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Isophenoxazine Synthase Model. Oxidation of o-Aminophenol by an Oxidation-Active Flavin Mimic in an Aqueous Solution

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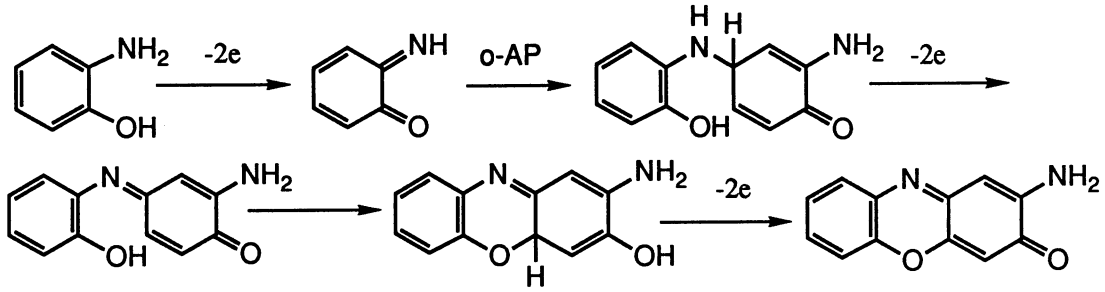
It was found that a flavin mimic, benzo-dipteridine (BDP), oxidizes o-aminophenol in an aqueous solution under anaerobic conditions. Under aerobic conditions, 2-amino-phenoxazine-3-one was isolated. This is the first example of an isophenoxazine synthase model.

There are many naturally occurring isophenoxazine derivatives such as actinomycin D,[1] xanthommatin,[2] and cinnabarinic acid,[3] which are formed via the oxidative coupling of two molecules of o-aminophenol derivatives catalyzed by isophenoxazine synthases as shown in Scheme 1.[4]



Scheme 1.

This oxidative condensation involves total 6e-oxidation. The first 2e-oxidation is an enzymatic process, since o-benzoquinone imine is established to react rapidly with o-aminophenol(o-AP) to afford 2-aminophenoxazine-3-one.[5] The phenoxazine ring is known to be formed via the oxidation of o-AP by cytochrome c and cytochrome oxidase,[6] and haemoglobin.[7] Similar oxidation of o-AP is also known to occur in human erythrocytes.[8] Meanwhile some isophenoxazine synthases are known to be flavoproteins.[9] However, there is no precedent in flavin model system probably because of low oxidation-activity of the conventional flavin model compounds so far synthesized. Thus very little is known about the oxidation mechanism. We have successfully exploited an oxidation-active flavin mimic [benzo-

dipteridine (BDP)],[10] which is quite useful for exploitation of new model reactions. Namely we have reported hitherto two new model reactions by employing BDP: a model of metabolic activation of N-nitrosamine derivatives[11] and an APS reductase model (oxidation of sulfite ion).[10b] Furthermore we have shown that BDP acts as a turnover catalyst for the oxidation of alcohols by N-hydroxyamine-BDP-O_2 in a two-phase system.[12] Here we wish to report the first example of an isophenoxazine synthase model by employing BDP in an aqueous solution.

Spectroscopic examination showed that BDP oxidizes o-AP to give reduced BDP in an aqueous solution under anaerobic conditions. Formation of the reduced BDP was kinetically investigated. Pseudo-first-order rate constants (k_{obsd}) were determined by following the absorption increase at 620 nm of the reduced BDP as described previously.[10b,11] A plot of k_{obsd} vs. $[o\text{-AP}]_o$ gave a straight line under the conditions of $[o\text{-AP}]_o/[BDP]_o$ = 80-400 and pH 6.42 (not shown), indicating that the rates are first-order in the concentration of o-AP. The pH-rate profile showed that the rates increase with the increase of pH and reach a plateau at pH which seems to correspond to pKa[13] of o-AP (Fig. 1). Buffer catalysis was not observed (0.0125-0.1 M phosphate, u = 0.3 with KCl).[14] The rate constants for o-AP derivatives are shown in Table 1. The rate is decelerated by electron-withrawing

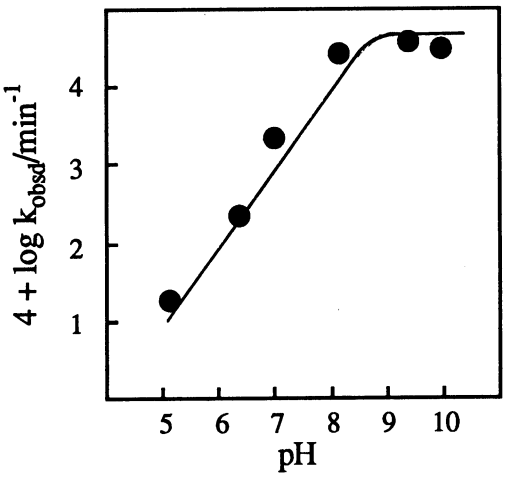
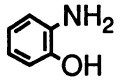
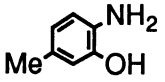
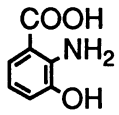
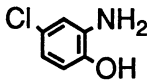


Fig. 1. pH-Rate profile
$[BDP]_o = 2.5 \times 10^{-5}$ M, $[o\text{-AP}]_o = 5.00 \times 10^{-4}$ M
[Buffer] = 0.1 M, μ = 0.3 (KCl), N_2, 25° C.

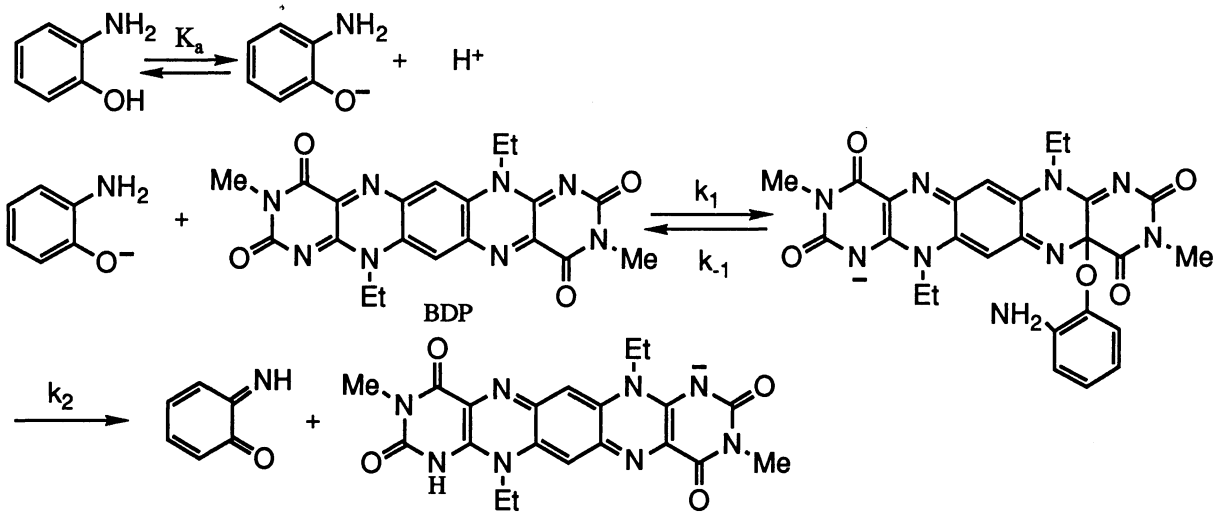
Table 1. Pseudo-first-order rate constants[a)]

Substrate	k_{obsd}/min^{-1}	Rel. rates
	5.60×10^{-2}	1.0
	1.71×10^{-1}	3.0
	7.35×10^{-3}	0.13
	Too slow	—

a) pH 6.35.

substituents. Taking account of the fact that BDP accelerates specifically the reactions involving nucleophilic attack at C(4a) position (ca. 10^7-fold),[10] the present kinetic results suggest the following reaction scheme

(Scheme 2). Namely the oxidation proceeds via a rate-determining nucleophilic attack of o-aminophenolate anion at the C(4a)-position followed by 1,4-elimination to give the reduced BDP and o-benzoquinone imine. By assuming the steady state to the adduct and $k_{-1} > k_1[\text{o-AP}]_o$, the rate equation is represented as Eq. 1, where $[\text{o-AP}]_o$ stands for initial concetration of o-AP.



Scheme 2.

$$k_{obsd} = \frac{K_a k_1 k_2 [\text{o-AP}]_o}{(k_{-1} + k_2)(K_a + [H^+])} \qquad (1)$$

Product analysis was performed under aerobic conditions as follows: A solution (0.1 M phosphate buffer pH 6.6, 150 ml) containing BDP (2.6 mg, 6.0×10^{-3} mmol dissolved in dimethylacetamide 5 ml) and o-AP (131 mg, 1.2 mmol) was stirred at room temperature for 24 h under air atmosphere. Crystals formed were collected by filtration, and recrystallized from EtOH; yield 80 mg (63% based on o-AP), mp 249 C.[15] All the data (mp, ^{1}H NMR, UV-vis) of this product were completely identical with those of an authentic sample of 2-aminophenoxazine-3-one. A control experiment without BDP showed a trace amount of 2-aminophenoxazine-3-one (detected on TLC). The result indicates that BDP can be used as a turnover oxidation catalyst for synthesis of 2-aminophenoxazine-3-one from o-AP under aerobic conditions. Although it was established that BDP oxidizes o-AP via 2e-oxidation, it is not clear whether BDP is concerned with the remaining oxidation steps in Scheme 1 or not.

The present study demonstrates that an oxidation-active flavin mimic (BDP) oxidizes o-AP in an aqueous solution, and is able to act as a turnover catalyst for formation of 2-aminophenoxazine-3-one from o-AP under aerobic conditions. This is the first example of an isophenoxazine synthase model.

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13) pKa of o-AP is reported to be 9.4; R. Kuhn and A. Wassermann, Helv. Chim. Acta, 11, 3 (1928).
14) 1 M = 1 mol dm^{-3}.
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