Kinetic Studies on Spin Trapping of Superoxide and Hydroxyl Radicals Generated in NADPH-Cytochrome P-450 Reductase-Parafuor 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-1-oxide (DMPO) were performed in NADPH-cytochrome P-450 reductase-paraquat 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) 2.8% 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 x 10⁶ M⁻¹ s⁻¹ for reduction of DMPO-OOH by Fe(II)-EDTA. The effect of diethylenetriamine pentaacetic acid (DETPAC) 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 superoxide dismutase, once isolated from erythrocytes on the basis of its activity, was found to be identical to erythrocuprein (3). Superoxide formed in biochemical systems has been assayed by following its reactions with horseradish peroxidase (4), lactoperoxidase (5), myeloperoxidase (6), diacetyldiethanol-substituted horseradish peroxidase (7), acetylated cytochrome c (8), succinylated cytochrome c (9), and some organic molecules (10, 11). Hydroxyl radicals formed in biochemical systems can be assayed chemically with p-nitrosodimethylaniline (12), benzoate (13), salicylate (14), 2-keto-4-thiobutylbutyrate (15), and circular DNA (16). However, electron spin resonance (ESR) has provided powerful and unambiguous techniques for detecting these oxygen radicals.

Superoxide is detected directly by ESR in frozen solutions (17, 18), but during the last decade spin-trapping methods have been extensively used to detect oxygen radicals with 5,5-dimethyl-1-pyrroline-1-oxide (DMPO) (19-22) and o-phenyl-N-tort-butylnitro (21-23).

Most of these spin-trapping studies, however, are qualitative. For the generation of oxygen radicals in biochemical systems, some investigators (20, 24-30) have reported kinetic data based on the relative intensity of ESR signals and only a few papers have reported a molar concentration for the amount of superoxide adduct of DMPO (DMPO-OOH) (31-32) or hydroxyl radical adduct of DMPO (DMPO-OH) (32-33). In spite of the fact that hydroxyl radical generation is closely related to superoxide formation, there is no kinetic and quantitative data that deal with the relationship between superoxide and hydroxyl radicals, except that of Finkelstein et al. (34) who reported the production of hydroxyl radical by decomposition of DMPO-OOH. Since the amount of such oxygen-radical adducts detected by ESR is only a part of the overall biochemical metabolites produced, it is necessary to elucidate kinetic meanings of the spin detection in the overall metabolism.

To clarify the quantitative relationships between enzymatic reactions and spin-trapping data of oxygen radicals, we have used the NADPH-cytochrome P-450 reductase-paraquat 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 the context of its chemical nature. The abbreviations and trivial names used are: DMPO, 5,5-dimethyl-1-pyrroline-1-oxide; DMPO-OOH, superoxide adduct of DMPO; DMPO-OH, hydroxyl radical adduct of DMPO; deferoxamine, desferoxamine B; DETAPAC, diethylenetriamine pentaacetic acid.

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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), diethylentriamine pentaacetic 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 \(k_d\), which is much higher than the value of \(3 \times 10^2 \text{ M} \cdot \text{s}^{-1}\) obtained using data of \(k_d = 3 \times 10^5 \text{ M}^{-1} \cdot \text{s}^{-1}\) (64) and \(k_d = 10 \text{ M}^{-1} \cdot \text{s}^{-1}\) (31) at pH 7.4. If one assumes that the \(k_d\) value is correct, the \(k_o\) value will be about \(1.2 \text{ M}^{-1} \cdot \text{s}^{-1}\), which is significantly less than that reported by Finkelstein et al. (31). This difference is probably, in part, ascribable to the differences in the experimental conditions (ionic strength, iron chelator, etc.). The DMPO concentration that traps one-half of the superoxide ions accumulated increases with the increase in the rate of superoxide formation as can be seen in Fig. 5 and Table II.

For the DMPO-OOH decay we conclude as follows: 1) DMPO-OOH decays with a half-life of 66 s (64) 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-OOH. 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, \(k_k[\text{DMPO-OOH}]\), 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 \(k_k\). The total amount of DMPO-OOH formed during the reaction will be greater than 29 \(\mu\text{M}\) since \(k_k\) 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). Equations 1 and 2 are 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 \(9 \times 10^4\) and \(10^9 \text{ M}^{-1} \cdot \text{s}^{-1}\), 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(III)-EDTA with superoxide, hydrogen peroxide, and DMPO-OOH. Fig. 14B shows a mechanism schematized using data so far reported

\[
\text{Fe(III)-EDTA} + \text{O}_2^- \rightarrow \text{Fe(IV)O}^--\text{EDTA}, \quad k_8 = 2 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}
\]

\[
\text{Fe(III)-EDTA} + \text{H}_2\text{O}_2 \rightarrow \text{Fe(III)-EDTA} + \text{H}_2\text{O}_2^-, \quad k_9 = 1.74 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}
\]

\[
\text{Fe(IV)O}^--\text{EDTA} + \text{H}_2\text{O}_2 \rightarrow \text{Fe(III)-EDTA} + \text{H}_2\text{O}_2^-, \quad k_9 = 1.74 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}
\]

\[
\text{Fe(III)-EDTA} + \text{H}_2\text{O}_2^-, \quad k_9 = 1.74 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}
\]

\[
\text{Fe(IV)O}^--\text{EDTA} + \text{H}_2\text{O}_2^-, \quad k_9 = 1.74 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}
\]

\[
\text{Fe(III)-EDTA} + \text{H}_2\text{O}_2^- \rightarrow \text{Fe(III)-EDTA} + \text{H}_2\text{O}_2, \quad k_9 = 1.74 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}
\]

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). \(k_1 = 0.011 \text{ s}^{-1}\) (28; this paper). \(k_o = 1 \times 10^4 \text{ M}^{-1} \cdot \text{s}^{-1}\) (31) and 1.2 \(\text{M}^{-1} \cdot \text{s}^{-1}\) (this paper). \(R_2\), reduction of O2 by paraquat radicals. \(k_2 = 7.7 \times 10^5 \text{ M}^{-1} \cdot \text{s}^{-1}\) (68). \(R_3\), reduction of Fe(III)-EDTA by paraquat radicals. The reaction is assumed to be fast (71). \(R_4\), reduction of Fe(III)-EDTA by superoxide. The reaction is pH dependent and \(k_4 = 1 \times 10^5 \text{ M}^{-1} \cdot \text{s}^{-1}\) (50, 69). The dotted line implies that \(k_4\) takes place through an unknown encounter complex (50). \(R_5\), reaction of Fe(II)-EDTA with a superoxide ion to form a peroxo-complex. \(k_5 = 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}\) (50, 69). \(R_6\), reversible complexing of Fe(III)-EDTA and \(k_6 = 1 \times 10^4 \text{ M}^{-1} \cdot \text{s}^{-1}\) (50, 69). The dotted line implies that \(k_6\) takes place through an encounter complex (50). \(R_7\), reaction of Fe(II)-EDTA with paraquat to form a nonspin compound. \(k_7 = 6 \times 10^5 \text{ M}^{-1} \cdot \text{s}^{-1}\) (this paper). \(R_8\), reaction of Fe(II)-EDTA with \(k_8 = 2 \times 10^6 \text{ M}^{-1} \cdot \text{s}^{-1}\) (50). 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), paraquat 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'_6′ 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 Digieese 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'_6′ 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 possibility that hydroxyl radicals are formed via Reaction 10. The efficiency of hydroxyl radical formation in the Fenton’s reaction is also a problem to be solved (72, 73). The increase of DMPO-OH formation in the presence of hydrogen peroxide (Table III) can be explained in terms of the decrease in the Fe(III)-EDTA concentration.

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] max 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] max 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 Kleinhans and Barefoot (33) in neutrophil systems. New kinetic approaches are necessary to solve this problem, which is now under investigation in this laboratory.

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Fig. 1. ESR spectra of DMPO-OH and DMPO-OH used for determination of the spin concentration. Reaction conditions: 

- Reaction mixture: 1.5 x 10^{-4} M DMPO
- Spin trap concentrations: 1 x 10^{-4} M DMPO
- Radical concentration: 1 x 10^{-4} M DMPO
- Temperature: 25°C

The reactions were carried out in a 1.5 ml volume of DMPO in a 10 mm quartz cell. The reaction mixture was irradiated with a 10 kW argon laser. The ESR spectra were recorded using a Varian E100 spectrometer.
Kinetics of Spin Trapping of Oxygen Radicals

The above kinetic studies on the formation of DMPD-OEt were carried out in the presence of deoxygenation in order to inhibit iron-catalyzed reaction. However, a number of systems have been reported in which the reaction was carried out in the presence of oxygen. The presence of oxygen can significantly affect the rate of spin trapping. In some cases, the rate of spin trapping can be inhibited by oxygen, while in others, the presence of oxygen can enhance the rate of spin trapping. The mechanism of these effects is not well understood, but it is likely that the presence of oxygen can affect the stability of the radicals or the rate of spin trapping. It has been suggested that the presence of oxygen can affect the rate of spin trapping by changing the rate of spin trapping in a way that is dependent on the specific radicals being trapped. It is important to consider the presence of oxygen when carrying out kinetic studies of spin trapping, as it can significantly affect the results.

![Diagram](http://www.jbc.org/content/38/1/657/F1.large.jpg)

**Figure 1.** Effect of spin trapping on the rate of DMPD-OEt formation. Plotted from data in Table II. DMPD-cyclopropane 0.40 M radicals and spin-trapping radicals. The reaction mixtures contained 0.1 M DMPD, 0.1 M cyclopropane, and 0.1 M DMPD-cyclopropane. The end of reaction and b continues to c and d, respectively.

| Time (min) | A | B | C |
|-----------|---|---|---|
| 0         | a | b | c |
| 4         | b | c | d |
| 7         | c | d | e |

**Table II.** Effect of spin trapping on the rate of DMPD-OEt formation from DMPD-cyclopropane 0.40 M radicals and spin-trapping radicals. The reaction mixtures contained 0.1 M DMPD, 0.1 M cyclopropane, and 0.1 M DMPD-cyclopropane 0.40 M radicals. The rate of DMPD-OEt was 0.005 M/s.

| DMPD | DMPD-cyclopropane 0.40 M radicals | Spin-trapping radicals | Rate of DMPD-OEt (M/s) |
|------|-----------------------------------|------------------------|------------------------|
| 100  | 0.005                             | 0.005                  | 0.005                  |
| 150  | 0.005                             | 0.005                  | 0.005                  |
| 250  | 0.005                             | 0.005                  | 0.005                  |

It seems reasonable that DMPD-OEt accumulation increased with the DMPD concentration in a species-generating system. DMPD-OEt had no inhibitory effect on the DMPD-cyclopropane 0.40 M radicals-radical reaction. The dependence of the rate of DMPD-OEt formation on DMPD concentration was analyzed using the following equation:

\[ v = \frac{k_D [DMPD]}{1 + k_D [DMPD] + k_{-1} [DMPD-OEt]} \]

where \( k_D \) is the rate constant for DMPD-OEt formation, and \( k_{-1} \) is the rate constant for DMPD-OEt decomposition.

In the presence of oxygen, the rate of spin trapping was significantly reduced. However, the presence of oxygen had no significant effect on the rate of DMPD-OEt formation from DMPD-cyclopropane 0.40 M radicals. It is possible that the presence of oxygen does not affect the rate of DMPD-OEt formation from DMPD-cyclopropane 0.40 M radicals, or that the rate of DMPD-OEt formation from DMPD-cyclopropane 0.40 M radicals is not affected by the presence of oxygen.

The results of these experiments suggest that the presence of oxygen can affect the rate of spin trapping, and that the rate of DMPD-OEt formation from DMPD-cyclopropane 0.40 M radicals is not affected by the presence of oxygen.

**Figure 2.** The effect of oxygen on the rate of DMPD-OEt formation. The reaction mixtures contained 0.1 M DMPD, 0.1 M cyclopropane, and 0.1 M DMPD-cyclopropane 0.40 M radicals. The rate of DMPD-OEt formation in the presence of oxygen was 0.005 M/s, and the rate of DMPD-OEt formation in the absence of oxygen was 0.005 M/s.
Kinetics of Spin Trapping of Oxygen Radicals

Fig. 3. Kinetics of spin trapping of oxygen radicals in the DMPO system. Each reaction mixture contained 100 μM DMPO, 10 μM Fe(II) as FeSO4, 20 μM DMPO-OOH, 10 μM NaNO2, and 1 μM Fe(II)-EDTA. The spectra were recorded according to the procedure described in the Materials and Methods section. The fraction of DMPO-OH was calculated using the relative intensities of the peaks at 0.85 and 0.86 ppm.

Fig. 4. Effect of DMPO-OOH on the formation of DMPO-OH. Each reaction mixture contained 100 μM DMPO, 10 μM Fe(II)-EDTA, 20 μM Fe(II), and 1 μM DMPO-OOH. The spectra were recorded according to the procedure described in the Materials and Methods section. The fraction of DMPO-OH was calculated using the relative intensities of the peaks at 0.85 and 0.86 ppm.

Similar experiments to those in Fig. 4 were performed in the presence of 5 μM Fe(II)-EDTA and 10 μM hydrogen peroxide. When deoxygenation was present, the addition of hydrogen peroxide did not alter the reaction pattern shown in Fig. 4B. However, with Fe(II)-EDTA and Fe(II) the formation of DMPO-OH was greatly diminished (Fig. 5). In particular, with Fe(II) only DMPO-OH was observed from the beginning of the reaction (Fig. 5A), even in this case the amount of DMPO-OH detected was less than one-third of that of DMPO-OH in the absence of deoxygenation (Table III).

Fig. 5. Effect of Fe(II)-mediated spin trapping of oxygen radicals in different systems. A. Effect of Fe(II)-mediated spin trapping of oxygen radicals in a mixture of 30% DMPO-OOH and 70% DMPO-OH. B. Effect of Fe(II)-mediated spin trapping of oxygen radicals in a mixture of 50% DMPO-OOH and 50% DMPO-OH.

In order to measure concentrations of DMPO-OH and DMPO 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 spectra (Fig. 6A). As the fraction of DMPO-OH decreased in the mixture, we observed a linear decrease in the height of peak c and linear decrease in the height of peak d and peak e. The relative intensity of peak d and peak e was constant throughout the reaction. The relative intensity of peak e was constant even when peak d was not present.

Fig. 6. Effect of Fe(II)-mediated spin trapping of oxygen radicals in a mixture of 50% DMPO-OOH and 50% DMPO-OH. A. Relative intensity of peaks d and e. B. Relative intensity of peak d. C. Relative intensity of peak e. D. Relative intensity of peak f. E. Relative intensity of peak g. F. Relative intensity of peak h. G. Relative intensity of peak i. H. Relative intensity of peak j. I. Relative intensity of peak k. J. Relative intensity of peak l. K. Relative intensity of peak m. L. Relative intensity of peak n. M. Relative intensity of peak o. N. Relative intensity of peak p. O. Relative intensity of peak q. P. Relative intensity of peak r. Q. Relative intensity of peak s. R. Relative intensity of peak t. S. Relative intensity of peak u. T. Relative intensity of peak v. U. Relative intensity of peak w. V. Relative intensity of peak x. W. Relative intensity of peak y. X. Relative intensity of peak z.

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Fig. 11. Effect of the Fe(III)-EDTA concentration on the DMPD-OOH formation. Reaction mixtures contained 10 μM EDTA, 10 μM DMPD, 20 μM potassium, 1 μM DMPO, 0.5 μM DMPO-DMPO, and 0.5 μM Fe(III)-EDTA. The concentration of DMPD-OOH formation was plotted against Fe(III)-EDTA concentration. A, spectra were recorded for the range of 0 to 5 μM Fe(III)-EDTA according to the procedure used for DMPO-DMPO (see MATERIALS AND METHODS). B, spectra were recorded 1 min after initiation of the reaction in A.

Fig. 12. Effect of Fe(III) concentration on the radical species trapped by DMPO in the presence of EDTA and DMPD. Reaction mixtures contained 1 μM EDTA, varying amounts of Fe(III) and 0 μM DMPO. The concentration of added Fe(III) ions was 5 μM for A, 5 μM for B, 5 μM for C, and 5 μM for D and E. The concentration of Fe(III)-EDTA was 2.5 μM for A, 2.5 μM for B, 2.5 μM for C, and 2.5 μM for D and E.

Table I

| Chelator added | DMPO-DMPO | DMPO-DMPO | DMPO-DMPO |
|----------------|------------|------------|------------|
| None           |            |            |            |
| 10             | 0          | 2.7        | 0.6        |
| 30             | 0          | 3.5        | 0.8        |
| 100            | 4.0        | 7.0        | 0.4        |
| Desferrioxamine| 300        | 8.0        | 0.7        |
| 300            | 100        | 4.0        | 0.7        |
| EDTA           | 500        | 100        | 0.2        |
| 1000           | 100        | 1.0        | 1.0        |
| 2000           | 100        | 1.0        | 1.0        |
| 3000           | 100        | 1.0        | 1.0        |
| 5000           | 100        | 1.0        | 1.0        |
Kinetic studies on spin trapping of superoxide and hydroxyl radicals generated in NADPH-cytochrome P-450 reductase-paraquat systems. Effect of iron chelates.

I Yamazaki, L H Piette and T A Grover

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