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A MODEL FOR METABOLIC ACTIVATION OF DIALKYLNITROSAMINES. OXIDATIVE DEALKYLATION OF N-NITROSO-2-(ALKYLAMINO)ACETONITRILE BY FLAVIN MIMIC IN AQUEOUS SOLUTION

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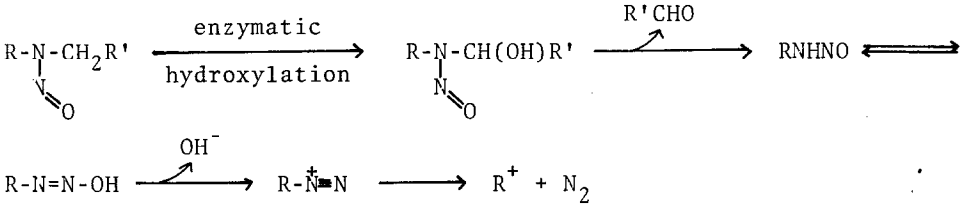
Summary: Oxidation-active flavin mimic, benzo[1,2-g, 5,4-g']dipteridine (BDP), is found to react with N-nitroso-2-(alkylamino)acetonitrile via oxidative dealkylation in aqueous solution. From the kinetic investigations, the oxidation mechanism is proposed.

Nitrosamines are known to be carcinogens which usually require metabolic activation in hepatic microsomal mixed function oxidase systems.[1] For example, a dialkylnitrosamine is oxidatively dealkylated through α-hydroxylation to afford aldehyde and monoalkylnitrosamine which decomposes sponteneously to the alkyl cation via the alkyl diazonium ion. The alkyl cation thus formed is believed to cause an initial process of carcinogenesis by alkylating cellular components and bases of DNA (Scheme 1).[1,2]

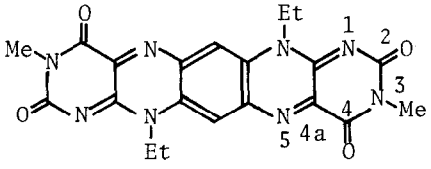


Scheme 1.

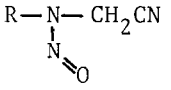
The enzymatic α-hydroxylation of dialkylnitrosamines is generally considered to involve cytochrome P-450 dependent monooxygenases,[3] although other enzymes are implicated.[4] In model systems, Smith et al. have shown that dibenzylnitrosamine is oxidized to give benzaldehyde and benzyl alcohol by tetraphenylporphinato iron(III) or manganese(III) chloride and oxidants such as iodosobenzene, m-chloroperbenzoic acid, and t-butyl hydroperoxide in benzene.[5] On the other hand, Lake et al. have proposed that a monoamine oxidase is responsible in part for the metabolic activation of the nitrosamines, based on the observations that the susbtrates and inhibitors of the monoamine oxidase repress the metabolism of the nitrosamines.[6]

Meanwhile we have shown that BDP exerts a remarkably high oxidizing activity towards the oxidation reactions which proceed through C(4a)-adduct formation such as thiol and phenylhydrazine oxidations in aqueous solution (ca. 10^7-fold more reactive than 3,10-dimethylisoalloxazine).[7]

In this letter, we wish to report that the oxidation-active flavin mimic (BDP) is able to oxidize N-nitroso-2-(alkyl or arylamino)acetonitriles[8] in a similar manner to the metabolic degradation of nitrosamines in biological systems.



BDP



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a: R = Me
b: R = n-Bu
c: R = Ph
d: R = p-ClPh
e: R = p-MePh
f: R = $PhCH_2$

Spectroscopic examination of the reaction of BDP and 1a in aqueous solution under anaerobic conditions showed formation of the 2e-reduced BDP,[7a] which regenerated BDP quantitatively on O_2 admittance. Dimethylnitrosamine, however, was not oxidized by BDP under the same conditions, implying the the acidity of α-hydrogen is a crucial factor for the reaction.

Product analysis was performed by employing 1f as follows. A mixture of BDP (40 mg, 0.092 mmol) and 1f (268 mg, 1.53 mmol) in aqueous solution (200 ml, 0.02 M phosphate buffer : MeCN = 1 : 1, pH 8.7) was stirred at room temperature for 17 h and at 75 °C for 4 h under air stream. After work-up, benzyl alcohol was obtained by distillation (110 mg, 65 %), indicating that BDP functions as a turnover catalyst. A control experiment without BDP showed no formation of benzyl alcohol.

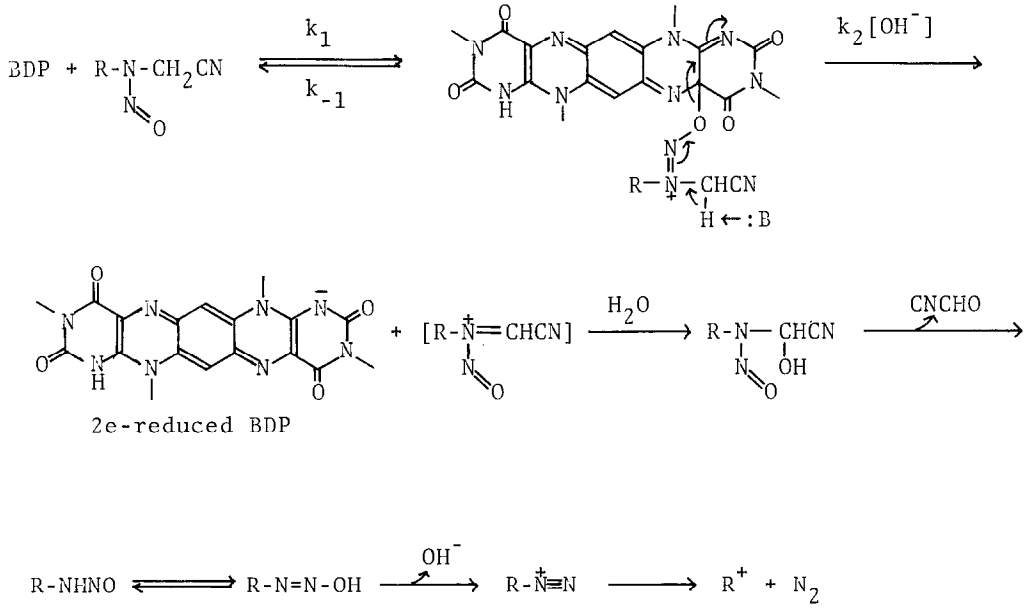
Pseudo first-order rate constants were determined by following the absorption increase of the 2e-reduced BDP at 700 nm under anaerobic conditions.[9] Kinetic isotope effects (k_H/k_D) were determined by employing 1a, 1f, and the corresponding deuterium derivatives [$RN(NO)CD_2CN$].[10] The rate constants (k_{obs}), k_H/k_D values, and the relative rate are summarized in Table 1.

Table 1. Pseudo first-order rate constants, kinetic isotope effects, and relative rates at 25 °C[a]

$RN(NO)CH_2CN$	$10^2\ k_{obs}/min^{-1}$	k_H/k_D	Rel. rate
1a	6.22 ± 0.20[b]		1.0
$MeN(NO)CD_2CN$	2.29 ± 0.08[b]	2.7	
1b	4.42		0.71
1c	6.92		1.1
1d	8.21		1.3
1e	5.90		0.94
1f	9.20 ± 0.12[b]		1.5
$PhCH_2N(NO)CD_2CN$	2.21 ± 0.14[b]	4.2	

a) [BDP] = 1.0 X 10^{-5}M, [$RN(NO)CH_2CN$] = 3.00 X 10^{-2}M, pH 8.76 (0.02 M phosphate buffer : MeCN = 1 : 1), N_2. b) Average of 3 or 4 runs.

The k_H/k_D values indicate that the deprotonation of the cyanomethyl moiety is involved in the rate-determining step. Since it is known that a nitroso oxygen is a fairly good nucleophile,[1b] and BDP enhances the oxidation reactions proceeding via C(4a)-adduct formation,[7] the mechanism of the oxidative degradation of the nitrosamines by BDP could be delineated as shown in Scheme 2.



Scheme 2.

Namely the nitroso oxygen attacks at the C(4a)-position of BDP to form an adduct, which affords the 2e-reduced BDP and $R\overset{+}{N}(NO)=CHCN$ by base-catalyzed 1,4-elimination. Moreover, the rates were found to be first-order with respect to [1a], and the log k_{obs} vs. pH plot for 1a gave a straight line with a slope of 1.0.[11] Thus, the rate equation is expressed by Eq. (1) by assuming the steady state to [4a-adduct], and $k_{-1} \gg k_1[RN(NO)CH_2CN]_o + k_2[OH^-]$, where $[RN(NO)CH_2CN]_o$ stands

$$k_{obs} = \frac{k_1 k_2}{k_{-1}}[RN(NO)CH_2CN]_o[OH^-] \qquad (1)$$

for the initial concentration of the nitrosamine. All the kinetic data obtained for the reaction of BDP and 1 are compatible with the proposed mechanism and Eq. (1).

To the best of our knowledge, it is the first example that a flavin mimic reacts with nitrosamine derivatives via oxidative dealkylation similar to the metabolic activation in vivo. The present study also suggests that a flavo-

enzyme may play an important role for the metabolic activation of nitrosamines in biological systems.

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9) The pseudo first-order rate constants were obtained from the initial slopes of the first-order plots, since the plots display downward curves due to the formation of the charge-transfer complex between the oxidized and reduced BDP.

10) The deuterium compounds were obtained by H-D exchange of 1a and 1f in acetone-d_6-D_2O at 80 °C for 3 h (3 times). No methylene protons were confirmed by ^{1}H-NMR spectra.

11) The pH-rate profile was obtained in the pH range of 7.50-8.76.

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