

OXIDATION ACTIVITY OF LONG CONJUGATIVE AND METAL-COORDINATIVE FLAVIN MODEL: BENT-BENZODIPTERIDINE

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Abstract - Redox behavior of 11,14-diethyl-3,8-dimethyl-1,3,5,6,8,10,11,14-octaazapentaphene-2,4,7,9 (14*H*,3*H*,8*H*,11*H*)-tetrone (bent-BDP) was studied in aqueous solution, and a metal ion effect was also examined for the oxidation of an NADH model in acetonitrile.

Well-invented flavin models¹ have made hitherto a great contribution to understanding of flavin chemistry. We have reported that linear conjugative benzodipteridine (BDP), possessing two isoalloxazine rings fused to a common benzo moiety in which all π -electrons are able to conjugate together, exhibits a remarkably high oxidation activity compared with the conventional flavin model (3,10-dimethylisoalloxazine, DMI), and BDP is quite useful for model study of flavin-mediated oxidation.² For design of more sophisticated model systems, functionalized oxidation-active flavin models would be indispensable. Meanwhile, we reported syntheses of "angular mixed flavins" which involve a flavin and deazaflavin moieties in the molecule, and "angular doubled flavins" (bent-BDP).³ Although bent-BDP is a new flavin model possessing a long conjugative system and a metal binding ability, the redox behavior and a metal ion effect have not yet been examined. Herein we wish to report (a) its redox potentials, (b) the oxidation activity for the oxidation of *N*-benzyl-1,4-dihydronicotinamide (BNAH) and 2-mercaptoethanol (ME) in aqueous solution, (c) binding constants for divalent metal ions (Ni^{2+} , Zn^{2+} , and Cd^{2+}) in MeCN, and a metal ion effect for BNAH oxidation in MeCN. The reference compounds are BDP and DMI as shown below.

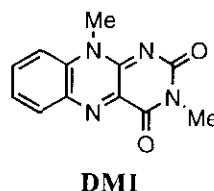
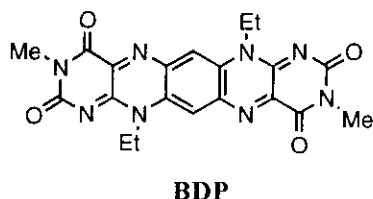
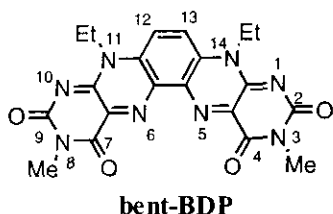
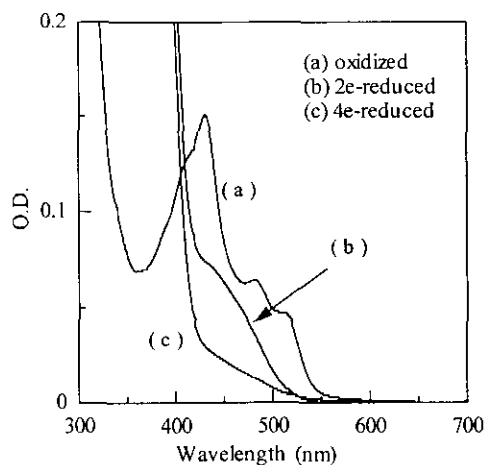
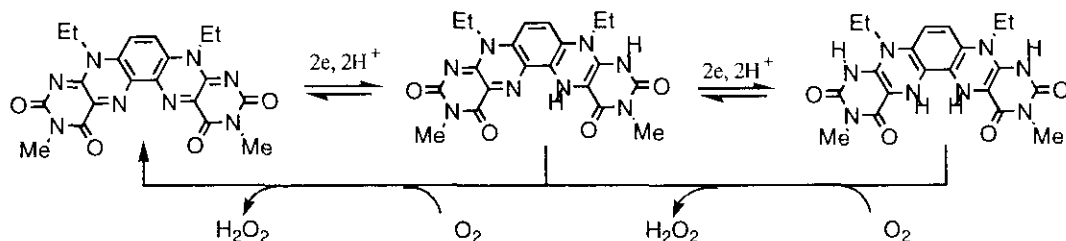


Figure 1. Absorption spectra of bent-BDP

[bent-BDP] = 1.0×10^{-5} M, [BNAH] = 2.0×10^{-4} M,
pH 7.0 (0.05 M HEPES, I = 0.05 with KNO_3), N_2 , 25 °C.

absorption changes were also observed for the oxidation of ME. These indicate that bent-BDP is able to accept total 4 electrons as shown below. It should be noted that such biphasic kinetics is not observed for BDP due to its larger negative E'' (-0.770 V).



Pseudo-first-order rate constants were determined by following the absorption decreases of bent-BDP at 432 nm under anaerobic conditions. Both the fast and subsequent slow reactions were confirmed to follow first-order kinetics. The rates were confirmed to be first-order with respect to [BNAH] and second-order to [ME]. Since the second reaction for ME oxidation was too slow to monitor the absorption decrease, the third-order rate constant was calculated by employing higher concentration of ME. The rate constants are summarized in Table 1 together with those of DMI and BDP. As expected from E'' , the oxidation activity of bent-BDP is higher than that of DMI, and lower than that of BDP. It should be noted that plots of $\log k$ vs. E'' gave linear correlations (correlation coefficient: 0.937 for BNAH; 0.998 for ME), implying that the oxidation proceeds *via* the same mechanisms for bent-BDP, DMI, and BDP.

Since a metal ion is known to be one of factors to control redox properties of flavins,⁶ the metal ion effect (Ni^{2+} , Zn^{2+} , and Cd^{2+}) was examined kinetically for the oxidation of BNAH in MeCN.⁷ Firstly, the binding constants were determined by absorption spectroscopy in MeCN. The absorption spectra of bent-

Redox potentials (E'') of bent-BDP were determined to be -0.325 and -0.540 V by cyclic voltammetry in aqueous buffer solution.⁴ This suggests that the first 2e-oxidation activity is in the order of BDP (E'' ; -0.120 V)⁵ > bent-BDP > DMI (E'' ; -0.439 V)⁵, and the second 2e-oxidation activity of bent-BDP is higher than that of BDP (E'' ; -0.770 V).⁵

Spectroscopic examination of bent-BDP for the oxidation of BNAH indicated that the reaction proceeds *via* a biphasic manner in aqueous solution. As shown in Figure 1, the absorption spectrum (a) of bent-BDP changes smoothly to the spectrum (b), which changes slowly to (c) with the reaction of BNAH. Both the spectra (b) and (c) were confirmed to regenerate the starting spectrum (a) by O_2 -bubbling. Similar

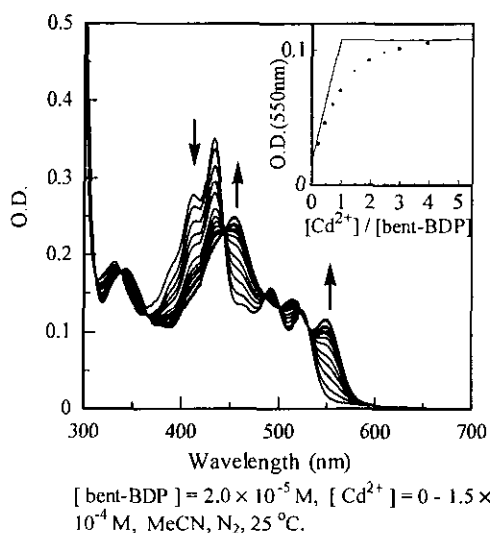
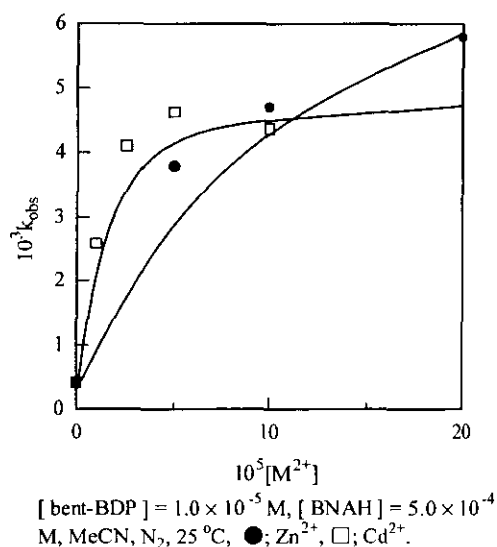
Table 1. Rate constants and relative rates

Flavin ^a	k ($M^{-1}sec^{-1}$)(rel. rate)		k ($M^{-2}sec^{-1}$)(rel. rate)	
	BNAH		ME	
DMI	1.39×10	(1.0)	4.9×10^{-4}	(1.0)
BDP	5.58×10^2	(40)	5.31×10^3	(1.1×10^7)
bent-BDP	2.61×10^2	(19)	3.98×10^{-1}	(8.1×10^2)
	5.89 ^b	(0.4)	$1.3 \times 10^{-6,b}$	(2.7×10^{-3})

a) [bent-BDP] = [BDP] = $1.0 \times 10^{-5} M$, [DMI] = $5.0 \times 10^{-5} M$, pH 7.0 (0.05 M HEPES, I = 0.5 with KNO_3), N_2 , 25 °C.

b) The rate constants for 2e-reduced bent-BDP to 4e-reduced one.

BDP were found to change passing through isosbestic points upon addition of metal ion (Figure 2). The inserted plot of O.D at 550 nm vs. $[Cd^{2+}]/[bent-BDP]$ suggests 1 : 1 complexation. The binding constants (K_F) were determined to be $\sim 0 M^{-1}$ (Ni^{2+}), $100 \pm 10 M^{-1}$ (Zn^{2+}), and $100,000 \pm 15,000 M^{-1}$ (Cd^{2+}), respectively. The larger size of the metal ion shows the larger binding

Figure 2. Spectral changes of bent-BDP with Cd^{2+} .**Figure 3.** Plots of k_{obs} vs. $[M^{2+}]$.

constant. Absorption spectrum of BDP was not changed upon addition of the metal ions. Meanwhile, 1 : 1 metal-binding ability of phenanthroline is known to be in the order of $Ni^{2+} > Zn^{2+} > Cd^{2+}$ in aqueous solution.⁸ The binding constants of bent-BDP, despite in MeCN, is much smaller than those of phenanthroline•metal complexes,⁸ and the order of the binding constants is reverse. These suggest that C(3)=O and C(7)=O are involved in the complexation probably due to weak electron donating ability of N(5) and N(6) of bent-BDP.

Pseudo-first-order rate constants were determined spectrophotometrically by following the absorption decrease at 432 nm. As shown in Figure 3, the rate accelerations were observed for Zn^{2+} and Cd^{2+} which are held by bent-BDP. The rate was little affected upon addition of Ni^{2+} . It was confirmed that the rate is first-order with respect to [BNAH] with or without the metal ions. The rate accelerations could be explained by formation of bent-BDP-metal ion complex (bent-BDP• M^{2+}), since almost no metal ion effect was observed for BDP.⁹ Although kinetic analysis was quite complex (especially for Zn^{2+}),¹⁰ the rate-

acceleration due to formation of bent-BDP•M²⁺ could be estimated to be *ca.* 400-fold for Zn²⁺ and *ca.* 10-fold for Cd²⁺.

The present study demonstrates that bent-BDP is able to accept total four electrons in the oxidation of BNAH and ME in aqueous solution, and the oxidation-activity is higher than that of the conventional flavin (DMI) and lower than that of BDP. However, the oxidation activity of bent-BDP is increased in the presence of the metal ion, suggesting that bent-BDP could be regarded as a potential flavin model for study of metal ion effects on flavin-mediated oxidation.

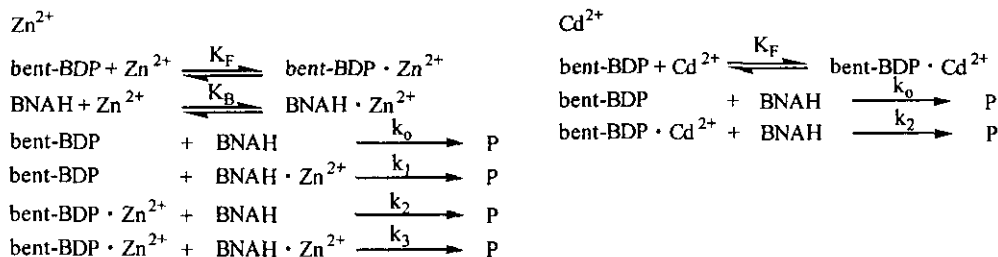
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4. Glassy carbon as a working electrode with an Ag/AgCl, 0.1 M KCl reference electrode [pH 7.0, 0.15 M McIlvaine buffer, *I* = 0.6 (Na₂SO₄) 25 °C], scan rate 100 mV s⁻¹.
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7. In an aqueous solution, no metal ion effect was observed. For the metal ion effect on ME oxidation, the rate was not determined due to precipitation. Metal ions were used as M(NO₃)₂•6H₂O.
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9. Value of *k*_{obs} for BDP was 4.1 x 10⁻³ s⁻¹ without a metal ion under the same conditions of Figure 3, and slightly decreased to 3.6 x 10⁻³ s⁻¹ with [Zn²⁺] = 2.0 x 10⁻⁴ M.
10. The kinetic data allow us to assume the following reaction scheme and rate equations. *K*_b's (binding constants for BNAH•M²⁺) were determined spectrophotometrically in MeCN; 3.1 x 10³ M⁻¹ for Zn²⁺ and 1.5 x 10² M⁻¹ for Cd²⁺. The rate constant (*k*_o) was 0.75 M⁻¹s⁻¹, which was determined without a metal ion. Because of *K*_f >> *K*_b for Cd²⁺, [BNAH • Cd²⁺] term was neglected. Furthermore, no metal ion effect for BDP may allow us to assume *k*_o=*k*_i. Using *k*_o and *K*_b values, non-linear

curve fitting gave the following computed values: For Zn^{2+} , $K_F \approx 330 \text{ M}^{-1}$, $k_2 = 300 \text{ M}^{-1}\text{s}^{-1}$, $k_3 = 45 \text{ M}^{-1}\text{s}^{-1}$; for Cd^{2+} , $K_F = 99,000 \text{ M}^{-1}$, $k_2 = 9 \text{ M}^{-1}\text{s}^{-1}$. The rate accelerations (k_2/k_0) are 400 for Zn^{2+} and 12 for Cd^{2+} .



$$k_{\text{obs}} = k_0[\text{BNAH}]_0 + \frac{[\text{Zn}^{2+}]_0[\text{BNAH} \cdot \text{Zn}^{2+}]}{[\text{Zn}^{2+}]_0[\text{BNAH} \cdot \text{Zn}^{2+}] + 1/K_F} \times \{k_2([\text{BNAH}]_0[\text{BNAH} \cdot \text{Zn}^{2+}]) + k_3[\text{BNAH} \cdot \text{Zn}^{2+}]\}$$

$$\text{where } [\text{BNAH} \cdot \text{Zn}^{2+}] = \frac{[\text{BNAH}]_0 + [\text{Zn}]_0 + 1/K_B - \{([\text{BNAH}]_0 + [\text{Zn}]_0 + 1/K_B)^2 - 4[\text{BNAH}]_0[\text{Zn}]_0\}^{1/2}}{2}$$

$$k_{\text{obs}} = k_0[\text{BNAH}]_0 + \frac{k_2 K_F [\text{Cd}^{2+}]_0 [\text{BNAH}]_0}{1 + K_F [\text{Cd}^{2+}]_0}$$

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