Kinetic Studies on Spin Trapping of Superoxide and Hydroxyl Radicals Generated in NADPH-Cytochrome P-450 Reductase-Pararquat Systems

EFFECT OF IRON CHELATES*

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Electron spin resonance (ESR) studies on spin trapping of superoxide and hydroxyl radicals by 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) were performed in NADPH-cytochrome P-450 reductase-pararquat systems at pH 7.4. Spin adduct concentrations were determined by comparing ESR spectra of the adducts with the ESR spectrum of a stable radical solution. Kinetic analysis in the presence of 100 μM desferrioxamine B (deferoxamine) showed that: 1) the oxidation of 1 mol of NADPH produces 2 mol of superoxide ions, all of which can be trapped by DMPO when extrapolated to infinite concentration; 2) the rate constant for the reaction of superoxide with DMPO was 1.2 M⁻¹ s⁻¹; 3) the superoxide spin adduct of DMPO (DMPO-OOH) decays with a half-life of 66 s and the maximum level of DMPO-OOH formed can be calculated by a simple steady state equation; and 4) 2.5% or less of the DMPO-OOH decay occurs through a reaction producing hydroxyl radicals.

In the presence of 100 μM EDTA, 5 μM Fe(III) ions nearly completely inhibited the formation of the hydroxyl radical adduct of DMPO (DMPO-OH) as well as the formation of DMPO-OOH and, when 100 μM hydrogen peroxide was present, produced DMPO-OH exclusively. Fe(III)-EDTA is reduced by superoxide and the competition of superoxide and hydrogen peroxide in the reaction with Fe(II)-EDTA seems to be reflected in the amounts of DMPO-OOH and DMPO-OH detected. These effects of EDTA can be explained from known kinetic data including a rate constant of 6 × 10⁴ M⁻¹ s⁻¹ for reduction of DMPO-OOH by Fe(II)-EDTA. The effect of diethylenetriamine pentaacetic acid (DETAPAC) on the formation of DMPO-OOH and DMPO-OH was between deferoxamine and EDTA, and about the same as that of endogenous chelator (phosphate).

Superoxide is produced in biological systems via various enzymatic and nonenzymatic reactions. Although superoxide itself produces some biological effects (1), it is now well accepted that the most deleterious effects of oxygen radicals are caused by hydroxyl radicals produced from iron-catalyzed Haber-Weiss reactions. This conclusion has been derived from a myriad of reports (2), which have accumulated since the publication of the Haber-Weiss reactions and spin-trapping data of oxygen radicals, we have used the NADPH-cytochrome P-450 reductase-pararquat system as a standard system, since the primary reduction product of oxygen is thought to be only superoxide. For instance, xanthine oxidase, a widely used superoxide-generating enzyme produces both superoxide ions and hydrogen peroxide as primary products (5, 35) and the kinetic analysis is considerably more complex.

Several papers have reported that the Haber-Weiss reaction is too slow to explain the formation of hydroxyl radicals in...
biological systems (36) and it is now well accepted that iron plays an essential role in the formation of hydroxyl radicals from superoxide-generating systems. Since the role of iron is greatly modified by its chelators, we have attempted to examine the effects of three typical iron chelators, desferrioxamine B (deferoxamine), diethylenetriaminepentaacetic acid (DETAPAC), and EDTA.

EXPERIMENTAL PROCEDURES AND RESULTS

Paraquat-mediated superoxide generation has been reported in NADPH-cytochrome P-450 reductase systems (58), glutathione reductase systems (41), animal systems (59, 60), plant systems (28, 61), and microorganisms (44, 62, 63). It is believed that reducing equivalents accepted by paraquat are all used to reduce oxygen to superoxide ions, but without any quantitative evidence. The kinetic data in Fig. 5 show that within experimental errors the following stoichiometry is established in the NADPH-cytochrome P-450 reductase-paraquat system,

\[
\text{NADPH} + 2 \text{O}_2 \rightarrow \text{NADP}^+ + \text{H}^+ + 2 \text{O}_2^-
\]

and also that all the resultant superoxide ions can be trapped by DMPO when extrapolated to infinite DMPO concentration. The slopes in Fig. 5 give a value of \(2.2 \times 10^5 \text{ M} \cdot \text{s}^{-1}\) for the rate of superoxide formation. The rate is nearly the same as that obtained in illuminated pea chloroplasts at pH 7 (28). The DMPO-OOH decay is accompanied by the appearance of a small amount of DMPO-OH. The conversion ratio is measured to be 2.8%, that is, 0.8 \(\mu\text{M}\) DMPO-OOH is formed per DMPO-OOH as shown in Fig. 4A. Here, a value of 0.011 s\(^{-1}\) is used for \(k_i\). The total amount of DMPO-OOH formed during the reaction will be greater than 29 \(\mu\text{M}\) since \(k_i\) appears to be greater than 0.011 s\(^{-1}\) under these experimental conditions (Table I). DMPO-OOH is assumed to be stable under these conditions. Since Fig. 6C shows that DMPO-OOH is formed through the reaction of DMPO with hydroxyl radicals, we conclude that 2.8% or less of DMPO-OOH decay occurs through a reaction producing hydroxyl radicals. Finkelstein et al. (34) have reported that the ratio is about 3%. The formation of DMPO-OOH from DMPO-OOH has been discussed also in neutrophil systems (65). 3) DMPO-OOH reaches a steady level a few minutes after initiation of the reactions in the presence of 5–10 \(\mu\text{M}\) paraquat (Fig. 2). Equa-

\[
k_d = \frac{2.8 \times 10^5 \text{ M} \cdot \text{s}^{-1}}{2.2 \times 10^5 \text{ M} \cdot \text{s}^{-1}} = 0.011 \text{ s}^{-1}
\]

tion 1 is valid under these experimental conditions.

The above conclusions are obtained from reactions in the presence of 100 \(\mu\text{M}\) deferoxamine. According to recent reports (66, 67), deferoxamine reacts with both superoxide ions and hydroxyl radicals, with rate constants of \(9 \times 10^9\) and \(10^{10} \text{ M}^{-1} \cdot \text{s}^{-1}\), respectively. These reactions may be negligible in our reaction systems since the concentration of DMPO is 1000 times higher than that of deferoxamine and we could not observe any nitroxide-free radical which is a product of the reaction of deferoxamine with superoxide (67). When deferoxamine is present, the mechanism of oxygen metabolism in the NADPH-cytochrome P-450 reductase-paraquat system is relatively simple as shown in Fig. 14A. The formation of hydroxyl radicals through reactions of hydrogen peroxide with superoxide and paraquat radicals is not detectable under our experimental conditions.

The reaction becomes complicated in the presence of Fe(III)-EDTA, which has been used most frequently as a Fenton reagent. The complication arises not only from the Fenton reaction, but also from reactions of Fe(II)-EDTA with superoxide, hydrogen peroxide, and DMPO-OH. Fig. 14B shows a mechanism schematized using data so far reported for the DMPO-OOH decay we conclude as follows: 1) DMPO-OOH decays with a half-life of 66 s (\(k_i = 0.011 \text{ s}^{-1}\)) at pH 7.4 according to first order kinetics. The rate is nearly the same as that obtained in illuminated pea chloroplasts at pH 7 (28). 2) the DMPO-OOH decay is accompanied by the production of a small amount of DMPO-OH. The conversion ratio is measured to be 2.8%, that is, 0.8 \(\mu\text{M}\) DMPO-OOH is formed during the decay of 29 \(\mu\text{M}\) DMPO-OOH, which is equal to the approximate integrated value, \(j_{k1}(\text{DMPO-OOH}) dt\), obtained from the kinetic trace for formation and decay of DMPO-OOH as shown in Fig. 4A. Here, a value of 0.011 s\(^{-1}\) is used for \(k_i\). The total amount of DMPO-OOH formed during the reaction will be greater than 29 \(\mu\text{M}\) since \(k_i\) appears to be greater than 0.011 s\(^{-1}\) under these experimental conditions (Table I). DMPO-OOH is assumed to be stable under these conditions. Since Fig. 6C shows that DMPO-OOH is formed through the reaction of DMPO with hydroxyl radicals, we conclude that 2.8% or less of DMPO-OOH decay occurs through a reaction producing hydroxyl radicals. Finkelstein et al. (34) have reported that the ratio is about 3%. The formation of DMPO-OOH from DMPO-OOH has been discussed also in neutrophil systems (65). 3) DMPO-OOH reaches a steady level a few minutes after initiation of the reactions in the presence of 5–10 \(\mu\text{M}\) paraquat (Fig. 2). Equa-

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\]

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Fig. 14. Proposed scheme for formation of DMPO-OOH and DMPO-OH in the NADPH-cytochrome P-450 reductase-paraquat system. The rate constants referenced are at neutral pH (mostly pH 7.4). A, reactions in the presence of deferoxamine and B, effect of Fe(III)-EDTA. \(k_i = 3 \times 10^9\) \text{ M}^{-1} \cdot \text{s}^{-1}\) (64). \(k_d\), decomposition of DMPO-OOH which yields mostly nonspin, not identified compound(s). The dotted line denotes a side reaction yielding hydroxyl radicals which can be spin trapped with DMPO. The molar ratio of DMPO-OOH formed per DMPO-OOH is about 0.03 (54; this paper). \(k_i = 0.011 \text{ s}^{-1}\) (28; this paper). \(R_p\), spin trapping of superoxide ions by DMPO. \(k_i = 10^3\) \text{ M}^{-1} \cdot \text{s}^{-1}\) (31 and 12 \text{ M}^{-1} \cdot \text{s}^{-1}\) (this paper). \(R_o\), reduction of DMPO-OH by paraquat radicals. \(k_o = 7.7 \times 10^{-5} \text{ M} \cdot \text{s}^{-1}\) (68). \(R_r\), reduction of Fe(III)-EDTA by paraquat radicals. The reaction is assumed to be fast (71). \(R_s\), reduction of Fe(III)-EDTA by superoxide. The reaction is pH dependent and \(k_s = 1-2 \times 10^9\) \text{ M}^{-1} \cdot \text{s}^{-1}\) (50, 69). The dotted line implies that \(R_s\) takes place through an unknown encounter complex (50). \(R_p\), reaction of Fe(II)-EDTA with a superoxide ion to form a peroxy-complex. \(k_p = 10^5\) \text{ M}^{-1} \cdot \text{s}^{-1}\) (50, 69). \(R_o\), reversible complexing of Fe(III)-EDTA and H,O_2 (50). \(k_o\) depends greatly on pH and the kind of buffers used (50). \(R_s\), reduction of DMPO-OOH by Fe(II)-EDTA to a nonspin compound. \(k_s = 6 \times 10^4\) \text{ M}^{-1} \cdot \text{s}^{-1}\) (this paper). \(R_r\), reaction of Fe(II)-EDTA with H,O_2 forming an oxidizing intermediate, which might be converted to a ferryl form, FeIV(OH)_2, or produce a hydroxyl radical (FeIV)_x = 2 \times 10^9\) \text{ M}^{-1} \cdot \text{s}^{-1}\) (50, 1.74 \times 10^9\) \text{ M}^{-1} \cdot \text{s}^{-1}\) (52). \(R_s\), reduction of Fe(II)-EDTA by Fe(II)-EDTA (52). \(R_p\), formation of a second oxidizing transient in the reaction of Fe(II)-EDTA with H,O_2. \(k_p = 3.2 \times 10^7\) \text{ M}^{-1} \cdot \text{s}^{-1}\) (52). The dotted line implies that the detailed mechanism is not yet clear.
(47–55, 68–71). The following features of Fe(III)-EDTA reactions may be explained by the calculated approximate rates of reactions.

1) Although Reaction 4 is reported to be rapid (71), perauratia radicals reduce exclusively oxygen (Reaction 3) rather than Fe(III)-EDTA (Reaction 4), when the Fe(III)-EDTA concentration is less than 5 μM.

2) When the superoxide generation is slow, 5 μM Fe(III)-EDTA completely inhibits the DMPO-OH accumulation (Fig. 8C). As seen in Fig. 14B, this inhibition may occur through two mechanisms, one is reductive decomposition of DMPO-OH by Fe(II)-EDTA (Reaction 7) and the other is a weak superoxide dismutase activity of iron-EDTA (Reactions 5, 6, and 6'). The superoxide dismutase activity of Fe(III)-EDTA is still controversial mostly because of the apparent slow dissociation of an iron-EDTA-peroxide complex (Reaction 6'). At pH 7.4, the value for \( k' \) is estimated to be at least 1 s⁻¹ or possibly larger (48, 50). The rate constant of 1 s⁻¹ would be very slow for iron-EDTA to catalyze the dismutation of superoxide ions at any significant rate under the usual assay conditions for O₂⁻ as described by Diguiseppi and Fridovich (64). Under the experimental conditions in Fig. 8C where the rate of superoxide formation is about 0.2 μM s⁻¹ (Table I) and the rate of reaction of DMPO with superoxide ions is very slow, calculations using the known rate constants shown in the legend of Fig. 14 and using a value of 1 s⁻¹ for \( k' \) clearly show that the maximal DMPO-OH accumulation is decreased from 6.2 (Table I) to 0.23 μM through Reactions 5, 6, and 6' in the presence of 5 μM iron-EDTA. Under the same conditions the DMPO-OH concentration would decrease to about one sixth the original concentration following Reaction 7 alone. Since these two mechanisms operate additively, the DMPO-OH accumulation following both decay mechanisms would be expected to decrease to a level below the ESR sensitivity (Table III). As the superoxide generation becomes faster, superoxide ions disappear mostly through dismutation and more Fe(III)-EDTA is needed for complete suppression of DMPO-OH formation (Fig. 11A).

3) Reaction of the Fe(II)-EDTA is switched from Reaction 6 to Reaction 8 by the presence of 100 μM hydrogen peroxide (Figs. 8C and 9B). Only a part of Reaction 8 may result in the formation of hydroxyl radicals (Reaction 8'). The other may be followed by hydrogen peroxide-consuming reactions (Reactions 9 and 10), the detailed mechanism remaining to be clarified. Rush and Koppenol (52) have suggested the increase to about one-sixth the original concentration following the ESR sensitivity (Table III). As the superoxide generation becomes faster, superoxide ions disappear mostly through dismutation and more Fe(III)-EDTA is needed for complete suppression of DMPO-OH formation (Fig. 11A).

4) In the presence of a certain amount of hydrogen peroxide, as shown in Table III, the increase in the rate of superoxide generation brings about a depression in hydroxyl radical production. This depression can be partially removed by increasing the concentration of hydrogen peroxide. Fig. 14B shows such competition between Reactions 6 and 8. However, the superoxide-induced destruction of DMPO-OH, recently reported by Samuni et al. (74) should also be considered. Although the results shown in Table III are somewhat complicated, those in the presence of EDTA can be explained by the known kinetic data (Fig. 14B). The results with DE-TAPAC and endogenous chelator, however, cannot be completely explained because of lack of detailed kinetic data. A slight increase of DMPO-OH formation by hydrogen peroxide in the presence of deferoxamine also remains unexplained. In Table III we consider that [DMPO-OH]₀ as measured by ESR is nearly equal to the total amount of DMPO-OH accumulated during the course of the reaction because of the inherent stability of the DMPO-OH adduct while the total amount of DMPO-OH actually formed is much greater than that measured by ESR as [DMPO-OH]₀ because of the inherent instability of the DMPO-OH adduct.

It is now clear that spin trapping by DMPO can be used effectively for kinetic analysis of oxygen radicals generated in enzyme reactions even though the reaction of DMPO with superoxide is slow and its product is unstable. The most important criticism to be raised might be that the generation of hydroxyl radicals following superoxide formation is modified by the trapping of superoxide by DMPO as discussed by Britigan et al. (55) and Kleinhaus and Barefoot (33) in neutrophil systems. New kinetic approaches are necessary to solve this problem, which is now under investigation in this laboratory.

REFERENCES


In the following three categories, (1) oxyg-en-reduced NADPH-cytochrome P-450 reductase was prepared from rat liver microsomes with a modification of the procedure of Boud and Brown, (2) for the oxidation of NADPH-cytochrome P-450 reductase solutions were obtained from Sigma Chemical Company. Deoxyhemoglobin was obtained from Sigma Biological, Inc. and 3,3'-diaminobenzidine-4-hydroxyphenylhydrazine (DAB) was obtained from Aldrich Chemical Company. H202 was used after re-lyophilizing.

Reactions were carried out at 35°C under anaerobic conditions in 0.1 M potassium phosphate buffer (pH 7.4) containing 0.18 M KCl. The volume of reaction solutions was 1.0 mL for both spectrophotometric and ESR assays. Hydrogen peroxide (H2O2) was employed as the experimental condition. Hydrogen peroxide was generated by the chemical reaction of hydrosulfite (H2S) and hydrogen peroxide (H2O2) in a reaction mixture containing H2S (2.0 mm) and H2O2 (2.0 mm). The reaction mixture was purged with oxygen as needed either before or after the reaction. After the reaction, the reaction mixture could be transferred to the ESR cavity. The ESR measurement was started about a 1 sec after initiation of the reaction. Room temperature was 25°C.

The data were collected according to the following three categories: (1) nitrogen-reduced NADPH-cytochrome P-450 reductase was prepared from rat liver microsomes with a modification of the procedure of Bowyer and Camilleri; (2) for the reduction of NADPH-cytochrome P-450 reductase, H2O2 was employed as the experimental condition. In both the reactions, the reaction mixture was purged with oxygen as needed either before or after the reaction. After the reaction, the reaction mixture could be transferred to the ESR cavity. The ESR measurement was started about a 1 sec after initiation of the reaction. Room temperature was 25°C.
Fig. 1. ESR spectra of DMPO-OH and DMPO-OH used for determination of the spin concentration. Estimation of spin concentration is based on peak areas and the band constant of 6.5, as a measure of the spin concentration. The spectra were obtained in the presence of 10 G magnetic field and 15 min at 25°C. a: control; b: 10 μg of paraquat; c: 100 μg of paraquat. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 2. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 3. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 4. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 5. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 6. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 7. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 8. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 9. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 10. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 11. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 12. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 13. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 14. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 15. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 16. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 17. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 18. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.

Fig. 19. ESR spectra of DMPO-OH at varying concentrations of paraquat. Spin concentration measurements were performed at a time t = 10 min in the presence of 15 G magnetic field. The spin concentration was estimated to be 3.5 x 10⁶ spins/g of paraquat.
Kinetics of Spin Trapping of Oxygen Radicals

The above kinetic studies on the formation of DMO-OH were carried out in the presence of deferoxamine in order to inhibit iron-mediated Fe(III) mediated reactions that of the spin trap radicals that are subsequently generated from radical. In the case of H2O2, the scheme that is involved does not involve Fe(III). However, it has been reported in a number of systems that H2O2-mediated quinone radicals that are generated from radical. In the presence of Fe(III), the rate of Fe(II)-OH formation would be enhanced. The rate of Fe(II)-OH formation would be enhanced.

![Image of Fig. 1: Effect of external Fe(III) on the radical (Fe(III)-OH) trapping of superoxide.](http://www.jbc.org)

In the presence of Fe(III), the rate of Fe(II)-OH formation would be enhanced. The rate of Fe(II)-OH formation would be enhanced.

![Image of Table II: Effect of DMO concentration on the rate of DMO-OH formation.](http://www.jbc.org)

<table>
<thead>
<tr>
<th>DMO concentration (mM)</th>
<th>Rate of DMO-OH formation (μmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.005</td>
</tr>
<tr>
<td>0.2</td>
<td>0.055</td>
</tr>
<tr>
<td>0.3</td>
<td>0.105</td>
</tr>
</tbody>
</table>

It seemed reasonable to assume that Fe(III) oxidation increased with the DMO concentration in a superoxide generating system. Fe(III) had no inhibitory effect on the H2O2-mediated quinone radicals that are subsequently generated from radical. In the presence of Fe(III), the rate of Fe(II)-OH formation would be enhanced. The rate of Fe(II)-OH formation would be enhanced.

![Image of Fig. 2: Graph showing the effect of Fe(III) concentration on the rate of DMO-OH formation.](http://www.jbc.org)

In the presence of Fe(III), the rate of Fe(II)-OH formation would be enhanced. The rate of Fe(II)-OH formation would be enhanced.

![Image of Fig. 3: Graph showing the effect of Fe(III) concentration on the rate of DMO-OH formation.](http://www.jbc.org)

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![Image of Fig. 4: Graph showing the effect of Fe(III) concentration on the rate of DMO-OH formation.](http://www.jbc.org)

In the presence of Fe(III), the rate of Fe(II)-OH formation would be enhanced. The rate of Fe(II)-OH formation would be enhanced.
Kinetics of Spin Trapping of Oxygen Radicals

Fig. 3. Kinetics of DMPO-OH formation in the HOCl-NaOH-peroxidase system. Each reaction mixture contained 100 μM HOCl, 100 μM NaOH, and 0.1 μM peroxidase. A, formation and decay of Fe(III) ions. B, DMPO/DMPO-OOH and 1 μM Fe(II), 0.1 μM HOCl, 10 mM ClO₄, or 44 μM Fe(III) ions. C, 10 mM NaOH and 0.1 μM Fe(II), 5 μM ClO₄, or 40 μM Fe(III) ions.

Fig. 4. Kinetics of DMPO-OH formation in the presence of reduced hydrogen peroxide. The conditions were as described in Fig. 3, except for the addition of 10 mM hydrogen peroxide. A, for Fe(II) and B, for Fe(III).

Fig. 5. Plots derived from computer-simulated dmPO-OOH spectrum of DMPO-OH and DMPO-OOH mixed in different ratios. A, upfield half of the ESR spectrum that can arise from a mixture of 50% DMPO-OH and 50% DMPO-OH. B, plots of the relative intensities of common positions a, b, c, d, e, and f (a fraction of total fraction of DMPO-OH at n). The relative intensity at each common position (a, b, c, d, e) can be calculated as 0.5 - 0.5 + 4.0 + 10.0 + 0.16 for f.

In order to measure concentrations of DMPO-OH and DMPO-OH in mixtures of the two, we used a computer simulation of the two species with varying mixture percentages using just the first portion of the spectrum (Fig. 5A). As the fraction of DMPO-OH increased in a mixture, we observed a linear increase in the height of peak c and a linear decrease in the height of peak b. The peak heights in Fig. 5 are described as a ratio of common positions a, b, c, d, e, and f (a fraction of total fraction of DMPO-OH at n). The relative intensity at each common position (a, b, c, d, e) can be calculated as 0.5 - 0.5 + 4.0 + 10.0 + 0.16 for f.

Similar experiments to those in Fig. 6 were performed in the presence of 5 μM Fe(II) ions and 0.1 μM hydrogen peroxide. When deferoxamine was present, the addition of hydrogen peroxide did not alter the reaction pattern shown in Fig. 6A. However, with deferoxamine and DMPO the annihilation of DMPO-OH was greatly accelerated (Fig. 6B). In particular, with DMPO, only DMPO-OH was observed from the beginning of the reaction (Fig. 6B), in contrast to the case where DMPO-OH detected was less than one-third of the DMPO-OH accumulated in the presence of deferoxamine (Table III).
Kinetics of Spin Trapping of Oxygen Radicals

Oxygen radicals can be trapped using spin traps, which form stable adducts that can be detected spectroscopically. The rate of spin trapping can be studied to understand the dynamics of oxygen radical reactions.

**Fig. 1a.** Effect of the Fe(III)-EDTA concentration on the DMPO-OOH formation.

**Fig. 1b.** Effect of Fe(III) ion concentration on the radical species trapped by DMPO.

**Table I.**

<table>
<thead>
<tr>
<th>Chelator added</th>
<th>% Added</th>
<th>[DMPO-OOH]</th>
<th>[DMPO-OOH]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(III)</td>
<td>0</td>
<td>2.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Deferoxamine</td>
<td>10</td>
<td>3.5</td>
<td>0.8</td>
</tr>
<tr>
<td>DESYMP</td>
<td>10</td>
<td>1.8</td>
<td>0.4</td>
</tr>
<tr>
<td>EDTA</td>
<td>10</td>
<td>4.0</td>
<td>0.7</td>
</tr>
<tr>
<td>1000</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

In the presence of EDTA and added hydrogen peroxide, the concentration of DMPO-OOH was measured.

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Kinetic studies on spin trapping of superoxide and hydroxyl radicals generated in NADPH-cytochrome P-450 reductase-paraquat systems. Effect of iron chelates.

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