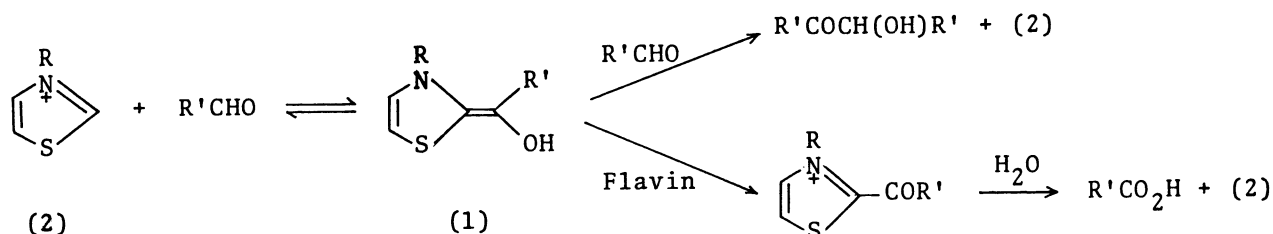


OXIDATIVE TRAPPING OF TRANSIENT THIAZOLIUM-ALDEHYDE ADDUCTS
 BY INTRAMOLECULAR FLAVIN. FLAVIN-THIAMINE BISCOENZYME MODEL

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A biscoenzyme which has within a molecular structure both flavin and thiazolium ion was synthesized. In the presence of the biscoenzyme and hexadecyltrimethylammonium bromide (CTAB) micelle, aldehydes were efficiently converted to carboxylic acids and the acyloin condensation products were scarcely detected. Thus, the biscoenzyme serves as an interesting model system for pyruvate dehydrogenase.

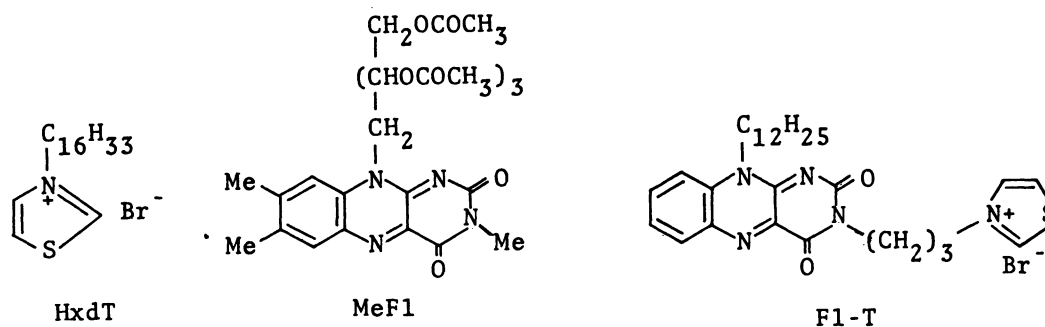
Recently, it was shown that the thiazolium-aldehyde adduct (active aldehyde: (1)) which is a key intermediate in the reaction sequence of acyloin condensation of aldehydes^{1,2)} is rapidly oxidized by flavin.³⁻⁶⁾ The reaction has a crucial similarity with the mechanistic feature of pyruvate dehydrogenase (E.C.1.2.2.2) which requires FAD and thiamine pyrophosphate as cofactors.



Scheme 1

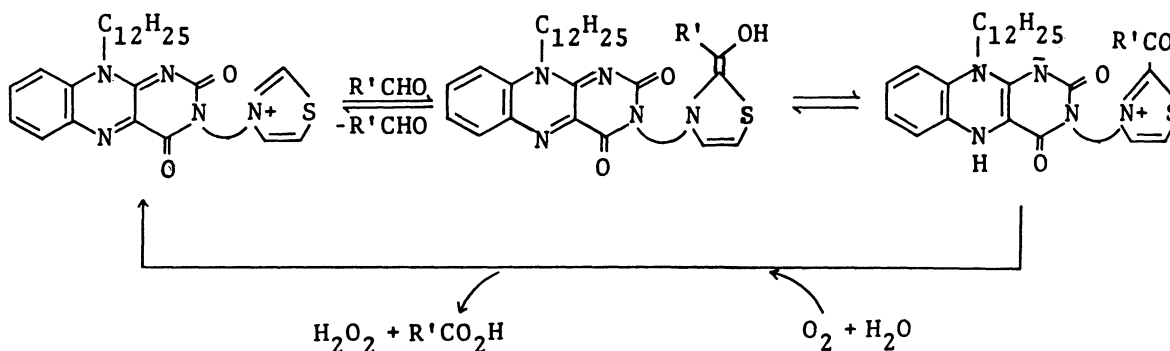
As shown in scheme 1, the flavin oxidation is competing with the conventional acyloin condensation. We found that the trapping efficiency (=oxidation product/sum of condensation products) is a function of aldehyde concentration: the higher is the aldehyde concentration, the lower becomes the trapping efficiency.⁴⁾ In order to apply this reaction to the synthetic purpose or to the enzyme model investigation, it would be desirable to suppress the competing condensation reaction completely. Therefore, we synthesized a biscoenzyme (Fl-T) which combine within a molecule both flavin and thiazolium ion⁷⁾ and compared the trapping efficiency with that of an intermolecular oxidation catalyzed by N-hexadecylthiazolium bromide (HxdT) and 3-methyltetra-O-acetylriboflavin (MeFl). To our knowledge, only one preceding example for biscoenzyme is reported, in which the charge-transfer interaction between flavin and nicotinamide is evaluated.⁸⁾ Probably, the present

study is the first example for the catalytic reaction mediated by a biscoenzyme.



As shown in Table, the trapping efficiency of intermolecular flavin oxidation (HxdT + MeFl) in the presence of CTAB (hexadecyltrimethylammonium bromide) micelle is a function of the aldehyde concentration. For example, the aerobic CTAB micellar solution containing MeFl (5 mM) and HxdT (1 mM) was able to convert 4-chlorobenzaldehyde (5 mM) to 4-chlorobenzoic acid in 63% yield and the condensation products such as 4,4'-dichlorobenzoin and 4,4'-dichlorobenzil (oxidation product from 4,4'-dichlorobenzoin) were not detected. However, when the concentration of MeFl was lowered to 1 mM and those of 4-chlorobenzaldehyde and HxdT were enhanced to 100 mM and 10 mM, respectively, the trapping efficiency was only 1.6 (=28/17). The result indicates that the rate of the formation of (1) is speeded up with increasing aldehyde concentration and it exceeds the trapping rate of intermolecular MeFl at 100 mM of 4-chlorobenzaldehyde.

On the other hand, examination of Table manifests that the condensation is suppressed almost completely in the presence of Fl-T, the trapping efficiency being always greater than 115. In particular, the condensation products were not detected at all in the presence of 5 mM of Fl-T. The remarkably high trapping efficiency should be attributed to the proximity effect of the intramolecular 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-type manner under aerobic conditions.



Scheme 2

Table. Product analysis for the flavin oxidation of 4-chlorobenzaldehyde(ArCHO)^a

ArCHO mM	MeFl mM	HxdT mM	Fl-T mM	Product (% yield)			Trapping Efficiency
				ArCO ₂ H	ArCOCH(OH)Ar	ArCOCOAr	
5	5	1	—	63	0	0	∞
50	5	1	—	26	0.4-0.7	0	37-65
100	1	10	—	28	2	15	1.6
100	—	—	5	34	0	0	∞
100	—	—	1	23	0.2	0	115
100	—	—	0.4	18	0.1	0	180
100	—	—	0	0	0	0	—

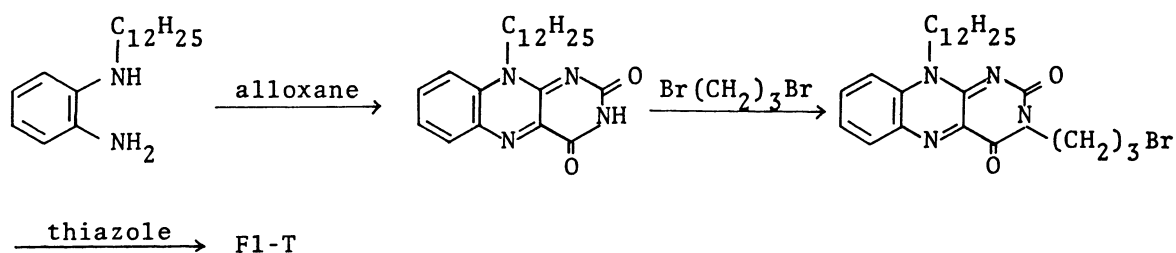
^a At 30°C and pH 8.0 with 0.05 M phosphate for 1 day in the dark. [CTAB] = 10 mM. The product analysis was carried out by using high-pressure liquid chromatography. The sum of the oxidation product, condensation products, and recovered 4-chlorobenzaldehyde was always better than 70%.

The kinetic measurements (30°C, anaerobic(N₂) conditions) were carried out by following spectrophotometrically the disappearance of the absorption band of MeFl (448 nm) or Fl-T (438 nm). When 4-chlorobenzaldehyde was used as substrate, the reaction mediated by MeFl + HxdT ([MeFl] = 5.00 × 10⁻⁵ M, [HxdT] = 1.00 × 10⁻⁴ M, [CTAB] = 10 mM, [4-chlorobenzaldehyde] = 2-50 mM) was zero-order in MeFl for up to 90% reaction. This fact implies that the oxidation of (1) by MeFl is not rate-determining and the formation of (1) should be assigned to the rate determining step. When pyridine-4-aldehyde (PyCHO), more activated aldehyde, was used as substrate, the reaction became first-order in MeFl, indicating the oxidation of (1) by intermolecular MeFl to be involved in the rate-determining step. The change in the rate-determining step is accounted for by the increase in the rate of the formation of (1) (HxdT-PyCHO adduct). On the other hand, the oxidation of PyCHO mediated by Fl-T under the comparable reaction conditions ([Fl-T] = (5-10) × 10⁻⁵ M) provided the zero-order disappearance for up to 60% reaction: that is, the formation of (1) is rate-determining. The result clearly demonstrates the high efficiency of intramolecular flavin trapping.⁹⁾

The high efficiency of intramolecular catalysis has frequently been regarded as a model system of enzymic catalysis. The present study showed that the combination of flavin and thiazolium ion into a molecular structure markedly improves the trapping efficiency by the flavin of the thiazolium-aldehyde adduct. We believe that this system would become a model for multi-enzymic catalysis and that the combination of various coenzymes would bear further interesting catalytic functions.

REFERENCES AND NOTES

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- 7) F1-T was synthesized according to the following reaction sequence.



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- 9) The detail of the kinetic examination will be submitted as a full manuscript.

(Received March 27, 1981)