Functional Flavin Receptors. Bis-melamine Derivatives Bearing a Guanidinium Ion Which Bind 6-Azaflavin and a Thymine-Linked Substrate through Hydrogen Bonds in Chloroform

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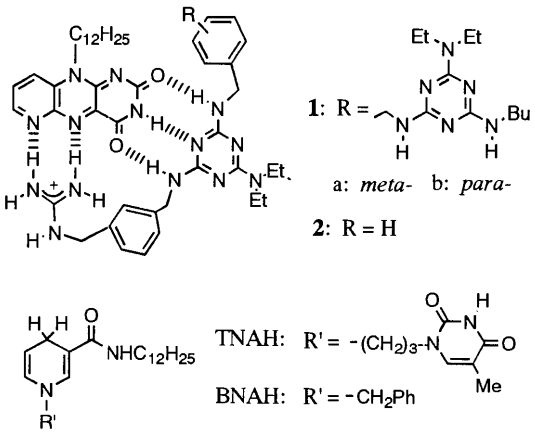
Bis-melamine derivatives bearing a guanidinium ion bind 6-azaflavin via five hydrogen bonds and an NADH model having a thymine moiety via three H-bonds in $CHCl_3$, resulting in the rate acceleration due to the proximity effect.

Incorporation of apoprotein functions into catalytic systems is inevitable for construction of artificial enzymes.[1] Synthetic receptors, which can hold catalyst and substrate in close proximity through noncovalent bonds, would be regarded to possess an apoprotein function. We have reported that a melamine derivative bearing a guanidinium ion acts as a flavin receptor binding tightly 6-azaflavin through five H-bonds in $CHCl_3$.[2] Functionalization of this receptor may provide the functionality close to 6-azaflavin by complexation. Since a melamine scaffold is known to form a complex with a thymine derivative via three H-bonds,[3] we designed bis-melamine derivatives bearing a guanidinium ion (**1**), which are expected to bind 6-azaflavin and a thymine-linked NADH model through H-bonds in $CHCl_3$.



We report herein that the rate of the oxidation of *N*-(3-thyminylpropyl)-1,4-dihydronicotinaminde derivative (TNAH) by 6-azaflavin is accelerated in the presence of **1** in $CHCl_3$. Receptors **1**[4] were synthesized by stepwise substitution of cyanuric chloride with the corresponding amines, followed by the reaction with *S*-ethylthiouronium bromide in EtOH, and anion exchange with $NaClO_4$. TNAH was prepared from 3-*N*-dodecylcarbamoyl-pyridine and 1-(3-bromopropyl)-thymine,[5] followed by $Na_2S_2O_4$ reduction.[6]

Binding constants (*K*) of **1** for 6-azaflavin were determined spectrophotometrically in $CHCl_3$ as described previously.[2] The 1 : 1 complexation was confirmed by the Job plot for 6-azaflavin and **1a** (Figure 1). As shown in Table 1, the smaller

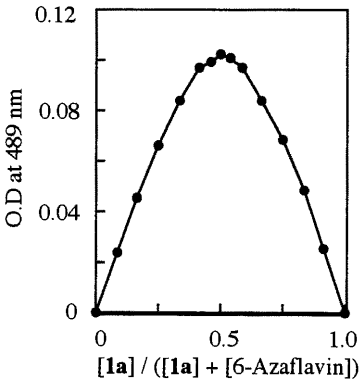


Figure 1. Job plot for complexation of **1a** and 6-azaflavin. [6-Azaflavin] + [**1a**] = 1.0 x 10^{-4} M, $CHCl_3$, 25 °C.

Table 1. Binding constants (*K*) for 6-azaflavin and free energy changes (ΔG)

Receptor[a]	K / M^{-1}	ΔG / kcal mol^{-1}
1a	4.7 ± 0.2 x 10^4	- 6.4
1b	6.8 ± 0.2 x 10^4	- 6.6
2	1.2 ± 0.1 x 10^5	- 6.9

[a] [6-Azaflavin] = 5.0 x 10^{-5} M, $CHCl_3$, 25 °C.

K values of **1** than that of **2** indicate that the second melamine part does not participate in the complex formation of 6-azaflavin and **1**, and rather disturbs the complexation presumably due to steric hindrance. This suggests that the second melamine part can be utilized as a thymine-binding site.

Pseudo-first-order rate constants (k_{obs}) of the oxidation of NADH models by 6-azaflavin were determined spectrophotometrically by following the absorption decreases of 6-azaflavin at 440 nm in $CHCl_3$ under anaerobic conditions. Effects of [TNAH] on k_{obs} in the presence of the receptor were shown in Figure 2. Under the conditions of Figure 2, complex formation of 6-azaflavin and **1** or **2** is more than 90%. In the presence of **1**, the rate saturation was observed, whereas linear plots are obtained in the absence of **1** and presence of **2**. The rate acceleration and saturation in Figure 2 can be explained by the following reaction scheme and eq (1). The curve fitting with eq (1) gave the computed rate constants as listed in Table 2. The rate accelerations due to five H-bonds (k_1/k_o) are small (3.1-fold for **1a**, 3.6-fold for **1b**, and 1.5-fold for **2**), which are comparable to those for BNAH. Namely, the rates for BNAH were first-order with respect to [BNAH] despite of the receptors to

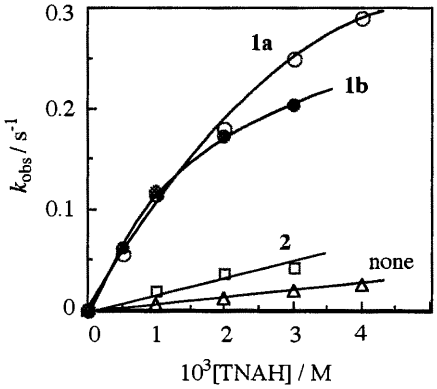


Figure 2. Plots of k_{obs} vs. [TNAH]. [6-Azaflavin] = 5.0×10^{-5} M, [**1a**] = 2.4×10^{-4} M, [**1b**] = 1.8×10^{-4} M, [**2**] = 1.3×10^{-4} M, $CHCl_3$, N_2, 25 °C.

$$\mathrm{F} + \mathrm{TNAH} \xrightarrow{k_o} \text{Products}$$
$$\mathrm{F} + \mathbf{1} \overset{K_1}{\rightleftharpoons} \mathrm{F}\cdot\mathbf{1} \xrightarrow{k_1[\mathrm{TNAH}]} \text{Products}$$
$$\mathrm{F}\cdot\mathbf{1} + \mathrm{TNAH} \overset{K_2}{\rightleftharpoons} \mathrm{F}\cdot\mathbf{1}\cdot\mathrm{TNAH} \xrightarrow{k_2} \text{Products}$$

$$k_{obs} = \frac{(k_o + k_1K_1[\mathbf{1}]_o + k_2K_1K_2[\mathbf{1}]_o)[\mathrm{TNAH}]_o}{1 + K_1[\mathbf{1}]_o + K_1K_2[\mathbf{1}]_o[\mathrm{TNAH}]_o} \quad (1)$$

where F represents 6-azaflavin, and $[\mathbf{1}]_o$ and $[\mathrm{TNAH}]_o$ are initial concentrations of **1** and TNAH, respectively.

Table 2. Computed rate constants and relative rates

	1a	**1b**	**2**
K_2 / M^{-1}	210	540	
k_1 / $M^{-1}s^{-1}$	29.4	25.7	12.3
k_2 / s^{-1}	0.52	0.29	
k_2K_2 / $M^{-1}s^{-1}$	109	157	
k_1 / k_o	3.6	3.1	1.5
k_2K_2 / k_o	13	19	

K values in Table 1 were used as K_1.
$k_o = 8.21$ M^{-1} s^{-1}

give the following second-order rate constants: 2.21 $M^{-1}s^{-1}$ (without receptor), 6.70 $M^{-1}s^{-1}$ (**1a**), 5.12 $M^{-1}s^{-1}$ (**1b**), and 3.47 $M^{-1}s^{-1}$ (**2**) under the conditions of Figure 2. The rate accelerations are 3.0-fold for **1a**, 2.3-fold for **1b**, and 1.6-fold for **2**. The rate accelerations (k_2K_2/k_o) due to the complex formation of 6-azaflavin·**1**·TNAH are 13-fold for **1a** and 19-fold for **1b**, respectively. The rate accelerations could be explained by that the receptor binds 6-azaflavin and TNAH to form a ternary complex in which both locate in an expedient position for the reaction as shown in Figure 3. It should be noted that no rate

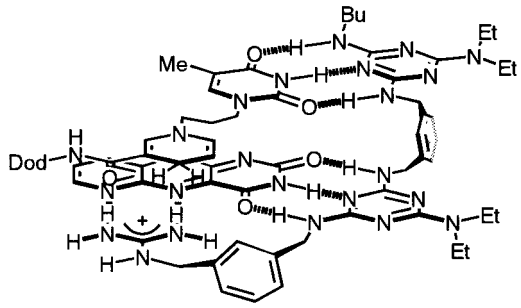


Figure 3. A possible structure of the ternary complex of 6-azaflavin, **1a**, and TNAH.

acceleration was observed when 6-aza-3-methylisoalloxazine was used under the same conditions. Meanwhile, we reported that the binding constant for a melamine and a thymine derivative due to three H-bonds is 43 M^{-1} in $CDCl_3$.[7] The much larger K_2 values due to three H-bonds may be explained by π–π stacking between 6-azaflavin and the dihydronicotinamide ring of TNAH in addition to the three H-bonds of the melamine and thymine moieties.

In summary, the present study has demonstrated that the receptors **1** bind 6-azaflavin and TNAH to form a ternary complex to result in the rate-accelerations due to a proximity effect. Such receptor molecules could be regarded to exhibit an apoprotein function.

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References and Notes

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4 **1a**; mp 86–88 °C (decomp.). Anal. Found: C, 53.80; H, 7.09; N, 26.45%. Calcd for $C_{35}H_{54}ClN_{15}O_4$: C, 53.59, H, 6.94; N, 26.79%. **1b**; mp 100–101 °C (decomp.). Anal. Found: C, 53.04; H, 6.95; N, 26.09%. Calcd for $C_{35}H_{54}ClN_{15}O_4 \cdot 2/3H_2O$: C, 52.79; H, 7.00; N, 26.38%. Receptor **2** and 6-azaflavin were supplied from our previous study.[2]
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6 TNAH was recrystallized from EtOH–H_2O. Mp 77–81 °C (decomp.). Anal. Found: C, 65.52; H, 9.30; N, 11.75%. Calcd for $C_{26}H_{42}N_4O_3 \cdot H_2O$: C, 65.86; H, 9.00; N, 11.75%.
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