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Coenzyme Models. 33. Evidence for Retro-acyloin Condensation as Catalyzed by Thiazolium Ion and Cationic Micelle. Oxidative Trapping of the "Active Aldehyde" Intermediates by Flavin

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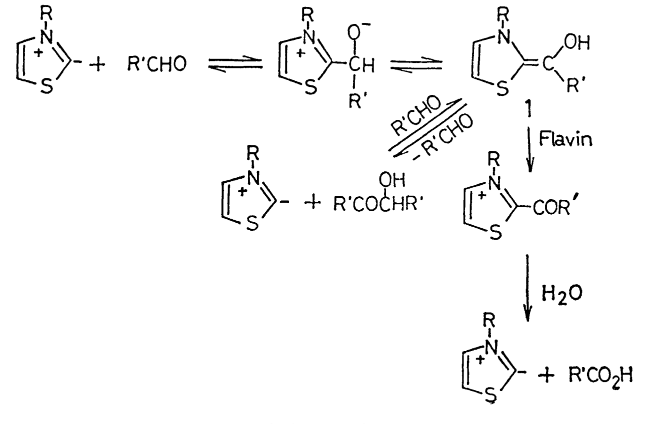
N-Hexadecylthiazolium bromide(HxdT) in the CTAB micelle, which is known as an excellent catalytic system for acyloin condensation of aldehydes, catalyzes the reverse reaction (*i.e.*, retro-acyloin condensation) to give aldehydes from α-ketols *via* the active aldehyde intermediates. The existence of the novel, HxdT-mediated process was proposed on the basis of an experimental discovery that flavin (3-methyltetra-*O*-acetylriboflavin: MeFl), which is capable of oxidatively trapping the active aldehyde intermediates, is reduced by α-ketols such as acetoin and 3-hydroxy-3-methyl-2-butanone in the micellized HxdT solution. It was further substantiated by detection of acetaldehyde in the final reaction mixture. Based on the disappearance rate of the absorbance of MeFl, we spectrophotometrically estimated the rate constants for the retro-acyloin condensation. Similarly, biacetyl, the monohydrated species of which is analogous to α-ketol, afforded acetaldehyde and acetic acid in the micellized HxdT solution, the rate constant being greater by factors of 10^2—10^3 than those for α-ketols. The relevance of the retro-acyloin condensation to biological systems (*e.g.*, the mechanism of transketolase catalysis) is discussed.

Recently, it has been established that some flavin-dependent enzymes employ carbanion intermediates during the course of the oxidation of bound substrates.[1-3] In previous publications of this series,[4-7] we have demonstrated that the application of the biochemical concept "flavin oxidation of carbanion" to organic chemistry is very useful in exploring a new class of oxidation reactions. For example, flavin is rapidly reduced according to zero-order kinetics in cyanide-catalyzed benzoin condensation,[4] thiazolium-catalyzed acyloin condensation,[5,7] and thiol-catalyzed rearrangement of glyoxals,[6] which all proceed *via* the carbanionic intermediates. The role of flavin oxidation in thiazolium-catalyzed reactions is also discussed by Yano *et al.*[8] The product analyses of these systems showed that the reactions are readily diverted to the oxidation reactions in the presence of flavin as an oxidative trapping agent. Similarly, the "active aldehydes" afforded from aldehydes and the conjugate bases of thiazolium ions are oxidized by nitrobenzene,[9] acridine and its analogues,[10] disulfides,[11] and nitrosobenzene.[12] In Scheme 1, we illustrated a typical example for flavin trapping in thiazolium-mediated acyloin condensation, in which the key intermediate **1** is rapidly oxidized by flavin to yield the corresponding carboxylic acid.[5]

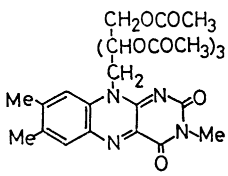


Scheme 1.

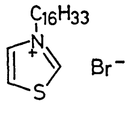
It is a well-known fact that the conjugate bases of thiazolium ions (vitamin B_1 analogues) catalyze acyloin condensation as cyanide ion catalyzes benzoin condensation.[13] To the best of our knowledge, however, there is no clear evidence as to whether the conjugate bases catalyze the reverse reaction (retro-acyloin condensation) to afford aldehydes from α-ketols. It occurred to us that if the retro-acyloin condensation is catalyzed by thiazolium ions, the transient intermediates **1** would be rapidly trapped by flavin. In the enzymatic system, the mechanism of transketolase which requires thiamine pyrophosphate(TPP) as a cofactor and catalyzes the cleavage of xylulose 5-phosphate or D-fructose 6-phosphate in its early stage is analogous to that of the retro-acyloin condensation.[14-16] Christen and Gasser[17] have reported that the 1,2-dihydroxyethyl–TPP intermediates (analogues of **1**) formed from these substrates in the active site of the enzyme can be oxidatively trapped by hexacyanoferrate(III). Based on flavin-trapping of **1** from α-ketols, we here report evidence that *N*-hexadecylthiazolium bromide(HxdT) bound to the CTAB(hexadecyltrimethylammonium bromide) micelle catalyzes not only the acyloin condensation[13,18] but also the retro-acyloin condensation.

Results and Discussion

Flavin Oxidation of α-Ketols and Analogues in Micellized HxdT Solution. We have used 3-methyltetra-*O*-acetylriboflavin(MeFl) and HxdT in the cationic CTAB solution. The kinetic measurements of the flavin oxidation were carried out at 30 °C under anaerobic conditions(N_2). The reaction was followed spectrophoto-



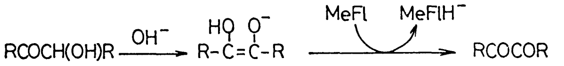
MeFl



HxdT

metrically by monitoring the disappearance of the absorption band of MeFl at 448 nm (ε 12200). Since the active aldehydes rapidly reduce MeFl,[5,8] the production of **1** from the substrates, if it occurs, can be immediately detected as a decrease in the absorption band.

In an anaerobic aqueous solution containing HxdT (1 mM) and CTAB (10 mM), MeFl was reduced by acetoin, benzoin, 3-hydroxy-3-methyl-2-butanone (HMB), 3-hydroxy-3-phenyl-2-butanone(HPB), and biacetyl, but the reaction with benzil and 4,4′-dichlorobenzil was not detected. As reported in the thiazolium-mediated flavin oxidation of aldehydes,[5] the zero-order decrease of the 448 nm band was observed for the initial stage of the reaction and lasted up to, for example, 40% reaction for acetoin (0.1 M). We noticed, however, that acetoin and benzoin in the CTAB micelle solution reduce MeFl even in the absence of HxdT. The reductant in this system is a 1,2-enediolate which is formed by base-catalyzed deprotonation of these "carbon acids" (Eq. 1).[19–21] Thus, the rates



$$\mathrm{RCOCH(OH)R} \xrightarrow{\mathrm{OH^-}} \mathrm{R{-}\overset{HO}{C}{=}\overset{O^-}{C}{-}R} \xrightarrow{\mathrm{MeFl}\ \ \mathrm{MeFlH^-}} \mathrm{RCOCOR} \qquad (1)$$

observed in the micellized HxdT solution (v_{obsd}) are expressed as the sum of the base-catalyzed term (v_{base}) and the HxdT-catalyzed term (v_{HxdT}) (Eq. 2),

$$v_{\mathrm{obsd}} = v_{\mathrm{base}} + v_{\mathrm{HxdT}}, \qquad (2)$$

where v_{base} is the rate of the flavin reduction in the CTAB micelle solution and v_{obsd} is that in the presence of both HxdT and the CTAB micelle.

We found that the base-catalyzed deprotonation of benzoin is so fast in the CTAB micelle that the accurate determination of v_{HxdT} from Eq. 2 is rather difficult. The facile deprotonation of benzoin is due to the acidity of benzoin as carbon acid. On the other hand, the v_{base} of acetoin was relatively slow, the v_{obsd} being greater by a factor of 6.1 than the v_{base}. From Eq. 2, we thus determined the v_{HxdT} which is responsible for the HxdT-mediated retro-acyloin condensation. More unequivocal evidence was obtained from the reaction with HMB and HPB since the base-catalyzed deprotonation is not the case in these substrates. In fact, MeFl was not reduced at all by HMB and HPB in the CTAB micelle. On the other hand, the reduction of MeFl occurred on the addition of HxdT to the micelle solution. We thus determined the v_{HxdT} ($=v_{\mathrm{obsd}}$) directly. Biacetyl, the monohydrated species of which is an analogue of α-ketol, also reduced MeFl in the micellized HxdT solution, the rate being much faster than that of α-ketols.

For the specification of the reaction order, the HxdT-catalyzed rates (v_{HxdT}) were determined as a function of the concentration of HxdT (0.2—2 mM) or substrates (for the concentration ranges see Table 1). It was found that the rates are first-order with respect to HxdT and substrates under these experimental conditions. The HxdT-catalyzed rates were thus given by Eq. 3. The $k_{2,\mathrm{app}}$ values are summarized in Table

$$v_{\mathrm{HxdT}} = k_{2,\mathrm{app}}[\mathrm{S}]^{1.0}[\mathrm{HxdT}]^{1.0}[\mathrm{MeFl}]^{0} \qquad (3)$$

1. The examination of this table reveals that (i) in aqueous solution, the thiazolium-mediated reduction of MeFl occurs only in the presence of the cationic micelle, (ii) the $k_{2,\mathrm{app}}$ for $\mathrm{CH_3COC(OH)(CH_3)X}$ series increases with increasing electron-withdrawing nature of X (*i.e.*, $\mathrm{CH_3 < H < C_6H_5}$), (iii) the reaction with biacetyl is much faster than that with analogous α-ketols, the rate constant being greater by factors of 10^2—10^3, and (iv) benzil and 4,4′-dichlorobenzil are totally unreactive. The effect of the substituents (ii) will be discussed later. The remarkable reactivity difference between biacetyl and benzil analogues is ascribed to the steric effect. For example, we have found that the micellized thiazolium ions is capable of catalyzing the decarboxylation of pyruvic acid but not that of 4-chlorobenzoylformic acid.[5] Both results suggest that the carbonyl group next to a phenyl ring is sterically deactivated in the thiazolium-mediated reactions.

As a summary of the foregoing results, one can safely conclude that the "active aldehydes" are produced from α-ketols and biacetyl in the micellized HxdT solution. In other words, it implies that the retro-acyloin condensation is mediated by micellized thiazolium ions. Subsequently, we carried out the product analyses of these reaction systems.

Product Analyses of the HxdT-mediated Retro-acyloin Condensation Systems. If HxdT-mediated retro-acyloin condensation takes place, 1 mol of acetoin afford

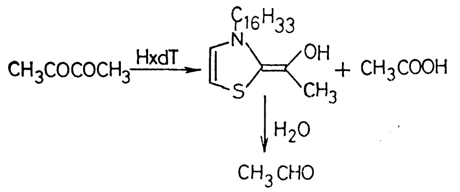
Table 1. Apparent second-order rate constants ($10^3 k_{2,\mathrm{app}}\ \mathrm{M^{-1}\,s^{-1}}$) for the oxidation of α-ketols and analogues[a)]

Substrate(S)	$\frac{[\mathrm{S}]}{\mathrm{mM}}$	$\frac{\mathrm{CTAB}}{\mathrm{mM}}$	pH	$\frac{10^3 k_{2,\mathrm{app}}}{\mathrm{M^{-1}\,s^{-1}}}$
$\mathrm{CH_3COCH(OH)CH_3}$	30—200	10	7.8	0.149
$\mathrm{CH_3COC(OH)(CH_3)_2}$	30—200	10	7.8	0.0116
$\mathrm{CH_3COC(OH)(CH_3)_2}$[b)]	30—200	0	7.8	No reaction
$\mathrm{CH_3COC(OH)(CH_3)C_6H_5}$	10— 50	10	7.8	2.05
$\mathrm{CH_3COCOCH_3}$	0.5—3.0	10	7.8	21.8
$\mathrm{CH_3COCOCH_3}$	0.5—3.0	10	8.0	27.6
$\mathrm{C_6H_5COCOC_6H_5}$	0.2—2.0	10	8.0	No reaction
$\mathrm{4\text{-}ClC_6H_4COCOC_6H_4Cl(4-)}$	0.2—2.0	10	8.0	No reaction

a) 30 °C, [HxdT] = 1.00 mM, [MeFl] = 5.00×10^{-5} M. pH was adjusted with 0.01 M phosphate. b) *N*-Benzylthiazolium bromide(1.00 mM) was used instead of HxdT.

2 mol of acetaldehyde, and 1 mol of 3-hydroxy-3-methyl-2-butanone affords 1 mol of acetaldehyde and 1 mol of acetone. The reactions were carried out at 30 °C in anaerobic (N_2) ampuls. After 1 d, we treated the final reaction solution with 2,4-dinitrophenylhydrazine[22] and analyzed the products as their hydrazone derivatives by using high-pressure liquid chromatography(HPLC). On the other hand, acetic acid was analyzed by GLC before the treatment with 2,4-dinitrophenylhydrazine. The results are summarized in Table 2. The examination of Table 2 reveals that the expected products are detected in the micellized HxdT solution, whereas no such product is afforded in the absence of HxdT. The yields of these products are relatively low, however. The low yields would be ascribed to the consumption of acetaldehyde (or acetone) by aldol-type condensation. In fact, we confirmed in a separate study that the concentration of acetaldehyde decreases with time in the CTAB micelle solution. These data support that HxdT in the CTAB micelle is capable of catalyzing the retro-acyloin condensation. It is now obvious, therefore, that the reduction of MeFl observed in the kinetic measurements is attributable to the reaction of MeFl with the active aldehydes (**1**) afforded from these substrates (see Scheme 1).

Table 3 shows that in the micellized HxdT solution biacetyl is converted to acetaldehyde and acetic acid. This reaction, the socalled biacetyl mutase reaction, has been found by Mizuhara and Oono.[23] The quantitative scission of 1 mol biacetyl gives 1 mol of acetic acid and 1 mol of acetaldehyde (Eq. 4). As shown in Table 3, however, the yield of acetic acid is much higher than that of acetaldehyde. The relatively low yield of acetaldehyde is again attributed to the consumption by aldol-type condensation. The yield of acetic acid calculated on the basis of HxdT was 190%. This implies that HxdT acts as a recycle catalyst. It is presumed that this reaction also proceeds *via* the active aldehyde intermediate.[23] In fact, biacetyl in the micellized HxdT solution reduces MeFl (*vide supra*), indicating the formation of the active aldehyde from biacetyl and HxdT. We added flavin to the reaction system, and carried out the product analysis. Entry 3 in Table 3 shows that in the presence of FMN(flavin mononucleotide) the yield of acetaldehyde is lowered, while that of acetic acid is significantly enhanced. It seems likely, therefore, that the added FMN diverts (at least partially) the reaction route from the biacetyl mutase to the oxidation. The incompleteness of the trapping efficiency is related to the following experimental difficulties. When the reaction is carried out under aerobic conditions where FMN serves as recycle oxidation catalyst, hydrogen peroxide formed through the reoxidation process of reduced FMN also oxidizes biacetyl to acetic acid (see entry 4 in Table 3). This makes the reaction system very complex. In order to preclude the oxidation by hydrogen peroxide, one has to carry out the reaction under anaerobic conditions and enhance the flavin concentration. However, MeFl is not so soluble in the reaction medium while water-soluble FMN caused the precipitation of CTAB at its high concentration. Thus, the flavin trapping in the present system could be demonstrated only qualitatively.



(4)

Table 2. Product analyses of HxdT-mediated retro-acyloin condensation of acetoin and HMB[a)]

Entry	Substrate	HxdT (mM)	Product(%)[b)]	
			CH_3CHO	$(CH_3)_2CO$
1	$CH_3COCH(OH)CH_3$	5	9.8	Trace(<1)
2	$CH_3COC(OH)(CH_3)_2$	5	7.8	14
3	$CH_3COC(OH)(CH_3)_2$	0	Trace(<0.1)	Trace(<1)

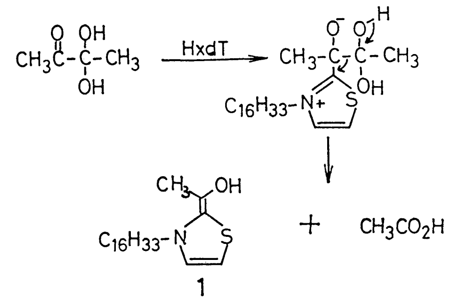
a) 30 °C, pH 7.4 with 0.01 M phosphate, 1 d, anaerobic (N_2), [substrate] = 100 mM, [CTAB] = 30 mM. b) Acetaldehyde and acetone were recovered as their 2,4-dinitrophenylhydrazone derivatives and the yields were determined by HPLC. The yields were calculated on the basis of HxdT.

Table 3. Product analyses of HxdT-mediated reaction of biacetyl[a)]

Entry	HxdT (mM)	Flavin (mM)	CTAB (mM)	Product(%)[b)]	
				CH_3CHO	CH_3CO_2H
1	0	0	30	0	0
2	5	0	30	10	190
3	5	10[c)]	30	6.4	510
4[d)]	0	0	10	0	(5.8 mM)

a) 30 °C, pH 7.4 with 0.01 M phosphate, 1 d, anaerobic (N_2), [biacetyl] = 100 mM. b) Acetaldehyde was recovered as its 2,4-dinitrophenylhydrazone derivative and analyzed by HPLC. Acetic acid was analyzed by GLC. The yields were calculated on the basis of HxdT. c) [FMN] = 10.0 mM. d) [$CH_3COCOCH_3$] = 30.0 mM, [H_2O_2] = 30 mM.

Comments on the Mechanism of Thiazolium-mediated Biacetyl Scission. Here, we wish to consider the mechanism of the HxdT-mediated biacetyl scission. Mizuhara and Oono,[23] who found for the first time that thiamine would catalyze the biacetyl mutase reaction, proposed that the mutase reaction begins with the reaction of thiamine and biacetyl enolate to give ketene and **1**. However, the mechanism involving the ketene production is now considered to be most improbable.[24] Here, two possible mechanisms come to mind: one is the reaction analogous to retro-acyloin condensation which occurs from the monohydrated form of biacetyl directly affording acetic acid and **1** (Eq. 5), and the other is the mechanism similar to that proposed for cyanide-ion catalyzed C–C bound scission of α,β-diketones (*e.g.*, benzil)[25,26] (Eq. 6). It is not easy to distinguish between these two mechanisms by conventional kinetic methods. Based on the flavin trapping of **1**, we could determine the rate constant for biacetyl scission as well as those of α-ketol scission. Since biacetyl is extensively hydrated in aqueous solution,[27] one may regard diacetyl as a substrate with X=OH in $CH_3COC(OH)(CH_3)X$ series. We noticed that the plot of Taft's σ_I[28] *vs.* $\log k_{2,app}$ of $CH_3COC(OH)(CH_3)X$ holds a good linear relationship (r=0.97) (Fig. 1). Here, one has to take it into account that $k_{2,app}$ in the micellar system is affected not only by the reactivity of the substrate but also by the partition of the substrate between the micelle and the bulk water phase. We carried out the same reaction in a homogeneous solution (methanol) in the presence of strong base (DBU: 1,8-diazabicyclo[5.4.0]-undec-7-ene). As shown in Fig. 1, the plot for X=CH_3, H, and phenyl again held a good straight line (r=0.99). The finding suggests that in the present case the partition would not provide a significant influence upon the rate constant. Thus, the involvement of the plot for X=OH in a single straight line implies that the mechanism of biacetyl scission would be similar to that of the retro-acyloin condensation.



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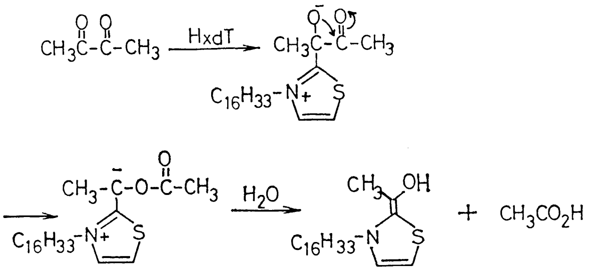

(6)

Conclusion. The present study establishes that the micellized thiazolium ion is capable of catalyzing not only the acyloin condensation but also the retro-acyloin condensation. Obviously, the thiazolium-mediated acyloin condensation is reversible. The finding suggests an important insight into the mechanism of the early stage of the transketolase catalysis. We are now devoting our research effort toward the application of this concept to transketone reaction in the model system.

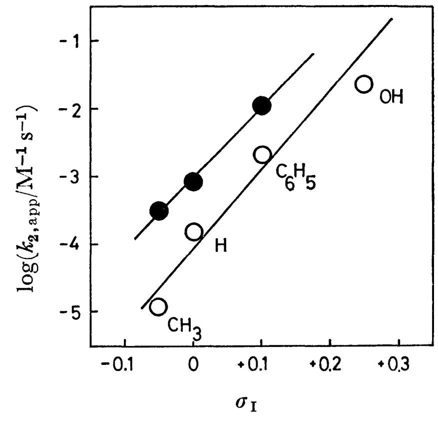

Fig. 1. Plot of σ_I for $CH_3COC(OH)(CH_3)X$ *vs.* log $k_{2,app}$. ○, Rate constants for the micellar system cited from Table 1. ●, Rate constants for the reaction in methanol, where [MeFl]$=3.30\times10^{-5}$ M, [*N*-benzylthiazolium bromide]$=1.10\times10^{-3}$ M, [DBU]$=5.30\times10^{-3}$ M. The $k_{2,app}$ is defined as v_{obsd}/[thiazolium ion][substrate]. In methanol, $k_{2,app}=3.08\times10^{-4}\,M^{-1}\,s^{-1}$ for X=CH_3, $8.09\times10^{-4}\,M^{-1}\,s^{-1}$ for X=H, and $1.10\times10^{-2}\,M^{-1}\,s^{-1}$ for X=C_6H_5.

Experimental

Materials. Preparations of the following compounds were described previously: MeFl,[29] HxdT,[5] *N*-benzylthiazolium bromide,[5] and 4,4′-dichlorobenzil.[5] Acetoin, 3-hydroxy-3-methyl-2-butanone, and biacetyl were purchased from Tokyo Kasei Co. Ltd. and were distilled before use. 3-Hydroxy-3-phenyl-2-butanone was prepared from biacetyl and benzene in the presence of $AlCl_3$ according to the method of Wegmann and Dahn.[30]

Kinetic Measurements. The kinetic measurements of the micellar system were carried out at 30 °C under anaerobic (N_2) conditions in 3 vol% of aqueous ethanol. The details of the method were described previously[4,5]. Similarly, the kinetic measurements in methanol were carried out at 30 °C under anaerobic (N_2) conditions. The details of the reaction conditions are recorded in a caption to Fig. 1.

Product Analyses. The reactions for the product analyses were carried out at 30 °C in anaerobic (N_2) sealed ampuls. The detailed conditions were recorded in Table 2. Acetic acid was analyzed by GLC (Shimadzu GC-Mimi I: column, Silicone GE SE-30). To analyze acetaldehyde and acetone, the solution was treated with an excess of 2,4-dinitrophenylhydrazine,[22] and both the precipitate and the filtrate were subjected to HPLC analysis (Shimadzu LC-3: column, Merck Lichrosorb RP-18). The hydrazone derivatives of acetaldehyde and acetone were mainly present in the filtrate. Their yields were determined by comparing the integrated intensity of the peaks with that of the authentic samples.

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