutions. The reduction was accelerated in the presence of cationic surfactant hexadecyltrimethylammonium bromide, but tetrabutylammonium bromide, which has no ability of micelle formation, does not show any effects on the reaction rate. The micellar effects are largely affected by pH of the medium, and the rate enhancing effects of the micelles are retarded by KCl. These results could be interpreted by the change in dissociation state of the substrate on the micelle surface and by the binding of the reaction product, leuco MB, to the micelles. The binding site of leuco MB was found to be outer core of the HTAB micelle by NMR. Similar results were obtained in the reduction of MB with L-cysteine, although the reduction did not proceed without the addition of cationic surfactant. The interaction between dissolved oxygen and the substrate in HTAB micellar solutions was also investigated.

Oxidation and reduction are fundamentally important reactions of metabolism in living systems catalyzed by oxidase and reductase. These enzymes are usually composed of two parts, coenzyme and apo-enzyme. Coenzymes are compounds which transfer electrons to substrates, whereas apo-enzymes are spherical proteins which provide the reaction sites. Associates of surfactant molecules are similar to spherical proteins in several respects.[1] (1) The micellar structure has many similar features to spherical proteins; (2) denaturants of proteins also destroy micellar structure; (3) the binding constants of substrates with micelles are in the same order as those with enzymes; (4) kinetics of micellar catalysis obeys the Michaelis-Menten equation which is applied extensively to the enzyme catalysis.

This report describes the results of micellar effects on the reduction of Methylene Blue (MB) with L-ascorbic acid or L-cysteine, and the oxidation of leuco MB with oxygen in the presence of surfactants.

Experimental

Materials. Methylene Blue was recrystallized three times from ethanol. L-Ascorbic acid and L-cysteine of reagent grade were used without further purification. Hexadecyltrimethylammonium bromide (HTAB) and sodium dodecyl sulfate (SDS) were recrystallized three times from ethanol–ether. Poly(oxyethylene) oleyl ether (POOE) was obtained commercially and used without further purification.

Reduction Rate Measurement of Methylene Blue. After mixing an aqueous solution of L-ascorbic acid (1.29×10^{-3} M) with an aqueous solution of MB (1.23×10^{-5} M) containing a certain amount of a surfactant, the decrease in the intensity of absorption peak of MB at 660 nm was followed. The pH of the solution was adjusted with a phosphate buffer, but sometimes sodium hydroxide or hydrochloric acid were used in order to avoid complexity caused by the buffer solution. The pH of reaction mixtures was measured with a Toa HM-5A pH meter. The reactions were carried out in UV cells of 10 or 2 mm path length and the decrease in absorption intensity of MB was recorded with a Union stopped-flow spectrophotometer model RA-1100 with a RA-1085 digital memory unit. For tracing slower reactions, a Union SM-401 spectrophotometer was employed. The reactions of MB with L-cysteine were examined by similar procedures.

Oxidation of Leuco Methylene Blue with Oxygen. Leuco MB was prepared by the electrolytic reduction of MB in 0.01 M hydrochloric acid solution. The solution was diluted with air-saturated water containing a given amount of surfactant. The oxidation rate was determined by measuring the increase in absorbance of MB at 660 nm in a UV cell of 10 mm path length at 25 °C.

Determination of Dynamic Solubilization Site of Leuco Methylene Blue. The ^{1}H NMR spectra were recorded on a Hitachi 60 MHz spectrometer and used for the determination of solubilization site of leuco MB. All spectra were measured on freshly prepared solutions at 35 °C. A 30% solution of tetramethylsilane in deuteriochloroform was used as an external standard.

Results and Discussion

Reduction of Methylene Blue with L-Ascorbic Acid. Under neutral conditions the reduction of MB with L-ascorbic acid is a reversible reaction as shown below and the position of equilibrium lies far to the left.

$$\mathrm{MB} + \mathrm{AsH} \rightleftarrows \mathrm{MBH} + \mathrm{As}$$

In the present investigation an excess amounts of L-ascorbic acid over those of MB were used, and the pseudo first order rate constants k_1 were determined from the relation shown below.

$$-\mathrm{d[MB]}/\mathrm{d}t = k_1[\mathrm{MB}]$$

The effects of the addition of various surfactants on the rate constant k_1 and the apparent equilibrium constant $K=[\mathrm{MBH}]/[\mathrm{MB}]$ were examined.

Figure 1 shows the effects of the addition of surfactants on k_1. The reduction of MB is accelerated by the addition of a cationic surfactant HTAB. The addition of a nonionic surfactant POOE also accelerates the reduction, but the effect is not so large as that of HTAB. On the other hand, an anionic surfactant SDS inhibits the reduction due to the formation of complex salt with MB. Tetrabutylammonium bromide, which is the compound of the same type as HTAB but has no ability of micelle formation, does not show any acceleration effect on the reaction. This indicates that micelle formation is essential for catalytic activity.

The dependence of k_1 on the HTAB concentration near critical micelle concentration (CMC) is shown in Fig. 2. Unlike the ordinary behavior of micellar

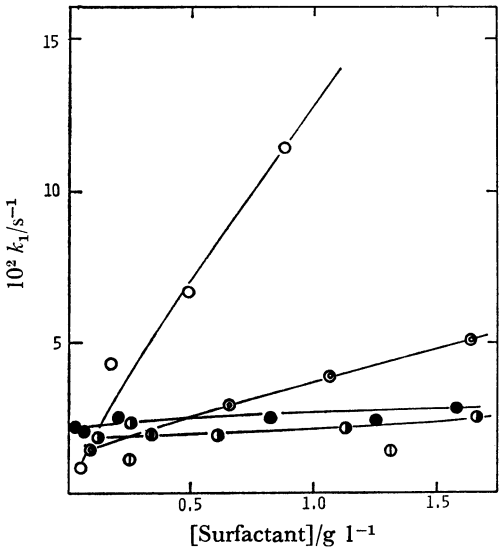

Fig. 1. Rate constant for the reaction of Methylene Blue with L-ascorbic acid as a function of surfactant concentration. Methylene Blue 1.23×10^{-5} M, ascorbic acid 1.29×10^{-3} M, 25 °C, pH 6.80.
○, HTAB; ●, POOE ($n=10$); ◑, POOE ($n=50$); ◎, poly(oxyethylene) *p*-nonylphenyl ether ($n=10$); ⊕, tetrabutylammonium bromide.

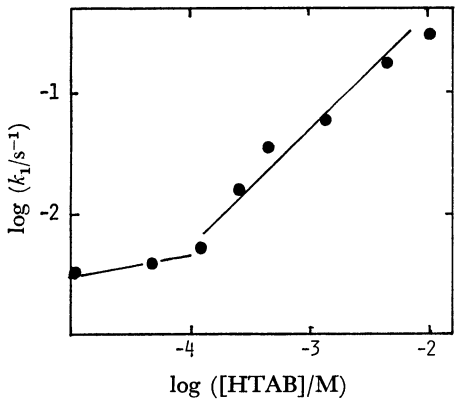

Fig. 2. Dependence of rate constant on HTAB concentration. Methylene Blue 1.23×10^{-5} M, ascorbic acid 1.29×10^{-3} M, 25 °C, pH 6.80.

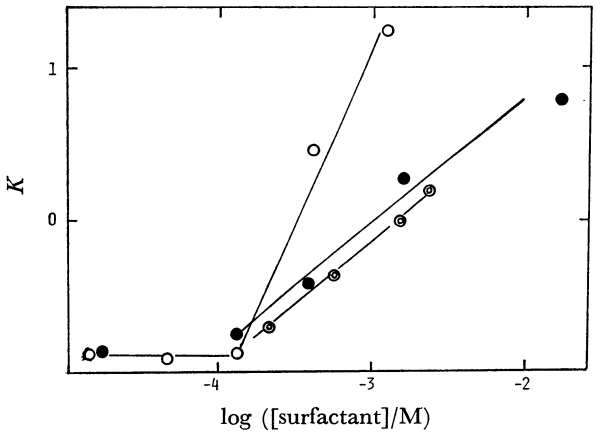

Fig. 3. Dependence of K on surfactant concentration. Methylene Blue 1.23×10^{-5} M, ascorbic acid 1.29×10^{-3} M, 25 °C, pH 6.80.
○, HTAB; ●, POOE ($n=10$); ◑, POOE ($n=50$); ◎, poly(oxyethylene) *p*-nonylphenyl ether ($n=10$).

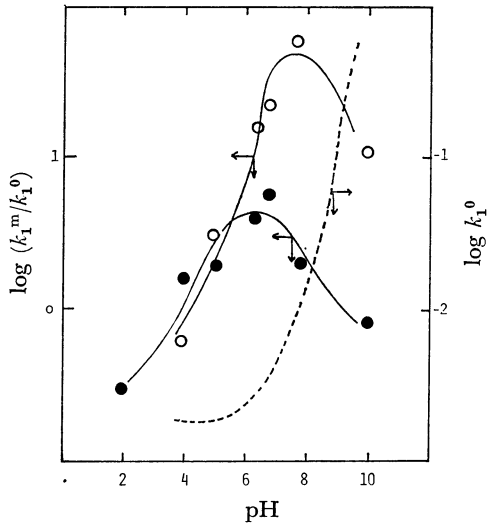

Fig. 4. Relationship between relative rate and pH. k_1^m and k_1^0 are rate constants with and without surfactant, respectively. Methylene Blue 1.23×10^{-5} M, ascorbic acid 1.29×10^{-3} M, 25 °C. ○, HTAB 1.37×10^{-3} M; ●, POOE ($n=10$) 1.97×10^{-3} M.

catalyses,[2] a linear relationship between k_1 and HTAB concentration is observed far above the CMC where the micelle concentration is much higher than those of the substrates. This lack of saturation in the rate profile suggests that the interaction between the cationic micelle and MB is weak, *i.e.* the binding constant is small. This seems reasonable because MB has the same positive charge as HTAB. The association between them is considered to be originated from hydrophobic interaction which competes with electrostatic repulsion between MB and the cationic head groups of the micelle.

Figure 3 shows the effect of surfactants on the apparent equilibrium constant K. HTAB has a large effect on K as well as on k_1, while POOE shows a medium effect on K. As will be discussed later, reduced MB (leuco MB) is most likely solubilized in the HTAB micelle core, and this would reduce the direct contact with oxidizing species leading to the increase in the apparent equilibrium constant.

The dependence of k_1 on pH is shown in Fig. 4. In these experiments, pH of the solutions was adjusted by using dilute aqueous solutions of hydrochloric acid or sodium hydroxide in order to exclude the possible effect of buffer solution.[3] The reduction rate in HTAB or POOE micellar solutions increases with increasing pH as well as in the absence of surfactants over most of the range of pH investigated. This implies that ascorbic acid reacts with MB faster in anionic form than in neutral form. The above observation suggests that the rate acceleration by HTAB is likely to be attributed to the concentration of MB and anionic ascorbic acid on the surface of the micelle where the pH is higher than

bulk water phase.

It should be noted that, as shown in Fig. 4, both HTAB and POOE have inhibitory effects on the reaction below pH 4. Under these acidic conditions ascorbic acid is considered to be neutral and binds easier with the micelles. The separate solubilization of MB and ascorbic acid into the micelles may be the reason for the rate retardation, although it is difficult to draw any definite conclusion from these pH studies.

It was reasonably expected that competitive binding of counter ions on the micellar surface occurs by the addition of inorganic salts, so that the local concentration of anionic substrates on HTAB micelle surface was expected to be reduced. Figure 5 shows the effect of potassium chloride on k_1 in HTAB solutions. The acceleration effect of HTAB is markedly reduced which may be due to the reduction of the binding of the substrate on the micelle surface.

To clarify the origin of nonionic micellar effects on the reduction rate shown above, the effect of solvent polarity was examined. The reactions were carried out in methanol–water mixed solvents. The reaction rate increases with increasing content of methanol (Fig. 6). This implies that less polar media favor the reaction and that the micellar effects of nonionic surfactants arise at least partly from the change of local polarity of the micellar systems.

The Dynamic Solubilization Site of Leuco Methylene Blue. Leuco MB is expected to be solubilized in surfactant micelles for its low solubility in water. NMR studies of surfactant solutions have been carried out in order to determine the dynamic solubilization site of organic compounds.[4] We studied the ^{1}H NMR of aqueous HTAB solutions in the presence of leuco MB in order to determine the solubilization site of leuco MB. Table 1 summarizes the observed chemical shifts and the results on pyrene reported by Grätzel *et al.*[5] Pyrene has been considered to be solubilized in HTAB micelle core causing higher shifts of resonance band of HTAB protons. As seen in Table 1, the protons of methyl groups attached to nitrogen exhibit upfield shifts by the addition of leuco MB which are comparable to that of pyrene of higher concentration. This seems to suggest that leuco MB is located in the outer core and near surface of the micelle.

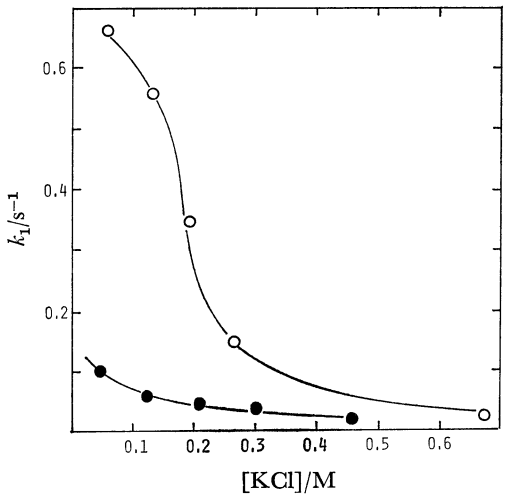

Fig. 5. Effect of KCl on the reduction rate of Methylene Blue with ascorbic acid in surfactant solutions. Methylene Blue 1.23×10^{-5} M, ascorbic acid 1.29×10^{-3} M, 25 °C, pH 6.80. ○, HTAB 8.05×10^{-3} M; ●, POOE ($n=10$) 5.00×10^{-3} M.

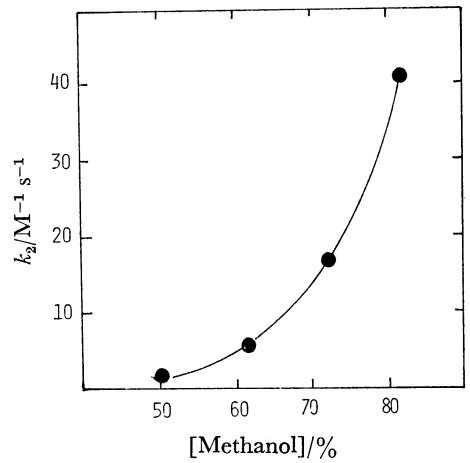

Fig. 6. Effect of methanol content on the reduction rate of Methylene Blue with ascorbic acid at 25 °C. Methylene Blue 8.05×10^{-5} M, ascorbic acid (Na salt) 5.82×10^{-3} M, without buffer.

Table 1. Change in chemical shift of HTAB protons by the addition of solubilizates[a]

Proton	$\Delta\delta$ MB 5.0×10^{-3} M	Leuco MB 1.3×10^{-4} M	Leuco MB 8.2×10^{-4} M	Pyrene[b] 1.0×10^{-2} M
$N(CH_3)_3$	0.00	0.027	0.039	0.033
$(CH_2)_n$	0.00	0.005	0.008	0.016

a) Ppm relative to an external TMS standard. [HTAB]= 0.1 M. $\Delta\delta$=Difference in chemical shifts of HTAB protons with and without solubilizates. MB=Methylene Blue. b) Ref. 5.

Reduction of Methylene Blue with L-*Cysteine.* Similar to the reduction with ascorbic acid, both acceleration by HTAB and inhibition by KCl were observed in the reduction of MB with L-cysteine as shown in Figs. 7

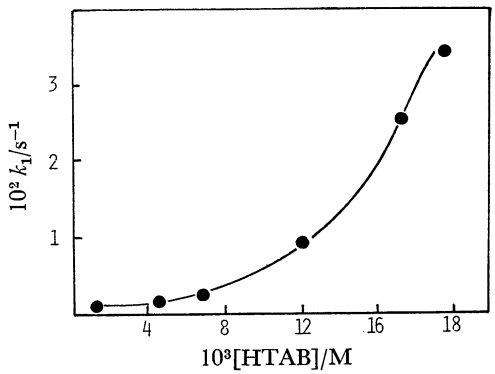

Fig. 7. Effect of HTAB on the reduction rate of Methylene Blue with L-cysteine. Methylene Blue 1.27×10^{-5} M, cysteine 3.12×10^{-3} M, 25 °C, pH 6.80.

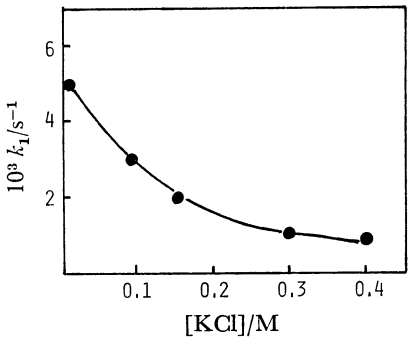

Fig. 8. Effect of KCl on k_1 in aqueous HTAB solutions. Methylene Blue 1.27×10^{-5} M, cysteine 3.12×10^{-3} M, HTAB 9.41×10^{-3} M, 25 °C, pH 6.80.

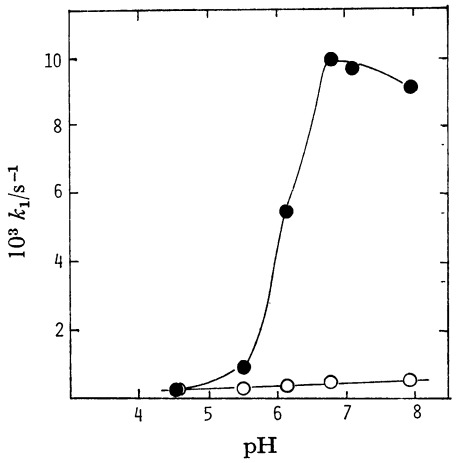

Fig. 9. Effect of pH on the reduction rate of Methylene Blue with L-cysteine at 25 °C. Methylene Blue 1.27×10^{-5} M, cysteine 8.25×10^{-3} M.
○, Without surfactant; ●, HTAB 1.13×10^{-2} M.

and 8. However, quite different results were obtained for the dependence of k_1 on pH as shown in Fig. 9. In HTAB micellar systems k_1 increases with the increase of pH followed by rate saturation above pH 7, whereas k_1 is almost independent of pH in the reaction without surfactants.

In general, there are two distinguished factors govering the micellar effects on organic reactions. One is the local concentration effect of substrates or catalysts at the reaction site, which is caused by electrostatic or hydrophobic interactions between micelles and the substrates. Another is the medium effect on the stability of transition states.[6] Many studies have revealed that the surface of cationic micelles is more basic than bulk water phase. Heitmann[7] suggested that the pK_a value of the SH group of *N*-dodecanoyl-DL-cysteine incorporated in cationic micelles is larger by unity than the value in micelle free solutions.

It is obvious that the results shown in Fig. 9 cannot be explained by pH effect of the cationic micelle surface and other factors must be effective in this case. It should be noted that, contrary to L-cysteine, the solubility of L-cystine in water is very low and in the presence of HTAB micelles it is likely to be solubilized in the micelles. In the present system, a large excess of HTAB micelles would solubilize the reaction products separately for each other, which would suppress the reverse reaction of leuco MB with L-cystine and increase the apparent k_1.

Another factor which should be taken into consideration is the effect of dissolved oxygen. It has been reported that the reduction of MB is inhibited by dissolved oxygen in aqueous solutions.[8] There is a possibility that oxygen participates in the reduction with L-cysteine through the following two processes. One is the inhibition of reduction by L-cysteine, and another is the rapid oxidation of leuco MB. Although we found it difficult to examine the effect of the former process, the latter was examined by the following method. Leuco MB was prepared electrolytically and the effect of HTAB micelle on its oxidation by dissolved oxygen was examined. Since the dissociation state and the micro environment of leuco MB would change by the addition of HTAB as describe above, the oxidation rate by dissolved oxygen was expected to be affected by HTAB. Actually however, the oxidation rate was found to be almost independent of HTAB concentration as seen in Fig. 10. It is interesting to note that different trends for the effect of added KCl on the oxidation rate

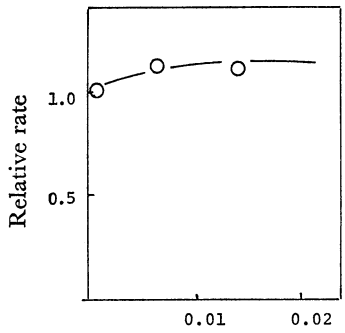

Fig. 10. Effect of HTAB on the relative oxidation rate of leuco Methylene Blue with dissolved oxygen in aqueous solutions.
Leuco Methylene Blue 1.33×10^{-5} M, 25 °C, pH 2.10.

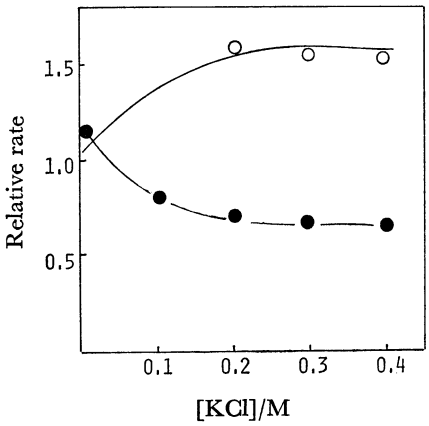

Fig. 11. Effect of KCl on the relative oxidation rate of leuco Methylene Blue with dissolved oxygen in aqueous HTAB solutions.
Leuco Methylene Blue 1.33×10^{-5} M, at 25 °C, pH 6.80. HTAB ○, 5.49×10^{-4} M; ●, 1.42×10^{-2} M.

were observed below and above cmc of HTAB (Fig. 11). The rate is lowered by the addition of KCl above cmc of HTAB, and this implies that cancelling the electric charge on the micelle surface would lead to the inhibition of the oxidation. The above observations tentatively lead to the conclusion that dissolved oxygen has, if any, small effect on the reduction system of MB by L-cysteine.

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