CHEMISTRY LETTERS, pp. 543 - 546, 1985.

NEW FLAVIN SEMIQUINONE RADICALS AS PRODUCED BY THE REACTION WITH NADH MODEL COMPOUNDS

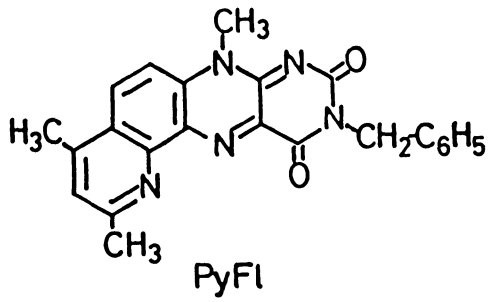
Seiji SHINKAI,* Noriaki HONDA, Yuichi ISHIKAWA, Franz MÜLLER,† and Osamu MANABE*

Department of Industrial Chemistry, Faculty of Engineering, Nagasaki University, Nagasaki 852

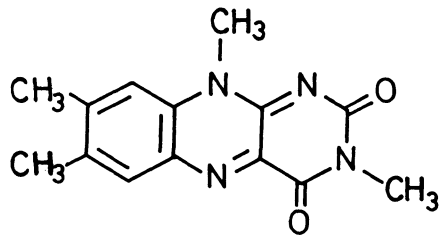
†Department of Biochemistry, Agricultural University, De Dreijen 11, 6703 BC Wageningen, The Netherlands

The oxidation activity of a flavin (PyFl) with a fused pyridine moiety as a metal-chelation site was enhanced in the presence of certain metal ions. The reaction of PyFl and 1-benzyl-1,4-dihydronicotinamide in the presence of Mg(II), Zn(II), and Cd(II) in acetonitrile gave a transient absorption band at around 500 nm and an ESR spectrum attributable to the semiquinone radical. This is the first example for the flavin semiquinone radical produced by the reaction with an NADH model compound.

Flavin coenzymes are versatile redox catalysts in many biological systems and serve as electron carriers, for example, to and from ion-sulfur proteins, molybdenum (xanthine oxidase), and heme proteins.[1] Thus, the interactions between metal ions and flavin coenzymes have drawn extensive interests of many flavin chemists. In spite of considerable attention to the metal-flavin interactions in biological systems, however, the model investigations have been very limited.[2-4] The difficulty of the model investigations mainly stems from the poor affinity of flavin molecules with metal ions. In fact, metal ions which are capable of binding to flavin molecules are limited to several low-valence metal ions, the complexes being stabilized by a metal→flavin charge transfer. Recently, we synthesized several new flavins which have additional metal-chelation sites, the metal complexes being stabilized probably by a flavin→metal charge transfer.[5-7] By this means, one can induce the positive shift of the redox potential leading to the activation as oxidizing agents.[3,7] In this communication, we address the effect of metal ions on the reactivity of 2,4,7-trimethyl-10-benzylquino[8,7-g]pteridine-9,11(7H, 10H)-dione(PyFl) which bears a fused pyridine moiety as a metal-chelation site.[5] As expected, the oxidation ability was enhanced through the complexation with certain metal ions. Furthermore, we have found unexpectedly that the reaction of some metal complexes with an NADH analogue affords the spectroscopically-detectable semiquinone radical. This is the first nonenzymatic example for the flavin semiquinone radical produced by the reaction with an NADH analogue.



PyFl



MeLFl

Table 1. Second-order rate constants(k_2) for the oxidation of BNAH by PyFl or MeLFl in acetonitrile at 303 K[a)]

Metal salt	$k_2/M^{-1}s^{-1}$	
	PyFl	MeLFl
None	0	0
$NaClO_4$	0	0
$Mg(ClO_4)_2$	0.486	ca. 4×10^{-3}
$Zn(NO_3)_2$	4.17	$<10^{-3}$
$Cd(NO_3)_2$	1.27	ca. 9×10^{-3}
$SbCl_3$[b)]	2.86	0.01

a) N_2, [flavin]=2.98×10^{-5} M(1 M=1 mol dm^{-3}), [BNAH]=1.50×10^{-3} M, [metal salt]=2.19×10^{-4} M, 0.33 vol% DMF. b) [2,6-lutidine]=1.40×10^{-3} M.

First, we evaluated the effect of complexed metal ions on the oxidation of 1-benzyl-1,4-dihydronicotinamide(BNAH) by PyFl and 3-methyllumiflavin(MeLFl) in acetonitrile at 303 K. The progress of the anaerobic reactions was followed spectrophotometrically by monitoring the disappearance of the absorption maxima of flavins (444 nm for PyFl; 446 nm for MeLFl). The rates were apparently first-order in flavins and BNAH. The second-order rate constants thus determined are summarized in Table 1. 2,6-Lutidine was added (when stated) as a proton scavenger to suppress the acid-catalyzed decomposition of BNAH. It is seen from Table 1 that (i) neither PyFl nor MeLFl (used as a reference) can oxidize BNAH in the absence of metal ions, (ii) oxidation by PyFl is efficiently catalyzed by several metal ions, but these metal ions are ineffective for the oxidation by MeLFl, and (iii) oxidation is not facilitated at all by the addition of $NaClO_4$ or Et_4NBr. It is known that some of these metal ions can interact with BNAH in acetonitrile (e.g., K_s(association constant)=ca. 10^3 M^{-1} for $Mg(ClO_4)_2$).[8)] Similarly, PyFl strongly binds these metal ions: the K_s values determined spectrophotometrically are 37 M^{-1} for Mg(II), 1.4×10^5 M^{-1} for Zn(II), and 4.4×10^5 M^{-1} for Cd(II) ([H_2O] in acetonitrile, (1-2) $\times$ 10^{-2} M). Since MeLFl showed no (or poor) metal affinity, the metal effect observed for PyFl should be attributed primarily to the PyFl-metal interaction which causes the electron-deficiency of PyFl.

The final absorption spectrum obtained in the presence of Sb(III) was similar to that of reduced PyFl. The authentic spectrum of reduced PyFl was prepared by photoreduction with EDTA or benzyl alcohol(Fig. 1). On the other hand, the reaction with BNAH in the presence of Mg(II), Zn(II), and Cd(II) produced a red color.

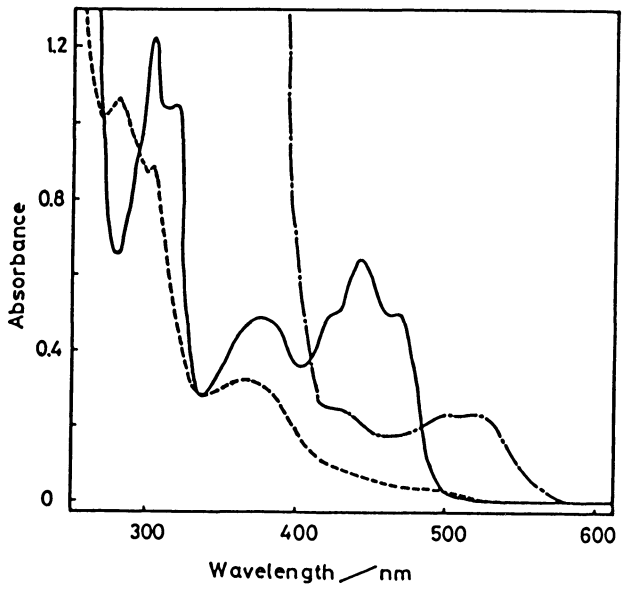


Fig. 1. Absorption spectra of PyFl(——), fully-reduced PyFl(---), and the semiquinone radical produced by the reaction with BNAH (2.50×10^{-3} M) in the presence of $Zn(NO_3)_2$ (2.19×10^{-4} M) (·– ·–) in MeCN at 303 K. [PyFl]=5.00×10^{-5} M.

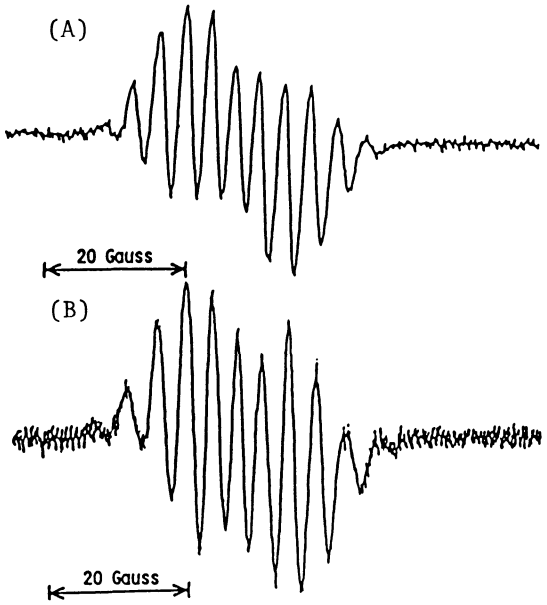
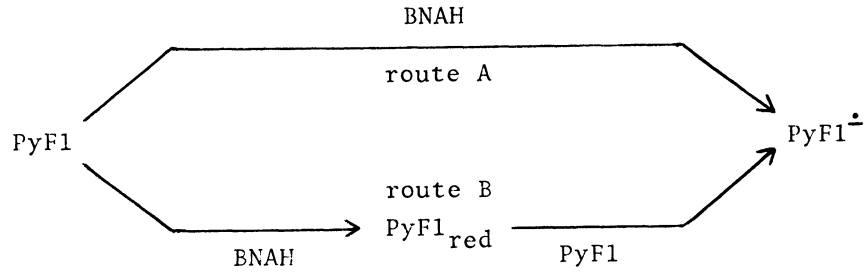


Fig. 2. ESR spectra obtained (A) by mixing equimolar amounts(5.00×10^{-4} M) of oxidized and reduced PyFl in the presence of $Zn(ClO_4)_2$(4-fold) and t-BuOK(4-fold) in DMF and (B) by the reaction of PyFl(2.99×10^{-4} M) and BNAH(1.50×10^{-3} M) in the presence of $Zn(NO_3)_2$(2.20×10^{-3} M) in MeCN.

The color is due to a new absorption band at around 500 nm. When O_2 was introduced into the red solution, oxidized PyFl was regenerated quantitatively. The new absorption spectrum, which is different from either PyFl or reduced PyFl, can be attributed to the flavin semiquinone radical.

In nonenzymatic systems, flavin semiquinone radicals are prepared only by mixing equimolar amounts of oxidized and reduced flavins in the presence of some metal ions(e.g., Zn(II)). Figure 2A shows the ESR spectrum obtained by mixing equimolar amounts of PyFl and reduced PyFl in the presence of Zn(II).[9,10] The red solution prepared from PyFl and BNAH in the presence of Zn(II) gave a similar ESR spectrum (Fig. 2B), and the time-dependence was well correlative to that of the absorption band at around 500 nm.[11] The similar ESR spectra were also observable in the presence of Cd(II) or Mg(II). These findings establish that the new absorption spectra are ascribed to the semiquinone radicals of PyFl. The maximum spin concentrations (determined by comparison with that of DPPH) were 34.4% for [BNAH]/[PyFl]=5.0 and 61.5% for [BNAH]/[PyFl]=50.0

There are two possible routes to reach the semiquinone radical $PyFl^{\dot{-}}$ (Eq. 1): the direct production in which BNAH acts as a one-electron donor (route A) and the two-step production in which PyFl is once converted by BNAH to fully-reduced PyFl ($PyFl_{red}$) followed by comproportionation with PyFl (route B).



(1)

Two mechanisms can be discriminated by the reaction with 4,4-dideuterio-BNAH.[12] The reaction of PyF1(2.98×10^{-5} M) and BNAH (or BNAH-d_2, 98.9% isotope purity: 1.50×10^{-3} M) in the presence of $Zn(NO_3)_2$ (2.19×10^{-4} M) was followed by the appearance of the absorption band at 500 nm. The kinetic isotope effect, $k_H/k_D = 2.6\pm0.1$, supports that PyF1· is produced via route B.

It is known that flavin semiquinone radicals are ubiquitously seen in flavoproteins and some of them are produced directly by the reaction with NAD(P)H.[9] They can serve as electron pools for electron transfers to and from other prosthetic groups. Hence, the present study suggests, for the first time, a possibility to mimic this class of electron transfers in nonenzymatic systems. Further investigation are currently under way in this laboratory.

We thank Professor U. K. Pandit for helpful discussions.

References

1) P. Hemmerich, G. Nagelschneider, and C. Veeger, FEBS Lett., 8, 69(1970).
2) P. Hemmerich, F. Müller, and A. Ehrenberg, "Oxidases and Related Redox Systems," John Wiley & Sons, New York (1965), p. 157 and references cited therein.
3) S. Fukuzumi, S. Kuroda, and T. Tanaka, Chem. Lett., 1984, 1375; S. Fukuzumi, Y. Kondo, and T. Tanaka, ibid., 1983, 485.
4) Y. Yano, T. Sakaguchi, and M. Nakazato, J. Chem. Soc., Chem. Commun., 1984, 595.
5) S. Shinkai, Y. Ishikawa, and O. Manabe, Bull. Chem. Soc. Jpn., 56, 1694(1983).
6) S. Shinkai, Y. Ishikawa, H. Shinkai, T. Tsuno, H. Makishima, K. Ueda, and O. Manabe, J. Am. Chem. Soc., 106, 1801(1984).
7) S. Shinkai, H. Nakao, K. Ueda, and O. Manabe, Tetrahedron Lett., 25, 5295(1984).
8) A. Ohno, H. Yamamoto, T. Okamoto, S. Oka, and Y. Ohnishi, Bull. Chem. Soc. Jpn., 50, 2385(1977). In more dehydrated acetonitrile, K_s is enhanced up to 1.21×10^4 M^{-1}(see ref. 3).
9) F. Müller, P. Hemmerich, and A. Ehrenberg, "Flavins and Flavoproteins," ed by H. Kamin, University Park Press, Baltimore (1971), p. 107.
10) A. Ehrenberg, F. Müller, and P. Hemmerich, Eur. J. Biochem., 2, 286(1967).
11) The ESR spectra and the absorption spectra were scarcely affected by the type of zinc salts(i.e., $ZnCl_2$, $Zn(ClO_4)_2$, and $Zn(NO_3)_2$), but the k_2 values changed significantly.
12) S. Fukuzumi, Y. Kondo, and T. Tanaka, Chem. Lett., 1983, 751.

(Received February 4, 1985)