

UNUSUAL KINETIC BEHAVIOR OF BIS-FLAVIN FOR N-BENZYL-
1,4-DIHYDRONICOTINAMIDE (BNAH) REDUCTION IN AN AQUEOUS SOLUTION

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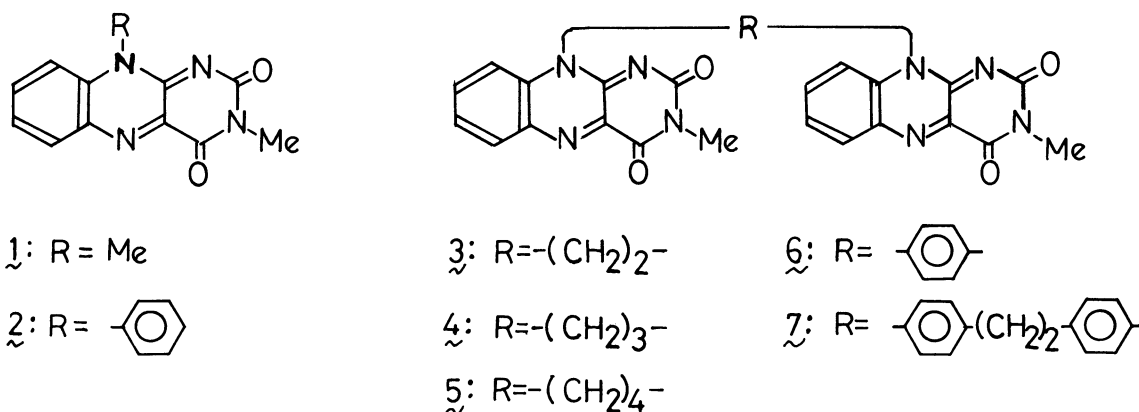
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Several bis-flavins linked at 10,10'-positions of isoalloxazine rings were synthesized. The reactivity of each flavin of a bis-flavin was found to be considerably influenced by another intramolecular flavin moiety, depending on the conformation of the bis-flavin.

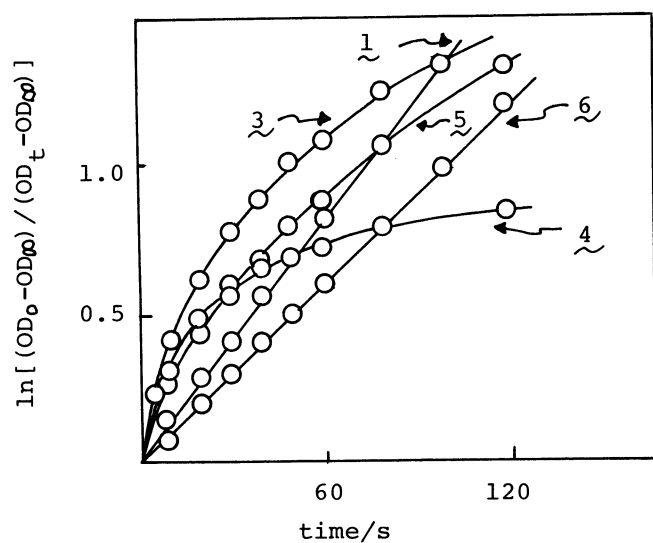
Flavins are known to form charge-transfer complexes with a variety of organic molecules in an aqueous solution, since oxidized flavins (Fox) act as electron acceptors and reduced flavins (FH₂) act as electron donors.¹⁾ For instance, the charge-transfer complex between FMN and FMNH₂ is observed by visible spectra at relatively high concentrations of FMN and FMNH₂ in an aqueous solution.²⁾ Meanwhile, Murakami et al. have reported that the reduction of hexachloroacetone by bis-1,4-dihydronicotinamides is remarkably enhanced due to an electronic interaction of intramolecular reduced and oxidized nicotinamide rings in CH₂Cl₂.³⁾ A similar electronic interaction may be conceivable in the case of flavin coenzyme model compounds. Thus, it is of interest to investigate the reactivity of the bis-flavins in which two intramolecular flavins are able to interact each other.

In this paper, we wish to report the reactivity of several bis-flavins linked at 10,10'-positions (F $\overline{\text{Ox}}$ Fox) for BNAH reduction in an aqueous solution.

The bis-flavins were prepared by condensation of the corresponding diamines and N-methylalloxan according to the essentially similar procedures described by Leonard et al.⁴⁾ Identification was performed by elemental analyses and absorption spectra. Vis-UV spectra of the bis-flavins were quite similar to those of mono-flavins (1 and 2) in an aqueous solution.



The reactivity of the flavins for BNAH reduction was estimated spectrophotometrically by following the decrease of the absorption of Fox (≈ 440 nm) in aqueous solution under anaerobic conditions.⁶⁾ Oxygen introduction into the cuvette after the reaction regenerated the starting oxidized flavin quantitatively in all the cases. First-order plots [$\ln(\text{OD}_0 - \text{OD}_\infty) / (\text{OD}_t - \text{OD}_\infty)$] vs. time] are shown in Fig. 1. The figure indicates that $\overline{\text{Fox}}$ Fox ($\underline{3}$, $\underline{4}$, and $\underline{5}$) does not follow first-order rate equation, whereas $\underline{1}$, $\underline{2}$, $\underline{6}$, and $\underline{7}$ obey first-order kinetics up to more than 90% of the reaction ($\underline{2}$ and $\underline{7}$; not shown in Fig. 1). The kinetic behaviors of $\underline{3}$, $\underline{4}$, and $\underline{5}$ are unusual. The CPK molecular model examination of $\overline{\text{FH}_2}$ Fox proved that intramolecular face-to-face interaction of two isoalloxazine rings is possible for $\underline{3}$, $\underline{4}$, and $\underline{5}$,



$[\text{Fox}] = 1.3 \times 10^{-5} \text{ M}$,
 $[\overline{\text{Fox}} \text{ Fox}] = 6.7 \times 10^{-6} \text{ M}$,
 $[\text{BNAH}] = 1 \times 10^{-3} \text{ M}$,
 pH 7.27 (0.1M, phosphate, $\mu = 0.5$),
 25°C.

Fig. 1. First-order plots.

whereas such an interaction is geometrically impossible for 6 and 7. Therefore, it is assumed that the nonlinear plots of 3, 4, and 5 in Fig. 1 may result from intramolecular charge-transfer complex. Namely, the charge-transferred oxidized flavin in $\overline{\text{FH}_2 \cdots \text{Fox}}$ is supposed to be hard to be reduced by BNAH, and the initial and the subsequent gentle slopes (3, 4, and 5) may correspond to the rate constants of the reactions of $\overline{\text{Fox}} + \text{BNAH}$ and $\overline{\text{FH}_2 \cdots \text{Fox}} + \text{BNAH}$. The rate constants thus obtained are summarized in Table 1. The table indicates; (a) the initial rates of 3, 4, and 5 are a few times larger than k_{obsd} of 1, 2, 6, and 7, (b) the rate

Table 1. The rate constants ^{a)}

| Flavins | $10^2 \times k_{\text{obsd}}/\text{s}^{-1}$ | Rel. rates |
|---------|---|------------|
| 1 | 1.34 | 1.0 |
| 2 | 0.880 | 0.66 |
| 3 | 4.7 ^{b)} , 0.70 ^{c)} | 3.5, 0.52 |
| 4 | 3.8 ^{b)} , 0.18 ^{c)} | 2.8, 0.13 |
| 5 | 3.2 ^{b)} , 0.72 ^{c)} | 2.4, 0.54 |
| 6 | 1.01 | 0.75 |
| 7 | 1.50 | 1.1 |

a) Average of two runs (\pm 5% error).

b) Estimated from the initial slopes.

c) Estimated from the slopes after 50% reaction.

retardation is most notable for 4, since the rate is diminished to 1/20 (0.18/3.8). This may be accounted for by formation of an intramolecular charge-transfer complex, since face-to-face orientation of two isoalloxazine rings in $\overline{\text{FH}_2 \cdots \text{Fox}}$ is most favorable for 4 according to the CPK molecular model construction.

Detection of a charge-transfer complex between FH_2 and Fox was attempted spectrophotometrically in EDTA-photoreduction⁶⁾ by employing 1, 4, and 6 under the same pH, since the present BNAH reduction was too fast to scan the whole spectra during the course of the reaction. The absorption spectrum of half-reduced 4 showed a broad absorption band at 700-900 nm ($\lambda \approx 600$), which disappeared when the reduction was completed. However, such a band was not observed for 1 and 6.

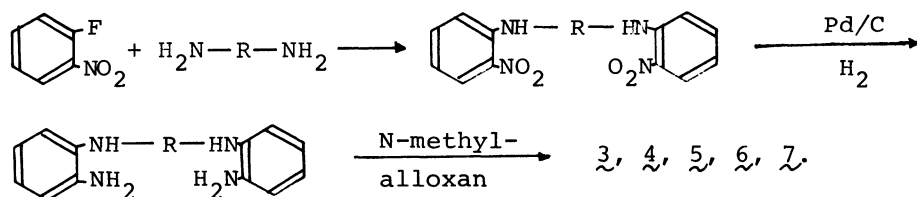
Hence, this band could be attributed to the intramolecular charge-transfer band of $\overline{\text{FH}_2\text{---Fox}}$.²⁾ This observation suggests that such a charge-transfer interaction is also operative in BNAH reduction.

The present study suggests that a reactivity of flavin is considerably influenced by another flavin located close to each other even in an aqueous solution.

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