CHEMISTRY LETTERS, pp. 1009 - 1012, 1984. © The Chemical Society of Japan 1984

ASSOCIATION AND REACTIVITY OF AMPHIPHILIC FLAVINS. 8-ALKYL MERCAPTOFLAVINS AND THE CORRESPONDING BIS-FLAVINS IN AQUEOUS SOLUTION

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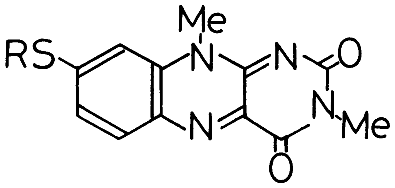
It was found that 8-octyl mercaptoflavin forms a dimer in aqueous solution, and the absorption spectrum is quite similar to that of the corresponding bis-flavin linked by $-(CH_2)_3-$ at 10,10'-positions of the isoalloxazine rings. The association mode and the reactivity for oxidation of N-benzyl-1,4-dihydronicotinamide(BNAH) are discussed.

In biological systems, flavin nuclei are more or less tightly bound to proteins, and in some cases they are closely located and interact with each other.$^{1)}$ Thus, flavin-flavin interactions in model systems have received considerable attention. An isoalloxazine ring is considered to be composed of hydrophobic and hydrophilic parts such as phenyl and pyrimidine moieties. Thus, the mode of aggregation would delicately depend on the positions of the additional hydrophobic groups attached to the isoalloxazine ring.$^{2)}$ Meanwhile, Shinkai et al. have proposed that 10-dodecylflavin aggregates in aqueous solution.$^{3)}$

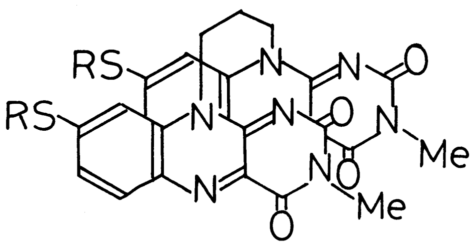
Recently, we showed that the bis-flavin($\overparen{\mathrm{Fox}\ \ \mathrm{Fox}}$) linked by $-(CH_2)_3-$ at 10,10'-positions of the isoalloxazine rings reveals enhanced reactivity of the first flavin for BNAH oxidation to give $\overparen{\mathrm{FH_2}\ \ \mathrm{Fox}}$, and diminished reactivity of the second flavin to give $\overparen{\mathrm{FH_2}\ \ \mathrm{FH_2}}$ due to intra-molecular charge-transfer complex formation ($\overparen{\mathrm{FH_2}\cdots\mathrm{Fox}}$) in aqueous solution.$^{4)}$ However, a question has remained as to the mode of the association of the intra-molecular isoalloxazine rings; that is (a) syn-association(phenyl-phenyl and pyrimidine and pyrimidine) or (b) anti-association(phenyl-pyrimidine).

In this paper, we describe association behaviors and reactivities of 8-octyl mercaptoflavin and the corresponding bis-flavin which is probably compelled to assume the syn-association due to hydrophobic interaction of the long alkyl groups in aqueous solution.



1; R = n-C_4H_9

2; R = n-C_8H_{17}



3; R = n-C_4H_9

4; R = n-$C_{12}H_{25}$

The flavins were prepared from 8-chloroflavins and thiols.[5)] Identification was performed by elemental analyses.[6)] The association behaviors were examined by the absorption spectra in ethanol and in aqueous solution. Remarkably different absorption spectra were observed for **1**, **2**, **3**, and **4** in aqueous solution as shown in Fig. 1, whereas the spectra of **1**, **2**, **3**, and **4** were almost same in EtOH.

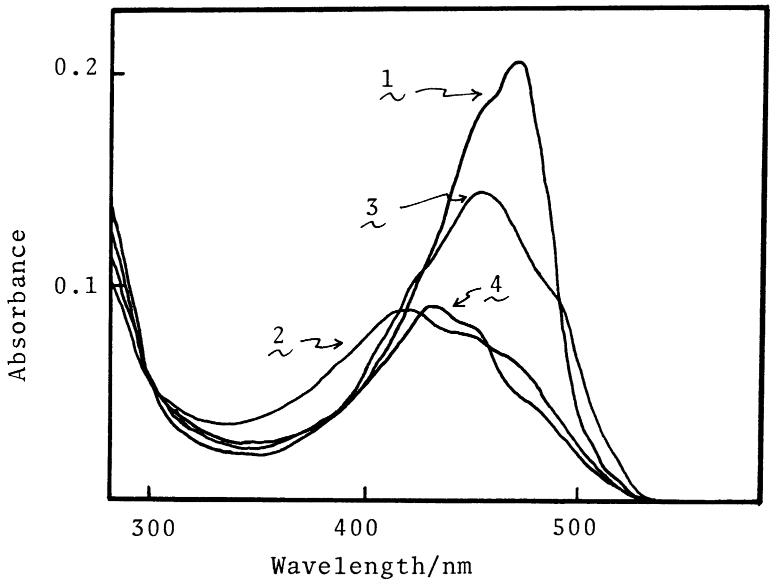


Fig. 1. Absorption spectra in aqueous solution(pH 7.30, 0.02 M phosphate, μ=0.06), [**1**]=[**2**]=6.67 x 10^{-6}M, [**3**]=[**4**]=3.33 x 10^{-6}M, 1% DMF, 25 °C.

Figure 1 indicates that the spectral shapes of the hydrophobic flavins(**2** and **4**) are unusual, whereas **1** displays a normal spectrum of 8-alkyl mercaptoflavin.[5)] The spectrum of **3** seems to be slightly changed from the normal one. The unusual spectra may be brought about by inter- or intra-molecular association of the isoalloxazine rings by the aid of hydrophobic interaction of 8-long alkyl groups in the aqueous solution. This is supported by the similarity of spectra of **1**, **2**, **3**, and 4 in EtOH, in which such a hydrophobic interaction is not achieved.

Furthermore, the spectral shape of 2 was found to be sensitive to concentration and temperature(Figs. 2 and 3). Figure 2 shows that the spectrum at 1 x 10^{-6}M is normal[λ_{max} 470 nm(ε; 23,000)],[5] and it shifts to 418 nm with the increase in the concentration of 2, with an isosbestic point at 425 nm. This suggests the presence of two species in equilibrium in the solution, as often observed for dye molecules in solution.[7] The aggregation number was determined to be 2.2 by the conventional method,[7] showing dimer formation. Similar spectral changes were also observed with rise of the temperature(Fig. 3), revealing deaggregation of dimer to monomer. The spectral changes due to temperature were found to be reversible. The phase-transition temperature was 43 °C, as obtained from the inserted figure in Fig. 3. For the bis-flavin(4), however, such spectral changes due to concentration and temperature were not observed, suggesting the intra-molecular association to be tighter than the inter-molecular one. In fact, addition of CTABr(1.3 x 10^{-3}M) was found to deaggregate the dimer of 2 to the monomer, whereas such deaggregation did not occur for 4.

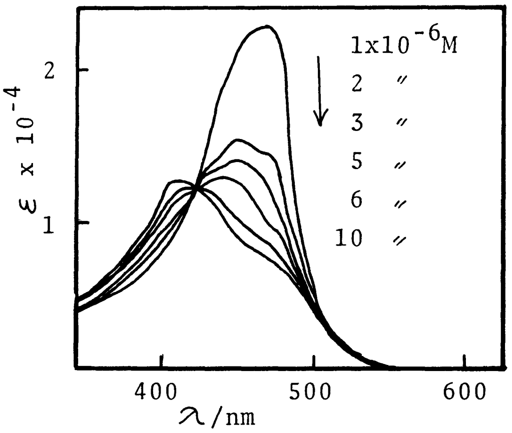


Fig. 2. Spectra of 2 at various concentrations.

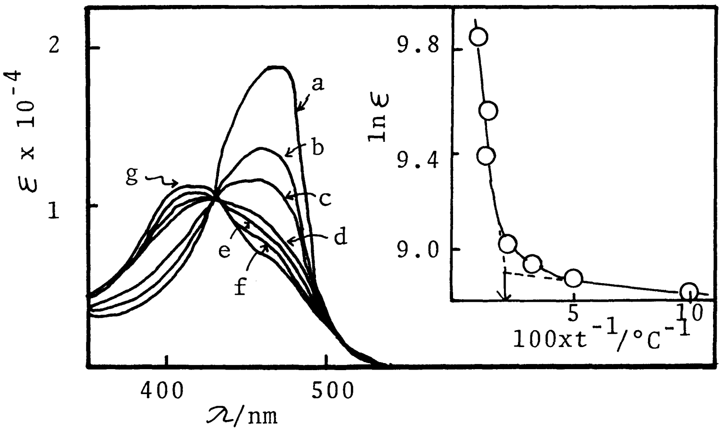


Fig. 3. Spectra of 2: a; 80, b; 70, c; 60, d; 40, e; 30, f; 20, g; 10 °C, [2]=1x10^{-5}M.

These observations together with the analogous spectral shapes of 2 and 4 allow us to propose a similar mode of association for 2 and 4. Namely, face-to-face syn-association of the isoalloxazine rings is conceivable: inter-molecular association for 2 and intra-molecular one for 4.

We have examined kinetically the reactivities of 1, 3, and 4 for oxidation of BNAH([1] = 6.67 x 10^{-6}M, [3] = [4] = 3.3 x 10^{-6}M, [BNAH] = 1 x 10^{-3}M, pH 7.54, 0.02 M phosphate, μ = 0.06, 25 °C). The rate for 2 could not be determined due to a nonlinear relation between absorbances and concentrations. The relative rates for **1**, **3**, and **4** were found to be 1 : 0.32 : 0.16, suggesting that the syn-association diminishes the reactivity. Thus, the rate enhancement previously obtained for unsubstituted bis-flavins may be explained by the intra-molecular charge-transfer complex formation, which is effectively achieved for the face-to-face anti-association of the isoalloxazine rings.

The present study demonstrates (a) a new type of association of amphiphilic flavins in aqueous solution, and that (b) the syn-association of the isoalloxazine rings diminishes the oxidizing reactivity, and also suggests that (c) spectral properties of 8-S-cysteinylflavins as probes of flavin-binding domains of flavoproteins$^{8)}$ must be treated carefully when two flavins are closely located.

References

1) H. Kamin, and J. D. Lambeth, and L. M. Siegel, "Flavins and Flavoproteins," ed by K. Yagi and T. Yamano, University Park Press, Baltimore (1980), p. 341.

2) W. Schmidt, J. Membrane Biol., **47**, 1 (1979); E. Frehland and H. W. Trissl, ibid., **21**, 147 (1975); R. Drabent and H. Grajek, Biochim. Biophys. Acta, **758**, 98 (1983).

3) S. Shinkai, A. Harada, Y. Ishikawa, and O. Manabe, J. Chem. Soc., Perkin Trans. 2, **1982**, 125.

4) Y. Yano and Eiichi Ohya, Chem. Lett., **1983**, 1281; J. Chem. Soc., Perkin Trans. 2, in press.

5) F. Yoneda, K. Shinozuka, K. Tsukuda, and A. Koshiro, J. Heterocyclic Chem., **16**, 1365 (1979); E. G. Moore, S. Ghisla, and V. Massey, J. Biol. Chem., **254**, 8173 (1979).

6) **1**; mp 260 °C(decomp). Found: C, 58.37, H; 5.50, N; 16.77% Calcd for $C_{16}H_{18}N_4O_2S$: C; 58.16, H; 5.49, N; 16.96%. **2**; mp 255 °C. Found: C; 59.62, H; 6.62, N; 14.15%. Calcd for $C_{20}H_{26}N_4O_2S \cdot H_2O$: C; 59.38, H, 6.97, N; 13.85%. **3**; mp 255 °C(decomp). Found: C; 56.54, H, 5.31, N; 16.00%. Calcd for $C_{33}H_{36}N_8O_4S_2 \cdot 1.5H_2O$: C; 56.64, H; 5.61, N; 16.01%. **4**; mp 271 °C(decomp). Found: C: 64.63, H; 7.48, N; 12.53%. Calcd for $C_{49}H_{68}N_8O_4S_2 \cdot 0.5H_2O$: C; 64.94, H; 7.67, N; 12.36%.

7) V. Zanker, Z. Phys. Chem., **200**, 250 (1952); N. Mataga, Bull. Chem. Soc. Jpn., **30**, 375 (1957).

8) V. Massey and P. Hemmerich, "Flavins and Flavoproteins," ed by V. Massey and C. H. Williams, Elsevier North-Holland, New York (1982), p. 83.

(Received February 25, 1984)