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Acid-Promoted Oxidations of an NAD(P)H Model with Hydrogen Peroxide and Molecular Oxygen

Seizo Tamagaki, Takashi Mimura, and Waichiro Tagaki*
Department of Bioapplied Chemistry, Faculty of Engineering, Osaka City University,
Sugimoto 3-3-138, Sumiyoshi-ku, Osaka 558
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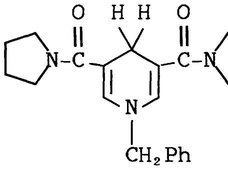
The $HClO_4$-catalyzed H_2O_2- and O_2-oxidations of a dihyropyridine in acetonitrile were mechanistically investigated; the H_2O_2-oxidation under nitrogen followed a heterolytic course to yield the corresponding pyridinium salt along with a monooxygenated product, while the O_2-oxidation produced the pyridinium salt and H_2O_2 via a radical-chain pathway in which H_2O_2 is not only merely the product but also an initiator.

NAD(P)H pyridine coenzyme is used as a versatile reductant in a living cell,[1] and numerous examples exist of reductions of many organic functional groups such as C=O, C=C, and N=O bonds with NAD(P)H and its model compounds.[2] Meanwhile, peroxides occur in a living body in connection with production or removal of peroxidic compounds and activation of oxygen.[3] Although the oxidations of NAD(P)H by peroxides are potentially important in biology, model studies on the oxidation of NAD(P)H with peroxidic compounds have been only a few. For examle, 1-methyl-1,4-dihydro-3,5-bis(ethoxycarbonyl)-2,6-lutidine was oxidized at high temperatures with diacyl peroxides by a free-radical chain mechanism to the corresponding pyridinium cation.[4] Thus, noncatalytic reactions are generally very slow despite the inherent reducing ability of an NAD(P)H ring itself. Recently, we have reported on the rate-enhancing effect of some bivalent metals in the O_2- and H_2O_2-oxidations of an NAD(P)H model (1-benzyl-3,5-bis(1-pyrrolidinylcarbonyl)-1,4-dihydropyridine (BPDH)); these reactions were found to proceed via a radical chain mechanism.[5]

In continuing our study of oxidations of a dihydropyridine ring, acid-catalyzed oxidation of BPDH with H_2O_2 under N_2 or O_2 has been thoroughly investigated in this work and we have found that, unlike bivalent metal-facilitated oxidations, the acid-catalyzed H_2O_2-oxidation proceeds via different courses depending on the reaction atmosphere. The acid-catalyzed oxidations of a closely-related NADH model with oxygen were previously studied under special conditions,[6] and by using an acid-stable NADH model (BPDH), where H_2O_2 is a final product.[7]



(BPDH)

Experimental

Materials. BPDH was prepared by reducing 1-benzyl-3,5-bis(1-pyrrolidinylcarbonyl)pyridinium chloride with $Na_2S_2O_4$ in a two-phase aqueous Na_2CO_3–CH_2Cl_2 system in a blanket of nitrogen as described in the preceding paper.[8] Acetonitrile was distilled from P_2O_{10}. H_2O_2 (30%) and $HClO_4$ (70%) were obtained from Wako Pure Chemical Industry and used as received.

Kinetics. The acid-catalyzed oxidations have been studied at 25 °C in CH_3CN in the presence of $HClO_4$ as the catalyst by monitoring the variation of the absorbance at 355 nm due to BPDH with time. Unless otherwise stated, the concentrations of BPDH, $HClO_4$, and H_2O_2 were 0.1 mM, 0.1—20 mM, and 0.5—15 mM, respectively. ($\mathrm{mM}=10^{-3}$ M, $1\,\mathrm{M}=1\,\mathrm{mol\,dm^{-3}}$). An $HClO_4$- and an H_2O_2-stock solution were prepared by diluting commercially available 70 and 30% aqueous solutions, respectively, with MeCN.

H_2O_2-Oxidation of BPDH. Product Analysis. BPDH (0.55 g, 1.5 mmol) was dissolved in 20 ml of CH_3CN. To this solution 2.0 mmol of H_2O_2 (30%) and 2.0 mmol of $HClO_4$ (70%) were added under nitrogen. After stirring for 24 h at room temperature, hydrochloric acid (1 equiv, 20 ml) was added. The acetonitrile was evaporated under reduced pressure, the resulting mixture was extracted with $CHCl_3$, and then the solvent was evaporated. The residue was subjected to preparative TLC on a silica plate eluting with 10:1 $CHCl_3$–MeOH to give the oxidized form of BPDH (BPD^+Cl^-) (50%) and 1-Benzyl-3,5-bis(1-pyrrolidinylcarbonyl)-3,4-dihydro-2(1*H*)-pyridinone (47%): Mp 188—192 °C; UV (MeCN) 274 (ε 8800); ^{1}H NMR ($CDCl_3$) δ=1.85 (8H, m, $N(CH_2)_2(C\underline{H}_2)_2$), 2.87 (2H, m($\underline{AB}$X), 4-$C\underline{H}_2$), 3.47 (8H, m, $N(C\underline{H}_2)_2(CH_2)_2$), 3.77 (1H, q(AB$\underline{X}$), 3-$\underline{H}$), 4.68 (2H, dd, Ph-$C\underline{H}_2$), 6.52 (1H, s, 6-$\underline{H}$), and 7.18 (5H, s, Ph); MS (70 eV) *m*/*z* (rel intensity) 381 (M^+; 0.7), 311 (1.1), 283 (73), and 91 (100). Elemental Anal: Found: C, 69.11; H, 7.10; N, 10.72%. Calcd for $C_{22}H_{27}N_3O_3$: C, 69.27; H, 7.13; N, 11.02%.

O_2-Oxidation of BPDH. Product Analysis. The reaction with O_2 was performed with 0.50 mmol BPDH and 0.5—50 mmol $HClO_4$ in pure oxygen-saturated MeCN (50 ml) for 24 h. To evaluate the yield of the H_2O_2 produced, excess PPh_3 and AcOH was added and the reaction mixture was kept standing at room temperature for another 24 h. An excess of hydrochloric acid was added, the solvent was evaporated, and the residue was subjected to preparative TLC eluting with 5:1 $CHCl_3$–MeOH, giving Ph_3P=O and the corresponding pyridinium chloride (BPD^+Cl^-, I). The results are collected in Table 1.

BPDH-$HClO_4$ 1:1 Adduct. Addition of an approximate excess of aqueous 70% $HClO_4$ to a solution of BPDH (0.5 mmol) in 25 mL of CH_3CN gave immediate precipitation of a yellow solid; decompn. 153—155 °C; its ^{1}H NMR spectrum

Table 1. O_2-Oxidation of BPDH in the Presence of Perchloric Acid

Catalyst	M	Isolated yield/%	
		H_2O_2[a]	BPD^+Cl^-
$HClO_4$	0.01	70.8	95.0
$HClO_4$	0.20	67.7	96.0
$HClO_4$	1.00	50.5	86.9
$Ca(ClO_4)_2$[5]	0.05	89.4	94.9

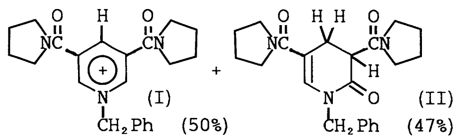
Conditions: $[BPDH]=1.0\times10^{-2}$ M; in O_2-saturated MeCN (50 mL) at 25 °C. a) The amount of produced H_2O_2 was determined as $Ph_3P=O$.

in DMSO-d_6 indicated that the adduct dissociated into the original components, $HClO_4$ and BPDH, in a molar ratio of 1 : 1. The resonance due to the liberated $HClO_4$ appeared at $\delta=10.0$.

Results and Discussion

The oxidation of BPDH by H_2O_2 under nitrogen in $HClO_4$-containing acetonitrile afforded the monooxygenated (II) as well as dehydrogenated (I) products being isolated in a 1 : 1 ratio. In order to disclose the reaction mechanism, the reaction was kinetically examined in detail. The progress of reaction was followed spectroscopically by monitoring the disappearance of BPDH. The BPDH oxidation of an equiv of H_2O_2 under nitrogen was so much slow that a 100-fold excess of H_2O_2 was employed to retain the practical reaction rate. The reaction followed the first-order kinetics in each [BPDH] and $[H_2O_2]$ (Fig. 1). The rate variation with changing $[HClO_4]$ (<10 mM) was complicated but interesting; the rate was linear with regard to $[HClO_4]$ in the region of less than 1.5 mM and, after passing through the maximum, the rate seems to approach slowly zero (Fig. 2); such a characteristic feature of acid-dependency of the rate can not be accounted for in terms of a decreasing activity of $HClO_4$ with its increasing concentrations because dissociation of $HClO_4$ to H^+ and ClO_4^- in acetonitrile is almost complete in such a low $[HClO_4]$ region ($pK_a<2$ in MeCN).[9] Another explanation might be possible by considering participation of water inevitably involved in 70% $HClO_4$, but it also seems unlikely, because no substantial influence was observed by addition of up to a 100-fold excess of water over $HClO_4$. Similarly, the abnormal rate-$[HClO_4]$ profile can not be interpreted, of course, by a mechanism, involving a protonation equilibrium between free BPDH and monoprotonated BPDH, where H^+ activates H_2O_2 and only the free BPDH is active for oxidation. Such a mechanism leads to yielding merely a saturation curve.

$$BPDH + H_2O_2 \xrightarrow{H^+}$$



(I) CH_2Ph (50%) + (II) CH_2Ph (47%)

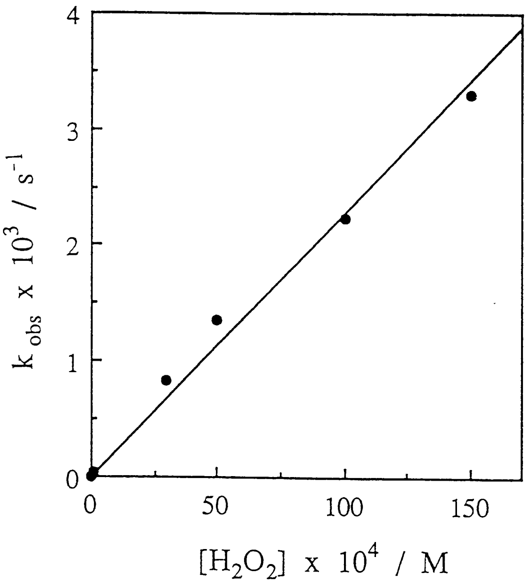


Fig. 1. Dependence of k_{obs} upon H_2O_2 concentration for H_2O_2-oxidation of BPDH under nitrogen in MeCN at 25 °C; $[BPDH]_0=0.1$ mM; $[HClO_4]=2.0$ mM.

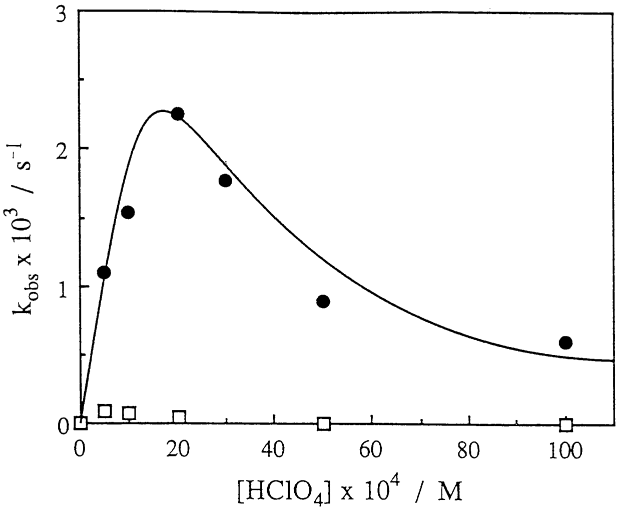


Fig. 2. H_2O_2-oxidation of BPDH in the presence of changing $HClO_4$ concentrations under nitrogen; $[BPDH]_0=0.1$ mM; ●: $[H_2O_2]_0=10$ mM, □: $[H_2O_2]_0=0.1$ mM.

Meanwhile, the UV-absorption maximum for BPDH (355 nm) shifted stepwise to higher wavelengths when $[HClO_4]$ was increased (Figs. 3a and 3b); the first shift to 390 nm occurring around $[HClO_4]=1$ mM is attributed to the formation of the BPDH–$HClO_4$ 1 : 1 adduct and the second shift to 410 nm around $[HClO_4]=3.5$ mM is attributed to that of the 1 : 2 adduct. The former adduct

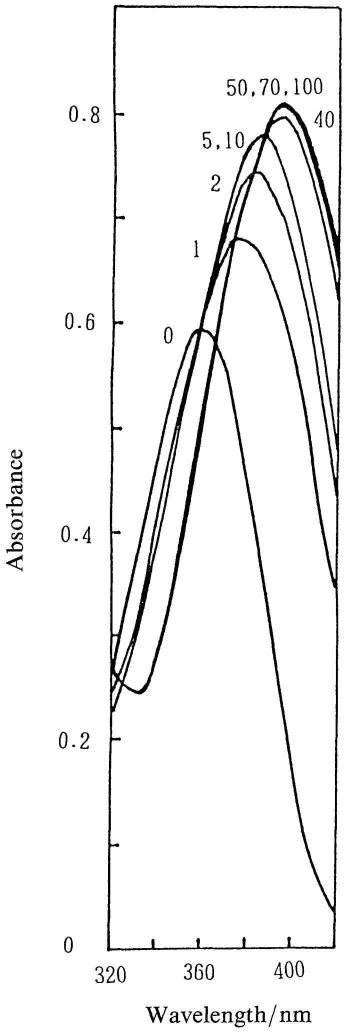


Fig. 3a. UV spectra of BPDH in the presence of $HClO_4$; the numbers indicate the concentrations of $HClO_4$ in 10^{-4} M.

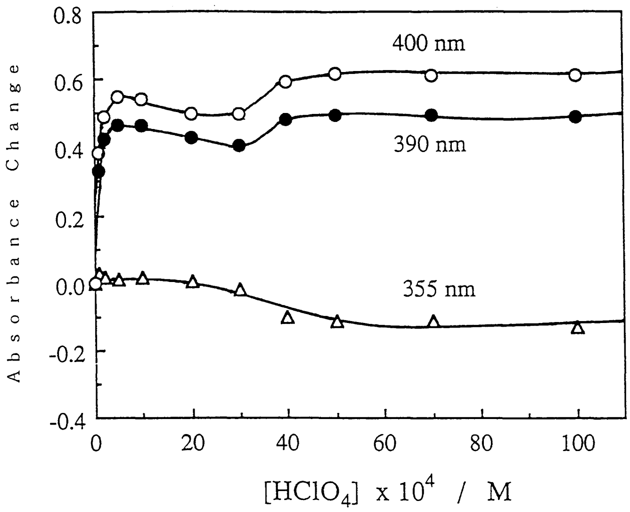
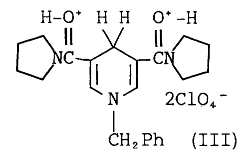


Fig. 3b. Change of absorbance due to BPDH with increasing $HClO_4$ concentrations at various wavelengths under nitrogen.

itself could be isolated quantitatively as a yellow precipitate from the acetonitrile solution of a mixture of BPDH and $HClO_4$. The complex exhibits two carbonyl stretching vibration frequencies with almost equal intensities at 1660 and 1690 cm^{-1} which are due to the protonated and the not protonated carbonyl groups in a BPDH molecule, respectively. The absorption due to BPDH itself appears at 1670 cm^{-1}. Thus, the overall protonation events may be expressed as equation 1.

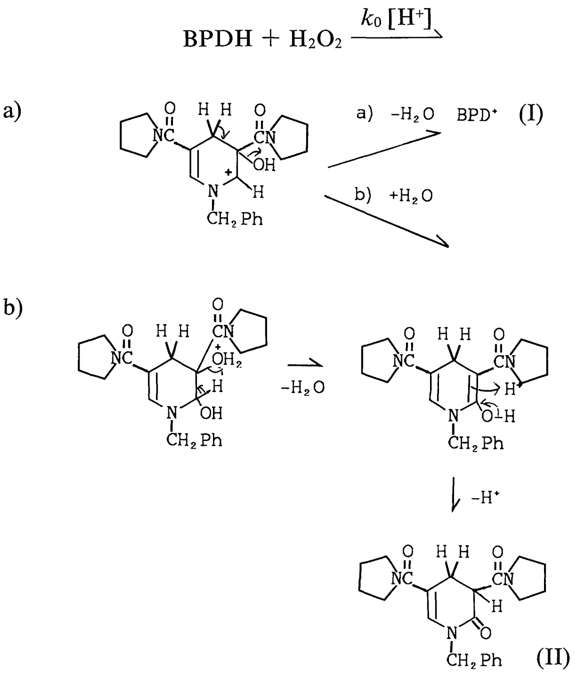
$$\text{BPDH} \underset{HClO_4}{\overset{K_{a1}}{\rightleftharpoons}} \text{BPDH}\cdot H^+ClO_4^-$$

$$\underset{HClO_4}{\overset{K_{a2}}{\rightleftharpoons}}$$



(1)

The site of the second protonation is not yet defined. However, we tentatively propose the structure III which bears a proton on each carbonyl oxygen in solution. We can safely say, therefore, that the singly and doubly protonated BPDHs have to coexist, both being virtually inactive toward this oxidation. Furthermore, no influence was observed by a 100-fold addition of a well-known radical chain inhibitor, 2,6-di-*t*-butyl-4-methylphenol (BHT). All these findings allow us to conclude that the oxidation proceeds through an ionic pathway (Scheme 1); namely, the reaction is initiated by rate-determining electrophilic attack of a protonated H_2O_2 at the 3-position of the BPDH ring with the highest



Scheme 1.

HOMO coefficient, followed by partition either to the pyridinium cation (I) via dehydration (path a) or to the monooxygenated compound (II) via hydration followed by dehydration (Path b).

Upon taking the acid-equilibria (1) into consideration explicitly, we obtain the rate equation (2) as a function of [$HClO_4$] well-accounting for the experimental data. From a set of data given in Fig. 2, K_{a1}, K_{a2}, and k' values were estimated by a nonlinear curve-fitting program to be 800 M^{-1}, 300 M^{-1}, and 4 M^{-1} s^{-1}, respectively. The rate constant k' means $k_0[H_2O_2]$, and is treated as a valuable independent of [H^+].

$$V = -\,d[BPDH]/dt = k_0[H_2O_2][BPDH]_T[H^+]/(1 + K_{a1}[H^+] + K_{a1}K_{a2}[H^+]^2) = k_{obs}[BPDH]_T \qquad (2)$$

$$k_{obs} = k'[H^+]/(1 + K_{a1}[H^+] + K_{a1}K_{a2}[H^+]^2)$$

In contrast to the slow oxidation between BPDH and an equiv of H_2O_2 under nitrogen, the same quantity of H_2O_2 under oxygen was found to remarkably enhance the BPDH oxidation. In order to disclose the reaction mechanism, the progress of the O_2-oxidation was followed spectroscopically by monitoring the disappearance of BPDH and the effect of H_2O_2 on rate was examined at first; the results thus obtained are given in Fig. 4, which shows that the rate increased with increasing added H_2O_2 but the addition of H_2O_2 was not necessarily required for the smooth oxidation to occur. Table 1 reveals also that the oxidation of BPDH under an oxygen atmosphere in $HClO_4$-containing acetonitrile afforded BPD^+ (I) and H_2O_2 in quantitative and good yields, respectively. Thus, from now on, we will discuss on the results of the reactions performed under oxygen with no H_2O_2 added. In Fig. 5 is shown a typical ln ($A-A_\infty$) vs. time plot, where a short lag period appeared, indicative of occurrence of a radical chain reaction; in fact, addition of a radical scavenger BHT strongly retarded the overall rate (Fig. 6). A following rapid BPDH consumption was first-order in BPDH; the linearity of the rate vs. $[BPDH]_0$ plot also confirmed this conclusion (data not shown). For simplifying rate evaluation, the rate constant (k_{obs}) for the BPDH-consuming phase was calculated by assuming pseudo-first-order kinetics with respect to BPDH throughout this work. As addition of a trace of H_2O_2 eliminated the lag period, one of the tasks of the H_2O_2 appears to be in enhancing the rate of the initiation step through its homolytic O–O cleavage at the start of the reaction.

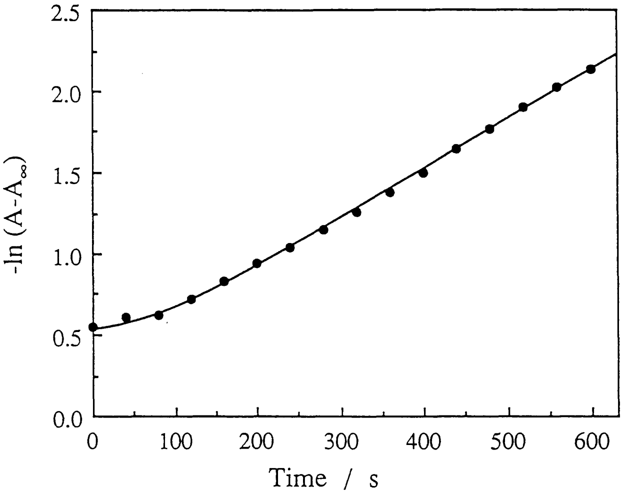


Fig. 5. ln ($A-A_\infty$) vs. time plot for pure O_2-oxidation of BPDH in the presence of $HClO_4$ in MeCN at 25 °C; [$HClO_4$]=5.0 mM; $[BPDH]_0$=0.1 mM.

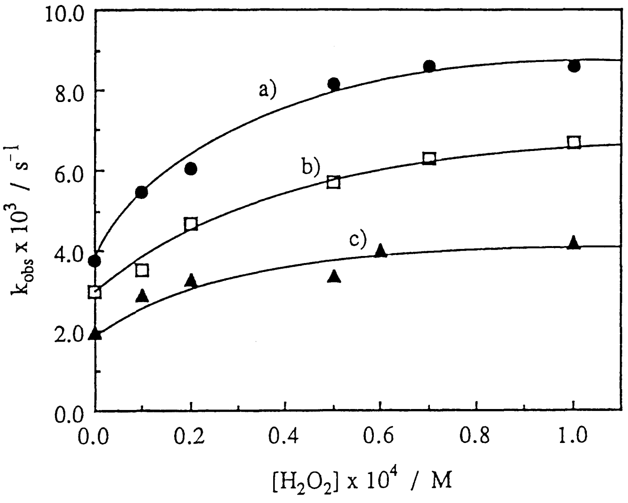


Fig. 4. Effect of H_2O_2 concentrations on the BPDH oxidation in oxygen-saturated MeCN at 25 °C; [$HClO_4$]=2.0 mM (a), 1.0 mM (b), 10 mM (c); $[BPDH]_0$=0.1 mM.

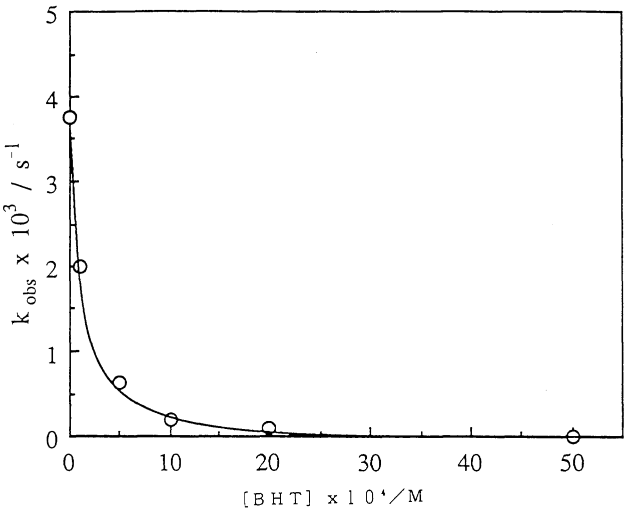


Fig. 6. Effect of altering concentrations of BHT on the rate of pure O_2-oxidation of BPDH; [$HClO_4$]= 2.0 mM; the other conditions were the same as those of Fig. 1.

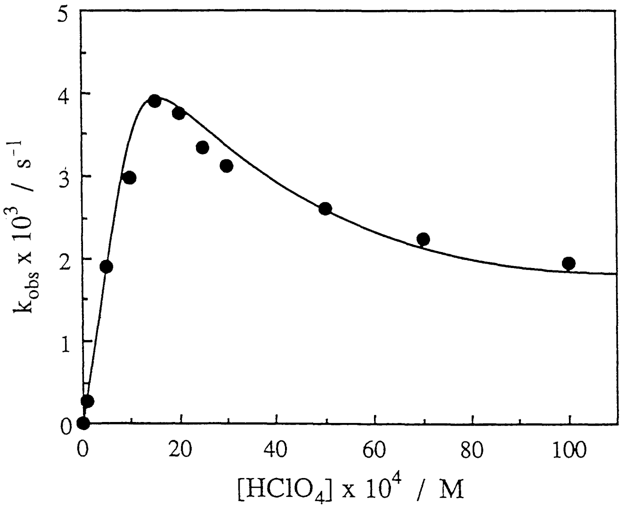


Fig. 7. Pure O_2-Oxidation of BPDH in the presence of various concentrations of $HClO_4$.

The effect of changing amounts of $HClO_4$ on rate exhibited the same characteristic feature as in the H_2O_2-oxidation under N_2 (Fig. 7), indicating that the virtually inactive singly- and doubly-protonated BPDHs are involved here also. When the rate constants were plotted as a function of the percent oxygen content ($\eta = P_{O_2}/(P_{O_2}+P_{N_2})$) of the O_2–N_2 mixture used to saturate the solution, a downward curvature was obtained; the rate was essentially independent of the concentration of the dissolved oxygen in solution at $\eta > 0.2$. Recently, Fukuzumi et al have kinetically studied closely related O_2-oxidation for an acid-stable NADH model, 10-methyl-9,10-dihydroacridine, in the presence of concentrated $HClO_4$; the reaction was first-order in each [$AcrH_2$] and [O_2]. They proposed a mechanism involving the rate-determining acid-catalyzed electron-transfer from $AcrH_2$ to O_2 followed by direct hydrogen transfer from $AcrH_2^{\cdot +}$ to $HO_2\cdot$ in the solvent cage to yield $AcrH^+$ and H_2O_2.7) In contrast with $AcrH_2$, BPDH undergoes an autoxidation. In order to explain the observed effects of concentrations of BPDH and O_2 on the rate, therefore, we will propose the following Scheme 2 consisting of a set of reactions (3),(4),(5),(6),(7),(8), and (9); H_2O_2-producing reaction (3) is taken into consideration to explain the appearence of the induction period in the absence of added H_2O_2. As for the initiation in the radical chain sequence, we are forced to assume a set of reactions (4), (5), and (6) being the only reactions of importance to be compatible with the experimental data. Solvents and reagents have been reported to be inevitably contaminated with adventitiuos metal ions,10) therefore, a reactive radical species capable of triggering the chain reaction might be produced according to the Fenton-like reaction. "M" here is used to denote an adventituous metal ion, maybe Fe^{2+}, which undergoes the Fenton reaction to give an HO· radical. This inference would be supported by the fact that addition of an 0.1 equiv of $Fe(ClO_4)_2$ to an O_2-saturated MeCN solution of BPDH removed the induction phase and accerelated the reaction by 1.5-fold.

At high O_2 concentrations ($\eta > 0.2$) the most important termination process must be the disproportionation of HOO· to H_2O_2 and O_2 since reaction (7) would become faster than reaction (8) under such conditions.

During the induction period:

$$\mathrm{BPDH} + O_2 \xrightarrow[\text{slow}]{H^+} \mathrm{BPD}^+ + H_2O_2 \qquad (3)$$

Chain-initiating reaction:

$$H_2O_2 + M \xrightarrow{k_1[H^+]\ (\sim 10^2)} HO\cdot + M^+ \qquad (4)^{11)}$$

$$2M^+ + \mathrm{BPDH} \xrightarrow{(>10^5)} 2M + \mathrm{BPD}^+ + H^+ \qquad (5)^{12)}$$

$$HO\cdot + \mathrm{BPDH} \xrightarrow{k_2\ (\sim 10^{10})} \mathrm{BPD}\cdot + H_2O \qquad (6)^{13)}$$

Chain-propagating reaction:

$$\mathrm{BPD}\cdot + O_2 + H^+ \xrightarrow{k_3\ (\sim 10^9)} \mathrm{BPD}^+ + HO_2\cdot \qquad (7)^{14)}$$

$$HO_2\cdot + \mathrm{BPDH} \xrightarrow{k_4\ (\sim 10^9)} H_2O_2\cdot + \mathrm{BPD}\cdot \qquad (8)^{15)}$$

Chain-terminating reaction:

$$2\,HO_2\cdot \xrightarrow{k_5\ (8\times 10^5)} H_2O_2\cdot + O_2 \qquad (9)^{16)}$$

Scheme 2. The figures in parentheses are presumed second-order-rate constants ($M^{-1}\ s^{-1}$) in water.

The combination of the individual steps, with assuming a long chain length for the major propagation process, affords the net reaction (Eq. 10):

$$\mathrm{BPDH} + O_2 \xrightarrow{H^+} \mathrm{BPD}^+ + H_2O_2 \qquad (10)$$

According to the steady state treatment for intermediates HO·, BPD·, and $HO_2\cdot$, we arrive at the following equation (11) for the overall rate at given time t (the protonation equilibria (Eq. 1) are not taken into account):

$$V = k_{obs}[\mathrm{BPDH}]$$

$$k_{obs} = k_4\{(k_1/k_5)[H_2O_2][Fe^{2+}][H^+]\}^{1/2} \qquad (11)$$

The proposed mechanism satisfactorily explains the

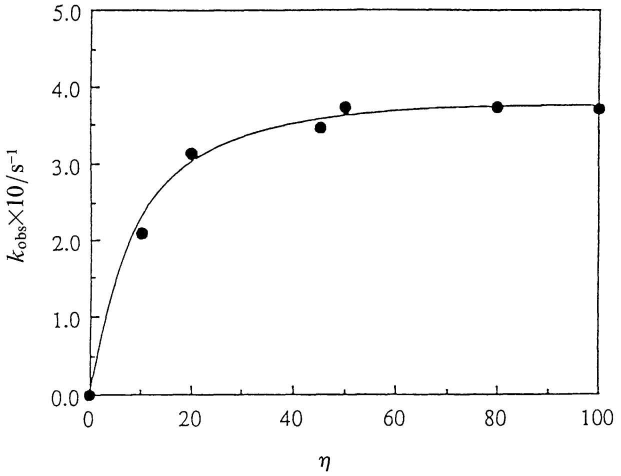


Fig. 8. Effect of partial pressures of oxygen (η) on k_{obs} for oxidation of BPDH; [$HClO_4$]=2.0 mM.

influence of changing O_2-concentrations; namely, the rate is independent of the percent O_2 content when the concentration of dissolved O_2 in solution is significantly high as compared with that of BPDH (Fig. 8). Furthermore, the equation (11) shows that the rate should increase with the square root of H_2O_2 concentrations. Actually, as Fig. 4 reveals, the plot of the observed rate constant (k_{obs}) vs. H_2O_2 concentrations affords a downward curvature.

Meanwhile, H_2O_2 not only caused the induction time to decrease but also H_2O_2 would be employed to initiate another series of chain reactions involving HO· radical as a chain-carrying species, which resulted in depression of the H_2O_2 yield (equations (12) and (13)).

$$\mathrm{BPD\cdot} + H_2O_2 \xrightarrow{H^+} \mathrm{BPD}^+ + \mathrm{HO\cdot} \qquad (12)$$

$$\mathrm{HO\cdot} + \mathrm{BPDH} \longrightarrow H_2O + \mathrm{BPD\cdot} \qquad (13)$$

The real overall mechanism might become so complicated accordingly that no quantitative evaluation of the rate constant for each step was not attempted.

In conclusion, 1-benzyl-3,5-bis(1-pyrrolydinylcarbonyl)-1,4-dihydropyridine (BPDH) underwent H_2O_2-oxidation in the presence of $HClO_4$ to give BPD^+ and the monooxygenated product in a 1 : 1 ratio through an ionic process, while the O_2-oxidation of the same substrate afforded the BPD^+ and H_2O_2 through a radical-chain pathway including HOO· and BPD· radicals as the chain carriers.

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