A NOVEL PROPERTY OF REDUCED BENZODIPTERIDINE TOWARD MOLECULAR OXYGEN

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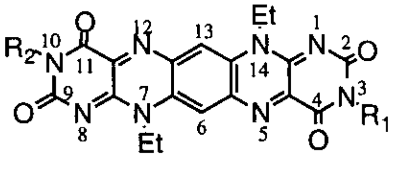
Abstract - Reactivity of reduced benzodipteridine toward O_2 was found to change dramatically, depending on the substituents at the N(3) and N(10) positions and pH in aqueous solution.

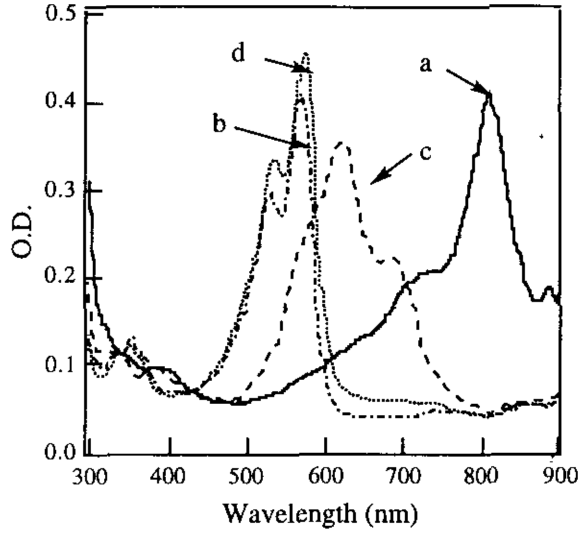
A flavin model-compound accepts two electrons and a proton(s) to form a reduced form which rapidly reacts with molecular oxygen to generate the starting compound. We have reported that benzodipteridine (BDP), consisting of two isoalloxazine skeletons with a benzo unit in common, accepts two electrons to form reduced one which regenerates BDP by O_2-bubbling in aqueous solutions, and BDP is a useful flavin model due to its high oxidation activity.[1] During the course of our investigation on BDP, we found unusual behavior of the reduced BDP toward O_2 in aqueous solutions. We report herein that the reduced H,H-BDP forms an O_2-stable compound by itself below pH 8.5, whereas O_2-unstable above pH 8.5. The following BDP's were employed.



H,H-BDP: $R_1 = R_2 = H$
Me,H-BDP: $R_1 = Me$, $R_2 = H$
Me,Me-BDP: $R_1 = R_2 = Me$

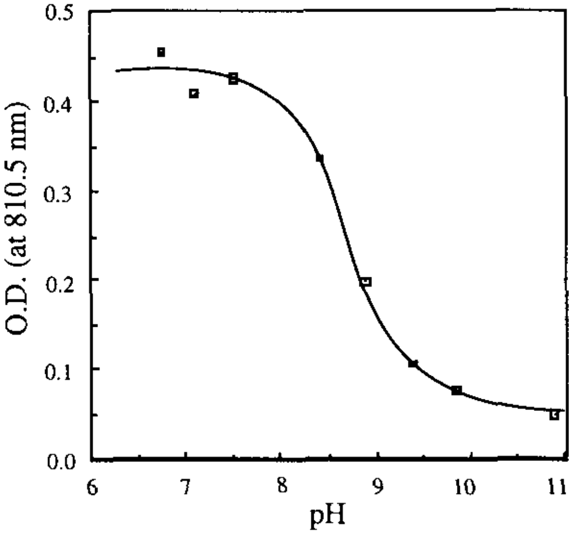
In the first place, we determined the redox potentials by cyclic voltammetry: H,H-BDP; -0.089 V, Me,H-BDP; - 0.102 V, Me,Me-BDP; - 0.104 V.[2] This order was reasonably explained by electron-donating Me group(s) at the N(3) and N(10) positions. Next, we examined the redox behavior of BDP spectrophotometrically by employing EDTA-photoreduction[1b] in aqueous solutions (pH 7.1 and 10.1) and in EtOH. The absorption spectra of the starting three BDP's were almost same [λmax 390 nm (log ε 4.30) and 548 nm (4.17)] (spectra not shown). The absorption spectra of H,H-BDP and Me,Me-BDP after the

Figure 1. Absorption spectra of BDP's after EDTA-photoreduction.



[BDP]=1.0 x 10^{-5}M, [EDTA•2Na]=5.0x10^{-4}M, 0.04M Britton-Robinson buffer (2 % DMF). H,H-BDP: a; pH 7.1, b; pH 10.1, Me,Me-BDP: c; pH 7.1, d; 10.1, N_2, 25 °C.

Figure 2. Plot of optical density at 810.5 nm vs. pH.



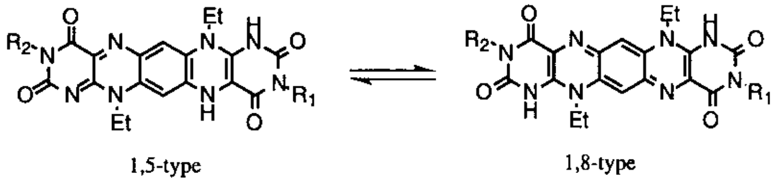
reduction in aqueous solutions were shown in Figure 1. Namely, Me,Me-BDPwas reduced to give the reduced Me,Me-BDP, which was oxidized by O_2 to regenerate the starting Me,Me-BDP despite of pH. However, the O_2-reactivity of the reduced H,H-BDP was markedly different from that of the reduced Me,Me-BDP at pH 7.1 (a and c), whereas similar at pH 10.1 (b and d). Namely, the absorption spectrum (a: λ_{max} 810.5 nm, log ε; 4.54) was not changed by O_2-bubbling, whereas that at pH 10.1 (b: λ_{max} 622 nm, log ε; 4.48) regenerated the starting spectrum by O_2-bubbling. When pH of the solution was changed from 7.1 to 10.1 on addition of alkaline solution, the spectrum (a) changed to the spectrum (b) and *vice versa*, indicating that the spectral changes are pH-interconvertible. The optical density at 810.5 of the spectrum (a) was plotted against pH (Figure 2), suggesting that the proton dissociation (pKa 8.5) disturbs formation of the O_2-stable compound. It should be noted that the redox behavior of Me,H-BDP was similar to that of Me,Me-BDP, and the reduction of H,H-BDP in EtOH was similar to that of other two.

The unique spectroscopic behavior of H,H-BDP at pH 7.1 was also observed for reduction by sodium dithionite, *N*-benzyl-1,4-dihydronicotin-amide, and 2-mercaptoethanol in aqueous solution (pH 7). Furthermore the formation of the O_2-stable compound was found to be quite sensitive to content of organic solvent in the aqueous solution. For example, spectroscopic examination of H,H-BDP with 2-mercaptoethanol in aqueous solution (pH 7.1) containing 4 % DMF showed that the absorption of the reduced H,H-BDP (622 nm) firstly appears and gradually shifts to that of the O_2-stable one (810.5 nm). This clearly indicates that the O_2-stable compound is composed of the reduced H,H-BDP by itself, and hydrophobic bonding is in part responsible for formation of the O_2-stable compound.

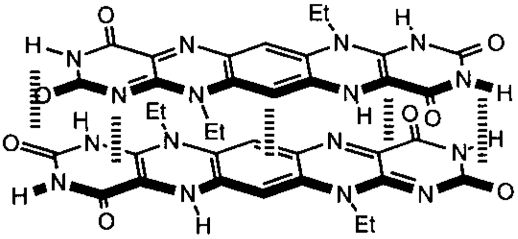
We have reported that the reduced Me,Me-BDP possesses two dissociative hydrogen (Me,Me-BDPH_2 $\rightleftharpoons$

Me,Me-BDPH$^-$ + H$^+$, pKa 3.6; Me,Me-BDPH$^-$ $\rightleftharpoons$ Me,Me-BDP^{2-} + H$^+$, pKa 8.5),[1b] and exists in two tautomeric forms, either 1,5- or 1,8-type, as shown in Scheme 1. Since pK$_a$ values are considered to be almost same for the reduced BDP's, the species in the solution are a monoanion at pH 7.1, and a dianion at pH 10.1. Meanwhile, the 1,5-type could be regarded to involve both oxidized and reduced isoalloxazine rings in the molecule with sharing a benzo moiety. Electrons in the dianionic form (H,H-BDP^{2-}) would be delocalized through the long conjugated system to avoid charge-charge repulsion at the N(1)- and N(5)-positions, thus adopting 1,8-type. Intermolecular attractive force between two molecules of the reduced BDP is conceivable to be stronger for 1,5- than 1,8-type, since the 1,5-type involves electron-donating reduced- and electron-accepting oxidized isoalloxazine rings in the molecule. It should be noted that a mixture of oxidized and reduced flavins forms a charge-transfer complex in an aqueous solution under anaerobic conditions.[3]

Scheme 1. Tautomeric forms of BDPH$_2$



We reported previously that pulse radiolysis of Me,Me-BDP shows a transient absorption spectrum (560 nm),[4] indicating that the compound absorbing at 810.5 nm is not a radical species. All the data suggest that the O$_2$-stable compound is composed of either H,H-BDPH$_2$ or H,H-BDPH$^-$. When one molecule is turned inside out and upside down, attractive forces between the molecules would be strengthened by (i) a charge-transfer interaction between the oxidized- and reduced-like isoalloxazine moieties in 1,5-type, (ii) interaction of anti-parallel dipoles[5] of N-H and C=O groups stacked each other. The numbers of the interactions of the anti-parallel dipoles (N-H and C=O) are six for H,H-BDPH$_2$ and four for H,H-BDPH$^-$, respectively. Thus we propose the following structural unit to be involved in the O$_2$-stable compound as shown below.



To get information about the structure, we tried to isolate the O$_2$-stable compound as follows: To a stirred solution (distilled water, 200 mL) containing H,H-BDP (10 mg, 0.24 mmol) in DMF (5 mL), 2-mercaptoethanol (0.2 mL) was added under N$_2$. After 2 h stirring, precipitates formed were collected by ultracentrifuge, washed with water (5 mL x 2) and ether (10 mL), and dried *in vacuo* to give violet powder (8 mg, mp > 300 °C), which was stable toward O$_2$ (at least several weeks). However, the powder is totally intractable for characterization. Namely, ^{1}H NMR spectrum could not be recorded due to solubility problem. In organic solvents such as DMSO and DMF, the compound generated reduced H,H-BDP which gradually gave H,H-BDP with dissolved O_2. All attempts to obtain crystals for X-Ray crystallographic analysis including electrocrystallization were unsuccessful. FAB and ESI MS spectra showed only a peak of the reduced H,H-BDP. Nevertheless it is worthy to note that a minor structural change of electron-donor and -acceptor molecules exerts a marked influence on formation of a π-molecular complex.

Meanwhile an air-stable π-organic complex is of interest from a viewpoint of organic electrical conductors.[6] For efficient formation of the complex, it is desirable that π-electron donor- and acceptor-molecules are planar and similar in size, and they possess hetero atoms outside the molecules.[7] The present system seems to satisfy in part these structural requirements. Preliminary measurement of electrical conductivity of the powder showed a semi-conductive property (2.3×10^{-6} Scm^{-1}).[8]

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REFERENCES AND NOTES

1 a) Y. Yano, T. Yokoyama, M. Ikuta, and K. Yoshida, *J. Org. Chem.*, 1987, **52**, 5606; b) Y. Yano, M. Nakazato, K. Iizuka, T. Hoshino, K. Tanaka, M. Koga, and F. Yoneda, *J. Chem. Soc., Perkin Trans. 2*, **1990**, 2179; c) Y. Yano, M. Ikuta, Y. Amemiya, and T. Nabeshima, *Chem. Lett.*, **1991**, 461; d) Y. Yano, K. Mitsui, Y. Ohsawa, T. Kobayashi, and T. Nabeshima, *J. Chem. Soc., Chem. Commun.*, **1993**, 1719; e) T. Kajiki, N. Tamura, T. Nabeshima, and Y. Yano, *Chem. Lett.*, **1995**, 1063.

2 pH 6.2 (0.1 M phosphate), Ag/AgCl (0.1 M KCl), N_2, 25 °C.

3 V. Massey and G. Palmer, *J. Biol. Chem.*, 1962, **237**, 2347; Y. Yano and E. Ohya, *J. Chem. Soc., Perkin Trans. 2*, **1984**, 1227.

4 P. F. Heelis, B. J. Parsons, and Y. Yano, *J. Chem. Soc., Perkin Trans. 2*, **1997**, 795.

5 C. Reichardt, 'Solvents and Solvent Effects in Organic Chemistry,' VCH, Weinheim, 1988, p. 11.

6 J. M. Williams, M. A. Beno, H. H. Wang, P. C. Leung, T. J. Emage, U. Gieser, and K. D. Carson, *Acc. Chem. Res.*, 1985, **18**, 261.

7 G. Saito and K. Yamaji, *Kagaku Sosetsu*, 1983, **42**, 59.

8 Measured on a compressed pellet with KBr at room temperature with a four-probe method.

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