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A Flavin Receptor. Effect of 2,6-Diaminopyridine Derivatives on the Reduction of Benzo-Dipteridine (Oxidation-Active Flavin Model)

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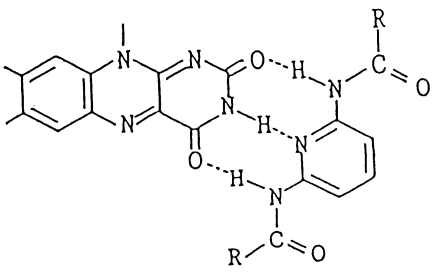
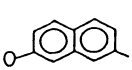
The Hamilton's macrocyclic receptor for thymine was found to act also as a flavin receptor; it forms a complex with 7,14-diethylbenzo[1,2-g; 5,4-g']dipteridine-2,4,9,11-(3H,7H,10H,14H)-tetrone (H-BDP) in $CHCl_3$ and depresses slightly its oxidation activity.

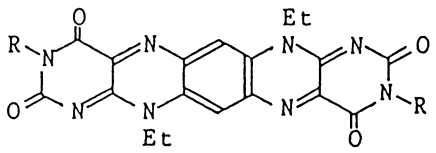
Functions of biological molecules generally appear through specific interactions with another molecule. A hydrogen bond is one of the significant factors in such interactions. Feibush et al. reported that barbiturates, glutarimides, and hydantoins, possessing similarity to uracil and thymine in structure, are able to interact with 2,6-diaminopyridine derivatives by triple hydrogen bond in solute-stationary phase of HPLC.[2] Recently, Hamilton et al. have shown that a macrocyclic receptor containing 2,6-diaminopyridine and naphthalene components (5) forms a molecular complex with a thymine derivative by 'molecular hinge'.[3] These prompted us to examine a possibility of 2,6-diaminopyridine derivatives (1-5) as a flavin receptor as shown in Scheme 1.



1: R = Me
2: R = n-$C_{12}H_{25}$
3: R = -$(CH_2)_{10}$-
4: R = -$(CH_2)_3O$- 
5: R = -$(CH_2)_3O$-  -$O(CH_2)_3$-

Scheme 1.

Benzo-dipteridines (BDP) were employed as flavin models because of the high oxidation activity,[4] the longer absorption wavelengths, and water solubility of H-BDP.



R = H; H-BDP
R = Me; Me-BDP

Effect of the receptors on the absorption spectra of BDP was firstly examined in $CHCl_3$. The absorption spectra of H-BDP in H_2O and $CHCl_3$ are shown in Fig. 1. The absorption band is splitted in $CHCl_3$ and is broadened in H_2O as seen for the conventional flavin models.[5] The spectral shape of H-BDP in $CHCl_3$ was found to change to similar one in H_2O with slight expand to longer wavelengths by addition of 5 (500 molar excess over H-BDP)(Fig. 2), whereas no effect for 1-4. In contrast, such a spectral change for Me-BDP was not observed. These results suggest formation of the triple hydrogen bond with aromatic stacking of the naphthalene moiety. In other words, 5 could be regarded as a flavin receptor to recognize H-BDP by forming a molecular complex. To ensure this further, an extraction experiment of H-BDP by the receptors was performed as follows. A glass-stoppered test tube (10 ml) containing H-BDP (2.0×10^{-5}M) in H_2O (4 ml) and $CHCl_3$ (4 ml) was shaken for 5 min and allowed to stand for 30 min, and the concentration of H-BDP was determined spectrophotometrically. H-BDP exists exclusively in H_2O layer in the absence of the receptors. H-BDP was found to transfer into the $CHCl_3$ layer with increase of 5,[6] and no transfer of H-BDP occurred by the other receptors. Thus, the transfer of water-soluble H-BDP to $CHCl_3$ layer by 5 could be explained

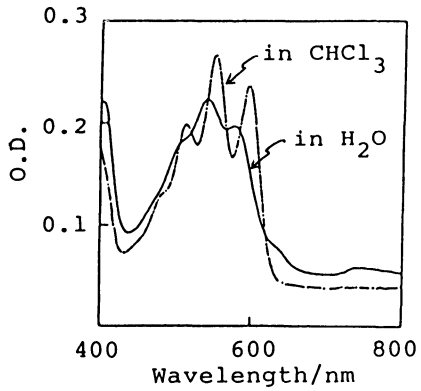


Fig. 1. Absorption spectra of H-BDP. [H-BDP]=2.0×10^{-5}M (in H_2O); 1.0×10^{-5}M (in $CHCl_3$).

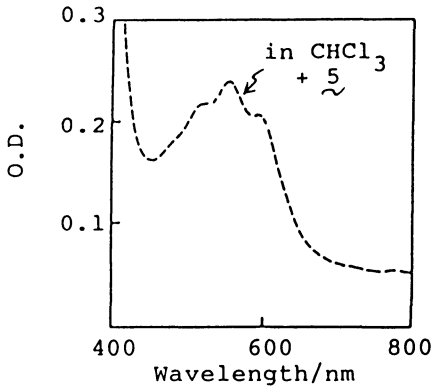


Fig. 2. Absorption spectrum of H-BDP in $CHCl_3$ with 5 (5.0×10^{-3}M).

by formation of the molecular complex which is soluble in $CHCl_3$.

The reactivity of H-BDP bound by the receptor is of interest in connection with that some flavin coenzymes are known to bind to specific sites of apoproteins by hydrogen bonds involving the triple hydrogen bond at the pyrimidine moiety to regulate activity.[7] Effects of the receptors on the oxidations of N-benzyl-1,4-dihydronicotinamide (BNAH) and $PhNHNH_2$ by BDP were examined kinetically in $CHCl_3$ under anaerobic conditions. Both oxidations occurred quite smoothly in the dark. Pseudo first-order rate constants were determined by following the absorption increase of the reduced BDP at 670 nm.[8] The results for H-BDP are presented in Table 1. A small rate-acceleration by 1-4 may be due to the hydrogen bonding to carobnyl groups of H-BDP in the transition states and/or the reduced state. The rate-retardation by 5 may be due to formation of the molecular complex which involves a charge-transfer complex between the naphthalene moiety and H-BDP to lower the oxidation activity. A little larger effect of the receptors on the rates for $PhNHNH_2$ than for BNAH could be accounted for by that the reaction site for the former [C(4a)-position][9] is more close to the hydrogen bonding sites than that for the latter [N(5)-position].[10] It should be emphasized that the rates for Me-BDP were not affected by any receptor.

Table 1. Effect of the receptors on the oxidations of BNAH and $phNHNH_2$ in $CHCl_3$

Flavin	Receptor	$10^2 \times k_{obsd}/s^{-1}$ (rel. rate) BNAH	$PhNHNH_2$
H-BDP	None	4.13 (1.0)	2.36 (1.0)
	1	5.90 (1.4)	6.11 (2.6)
	2	6.93 (1.7)	4.91 (2.1)
	3	4.10 (1.0)	3.10 (1.3)
	4	8.82 (2.1)	11.5 (4.9)
	5	3.65 (0.9)	1.23 (0.5)

[BDP] = 1.0×10^{-5}M (1 M = 1 mol dm^{-3}), [Receptor] = 5.0×10^{-3}M, [BNAH] = 2.00×10^{-3}M, [$PhNHNH_2$] = 2.00×10^{-4}M, N_2, 25 °C.

The present study demonstrates that the Hamilton's receptor for a thymine can be used as a flavin receptor, that would afford promise of molecular models for flavin apoproteins. The work in this line is currently in progress in our laboratory.

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