

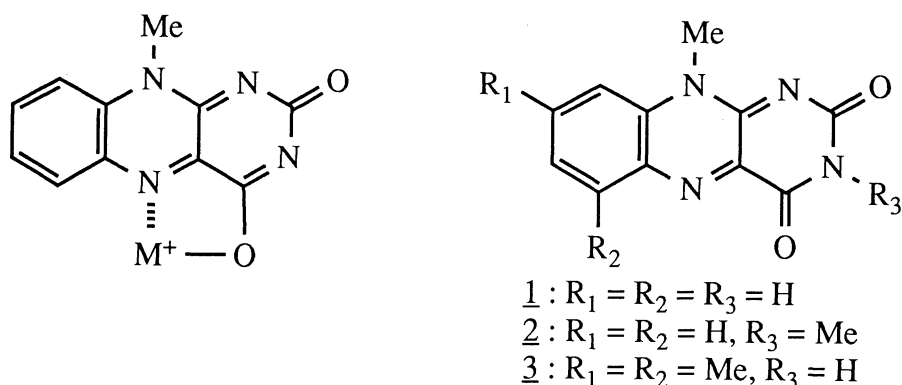
Importance of N(3)-H of an Isoalloxazine Ring. Large Metal Ion Effect on  
Oxidation of an NADH Model by the N(3)-H Dissociated Flavin

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It was found that N(3)-H dissociation of 10-methyl-isoalloxazine plays a crucial role for a rate-accelerating metal ion effect on the oxidation of N-benzyl-1,4-dihydro-nicotinamide (BNAH) in MeCN.

Naturally occurring isoalloxazine derivatives such as FMN and FAD possess no substituent at the N(3)-position. The N(3)-H moiety of the isoalloxazine ring is known to be one of hydrogen-bonding sites for apoproteins.<sup>1)</sup> In model systems, however, the N(3)-position is usually blocked by an alkyl group to avoid kinetic complexity due to the proton dissociation. Thus little has been known for the roles of the N(3)-H of the isoalloxazine ring.

Meanwhile some flavoenzymes contain metal ions as prosthetic groups.<sup>2)</sup> The N(3)-H dissociated isoalloxazine ring is able to adopt a structure like



Scheme 1.

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an 8-quinolinol anion which is a good ligand for metal ions (Scheme 1). This prompted us to examine the metal ion effects ( $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ , and  $\text{Zn}^{2+}$ ) on the oxidation of BNAH by isoalloxazine derivatives (1, 2, and 3)<sup>3)</sup> in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in MeCN.<sup>4)</sup> To our knowledge, there has been no precedent from this point of view in model systems, although the metal ion effects have been studied by employing a metal-coordinative flavin model<sup>5)</sup> or a conventional flavin model with very high concentrations of the metal ions (e.g. 0.1 M)<sup>6)</sup> (1 M = 1 mol dm<sup>-3</sup>).

Firstly, interactions of the flavins and the metal ions were examined spectrophotometrically in the presence and absence of DBU in MeCN. In the case of  $\text{Ni}^{2+}$ , for instance, no substantial change of the absorption spectrum of 1 ( $\lambda_{\text{max}}$  322 and 430 nm) was observed by addition of either DBU ( $1.0 \times 10^{-3}\text{M}$ ) or  $\text{Ni}^{2+}$  ( $1.0 \times 10^{-3}\text{M}$ ). However, a red shift ( $\lambda_{\text{max}}$  337 and 443 nm) was observed on addition of DBU and  $\text{Ni}^{2+}$  together, whereas such a spectral shift was not observed for 2 and 3 under the same conditions. No spectral change for 3 indicates clearly that the metal ion is unable to bind C(4)-O<sup>-</sup> and N(5) positions due to steric hindrance of 6-methyl group. This is also supported by examination of CPK molecular models of 3. These observations suggest that the spectral shift is due to metal-binding at C(4)-O<sup>-</sup> and N(5) atoms as shown in Scheme 1.<sup>7)</sup>

In the next place, pseudo-first-order rate constants for the oxidation of BNAH by the flavins were determined by following absorption decreases of the flavins at 440 nm as described previously.<sup>8)</sup> Plots of  $k_{\text{obsd}}$  vs.  $[\text{M}^{2+}]$  or  $[\text{BNAH}]$  are shown in Figs. 1 and 2. The figures show that the rates increase considerably with the increase of  $[\text{M}^{2+}]$  or  $[\text{BNAH}]$  to reach saturation. The presence of DBU is essential for the rate-accelerations and the saturation kinetics. Namely, in the absence of DBU, noticeable rate accelerations due to the metal ions, and the rate-saturation with the increase of  $[\text{BNAH}]$  were not observed (data not shown). Furthermore, in the cases of 2 and 3, no substantial rate-accelerating metal ion effect was observed even in the presence of DBU. These results clearly indicate that (i) proton removal of the N(3)-H by DBU is crucial for the rate-accelerating metal ion effect and (ii) the oxidation proceeds via a ternary complex (C) of 1<sup>-</sup>,  $\text{M}^{2+}$ , and BNAH (Scheme 2). The rate equation is derived by assuming a steady state for [C] and  $k_{-1} \gg k_2$  under the conditions of  $[\text{BNAH}]_0$ ,  $[\text{M}^{2+}]_0 \gg [\text{1}]_0$  and  $K = k_1/k_{-1}$ . From double reciprocal plots of  $1/k_{\text{obsd}}$  vs.  $1/[\text{M}^{2+}]$ , the rate constants were calculated (Table 1). The theoretical lines (solid lines in Fig. 1) obtained from K and  $k_2$  in Table 1 are consistent with the experimental data. The theoretical lines in Fig. 2 were also obtained in a similar treatment.<sup>9)</sup> As shown in Table 1, the metal ions accelerate the rates  $10^2$ - $10^3$  fold. The large rate enhancement

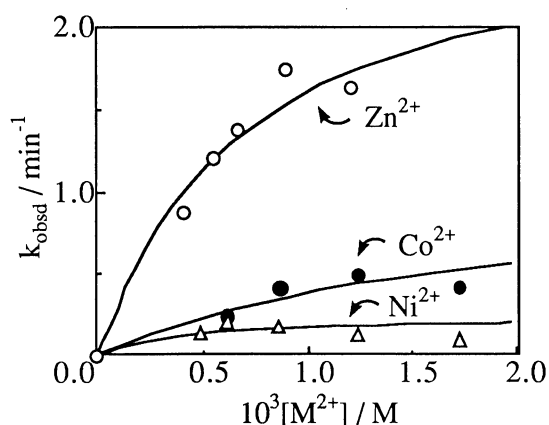


Fig. 1. Plots of  $k_{\text{obsd}}$  vs.  $[M^{2+}]$ .  
 $[1]_0 = 4.5 \times 10^{-5} \text{M}$ ,  $[\text{BNAH}]_0 = 1.82 \times 10^{-3} \text{M}$   
 $[\text{DBU}]_0 = 9.08 \times 10^{-4} \text{M}$ ,  $\text{N}_2$ ,  $25^\circ \text{C}$ .

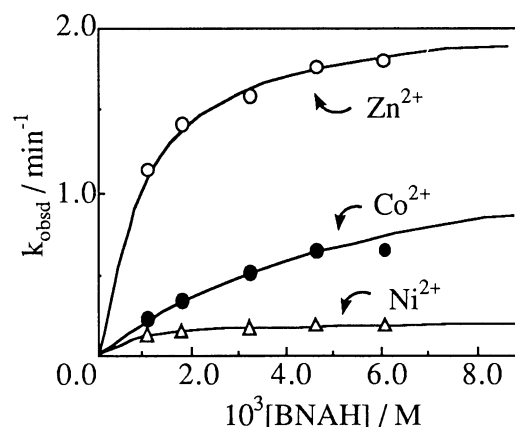
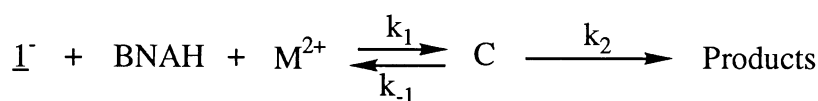


Fig. 2. Plots of  $k_{\text{obsd}}$  vs.  $[\text{BNAH}]$ .  
 $[1]_0 = 4.5 \times 10^{-5} \text{M}$ ,  $[M^{2+}]_0 = 5.97 \times 10^{-4} \text{M}$   
 $[\text{DBU}]_0 = 9.24 \times 10^{-4} \text{M}$ ,  $\text{N}_2$ ,  $25^\circ \text{C}$ .



$$k_{\text{obsd}} = \frac{k_2 K [\text{BNAH}]_0 [M^{2+}]_0}{1 + K [\text{BNAH}]_0 [M^{2+}]_0} \quad K = k_1 / k_{-1}$$

Scheme 2.

Table 1. Rate constants and relative rates

$M^{2+}$ a)	$k_{\text{obsd}} / \text{min}^{-1}$	$K / \text{M}^{-2}$	$k_2 / \text{min}^{-1}$	Rel. rates
None b)	$< 1 \times 10^{-3}$	-	-	1.0
$\text{Co}^{2+}$		$2.6 \times 10^5$	1.1	$> 1.1 \times 10^3$
$\text{Ni}^{2+}$		$1.7 \times 10^6$	0.24	$> 2.4 \times 10^2$
$\text{Zn}^{2+}$		$8.3 \times 10^5$	2.7	$> 2.7 \times 10^3$

a)  $\text{M}(\text{NO}_2)_3 \cdot 6\text{H}_2\text{O}$ . b)  $[1]_0 = 4.5 \times 10^{-5} \text{M}$ ,  $[\text{BNAH}]_0 = 1.82 \times 10^{-3} \text{M}$ ,  
 $[\text{DBU}]_0 = 9.08 \times 10^{-4} \text{M}$ ,  $\text{N}_2$ ,  $25^\circ \text{C}$ .

may be accounted for by that the metal ion increases the oxidation activity of N(3)-H deprotonated flavin by complexation and the complexed metal ion acts as a substrate-binding site to form the ternary complex (C), although the detail such as its structure is not clarified yet.

In addition to the role as the metal ligand, the N(3)-H dissociated flavin resists alkaline hydrolysis.<sup>10)</sup> Namely the rate of alkaline hydrolysis ( $\text{pH} > 10$ ) of 1 is depressed by a factor of two orders of magnitude by the N(3)-H deprotonation; the rate constants of the hydrolysis were  $4.57 \times 10^{-4} \text{min}^{-1}$  for 1 and  $5.10 \times 10^{-2} \text{min}^{-1}$  for 2 at  $\text{pH} 12.0$  and  $25$

°C, In other words, the N(3)-H of isoalloxazine rings could function as a self-protective moiety by proton dissociation under alkaline conditions.

In summary, the present study demonstrates that an N(3)-H dissociated isoalloxazine ring not only acts as a metal-ligand but also protects itself from alkaline hydrolysis. These facts suggest intrinsic significance of N(3)-H of naturally occurring isoalloxazine rings.

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