Coenzyme Models. 42.[1]

# Facile Photo-Oxidation of Alcohols by a Flavin with a Metal-Chelation Site

Seiji SHINKAI*, Hideki NAKAO*
Kaori UEDA*, and Osamu MANABE*

A new flavin molecule with a metal-chelation site has been applied to photo-oxidation of alcohols: it is 2, 4, 7-trimethyl-10-benzylquino [8, 7-g] pteridine-9, 11 (7H, 10H)-dione (1) which has both an isoalloxazine structure and a phenathroline-like structure within a molecule. In contrast to conventional flavins which do not exhibit any measurable affinity toward redox-inactive transition metal ions, 1 formed stable complexes with most heavy metal ions in acetonitrile probably by means of a flavin → metal charge transfer. Photo-oxidation of alcohols by 1 was efficiently accelerated in the presence of Mg(II) and Zn(II), the k_1' (pseudo-first-order rate constant) being greater by 5.1-71 fold than those in the absence of metal ions. Such a marked rate increase was not observed for 3-methyl-10-ethylisoalloxazine used as a reference flavin. The aerobic photo-oxidation of benzyl alcohol by the 1·Zn(II) complex served as a light-mediated recycle oxidation catalyst. These results indicate that the flavin with a metal-chelation site is not only useful synthetically as a recycle-type oxidation catalyst but also capable of mimicking the flavin-metal interactions important in metalloflavoproteins.

Flavin and NAD(P)+ are versatile redox "catalysts" in many biological systems.[2-4] In the past, biomimetic studies to apply these coenzymes to mediate redox reactions in nonenzymatic systems have been of much concern.[5] In particular, flavin coenzymes and their analogs have been expected to be useful as "recycle oxidation catalysts", because the oxidized forms can be readily regenerated by air oxidation of the reduced forms (*i.e.*, a ping-pong mechanism).[6-11] For example, Yoneda and co-workers[6,12] have synthesized a number of flavin analogs and demonstrated their utility in synthetic chemistry. We also found that an efficient model system for the oxidative electron bridge from NAD(P)+ to flavin in a respiratory chain can be constructed by using 5-deazaflavin and flavin mononucleotide.[8] This electron bridge is effected by the compensatory action of two different cofactors: that is, 5-deazaflavin oxidizes alcohols and amines but the reduced form is rather insensitive to molecular oxygen, whereas flavin can oxidize the reduced form of 5-deazaflavin and the reduced flavin is rapidly reoxidized by molecular oxygen. In overall, the substrates are oxidized by molecular oxygen through the electron bridge. In these examples the yields largely exceed 100%, indicating that the systems have the characteristics of recycle oxidation catalysts.

Meanwhile, it is known that photo-excited flavins act as electron-acceptors to oxidize phenols, amines, *etc.*, and the oxidizability is much stronger than that of ground-state flavins.[13-15] It occurred to us that synthetic application of photo-excited flavins may be an escaped attention. We have already found that the oxidizabilitly of ground-state flavins is efficiently improved through complexation with certain metal ions.[16-18] One may thus expect that the oxidizability of flavins would be enhanced synergistically by light

and metal complexation. In order to examine this hypothesis, we have synthesized a new flavin with a metal-chelation site because conventional flavins mostly lack the metal affinity. Flavin 1(2, 4, 7-trimethyl-10-benzylquino [8, 7-*g*] pteridine-9, 11 (7H, 10H)-dione) has a phenanthroline-like structure as a chelation site and therefore is expected to show the affinities for heavy metal ions. As a reference flavin, we used 2 (3-methyl-10-ethylisoalloxazine).
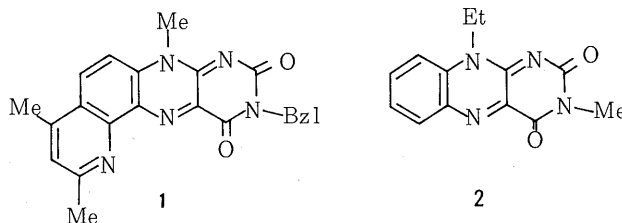
Experimental Section
Methods of Photo-Oxidation and Product Analyses.

Light-mediated oxidation was carried out anaerobically in a thermostated (30 °C) water-bath using a Thunberg cuvete and a 17-W fluorescent lamp. The distance between the lamp and the cuvette was 10 cm. Acetonitrile used as reaction solvent was carefully purified under a N_2 stream. The water concentration (determined by a Karl-Fischer titration) was $(1-2) \times 10^{-2}$M. The photo-oxidation was followed spectrophotometrically by monitoring the disappearance of the absorption maxima of 1. The time-dependences were apparently approximated (for up to two half-lives) by the first-order rate equation. Introduction of O_2 into the final solution regenerated oxidized flavins quantitatively (except for a few entries; see text). We discussed the oxidizability of the flavins by the pseudo-first-order rate constants (k_1') thus obtained.

The preparative-scale photo-oxidation of benzyl alcohol was carried out aerobically in a 300 ml flask covered with a sheet of aluminium foil. The acetonitrile solution containing 1, $Zn(ClO_4)_2 \cdot 6H_2O$, and benzyl alcohol was photoirradiated by a 17-W fluorescent lamp. The yield of produced benzaldehyde was determined by a GLC method.

Results and Discussion
Photo-Oxidation of Alcohols by Flavin-Metal Complexes.

We found that 1 and 2 can photo-oxidize benzyl alcohol to benzaldehyde in acetonitrile through excitation by a fluorescent lamp. The rates were efficiently speeded up by Mg(II) and Zn(II), whereas the reaction was strongly inhibited by Co(II). The deactivation caused by Co(II) is attributable either to a Co (II) → flavin charge transfer or to quenching of the flavin triplet state. In general, the photo-oxidation by flavins proceeds *via* the tripled state.[13-15, 19] Co(II), the heaviest metal ion among tested herein, may quench the triplet state most strongly.

Here, we tried to determine the rate constants for the photo-oxidation. Under anaerobic conditions, the absorption bands of 1 and 2 changed with photoirradiation time and finally reached the absorption spectra attributable to the reduced flavins. Introduction of O_2 into the cell regenerated the oxidized flavins quantitatively. It is known that the kinetic analysis of the light-mediated reactions is fairly complicated because they usually consist of several successive reactions. We found, however, that in the present system the disappearance of the absorption maxima can be approximated by the first-order rate equation with r>0.99 (for up to two-half lives). Hence, we discuss the reactivity by the pseudo-first-order rate constants (k_1') thus determined (Table 1).

The k_1' values for 1 and 2 were comparable each other in the absence of metal ions. The addition of

Table 1. Apparent Pseudo-First-Order Rate Constants (k_1') for Photo-Oxidation of Benzyl Alcohol by **1** and **2**[a]

metal (mM)	$10^2 \cdot k_1'/min^{-1}$	
	1	2
none	0.85	0.58
KClO$_4$(1.0)	0.80	0.55
Mg(ClO$_4$)$_2$(5.11)	13.5	2.2
Zn(ClO$_4$)$_2 \cdot$6H$_2$O(0.101)	14.9	4.0
Co(ClO$_4$)$_2 \cdot$6H$_2$O(0.102)	0.56	0.054

a) 30 °C, acetonitrile, [flavin]$=2.00 \times 10^{-5}$M, [benzyl alcohol]$=0.200$M.

KClO$_4$ was totally ineffective. The k_1' for **1** increased efficiently with increasing metal concentrations and saturated at 1×10^{-2}M for Mg(II) and at 1×10^{-4}M for Zn(II) (Figure 1). These k_1' values were greater by 16-18 fold than that in the absence of metal ions. Similar rate increase was observe for **2**, but the rate augmentations were much smaller (4-7 fold). We estimated the association constants (K) for **1**+Zn(II) from the plot in Figure 1. When the reaction proceeds according to scheme 1, the plot can be analyzed by using eq 1,[20] which holds for the formation of a 1 : 1 complex,
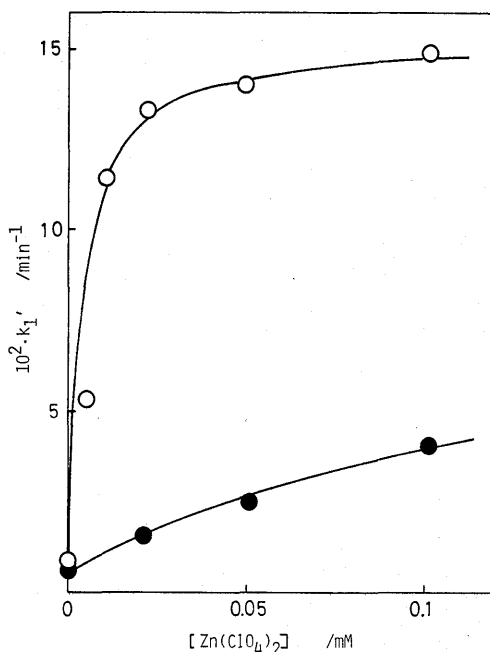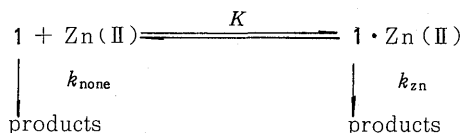

Figure 1. Photo-oxidation of benzyl alcohol by **1** (○) or **2**(●) at 30 °C in acetonitrile: [flavin]$=2.00 \times 10^{-5}$M, [benzyl alcohol]$=0.200$M.
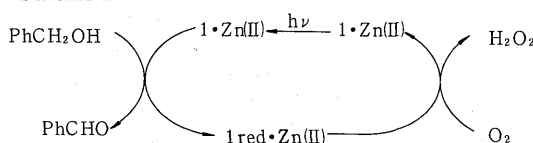
Scheme 1

$$\mathbf{1} + Zn(II) \xrightleftharpoons{\quad K \quad} \mathbf{1} \cdot Zn(II)$$

$$\downarrow k_{none} \qquad\qquad\qquad \downarrow k_{zn}$$

$$\text{products} \qquad\qquad\qquad \text{products}$$

$$\frac{k_{none}}{k_1' - k_{none}} = \frac{1}{qK} \cdot \frac{1}{[Zn(II)]} + \frac{1}{q} \qquad (1)$$

where $q=(k_{zn}/k_{none})^{-1}$ and k_{none} and k_{zn} denote the pseudo-first-order rate constants in the absence and the presence of Zn(II), respectively. From a plot of $k_{none}/(k_1' - k_{none})$ against $[Zn(II)]^{-1}$, we determined $(qK)^{-1}$ (slope) and q^{-1} (intercept) by the least-squares procedure ($r=0.99$). K and k_{zn} were calculated from these two terms: $K=2.6 \times 10^5$M^{-1} and $k_{zn} =0.153$ min^{-1}. As this K value (determined in acetonitrile containing 0.2 M benzyl alcohol) is close to that determined spectrophotometrically in pure acetonitrile $(1.40 \times 10^5$M$^{-1})$, one may conclude that the active species in the photo-oxidation is the **1**$\cdot$Zn(II) complex.

Since the **1**$_{red} \cdot$Zn(II) complex was reoxidized instantaneously to **1**$\cdot$Zn(II) by molecular oxygen, one may expect the ping-pong-type recycle oxidation in aerobic acetonitrile (scheme 2).

Scheme 2

There is a latent problem in scheme 2, however. It is known that molecular oxygen acts, although not an efficient one, as a quencher for triplet state flavins,[19] and photo-oxidation by flavins proceeds in most cases *via* the triplet state.[13-15, 19] We evaluated the influence of Zn(II) on the relative fluorescence intensity (I/I_0) for 1 and 2 (Figure 2). It is seen from Figure 2 that in contrast to a weak quenching effect on 2, the I/I_0 for 1 is very efficiently decreased and the fluorescence was scarcely detected at $[Zn(II)] > 1 \times 10^{-6}$M. The experimental fact that the photo-oxidation is accelerated in spite of the efficient fluorescence quenching suggests that the photo-oxidation by the 1·Zn(II) complex takes place *via* the triplet state.

We carried out a preparative-scale photo-oxidation under the following reaction conditions: $[1] = 9.50 \times 10^{-4}$M, $[Zn(ClO_4)_2 \cdot 6H_2O] = 4.60 \times 10^{-3}$M, [benzyl alcohol] = 0.483 M in O_2-saturated acetonitrile. As shown in Figure 3, the concentration of benzaldehyde increased linearly with the reaction time. The yield calculated on the basis of 1 after 20 h amounted to 6150%. Hence, the 1·Zn(II) complex acts as a light-mediated recycle oxidation catalyst even in the presence of O_2.

The 1·(II) complex which showed the highest catalytic activity was applied to the anaerobic photo-oxidation of several alcohols. The results are summarized in Table 2. In the absence of Zn(II), 1-phenylethanol and 1-butanol were also photo-oxidized by 1. Introduction of O_2 into the final reaction solution regenerated oxidized 1 almost quantitatively. On the other hand, the photo-oxidation of cyclohexanol hardly took place and the fraction of oxidized 1 regenerated by O_2 introduction was about 20%. The result suggests that photoirradiation induces partial decomposition of 1 because of inefficiency of the photo-oxidation process. In the presence of Zn(II), in contrast, the rate of the photo-oxidation was speeded up by 5.1-71 fold and O_2 introduction regenerated oxidized 1 almost quantitavively (>95%). Paricularly, the rate enhancement for the photo-oxidation of cyclohexanol was outstanding.

Conclusion

In the present study, we have demonstrated that introduction of an additional metal chelation site into the isoalloxazine skeleton leads to several novel phenomena: (i) changes in the absorption spectra[21] and the reactivities by added metal ions, (ii) a flavin → metal charge transfer which has never been realized in
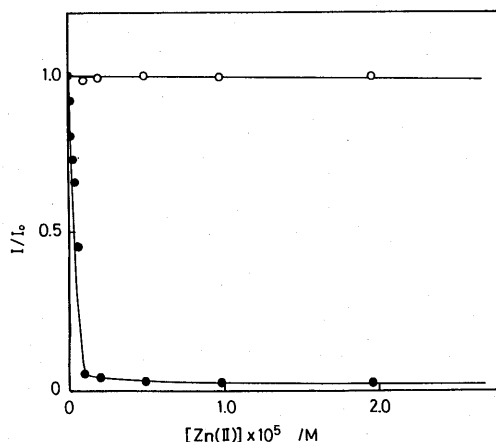

Figure 2. Influence of added $Zn(ClO_4) \cdot 6H_2O$ on the fluorescence intensity of 1 (●) and 2 (○) at 30 °C in acetonitrile. [Flavin] = 9.97×10^{-8}M; excitation, 393 nm for 1 and 338 nm for 2; emission, 502 nm for 1 and 504 nm for 2.

Table 2. Photo-Oxidation of Several Alcohols by the $1 \cdot Zn(II)$ Complex[a]

alcohol	$10^2 \cdot k_1/min^{-1}$	
	no metal	$[Zn(II)] = 1.01 \times 10^{-4}M$
benzyl alcohol	0.85	14.9
1-phenylethanol	1.34	6.89
1-butanol	0.15	2.30
cyclohexanol	ca. 0.03	2.13

a) 30 °C, acetonitrile, $[1] = 2.00 \times 10^{-5}M$, $[alcohol] = 0.200M$.

Figure 3. Photo-oxidation of benzyl alcohol (0.483 M) by 1 ($9.50 \times 10^{-4}M$) $+ Zn(ClO_4)_2 \cdot 6H_2O$ ($4.60 \times 10^{-3}M$) in acetonitrile at 30 °C.

conventional flavins, and (iii) application of the flavin-metal complexes as recycle oxidation catalysts. These phenomena not only imitate several biological concepts important in enzyme chemistry (particularly, in metalloflavoproteins) but also suggest potential applications of these complexes as synthetic reagents. Since a complexone-type ligand family has a wide variety of association abilities, we believe that modification of the metal-chelation site would lead to further development of novel flavin chemistry.

References and Notes

1) Part 41: S. Shinkai, N. Honda, Y. Ishikawa, and O. Manabe, *J. Am. Chem. Soc.,* 107, 6286 (1985).

2) P. Hemmerich, G. Nagelschneider, and C. Veeger, *FEBS Lett.,* 8, 69 (1970).

3) T. C. Bruice, *Acc. Chem. Res.,* 13, 256 (1980).

4) C. Walsh, *Acc. Chem. Res.,* 13, 148 (1980).

5) For comprehensive reviews see (a) S. Shinkai, "Enzyme Chemistry", C. J. Suckling, Ed., Chapman & Hall, London, 1984, p. 40. (b) S. Shinkai, *Progr. Polym. Sci.,* 8, 1, (1982).

6) (a) F. Yoneda, H. Yamato, and M. Ono, *J. Am. Chem. Soc.,* 103, 5943 (1981). (b) F. Yoneda, Y. Sakuma, Y. Kadokawa, and A. Koshiro, *Chem. Lett.,* 1979, 1467. (c) T. Nagamatsu, E. Matsumoto, and F. Yoneda, *ibid.,* 1982, 1127.

7) (a) S. Shinkai, H. Hamada, H. Kuroda, and O. Manabe, *J. Org. Chem.,* 46, 2333 (1981). (b) S. Shinkai, K. Mori, Y. Kusano, and O. Manabe, *Bull. Chem. Soc. Jpn.,* 52, 3606 (1979).

8) S. Shinkai, H. Kuroda, O. Manabe, and F. Yoneda, *J. Chem. Soc., Chem. Commun.,* 1981, 391.

9) (a) Y. Yano, I. Yatsu, E. Oya, and M. Ohshima, *Chem. Lett.,* 1983, 775. (b) Y. Yano, M. Ohshima, and S. Sutoh, *J. Chem. Soc., Chem. Commun.,* 1984, 695.

10) (a) I. Yokoe and T. C. Bruice, *J. Am. Chem. Soc.,* 97, 450 (1975). (b) T. C. Bruice, T. W. Chan, J. P. Taulane, I. Yokoe, D. L. Elliot, R. F. Williams, and M. Novak, *ibid.,* 99, 6713 (1977).

11) W.-R. Knappe "Flavins and Flavoproteins", K. Yagi and T. Yamano, Ed., Japan Scientific Societies Press, Tokyo, 1980, p. 469.

12) For a comprehensive review see F. Yoneda, *Yakugaku Zasshi,* 97, 104 (1984).

13) R. Traber, T. Werner, S. Schreir, H. E. Kramer, W.-R. Knappe, and P. Hemmerich, "Flavins and Flavoproteins", K. Yagi, T. Yamano, Ed., Japan Scientific Societies Press, Tokyo, 1980, p. 431.

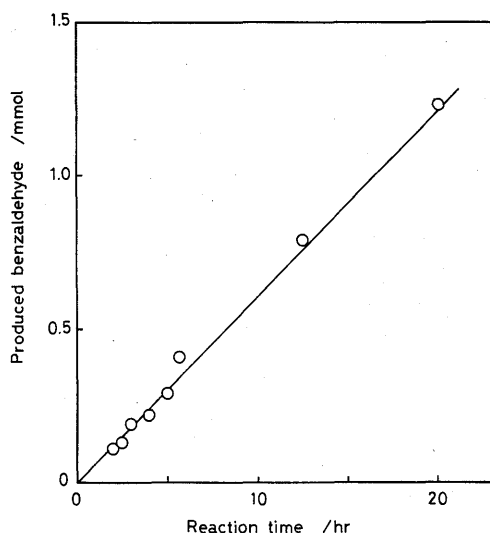14) (a) K. Maruyama and T. Otsuki, *Chem. Lett.,* 1983, 847. (b) S. Fukuzumi, S. Kuroda, and T. Tanaka,

Chem. Lett., 1984, 417.

15) G. A. Eberlein and M. F. Powell, *J. Am. Chem. Soc.,* 106, 3309 (1984).

16) (a) S. Shinkai, Y. Ishikawa, and O. Manabe, *Chem. Lett.,* 1982, 809. (b) Idem, *Bull. Chem. Soc. Jpn.,* 56, 1694 (1983).

17) S. Shinkai, Y. Ishikawa, H. Shinkai, T. Tsuno, H. Makishima, K. Ueda, and O. Manabe, *J. Am. Chem. Soc.,* 106, 1801 (1984).

18) S. Shinkai, N. Honda, Y. Ishikawa, and O. Manabe, *J. Chem. Soc., Perkin Trans. 1,* 1985, 565.

19) S. E. Rokita and C. T. Walsh, *J. Am. Chem. Soc.,* 106, 4589 (1984).

20) J. A. Mollica. Jr. and K. A. Connors, *J. Am. Chem. Soc.,* 89, 308 (1967).

21) S. Shinkai, H. Nakao, N. Honda, O. Manabe, and F. Müller, *J. Chem. Soc., Perkin Trans. 1,* 1986, 1825.