Chain Decomposition of Hydrogen Peroxide Induced by Catalytic Levels of Reduced 5-Methylphenazinium and Metal Ions: A Nonclassical Fenton System

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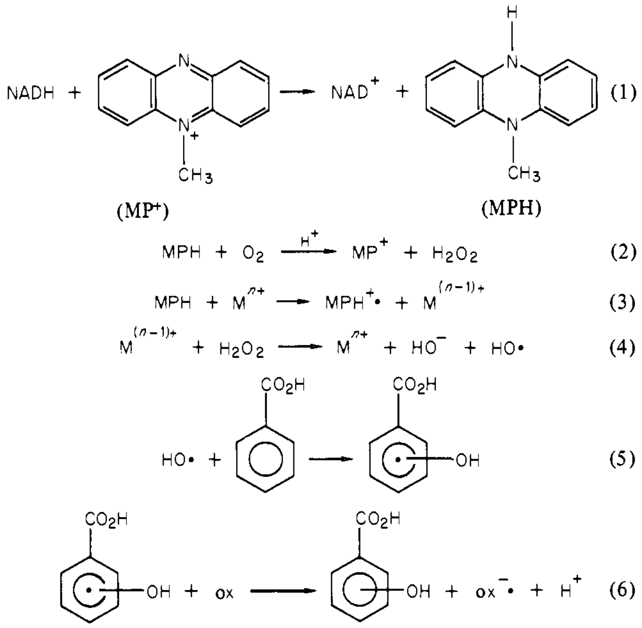
Abstract: Catalytic levels of fully reduced 5-methylphenazinium (MPH) and metal ions induce a chain decomposition of H_2O_2 in N_2-saturated aqueous benzoic acid solution, with concomitant production of *o*-, *m*-, and *p*-hydroxybenzoic acids. The oxidant inducing the aromatic hydroxylations is the hydroxyl radical, produced in a one-electron, metal ion mediated reduction of H_2O_2. The rate of decomposition is zero order with respect to $[H_2O_2]$ when the reaction is catalyzed by adventitious metal ions or by added Fe^{III} or Fe^{II}. For Fe^{III} in citrate buffer, the rate expression is $-d[H_2O_2]/dt = k[MPH]_0([Fe^{III}]_0 + a)$ where $k = 2.7 \times 10^3$ M^{-1} s^{-1} and $a = 7.0 \times 10^{-7}$ M. The reaction mechanism is essentially the same as for the classical Fenton system except that an added reducing agent is required to return the oxidized catalytic metal ion to its reduced state. Thus, the present system can be grouped with a number of other "nonclassical Fenton systems" such as the Udenfriend and Hamilton systems, which are capable of aromatic hydroxylations and which require both a catalytic metal ion and a reducing agent.

It is well-known that addition of the 5-methylphenazinium cation (MP^+) and reduced β-nicotinamide adenine dinucleotide (NADH) to oxygenated aqueous solutions generates a species which can hydroxylate aromatic substrates.[1-3] It is now clear that this reactive species is the hydroxyl radical ($HO\cdot$)[2,3] produced by an adventitious metal ion catalyzed decomposition of hydrogen peroxide (H_2O_2) also produced by the system.[3] The essential steps in the hydroxylation mechanism (benzoic acid as substrate) are given in Scheme I. $MPH^{+}\cdot$ is the protonated, one-electron reduced form of MP^+; MPH denotes the two-electron reduced (and protonated) form of MP^+. Adventitious metal ions M^{n+} and $M^{(n-1)+}$ (probably Fe^{III} and Fe^{II})[4] would be complexed by buffer ions.

The mechanism suggests that in the presence of excess H_2O_2 a chain decomposition could occur if the hydroxycyclohexadienyl radicals ($HO\cdot$ adducts produced in reaction 5) were oxidized by $MPH^{+}\cdot$ (reaction 6) to re-form MPH. The chain would be initiated by reaction 3 and propagated by reactions 3–6. Termination of the chain reaction would occur when the $HO\cdot$ adducts undergo reactions other than oxidation by $MPH^{+}\cdot$, e.g., oxidation by another system species, reduction, dimerization, or disproportionation. The proposed chain sequence involving the metal ions resembles the mechanisms for hydroxyl radical production by the Fenton system, recently discussed by Walling.[5] The major difference is the requirement of an added reducing agent for the oxidized catalytic metal ion, which is reduced by the product organic radical in the classical Fenton system. This report presents results which demonstrate that a chain decomposition of H_2O_2 does indeed occur and that the decomposition can be catalyzed either by adventitious metal ions or by added iron ions.

Scheme I



$$MPH + O_2 \xrightarrow{H^+} MP^+ + H_2O_2 \quad (2)$$

$$MPH + M^{n+} \longrightarrow MPH^{+}\cdot + M^{(n-1)+} \quad (3)$$

$$M^{(n-1)+} + H_2O_2 \longrightarrow M^{n+} + HO^- + HO\cdot \quad (4)$$

Experimental Section

Materials. 5-Methylphenazinium methyl sulfate (phenazine methosulfate; Aldrich and Sigma), β-nicotinamide adenine dinucleotide, reduced form (NADH; Sigma Stock No. N8129), *N,N*-bis[2-[bis(carboxymethyl)amino]ethyl]glycine (DETAPAC; Baker grade, ≳98%), sodium acetate (Fluka AG, puriss pa), tetrabutylammonium perchlorate (Alfa Products, Baker, and Eastman Kodak), hydrogen peroxide (Ultrex grade, Baker Chemical), nitrogen (ultrapure; ≥99.999% N_2, Airco), and oxygen ("Zero 0.5"; total hydrocarbon <0.5 ppm, Airco) were used as received. Water was distilled serially from acid dichromate, alkaline permanganate, and pure water. *m*-Toluic acid (Aldrich) was recrystallized from triply distilled water until LC-detectable impurities were eliminated. 5-Methylphenazinium (MP^+) in the fully reduced, dihydrophenazine oxidation state (MPH)[6] was prepared by reduction of MP^+ with ascorbic acid in N_2-saturated solutions, followed by addition of KH_2PO_4 and K_2HPO_4 to adjust the solution to ca. pH 6. MPH was collected by centrifugation, washed several times with N_2-saturated, triply distilled water, and dried under a stream of N_2 at room temperature. All other chemicals were reagent grade from Baker Chemical or Fisher Scientific.

Experiments. All reactions were carried out at room temperature, and solutions were protected from light, either by use of dim red room lighting or by covering vessels with a black cloth. In experiments with the externally prepared MPH, weighed amounts of the reagent were added in powder form to aliquots of the reaction solution containing all other reagents, as necessitated by the very low solubility of MPH in water. For O_2-free experiments, reaction solutions were bubbled with N_2 throughout the reaction period. Hydrogen peroxide concentrations were determined by using a modification of the Ghormley iodometric method.[7] UV

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(1) Prema Kumar, R.; Ravidranath, S. D.; Vaidyanathan, C. S.; Appaji Rao, N. *Biochem. Biophys. Res. Commun.* **1972**, *48*, 1049–54. Ravidranath, S. D.; Ashok Kumar, A.; Vaidyanathan, C. S.; Appaji Rao, N. *Arch. Biochem. Biophys.* **1974**, *165*, 478–84.

(2) Halliwell, B. *Biochem. J.* **1977**, *167*, 317–20.

(3) Richter, H. W.; Fetrow, M. A.; Lewis, R. E.; Waddell, W. H. *J. Am. Chem. Soc.* **1982**, *104*, 1666–71.

(4) As determined by atomic absorption spectroscopy,[3] the reagents employed contained low levels of adventitious iron ions. In addition, ions of other metals can serve in such catalytic reactions (see, e.g., Czapski, G.; Samuni, A.; Meisel, D. *J. Phys. Chem.* **1971**, *75*, 3271–80).

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Table I. Yields of Products from MP$^+$, NADH, and H_2O_2 in N_2-Saturated Solutions[a]

[NADH]$_0$/ μM	[HBA]$_f$/ [NADH]$_0$	relative yields		
		oHBA	mHBA	pHBA
30.2	11.5	1.00	0.993	0.567
75.0	11.1	1.00	0.801	0.451
150	8.71	1.00	0.666	0.438

[a] Initial reactant concentrations: citrate buffer, 50 mM, pH 4.4; BA, 10 mM; H_2O_2, 42.5 mM; N_2 saturated. [MP$^+$]$_0$/[NADH]$_0$ = 1.09 in all cases.

Table II. Yields of Products from MPH and H_2O_2 in N_2-Saturated Solutions[a]

[H_2O_2]$_0$/ mM	[MPH]$_0$/ μM	buf-fer	[O_2]/ mM	[HBA]$_f$/ [MPH]$_0$	relative yields		
					oHBA	mHBA	pHBA
42	468	*b*	0	5.00	1.00	0.989	0.475
42	266	*b*	0	8.71	1.00	0.933	0.465
42	125	*b*	0	11.4	1.00	1.06	0.510
42	119	*b*	0	13.2	1.00	1.08	0.504
42	117	*b*	0	13.8	1.00	1.02	0.507
10.5	141	*b*	0	14.0	1.00	1.01	0.518
5.2	127	*b*	0	15.9	1.00	1.07	0.505
42	128	*c*	0	4.63	1.00	0.967	0.473
42	121	*b*	1.29[d]	$\geqslant$0.06	*e*	1.00	1.26

[a] All solutions contained [BA]$_0$ = 10 mM and were adjusted to pH 4.3–4.6. [b] 50 mM citrate buffer. [c] No added buffer.[8] [d] Solution was bubbled continuously with O_2. [e] Not determined.

Table III. Yields of Products from MPH in Oxygenated Solutions[a]

[MPH]$_0$/ μM	[O_2]/ mM	buf-fer	[HBA]$_f$/ [MPH]$_0$	relative yields		
				oHBA	mHBA	pHBA
117	0.27[b]	*d*	0.0408	1.00	0.764	0.534
115	0.27[b]	*e*	0.152	1.00	0.582	0.540
500	0.27[b]	*e*	0.262	1.00	0.761	0.556
468	1.29[c]	*e*	0.0466	1.00	0.903	1.17

[a] All solutions contained [BA]$_0$ = 10 mM and were adjusted to pH 4.3-4.4. [b] Initially air-saturated samples were capped upon initiation of reaction. [c] Solution was bubbled continuously with O_2. [d] No added buffer.[8] [e] 50 mM citrate buffer.

absorption spectra were obtained on a Perkin-Elmer Model 575 absorbance spectrophotometer. Unreacted benzoic acid and reaction products were acidified with HCl and, after addition of *m*-toluic acid for internal standardization, were extracted with ether, stripped of solvent, and subjected to high-pressure LC analysis.

Chromatography. Analyses were performed on a high-pressure LC system consisting of a Perkin-Elmer Series 1 LC pump, Rheodyne loop injector, Waters Model 440 absorbance detector, Leeds & Northrup Speedomax Type G recorder with disc integrator, and Hewlett-Packard Model 3390-A reporting integrator. Reversed-phase ion-pair chromatography was employed by using a Waters μ-Bondapak C_{18} analytical column (0.39 × 30 cm) and a mobile phase of water/methanol, 50 mM phosphate buffer, pH 7.5, and 10 mM tetrabutylammonium perchlorate. Eluted species were identified by comparison of high-pressure LC retention times with those of commercial samples. Peaks were monitored with 254- or 280-nm UV absorbance and their areas determined by disc or electronic integration.

Results

The hydroxylation of benzoic acid (BA) by the addition of the 5-methylphenazinium cation (MP$^+$), reduced β-nicotinamide adenine dinucleotide (NADH), and O_2 to aqueous benzoic acid solutions has been demonstrated previously.[3] All three isomeric hydroxybenzoic acids (HBA) are produced, where the number of HBA molecules formed per molecule of NADH added ([HBA]$_f$/[NADH]$_0$) was ≤0.65; the theoretical limit is one. The present experiments show that benzoic acid is also hydroxylated by (1) MP$^+$, NADH, and H_2O_2 in N_2-saturated solution, (2) fully reduced MP$^+$ (i.e., MPH) and H_2O_2 in N_2-saturated solution, and (3) MPH in O_2-saturated solution. Under all conditions, *o*-, *m*-, and *p*-hydroxybenzoic acids (oHBA, mHBA and pHBA, respectively) were obtained. In the presence of added H_2O_2 (with O_2 excluded), [HBA]$_f$/[NADH]$_0$ and [HBA]$_f$/[MPH]$_0$ (the number of HBA molecules formed per molecule of MPH added) were >1, indicating the presence of a chain reaction. Similarly, the added H_2O_2 was decomposed with $-\Delta[H_2O_2]/[MPH]_0 > 1$. The behavior of [HBA]$_f$/[NADH]$_0$ and [HBA]$_f$/[MPH]$_0$ and kinetic aspects of the chain decomposition of the H_2O_2 were examined. In all cases, benzoic acid was present in excess (with respect to NADH or MPH) and conversions of <25% of the benzoic acid were employed.

Hydroxylation of Benzoic Acid. The addition of MP$^+$, NADH, and H_2O_2 to N_2-saturated, citrate-buffered solutions of benzoic acid produced hydroxylated benzoic acids with [HBA]$_f$/[NADH]$_0$ > 1 (Table I). As [NADH]$_0$ was increased, [HBA]$_f$ increased, but [HBA]$_f$/[NADH]$_0$ decreased. The yields of mHBA and pHBA, relative to oHBA, decreased with increasing [NADH]$_0$.

The addition of MPH and H_2O_2 to N_2-saturated solutions of benzoic acid also yielded hydroxylated benzoic acids with [HBA]$_f$/[MPH]$_0$ > 1 (Table II). When [MPH]$_0$ was increased, [HBA]$_f$ increased while [HBA]$_f$/[MPH]$_0$ decreased. There was a concomitant increase in the areas of chromatographic peaks corresponding to dihydroxybenzoic acids and other, unidentified, products ([HBA]$_f$ was 23% of [BA]$_0$ in the experiment with the highest [MPH]$_0$). When [H_2O_2]$_0$ was decreased, [HBA]$_f$/[MPH]$_0$ increased slightly (a fivefold decrease produced an increase of 25% in [HBA]$_f$/[MPH]$_0$). Saturation with O_2 (instead of N_2) dramatically lowered the product yields; [pHBA]$_f$/[MPH]$_0$ dropped by a factor of ca. 80. Elimination of the citrate buffer decreased [HBA]$_f$/[MPH]$_0$ by a factor of 3.[8] The relative product yields obtained with [MPH]$_0$ = 120 μM were oHBA/mHBA/pHBA = 1.00/(1.05 ± 0.01)/(0.509 ± 0.002), independent of [H_2O_2]. These ratios changed only slightly with increasing [MPH]$_0$.

The addition of MPH to oxygenated solutions of benzoic acid produced hydroxylated benzoic acids (Table III). In essence, these experiments are the same as the MP$^+$/NADH/O_2 experiments,[3] except that reduction of MP$^+$ occurs externally (by ascorbic acid) rather than in situ by NADH, and the results paralleled those of the MP$^+$/NADH/O_2 experiments. In each case [HBA]$_f$/[MPH]$_0$ was <1, as anticipated in the absence of added H_2O_2. The [HBA]$_f$/[MPH]$_0$ values obtained with O_2 saturation were a factor of 5 lower than those values obtained upon air saturation. With constant [O_2]$_0$, increasing [MPH]$_0$ gave increased [HBA]$_f$/[MPH]$_0$, in correspondence with the MP$^+$/NADH/O_2 experiments in which the ratio of [NADH]$_0$ to [O_2] is a controlling factor in the value of [HBA]$_f$/[NADH]$_0$. Omission of citrate buffer decreased [HBA]$_f$/[MPH]$_0$ (by a factor of 4), as in the MPH/H_2O_2 and MP$^+$/NADH/O_2 experiments.

Dynamics of H_2O_2 Decomposition in MPH/H_2O_2 Experiments. The rate of decomposition of H_2O_2 in citrate-buffered solutions was zero order with respect to [H_2O_2], i.e., $-d[H_2O_2]/dt \neq fn([H_2O_2])$, under a wide variety of conditions (Figure 1a). Generally, $-d[H_2O_2]/dt$ was constant for ≥80% of the decomposition, which was complete, even with ratios of [H_2O_2]$_0$/[MPH]$_0$ as large as 70. The observed rate of decomposition varied somewhat with different preparations of reaction solution and appeared to increase with increasing [MPH]$_0$.

The addition of catalytic levels of iron ions (Fe^{III} or Fe^{II}) to citrate-buffered MPH/H_2O_2 (where $[Fe^{III}] < [MPH] \ll [H_2O_2] < [BA]$) substantially increased the rate of H_2O_2 decomposition. $-d[H_2O_2]/dt$ was once again constant with respect to time (Figure 1b), and the decomposition proceeded to completion. There was essentially no difference in the results obtained with Fe^{II} or Fe^{III}.

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(7) Allen, A. O.; Hochanadel, C. J.; Ghormley, J. A.; Davis, T. W. *J. Phys. Chem.* **1952**, *56*, 575–86. Schwarz, H. A.; Salzman, A. J. *Radiat. Res.* **1958**, *9*, 502–8. Bielski, B. H. J.; Allen, A. O. *Int. J. Radiat. Phys. Chem.* **1969**, *1*, 153–63.

(8) At the pH of these experiments, benzoic acid itself exerts a buffering action since it has a pK_a of 4.20 (Harned, H. S.; Owen, B. B. *Chem. Rev.* **1939**, *25*, 31–65.). Thus, solutions with or without citrate added will be buffered equally well.

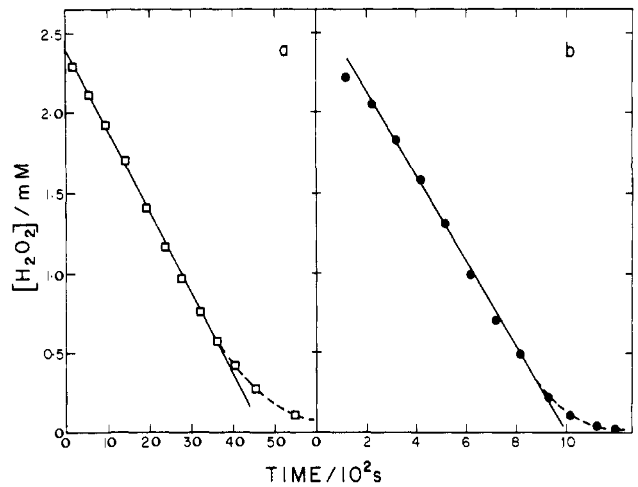

Figure 1. Decomposition of H_2O_2 in the citrate-buffered MPH/H_2O_2 experiments. Initial reactant concentrations: citrate buffer, 10 mM, pH 4.40; benzoic acid, 20 mM; H_2O_2, 2.4 mM; MPH, 200 μM; N_2 saturated. (●) Fe^{III}, 0 μM; (□) Fe^{III}, 7.2 μM. Solid lines are linear least-squares fits of the data (points in the later nonlinear region are excluded).

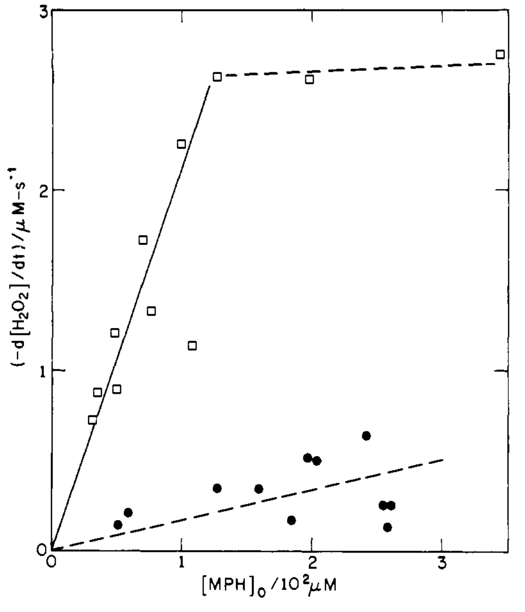

Figure 2. Dependence of the H_2O_2 decomposition rate on $[MPH]_0$ in the citrate-buffered MPH/H_2O_2 experiments. Initial reactant concentrations: citrate buffer, 10 mM, pH 4.3–4.6; benzoic acid, 20 mM; H_2O_2, 2.4 mM; N_2 saturated. (●) Fe^{III}, 0 μM; (□) Fe^{III}, 7.2–7.4 μM.

With constant $[Fe^{III}]_0$, $-d[H_2O_2]/dt$ increased linearly with $[MPH]_0$, radiating from the origin (Figure 2). A plateau was obtained for $[MPH]_0 \geq 125$ μM corresponding to saturation with MPH. With constant $[MPH]_0$, $-d[H_2O_2]/dt$ increased linearly with $[Fe^{III}]_0$, with nonzero intercepts (Figure 3). Thus, for $[MPH]_0 < 125$ μM, the rate expression for H_2O_2 decomposition in citrate-buffered solution is

$$-d[H_2O_2]/dt = k[MPH]_0([Fe^{III}]_0 + a) \qquad (7)$$

where the constant a adjusts for the decomposition in the absence of added iron ions. The solid lines in Figures 2 and 3 were obtained from eq 7 by using $k = 2.66 \times 10^3\ M^{-1}\ s^{-1}$ and $a = 7.0 \times 10^{-7}$ M (the upper curve in Figure 3 assumes $[MPH]_0 = 125$ μM).

When the pH was varied from 3.4 to 5.8 for citrate-buffered MPH/H_2O_2 experiments (Fe^{III} added), $-d[H_2O_2]/dt$ was constant with time for pH ≥4.3 and was independent of pH. In more acidic media, the rates accelerated, although initial rates were the same as in the pH-independent region. The addition of 100 μM of MP^+ to a solution containing 20 mM benzoic acid, 2.4 mM H_2O_2, 4.7 μM Fe^{III}, 250 μM MPH, and 10 mM citrate buffer at pH 4.5, resulted in a doubling in $-d[H_2O_2]/dt$.

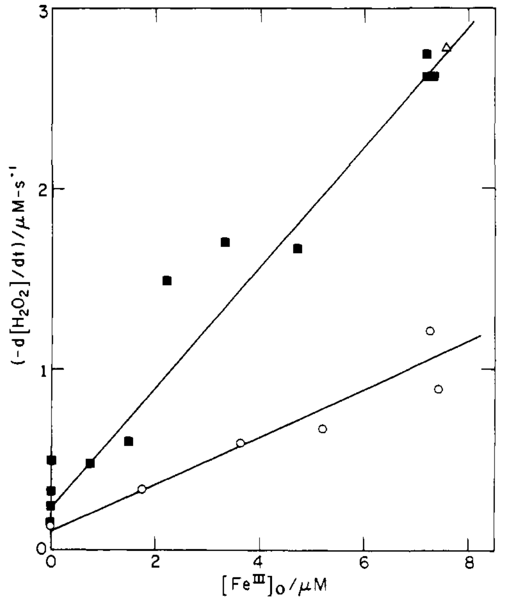

Figure 3. Dependence of the H_2O_2 decomposition rate on $[Fe^{III}]_0$ in the MPH/H_2O_2 experiments. Initial reactant concentrations: benzoic acid, 20 mM; H_2O_2, 2.4 mM; N_2 saturated. (■, ○) citrate buffer, 10 mM, pH 4.3–4.6; (△) acetate buffer, 10 mM, pH 4.5; EDTA, 64 μM; (■, △) MPH, 125–330 μM; (○) MPH, 50 μM.

Substitution of acetate buffer for citrate buffer in MPH/H_2O_2 experiments (no added iron ions) yielded a 20-fold decrease in $-d[H_2O_2]/dt$. Upon addition of Fe^{II}, the decomposition rate increased as with citrate buffer; however, the value of the constant k (eq 7) was smaller by a factor of 3 and decomposition of H_2O_2 was incomplete (cessation of decomposition was accompanied by the appearance of the characteristic yellow color of MP^+). Upon addition of EDTA to acetate-buffered solution (containing Fe^{III}), 100% decomposition of the H_2O_2 was once again observed, with the rate virtually the same as obtained with citrate buffer (Figure 3).

Discussion

The present results confirm that in the hydroxylation system composed of the 5-methylphenazinium cation (MP^+) and reduced β-nicotinamide adenine dinucleotide (NADH) in oxygenated aqueous solution,[3] the crucial species in the production of hydroxyl radical are MPH, the two-electron-reduced MP^+ (produced in reaction 1), H_2O_2 (produced from O_2 in reaction 2), and suitably complexed adventitious metal ions. Thus, in the $MP^+/NADH/O_2$ system, the requirement for NADH is that of a reducing agent for MP^+, while O_2 is needed as a progenitor of H_2O_2. In addition, it has been demonstrated that in the presence of added H_2O_2, catalytic levels of MPH and adventitious metal ions (or added iron ions) support a chain decomposition of H_2O_2 in benzoic acid solution (in the absence of O_2) with a concomitant production of hydroxybenzoic acids (HBA). Thus, the chain reaction postulated from the reaction mechanism in the $MP^+/NADH/O_2$ system does occur, and the results from the kinetic and product-yield studies indicate the mechanism given in Scheme II for MPH/H_2O_2 experiments in citrate buffer with added iron ions.[9] Reactions 3′ and 8 both initiate the chain and participate in its propagation; reactions 4′, 5, and 6′ are propagation steps. The concentrations of $MPH^{+}\cdot$ and $MP\cdot$ (the one-electron reduced forms of MP^+) are regulated by the acid–base equilibrium 9 and by the disproportionation equilibrium 10. Termination can occur when the HO· adduct undergoes reactions other than oxidation by MP^+ (reaction 6′).

Hydroxylation of Benzoic Acid. The behavior of all three hydroxylation systems reported here resembles closely the behavior of the $MP^+/NADH/O_2$ system[3] with regard to hydroxylation of

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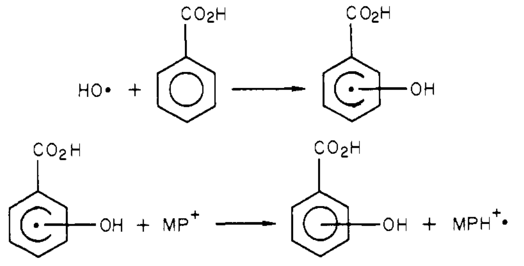
[9] Scheme II is the simplest representation of the mechanism, which could include other reactions such as oxidation of the HO· adducts by $MPH^{+}\cdot$.

Scheme II

$$MPH + Fe^{III}(citrate) \longrightarrow MPH^{+}\cdot + Fe^{II}(citrate) \qquad (3')$$

$$MP\cdot + Fe^{III}(citrate) \longrightarrow MP^{+} + Fe^{II}(citrate) \qquad (8)$$

$$Fe^{II}(citrate) + H_2O_2 \longrightarrow Fe^{III}(citrate) + HO^{-} + HO\cdot \qquad (4')$$



(5)

(6′)

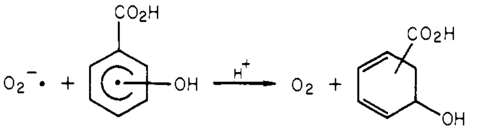
$$MPH^{+}\cdot \rightleftharpoons MP\cdot + H^{+} \qquad (9)$$

$$MPH^{+}\cdot + MP\cdot \rightleftharpoons MPH + MP^{+} \qquad (10)$$

benzoic acid: (1) in the present experiments, the relative yields of isomers under optimal conditions (no O_2, low percent conversion of benzoic acid) in the MPH/H_2O_2 experiments (i.e., oHBA/mHBA/pHBA = 1.00/1.05/0.51) were similar to the relative product yields of reaction with HO· generated radiolytically under conditions of quantitative oxidation of the hydroxycyclohexadienyl radicals (i.e., 1.00/1.02/0.59);[3] (2) elimination of the citrate buffer resulted in a substantial reduction in product yields (where $[HBA]_f/[MPH]_0 > 1$ under O_2-free conditions); (3) saturation with O_2 greatly reduced product yields; and (4) comparison of the MPH/H_2O_2 and MPH/O_2 experiments shows that the presence of O_2 leads to a reduction in the relative yield of mHBA, just as is seen with increasing $[O_2]$ in the $MP^+/NADH/O_2$ experiments. Thus, the character of the hydroxylation reactions in the present experiments is virtually the same as that in the $MP^+/NADH/O_2$ experiments, demonstrating that benzoic acid is hydroxylated by the same basic mechanism in all the reagent combinations tested. The experiments show conclusively that NADH, O_2, and MP^+ are not required per se; the essential reagents are MPH and H_2O_2.

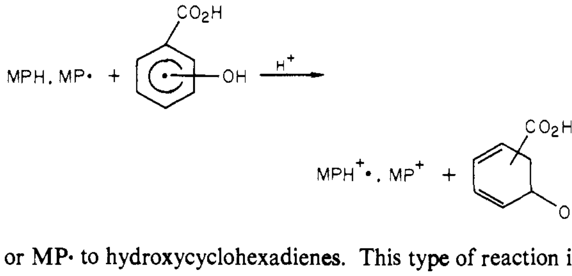
In the absence of O_2, up to 16 molecules of HBA were obtained for each MPH molecule added; saturation with O_2 reduced $[HBA]_f/[MPH]_0$ by a factor of 220. The quenching effect of O_2 suggests that reaction 2 is rapid. Removal of MPH via reaction 2 reduces the frequency of chain initiation via reaction 3′, favoring Fe^{III} and reducing the production of HO·. In addition, the introduction of O_2 stimulates production of the superoxide radical ($O_2^{-}\cdot$) via reaction 11; $O_2^{-}\cdot$ can reduce an HO· adduct to the corresponding hydroxycyclohexadiene[3] (reaction 12) via a chain-terminating reaction.

$$MP\cdot + O_2 \rightarrow MP^{+} + O_2^{-}\cdot \qquad (11)$$



(12)

The decrease in $[HBA]_f/[MPH]_0$ (or $HBA]_f/[NADH]_0$) with increasing additions of MPH (or NADH) indicates the occurrence of reaction 13 in which the HO· adducts are reduced by MPH



(13)

or MP· to hydroxycyclohexadienes. This type of reaction is chain terminating, which multiplies its effectiveness. $[HBA]_f/[MPH]_0$ is further decreased by attack of HO· on the HBA products. Benzoic acid conversions of up to 25% were employed, and rate constants for HO· reaction with HBA and benzoic acid are about the same;[10] however, these reactions cannot account completely for the losses in HBA. The observed increases in side-reaction products with increasing $[MPH]_0$ are consistent with both processes described above. The slight variations in relative product yields as $[MPH]_0$ was increased may be attributed to slightly different rate constants for the reactions with the different isomeric HBA and HO· adducts.

The slight increases in $[HBA]_f/[MPH]_0$ obtained in the MPH/H_2O_2 experiments with *decreasing* $[H_2O_2]_0$ can be attributed to a decrease in scavenging of HO· by H_2O_2[10] (reaction 14). Each time reaction 14 occurs, two chain terminations can

$$HO\cdot + H_2O_2 \rightarrow O_2^{-}\cdot + H^{+} + H_2O \qquad (14)$$

result via loss of chain carriers in reactions 14 and 12. It should be noted that in the product-study experiments values of $[H_2O_2]_0/[MPH]_0$ were generally ca. 350, while for the kinetic studies, $[H_2O_2]_0/[MPH]_0 \leq 70$. Thus, in most cases with the product-study experiments H_2O_2 was not totally consumed; however, in the experiment with the lowest $[H_2O_2]$, it appeared that all the H_2O_2 was consumed, and in this case $[HBA]_f$ was 40% of $[H_2O_2]_0$.

Decomposition of H_2O_2. The experimental kinetic expression for the rate of H_2O_2 decomposition in citrate-buffered solutions containing MPH and Fe^{III} (eq 7) yields the following: (1) lack of dependence on $[H_2O_2]$ demonstrates that reaction 4′ is not the rate-determining step; (2) the first-order dependence of the rate on $[MPH]_0$ and $[Fe^{III}]_0$ indicates that the initiation reaction 3′ is the rate-determining step; and (3) the proportionality to the *initial* concentrations of MPH and Fe^{III} and the constancy of the rate over the major portion of the H_2O_2 decomposition require that the MPH and Fe^{III} consumed in reaction 3′ are rapidly and efficiently restored in subsequent reactions. The catalytic character of MPH and Fe^{III} are clearly shown by the number of H_2O_2 molecules decomposed: up to 70 per MPH and as many as 3200 per Fe^{III}.

The participation of adventitious metal ions in the chain reaction in experiments with no added iron is clear. If the alternative reaction 15 were the only (or major) initiation step, $-d[H_2O_2]/dt$

$$MPH + H_2O_2 \rightarrow MPH^{+}\cdot + HO^{-} + HO\cdot \qquad (15)$$

would be first order with respect to $[H_2O_2]$, which was not the case. Indeed, the kinetic behavior with or without added iron ions was essentially the same. Since reaction 15 is unaffected by buffer, the very low rate of H_2O_2 decomposition in acetate buffer puts an upper limit of 0.07 $M^{-1}\ s^{-1}$ on k_{15}.

Initiation reaction 8 rationalizes the effect of MP^+ on the MPH/H_2O_2 system. Upon addition of MP^+, $[MP\cdot]$ and $[MPH^{+}\cdot]$ increase via equilibrium 10, increasing the rate of reaction 8 and generating the observed increase in $-d[H_2O_2]/dt$ ([MPH] remained constant at the saturation level since undissolved material was present). Reduction of Fe^{III}(citrate) by MP·, instead of by $MPH^{+}\cdot$, is included because of the lower redox potential of MP·;[11] however, reduction by $MPH^{+}\cdot$ cannot be excluded. Similar considerations explain the acceleration of $-d[H_2O_2]/dt$ at lower

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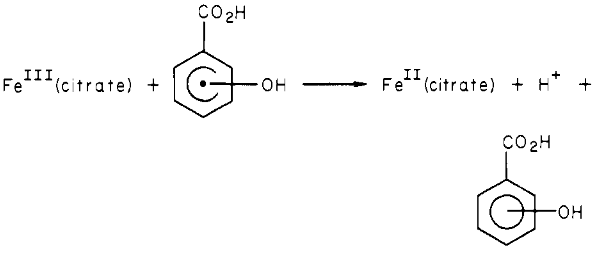
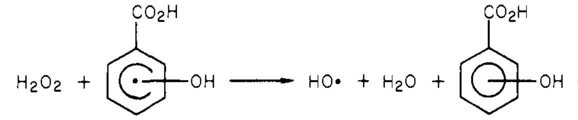
[10] Farhataziz; Ross, A. B. *Natl. Stand. Ref. Data Ser.* (*U.S.*), *Natl. Bur. Stand.* **1977**, *No. 59*.

[11] The influence of pH on the disproportionation of the semiquinoid forms of MP^+ (i.e., $MPH^{+}\cdot$ and MP·) has been discussed by Zaugg.[6] In acid pH, $MPH^{+}\cdot$ is stable; however, with increasing pH, $MPH^{+}\cdot$ deprotonates and disproportionation ensues. Over the pH range investigated, there is a substantial change in the equilibrated $[MPH^{+}\cdot]$. This phenomenon is exhibited in the preparation of MPH used here, i.e., upon addition of ascorbic acid to a N_2-saturated, *unbuffered* solution of MP^+, the deep green color of $MPH^{+}\cdot$ developed, and MPH was formed (and precipitated) only when the solution pH was increased by buffer addition. A corollary of this behavior is that the redox potential of MP· is lower than that of $MPH^{+}\cdot$; thus, $MPH^{+}\cdot$ can be reduced by MP· (disproportionation reaction 10) but not by $MPH^{+}\cdot$. A similar relationship between the redox potentials of acid/base pairs is often encountered with reducing agents containing a labile proton, e.g., ascorbic acid/ascorbate, $H_2O\cdot/O_2^{-}\cdot$, and catechols/catechol anions.

pH. Reaction 3′, per se, is pH independent, as demonstrated at pH ≥4.3; however, as pH is decreased, the MP^+ generated by chain terminations is increasingly converted to MP· and $MPH^{+}\cdot$ (via a shift of equilibrium 10 to the left)[11] and $-d[H_2O_2]/dt$ accelerates via reaction 8.

Oxidation of the HO· adducts by MP^+, reaction 6′, fulfills the kinetic requirements of the MPH/H_2O_2 system, as would oxidation by Fe^{III}(citrate) or H_2O_2 (reactions 16 and 17, respectively).


(16)


(17)

Chain decompositions of H_2O_2 would occur with reaction 4′ initiating and reactions 4′, 5, and 16 or reactions 5 and 17 propagating the chain. However, if the chain involving reaction 16 (or reaction 17) were a major path, addition of Fe^{II} to citrate-buffered reaction solutions would result in the chain decomposition of H_2O_2 even in the absence of MPH, and this was not the case.[12]

The change in the character of the H_2O_2 decomposition when acetate buffer was employed clarifies the decreases in product yields obtained when acetate-buffered or "unbuffered" solutions were compared with citrate-buffered solutions. These differences can be correlated with the effect of the buffering agent (i.e., complexing agent) on the redox potential of the added or adventitious metal ions.[13] It has been recognized for a long time[14] that the potential of the Fe^{III}/Fe^{II} pair can vary from that of a strong oxidant to that of a strong reductant, depending upon the nature of the complexing ligand. Thus, complexes of iron ions with citrate,[15] EDTA[16,17] (ethylenediaminetetraacetic acid), and DETAPAC[17] (diethylenetriaminepentaacetic acid) all have low redox potentials, while complexes with acetate,[14] cyanide,[18] and benzoate (which essentially does not complex iron ions) give high redox potenials. High product yields and rapid, complete decomposition of H_2O_2 are associated with the first group, while lowered product yields and slower, incomplete H_2O_2 decomposition are found with the second group.

All of the kinetic and product yield studies are consistent with the mechanism given in Scheme II for the chain decomposition of H_2O_2 in N_2-saturated benzoic acid solution, with concomitant hydroxylation of the benzoic acid. The chemistry is essentially that of the Fenton reagent as discussed by Walling,[5] except that the catalyzing metal ion is returned to its reduced state via reduction by MPH instead of by the product organic radical; in the present system, the complexed metal ion is either energetically or kinetically incapable of oxidizing the HO· adduct. The MPH/H_2O_2/metal ion system described here resembles the chemical systems of Udenfriend,[19] Hamilton,[20] Ullrich,[21] McCord,[22] and Halliwell,[23] recently reviewed by Fee[24] and by Rigo and Rotilio.[25] In each of these systems, a strong oxidant is produced in the presence of H_2O_2 (added directly or produced in situ via autoxidation), a reducing agent ($O_2^{-}\cdot$ produced in situ in the case of McCord[22] and Halliwell[23]), catalytic levels of metal ions, and chelating agents (added as buffers in some cases). An essential difference between all these systems and the "classical" Fenton system is the requirement for addition or generation of a reducing agent. Because of this crucial difference, it is appropriate that they be considered together as "nonclassical Fenton systems". It is conceivable that the basic mechanism in all these cases is the same and that apparent differences result from the presence of different chelating agents, which alter the redox potentials of the catalytic metal ions. Indeed, it is the alteration of the redox potentials of the metal species engendered by the switch from the acid pH of Fenton's reagent and the addition of chelating agents (necessitated, in a sense, by the hydrolysis and subsequent precipitation of Fe^{III} in nonacid solution) which introduces the requriement for an added reducing agent in these nonclassical Fenton systems. The lowered redox potentials of the complexed metal ions renders them incapable of oxidizing the organic radicals produced by HO· reactions and thus prevents the chain decomposition of H_2O_2 as it occurs with Fenton's reagent.

By analogy to the present system, it is probable that hydroxyl radicals are generated in all the nonclassical Fenton systems listed, as has been suggested.[24] The ubiquitous nature of these systems adds further substance to current theories that many of the in vivo cytotoxic effects of oxygen arise from long-known, Fenton-type chain reactions.[22-25] The chain decomposition of H_2O_2 under anaerobic conditions, seen in the present system, demonstrates that $O_2^{-}\cdot$ is not required for the deleterious cytotoxic effects of O_2 to be expressed. In large part, normal metabolism utilizing O_2 as the ultimate electron acceptor proceeds via direct two-electron reduction to produce H_2O_2 without the intermediacy of $O_2^{-}\cdot$, and under these conditions nonclassical Fenton chemistry of the type described by McCord[22] and Halliwell[23] could not occur. Nonetheless, nonclassical Fenton chemistry of the type seen in the present system could occur by utilizing the H_2O_2 produced, a natural biological reducing agent, and the ever-present chelated metal ions.

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Registry No. MPH, 20057-16-3; Fe, 7439-89-6; BA, 65-85-0; H_2O_2, 7722-84-1.

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(12) In a N_2-saturated solution containing 20 mM benzoic acid, 1 mM H_2O_2, and 10 mM citrate buffer for pH 4.5, the addition of ferrous ion ($[Fe^{II}] = 7\ \mu M$) did not yield any decomposition of the H_2O_2 over the time period of the present experiments. The requirement for MPH is further demonstrated by the zero intercepts in the $-d[H_2O_2]/dt$ vs. $[MPH]_0$ plots (Figure 2).

(13) The reactions of acetic acid and acetate ion with HO· produce radicals which are not readily oxidized, so that the scavenging of HO· by the acetate buffer would be a chain-terminating event (i.e., return of the electron to MP^+, as in reaction 6′, is prevented). However, k(HO· + benzoic acid, benzoate) ~ $100k$(HO· + acetic acid, acetate),[10] and [benzoic acid, benzoate] = 2[acetic acid, acetate] in the acetate-buffered experiments cited; thus, only 0.5% of the HO· reacts with the buffer. This calculation and the effect of EDTA on the H_2O_2 decomposition (i.e., restoration of the rate to that seen with citrate) demonstrate that the acetate buffer depresses the decomposition rate via complexation of the metal ions and not via the scavenging of HO·.

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