10c, 56905-05-6; **10d**, 53045-66-2; **11a**, 21430-05-7; **11a** 3,5-dinitrobenzoate, 21430-06-8; **11b**, 79828-24-3; **11c**, 79828-25-4; **11d**, 79828-26-5; **12a**, 21430-07-9; **12a** 3,5-dinitrobenzoate, 21430-08-0; **12b**, 79828-27-6; **12c**, 79828-28-7; **12d**, 79828-29-8; **13a**, 13668-59-2; **13b**, 3309-97-5; **14a**, 79828-30-1; **14b**, 77369-76-7; **14b** 3,5-dinitrobenzoate, 79828-31-2; **15a**, 1120-80-5; **15b**, 4845-04-9; **16a**, 52829-98-8; **16b**, 1193-81-3; **18**, 79828-32-3; **19**, 51238-57-4; **20**, 79828-33-4; **21**, 79828-34-5; (*Z*)-**22**, 20125-84-2; (*E*)-**22**, 20125-85-3; **23**, 79828-35-6; **24**, 79828-36-7; **30a**, 1803-71-0; **30b**, 76019-22-2; **30c**, 40760-35-8; **31a**, 79828-37-8; **31b**, 74126-47-9; **31c**, 16933-29-2; **32a**, 79828-38-9; **32c**, 39149-97-8; **33**, 77103-98-1; **34**, 3778-92-5; **35a**, 79828-39-0; **35b**, 78426-31-0; **36a**, 63791-10-6; **36b**, 78426-32-1; **37**, 79828-40-3; Me_2AlCl, 1184-58-3; methylenecyclohexane, 1192-37-6; paraformaldehyde, 30525-89-4; paraldehyde, 123-63-7; isovaleraldehyde, 590-86-3; pivalaldehyde, 630-19-3; benzaldehyde, 100-52-7; limonene, 5989-27-5; isoprene, 78-79-5; methylenecyclobutane, 1120-56-5; 2-methyl-2-butene, 513-35-9; nonanal, 124-19-6; 1-methylcyclohexene, 591-49-1; 2,3-dimethyl-2-butene, 563-79-1; *trans*-2-butene, 624-64-6; *cis*-2-butene, 590-18-1; *trans*-4-octene, 14850-23-8; *cis*-4-octene, 7642-15-1; *trans*-2-heptene, 14686-13-6; *cis*-2-heptene, 6443-92-1; cyclopentene, 142-29-0; *trans*-2-chlorocyclopentanol, 20377-80-4; *trans*-2-chlorocyclopentanemethanol, 25236-94-6; 3-chlorocyclopentanemethanol, 79828-41-4; cyclohexene, 110-83-8; 1,4-cyclohexadiene, 628-41-1; 1,5-cyclooctadiene, 111-78-4; 1-octene, 111-66-0; vinylcyclohexane, 695-12-5; 2-methyl-1-pentene, 763-29-1; 2,3-dimethyl-1-butene, 563-78-0; 3-methyl-3-buten-1-ol, 763-32-6; isobutylene, 115-11-7; (*E*)-3-methyl-2-pentene, 616-12-6; (*Z*)-3-methyl-2-pentene, 922-62-3; 2,3,3-trimethyl-1-pentanol, 66576-25-8; 1-methylcyclopentane, 693-89-0; 3-methyl-2-buten-1-ol, 556-82-1.

Coenzyme Models. 31. Efficient Trapping of Transient Thiazolium–Aldehyde Adducts (Active Aldehydes) by Intramolecular and Quasi-Intramolecular Flavins. Flavin–Thiamin Biscoenzyme

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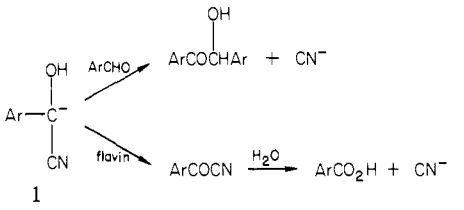
Abstract: The reaction sequence of acyloin condensation of aldehydes, catalyzed by thiazolium ion bound to the CTAB micelle, can be diverted by the addition of flavin to the oxidation reaction to afford the corresponding carboxylic acids. It was found, however, that when the aldehyde concentration is elevated or the aldehyde is relatively reactive, intermolecular flavin (3-methyltetra-*O*-acetylriboflavin, MeFl) cannot trap the intermediates (active aldehydes) formed from thiazolium ion and aldehydes completely, leading to a competition between the conventional acyloin condensation and the flavin oxidation. We have applied the concept of intramolecular catalysis to this system by two methods in order to suppress the acyloin condensation relative to the flavin oxidation. The first utilizes quasi-intramolecular flavin oxidation in which hydrophobic 10-dodecylisoalloxazine (10-DodFl) and *N*-hexadecylthiazolium bromide (HxdT) are bound to a CTAB micelle aggregate. The second is a flavin–thiazolium biscoenzyme (Fl–T) oxidation in which the intermediates on the thiazolium moiety are oxidized efficiently by the intramolecular flavin. When 4-chlorobenzaldehyde (100 mM) was employed as substrate, the trapping efficiency (=flavin oxidation product/sum of acyloin condensation products) for MeFl was 1.6. The trapping efficiency for the quasi-intramolecular flavin oxidation was improved up to 15–33-fold owing to the enhanced local concentration of 10-DodFl in the micelle phase; efficiency for the biscoenzyme system was further enhanced (>115-fold). A kinetic examination has established that the reaction is zero order in MeFl for the intermolecular flavin oxidation of 4-chlorobenzaldehyde, whereas it becomes first order in MeFl for the oxidation of more reactive pyridine-4-carboxaldehyde (pyCHO). This indicates that the rate-limiting step changes depending on the reactivity of aldehyde: the deprotonation from the thiazolium–aldehyde adduct is rate limiting in the oxidation of 4-chlorobenzaldehyde, whereas the oxidation of the deprotonated active aldehyde by MeFl becomes rate limiting in the oxidation of pyCHO. On the other hand, quasi-intramolecular flavin oxidation of pyCHO was zero order in 10-DodFl at low pyCHO concentrations (<10 mM) and was approximated by a first-order equation at high pyCHO concentrations (>50 mM). In the biscoenzyme oxidation of pyCHO, the zero-order decrease was always observed for up to 60% reaction, indicating the high efficiency of intramolecular flavin as a trapping agent. The present system is a relevant model for pyruvate oxidase which requires FAD and thiamine pyrophosphate as cofactors and catalyzes the convension of pyruvic acid to acetic acid.

Recently, it has become obvious that carbanions are employed as substrates in some biological oxidation reactions. In particular, it has been established unequivocally that the mechanisms by which flavoenzymes such as amino acid oxidases and lactate oxidases oxidize their specific substrates involve the flavin oxidation of carbanions.[1-4] This concept is also supported by model studies in nonenzymatic systems.[5-7] However, the investigation of the application of the newly established concept—"flavin oxidation of carbanions"—has been very limited. In previous publications of this series, we demonstrated that the application of the concept to organic chemistry is very useful in exploiting a new class of oxidation reactions.[8-10] For example, the benzoin (or acyloin)

Scheme I



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(1) (a) Bruice, T. C. *Prog. Bioorg. Chem.* **1976**, *4*, 1. (b) Bruice, T. C. *Acc. Chem. Res.* **1980**, *13*, 256.

(2) (a) Walsh, C. *Annu. Rev. Biochem.* **1978**, *47*, 881. (b) Walsh, C. *Acc. Chem. Res.* **1980**, *13*, 148. (c) Walsh, C. T.; Schonhrunn, A.; Abeles, R. H. *J. Biol. Chem.* **1971**, *246*, 6855. (d) Walsh, C. T.; Krodel, E.; Massey, V.; Abeles, R. H. *Ibid.* **1973**, *248*, 1946.

(3) Kosman, D. J. "Bioorganic Chemistry"; Van Tammelen, E. E., Ed.; Academic Press: New York, 1977; Vol. 2, p 175.

(4) (a) Cogoli-Greuter, M.; Hausner, V.; Christen, P. *Eur. J. Biochem.* **1979**, *100*, 295. (b) Lubini, D. G. E.; Christen, P. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2527. (c) Christen, P.; Gasser A. *Eur. J. Biochem.* **1980**, *107*, 73.

(5) Shinkai, S.; Kunitake, T.; Bruice, T. C. *J. Am. Chem. Soc.* **1974**, *96*, 7140.

(6) Williams, R. F.; Shinkai, S.; Bruice, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 1763.

(7) Bruice, T. C.; Taulane, J. P. *J. Am. Chem. Soc.* **1976**, *98*, 7769.

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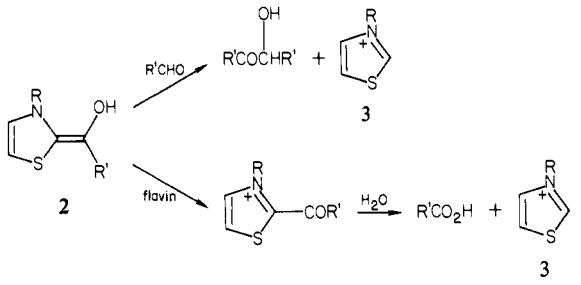
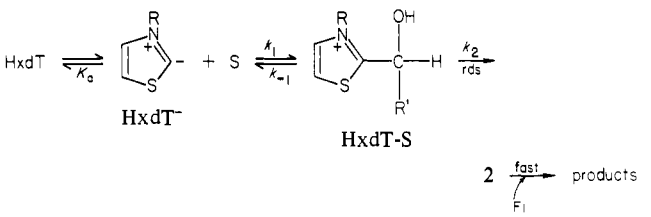
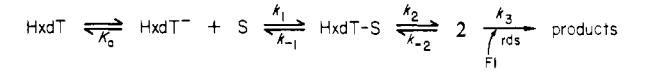
Scheme II



Scheme III

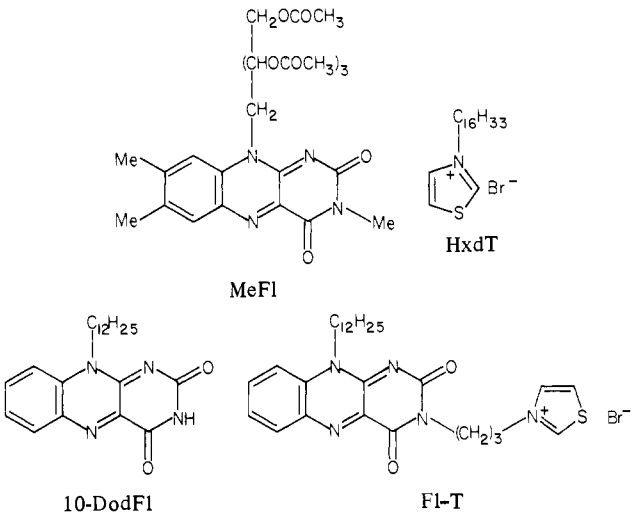


Scheme IV



condensation catalyzed by cyanide ion (or thiazolium ion, **3**) employs carbanionic intermediates such as **1** (or **2**).[11-13] Added flavin is capable of efficiently oxidizing these carbanionic intermediates, diverting the reaction sequence of conventional condensation to that of oxidation.[8,9,14] Oxidative decarboxylation of α-keto acids such as pyruvic acid and benzoylformic acid to the corresponding carboxylic acids is also catalyzed by flavin plus cyanide ion (or **3**) in a similar manner. The thiazolium-mediated flavin oxidation of α-keto acids is of particular interest in connection with the mechanism of pyruvate oxidase, since it requires FAD and thiamin pyrophosphate as cofactors and catalyzes the conversion of pyruvic acid to acetic acid and CO_2.

In Schemes I and II, oxidation competes with conventional acyloin condensation. In order to apply the above flavin oxidations to a synthetic purpose or to an enzyme model investigation, it becomes desirable to suppress the competing condensation reaction as much as possible. It occurred to us that the efficient flavin trapping of **2** would be achieved by a biscoenzyme system which combines within a single molecule both flavin and thiazolium ion. In this paper, we report that the condensation of **2** with aldehyde substrates is suppressed *almost completely* by a quasi-intramolecular system consisting of *N*-hexadecylthiazolium bromide (HxdT) and 10-dodecylisoalloxazine (10-DodFl) bound to hexadecyltrimethylammonium bromide (CTAB) micelle and *completely* by a biscoenzyme (Fl-T) containing intramolecularly both isoalloxazine and thiazolium ion.



MeFl HxdT

10-DodFl Fl-T

Results and Discussion

Kinetics. The progress of the reaction was followed by monitoring the disappearance of the absorption maximum of the flavin.

It is known that when the disappearance is zero order in flavin (Fl), the reaction involves rate-limiting formation of a reactive species followed by the immediate oxidation by Fl.[5-10,14,15] The reaction sequence for the zero-order disappearance is thus expressed by Scheme III, where K_a is the acid dissociation constant of HxdT and S is substrate aldehyde (R'CHO). We have shown that the reaction rate (v_{obsd}) for Scheme III is expressed by eq 1 ($K_1 = k_1/k_{-1}$).[9] Equation 1 indicates that the reaction occurring

$$v_{obsd} = -\frac{d[Fl]}{dt} = \frac{k_2 K_1 K_a [S]_0 [HxdT]_0}{[H^+] + K_1 K_a [S]_0} \quad (1)$$

according to Scheme III is first order in HxdT and shows a saturation phenomenon at high substrate concentrations. We have found, however, that the v_{obsd} is apparently first order in S under the usual reaction conditions ([S] < 20 mM),[9] suggesting the relation $[H^+] >> K_1 K_a [S]_0$ in eq 1.

When the disappearance is first order in flavin, the oxidation of **2** by flavin must be involved in the rate-determining step (Scheme IV). The corresponding rate equation is given by eq 2 ($K_2 = k_2/k_{-2}$), which indicates the reaction to be first order in HxdT and Fl and to be saturated in S.

$$v_{obsd} = -\frac{d[Fl]}{dt} = k_{obsd}[Fl] = \frac{k_3 K_1 K_2 K_a [S]_0 [HxdT]_0 [Fl]}{[H^+] + (K_1 K_a + K_1 K_2 K_a)[S]_0} \quad (2)$$

Schemes III and IV show that the oxidation path is favored relative to the condensation path when (i) the concentration of flavin is enhanced and the flavin is activated (for example, by electron-withdrawing substituents) and (ii) the concentration of substrate is lowered and the substrate is inactivated (for example, by electron-donating substituents).

Intermolecular Flavin Oxidation. In a previous publication of this series, we showed that in a CTAB (10 mM) micellar solution containing 3-methyltetra-*O*-acetylriboflavin (MeFl 5 mM) and HxdT (1 mM), 4-chlorobenzaldehyde (5 mM) is converted into 4-chlorobenzoic acid in 63% yield and condensation products such as 4,4'-dichlorobenzoin and 4,4'-dichlorobenzil (oxidation product from 4,4'-dichlorobenzoin) are not detected.[9] The disappearance of the absorbance of MeFl is zero order up to 90% reaction. This indicates that the oxidative trapping by intermolecular flavin is relatively efficient and is not involved in the rate-limiting step under these reaction conditions.

We found, however, that when the concentration of MeFl is lowered to 1 mM and those of 4-chlorobenzaldehyde and HxdT are enhanced to 100 and 10 mM, respectively, the acyloin condensation occurs competitively and the trapping efficiency (ratio of oxidation product vs. condensation products) is 1.6 (=28/17) (Table I). Under comparable reaction conditions, the disappearance of the absorbance of MeFl changed from zero order to first order at about 30% reaction. These results show that the elevated concentration of 4-chlorobenzaldehyde results in an acceleration of the formation of **2** and that a considerable fraction of **2** is consumed by the condensation reaction without being

(8) Shinkai, S.; Yamashita, T.; Kusano, Y.; Ide, T.; Manabe, O. *J. Am. Chem. Soc.* **1980**, *102*, 2335.
(9) (a) Shinkai, S.; Yamashita, T.; Kusano, Y.; Manabe, O. *Tetrahedron Lett.* **1980**, 2543. (b) *J. Org. Chem.* **1980**, *45*, 4947.
(10) Shinkai, S.; Yamashita, T.; Kusano, Y.; Manabe, O. *J. Am. Chem. Soc.* **1981**, *103*, 2070.
(11) Lapworth, A. *J. Chem. Soc.* **1903**, *83*, 995.
(12) Breslow, R. *J. Am. Chem. Soc.* **1957**, *79*, 1762.
(13) Tagaki, W.; Hara, H. *J. Chem. Soc., Chem. Commun.* **1973**, *891*.
(14) Yano, Y.; Hoshino, Y.; Tagaki, W. *Chem. Lett.* **1980**, 749.
(15) (a) Main, L.; Kasperek, G. J.; Bruice, T. C. *Biochemistry* **1972**, *11*, 3991. (b) Chan, T. W.; Bruice, T. C. *J. Am. Chem. Soc.* **1977**, *99*, 2389.

Table I. Product Analysis for the Flavin Oxidation of 4-Chlorobenzaldehyde (ArCHO)[a]

[ArCHO], mM	[HxdT], mM	[MeFl], mM	[10-DodFl], mM	[Fl-T], mM	yield, % $ArCO_2H$	yield, % ArCH(OH)COAr	yield, % ArCOCOAr	trapping efficiency
5	1	5			63	0	0	∞
50	1	5			26	0.4-0.7	0	37-65
100	10	1			28	2	15	1.6
100	10		0.1		23	1.4	0.1	15
100	10		0.4		26	0.6	0.2	33
100				0.4	18	0.1	0	180
100				1	23	0.2	0	115
100				5	34	0	0	∞

[a] At 30 °C and pH 8.0 with 0.05 M phosphate for 1 day in the dark.

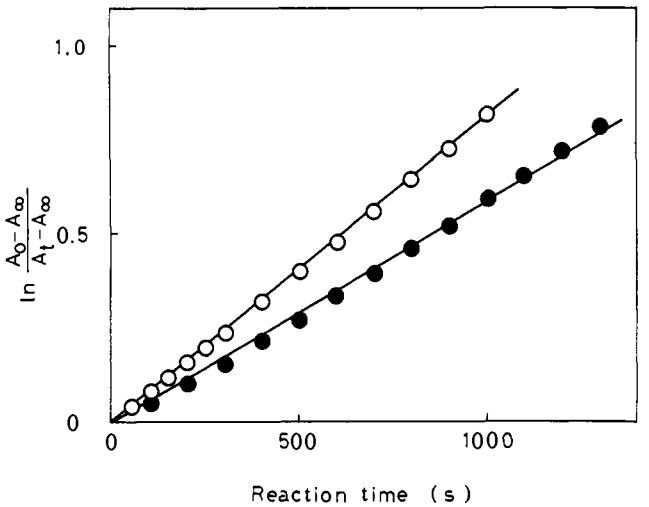


Figure 1. First-order plots for the oxidation of pyCHO by MeFl (pH 7.50 with 0.02 M phosphate, [MeFl] = 5.00×10^{-5} M, [HxdT] = 1.00×10^{-4} M, [CTAB] = 10 mM): ○, [pyCHO] = 5.00×10^{-2} M; ●, [pyCHO] = 1.00×10^{-2} M.

trapped oxidatively by intermolecular flavin.

The trapping efficiency of intermolecular flavin was further evaluated kinetically with a more activated substrate, pyridine-4-carboxaldehyde (pyCHO). Being different from the linear zero-order decrease observed for 4-chlorobenzaldehyde, the reaction was apparently first-order in MeFl at 2–50 mM pyCHO (Figure 1). The result implies that the rate-determining step for 4-chlorobenzaldehyde is k_2 (deprotonation), while that for the more activated pyCHO is k_3 (flavin oxidation). The change in the rate-determining step is ascribed to the difference in the reactivity of two substrates. The plot of the pseudo-first-order rate constant (k_{obsd}) versus the concentration of pyCHO (Figure 2A) clearly shows a saturation phenomenon, indicating that in eq 2 ($K_1K_a + K_1K_2K_a$)[S]$_0$ is not negligibly smaller than [H$^+$]. As described later, one may assume [H$^+$] >> K_1K_a[pyCHO]$_0$, so that eq 2 can be rewritten as eq 3 which suggests a linear dependence between

$$\frac{1}{k_{obsd}} = \frac{1}{k_3[\mathrm{HxdT}]_0} + \frac{[\mathrm{H^+}]}{k_3[\mathrm{HxdT}]_0 K_1 K_2 K_a}\left[\frac{1}{[\mathrm{pyCHO}]}\right] \quad (3)$$

$1/k_{obsd}$ and 1/[pyCHO]. Figure 2B clearly substantiates a linear dependence which has a correlation coefficient of 0.999. From the slope ($[H^+]/k_3[HxdT]_0K_1K_2K_a$) and the intercept ($1/k_3$-$[HxdT]_0$) we obtained $k_3 = 8.88$ M^{-1} s^{-1} and $K_1K_2K_a = 5.00 \times 10^{-6}$. Since [H$^+$] = 3.16×10^{-8} M and $K_1K_2K_a$[pyCHO]$_0$ = $(1\text{–}25) \times 10^{-8}$ M, the latter term cannot be negligibly smaller than the former term. This situation provides the saturation phenomenon in the k_{obsd} vs. [pyCHO] plot (Figure 2A).

Quasi-Intramolecular Flavin Oxidation. It has been established that micellar catalysis of bimolecular reactions largely stems from the concentration of reactants into the limited micellar phase.[16]

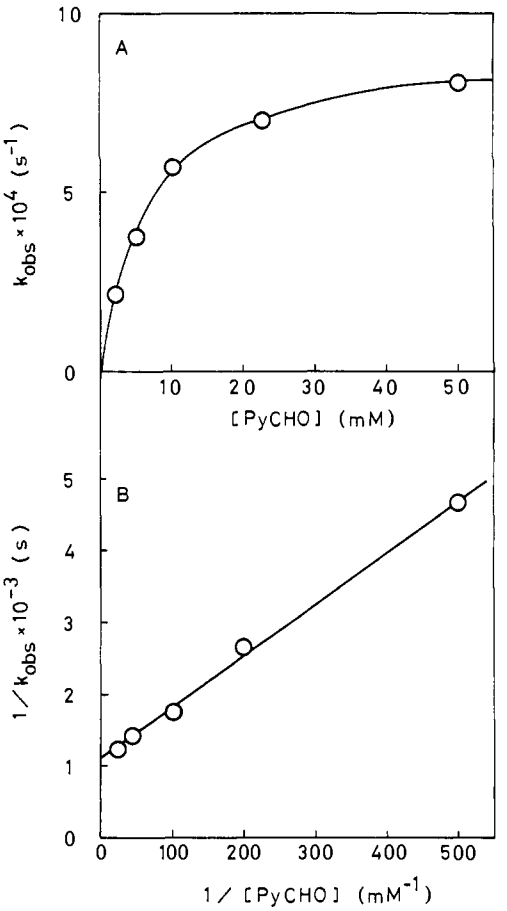


Figure 2. Pseudo-first-order rate constant for the oxidation of pyCHO by MeFl as a functions of pyCHO concentration (pH 7.50 with 0.02 M phosphate, [MeFl] = 5.00×10^{-5} M, [HxdT] = 1.00×10^{-4} M, [CTAB] = 10 mM.

For example, Bunton et al.[17] indicate on the basis of their investigation of the pseudophase model of micellar catalysis that k_ψ^{max} (maximum rate constant in the micellar system) is approximately equal to $7nk_w$ (n, mole fraction of reactants in the micelle; k_w, rate constant in bulk water). Thus, trapping efficiency in our system may be improved by enhancing the mole fraction of flavin in the micelle phase, since **2** from HxdT and aldehyde is efficiently formed in the micellar phase.[13] The most expeditious way to improve the partition coefficient is to adopt a hydrophobic flavin such as 10-DodFl. It is expected, therefore, that the oxidation of **2** by 10-DodFl would proceed quasi-intramolecularly in a micelle aggregate.

Examination of Table I reveals that the use of 10-DodFl is effective in suppressing the condensation of 4-chlorobenzaldehyde. The trapping efficiency in the presence of 0.4 mM 10-DodFl is

(16) Fendler, J. H.; Fendler, E. J. "Catalysis in Micellar and Macromolecular Systems"; Academic Press: New York, 1975.

(17) (a) Bunton, C. A.; Hamed, F.; Romsted, L. S. *Tetrahedron Lett.* **1980**, 1217. (b) Bunton, C. A.; Romsted, L. S.; Thamavit, C. *J. Am. Chem. Soc.* **1980**, *102*, 3900.

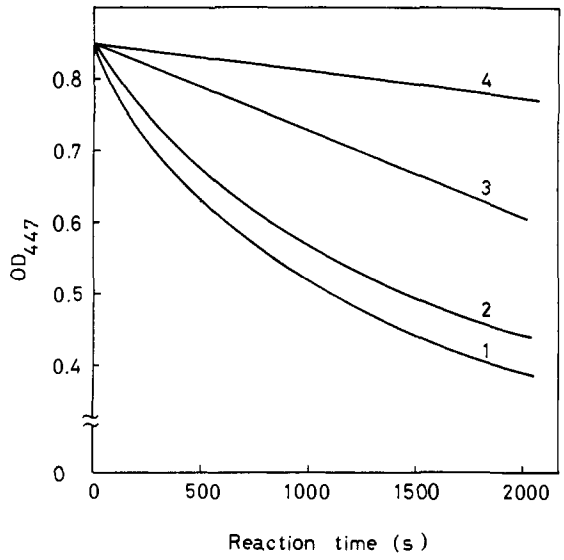


Figure 3. Absorbance of 10-DodFl (OD_{447}) vs. reaction time (pH 7.50 with 0.02 M phosphate, [10-DodFl] = 1.00×10^{-4} M, [HxdT] = 1.00×10^{-4} M, [CTAB] = 10 mM): 1, [pyCHO] = 100 mM; 2, 50 mM; 3, 10 mM; 4, 3 mM.

33 (=26/0.8) and is much better than that of MeFl, even in the presence of only 0.1 mM 10-DodFl (15 = 23/1.5). Clearly, 10-DodFl acts as an efficient oxidant of **2** due to the enhanced proximity effect. The improved trapping efficiency thus is attributed to the favorable partitioning of 10-DodFl into the micelle phase in preference to the substrate.

Kinetic examination using pyCHO as a substrate is illustrated in Figure 3. At low substrate concentrations (3–10 mM), a zero-order decrease was observed initially, followed by a relatively slow first-order decrease. In lines 3 and 4, the first-order decrease, which is not shown in Figure 3, began above ca. 50% reaction. On the other hand, at high substrate concentrations (50–100 mM) the reaction wholly satisfied a first-order equation. Hence, in this system the concentration of pyCHO controls which step of the overall process is rate determining. Because of the kinetic complexity, this system was not further investigated.

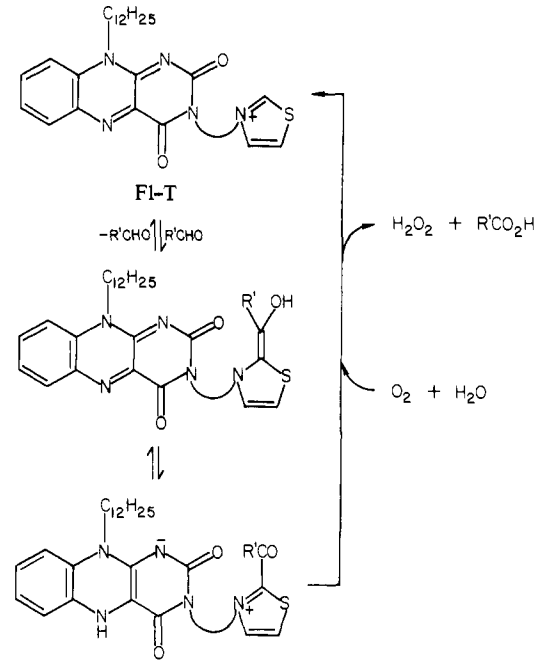
Intramolecular Flavin Oxidation (Biscoenzyme Catalysis). To the best of our knowledge, only one preceding example of a biscoenzyme is reported: Blankenhorn[18] synthesized flavin–nicotinamide biscoenzymes covalently linked by two, three, or four methylene groups through positions $N(10)$ of the flavin and $N(1)$ of the nicotinamide. He demonstrated that the biscoenzymes form long-wavelength-absorbing, intramolecular complexes between oxidized flavin and reduced nicotinamide and that the rate of intramolecular flavin-dependent dihydronicotinamide dehydrogenation is highest for a biscoenzyme with three methylene groups. However, he did not use these biscoenzymes as "catalysts" for flavin (or nicotinamide)-dependent redox reactions. Probably, this paper is the first example of a biscoenzyme-mediated redox reaction.

A thiazolium ion can be attached either to $N(10)$ or to $N(3)$ of the isoalloxazine nucleus. For the sake of the synthetic simplicity, we chose the 3-substituted isoalloxazine with a three methylene spacer. A CPK model of Fl–T suggests that when Fl–T adopts the folded conformation, the 2-position of the thiazolium ion residues proximate to the 4a-position of the isoalloxazine. Since these two positions can be involved in the reaction of each coenzyme,[1,2,12,13] intramolecular flavin oxidation of **2** is expected.

Examination of Table I shows that the condensation reaction is suppressed almost completely in the presence of Fl–T, the trapping efficiency being better than 115. In particular, condensation products were not detected at all in the presence of 5 mM of Fl–T. The remarkably high trapping efficiency should be accounted for by the high proximity of the intramolecular

(18) Blankenhorn, G. *Eur. J. Biochem.* **1975**, *50*, 351.

Scheme V



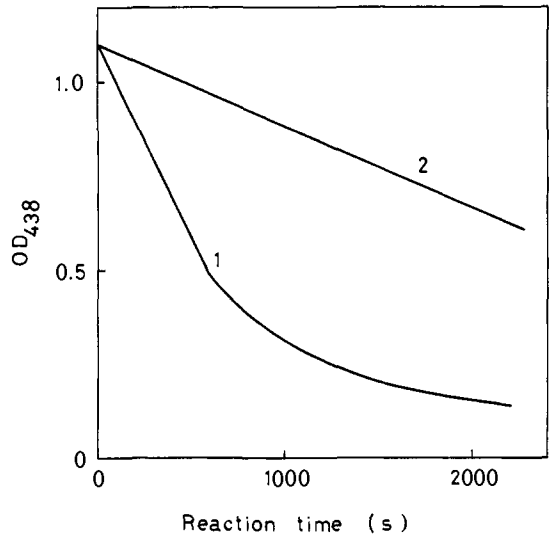


Figure 4. Absorbance of Fl–T (OD_{438}) vs. reaction time (pH 7.50 with 0.02 M phosphate, [Fl–T] = 1.00×10^{-4} M, [CTAB] = 10 mM): 1, [pyCHO] = 50 mM; 2, 10 mM.

isoalloxazine. Since the yields of 4-chlorobenzoic acid calculated on the basis of Fl–T amount to 680–4500%, the biscoenzyme is recycled in a pingpong manner under the conditions studied.

The typical examples of the time dependence for the oxidation of pyCHO by Fl–T under anaerobic conditions are shown in Figure 4. A clear zero-order decrease of Fl–T was observed during the initial stage of the reaction followed by a relatively slow, first-order decrease (above ca. 60% reaction). First-order plots of $(\ln (A_0 - A_\infty)/(A_t - A_\infty)$ vs. reaction time (data not shown) did not give straight lines during the initial 60% of the reaction, but linear plots were obtained beyond this point. Thus, the rate-determining step of the initial stage of the reaction is the deprotonation of HxdT–S to yield reactive **2** (i.e., k_2) and that of the second stage is the isoalloxazine oxidation of **2**. The initial zero-order decrease was analyzed by eq 1. As shown in Figure 5, the reaction is first order in pyCHO under the experimental conditions and $[H^+] >> K_1K_a[S]_0$ is assumed. One may thus rewrite eq 1 as eq 4. Hence,

$$v_{obsd} = \frac{k_2K_1K_a[S]_0[HxdT]_0}{[H^+]} = k_{2,app}[S]_0[HxdT]_0 \quad (4)$$

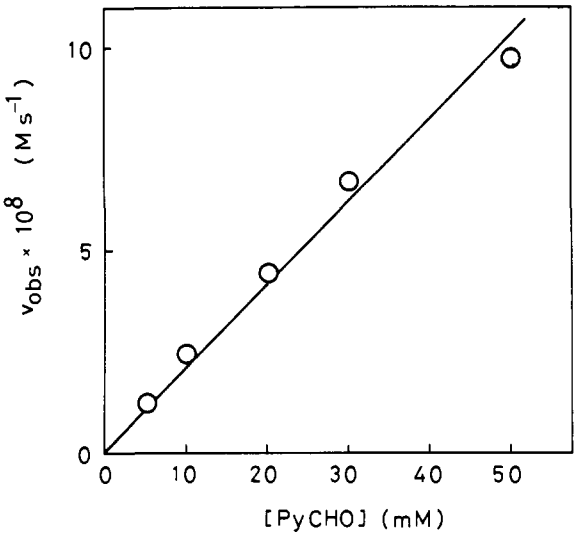


Figure 5. Plot of reaction rate for the initial zero-order stage vs. pyCHO concentration (pH 7.50 with 0.02 M phosphate, [Fl-T] = 1.00×10^{-4} M, [CTAB] = 10 mM.

the apparent second-order rate constant ($k_{2,app}$ = slope of Figure 5/[HxdT]$_0$) is equivalent to $k_2K_1K_a/[H^+]$. We thus obtained $k_{2,app}$ = 2.08×10^{-2} M^{-1} s^{-1} and $k_2K_1K_a = 6.57 \times 10^{-10}$ s^{-1}. In order to derive eq 3 from eq 2, we assumed that $K_1K_a[S]_0$ is sufficiently smaller than $[H^+]$. That in fact $[H^+] >> K_1K_a[S]_0$ is verified by eq 4 (cf. eq 1) and the linear dependence of v_{obsd} on $[S]_0$ (see Figure 5). On the other hand, we could not neglect $K_1K_2K_a$ in comparison to $[H^+]$ in eq 3. These facts suggest that K_2 (deprotonation–protonation equilibrium of HxdT-S in Scheme IV) is relatively greater than K_1 or K_a.

The pseudo-first-order rate constants for the second stage of the reaction are plotted against the concentration of pyCHO in Figure 6A. The rate constants clearly indicate a saturation phenomenon, so that the data were again analyzed according to eq 3. From the least-squares computation of the plot of $1/k_{obsd}$ vs. 1/[pyCHO] (Figure 6B), we obtained the slope (12.3 M s) and the intercept (690 s): Thus, $k_3 = 14.5$ M^{-1} s^{-1} and $K_1K_2K_a = 1.77 \times 10^{-6}$. Surprisingly, the k_3 value for the second stage is greater only by a factor of 1.6 than that for the intermolecular flavin oxidation. This seems apparently incompatible with the result of the product analysis. One should note, however, that the kinetic measurements were carried out under anaerobic conditions. The product analysis was performed under aerobic conditions where the isoalloxazine molecule of Fl-T is always renewed as its oxidized form through a pingpong-type reoxidation by molecular oxygen. Under these conditions, the reactive species **2** in the thiazolium moiety of Fl_{ox}-T is rapidly trapped by the intramolecular isoalloxazine. In the second-stage of the anaerobic kinetic conditions, however, a considerable fraction of the isoalloxazine has been converted to its reduced form. The reactive species **2** on the thiazolium moiety of Fl_{red}-T thus will be oxidized by intermolecular isoalloxazine in the Fl_{ox}-T form. This rationalizes why k_3 for Fl-T is not much greater than that for MeFl plus HxdT. With the biscoenzyme Fl-T, we believe that the initial, zero-order stage reflects intramolecular flavin trapping and the second, first-order stage reflects intermolecular flavin trapping. Probably, the zero-order-like conditions are maintained throughout the aerobic oxidation of 4-chlorobenzaldehyde by Fl-T.

Concluding Remarks. The present study establishes that the efficiency of flavin trapping of active aldehydes of thiazolium ions is remarkably improved by combining thiazolium ion and flavin within a single molecular structure or within a micelle aggregate. This is an interesting application of the concept of intramolecular catalysis to biscoenzymes. Also significant is that biscoenzymes may serve as interesting models not only for pyruvate oxidase but also for multienzyme complexes such as pyruvate dehydrogenase. The high efficiency of intramolecular catalysis has frequently been regarded as model system of enzymic catalysis.[19,20] The combination of various coenzymes within a molecule shows further interesting catalytic functions.

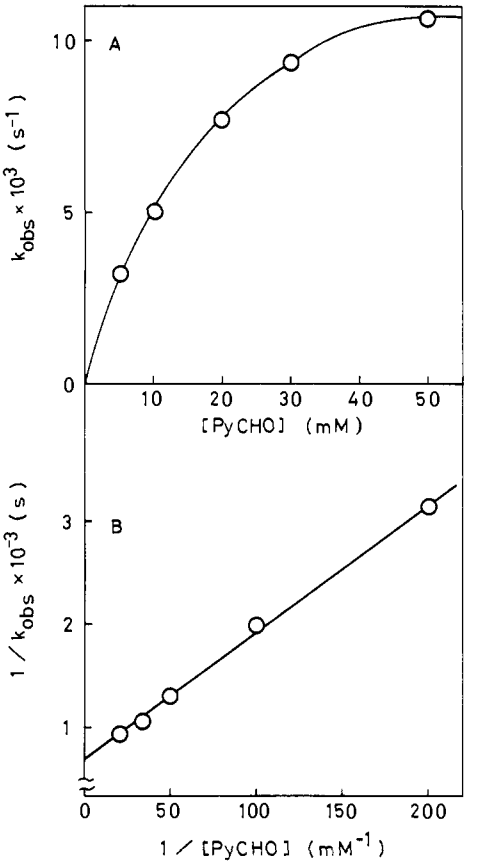


Figure 6. Pseudo-first-order rate constant for the second first-order stage as a function of pyCHO concentration (pH 7.50 with 0.02 M phosphate, [Fl-T] = 1.00×10^{-4} M, [CTAB] = 10 mM).

Experimental Section

Materials. Preparations of HxdT, MeFl, 10-DodFl, and authentic samples for high-ressure LC analysis were described previously.[8,9,21]

Fl-T was synthesized from 10-DodFl via the alkylation of the *N*(3) position with 1,3-dibromopropane. To 60 mL of *N*,*N*-dimethylformaldehyde (DMF) solution of 10-DodFl (1.0 g, 2.6 mmol) were added powdered potassium carbonate (17 g, 0.12 mol) and 1,3-dibromopropane (50 g, 0.25 mol), and the solution was stirred at 80–85 °C for 45 min. Potassium carbonate was filtered off, DMF and excess 1,3-dibromopropane in the filtrate being evaporated in vacuo. The residue which contained three compounds was subjected to thin-layer chromatography (silica gel and MeOH–$CHCl_3$ in 2:98 v/v). Three colored bands on the thin-layer plate were collected and then extracted with methanol. After evaporation, each residue was recrystallized from hexane-saturated ethanol. Based on IR and elemental analysis, we identified them (in the order of low R_f to high R_f) to be 10-DodFl, 3-(γ-hydroxylpropyl)-10-dodecyliosalloxazine (yield, 13%; mp 125–126 °C; IR (KBr disk) ν_{OH} 3440 cm^{-1}, $\nu_{C=O}$ 1705 cm^{-1}), and 3-(γ-bromopropyl)-10-dodecylisoalloxazine (yield, 56%; mp 138–139 °C; IR (KBr disk) $\nu_{C=O}$ 1705 cm^{-1}). The results of the elemental analysis for the latter two isoalloxazines were in satisfactory agreement with those of the required structures.

Treatment of 3-(γ-bromopropyl)-10-dodecylisoalloxazine (0.73 g, 1.4 mmol) with thiazole (0.5 mL, 7.5 mmol) in 40 mL of benzene under reflux gave the yellow precipitate (Fl-T), which was collected by suction, washed with isopropyl ether, and recrystallized from ethanol–isopropyl ether: yield 34%; mp 165–166 °C; IR (KBr disk) $\nu_{C=O}$ 1705 cm^{-1}. Anal. ($C_{28}H_{38}N_5O_2SBr \cdot H_2O$): C, H, N.

Product Analysis. The aerobic oxidation of 4-chlorobenzaldehyde was carried out at 30 °C and pH 8.0 with 0.02 M phosphate buffer for 1 day in the dark. Oxygen was bubbled into the reaction mixture at a constant

(19) Fife, T. H. "Bioorganic Chemistry"; Van Tammelen, E. E., Ed.; Academic Press: New York, 1977; Vol. 1, p 93.
(20) Bruice, T. C.; Benkovic, S. "Bioorganic Mechanisms"; W. A. Benjamin: New York, 1966; Chapter 1.
(21) (a) Shinkai, S; Harada, A.; Ishikawa, Y.; Manabe, O.; Yoneda, F. *Chem. Lett.* **1981**, 479. (b) *J. Chem. Soc., Perkin Trans. 2*, in press.

rate, confirming that the yellow color of flavin does not disappear. The products were analyzed by using high-pressure LC (Shimadzu LC-3). Four peaks appeared, which were identified to be 4-chlorobenzoic acid, 4,4'-dichlorobenzoin, 4,4'-dichlorobenzil, and 4-chlorobenzaldehyde by using each authentic sample. The sum of these four compounds was always better than 70%. The details of the treatment of the reaction products and the product analysis were described previously.[8,9]

Kinetics. The kinetic measurements of the flavin oxidation of aldehydes were carried out at 30 °C under anaerobic (N_2) conditions in 3 vol % aqueous ethanol. A Thunberg cuvette was used to provide the anaerobic reaction conditions. The progress of the reaction was monitored spectrophotometrically by following the disappearance of each absorption maximum of flavin: 448 nm for MeFl, 447 nm for 10-DodFl, and 438 nm for Fl-T. The detailed procedure was described previously.[8,9]

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Registry No. 4-Chlorobenzaldehyde, 104-88-1; 3-(*r*-hydroxypropyl)-10-dodecylisoalloxazine, 79828-14-1; 3-(*r*-bromopropyl)-10-dodecylisoalloxazine, 79828-15-2; HxdT, 75066-49-8; MeFl, 79839-37-5; 10-DodFl, 79828-16-3; Fl-T, 79828-17-4; PyCHO, 872-85-5.

Solvent Effects on Rates of Free-Radical Reactions. Addition of the *p*-Aminobenzenethiyl Radical to Styrene[1]

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Abstract: Absolute rate constants (k_1) for addition of the *p*-aminobenzenethiyl radical (*p*-$NH_2C_6H_4S\cdot$) to styrene in 26 kinds of solvents have been determined by the flash photolysis technique. The relative rate constants for the reverse reactions and the relative equilibrium constants have also been estimated. A large bathochromic shift of the transient absorption maxima of *p*-$NH_2C_6H_4S\cdot$ was observed with an increase in solvent polarity, which suggests the stabilization of *p*-$NH_2C_6H_4S\cdot$ by the solvation of polar solvents. The k_1 values decreased largely with solvent polarity, whereas the relative reverse rate constants were practically invariant. This suggests that the solvated thiyl radicals in the reactant lose many of the solvent molecules in the transition state. In the plots of log k_1 vs. empirical solvent polarity parameters such as Dimroth-Reichardt's $E_T(30)$, a linear relationship was observed for most solvents. Solvents having hydrogen-bonding ability and electron-accepting ability accelerate the rates, and alkylamines reduce the rates; the solvent polarity is a main factor governing the solvent effect on the reaction and the specific interactions also play important roles.

An extensive study has revealed that the medium affects the reactivity and selectivity of the free-radical reacitons.[2] The solvent effects in atom transfer, disproportionation, and addition of the free radicals have mainly been interpreted in terms of specific interactions between the free radicals and solvents such as electron donor-acceptor interaction[3-5] and hydrogen bonding.[6,7] The electron donnor-acceptor complexes between halogen atoms and π-electron donors have been directly detected by means of the flash-photolysis technique by Strong.[8] The importance of sovlent polarity in the free-radical reactions was first pointed out by Walling and Wagner.[7] The effects of solvent polarity on the hyperfine structures of the ESR spectra of stable free radicals[9-12] suggested that large solvent polarity effects would be anticipated in the free-radical reactions if polarizable free radicals are chosen. We will report in this paper our finding that the introduction of the amino group in the para position on the benzenethiyl radical makes the thiyl radical polarizable and that both the absorption bands of *p*-$NH_2C_6H_4S\cdot$ and the absolute addition rate constants of *p*-$NH_2C_6H_4S\cdot$ to styrene, which can be determined by the flash-photolysis technique,[1,13] vary greatly with the change of solvent polarity.

Results and Discussion

Assignment and Solvent Effect of the Absorption Band of *p*-$NH_2C_6H_4S\cdot$. Figure 1 shows the transient absorption spectra observed by the flash photodecomposition of $(p\text{-}NH_2C_6H_4)_2S_2$ in various solvents. These absorption bands were ascribed to *p*-$NH_2C_6H_4S\cdot$ since the same absorption band was observed by the photolysis of *p*-aminobenzenethiol in each solvent. A similar spectrum was reported by Thyrion.[14]

The bathochromic shift of the absorption maxima and the broadening of the bandwidths were observed with an increase in solvent polarity. Both the shift and broadening correlate roughly to some empirical parameters of solvent polarity such as Kosower's Z values[15] and Dimroth-Reichardt's $E_T(30)$ values,[16] but definite

(1) Evaluation of Addition Rates of Thiyl Radicals to Vinyl Monomers by Flash Photolysis. 4. For part 3 see: Ito, O.; Matsuda, M. *J. Am. Chem. Soc.* **1981**, *103*, 5871.
(2) Reviews and references therein: (a) Huyser, E. S. In "Advances in Free-Radical Chemistry", Williams, G. H., Ed., Logos Press: New York, 1965; Vol. I, p 77. (b) Martin, J. C. In "Free Radicals", Kochi, J. K., Ed., Wiley: New York, 1973; Vol. I, Chapter 20. (c) Reichardt, D. "Solvent Effects in Organic Chemistry"; Verlarg Chemie: Weinheim, 1979; p 110.
(3) Russell, G. A. *J. Am. Chem. Soc.* **1958**, *80*, 4987.
(4) Walling, C., Wagner, P. *J. Am. Chem. Soc.* **1963**, *85*, 2333.
(5) Huyser, E. S.; Kim, L. *J. Org. Chem.* **1967**, *32*, 618.
(6) Bacha, J. D.; Kochi, J. K. *J. Org. Chem.* **1965**, *30*, 3272.
(7) Walling, C.; Wagner, P. *J. Am. Chem. Soc.* **1964**, *86*, 3368.
(8) Strong, R. S. *J. Phys. Chem.* **1962**, *66*, 2423.
(9) Gendell, J.; Freed, J. H.; Frenkel, G. K. *J. Chem. Phys.* **1962**, *37*, 2832.
(10) Hayat, H.; Silver, B. L. *J. Phys. Chem.* **1973**, *77*, 72.
(11) Reddoch, A. H.; Konishi, S. *J. Chem. Phys.* **1979**, *70*, 2121.
(12) Kubota, S.; Ikegami, Y. *J. Phys. Chem.* **1979**, *82*, 2739.
(13) Ito, O.; Matsuda, M. *J. Am. Chem. Soc.* **1979**, *101*, 1815.
(14) Thyrion, F. C. *J. Phys. Chem.* **1973**, *77*, 1478.
(15) Kosower, E. M. *J. Am. Chem. Soc.* **1958**, *80*, 3253.
(16) Dimroth, K.; Reichardt, C.; Siepmann, T.; Bohlmann, F. *Justus Liebigs Ann. Chem.* **1963**, *661*, 1.

0002-7863/82/1504-0568$01.25/0