Metal-ion-facilitated Oxidations of Dihydropyridines with Molecular Oxygen and Hydrogen Peroxide

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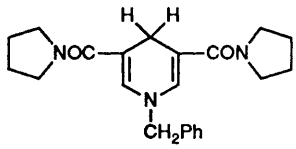
Some bivalent metal ions such as alkaline-earth metal ions promoted the O_2- and H_2O_2-oxidations of 3,5-disubstituted 1,4-dihydropyridines in acetonitrile; particularly, the presence of Ca^{2+} in excess over the dihydropyridines completed these reactions within a few seconds, producing calcium hydroperoxide and hydroxide, respectively. The absorbance *vs.* time plot and the effects of various additives on these rates show that each reaction takes place through a free-radical chain mechanism.

NADH, a reduced nicotinamide adenine dinucleotide coenzyme, has aroused much interest because of the broad spectrum of its biological activities as an electron transport couple in cellular respiratory energy metabolism, including mixed function oxidoreductase enzymes such as alcohol- and pyruvate-dehydrogenases, cytochrome- and quinone-reductases, and FAD-dependent amino acid-oxidases and mono-oxygenases. NADPH functions mainly as a hydrogen donor in biosynthetic reactions such as fatty acid and steroid syntheses.[1] Unlike dihydroflavins,[2] NADH [$E^{\circ\prime} = -0.325$ V *vs.* normal hydrogen electrode (NHE)],[3] as well as its typical model 1-benzyl-1,4-dihydronicotinamide (BNAH), has been firmly believed not to react with O_2 to give hydrogen peroxide ($E^{\circ\prime} = +0.27$ V) in spite of its lower two-electron redox potential than that of dihydroflavins ($E^{\circ\prime} = -0.19$ V).[4] There are a number of precedents in the literature for molecular oxygen oxidation of organic substrates in the presence of NADH. However, we have been unable to find any report of the direct reduction of molecular oxygen to hydrogen peroxide with 1,4-dihydropyridines, although there are many examples of enzymic and non-enzymic formation of H_2O_2 from O_2 by a combination of reducing substrates and catalytic transition metal ions such as NADH/1,10-phenanthroline/Cu^{II},[5a] ascorbic acid/Cu^{II},[5b] and H_2S/Ru^{II},[5c] where reduced metal ions are assumed to be first formed.

We report herein the results of our investigations of metal ion-, especially calcium ion-induced H_2O_2-formation from 1-benzyl-3,5-bis(pyrrolidinylcarbonyl)-1,4-dihydropyridine BPDH (**1**) and dissolved oxygen in acetonitrile solvent. *In vivo*, 3,5-disubstituted 1,4-dihydropyridines such as nifedepine (**2**), a derivative of the Hantzsch ester (**3**), are important in modulating calcium-ion flux across cellular membranes,[6] while calcium ions are known to stimulate and control neutrophiles and macrophages to produce superoxide-free radicals and hydrogen peroxide, which are involved in the killing of invading microbes.[7] In this sense, the chemical action of 1,4-dihydropyridines toward calcium ions is of interest.



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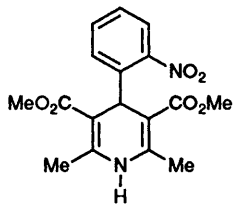

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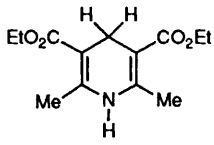

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Experimental

Materials.—BPDH was prepared according to the method described in a previous paper.[8] Metal perchlorate hydrates (special grade, Kishida Chemicals Co.) were employed without further purification. Acetonitrile was distilled from P_4O_{10}.

Kinetic Procedure.—All experiments were performed at 25 °C in acetonitrile. The reaction with oxygen was started by injecting the required volume of a 75 mmol dm^{-3} acetonitrile solution of metal perchlorates with a microsyringe into 3 cm^3 of a BPDH solution (0.1 mmol dm^{-3}) in a 1 cm UV cell capped with a rubber septum. The dissolved oxygen concentration in acetonitrile is 13 mmol dm^{-3} under O_2 at 1 atm pressure.[9] Meanwhile, the reaction with H_2O_2 was started under nitrogen by injecting, for example, 20 mm^3 of a 75 mmol dm^{-3} acetonitrile solution of $Ca(ClO_4)_2$ into a cell containing 3 mm^3 of a 0.1 mmol dm^{-3} acetonitrile solution of BPDH and 20 mm^3 of a 15 mmol dm^{-3} acetonitrile solution of H_2O_2 (equivalent to BPDH) which was prepared by diluting a 30% aqueous H_2O_2 solution (H_2O:H_2O_2 molar ratio 4.4) with acetonitrile. The progress of the reaction was followed by monitoring the disappearance of absorbance at 355 nm, or 320 nm in the case of Pb^{II}. The UV–VIS spectra were recorded on a Hitachi 220 spectrophotometer.

Under the same kinetic conditions BNAH and the Hantzsch ester (**3**) did not react with molecular oxygen at all and the decreasing absorbance observed for the BNAH substrate was attributable to its hydration rather than oxidation.

Product Analysis.—Oxygen gas was bubbled through 20 cm^3 of an acetonitrile solution of BPDH (0.5 mmol) in the presence of 1 equiv. of calcium perchlorate until the dihydropyridine could not be seen on TLC. To this was added acetic acid (2 equiv.) and triphenylphosphine (2 equiv.). The solution was left to stand overnight and then treated with a large excess of hydrochloric acid. The mixture was thoroughly extracted with chloroform and subjected to preparative TLC using

Kieselgel 60 GF_{254} and $CHCl_3$–MeOH (50:1) as the eluant, affording the corresponding BPD^+Cl^- salt and triphenylphosphine oxide in 90 and 81% isolated yields (±2), respectively (average of duplicate runs). In a similar manner, 98% of the salt and 72% of the phosphine oxide were obtained with zinc perchlorate.

A separate experiment for the qualitative detection of hydrogen peroxide was performed by the conventional spectroscopic method at 350 nm using potassium iodide and catalytic ammonium molybdate which clearly showed the formation of hydrogen peroxide during the reaction.[10]

The reaction of BPDH (0.5 mmol) with H_2O_2 in the presence of calcium perchlorate (0.5 mmol) in acetonitrile under nitrogen, after the same work-up as above, afforded 97% BPD^+Cl^-.

Electrochemistry.—Cyclic voltammograms were recorded on a potentiostat/galvinostat HLV-151 with a function generator (Hokuto Denko) in a three-electrode, one-compartment cell with a Luggin capillary. The working and counter electrodes used were a platinum plate and a platinum wire, respectively, in the same compartment cell. The potentials were referred to Ag/AgCl. All acetonitrile solutions were deoxygenated with a stream of nitrogen. The concentrations of substrates and the supporting electrolyte (Bu_4NClO_4) were 1 mmol dm^{-3} and 0.1 mol dm^{-3}, respectively. The sweep rate was 200 mV s^{-1}. Under these conditions the anodic peak potentials (E_p^a) for BNAH and BPDH were +0.66 and +0.70 V, respectively. The corresponding values in the presence of $Ca(ClO_4)_2$ (10 mmol dm^{-3}) were +0.82 and +0.83 V, respectively. The cathodic peak potential (E_p^c) of BPD^+Cl^- was −0.98 V.

Results and Discussion

Figure 1 shows representative plots of decreasing absorbance *vs.* time for the oxidation of BPDH with O_2 in the presence (fivefold excess *vs.* BPDH) of a variety of bivalent metal perchlorates (0.5 mmol dm^{-3}) at 25 °C in acetonitrile. Bivalent transition-metal ions investigated here, such as Mn^{II}, Co^{II}, and Ni^{II}, are all practically ineffective unlike the catalytic reduction of nitrosobenzene with BPDH[11] and the reaction rates are too slow to measure. Mg^{II} and Zn^{II} are slightly effective. Pb^{II} is fairly effective, but the time plots, which do not obey the usual simple kinetic equations, consist of two zones; the first slow one, referred to as an induction phase, which is followed by the fast one referred to as a chain-propagation phase. Table 1 summarizes values for the length of the induction phase and the mean velocity (mmol dm^{-3} s^{-1}) of the induction and propagation phases, which define and characterize the nature of the reaction courses. It can be seen from Figure 1 that alkaline-earth metal cations such as Ca^{II}, Sr^{II}, and Ba^{II} exert a pronounced effect on the rate; in particular, Ca^{II} facilitates the reaction most efficiently. In these cases, the plots are all biphasic as in the case of Pb^{II}. The observation of an induction period suggests the possibility that the reaction proceeds by a free-radical chain mechanism. In fact, addition of an equimolar amount of catechol, a known free-radical scavenger, completely prohibits all the reactions investigated here. Hydroquinone and α-tocopherol remarkably increase the induction period of the Ca^{II}-facilitated reaction, and completely inhibit the Zn^{II}-facilitated reaction.

Figure 2 shows the results obtained using different concentrations of Ca^{II} ranging from 0.02 to 1 mmol dm^{-3}. It is interesting to note that the reactions in the presence of less than equimolar amounts of Ca^{II} *vs.* BPDH result in only slow consumption of BPDH as compared with the reaction using excess Ca^{II} over BPDH. This is most clearly shown using a Ca^{II} concentration of 0.05 mmol dm^{-3}, where the progress of the

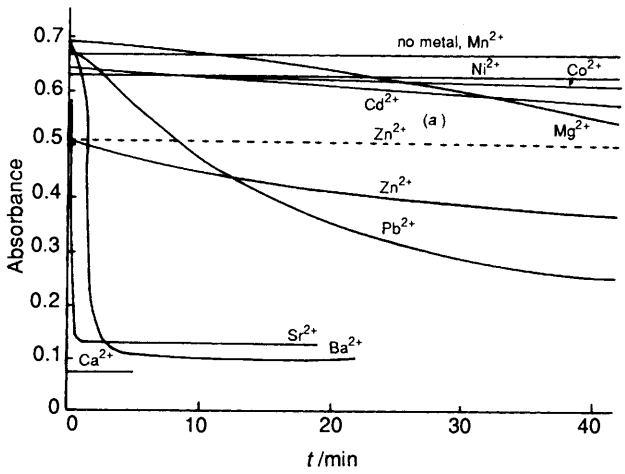


Figure 1. Time course of the oxidation of BPDH (0.1 mmol dm^{-3}) with molecular oxygen in MeCN at 25 °C in the presence of various bivalent metal ions (0.5 mmol cm^{-3}): (*a*) in the presence of hydroquinone (10 mmol dm^{-3}).

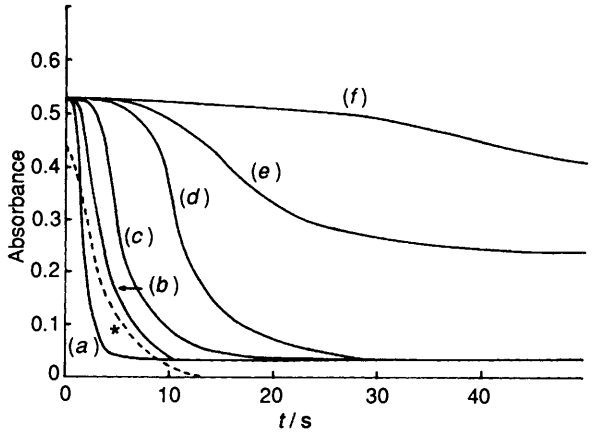


Figure 2. Time courses of the oxidation of BPDH (0.1 mmol dm^{-3}) with molecular oxygen in MeCN at 25 °C in the presence of different amounts of calcium perchlorate: (*a*) 1.0; (*b*) 0.5; (*c*) 0.2; (*d*) 0.1; (*e*) (0.05; (*f*) 0.02 mmol dm^{-3}. (*) The oxidation of MPDH in the presence of calcium perchlorate (0.5 mmol dm^{-3}).

reaction virtually stops when only 50% of the initially added BPDH (0.1 mmol dm^{-3}) has been consumed; *i.e.*, excess Ca^{II} over BPDH is required for all BPDH being consumed. From this we conclude that calcium hydroperoxide, accumulating as the reaction progresses, suppresses the intrinsic ability of calcium cations to enhance the rate, since a more basic counter anion HOO^- *vs.* ClO_4^- forms a more stable complex with Ca^{II} and hence decreases the Lewis acidity of Ca^{II}.

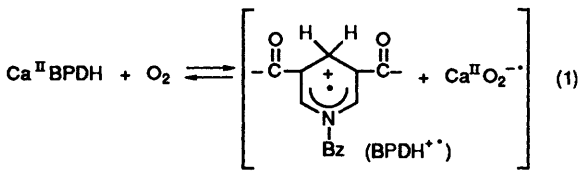
These observations can be reasonably accounted for by the following mechanism involving firstly reversible electron transfer (ET) between Ca^{II}-complexed BPDH and dioxygen to produce the highly unstable BPDH radical cation ($BPDH^{+\bullet}$) and calcium superoxide [equation (1)]. Pre-equilibrium complexation between BPDH and Ca^{II} is assumed, but the site or type of this is unspecified.[12] 1-Methyl-3,5-bis-(pyrrolidinylcarbonyl)-1,4-dihydropyridine (MPDH) shows similar behaviour to that of BPDH as predicted from the similarity of their structures [Figure 2(*a*)]. In sharp contrast with reactive BPDH and MPDH, the lack of reactivity of BNAH could be attributed to its stronger complexation with Ca^{II}, although this has not been confirmed.

On the basis of electrochemical data for $BPDH/BPDH^{+\bullet}$

Table 1. Oxidation of BPDH with oxygen in the presence of calcium perchlorate in oxygen-saturated acetonitrile. Effects of changing amounts of Ca^{2+}, various additives, and deuterium substitution.[a]

Ca^{2+}/ mmol dm^{-3}	Additive/mmol dm^{-3}	Induction period/s	Velocity of induction/ 10^{-4} mmol dm^{-3} s^{-1}	Velocity of propagation[b]/ 10^{-2} mmol dm^{-3} s^{-1}
1.0		—[c]	—[c]	2.7
0.5		—[c]	—[c]	1.8 (1.15)
0.5[d]		—[c]	—[c]	0.64 (0.44)
0.2		2.6	4.8	1.5
0.1		7.4 (8.1)	3.3 (2.7)[e]	1.3 (0.87)
0.05		11.8	1.9	0.40
0.02		33.8	1.9	0.12
0.5	α-Tocopherol, 10	17.3	1.4	0.51
0.5	Hydroquinone, 10	7.8	9.1	1.8
0.5	Hydroquinone, 20	12.3	3.1	1.6
0.1	Catechol, 0.1	2 000	0.1	0.002
0.5	DMSO, 10	—[c]	—[c]	1.3
0.5	NBT, 0.1	430	0.6	0.17
0.5	NBT, 0.05	80	1.6	0.25
0.5		1 460[f]	0.04[f]	0.007[f]

Unless otherwise noted, the reactions were conducted under the same conditions of BPDH concentration (0.1 mmol dm^{-3}) and O_2 pressure (1 atm). [a] Figures in parentheses denote values for mono-deuterium-substituted-[$^2H_1$]BPDH. [b] Mean velocity derived from the slope at the midpoint of a propagation zone. [c] Too fast to measure. [d] O_2 = 0.2 atm. [e] Apparent kinetic isotope effect, 1.2. [f] Value for 1-benzylpyrrolidin-3-ylcarbonyl-1,4-dihydropyridine.



(1)

(E_p^a = +0.70 V *vs.* Ag/AgCl, associated with +0.90 V *vs.* NHE)[13a] and for $O_2/O_2^{-\bullet}$ ($E°$ = −0.82 V *vs.* NHE)[13b] in MeCN, the simple ET process is unlikely owing to the highly unfavourable free energy change (ΔE = *ca.* −1.7 V). Nevertheless, the presence of suitable Lewis acids is expected to make the ET process less endothermic; protonation, for example, is known to increase the redox potential of the dioxygen/superoxide couple to $E°$ = +0.12 V *vs.* NHE.[13b] Hence, one possible interpretation of the major role of Ca^{II} would be in terms of its ability to lower the activation energy of the ET process.

However, the ET between Ca^{II} BPDH and O_2 still seems highly endothermic and appears to be on the borderline of feasibility; the ionic radical-pair thus formed appears to be incapable of triggering a subsequent radical-chain reaction during its short lifetime. Therefore, it is reasonable to assume that step (1) is followed by an exothermic proton-transfer process to produce free radicals, BPD$^\bullet$ and a hydroperoxyl radical [equation (2)]; *i.e.*, the superoxide anion can undergo facile proton abstraction from $BPDH^{+\bullet}$ since $BPDH^{+\bullet}$ is more acidic than the conjugate acid of superoxide on the basis of the data in water.[14,15]

Thus the most probable initiation mechanism is given in equations (1) and (2). A small, but positive isotope effect (k_H/k_D = 1.2) in the induction phase, observed on mono-deuterium substitution of the ring methylene hydrogen in BPDH, indicates that equation (2) is partially rate-determining

$$[BPDH^{+\bullet} + Ca^{II}O_2^{-\bullet}] \longrightarrow Ca^{II}BPD^\bullet + HOO^\bullet \quad (2)$$

(Table 1). This would be followed by a propagation sequence [equations (3)–(7)]. Each step has been generally accepted as reasonable; the hydroperoxyl radical abstracts an electron from Ca^{II}BPDH by a diffusion process yielding products HOO^-Ca^{II} and $BPDH^{+\bullet}$ or $Ca^{II}BPD^\bullet$ according to equation (3) followed by equations (4) and (5), while the strongly reducing radical Ca^{II}-complexed BPD$^\bullet$ (E_p^c = *ca.* −0.98 V *vs.* Ag/AgCl in MeCN)[16] transfers its electron to dissolved molecular oxygen giving rise to a superoxide anion [equation (6)] which is readily converted into its protonated equivalent [equation (7)] or a hydroperoxyl radical.

$$HOO^\bullet + Ca^{II}BPDH \longrightarrow HOO^-Ca^{II} + BPDH^{+\bullet} \quad (3)[17]$$

$$BPDH^{+\bullet} \longrightarrow BPD^\bullet + H^+ \quad (4)[18]$$

$$BPD^\bullet + Ca^{II} \rightleftharpoons Ca^{II}BPD^\bullet \quad (5)$$

$$Ca^{II}BPD^\bullet + O_2 \longrightarrow BPD^+ + Ca^{II}O_2^{-\bullet} \quad (6)[19]$$

$$Ca^{II}O_2^{-\bullet} + H^+ \rightleftharpoons HOO^\bullet + Ca^{II} \quad (7)$$

Termination

$$Ca^{II}O_2^- + HOO^\bullet \longrightarrow O_2 + HOO^-Ca^{II} \quad (8)[17]$$

Thus, the superoxide must be a key intermediate on the major path because the reaction suffers significant rate retardation upon addition of Nitro Blue Tetrazolium (NBT), a well known diagnostic reagent for the presence of superoxide anion. However, we cannot rule out an alternative possibility that NBT picks up the BPD$^\bullet$ radical rather than the superoxide anion, since BPD$^\bullet$ has a large negative oxidation potential as $O_2^{-\bullet}$. In contrast, the addition of a large excess of dimethyl sulphoxide (DMSO), an OH radical scavenger, did not significantly affect the initiation and propagation steps, suggesting that the radical chain pathway involving OH radicals is of minor importance. An OH radical, if generated, would be used to produce a BPD$^\bullet$ radical according to equation (14) (see further on).

Figure 3 reveals that dependence of the propagation rate on the concentration of Ca^{II} is less when Ca^{II} is present in excess over BPDH, *i.e.*, only twofold rate increase with tenfold increase in Ca^{II} concentration, indicating that BPDH complexes

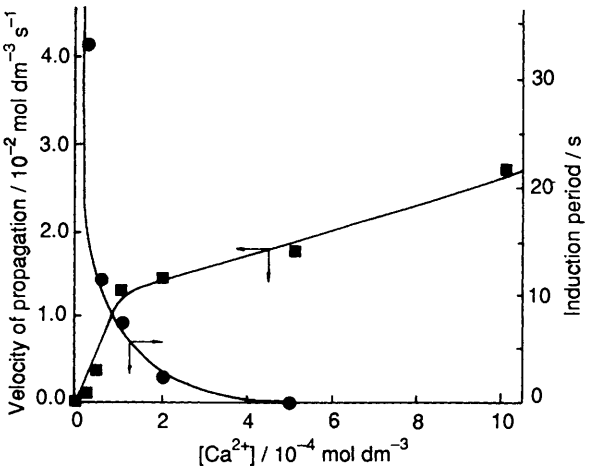


Figure 3. Plots of propagation velocity and induction period *vs.* concentration of calcium perchlorate for O_2 oxidation of BPDH (0.1 mmol dm^{-3}) in MeCN at 25 °C.

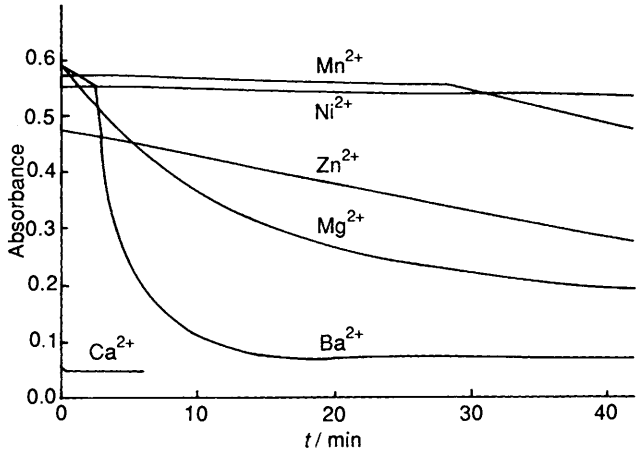


Figure 4. Oxidation of BPDH with H_2O_2 in the presence of bivalent metal ions in MeCN under nitrogen at 25 °C. Effects of different metal ions and their concentrations on absorbance–time plots. [BPDH] = 0.1 mmol dm^{-3}; [M^{2+}] = 0.5 mmol dm^{-3}.

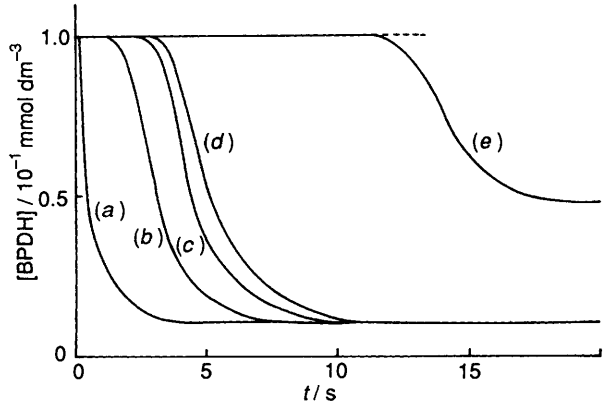


Figure 5. Time plot for oxidation of BPDH with H_2O_2 in the presence of different concentrations of calcium perchlorate under nitrogen, in MeCN. [BPDH] = 0.1 mmol dm^{-3}; [H_2O_2] = 0.1 mmol dm^{-3}. Concentration of calcium perchlorate: (*a*) 1.0; (*b*) 0.5; (*c*) 0.1; (*d*) 0.05; (*e*) 0.025; (*f*) 0.01 mmol dm^{-3}.

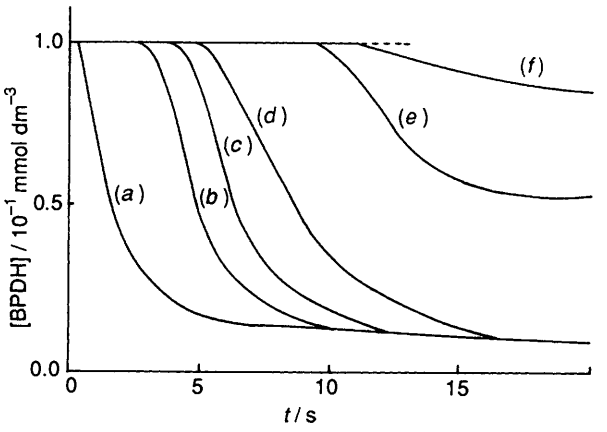


Figure 6. Time plot for oxidation of BPDH with different amounts of H_2O_2 in the presence of calcium perchlorate under nitrogen. [BPDH] = 0.1 mmol dm^{-3} [$Ca(ClO_4)_2$] = 0.5 mmol dm^{-3}. Concentration of H_2O_2: (*a*) 1.0; (*b*) 0.5; (*c*) 0.2; (*d*) 0.1; (*e*) 0.05 mmol dm^{-3}.

Ca^{II} in a 1:1 stoicheiometric ratio, but excess Ca^{II} takes part to a minor extent in accelerating the propagation rate. Calcium ion serves to protect the final product OOH^- against reductive decay through complexation (see further on). Equation (8) would be a probable termination reaction. The overall stoicheiometry of the reaction is given in equation (9).

$$BPDH + O_2 + Ca^{II} \longrightarrow BPD^+ + HO_2{}^-Ca^{II} \quad (9)$$

Introduction of a trace amount of hydrogen peroxide into the oxygen-saturated reaction solution at the initial stage of the reaction markedly reduced the induction period (data not shown), which implies that a new initiation process is operative; reaction of the hydrogen peroxide with BPDH in the presence of calcium ion would allow generation of BPD$^\bullet$ and OH$^\bullet$ radicals according to equations (10) and (11), which could also serve to start the propagation reaction.

Thus, we must now discuss the role of H_2O_2 by examining the BPDH oxidation with H_2O_2 in the presence of various bivalent metal ions under nitrogen, since H_2O_2 could be a good oxidant. The results are shown in Figures 4–6 and Table 2. Some characteristic features are as follows. (*i*) Ca^{II} was the most efficient of the metal cations examined (Figure 4). (*ii*) The time profiles were all biphasic, exhibiting an induction period. This type of time profile strongly rules out the possibility that the reaction mechanism is a consecutive one. (*iii*) The reaction was mildly and strongly inhibited by the presence of hydroquinone and catechol, respectively, in an analogous manner to the oxygenation. However, the inhibitory effect of an OH radical scavenger, DMSO, was much more pronounced in the H_2O_2 *vs.* O_2 oxidation. This inhibition is significant, strongly indicating that OH radicals should be a key intermediate on the major path. The moderate inhibitory effect by NBT is indicative of the predominant scavenging of BPD$^\bullet$ rather than superoxide ion by NBT (Table 2). As one can see from Figures 5 and 6, the reaction stoicheiometry is 1:1:1/2 for the molar ratio of BPDH:H_2O_2:Ca^{II}.

On the basis of these results, the reaction mechanism given by equations (10)–(16) can be proposed as most plausible, where efficient formation of OH radicals propagates a reaction. A

Initiation

$$Ca^{II}BPDH + H_2O_2 \rightleftharpoons BPDH^{+\bullet} + Ca^{II}OH^- + OH^\bullet \quad (10)$$

Table 2. Oxidation of BPDH with hydrogen peroxide in the presence of calcium perchlorate in acetonitrile under nitrogen. Effects of changing amounts of hydrogen peroxide and calcium perchlorate, and various kinds of additives.[a]

H_2O_2/ mmol dm^{-3}	Ca^{2+} Additive/mmol dm^{-3}	Induction period/s	Velocity of propagation[b]/ 10^{-2} mmol dm^{-3} s^{-1}	BPDH consumption (%)
1.0	0.5	—[c]	4.1	
0.5	0.5	1.9	4.1	
0.2	0.5	3.3 (19)[d]	3.1 (1.7)[d]	
0.1	0.5	3.6	2.6	
0.05	0.5	12.4	1.7	50
0.1	1	—[c]	2.9	100
0.1	0.1	4.4	2.3	100
0.1	0.05	5.1	1.8	100
0.1	0.025	9.9	1.1	55
0.1	0.01	11.9	0.2	19
0.1	0.5 DMSO, 10	7.1	0.66	
0.1	0.5 HQ[e] 10	6.0	0.27	
0.1	0.5 HQ, 20	13.3	0.13	
0.1	0.5 Cat,[f] 0.1	30	0.034	
0.1	0.5 NBT, 0.1	1 850	0.027	
0.1	0.5 NBT, 0.05	1 060	0.13	

[a] [BPDH] = 0.1 mmol dm^{-3}. [b] See footnote *f* of Table 1 for definition of the velocity. [c] Too fast to measure. [d] Value for [$^2H_1$]BPDH. [e] Hydroquinone. [f] Pyrocatechol.

$$BPDH^{+\bullet} + Ca^{II}OH^- \longrightarrow Ca^{II}BPD^\bullet + H_2O \quad (11)$$

Propagation

$$Ca^{II}BPD^\bullet + H_2O_2 \longrightarrow BPD^+ + HO^\bullet + Ca^{II}OH^- \quad (12)$$

$$Ca^{II}OH^- \rightleftharpoons 1/2Ca(OH)_2 + 1/2Ca^{II} \quad (13)$$

$$HO^\bullet + Ca^{II}BPDH \longrightarrow Ca^{II}OH^- + BPDH^{+\bullet} \quad (14)$$

$$BPDH^{+\bullet} \longrightarrow BPD^\bullet + H^+ \quad (4)$$

$$Ca^{II}OH^- + H^+ \rightleftharpoons Ca^{II} + H_2O \quad (15)$$

$$BPD^\bullet + Ca^{II} \rightleftharpoons Ca^{II}BPD^\bullet \quad (5)$$

Termination

$$Ca^{II}BPD^\bullet + HO^\bullet \longrightarrow BPD^+ + Ca^{II}OH^- \quad (16)$$

Overall reaction

$$BPDH + H_2O + 1/2Ca^{II} \longrightarrow BPD^+ + H_2O + 1/2Ca(OH)_2 \quad (17)$$

hydrogen atom transfer process [equation (18)] could be considered in place of equations (14), (4), (15), and (5), involving sequential electron and proton transfers.

$$HO^\bullet + Ca^{II}BPDH \longrightarrow Ca^{II}BPD^\bullet + H_2O \quad (18)$$

The summation of equations (12)–(14), (4), (15), and (5) provides equation (17) for the net reaction; in order to account for the observed molar ratio, $Ca^{II}OH$ unlike $Ca^{II}OOH^-$ is assumed to disproportionate to give $Ca(OH)_2$ and Ca^{II} [equation (13)]. Here a question arises as to why $Ca^{II}OOH^-$ could be a stable product in the O_2 oxidation, but not in the H_2O_2 oxidation. A possible answer to this question might be that lack of H_2O shifts the equilibrium in equation (19) to the left in the O_2-oxidation, while in the H_2O_2-oxidation the existence of H_2O inevitably introduced with H_2O_2 shifts the equilibrium to the right to leave H_2O_2 uncomplexed; hence reaction of added H_2O_2 with $Ca^{II}OH^-$ to yield $Ca^{II}OOH^-$ might not occur.

$$Ca^{II}OOH^- + H_2O \rightleftharpoons Ca^{II}OH^- + H_2O_2 \quad (19)$$

In conclusion, calcium ion is an excellent promoter for the oxidation of certain dihydropyridines with dioxygen. The net reaction is simple, but to our knowledge, this is the first time that anyone has demonstrated such a reaction occurring at a rapid rate. Pathologically, O_2^- or H_2O_2-producing NADPH oxidases are important enzymes, since their deficiency in the cellular membrane of phagocytes causes a chronic granulomatous disease. Although it has been reported recently that well purified NADPH oxidases consist of a single polypeptide chain containing neither FAD nor cytochrome b_{558},[20] there is still much debate about the structural characterization of NADPH oxidases. Thus, the actual mechanism for NADPH-oxidase-catalysed reduction of dioxygen remains incompletely understood. We feel that the present reaction may partly mimic the chemical events of NADPH oxidases, which are known to be stimulated by Ca^{II} inside cells.

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