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HYDRIDE TRANSFER REACTIONS OF LEUCO METHYLENE BLUE AND LEUCO THIONINE WITH SOME *p*-BENZOQUINONES

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The kinetics of the oxidation of leuco methylene blue and leuco thionine by some *p*-benzoquinones were investigated spectrophotometrically by means of the stopped-flow technique. Leuco methylene blue and leuco thionine were produced by photoreductions of methylene blue and thionine with triethylamine in ethanol in the drive syringe of the stopped-flow apparatus and their solutions were used as such in the measurement of the oxidation rates. The kinetic isotope effect on the oxidation rate was observed. The results can be explained by the general mechanism with stepwise electron–proton––electron transfers.

INTRODUCTION

There has been considerable interest in the reaction mechanism of hydride transfer from various substrates to π-acceptors.[1–5] The key mechanistic question has been whether the hydride transfer occurs in a direct one-step hydride transfer[6–9] or in stepwise electron–proton–electron (EPE) transfers.[10–13]

Previously, we studied the hydride-equivalent transfer reactions of leuco crystal violet(LCV),[14,15] leuco malachite green (LMG)[16] and leuco Bindschedler's green (BGH)[17] with different *p*-benzoquinones (Qs) and concluded that the reactions can be interpreted in terms of the general EPE mechanism.

Recently, we have studied the hydride transfer reactions of Michler's hydride (MH_2) with different types of π-acceptors.[18] We found that the role of cyanomethylenes as an acceptor is essentially different from that of *p*-benzoquinones, and pointed out that benzoquinones can successively accept an electron and a proton from MH_2 in the solvent cage, whereas cyanomethylenes can accept an electron but not a proton. Therefore, π-acceptors can be divided into two groups.

Since leuco methylene blue (MBH) and leuco thionine (TH) are known to be easily oxidized with dissolved oxygen, kinetic studies on the reactions of these compounds with π-acceptors have not previously been reported. In this study, we have found that the reactions between MBH or TH and some Qs can be followed by producing MBH and TH by means of photoreduction with triethylamine in a closed system and by mixing a solution of MBH (or TH) with those of Qs. This paper reports a kinetic study of the reactions of MBH and TH with some *p*-benzoquinones.

EXPERIMENTAL

Materials. Methylene blue (MB^+Cl^-) was purchased from Wako Pure Chemicals Industries, and was used as received. Thionine (T^+Cl^-) was purchased from Tokyo Kasei Kogyo and recrystalized from ethanol. 2-Methyl-*p*-benzoquinone (MBQ), 2-methoxy-*p*-benzoquinone (MOBQ), 2,5-dimethyl-*p*-benzoquinone (2,5-DMBQ), 2,6-dimethyl-*p*-benzoquinone (2,6-DMBQ), 2,5-di-*tert*-butyl-*p*-benzoquinone (2,5-DBBQ) and tetramethyl-*p*-benzoquinone (TMBQ) were purchased commercially and purified by sublimation. Triethylamine was used as received. Ethanol was dried over molecular sieves 3A-1/16 and distilled. Ethanol-*d* (99·5%) was purchased from Aldrich and used as received.

Kinetic measurements. Ethanolic solution of benzoquinones and of MB^+ (or T^+) and triethylamine were separately charged in drive syringes of the stopped-flow apparatus which were thermostatically regulated within ±0·1 °C. Two solutions were deaerated by bubbling with nitrogen gas before they were charged. The solution of MB^+ was photoreduced by irradiation with visible light supplied by a 650 W tungsten projection lamp and a solution of leuco methylene blue

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(MBH) was produced. The mixing was performed within 1 ms by means of nitrogen gas pressure. After mixing, the change in the absorbance at 655 nm for MB^+ (605 nm for T^+) was monitored by using a Unisoku USP–519 spectrophotometer. The run were repeated 10–30 times and curve smoothing was performed by means of an attached computer. All the runs were performed with excess quinone concentrations.

RESULTS

Oster and Wotherspoon[19] reported that MB^+ and T^+ are photoreduced by visible light in the presence of various reducing agents. Obata and Koizumi[20] also observed the photobleaching of a solution of MB^+ by trimethylamine and identified the reaction products as the leuco dye and amine oxide. Figure 1 shows the spectra of MB^+ taken before and after the photoreduction. Figure 2 shows the spectrum of a reaction mixture between MBH and 2,6-DMBQ. The findings shown in Figures 1 and 2 can be explained by the formation of MBH by photoreduction and the re-production of MB^+ by oxidation with benzoquinones.

A time-dependent curve of the absorption at 655 nm after averaging the repeated runs is shown in Figure 3. The absorbance at 655 nm increased rapidly after mixing of the MBH solution with 2,6-DMBQ solution, whereas it did not increase when MBH solution was mixed with the deaerated solvent. This shows that the oxidation of MBH by oxygen remained in the solvent after deaeration and oxygen which was dissolved on mixing and during the reaction can be neglected. By setting an appropriate time region in the initial stage, the curve was analysed by the Damping Gauses–Newton method combined with the aid of an attached computer program. The apparent first-order rate constants (k_{obsd}) were estimated with several concentrations of Qs at different temperatures.

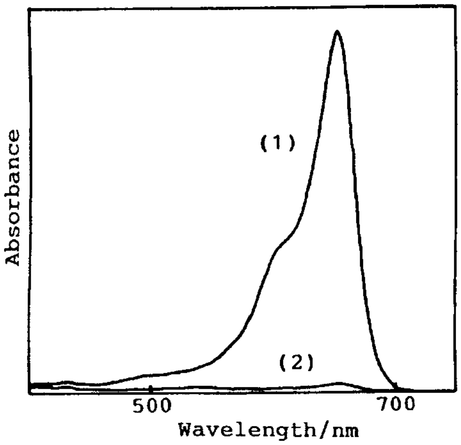

Figure 1. Absorption spectra of MB^+ taken (1) before and (2) after photoreduction

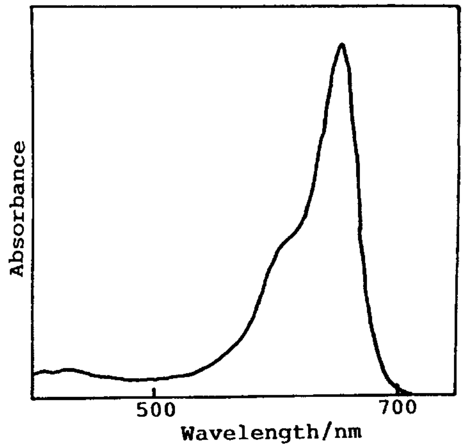

Figure 2. Absorption spectrum of reaction mixture of MBH and 2,6-DMBQ

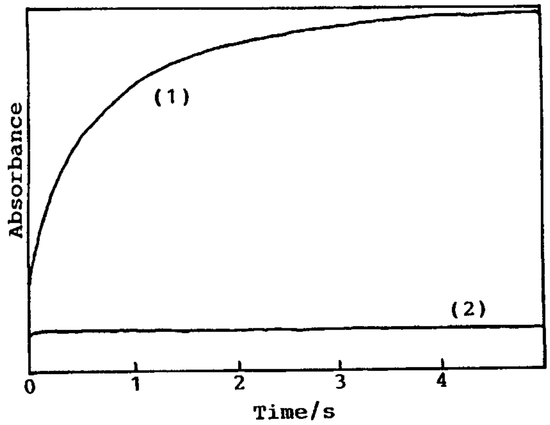

Figure 3. Time dependence of absorbance at 655 nm after mixing (1) MBH solution with 2,6-DMBQ solution and (2) MBH solution with ethanol

Since we used a large excess of TEA as a reducing agent, it is necessary to examine the influence of TEA on the rate of the hydride transfer reaction. Table 1 shows the k_{obsd} value obtained with various amounts of TEA. As can be seen, k_{obsd} is independent of the concentration of TEA. The rate constants (k_{obsd}) for the reaction of TH and some *p*-benzoquinones were also obtained in a similar manner.

Since MBH and TH are very unstable, as mentioned above, we could obtain for the first time the rate constants for the hydride transfer reactions from MBH or TH to *p*-benzoquinones by the method described above.

Table 1. Dependence of the concentration of TEA on the apparent rate constant for the reaction of MBH with 2,6-DMBQ

TEA/mmol dm^{-3}	1·12	2·25	3·37	4·49	5·62
k_{obsd}/s^{-1}	1·46	1·48	1·51	1·49	1·51

DISCUSSION

According to the reaction mechanism widely accepted for the hydride-transfer reaction, the reactants MBH and Q are in rapid equilibrium with a charge-transfer (CT) complex [MBH–Q] which is also in rapid equilibrium with the radical ion pair [MBH$^{+\cdot}$–Q$^{-\cdot}$] formed by the reversible electron transfer from MBH to Q in the CT complex [equation (1)]. This reversible electron transfer is followed by the irreversible proton transfer from MBH$^{+\cdot}$ to Q$^{-\cdot}$ in the radical ion pair and the subsequent electron transfer to form MB$^+$ and QH$^-$ [equation (2)]. These reactions are followed by a fast electron transfer reaction between QH$^-$ and Q [equation (3)] and the subsequent disproportionation of QH [equation (4)].

$$\mathrm{MBH} + \mathrm{Q} \overset{K_1}{\rightleftharpoons} [\mathrm{MBH{-}Q}] \overset{K_2}{\rightleftharpoons} [\mathrm{MBH}^{+\cdot}{-}\mathrm{Q}^{-\cdot}] \quad (1)$$

$$[\mathrm{MBH}^{+\cdot}{-}\mathrm{Q}^{-\cdot}] \xrightarrow{k_f} [\mathrm{MB}^{\cdot}{-}\mathrm{QH}^{\cdot}] \xrightarrow{\text{fast}} \mathrm{MB}^+ + \mathrm{QH}^- \quad (2)$$

$$\mathrm{QH}^- + \mathrm{Q} \xrightarrow{\text{fast}} \mathrm{QH} + \mathrm{Q} \quad (3)$$

$$2\mathrm{QH}^+ \xrightarrow{\text{fast}} \mathrm{QH}_2 + \mathrm{Q} \quad (4)$$

From the above scheme, the rate of formation of MB$^+$ is given by

$$\frac{d[\mathrm{MB}^+]}{dt} = \frac{k_f K[\mathrm{Q}]}{1 + K[\mathrm{Q}]}([\mathrm{MB}^+]_\infty - [\mathrm{MB}^+]) \quad (5)$$

where $K = K_1K_2$. The pseudo-first-order rate constant k_{obsd} in the presence of excess Q may be expressed by

$$k_{obsd} = \frac{k_f K[Q]}{1 + K[\mathrm{Q}]} \quad (6)$$

which can be rearranged to

$$\frac{1}{k_{obsd}} = \frac{1}{k_f} + \frac{1}{k_f\mathrm{K}}\frac{1}{[\mathrm{Q}]} \quad (7)$$

The validity of equation (7) is shown by the plot of $1/k_{obsd}$ against $1/[\mathrm{Q}]$, which gives a linear correlation (Figure 4). The values of k_f and k_fK at several temperatures can be obtained from the intercepts and the slopes in Figure 4. The values of k_f and K for the reaction of MBH with other benzoquinones were obtained in a similar manner. The plots of $1/k_{obsd}$ against $1/[\mathrm{Q}]$ for TH are shown in Figure 5. In this case, the intercepts are very small and could not be determined precisely. Therefore, for TH only the k_fK values was obtained. These values are given in Table 2. From the temperature dependences of k_fK for MBH and TH, the observed activation enthalpy and entropy were determined and are given in Table 2. The values of ΔH^* and ΔS^* for MBH are very similar to those for TH. Fukuzumi *et al.*[13] obtained the activation enthalpy and entropy for the

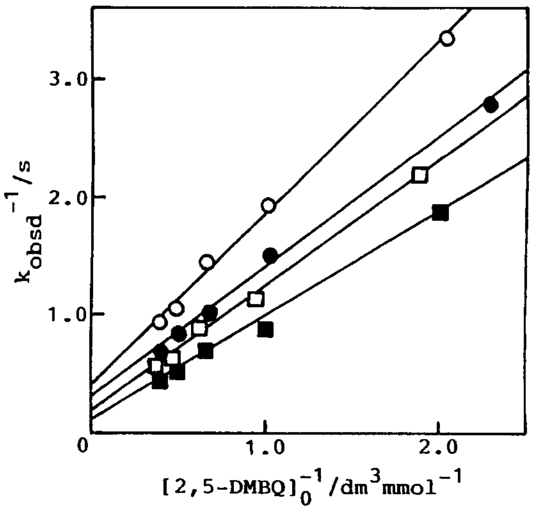

Figure 4. Plots of $1/k_{obsd}$ vs $1/[\mathrm{Q}]_0$ for the reaction of MBH with 2,5-DMBQ at (○) 34·5, (●) 40·5, (□) 44·0 and (■) 50·0 °C

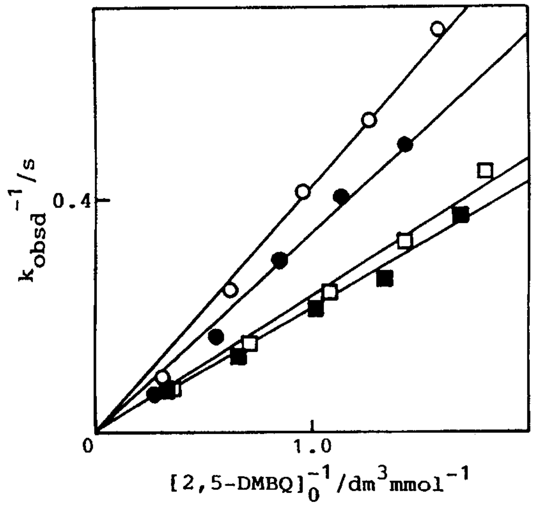

Figure 5. Plots of $1/k_{obsd}$ vs $1/[\mathrm{Q}]_0$ for the reaction of TH with 2,5-DMBQ at (○) 25·0, (●) 35·0, (□) 45·0 and (■) 50·0 °C

Table 2. Rate data and activation parameters for the reactions of MBH and TH with *p*-benzoquinones

Quinones	MBH				TH	
	T/°C	k_fK/dm^3 mol^{-1} s^{-1}	k_f/s^{-1}	K/dm^3 mol^{-1}	T/°C	k_fK/dm^3 mol^{-1} s^{-1}
2,5-DMBQ	34·5	679	2·59	260	25·0	1730
	40·5	846	4·39	192	35·0	2110
	44·0	916	6·30	145	45·0	3170
	50·0	1120	10·6	106	50·0	3670
2,6-DMBQ	26·5	539	5·31	103	25·0	1430
	31·0	742	8·00	92·8	35·0	2000
	40·5	898	16·8	53·5	45·0	2920
	51·0	1500	36·6	40·9	50·0	2920
2,5-DBBQ	26·0	48·4	0·115	424	—	—
	30·5	60·9	0·221	276		
	34·5	65·4	0·249	262		
	40·0	73·0	0·391	187		

	ΔH^*/kJ mol^{-1}	$\Delta S^*_{298\,K}$/J K^{-1} mol^{-1}	ΔH^*/kJ mol^{-1}	$\Delta S^*_{298\,K}$/J K^{-1} mol^{-1}
2,5-DMBQ	22·9	−124	22·3	−109
2,6-DMBQ	27·8	−108	21·6	−112
2,5-DBBQ	19·4	−156	—	—

reaction of BNAH with *p*-chloranil, the mechanism of which is very similar to that for the present system. The values of ΔH^* and ΔS^* are 11 kJ mol^{-1} and −126 J mol^{-1}K^{-1}, respectively. The values of ΔS^* for MBH and TH are very similar to that for BNAH, while the values of ΔH^* for MBH and TH are slightly larger than that for BNAH. The smaller activation enthalpy for BNAH may be responsible for the use of the more reactive benzoquinone.

The k_fK values for the hydride equivalent transfer from MBH or TH to a series of *p*-benzoquinone derivatives in ethanol at 298 K are plotted against the redox potential $E°(Q/Q^{-\cdot})$ in Figure 6. Since the $E°(Q/Q^{-\cdot})$-values for quinones used here in ethanol were not available, we used the values in acetonitrile. Smooth correlations between ln k_fK and $E°(Q/Q^{-\cdot})$ are seen. Similar relationships have been reported for the hydride equivalent transfer from several hydride donors to *p*-benzoquinone derivatives.[13,17,22] Fukuzumi *et al.*[13] showed a smooth correlation between log k_fK for the hydride equivalent transfer reactions from BNAH to a series of *p*-benzoquinone derivatives and $E°(Q/Q^{-\cdot})$. They pointed out that reasonable agreement of the experimental results with the curve drawn by the simulation based on the reaction mechanism shown above by using the free energy relationships for the electron transfer and the proton transfer was obtained. Carlson and Miller[22] studied the kinetics of NADH oxidation by o- and *p*-benzoquinones in buffered aqueous solution and also found a good correlation between log k (second-order rate constant for hydride transfer) and $E^7_{H^-}$ which is the Q/QH$^-$ potential at pH 7, calculated by using E°_H (Q/QH$_2$ potential at pH 0) and pK_a. As mentioned above, we used $E°(Q/Q^{-\cdot})$-values in acetonitrile instead of those in ethanol. Smooth relationships are expected between redox potentials in various media. Indeed, a smooth relationship can be seen for $E^7_{H^-}$ obtained by Carlson and Miller[22] and $E°(Q/Q^{-\cdot})$ in acetonitrile. The correlation seen in Figure 6, therefore, shows the general tendency that the rate constants for

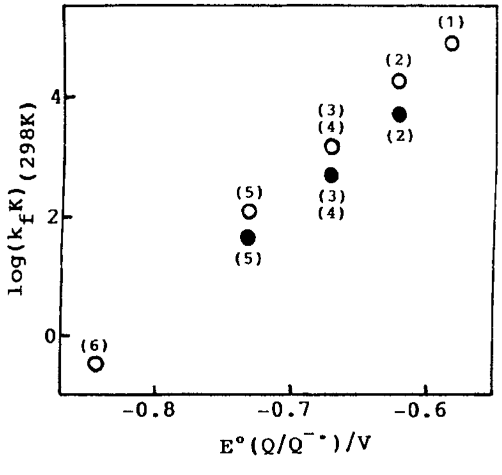

Figure 6. Correlation between log k_fK at 298 K in ethanol and the redox potential of *p*-benzoquinones, $E°(Q/Q^{-\cdot})$ in acetonitrile.[21] (1) MBQ, (2) MOBQ, (3) 2,5-DMBQ; (4) 2,6-DMBQ; (5) 2,5-DBBQ; (6) TMBQ. (●) MBH; (○) TH

Table 3. Isotope effects on rate constant and equilibrium constant for the reaction of MBH and MBD with 2,6-DMBQ

System	k_f^a/s^{-1}	K^a/dm^3 mol^{-1}
MBH–2,6-DMBQ	6·71	94·3
MBD–2,6-DMBQ	3·97	99·6

[a] $k_f^H/k_f^D = 1{\cdot}7$; $K^H/K^D = 0{\cdot}95$.

the hydride transfer reactions decrease with decrease in the redox potentials of quinones.

Since a proton is transferred in a rate-determining step involving an intermediate in equilibrium with reactants in the above mechanism, a kinetic isotope effect is expected. We produced leuco methylene blue-*d* (MBD) by using ethanol-*d* instead to ethanol as solvent and measured the rate of the reaction between MBD and 2,6-DMBQ. In Table 3, the rate data for the reactions of MBH and MBD with 2,6-DMBQ are shown. The ratio of k_f^H/k_f^D is about 1·7, while K^H/K^D is nearly unity. These results are in agreement with the contention that no primary kinetic isotope effect is expected for the formation of the radical ion pair (K), but for the proton transfer from $MBH^{+\cdot}$ to $Q^{-\cdot}$ in the radical ion pair (k_f) a primary isotope effect is expected. Fukuzumi *et al.*[13] observed the isotope effect k_H/k_D in the range 1·5–6·2 and a bell-shaped dependence of the k_H/k_D-values on the $E°(Q/Q^{-\cdot})$-values for the reactions of BNAH with *p*-benzoquinones. For a strong electron acceptor (DDQ), the rate constant k_H was large and the isotope effect observed was small. The isotope effect for k_f observed is not high but is not negligible. This shows that the hydrogen atom which is involved in leuco dye comes from the solvent molecule (from the hydroxy group of ethanol). The small isotope effect also shows that the transition state in the proton transfer in the radical ion pair $[MBH^{+\cdot}–Q^{-\cdot}]$ is strongly reactant-like or product-like.[23]

CONCLUSIONS

We obtained the rate constants for hydride transfer reactions between MBH or TH and some *p*-benzoquinones for the first time by means of a combination of the formation of MBH and TH by photoreduction of methylene blue and thionine and the stopped-flow technique. We have demonstrated that the general mechanism with stepwise electron–proton–electron transfers holds in the present reactions from the results of the dependence of the rate constants on the redox potential of benzoquinones and the kinetic isotope effect.

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