Rate Acceleration of the Oxidation of an NADH Model by Flavin with a Functionalized Flavin Receptor in Chloroform

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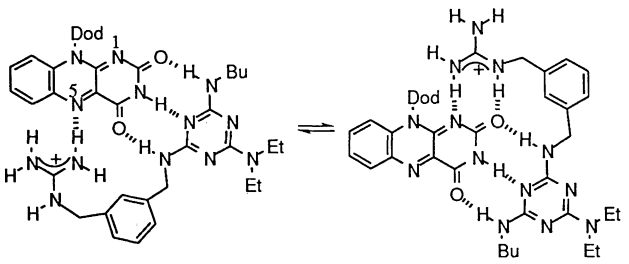
A melamine derivative bearing a guanidinium ion was found to enhance the oxidation-activity of flavin via hydrogen bonding at the N(1) position of the isoalloxazine ring for the oxidation of N-benzyl-1,4-dihydronicotinamide (BNAH) in $CHCl_3$.

Diverse functions of flavin coenzymes are known to be regulated by interaction with apoproteins.[1] Thus, introduction of the apoprotein functions into model systems would be inevitable for construction of artificial flavoenzymes. From this point of view, we have reported that 2,6-diaminopyridine derivatives act as a flavin receptor via a triple hydrogen bond toward C(2)=O, N(3)-H, and C(4)=O of the isoalloxazine ring, in which, however, the binding ability due to the triple hydrogen bond is fairly week,[2] and the reactivity of the flavin for BNAH is little affected by the triple hydrogen bond.[2c] We have also reported that melamine derivatives (**1**) bind **2** with the binding constants (K) of 2,000 M^{-1} for **2•1a** and 150 M^{-1} for **2•1b**.[3] The larger K value of **2•1a** suggests that the guanidinium ion of **1a** participates in an additional hydrogen bonding at the N(1) or N(5) position as shown below, although experimantal evidence has been lacking.



We report herein the binding ability of **1a** toward 5-deaza-flavin (**3**) which is unable to adopt the N(5)-hydrogen bonding, and also a rate acceleration of the oxidation of BNAH by **4** in the presence of **1a** in $CHCl_3$.[4]

The binding constants were determined by fluorescence spectroscopy in $CHCl_3$.[2c] The results are summarized in Table 1. The stronger binding ability of **1a** for **2** and **4a** than for **3** could be explained by that **2** and **4a** possess both the N(1) and N(5) as hydrogen bonding sites, whereas only the N(1) for **3**. No quenching was observed for **4b•1a** ($K = \sim 0\ M^{-1}$). The larger K value of **3•1a** than that of **3•1b** clearly indicates an additional hydrogen bond at the N(1) position by the guanidinium moiety.[5] This prompted us to examine the effect of the hydrogen bonding on the reactivity of a flavin. Meanwhile Massey et al. have proposed that reactivities of flavoenzymes are regulated by the hydrogen bondings at the N(1) and N(5) positions of an isoalloxazine ring.[6] In fact, it has been reported that intra-

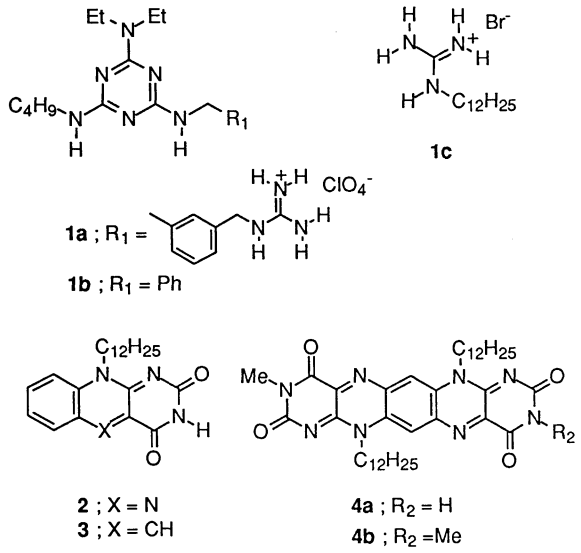


Table 1. Binding constants (K / M^{-1}) in $CHCl_3$

Receptors	Flavins 2[a]	3	4a[b]
1a	2,000 ± 100	840 ± 10[b]	1,700 ± 20
1b	140 ± 10	130 ± 0[c]	260 ± 10
1c	17 ± 1	—[d]	—[d]

[a] From ref.3. [b] [3] = [4a] = 1.0 x 10^{-5} M, [Receptor] = 0 - 5.0 x 10^{-4} M, excited at 400 nm (**3**) and 560 nm (**4a**), 20 °C. [c] ^1H NMR (500 MHz, $CDCl_3$), [3] = 2.5 x 10^{-3} M, [Receptor] = 0 - 1.0 x 10^{-2} M, 25 °C. [d] Not determined.

molecular hydrogen bonding at the N(5) position of the isoalloxazine ring promotes the reactions proceeding via the C(4a) attack,[7] whereas it shows no effect on BNAH oxidation.[7a]

The effect of the receptor on the oxidation of BNAH was examined kinetically by employing the oxidation-active flavin model (**4**) in $CHCl_3$ as described previously,[2c] since the conventional flavin models such as **2** and **3** are unable to oxidize BNAH in $CHCl_3$. Even with **1a**, they could not oxidize it. The rate for **4a** was found to increase with increase of [**1a**], following the Michaelis-Menten kinetics, whereas the rates for **4a**/(**1b** +**1c**) and **4b**/**1a** were little affected (Figure 1). The rates were confirmed to be first-order with respect to [BNAH] in the presence and absence of **1a**. These results allow us to assume the following reaction scheme and rate equations, where $[BNAH]_0$ and $[\mathbf{1a}]_0$ represent the initial concentrations, and k_0 represents the pseudo first-order rate constant for the N(5)

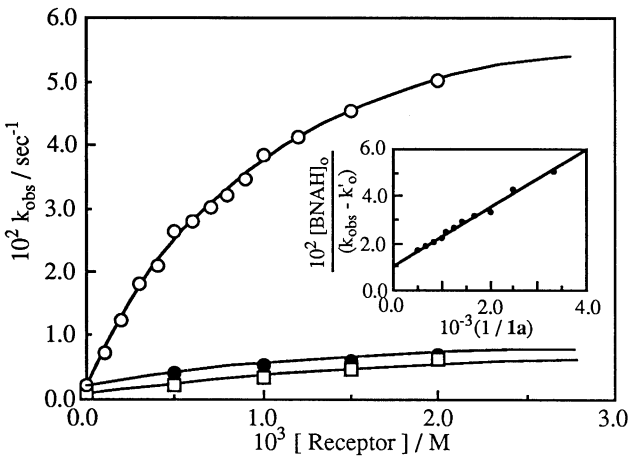
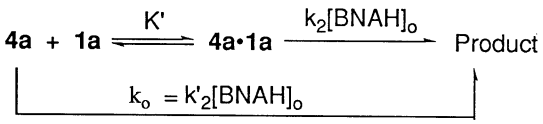


Figure 1. Plots of k_{obs} vs [Receptor]. [**4a**] = [**4b**] = 1.0 x 10^{-5} M, [BNAH] = 8.0 x 10^{-4} M, [**1a**] = [**1b**] = 0 - 2.0 x 10^{-3} M, 25°C, $CHCl_3$. o ; **4a/1a**, ● ; **4a/(1b + 1c)**, □ ; **4b/1a**.

hydrogen-bonded **4a** and free **4a** (k_O= k'_2[BNAH]$_O$). From the reciprocal plots (inset in Figure 1), K'and k_2 were calculated to



$$k_{obs} = k_o + \frac{k_2K'[BNAH]_o[\mathbf{1a}]_o}{1 + K'[\mathbf{1a}]_o}$$

$$\frac{[BNAH]_o}{k_{obs} - k_o} = \frac{1}{k_2K'[\mathbf{1a}]_o} + \frac{1}{k_2}$$

be 870 M^{-1} and 94 $M^{-1}sec^{-1}$, respectively. The K' value is in good accord with the binding constant of **3•1a** (K = 840 M^{-1}), suggesting that the N(1) is involved as the hydrogen bonding site. From the plots of k_O vs. [BNAH] (not shown), k'_2 was calculated to be 3.5 $M^{-1}sec^{-1}$. Thus, the rate acceleration (k_2/k'_2) due to the receptor is 27-fold (94/3.5). Since the N(5)-hydrogen bonding is known to exhibit no effect, this rate enhancement due to the receptor is accounted for by the hydrogen bonding at the N(1) position, which may facilitate hydride (or its equivalent) transfer from BNAH to the isoalloxazine ring. To our knowledge, it is the first example that the oxidation of an NADH model by a flavin is enhanced in the presence of a functionalized flavin receptor. The receptor molecule could be regarded to exhibt a function of apoproteins.

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References and Notes

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3 N. Tamura, T. Kajiki, T. Nabeshima, and Y. Yano, *J. Chem. Soc., Chem. Commun.*, **1994**, 2583; 1 M = 1 mol dm^{-3}.

4 Compounds **1**, **2** and **4** were supplied from our previous study.[2c, 3] 5-Deazaflavin (**3**) was prepared according to the method of Yoneda (F. Yoneda, Y. Sakuma, S. Mizumoto, and R. Ito, *J. Chem. Soc., Perkin 1*, **1976**, 1805). Mp. 201-2 °C, elemental analysis: Found: C; 70.91, H; 8.05, N; 10.64 %. Calcd for $C_{23}H_{31}N_3O_2$•0.5H_2O: C; 70.74, H; 8.26, N; 10.76 %.

5 The participation of the guanidinium protons into the hydrogen bonding in **3•1a** was not clearly recognized by ^1H NMR spectrum of a 1 : 1 mixture of **3** and **1a** due to broadening.

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