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Coenzyme Models 27
Degradative Oxidation of Glyoxals to Carboxylic Acids Mediated by Flavin and Cyanide Ion

by

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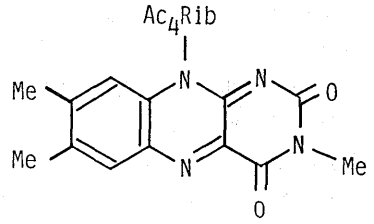
In an aerobic aqueous solution containing flavin and cyanide ion, phenylglyoxal and 4-chlorophenylglyoxal were easily oxidized to benzoic acid and 4-chlorobenzoic acid, respectively (49–61 % yield). The product analysis indicates that the initial step (ArCOCHO → $ArCOCO_2H$) is the "flavin-trapping" mediated by flavin and cyanide ion and the second step ($ArCOCO_2H$ → $ArCO_2H$) is the oxidation by hydrogen peroxide which is yielded through the ping-pong-type regeneration of flavin. Methylglyoxal was also oxidized degradatively to acetic acid. The oxidation of glyoxal was more complicated: oxalic acid was afforded in the nonmicellar system, while formic acid was the sole detectable product in the CTAB micellar system. The reactions reported here demonstrate a novel facet of flavin catalyses.

One of the most noticeable findings in recent flavin chemistry is that a considerable number of flavoenzymes employ carbanions as substrates in the course of their biological oxidation reactions. The concept has been reviewed by Kosman[1] from a biochemical point of view. It occurred to us that this concept-"oxidation of carbanions by flavin"-is readily applicable to organic chemistry, since a carbanion intermediate is proposed for a great number of organic reactions[2] . If the carbanion intermediate is oxidatively trapped by flavin, the reaction can be readily diverted to the oxidation reaction, leading to a development of new synthetic procedures. As an application, we herein report the degradative oxidation of glyoxals to carboxylic acids mediated by flavin and cyanide ion.

We have used 3-methyltetra-0-acetylriboflavin (MeFl), and the aerobic oxidation of phenylglyoxal and 4-chlorophenylglyoxal was carried out at 50℃ for 24 hr in the dark. Neutralization with aqueous HCl precipitated benzoic acid, which was collected by suction. The filtrate was extracted with chloroform and analyzed by high-pressure liquid chromatography. The yields summarized in Table 1 are the sum of the precipitated and extracted material.

MeFl:



Examination of Table 1 reveals that (1) the main product in alkaline solution is, of course, mandelic acid (i.e., Cannizzaro reaction)[3] , while in the presence of MeFl and KCN the reaction is diverted to the oxidation reaction to give benzoic acid in relatively high yields (>49%), (2) in MeFl-containing systems the formation of benzoylformic acids is not or hardly detected, whereas in case MeFl was replaced with methylene blue 21% of benzoylformic acid resulted, and (3) the cationic micelle of CTAB (hexadecyltrimethylammonium bromide) was not so efficient a catalyst as observed in related decarboxylation reactions[4] .

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Table 1 Flavin oxidation of phenylglyoxals(ArCOCHO) [a]

Substratc	Reactioh Conditions			Product(%)		
	KCN (mM)	MeFl (mM)	CTAB (mM)	$ArCHCO_2H$ | OH	$ArCOCO_2H$	$ArCO_2H$
C_6H_5COCHO[b]	0[c]	1.3	0	90	0	7
	13	1.3	0	0.6	3.5	59
	13	1.3	3.0	2.0	1.7	61
	13	0[d]	0	1.1	21	38
4-ClC_6H_4COCHO[e]	0[f]	1.6	3.0	23	0	12
	16	1.6	0	6.0	0	49
	16	1.6	3.0	4.9	0	55

a) 50° C, 24hr in aerobic aqueous solution. b) [C_6H_5COCHO] =13mM. c) 68 mM of KOH were present instead of KCN. d) Methylene blue(6.6 mM) was added instead of MeFl
e) [4-ClC_6H_4COCHO] = 5.4 mM. f) Thirty mMof KOH were present instead of KCN.

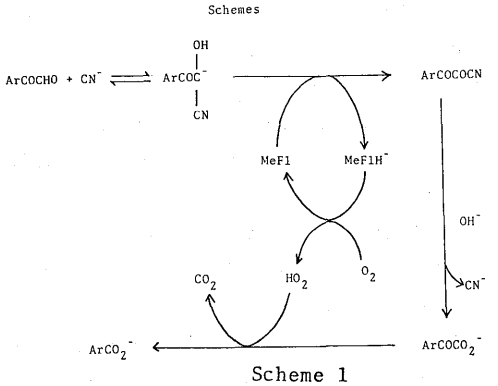
Franzen[5] previously reported the reaction of glyoxals (RCOCHO) with cyanide ion. He proposed that the benzoin-type condensation via the carbanion intermediate, $RCO\bar{C}(CN)OH$, occurs in the primary step and the product, RCOCH(OH)COCOR,decomposes further to complex molecules. As shown in Table 1, the MeFl-containing system is considerably simplified, the detectable products being only mandelic acid, benzoylformic acid, and benzoic acid. This suggests that added MeFl is able to divert the reaction route.

The decarboxylation is very sensitive to the reaction media. For example, a cationic micelle which provides the hydrophobic reaction environment becomes a suitable catalyst for thedecarboxylation[4]

In a previous publication of this series[6] we showed that the CN^- catalyzed decarboxylation of benzoylformic acid hardly occur without the CTAB micelle. This means that benzoylformic acid which corresponds to an intermediary product is accumulated in the absence of the CTAB micelle. As shows in Table 1, however, phenylglyoxal and 4-chlorophenylglyoxal were directly oxidized to the corresponding carboxylic acids.

A clue to the reaction mechanism is provided by the fact that the accumulation of benzoylformic acid occurred when MeFl was replaced by methylene blue(Table 1). The CN^- mediated oxidation of glyoxals to α-keto acids results in the equimolar amount of the leuco-form of flavin or methylene blue. The leuco-form of flavin is immediately reoxidized by oxygen to yield hydrogen peroxide, whereas that of methylene blue is fairly stable against oxygen. Since hydorgen peroxide is a well-known reagent for the decarboxylation of α-keto acids[7] , it is most likely that hydrogen peroxide oxidizes benzoylformic acid in the flavin-containing system. In fact, benzoylformic acid(13mM) was oxidized by hydrogen peroxide(13mM) to benzoic acid almost quantitatively (97% yield) under the same reaction conditions (50℃, 24hr).

The foregoing results implecate that the oxidation of glyoxals to α-keto acids is mediated by MeFl and CN^- and that of α-keto acids to carboxylic acids by hydrogen peroxide yielded from reduced MeFl. As a summary one can depict Scheme 1.



Scheme 1

Since MeFl and cyanide ion behave as turn-over catalysts, the overall reaction corresponds to oxygen oxidation of glyoxals to carboxylc acids, carbon dioxide, and water.

Aliphatic glyoxals such as methyl glyoxal and glyoxal were also oxidized to the corresponding carboxylic acids. The paper chromatographic study was summarized in Table 2. The oxidation of methylglyoxal gave two observable spots: one spot at R_f = 0.10 was acetic acid, but another, much weaker spot at R_f = 0.01 could not be identified. The oxidation of glyoxal was more complicated. As shown in Scheme 2, the flavin oxidation of glyoxal initially yields glyoxalic acid, and it can be further oxidized either to oxalic acid through oxidation of the aldehyde moiety or to formic acid through oxidative decarboxylation of the carboxylate moiety.

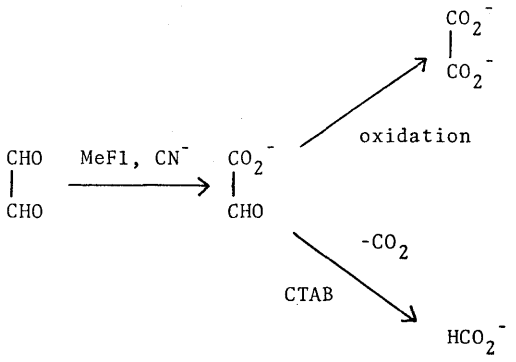
Table 2. Paper chromatographic study of the oxidation of aliphatic glyoxals.[a]

Substrate	Reaction Conditions				R_f	
	M	KCN (mM)	MeFl (mM)	CTAB (mM)		
CH_3COCHO	0.11	310	7.2	3.0	0.10[b]	0.01 (weak)
OHCCHO	0.11	310	7.2	0	0.04[c]	
OHCCHO	0.11	310	7.2	3.0	0.56[c]	

a) 50° c, 24 hr.

b) Developing solvent, 1-butanol (20ml) +1.5 N NH_3 (2ml). The R_f value of authentic sample (CH_3CO_2H) was 0.11.

c) Developing solvent, ethanol (16ml) + conc. NH_3 (0.8ml) + water (12ml). The R_f value of anthentic samples: formic acid, 0.56; oxalic acid, 0.05.



Scheme 2

The product analysis (Table 2) indicates that the treatment of glyoxal in the CTAB micelle solution afforded acid, whereas oxalic acid was given in the absence of the CTAB micelle. The marked change in the reaction route suggests that the CTAB micelle excusively facilitates the decarboxylation reaction. The result is in line with our previous observation on the CTAB micellar catalysis[6].

In summary, the present study establishes that glyoxals are directly converted to carboxylic acids in an aerobic solution containing flavin and cyanide

ion. Apparently, one-step oxidation occurs due to the pingpong nature of flavin. This would be useful as a convenient oxidation method under mild reaction conditions. The present results also involue significant biological implications. Hydrogen peroxide is formed wherever reduced flavin is reoxidized by oxygen. Unless hydrogen peroxide is decomposed rapidly, it would cause undesired, additional reactions in the flavin-mediated metabolic cycles. This suggests that flavin mediated cycles may reside in conjugation with H_2O_2-decomposing enzymes such as catalase and peroxidase.

Experimental

The preparation of MeFl was describedpreviously[6]

CTAB was purchased from Wako Pure Chem. Ind., and recrystallized from ethanol before use. 4-Chlorophenylglyoxal was prepared from 4-chloroacetophenone according to the method of Karrer and Musante[8] :mp 127-8℃(lit[8] . 122℃).

The oxidation of glyoxals by MeFl was carried out under aerobic conditions at 50℃. The procedure has been described[6] . The details of the reaction conditions are recorded in Tables 1 and 2. The aromatic products such as mandelic acid, benzoylformic acid, and benzoic acid were analyzed using high-pressure liquid chromatography (Shimadzu LC-2), and their yields were estimated by comparing the peak intensities with those of authentic samples. Aliphatic products were identifeid qualitatively by paper chromatography (the detail of the analysis conditions was recorded in Table 2).

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