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# FACILE OXIDATION OF THIOPHENOL BY ISOALLOXAZINE(FLAVIN) BOUND TO A CATIONIC MICELLE

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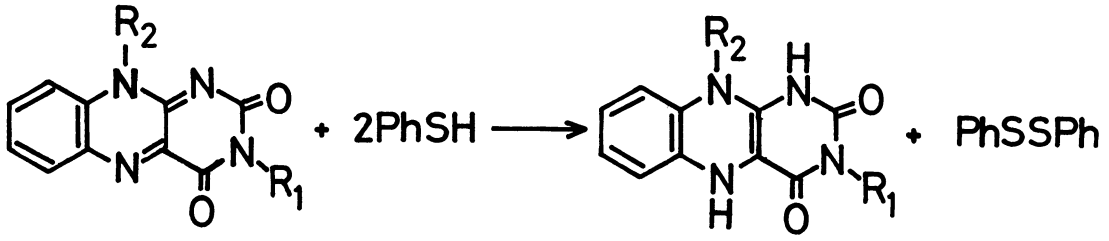
Oxidation of thiophenol by isoalloxazines(flavin analogs) was found to proceed readily in a cationic micelle solution. The observed rate enhancement was related to the activation of the thiophenol anion in the hydrophobic environment of the micelle.

The conversion of thiols to disulfides coupled with reduction of electron-deficient organic agents has been of much concern in connection with the enzymatic oxidation-reduction process.[1,2] Recently, it has been reported that the nucleophilicity of thiol groups bound to cationic hydrophobic aggregates(micelles, polysoaps, etc.) is remarkably enhanced,[3-6] probably due to the formation of desolvated "hydrophobic ion pairs".[7-9] It was also noticed that such thiol groups undergo air oxidation very readily.[5,6] This suggests that the efficiency of biologically important oxidation-reduction reactions involving thiol groups would be improved in the presence of cationic hydrophobic aggregates. The active site of enzymes is situated in relatively hydrophobic regions and the micelle is said to possess the hydrophobic environment similar to enzymes.[10] Therefore, facilitation of oxidation-reduction reactions by the micellar environment may have important bearing with microenvironmental effects in the corresponding enzymatic reactions.

Although aliphatic thiols are slowly oxidized by flavins,[11,12] thiophenol is not oxidized under ambient conditions unless very electron-deficient isoalloxazine, 3,10-dimethyl-8-cyanoisoalloxazine is employed.[13] We report, herein, that isoalloxazines bound to a cationic micelle do oxidize thiophenol to diphenyl disulfide quite readily.

3-Hexadecyl-10-butylisoalloxazine(I), mp 77-80°C, was prepared from hexadecyl iodide and 10-butylisoalloxazine[14] in the presence of $K_2CO_3$, and identified by NMR spectroscopy and elemental analysis. The preparation of 3-methyl-10-ethylisoalloxazine(II) was reported previously.[14]




(I): $R_1 = CH_3(CH_2)_{15}-$, $R_2 = CH_3(CH_2)_3-$

(II): $R_1 = CH_3-$, $R_2 = CH_3CH_2-$

The reactions of isoalloxazines(ca. $4 \times 10^{-5}$M) with thiophenol($10^{-3}$M) were performed in water at 30°C under anaerobic($N_2$) conditions, and were monitored at 440 nm for I and at 433 nm for II. The reduction of II by thiophenol was not detected in nonmicellar systems. In contrast, both isoalloxazines(I and II) underwent facile reduction in the presence of a cationic micelle of hexadecyltrimethylammonium bromide(CTAB: 3 mM). When excess thiophenol was used, final spectra(> 300 nm) of the reaction mixtures were identical with those of reduced isoalloxazines which had been separately prepared by photoreduction with ethylenediaminetetraacetic acid.[15] Introduction of oxygen into the final reaction mixtures regenerated I and II quantitatively, and, in the reaction of II and thiophenol, diphenyl disulfide was detected by high pressure liquid chromatography in 93 % yield based on II. These results clearly indicate that the reaction occurred according to the above scheme in the cationic micellar system. The pseudo-first-order rate constant, $k_{obsd}$, was in the range of $(1 \sim 5) \times 10^{-2}s^{-1}$. Since $k_{obsd}$ in the nonmicellar system is estimated to be less than $10^{-5}s^{-1}$, the cationic micelle can increase the reduction rate by more than three orders of magnitude. This rate difference corresponds to the lowering of the activation energy by at least 4 kcal/mol.

The kinetic study in the micellar system(30°C, $\mu$ = 0.02, 0.02 M phosphate buffer) was carried out under the pseudo-first-order condition($[PhSH]_T$(total thiophenol) $\cong 10^{-3}$M). Plots of $k_{obsd}$ vs. $[PhSH]_T^2$ give straight lines(Fig. 1). Therefore, the reaction is second-order in thiophenol(Eq. 1). At given thiophenol concentrations, plots of log $k_3'$(apparent third-order rate constant; the slope of Fig. 1) vs. pH produced bell-shaped curves(Fig. 2). Thus, Eq. 2 is derived,

$$v_{obsd} = k_3'[PhSH]_T^2\,[\text{isoalloxazine}] \quad (1)$$

$$k_3'[PhSH]_T^2 = k_3[PhSH][PhS^-] = \frac{k_3 K_{app} a_H}{(K_{app} + a_H)^2}[PhSH]_T^2 \quad (2)$$

where $K_{app}$ is the apparent dissociation constant of thiophenol. Curve-fitting of Eq. 2 for the data of Fig. 2 provides the following values: for I + PhSH, $pK_{app}$ = 6.6, $k_3 = 5.78 \times 10^4 M^{-2}s^{-1}$; for II + PhSH, $pK_{app}$ = 6.6, $k_3 = 3.20 \times 10^4 M^{-2}s^{-1}$.

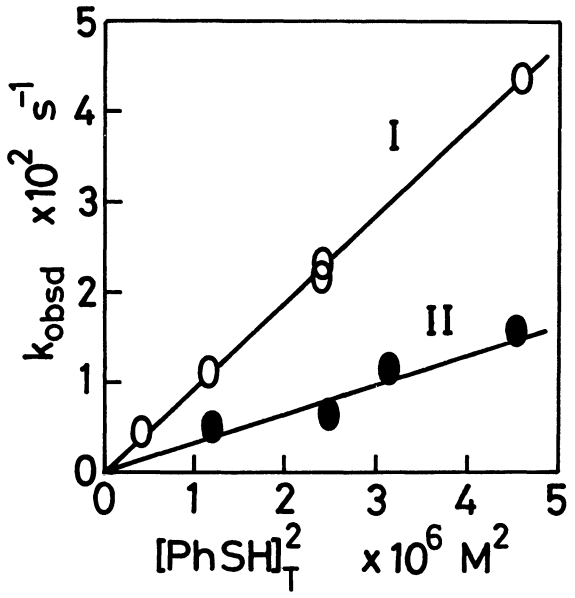



Fig. 1. $k_{obsd}$ vs. $[PhSH]_T^2$
pH 7.0, [CTAB] = 3.0 x $10^{-3}$M, 3 vol% ethanol, [I] = 4.02 x $10^{-5}$M, [II] = 4.28 x $10^{-5}$M.

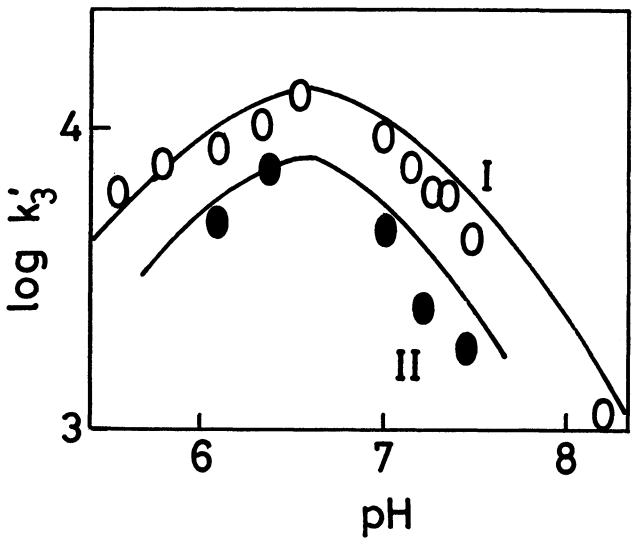



Fig. 2. log $k_3'$ vs. pH
[CTAB] = 3.0 x $10^{-3}$M, $[PhSH]_T$= 1.06 x $10^{-3}$M, 3 vol% ethanol, [I] = 4.02 x $10^{-5}$M, [II] = 4.28 x $10^{-5}$M.

The reaction rates for both isoalloxazines were suppressed with increasing KCl and phosphate buffer concentrations(for the reaction of thiophenol and II, $k_3'$ at 0.2 M phosphate/ $k_3'$ at 0.02 M phosphate = 0.12). Probably, thiophenol oxidation by isoalloxazines would not be subject to general catalysis.

This kinetic situation is in complete accord with that reported by Yokoe and Bruice[13] for the reaction of 3,10-dimethyl-8-cyanoisoalloxazine and thiophenol in a nonmicellar system. The reaction mechanisms proposed by these authors involve the rate-limiting nucleophilic attack of the thiophenoxide anion. Since the nucleophilicity of the thiolate anion is known to be enhanced in cationic micellar system,[16] the large enhancement of the reduction rate observed in the present study may also be explained by the enhanced nucleophilicity of the thiophenoxide anion. The kinetically determined $pK_{app}$ value(= 6.6) is very close to $pK_a$ of thiophenol(= 6.5) obtained by spectrophotometric titration in the presence of 3 mM CTAB. These values are 0.7 pK unit lower than that estimated kinetically in a nonmicellar system.[13]

We recently found that oxidation of the nitroethane anion by flavins, which is usually not possible in the nonenzymatic system, does take place with I in the CTAB micelle.[17] However, the reaction did not proceed with II(less hydro-

phobic isoalloxazine) under comparable conditions. We concluded from the spectroscopic evidence that binding of the isoalloxazine ring at the micelle interior promoted the oxidation reaction. The nitroethane anion may be loosely associated with the micelle by electrostatic interaction. On the other hand, the rate-CTAB concentration profile for the reaction of II and thiophenol shows the association constant between the CTAB micelle and the thiophenoxide ion to be considerably large(ca. $3 \times 10^3$ $M^{-1}$). This is consistent with the lowering of $pK_{app}$(0.7 pK unit) in the micellar system relative to the nonmicellar system.

In conclusion, the present study established that the micellar environment is crucial for the oxidation of thiophenol by conventional flavins. Preliminary experiments similarly indicate that the reactions of 2-mercaptoethanol and 1,4-butanedithiol with flavins are remarkably accelerated by cationic micelles.

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References

1) N.S. Kosower, G.A. Vanderhoff, E.M. Kosower, and P.K.C. Huang, Biochem. Biophys. Res. Commun., 20, 469(1965).
2) F. Yoneda, K. Suzuki, and Y. Nitta, J. Org. Chem., 32, 729(1967).
3) P. Heitmann, Eur. J. Biochem., 5, 305(1968).
4) W. Tagaki, T. Amada, Y. Yamashita, and Y. Yano, J. Chem. Soc., Chem. Commun., 1131(1972).
5) S. Shinkai and T. Kunitake, Makromol. Chem., in press.
6) S. Shinkai and T. Kunitake, Bull. Chem. Soc. Japan, 49, 3219(1976).
7) T. Kunitake, S. Shinkai, and Y. Okahata, Bull. Chem. Soc. Japan, 49, 540(1976).
8) S. Shinkai and T. Kunitake, Chem. Lett., 109(1976).
9) S. Shinkai and T. Kunitake, J. Chem. Soc., Perkin II, 980(1976).
10) E.H. Cordes and C. Gitler, Progr. Bioorg. Chem., 2, 1(1973).
11) M.J. Gibian and D.V. Winkelman, Tetrahedron Lett., 3901(1969).
12) E.L. Loechler and T.C. Hollocher, J. Am. Chem. Soc., 97, 3236(1975).
13) I. Yokoe and T.C. Bruice, J. Am. Chem. Soc., 97, 450(1975).
14) F. Yoneda, Y. Sakuma, M. Ichiba, and K. Shinomura, J. Am. Chem. Soc., 98, 830 (1976).
15) G. Blankenhorn, S. Ghisla, and P. Hemmerich, Z. Naturforsch. 27 b, 1038(1972).
16) H. Chaimovich, A. Blanco, L. Chayet, L.M. Costa, P.M. Monteiro, C.A. Bunton, and C. Paik, Tetrahedron, 31, 1139(1975).
17) S. Shinkai, Y. Sakuma, and F. Yoneda, J. Chem. Soc., Chem. Commun., in press.

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