Regulation of Redox Properties of 6-Azaflavin by Hydrogen Bonding with a Receptor in Chloroform-Acetonitrile

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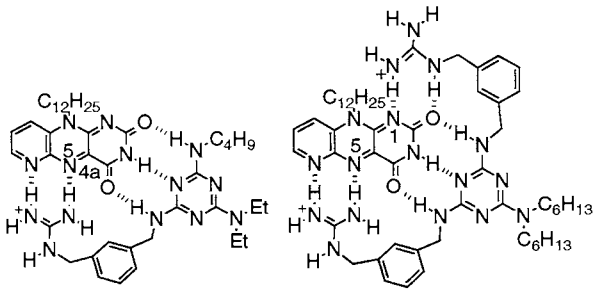
(Received February 1, 1999; CL-990062)

A melamine derivative bearing two guanidinium ions binds 6-azaflavin via hydrogen bonds at N(1), C(2)=O, N(3)-H, C(4)=O, N(5), and N(6) positions, and affects considerably the redox properties in $CHCl_3$ containing 20% (v/v) CH_3CN.

Flavin coenzymes such as FMN and FAD exhibit diverse redox functions through interactions with apoproteins, in which hydrogen bonding plays crucial roles on manifestation of the specific functions.[1] We have reported that a melamine derivative bearing a guanidinium ion (**1**) strongly binds 6-aza-10-dodecylisoalloxazine (6-azaflavin) via five hydrogen bonds in $CHCl_3$ as shown in Scheme 1(a),[2] and the acidity of the guanidinium hydrogen plays a crucial role on stabilization of anionic semiquinone flavin radical in $CHCl_3$.[3] Yoneda et al. have reported that an anionic semiquinone radical of flavin-6-carboxylate is stabilized by intramolecular N(5)-hydrogen bonding even in aqueous solution.[4] These results suggest that acidity of the H-bond donor at the N(5)-position is essential for stabilization of the anionic flavin radical. We wish to report herein that a melamine receptor (**2**) shows remarkable hydrogen-bonding effects on redox potentials, stabilization of anionic semiquinone radical, and oxidation activity of 6-azaflavin in $CHCl_3$-MeCN (20%).



(a) 6-Azaflavin·**1** (b) 6-Azaflavin·**2**

Scheme 1. Complexes of 6-azaflavin and receptors.

Receptor **2**[5] was prepared according to procedures similar to those of **1**.[2] Binding constants of 6-azaflavin•**2** were determined spectrophotometrically as described previously for 6-azaflavin•**1**.[2] Because of solubility problem of **2** in $CHCl_3$, MeCN was added. The 1 : 1 stoichiometry for the complex formation was confirmed by the Job plot (data not shown). 6-Azaflavin•**2** was isolated as powder, and the 1 : 1 ratio was also confirmed by ESI MS.[6] As shown in Table 1, the larger K values of **2** than those of **1** suggest that both guanidinium moieties of **2** are involved in the hydrogen bonding as shown in Scheme 1(b). However, the binding abilities of **1** and **2** become close with increase of MeCN content, implying that hydrogen bonding due to the second guanidinium ion is less responsible for the complexation.

Redox potentials of 6-azaflavin were determined by cyclic voltammetry in CH_2Cl_2-MeCN (20%).[7] Without the receptors, 6-azaflavin showed a reversible redox couple ($E_{1/2}$ = -926 mV vs. ferrocene/ferrocenium). Upon increasing the concentration of the receptors, the redox potential shifted to a positive direction, leading to fixed potentials; $E_{1/2}$ = -706 mV for **1** and -609 mV for **2**. Namely the shifts of the potentials due to the receptors ($\Delta E_{1/2}$) are 220 mV for **1** and 317 mV for **2**, corresponding to stabilization of anionic radical of 6-azaflavin by -5.1 and -7.3 kcal/mol, respectively.

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

$CHCl_3$ / % (v/v)	1	2
100	$1.4 \pm 0.1 \times 10^5$	—— [b]
90	$1.8 \pm 0.5 \times 10^4$	$3.8 \pm 0.6 \times 10^4$
80	$1.3 \pm 0.3 \times 10^4$	$1.9 \pm 0.2 \times 10^4$
50	$6.2 \pm 0.2 \times 10^3$	$6.5 \pm 0.8 \times 10^3$

[a] [6-Azafavin] = 5.0×10^{-5} M, [Receptor] = 0 - 2.0×10^{-4} M, 25 °C. [b] Not determined due to insolubility of **2**.

Formation of the anionic radical of 6-azaflavin was detected spectrophotometrically by employing the oxidation of N-benzyl-1,4-dihydronicotinamide (BNAH) and thiophenol (with Bu_3N) in the presence of the receptors in $CHCl_3$-CH_3CN (20%) under anaerobic conditions. The absorption spectrum of the anionic semiquinone radical, which was formed by comproportionation of the oxidized and reduced 6-azaflavins,[3] was observed for the both oxidation with **2**, whereas not with **1** (Figure 1). Use of 6-aza-3-methyl-isoalloxazine did not give the absorption spectrum of the radical, but its reduced spectrum. It is the first example that the anionic semiquinone radical is stabilized by hydrogen bonding of the guanidinium ions at both N(1) and N(5)-positions despite the presence of reducing reagents such as BNAH and PhSH. It can be said that an anionic radical of 6-azaflavin is stabilized by N(5) hydrogen bonding of acidic H-bond donor or by both N(1) and N(5) hydrogen bondings as shown in Scheme 1(b).

Effects of hydrogen bonding on the oxidation activity of 6-

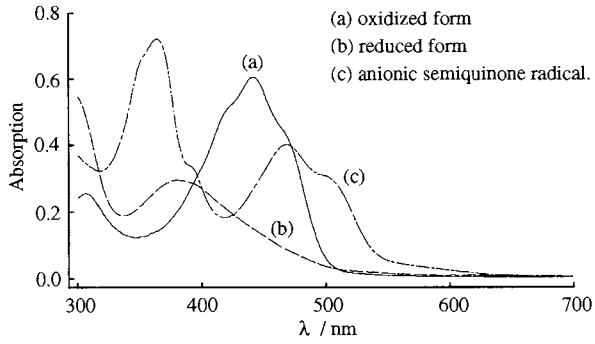


Figure 1. Absorption spectra of 6-azaflavin in the reaction with PhSH. [6-Azaflavin] = 5.0×10^{-5} M, [PhSH] = [Bu_3N] = 2.0×10^{-4} M or 1.0×10^{-3} M, [**2**] = 1.0×10^{-4} M in $CHCl_3$ - MeCN (20%) at 25 °C.

azaflavin were kinetically examined in reactions with BNAH and PhSH (with Bu_3N). Pseudo-first-order rate constants were determined by following the absorption decreases at 440 nm. The rates were confirmed to be first-order with respect to [BNAH], and second-order with [PhSH] and first-order with [Bu_3N], respectively. This suggests that the both oxidation proceeds via the mechanisms established in aqueous solutions.[8] The effects of the concentrations of the receptors on the rates were shown in Figure 2. The curve fitting with the rate equations (eqs. 1 and 3)[9] gave the computed rate constants as shown in Table 2.

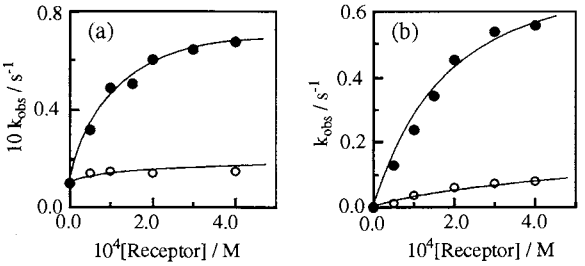


Figure 2. Plots of k_{obs} vs. [Receptor]. [6-Azaflavin] = 5.0 x 10^{-5} M, [Receptor] = 0 - 4.0 x 10^{-4} M, N_2, 25 °C, $CHCl_3$ - MeCN (20%). (a) [BNAH] = 2.0 x 10^{-3} M, (b) [PhSH] = 2.0 x 10^{-3} M, [Bu_3N] = 0.01 M. ○; **1**, ● ; **2**.

Table 2. Computed binding constants (K') and rate constants

Substrate		1	2
BNAH	K' (M^{-1})	1.0 x 10^4	1.7 x 10^4
	k_2 ($M^{-1}\cdot s^{-1}$)	1.3	6.4
	k_2 / k_o	1.6	8.0
PhSH	K' (M^{-1})	3.7 x 10^3	4.5 x 10^3
	k_2 ($M^{-3}\cdot s^{-1}$)	1.0 x 10^8	4.7 x 10^8
	k_2 / k_o	1.7 x 10^3	7.8 x 10^3

k_o = 0.8 $M^{-1}s^{-1}$ for BNAH, k_o = 6.0 x 10^4 $M^{-3}s^{-1}$ for PhSH.

The K' values kinetically obtained are in fairly good agreement with those determined spectrophotometrically. The rate accelerations (k_2/k_o) for BNAH are 1.6-fold for **1** and 8-fold for **2**, indicating that the N(5)-hydrogen bonding affects little the rate, but the N(1)-hydrogen bonding accelerates the rate for the reaction at N(5)-position.[10] For PhSH oxidation, the K' value for **2** (4.5 x 10^3 M^{-1}) is in good agreement with that in the presence of [Bu_3N] = 0.01 M (K = 4.1 x 10^3 M^{-1}).[11] The rate accelerations are 1.7 x 10^3-fold for **1** and 7.8 x 10^3-fold for **2**, respectively. The larger rate acceleration for **2** can be explained by that the N(5)-hydrogen bonding facilitates a nucleophilic attack of PhS^- at C(4a)-position and N(1)-hydrogen bonding facilitates nucleophilic attack of second PhS^- at the sulfur atom of the C(4a)-adduct. It should be noted that intramolecular N(5)-hydrogen bonding is known to facilitate a nucleophilic attack at C(4a)-position.[4,12]

In summary, the present study provides experimental evidence that intermolecular both N(1)- and N(5)-hydrogen bonding stabilizes an anionic radical of 6-azaflavin, the N(1)-hydrogen bonding facilitates the reaction at N(5), and the N(5)-hydrogen bonding promotes the reaction involving a nucleophilic attack at C(4a)-position. The effect of N(5)-hydrogen bonding, however, is much more effective than N(1)-hydrogen bonding for the reactions involving nucleophilic attack at C(4a)-position, which would be useful for understanding of the roles of H-bonds seen in X-ray crystallographic data of flavoenzymes.[13]

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan. We are grateful to Professor V. M. Rotello for sending us a manuscript prior to publication.[14]

References and Notes

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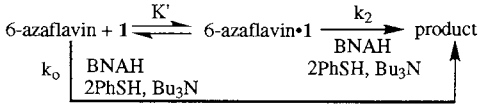
5 Mp 124-127 °C (MeCOMe-$CHCl_3$). ^{1}H NMR (δ, $CDCl_3$, 200 MHz), 0.87 (t, 3H, J = 7.5 Hz, -$(CH_2)_3\underline{CH_3}$), 1.26 (m, 12H, -$NCH_2\underline{CH_2}(\underline{CH_2})_3CH_3$), 1.51 (bs. 4H, $NCH_2\underline{CH_2}$-), 3.42 (t, 4H, -$N\underline{CH_2}$-), 4.34 (d, 4H, J = 3.5 Hz, melamino-$NH\underline{CH_2}C_6H_4$), 4.52 (d, 4H, J = 3.25 Hz, guanidino-$NH\underline{CH_2}C_6H_4$-), 4.71 (bs. 2H, melamino-NH), 6.78 (bs. 8H, guanidino-NH_2), 7.22-7.33 (m, 8H, C_6H_4), 7.60 (bs. 2H, guanidino-NH). Found: C, 48.73; H, 6.38; N;20.04%. Anal. Calcd for $C_{33}H_{54}N_{12}Cl_2O_8$: C, 48.47; H, 6.66; N, 20.55%.
6 The complex was obtained from a mixture of 6-azaflavin and **2** in acetone on spontaneous vaporization. The powder dissolved in MeCN was subjected to ESI MS; m/z 1100.7 (M-ClO_4), 501.1(M-2ClO_4).
7 Platinum electrode, [6-azaflavin] = 5.0 x 10^{-4} M, [$Bu_4N^+ClO_4^-$] = 0.1 M. Scan rate; 100 mV/s^{-1}, 25 °C. 1 M = 1 mol dm^{-3}
8 For oxidation of NADH models: M. F. Powell, W. H. Wong, and T. C. Bruice, *Proc. Natl. Acad. Sci., U.S.A.*, **79**, 4604 (1982); M. F. Powell, and T. C. Bruice, *J. Am. Chem. Soc.*, **105**, 1014 (1983). For thiol oxidation: E. L. Loechler and T. C. Hollocher, *J. Am. Chem. Soc.*, **102**, 7312, 7322 (1980).
9 The rate equations were derived from the following reaction scheme:



$$[C] = \frac{(K'[Fl]_o + K'[1]_o + 1)}{2K'} - \frac{\{([Fl]_o^2 + [1]_o^2 - 2[Fl]_o[1]_o)K'^2 + 2K'([Fl]_o + [1]_o) + 1\}^{1/2}}{2K'} \quad (1)$$

$$\text{Rate} = k_{obs}[Fl]_o = k_o([Fl]_o - [C])[S]_o + k_2[C][S]_o \quad (2)$$

$$k_{obs} = \frac{\{k_o[Fl]_o + (k_2 - k_o)[C]\}[S]_o}{[Fl]_o} \quad (3)$$

$[Fl]_o$ and $[1]_o$ represent the initial concentrations of 6-azaflavin and **1**. $[S]_o$ =[BNAH]$_o$ or [PhSH]^2[Bu_3N], [C] = 6-azaflavin•**1**.

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11 The binding constants of 6-azaflavin•**2** were determined separately in the presence of Bu_3N: K = 7.4 x 10^3M^{-1} ([Bu_3N] = 5.0 x 10^{-3} M), 2.3 x 10^3M^{-1} (1.5 x 10^{-2} M).
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14 A. Niemz and V. M. Rotello, *Acc. Chem. Res.*, in press. Flavin receptors using H-bonds at C(2)=O, N(3)-H, and C(4)=O are discussed