Catalyses by Polymer Complexes. Part 3.[†] Polymer Micellar Catalysis of Isoalloxazine (Flavin) Oxidation of Thiols

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The effect of cationic polymer micelles on the isoalloxazine (flavin) oxidation of thiols is reported: the polymers employed were poly-(2-ethyl-1-vinylimidazole) quaternized with ethyl bromide and lauryl bromide [lauryl group content: 8.8 mol % (L-9), 28.9 mol % (L-29), and 40.9 mol % (L-41)]. Addition of L-29 and L-41 polymers caused a red shift of the u.v. absorption maximum of thiophenoxide ion and an increase in its acid dissociation constant, while L-9 polymer scarcely affected these values. Under anaerobic conditions, the oxidation of thiophenol and 2-mercaptoethanol by 10-ethyl-3-methylisoalloxazine in the presence of micelle-like polymers (L-29 and L-41) was 10^2—10^5 times faster than the corresponding reaction in a non-polymeric system, whereas L-9, a polyelectrolyte-like polymer, produced almost no acceleration. The thiolate anion bound to the polymer domain is activated probably due to the formation of a hydrophobic ion pair. On the other hand, the oxidation of butane-1,4-dithiol was not much affected by the polymer micelle. The difference in mechanism of dithiol oxidation is discussed in connection with the microenvironmental effect.

THE reaction environment produced by microheterogenous systems is of great interest in relation to enzymic catalysis.[1,2] Particularly, coenzyme catalysis depends very much upon the microenvironment of apoenzymes. We recently found that the reactivity of some aqueous coenzymes and their analogues is remarkably affected by the microenvironment of synthetic hydrophobic aggregates and polymers, and emphasized the crucial role of the hydrophobic domain in some coenzyme-dependent reactions.[3-7] Since the coenzyme binding site of apoenzymes is said to be situated in relatively hydrophobic regions,[8] inspection of the environmental effect in the model system would have a useful bearing upon understanding of the coenzyme–apoenzyme interaction.

The conversion of thiol to disulphide coupled with reduction of flavin is interesting as a model reaction for the catalysis of vitamin B_2 (riboflavin) requiring proteins. Several model studies established that this oxidation–reduction reaction with aliphatic (not aromatic) mono- and di-thiols is possible even in the absence of apoenzymes. These investigations, however, have been limited to simple aqueous systems, and the environmental effect on the reaction has never been assessed.

Recently, it has been found that the flavin oxidation of thiophenol to diphenyl disulphide, usually not possible in nonenzymatic systems, does take place with an isoalloxazine bound to a cationic micelle.[7] The result suggests that the micellar hydrophobic environment is crucial for the flavin-mediated oxidation–reduction reaction. In an attempt to establish the relationship between the efficiency of this reaction and the hydrophobic environment, we examined the reaction of thiophenol, 2-mercaptoethanol, and butane-1,4-dithiol with a flavin compound, 10-ethyl-3-methylisoalloxazine (1) in the presence of polymer micelles. Polymer micelles can provide the structural characteristics of both conventional polyelectrolytes and mi-

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[†] Part 2, S. Shinkai and T. Kunitake, *Makromol. Chem.*, 1977, **178**, 1613.

[‡] The abbreviation L-9 stands for polymer containing 9 mol % laurylated imidazole unit.

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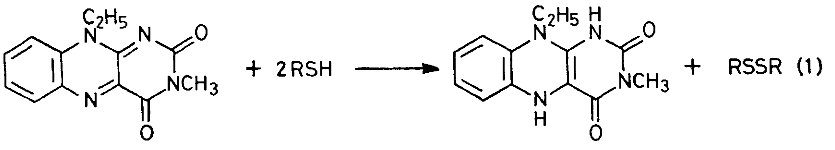
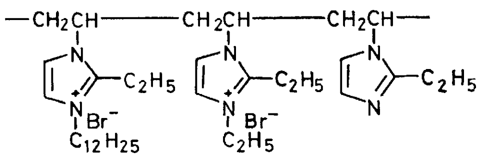
celles,[9-12] and the hydrophobicity is readily adjustable through the content of the pendent aliphatic groups. Therefore, this is one of the most convenient model systems for investigating the effect of the environment on the coenzyme reactivity.



(1)

Polymers:*



EXPERIMENTAL

Materials.—10-Ethyl-3-methylisoalloxazine (1) was a gift from Professor F. Yoneda (for preparation see ref. 13). Thiols were distilled under nitrogen before use. Thiophenol had b.p. 70—72 °C at 29 mmHg (lit.,[14] 169 °C), 2-mercaptoethanol b.p. 154—156 °C (lit.,[14] 157 °C), and butane-1,4-dithiol b.p. 98—102 °C at 17 mmHg (lit.,[15] 74.5 °C at 10 mmHg). Preparations of polymer micelles from poly-(1-vinyl-2-ethylimidazole) (DP 73) have been described.[12] The composition of the polymer micelles is recorded in Table 1.

TABLE 1

Composition of polymers

Polymer	Lauryl group content (mol %)	Ethyl group content (mol %)
L-9	8.8	83
L-29	28.9	67
L-41	40.9	49

Titration of Thiols.—The spectrophotometric titration was carried out at 30 °C under anaerobic(N_2) conditions by using a modified Thurnberg cuvette. The wavelengths employed are 270 nm for thiophenol and 240 nm for butane-1,4-dithiol.

Kinetics.—All kinetic measurements were carried out anaerobically at 30 ± 0.1 °C at a calculated ionic strength (μ 0.02 with KCl) unless otherwise stated. A modified Thunberg cuvette was employed to achieve the anaerobic conditions. In all cases excess of thiols was present, so that pseudo-first-order rate behaviour was observed up to three half-lives. The detailed procedure has been described elsewhere.[5]

RESULTS

Spectra of Polymer-bound Thiophenol Anion.—Prior to the kinetic study, the effect of aqueous polymer micelles upon the u.v. spectra of thiophenoxide ion was examined. Thiophenoxide ion possesses an absorption maximum at 262 nm (ε 12 300 l mol^{-1} cm^{-1}) in an aqueous system which shifts considerably to longer wavelengths in dipolar aprotic solvents (*e.g.* 308 nm in dimethylformamide and 285—299 nm in tetrahydrofuran).[16] It was expected, therefore, that the u.v. spectrum of thiophenoxide ion would reflect the medium polarity.[17]

Addition of L-9 (5×10^{-3} unit M), a polyelectrolyte-like polymer, had little effect on the spectrum of thiophenoxide ion, but there were marked spectral changes upon addition of L-29 and L-41 micelle-like polymers (Table 2). The $\lambda_{max.}$ value plotted against the concentration of L-29 gave a saturation curve with a maximal shift of 274 nm. This corresponds to the red shift of 12 nm from the typical aqueous value. A similar red shift of thiophenoxide ion has been noticed in the presence of conventional cationic surfactants at concentrations above the critical micelle concentration (c.m.c.).[17] The $\lambda_{max.}$ value observed at [hexadecyltrimethylammonium bromide] 1.5mM was 271 nm, and thiophenoxide ion was considered to be incorporated wholly into the micelle phase at a surfactant concentration greater than this. These results suggest that micelle-like polymers (L-29 and L-41) interact strongly with thiophenoxide ion, and that the medium polarity therein is considerably lowered.

TABLE 2

U.v. spectra of thiophenoxide ion in the presence of polymer micelles at 30 °C [a]

Polymer	10^3 Concentration (unit M)	$\lambda_{max.}$/nm	$\varepsilon_{max.}$/ l mol^{-1} cm^{-1}
None		262	12 300
L-9	5.00	263	12 300
L-29	1.21	265	11 000
L-29	2.12	266	9 600
L-29	3.03	268	9 300
L-29	5.00	269	9 200
L-29	6.06	270	9 000
L-29	8.00	271	8 800
L-41	5.00	268	9 360

[a] [PhS$^-$] 1.16×10^{-4}M, μ 0.02 with KCl, pH 10.0.

Spectrophotometric Titrations.—The acid dissociation constants (K_a) for thiophenol and butane-1,4-dithiol were determined in the absence and presence of L-9 and L-29. Absorption maxima at 263 and 240 nm were employed for

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[10] T. Rudolfo, J. A. Hamilton, and E. H. Cordes, *J. Org. Chem.*, 1974, **39**, 2281.

[11] T. Okubo and N. Ise, *J. Org. Chem.*, 1973, **38**, 3120.

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[15] W. P. Hall and E. E. Reid, *J. Amer. Chem. Soc.*, 1943, **65**, 1466.

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thiophenol and butane-1,4-dithiol, respectively. Titration of butane-1,4-dithiol at pH 9.0—11.3 resulted in only one inflection. The molar absorption coefficient observed (3 800 l mol^{-1} cm^{-1}) is close to those of aliphatic thiolate anions (4 000—6 000 l mol^{-1} cm^{-1}).[18] Therefore, the observed inflection will correspond to the first dissociation of the two SH groups.*

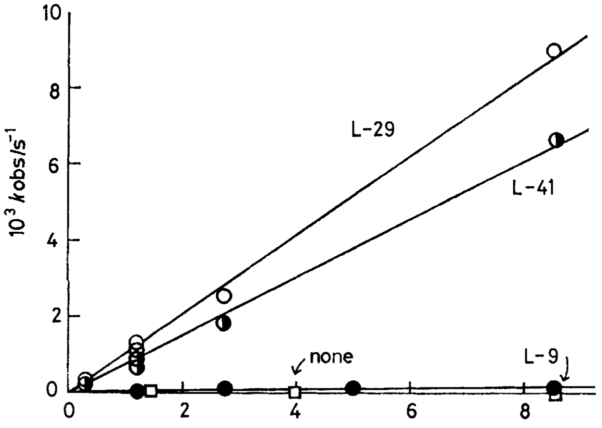

FIGURE 1 Pseudo-first-order rate constant ($k_{\text{obs.}}$) *versus* [2-mercaptoethanol].^{2} [(1)] 2.01 × 10^{-5}M, [L-9] 1.18 × 10^{-2} unit M, [L-29] 1.21 × 10^{-2} unit M, [L-41] 1.02 × 10^{-2} unit M, pH 9.0 ± 0.07, 0.02M-borate, μ 0.02, 3 vol % ethanol

The titration results are summarized in Table 3. L-9 and L-29 polymers exerted different influences upon the dissociation constants. The pK_a values were hardly changed by L-9 polymer, while they were lowered by 0.3 pK unit in the presence of L-29 polymer. Since the conventional cationic micelle generally lowers the pK_a of bound acidic species,[5,20,21] the trend observed here again supports that L-29 is a micelle-like polymer and L-9 a polyelectrolyte-like polymer.

TABLE 3

pK_a of thiols [a]

Thiol	No polymer	L-9	L-29
PhSH	6.8	6.8 (10.4 × 10^{-3} unit M)	6.5 (12.1 × 10^{-3} unit M)
HS[CH$_2$]$_4$SH	10.7	10.6 (3.00 × 10^{-3} unit M)	10.4 (3.03 × 10^{-3} unit M)

[a] 30 °C, μ 0.02 with KCl. Values in parentheses are polymer concentrations calculated based on the total monomeric unit.

Determination of Reaction Order and Rate Constant.—The reactions of isoalloxazine (1) with thiols were carried out in water at 30 °C under anaerobic conditions (N$_2$). Final spectra (>300 nm) of the reaction mixture were identical with that of reduced (1) which had been separately prepared by photoreduction with ethylenediaminetetra-acetic acid.[22] Introduction of oxygen into the final reaction mixture immediately regenerated (1) quantitatively.

When excess of thiol was used, the disappearance of (1) monitored at 433 nm [$\lambda_{\text{max.}}$ of (1)] gave good pseudo-first-order plots (see Experimental section). Figure 1 shows plots of the pseudo-first-order rate constant, $k_{\text{obs.}}$, against the square of the total concentration of 2-mercaptoethanol {[HO(CH$_2$)$_2$SH]$_T$}. Linear plots which pass through the origin establish that the reaction is second order in 2-mercaptoethanol.

The reduction of (1) by thiophenol was not detected in the absence of polymers, and addition of L-9 polymer (11.8 × 10^{-3} unit M) was also ineffective. Thus, $k_{\text{obs.}}$ in these systems is estimated as $<10^{-5}$ s^{-1}. On the other hand, isoalloxazine (1) underwent ready reduction in the presence of L-29 and L-41 polymers, and plots of $k_{\text{obs.}}$ *versus* [PhSH]$_T{}^2$ gave good straight lines {data not shown, [PhSH]$_T$ = (3—15) × 10^{-4}M}. Therefore, reduction with thiophenol is also second order in thiophenol. These results establish the kinetic expression (2) where [RSH]$_T$ is the total concentration of 2-mercaptoethanol or thiophenol, and k_3' is the apparent third-order rate constant (slope of Figure 1).

TABLE 4

Rate constants for the reaction of (1) and thiols at 30 °C

10^3 Polymer Concentration (unit M)		k_3' for PhSH [a] l^2 mol^{-2} s^{-1}	k_3' for HO[CH$_2$]$_2$SH [b]/ l^2 mol^{-2} s^{-1}	k_2' for HS[CH$_2$]$_4$SH [c] l mol^{-1} s^{-1}
None		No reaction	0.047	1.02
L-9	11.8	No reaction	0.18	2.02
L-29	12.1	11 500	11.1	3.41
L-41	10.2	9 750	7.80	3.37

[a] [(1)] 2.01 × 10^{-5}M, [PhSH]$_T$ (3—15) × 10^{-4}M, pH 6.77 ± 0.03 (0.02M-phosphate), 3 vol % ethanol. [b] [(1)] 2.01 × 10^{-5}M, [HO(CH$_2$)$_2$SH]$_T$ (5—29) × 10^{-3}M, pH 9.00 ± 0.07 (0.02M-borate), 3 vol % ethanol. [c] [(1)] 4.02 × 10^{-5}M, [HS(CH$_2$)$_4$SH]$_T$ (5—23) × 10^{-4}M, pH 10.00 ± 0.06 (0.02M-borate), 3 vol % ethanol. [L-9] 3.00 × 10^{-3} unit M, [L-29] 3.03 × 10^{-3} unit M, [L-41] 3.00 × 10^{-3} unit M.

$$v_{\text{obs.}} = k_{\text{obs.}}[(1)] = k_3'[\text{RSH}]_T{}^2[(1)] \qquad (2)$$

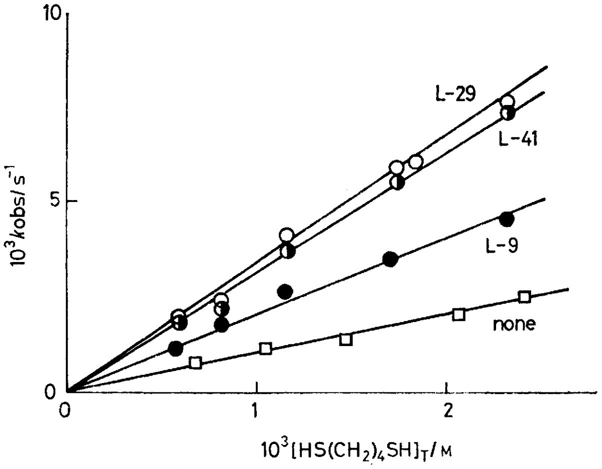

FIGURE 2 Pseudo-first-order rate constant ($k_{\text{obs.}}$) *versus* [butane-1,4-dithiol]. [(1)] 4.02 × 10^{-5}M, [L-9] 3.00 × 10^{-3} unit M, [L-29] 3.03 × 10^{-3} unit M, pH 10.0 ± 0.06, 0.02M-borate, μ 0.02, 3 vol % ethanol

The k_3' values thus determined are recorded in Table 4. The reaction with 2-mercaptoethanol was accelerated only

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* However, *cf.* related systems, ref. 19 and G. M. Whitesides, J. E. Lilburn, and R. P. Szajewski, *J. Org. Chem.*, 1977, **42**, 332.

[18] R. E. Benesch and R. Benesch, *J. Amer. Chem. Soc.*, 1955, **77**, 5877.

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[22] G. Blankenhorn, S. Ghisla, and P. Hemmerich, *Z. Naturforsch.* 1972, **27b**, 1038.

3.8-fold by addition of L-9 polymer. In contrast, marked rate increases were achieved on addition of L-29 and L-41 polymers (236- and 116-fold, respectively). A more pronounced polymer-micellar effect was observed for the reaction of (1) and thiophenol. Since the lower limit for detectable k_3' value is 1 l^2 mol^{-2} s^{-1} under the reaction condition employed ([PhSH] $\lll$ [HOC_2H_4SH]), the rate augmentation observed for thiophenol is more than four orders of magnitude.

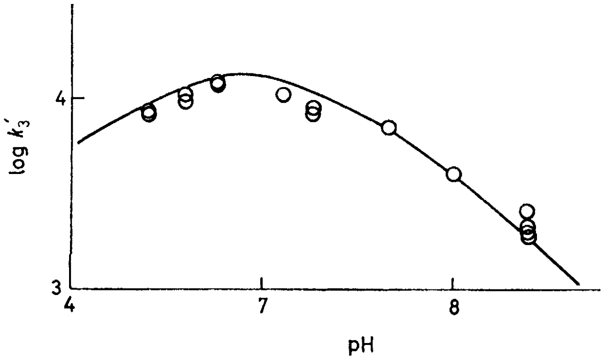

FIGURE 3 pH Dependence of the oxidation of thiophenol in the presence of L-29 (1.21 × 10^{-2} unit M). [(1)] 2.01 × 10^{-5}M [PhSH]$_T$ 1.06 × 10^{-3}M. The solid curve was obtained from equation (3) for K_{app} 1.26 × 10^{-7}M and k_3 5.40 × 10^4 l^2 mol^{-2} s^{-1}

As demonstrated already,[19,23,24] flavin oxidation of dithiol is first order in dithiol. In Figure 2, $k_{obs.}$ for the reaction of (1) and butane-1,4-dithiol was plotted against the concentration of butane-1,4-dithiol. The linear relationship observed proves the reaction to be first order in butane-1,4-dithiol. The second-order rate constants (k_2'; slope in Figure 2) are collected in Table 4. L-9 polymer was again ineffective. Contrary to expectation, neither L-29 nor L-41 polymer accelerated the reaction appreciably. The largest rate augmentation which was achieved in the presence of L-29 was only 3.4-fold.

TABLE 5

Rate constants, association constants, and pH at the rate maximum

	PhSH				HS[CH$_2$]$_4$SH			
Polymer	pH$_{max.}$[a]	k_3' at pH$_{max.}$[b]/ l^2 mol^{-2} s^{-1}	k_{3p}[c]/ l^2 mol^{-2} s^{-1}	K_{PhS}[c]/l mol^{-1}	pH$_{max.}$[d]	k_2' at pH$_{max.}$[d]/ l mol^{-1} s^{-1}	k_{2}',bound[e]/ l mol^{-1} s^{-1}	K[e]/l mol^{-1}
None					10.9	1.78		
L-9					10.4	2.40	3.70	185
L-29	6.9	13 500	236 000	1 730	10.3	4.07	5.61	340
L-41							4.81	351

[a] pH$_{max.}$ was obtained from Figure 3, and is equal to pK_{app} in equation (3). [b] Apparent third-order rate constant at pH$_{max.}$ (6.9), and is equal to 0.25k_3. [c] Determined from equation (5). [d] Obtained from Figure 4. [e] Determined from equation (6).

Effects of Ionic Strength and Buffer Concentration on the Reaction with Thiophenol.—The rate of oxidation of thiophenol in the presence of L-29 polymer was markedly suppressed with increasing salt (KCl) concentration. The apparent third-order rate constant (k_3' at pH 6.77) at μ 0.3 was evaluated to be 1 010 l^2 mol^{-2} s^{-1}, which is >10 times smaller than that at μ 0.02 (11 500 l^2 mol^{-2} s^{-1}).

Subsequently, buffer (borate) concentration was varied (0.02—0.12M) at a fixed ionic strength (μ 0.3). However, the apparent third-order rate constants were the same within the experimental error (1 100 ± 100 l^2 mol^{-2} s^{-1}). Thus, the oxidation of thiophenol is not subject to general catalysis. This is in accord with the observation in related flavin oxidation reactions.[7,25]

pH-*Rate Profiles.*—pH–Rate profiles for the reaction of (1) with thiophenol (Figure 3) and butane-1,4-dithiol

$$k_3' = k_3\frac{[PhSH][PhS^-]}{[PhSH]_T^2} = k_3\frac{K_{app}a_H}{(K_{app} + a_H)^2} \qquad (3)$$

(Figure 4) gave bell-shaped curves. Similar profiles have been obtained in related systems.[7,23,25] The bell-shaped profile is most probably expressed by equation (3) where K_{app} is the apparent dissociation constant of thiophenol, and k_3 is the third-order rate constant. Curve fitting of equation (3) for the data of Figure 3 provides pK_{app} 6.9 and k_3 5.40 × 10^4 l^2 mol^{-2} s^{-1} (see caption to Figure 3). The solid curve was depicted based on these values.

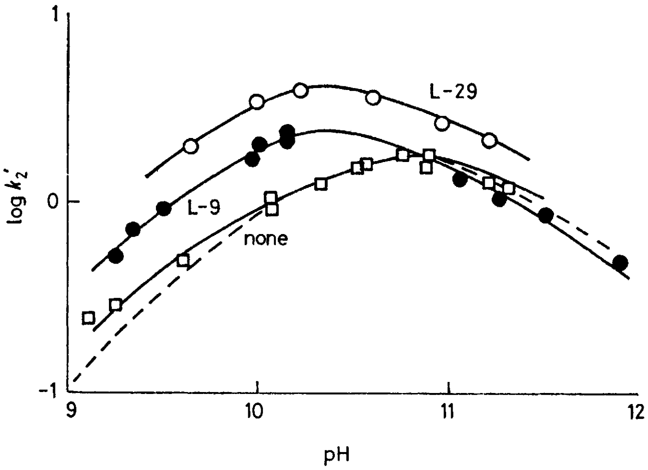

FIGURE 4 pH Dependence of the oxidation of butane-1,4-dithiol in the presence of L-9 (3.00 × 10^{-3} unit M) and L-29 (3.03 × 10^{-3} unit M) and without polymer. [(1)] 4.02 × 10^{-5}M, [$HS(CH_2)_4SH$]$_T$ 2.31 × 10^{-3}M. The dotted curve was obtained from equation (4) for the following best fit values: K_{a1} 5.01 × 10^{-11} mol l^{-1} (pK_{a1} 10.3), K_{a2} 5.01 × 10^{-12} mol l^{-1} (pK_{a2} 11.3, pK_{a2} ≈ pK_{a1} + 1.0[24]), and k_2 2.60 l mol^{-1} s^{-1}

On the other hand, bell-shaped profiles obtained for the reaction of (1) and butane-1,4-dithiol cannot be completely described by a single kinetic equation. As shown in Figure 4 for the data without polymer, the experimental plots showed positive deviations from the theoretical curve (dotted curve) which was drawn from equation (4) in the less alkaline region. The theoretical curve was obtained based on the most reasonable values of K_{a1} and K_{a2} (see

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[23] T. C. Bruice, L. Main, S. Smith, and P. Y. Bruice, *J. Amer. Chem. Soc.*, 1971, **93**, 7327.

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[25] I. Yokoe and T. C. Bruice, *J. Amer. Chem. Soc.*, 1975, **97**, 450.

caption of Figure 4). A similar result was reported by Loechler and Hollocher,[24] and they suggested the involvement of the general acid catalysis.

$$k_2' = k_2 \frac{[HS(CH_2)_4S^-]}{[HS(CH_2)_4SH]_T} = \frac{k_2 a_H K_{a1}}{a_H^2 + a_H K_{a1} + K_{a2} K_{a1}} \quad (4)$$

The pH value where the rate maximum is observed is designated as $pH_{max.}$ and collected in Table 5. Addition of polymers L-9 and L-29 lowered $pH_{max.}$ for the oxidation of butane-1,4-dithiol by 0.5—0.6 pH unit. Although $pH_{max.}$ conceivably reflects the maximum concentration of the butane-1,4-dithiol monoanion, pK_{a1} (for the first ionization) as determined by titration was not affected by addition of L-9 polymer.

Reaction Rate versus *Polymer Concentration*.—At given thiol concentrations, rate constants (k_2' for butane-1,4-dithiol and k_3' for thiophenol) were determined as a function of polymer concentrations (Figure 5). Conventional micellar catalysis generally features sigmoidal dependence on the surfactant concentration, *i.e.* the rate constant is almost independent of the surfactant concentration below the c.m.c., whereas it rapidly rises or descends above the c.m.c. This pattern was also true in the coenzyme-catalysed systems we have investigated.[3-5] On the other hand, the rate constant showed rapid increase at very low concentrations of L-29 and L-41, followed by gradual saturation (Figure 5). Thus, these polymers must possess a micelle-like domain at very low concentrations.

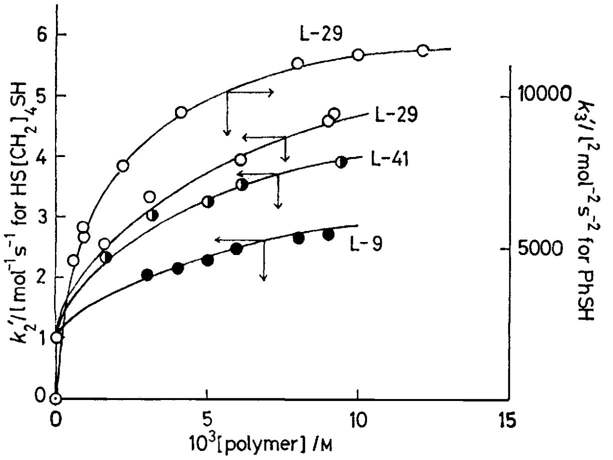
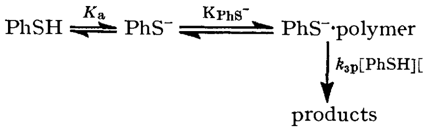


FIGURE 5 Apparent second-order rate constants for butane-1,4-dithiol and apparent third-order rate constants for thiophenol plotted against the polymer concentration. For butane-1,4-dithiol: [(1)] 4.02×10^{-5}M, $[HS(CH_2)_4SH]_T$ 2.31×10^{-3}M, pH 10.0 ± 0.07 with 0.02M-borate. For thiophenol: [(1)] 2.01×10^{-5}M, $[PhSH]_T$ 1.06×10^{-3}M, pH 6.77 ± 0.05 with 0.02M-phosphate

The saturation kinetics observed strongly suggest the formation of complexes between polymer micelle and thiol or isoalloxazine (1). It is known that, if the isoalloxazine ring is present in the hydrophobic region of the micelle, $\lambda_{max.}$ shifts from *ca.* 440 nm to longer wavelengths by 7—10 nm and a distinct shoulder appears at 460—470 nm.[26] This is characteristic of flavins in organic solvents. However, this spectral change was not detected under the kinetic conditions. The binding constant of neutral species to the cationic micellar phase is much smaller than that of anionic species (>10^2-fold difference).[27] Thus, the association of undissociated thiols (neutral species) with the polymer micelle seems to be negligible, and the binding of thiolate anion onto the polymer micelle as supported by the spectral change (see above) must be responsible for the rate saturation. Then the saturation profile for thiophenol (Figure 5) can be accounted for by Scheme 1, where K_{PhS^-}



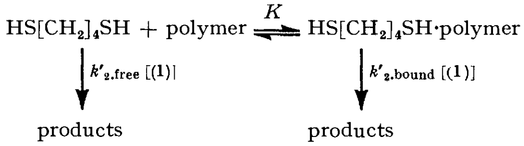
SCHEME 1

is the equilibrium constant for the association of PhS$^-$ with polymer and k_{3p} the third-order rate constant calculated on the basis of the concentration of polymer-bound PhS$^-$. Since an excess of polymer is present in most cases, the concentration of polymer is assumed to be constant. From the material balance ($[PhSH]_T = [PhSH] + [PhS^-] + [PhS^-\cdot polymer]$) and the equilibrium constant ($K_{PhS^-} = [PhS^-\cdot polymer]/[PhS^-][polymer]$), equation (5) is derived in which the polymer concentration is linearly correlated with $([polymer]/k_3')^{1/2}$. K_{PhS^-} 1 730 l mol^{-1} and k_{3p} 2.36×10^5 l^2 mol^{-2} s^{-1} were obtained with correlation coefficient 0.992 from the data in Figure 5. The excellence of the correlation indicates that the assumption, made in deriving equation (5) is allowable.

$$\left(\frac{[polymer]}{k_3'}\right)^{1/2} = \left(\frac{a_H}{k_{3p} K_a K_{PhS^-}}\right)^{1/2} \left(1 + \frac{K_a}{a_H} + \frac{K_a K_{PhS^-}}{a_H}[polymer]\right) \quad (5)$$

It was suggested for the polymer-micelle-catalysed decarboxylation reaction that added aromatic anions associate with the head group of the laurylated unit.[28] If K_{PhS^-} is calculated on the basis of the laurylated unit concentration, a value of 6.0×10^3 l mol^{-1} is obtained. This value is comparable with that obtained in the conventional cationic micellar system (3.0×10^{-3} l mol^{-1}).[7]

The reaction mechanism for the oxidation of butane-1,4-dithiol seems to be complex. We thus evaluated the apparent binding constants K based on Scheme 2 where



SCHEME 2

$k'_{2,free}$ is the apparent second-order rate constant for the nonpolymeric system and $k_2'_{,bound}$ the apparent second-order rate constant for polymer-bound species.

$$\frac{k'_{2,free}}{k_2' - k'_{2,free}} = \frac{1}{qK[polymer]} + \frac{1}{q} \quad (6)$$

Equation (6) where $q = (k'_{2,bound}/k_{2,free}) - 1$ can be derived for Scheme 2.[29] This treatment provided linear rel-

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[26] S. Shinkai, Y. Sakuma, and F. Yoneda, *J.C.S. Chem. Comm.*, 1976, 986.

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ationships with correlation coefficient 0.992—0.999. $k'_{2,\mathrm{bound}}$ and K calculated from the slope and the intercept are summarized in Table 5.

DISCUSSION

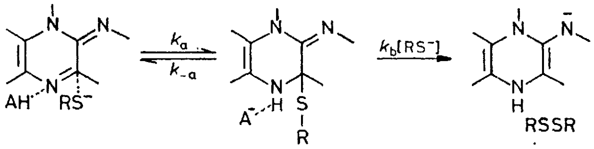
Polymer Micellar Effects.—It has been established that the nucleophilicity of anionic species (involving thiolate anions) is unusually enhanced in cationic hydrophobic aggregates (see refs. 6 and 21, and references cited therein). The cause of activation is considered to be desolvation, *i.e.* the anion included in the hydrophobic region as a ' hydrophobic ion pair ' attains its high reactivity from dehydration relative to an anion in an aqueous environment. The efficiency of flavin oxidation of thiols is greatly improved by employing such activated thiols, since the reaction involves the rate-limiting nucleophilic attack of thiolate anion on an isoalloxazine ring.[24,25]

Spectral data (Table 2), lowering of the pK_a (Table 3), and the relatively large association constants (Table 5) observed in the presence of L-29 and L-41 polymers all suggest the formation of hydrophobic ion pairs between the thiolate anion and the ionic head group of the polymer micelles. On the other hand, addition of L-9 polymer hardly affected these values, indicating that L-9, a polyelectrolyte-like polymer, is not able to form the hydrophobic ion pair. This contrasting behaviour is related to the oxidation rate (Table 4): the reaction is markedly accelerated by L-29 and L-41 (except for butane-1,4-dithiol), but not by L-9 polymer. It is thus concluded that the hydrophobic nature of the polymer is primarily responsible for the activation of thiol anions and that the polymeric charges are probably utilized only for binding the anion to the polymer micellar phase.

Flavins are not able to oxidize thiophenol in nonenzymatic system.[25] Oxidation of thiolphenol by (1) is not detectable in the nonpolymeric system and also in the presence of L-9 polymer. According to Yokoe and Bruice,[25] an electron-deficient isoalloxazine, 8-cyano-3,10-dimethylisoalloxazine is able to perform this reaction with k_3 1.5×10^5 $\mathrm{l^2\ mol^{-2}\ s^{-1}}$ at 30 °C. Since the k_{3p} value obtained for the present system (Table 5) exceeds this value, it can be said that the polymer micellar environment lowers the free energy of activation more effectively than the cyano-substituent. It is important that such a rate acceleration can be achieved by the secondary valence force, and that the thiolate anion, but not isoalloxazine, is activated by polymer micelles.

The pronounced rate enhancement for thiophenol relative to that for 2-mercaptoethanol may be ascribed to favourable partitioning of thiophenol in the polymer micellar phase. It was found from kinetic measurements that the L-29 solution becomes turbid upon addition of $>1.5 \times 10^{-3}$M-thiophenol. On the other hand, 2-mercaptoethanol could be used at concentrations one order of magnitude larger (see Figure 1).

Reaction Mechanisms in Relation to Environmental Effects.—The kinetic characteristics of the oxidation of monothiols (second-order in thiol and bell-shaped pH-dependence) are similar to those reported by Yokoe and Bruice.[25] The rate law (3) is in accord with general acid-catalysed addition to C-4a followed by the nucleophilic attack of the second RS^- anion upon the adduct [equation (7)]. Provided that the breakdown step (k_b) is rate limiting as claimed by Loechler and Hollocher,[24] it follows that the polymer micelles facilitate the reaction: $RS^- + (1)\text{-}SR \longrightarrow RSSR + (1)^-$. This is analogous to the Ellman reaction,[30] the rate of which is markedly accelerated by cationic hydrophobic aggregates (unpublished result).



(7)

As illustrated in Figure 3, the theoretical curve derived from equation (3) satisfactorily describes the experimental data. However, pK_{app} (kinetic pK_a of thiophenol, 6.9) does not agree with the titration pK_a (6.5). A similar discrepancy (pK_{app} > titration pK_a) has been noticed in Yokoe and Bruice's system [25] and in a conventional cationic micellar system.[7] Conceivably, K_{app} consists of a number of kinetic parameters.[31]

Strangely, the reaction with butane-1,4-dithiol was not subject to the polymer micellar effect. In our previous investigation, a conventional cationic micelle was shown to accelerate the reaction of (1) and monothiol by factors of 10^2—10^5-fold, while the reaction with butane-1,4-dithiol was accelerated only by 18-fold.[32] On the other hand, the lowered pK_a values (Table 3 and ref. 32) indicate that dissociated species of butane-1,4-dithiol is bound to the (polymer) micellar phase. The reactivity difference may be explained by the following factors. First, the difference could arise from a change in the rate-limiting step. According to the reaction mechanism proposed for dithiols,[24] addition to C-4a [k_a in equation (7)] becomes rate limiting in the pH region near $\mathrm{pH_{max.}}$. Since the kinetic measurements were carried out at pH 10.0 and $\mathrm{pH_{max.}}$ is 10.3—10.9 (Table 5), the rate would reflect the k_a process. In fact, the reaction of (1) and butane-1,4-dithiol at pH 10.0 is accelerated by increasing buffer concentration.[32] The (general) acid catalysed reactions (*e.g.*, acetal hydrolysis, imine formation, and hydration of 1,4-dihydronicotinamide) are usually accelerated by anionic micellar environments, and cationic micelles tend to suppress the reaction.[1,3,4] Since the environment of cationic polymer micelles is presumably similar to that of conventional cationic micelles, it is unlikely that the k_a process including general acid catalysis is accelerated by these polymer micelles. The small rate acceleration may have been observed because the enhancement in nucleo-

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philicity of RS^- is cancelled by the inefficiency of the general acid catalysis.

The second possibility is that monoanionic butane-1,4-dithiol is not subject to micellar activation due to intramolecular hydrogen bonding with the undissociated SH group. It is well known that the reactivity of anions is markedly reduced in protic solvents due to hydrogen-bonding with solvent protons.[33] Since micellar activation of thiolate anion largely stems from desolvation of the anion, the nucleophilicity of monoanionic species of butane-1,4-dithiol would not be enhanced despite the formation of a hydrophobic ion pair.

In conclusion, the present study has established the importance of hydrophobic environments in the flavin-mediated oxidation of thiols. It has also been found that the susceptibility of mono- and di-thiols to the environment is fundamentally different. Further applications of these concepts would provide useful model systems for understanding the function of flavin-dependent enzymes.

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