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KINETICS OF THE COMPETITIVE DEGRADATION OF DEOXYRIBOSE AND OTHER MOLECULES BY HYDROXYL RADICALS PRODUCED BY THE FENTON REACTION IN THE PRESENCE OF ASCORBIC ACID

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The competition method in which the Fenton reaction is employed as an ·OH radical generator and deoxyribose as a detecting molecule, has been used to determine the rate constants for reactions of the ·OH radical with its scavengers. Nonlinear competition plots were obtained for those scavengers which reacted with the Fenton reagents (Fe^{2+} or H_2O_2). Ascorbic acid is believed to overcome this problem. We have investigated the kinetics of deoxyribose degradation by ·OH radicals generated by the Fenton reaction in the presence of ascorbic acid, and observed that the inclusion of ascorbic acid in the Fenton system greatly increased the rate of ·OH radical generation. As a result, the interaction between some scavengers and the Fenton reagents became negligeable and linear competition plots of A°/A vs scavenger concentrations were obtained. The effects of experimental conditions such as, the concentrations of ascorbic acid, deoxyribose, H_2O_2 and Fe^{2+}-EDTA, the EDTA/Fe^{2+} ratio as well as the incubation time, on the deoxyribose degradation and the determination of the rate constant for mercaptoethanol chosen as a reference compound were studied. The small standard error, $(6.76 \pm 0.21) \times 10^9\ M^{-1}s^{-1}$, observed for the rate constant values for mercaptoethanol determined under 13 different experimental conditions, indicates the latter did not influence the rate constant determination. This is in fact assured by introducing a term, k_x, into the kinetic equation. This term represents the rate of ·OH reactions with other reagents such as ascorbic acid, Fe^{2+}-EDTA, H_2O_2 etc. The agreement of the rate constants obtained in this work with that determined by pulse radiolysis techniques for cysteine, thiourea and many other scavengers, suggests that this simple competition method is applicable to a wide range of compounds, including those which react with the Fenton reagents and those whose solubility in water is low.

KEY WORDS: Hydroxyl radical, Fenton reaction, Ascorbic acid, Deoxyribose, Mercaptoethanol, Kinetics.

INTRODUCTION

The rapid and nonspecific reactivity of the ·OH radical renders this free radical particularly dangerous. It may abstract hydrogen from, or hydroxylate, most biomolecules, causing cell injury or death. Hydroxyl radical is believed to be the etiological agent for a large number of diseases and may also be involved in natural aging.[1–3] It has been suggested that a number of agents of therapeutic use, such as allopurinol,[4] non-steroidal anti-inflammatory drugs,[5] and amygdalin,[6] might exert some of their beneficial effects by scavenging ·OH radicals. Such an effect depends on,

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among other parameters, the rate constants for the reactions of the agents with ·OH radicals.[7] To determine such rate constants, Halliwell *et al.* have proposed a simple 'test-tube' assay using the Fenton reaction (in the presence of ascorbic acid) as a source of ·OH radicals and deoxyribose as a detecting molecule.[8] In this method, deoxyribose is degraded by ·OH radicals generated by the reaction between H_2O_2 and Fe^{2+}-EDTA in the presence of ascorbic acid. One of the deoxyribose degradation products is malondialdehyde[9] which is quantitatively detected by its ability to react with thiobarbituric acid to form a chromogen absorbing at 532 nm.[10,11] Any ·OH scavenger added to the reaction mixture should compete with deoxyribose for ·OH to an extent depending on its rate constant for reaction with ·OH and its concentration relative to deoxyribose; hence, it will decrease the rate of deoxyribose degradation. In a previous report,[12] we analyzed the kinetics of the Fenton reaction and the competitive degradation of deoxyribose by ·OH radicals, and established a kinetic equation which allowed us to use any concentration of deoxyribose to determine the rate constants for the reactions of scavengers with ·OH radicals. This competition method was applied to various scavengers, and for some of them for example, thiourea, cysteine and mercaptoethanol, the kinetic competition plots deviated from linearity, most likely due to their interference with the Fenton reagents. It is known that thiourea reacts with H_2O_2 and diminishes the rate of ·OH generation,[13,14] whereas cysteine and mercaptoethanol regenerate Fe^{2+} from Fe^{3+} and increase the rate of ·OH generation.[15,16] Ascorbic acid has been widely used in the Fenton system to increase the rate of ·OH production owing to its reducing properties:[17,8]

$$\text{Ascorbic acid} + Fe^{3+} \longrightarrow \text{Oxidized ascorbic acid} + Fe^{2+}$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + \cdot OH \text{ (Fenton reaction)}$$

Thus, the inclusion of ascorbic acid in the Fenton system may probably minimize, even suppress, the interferences mentioned above. In this report, this hypothesis has been examined by determining the rate constants for reactions of a wide range of different substances, and found to be valid.

MATERIALS AND METHODS

Reagents

2-Deoxy-D-ribose was from Aldrich. All other chemicals used were of the highest grade available.

Degradation of Deoxyribose

The assay procedure was essentially that described by Zhao *et al.*[12] with a few modifications introduced. Reaction mixtures contained, in a final volume of 3 ml, the following reagents added in the order stated and at the final concentrations as noted under each figure: phosphate-saline buffer, pH 7.4 (24 mM NaH_2PO_4-Na_2HPO_4 in 15 mM NaCl); deoxyribose; ascorbic acid (AA); hydrogen peroxide (H_2O_2); EDTA and $(NH_4)_2Fe(SO_4)_2$. $(NH_4)_2Fe(SO_4)_2$ and EDTA were premixed just before addition to the reaction mixture. Solutions of $(NH_4)_2Fe(SO_4)_2$ and ascorbic acid were prepared in de-aerated water immediately before use. EDTA-$(NH_4)_2Fe(SO_4)_2$ solution was added

to set off the reaction. Reaction mixtures were incubated at 37°C for a given time noted in each experiment. 1.5 ml of 2.8% cold trichloroacetic acid were then added and 1.0 ml of the mixture obtained was drawn. Thiobarbituric acid (TBA) reactivity was developed by adding 1 ml of TBA reagent (1%, w/v, in 0.05 M NaOH), followed by heating at 100°C for 15 min. When the mixture was cool, the absorbance at 532 nm was measured against appropriate blanks.

RESULTS AND DISCUSSION

Kinetics of Deoxyribose Degradation

The kinetics of deoxyribose degradation by ·OH radicals generated by the Fenton reaction in the absence of ascorbic acid has previously been described,[12] and the main kinetic equation is given by

$$\frac{A^\circ}{A} = 1 + \frac{k_S\,[S]}{k_D[D] + k_x} \tag{1}$$

where A° and A are the absorbances at 532 nm, A° being in the absence and A in the presence of a scavenger (S); k_D and k_S are the rate constants for the reactions of deoxyribose and S respectively with ·OH radicals; and k_x represents the part of ·OH that reacts with other reagents with the exclusion of deoxyribose, such as Fe^{2+}-EDTA (rate constant $k_{Fe^{2+}\text{-EDTA}}$), H_2O_2 (rate constant $k_{H_2O_2}$) etc:

$$k_x = k_{Fe^{2+}\text{-EDTA}}\ [Fe^{2+}\text{-EDTA}] + k_{H_2O_2}[H_2O_2] + \ldots \tag{2}$$

It should be noted that equation (1) can be obtained only when the rates of ·OH formation in the absence ($V_{\cdot OH}$) and in the presence ($V^S_{\cdot OH}$) of S are the same. Otherwise, the competition kinetic equation should be expressed as:

$$\frac{A^\circ}{A} = \frac{V_{\cdot OH}}{V^S_{\cdot OH}}\left(1 + \frac{k_S\,[S]}{k_D[D] + k_x}\right) \tag{3}$$

In the presence of ascorbic acid (AA, rate constant k_{AA}), the competition kinetic equation is the same as that in the absence of ascorbic acid, but the k_x term should contain, in addition, the part of ·OH that reacts with ascorbic acid:

$$k_x = k_{AA}[AA] + k_{Fe^{2+}\text{-EDTA}}\,[Fe^{2+}\text{-EDTA}] + k_{H_2O_2}[H_2O_2] + \ldots \tag{4}$$

k_x is a constant depending on the experimental conditions, and can be determined by drawing the line 1/A° vs 1/[D] from the following equation:[12]

$$\frac{1}{A^\circ} = \frac{1}{V_{\cdot OH}}\left(1 + \frac{k_x}{k_D[D]}\right) \tag{5}$$

Effect of Experimental Conditions on Deoxyribose Degradation

In order to determine a range of experimental conditions which may be used for the study of various kinds of ·OH scavengers, we first examined the main parameters which may influence the production of ·OH radicals or deoxyribose degradation, such as the incubation time, the concentrations of ascorbic acid, deoxyribose, H_2O_2 and Fe^{2+}-EDTA, and the EDTA/Fe^{2+} ratio.

Effect of ascorbic acid concentration The effect of the ascorbic acid concentration on the deoxyribose degradation was examined using [D] 3 mM, [H_2O_2] 0.85 mM, [Fe^{2+}] 0.03 mM, [EDTA] 0.045 mM, with a reaction time of 15 min (Figure 1). It was found that a low concentration of ascorbic acid greatly enhanced the deoxyribose degradation which, at a concentration of about 0.65 mM, reached a maximum higher than that obtained in the absence of ascorbic acid, by a factor of 9. Whereas a high concentration of ascorbic acid exerted an inhibitor effect. In fact, ascorbic acid has two opposing effects in the Fenton system. Firstly, it increases the ·OH production through the generation of Fe^{2+} from Fe^{3+} (oxido- reduction) and the formation of H_2O_2, according to the following mechanism:[18,19]

$$AA + O_2 \xrightarrow[Fe^{2+}]{\text{catalyst}} O_2^{\cdot -} + \text{oxidized AA}$$

$$2O_2^{\cdot -} + 2H^+ \longrightarrow H_2O_2 + O_2$$

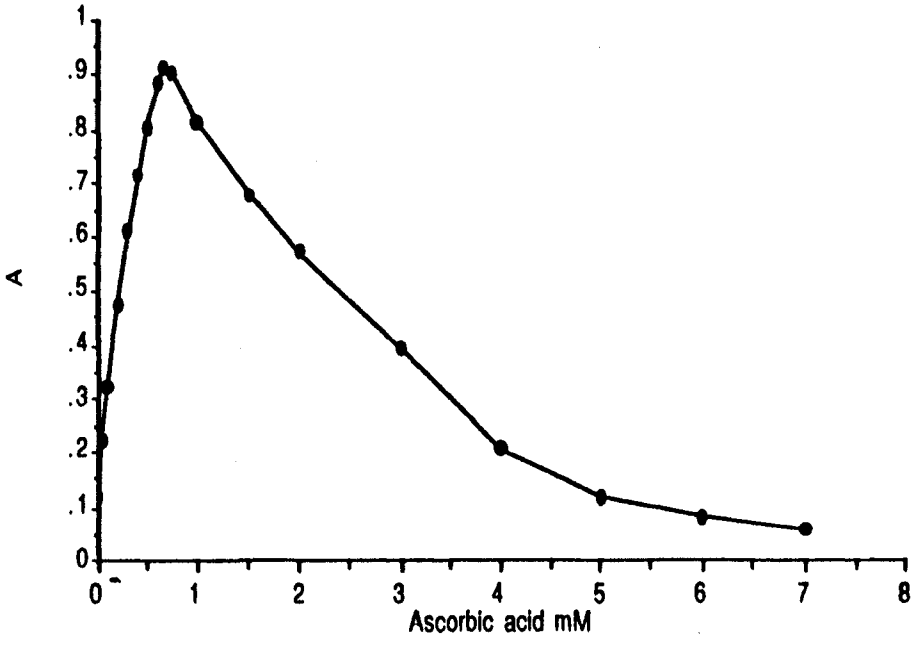

FIGURE 1 Effect of ascorbic acid concentration on deoxyribose degradation. [D] = 3 mM, [H_2O_2] = 0.85 mM, [Fe^{2+}] = 0.03 mM and [EDTA] = 0.045 mM. The reaction was carried out in phosphate buffer, pH 7.4 at 37°C for 15 min. A represents the absorbance at 532 nm of the chromogen formed between deoxyribose degradation product (malondialdehyde) and thiobarbituric acid.

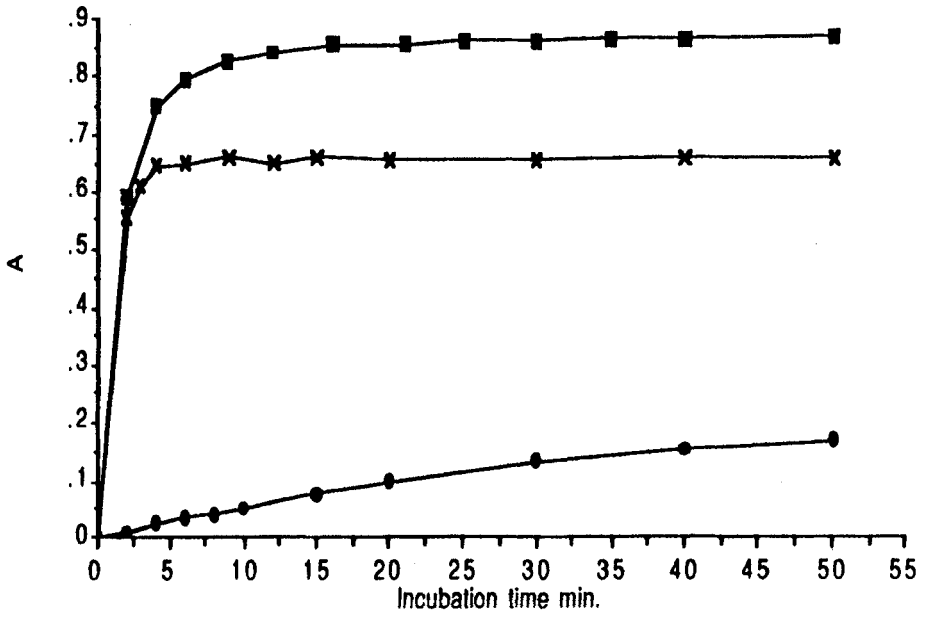

FIGURE 2 Deoxyribose degradation by ·OH radicals vs incubation time for different ascorbic acid concentrations: ●, without ascorbic acid, ■ 0.6 mM and x, 1.5 mM. The other conditions were similar to those of Figure 1.

Secondly, ascorbic acid intercepts ·OH with a rate constant of 1.2×10^{10} $M^{-1}s^{-1}$.[20] Figure 1 indicates that the former effect predominates at low concentrations and the latter at high concentrations (above 5 mM under our experimental conditions).

Effect of incubation time Figure 2 shows the deoxyribose degradation in the absence or in the presence of two different concentrations of ascorbic acid with incubation time. As wé can see, the presence of ascorbic acid increased the rate as well as the quantity of deoxyribose degradation, which reached a maximum within a few minutes in contrast to the time needed in the absence of ascorbic acid (>50 min). The concentration of ascorbic acid significantly modified the quantity but had little effect on the rate of deoxyribose degradation.

Effect of EDTA/Fe^{2+} ratio Deoxyribose degradation was studied using [D] 3 mM, [H_2O_2] 0.85 mM, [AA] 1.5 mM, [Fe^{2+}] 0.03 mM and various concentrations of EDTA (Figure 3). As expected, the addition of EDTA led to strong increases in the deoxyribose degradation which reached a maximum at an EDTA/Fe^{2+} ratio of 1.5. This effect of EDTA on the deoxyribose degradation or the ·OH production was due to the lower redox potential of EDTA-Fe^{2+} compared to free Fe^{2+}.[21] The fact that a plateau instead of a peak (as the case in the absence of ascorbic acid) was obtained in this experiment, indicates that although EDTA can react with ·OH radical (rate constant of 2.7×10 $M^{-1}s^{-1}$ [22]), its scavenging effect is negligeable compared with that of ascorbic acid.

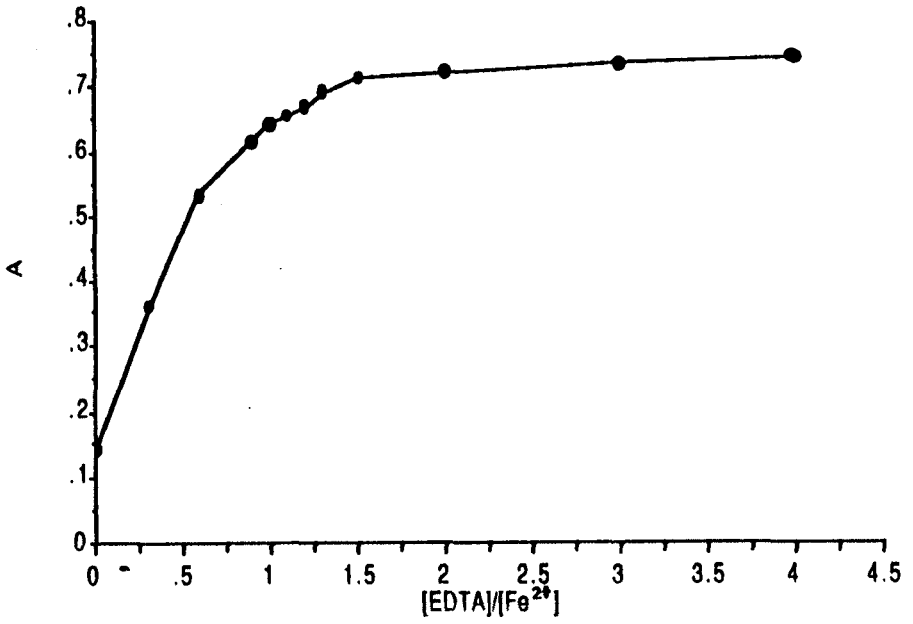

FIGURE 3 Effect of [EDTA]/[Fe^{2+}] ratio on deoxyribose degradation. [AA] = 1.5 mM and the other conditions were similar to those of Figure 1.

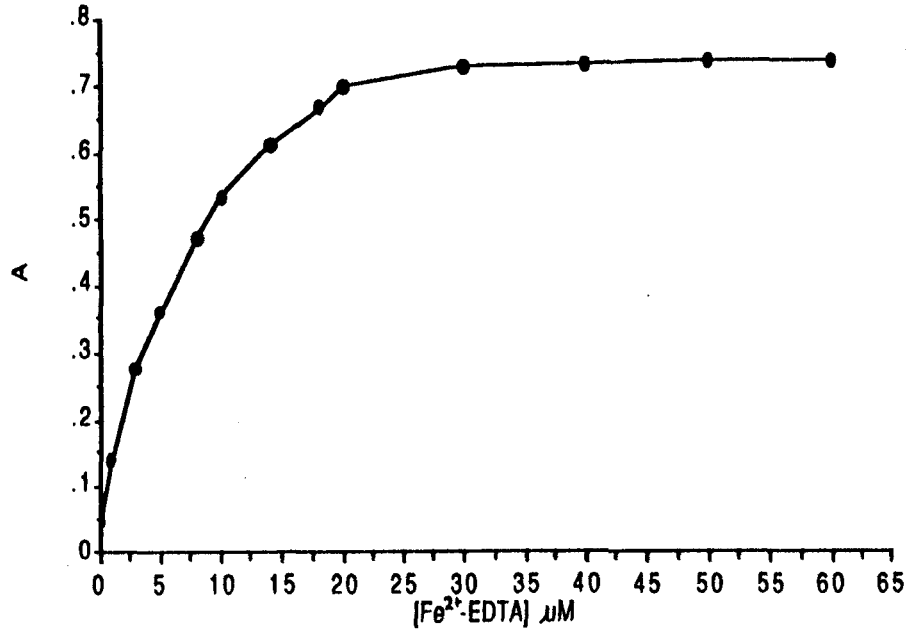

FIGURE 4 Effect of Fe^{2+}-EDTA concentration on deoxyribose degradation. Variable EDTA concentrations were used to hold up the ratio [EDTA]/[Fe^{2+}] = 1.5. The other conditions were similar to those of Figure 1 and the ascorbic acid concentration was 1.5 mM.

Effect of Fe^{2+}-EDTA concentration Deoxyribose degradation increased with increasing concentrations of Fe^{2+}-EDTA, reaching a maximum at a Fe^{2+}-EDTA concentration of about 0.03 mM, almost 28 times lower than the H_2O_2 concentration used (Figure 4). This result suggests that the concentration of H_2O_2 and not Fe^{2+}-EDTA restrains the deoxyribose degradation under our experimental conditions. The reason for this is that although the initial Fe^{2+}-EDTA concentration used was much lower than that of H_2O_2, Fe^{2+}-EDTA could be regenerated from its reaction product, Fe^{2+}-EDTA, through the action of ascorbic acid present in the reaction medium.

Effect of H_2O_2 concentration When ascorbic acid incubated with Fe^{2+}-EDTA in the absence of H_2O_2, a deoxyribose degradation was observed, equivalent to about 20% of that produced when 0.85 mM H_2O_2 was present in the incubation medium (Figure 5). This result confirms that ascorbic acid itself can produce ·OH radicals in the presence of Fe^{2+}-EDTA via the mechanism described above. The fact that no plateau was obtained up to a H_2O_2 concentration of 1.6 mM, indicates that the latter is the factor restricting the deoxyribose degradation under the employed experimental conditions.

Effect of deoxyribose concentration A correlation between deoxyribose degradation and its concentration was observed as shown in Figure 6, suggesting that deoxyribose, even at a concentration of 10 mM, could not accept all ·OH radicals generated under the used experimental conditions. The plot of $\frac{1}{A^\circ}$ vs $\frac{1}{[D]}$ gave a straight line (Figure 7) from which k_x was calculated as 2.94 mMk_D.

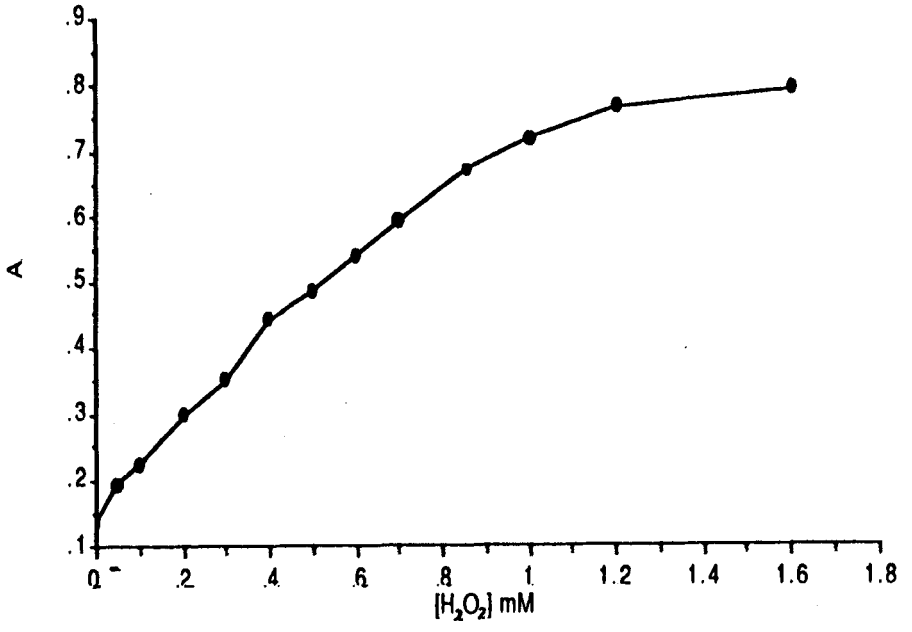

FIGURE 5 Effect of H_2O_2 concentration on deoxyribose degradation. The other conditions were similar to those of Figure 1 and the ascorbic acid concentration was 1.5 mM.

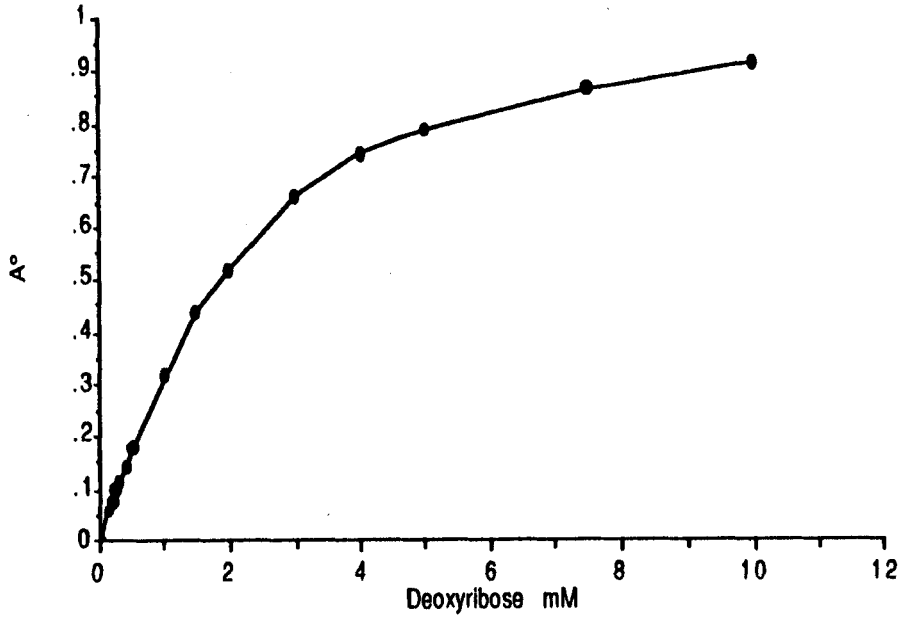

FIGURE 6 Effect of initial deoxyribose concentration on its degradation by ·OH radicals. The other conditions were similar to those of Figure 1 and the ascorbic acid concentration was 1.5 mM.

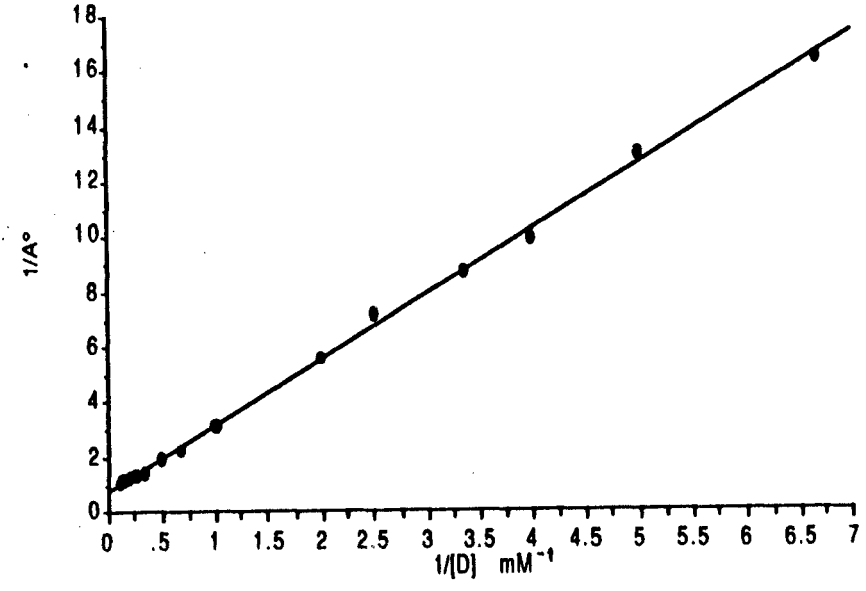

FIGURE 7 Relationship between 1/A° and 1/[D] from the data shown in Figure 6. Determination of parameter k_x.

On the basis of the results obtained above, we selected [D] = 3 mM (or 0.6 mM), [AA] = 1.5 mM (or 0.6 mM when [D] = 0.6 mM), [H_2O_2] = 0.85 mM, [Fe^{2+}] = 0.03 mM, [EDTA] = 0.045 mM, with a reaction time of 15 min as the essential experimental conditions for all subsequent experiments.

Determination of the Rate Constant for the Reaction of Mercaptoethanol with ·OH Radicals under Different Experimental Conditions

Mercaptoethanol was chosen as a reference substance to study the role of ascorbic acid in the Fenton system. Figure 8 clearly shows that the plot A°/A vs mercaptoethanol concentrations is linear, in contrast to that obtained in the absence of ascorbic acid.[12] The fact that the offset of the line is practically equal to unity, indicates that the rates of ·OH formation, in the absence and in the presence of mercaptoethanol, are the same: $V_{\cdot OH} = V^{S}_{\cdot OH}$. This result suggests that the presence of ascorbic acid in the Fenton system suppresses the effect of mercaptoethanol on the regeneration of Fe^{2+} from Fe^{3+}. The rate constant for mercaptoethanol is calculated to be 6.75×10^9 $M^{-1}s^{-1}$ using a rate constant value of 1.9×10^9 $M^{-1}s^{-1}$ for deoxyribose.[8] This value agrees closely with the value determined by pulse radiolysis techniques (6.8×10^9 $M^{-1}s^{-1}$).[20]

The rate constant of mercaptoethanol under different experimental conditions was determined in order to study the effects of the parameters, such as the concentrations of AA, D, Fe^{2+}-EDTA and H_2O_2 as well as the incubation time, on the linearity of the plots A°/A vs [S] and the rate constant value of mercaptoethanol. Since the parameter k_x depends on experimental conditions, its value in each case was at first determined

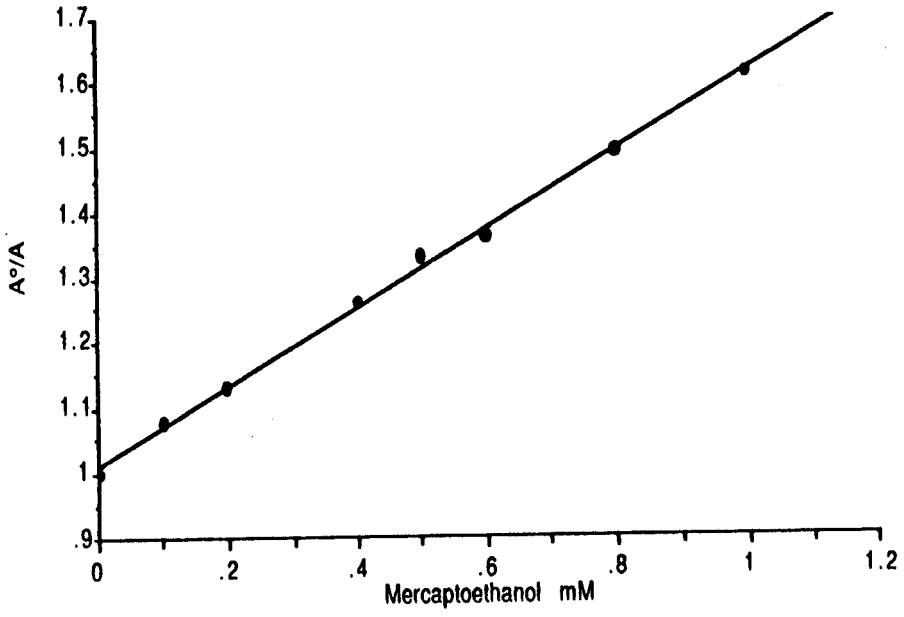

FIGURE 8 Relationship between A°/A and mercaptoethanol concentration. The other conditions were similar to those of Figure 1 and the ascorbic acid concentration was 1.5 mM.

TABLE 1
Determination of the parameter k_x under different experimental conditions. The reaction was carried out in phosphate buffer (pH 7.4) at 37°C for 15 min.

[AA] (mM)	[Fe^{2+}] (mM)	[EDTA] (mM)	[H_2O_2] (mM)	k_x (mM·k_D)
1.5	0.03	0.045	0.85	2.94
0.6	0.03	0.045	0.85	1.67
2.5	0.03	0.045	0.85	4.19
1.5	0.01	0.015	0.85	2.61
1.5	0.05	0.075	0.85	2.51
1.5	0.03	0.045	0.4	3.24
1.5	0.03	0.045	1.4	2.80

by drawing the plots 1/A° vs 1/[D]. The results obtained show that all the plots gave straight lines. The k_x values calculated from the slopes and the offsets are summarized in Table 1. It is observed that the k_x values mainly depend on the ascorbic acid concentrations, but less on that of Fe^{2+}-EDTA and H_2O_2. Although the complex Fe^{2+}-EDTA has a reactivity towards ·OH not far from that of ascorbic acid, its concentration is much smaller. The opposite case is true for H_2O_2. As a result, the two latter terms ($k_{Fe^{2+}\text{-EDTA}}$[Fe^{2+}-EDTA] and $k_{H_2O_2}$[H_2O_2]) in equation (4) are less important than the first one (k_{AA}[AA]) for the k_x value.

The plots A°/A vs mercaptoethanol concentration under different experimental conditions were drawn and all the plots gave straight lines with offsets close to unity. The rate constant values for mercaptoethanol, calculated by using the corresponding values of k_x, are given in Table 2. The mean value obtained under 13 different experimental conditions is $(6.76 \pm 0.21) \times 10^9$ $M^{-1}s^{-1}$. This value is in good agreement with the data of the literature.[20] These results show that the experimental conditions did not affect the rate constant determination of mercaptoethanol. The fact that the plot A°/A vs mercaptoethanol concentration drawn at an ascorbic acid concentration of 0.6 mM gave a straight line with an offset of unity, indicates that this ascorbic acid concentration is sufficient to suppress the interference of mercaptoethanol with the Fenton reagents under our experimental conditions. Although the ascorbic acid concentrations significantly affected the quantity of ·OH produced, it did not influence the *k* determination of mercaptoethanol owing to the k_x term in the kinetic competition equation. The fact that the *k* values of mercaptoethanol determined at different concentrations of deoxyribose are very similar, indicates the lack of dependence of *k* determination on [D]; this is also due to the k_x term.

Determination of the Rate Constants for Other Substances

It was previously shown that the kinetic competition plots drawn in the absence of ascorbic acid for cysteine and thiourea (A°/A vs their concentrations) deviated from linearity as that obtained for mercaptoethanol. In the presence of ascorbic acid, their kinetic competition plots are given in Figures 9, 10 and 11. It was found that the plots A°/A vs cysteine concentration drawn at ascorbic acid concentrations of 1.5 and 2.5 mM (Figure 10) gave straight lines. This case was not true for a concentration of 0.6 mM (Figure 9), meaning that this latter is not sufficient to suppress the effect of cysteine on the rate of ·OH radical production under the experimental conditions used, i.e., $V_{\cdot OH} \neq V^S_{\cdot OH}$. The rate constant values for cysteine, determined by the use of 1.5 mM or 2.5 mM of ascorbic acid, are 1.13×10^{10} and 1.17×10^{10} $M^{-1}s^{-1}$ respectively, indicating the independence of the rate constant values on the ascorbic acid concentration, as well

TABLE 2
Effects of different experimental conditions on the measurement of the rate constant of mercaptoethanol. The reaction was carried out in phosphate buffer (pH 7.4) at 37°C for 15 min.

[D] (mM)	[AA] (mM)	[Fe^{2+}] (mM)	[EDTA] (mM)	[H_2O_2] (mM)	Incubation Time (min)	$k \times 10^{-9}$ ($M^{-1}s^{-1}$)
3.0	1.5	0.03	0.045	0.85	15	6.75
3.0	0.6	0.03	0.045	0.85	15	6.68
3.0	2.5	0.03	0.045	0.85	15	6.58
0.6	0.6	0.03	0.045	0.85	15	6.76
0.6	1.5	0.03	0.045	0.85	15	7.08
0.6	2.5	0.03	0.045	0.85	15	6.41
5.0	1.5	0.03	0.045	0.85	15	6.67
3.0	1.5	0.01	0.015	0.85	15	7.02
3.0	1.5	0.05	0.075	0.85	15	6.89
3.0	1.5	0.03	0.045	0.4	15	6.91
3.0	1.5	0.03	0.045	1.4	15	6.51
3.0	1.5	0.03	0.045	0.85	7	6.89
3.0	1.5	0.03	0.045	0.85	30	6.48

as the agreement of the obtained values with the value of literature ($k = 1.3 \times 10^{10}$ $M^{-1}s^{-1}$).[23] Figure 11 displays a linear relationship between A°/A and the thiourea concentration obtained at an ascorbic acid concentration of 1.5 mM, signifying that the presence of ascorbic acid suppresses the interference of thiourea with the Fenton

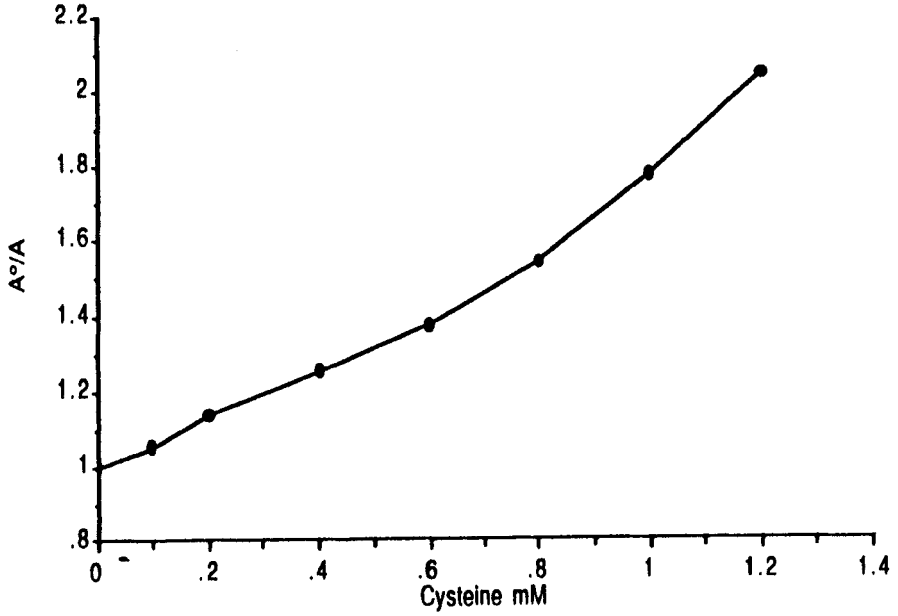

FIGURE 9 Competition experiment between ·OH reactions with deoxyribose (D) and cysteine (S). Variation with [S] of the relative extent $\frac{A^\circ}{A}$ of deoxyribose degradation in the absence (A°) and in the presence (A) of cysteine. The other conditions were similar to those of Figure 1 and the ascorbic acid concentration was 0.6 mM.

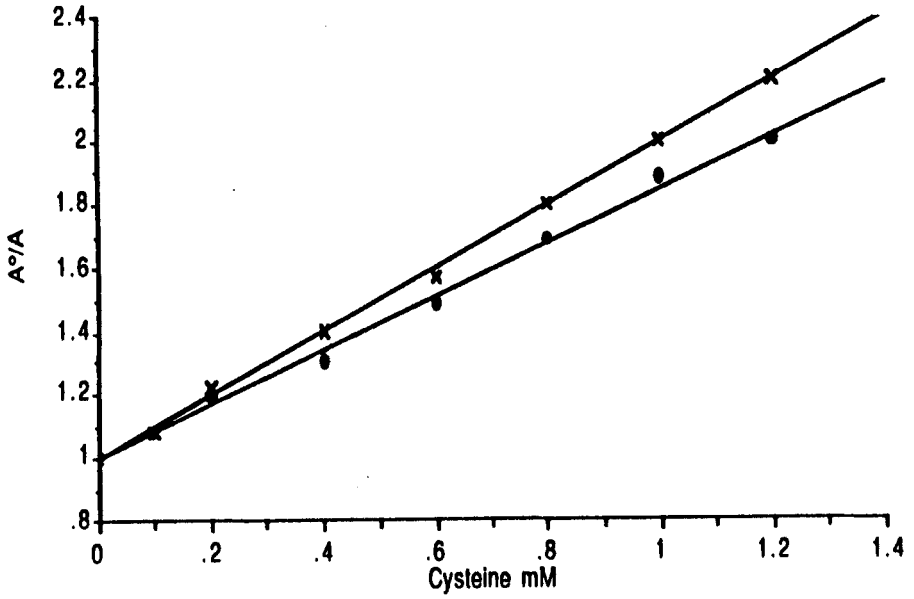

FIGURE 10 Relationship between A°/A and cysteine concentration for two ascorbic acid concentrations: x, 1.5 mM and ●, 2.5 mM. [D] = 3 mM, [H_2O_2] = 0.85 mM, [Fe^{2+}] = 0.03 mM and [EDTA] = 0.045 mM. The reaction was carried out in phosphate buffer pH 7.4, at 37°C for 15 min.

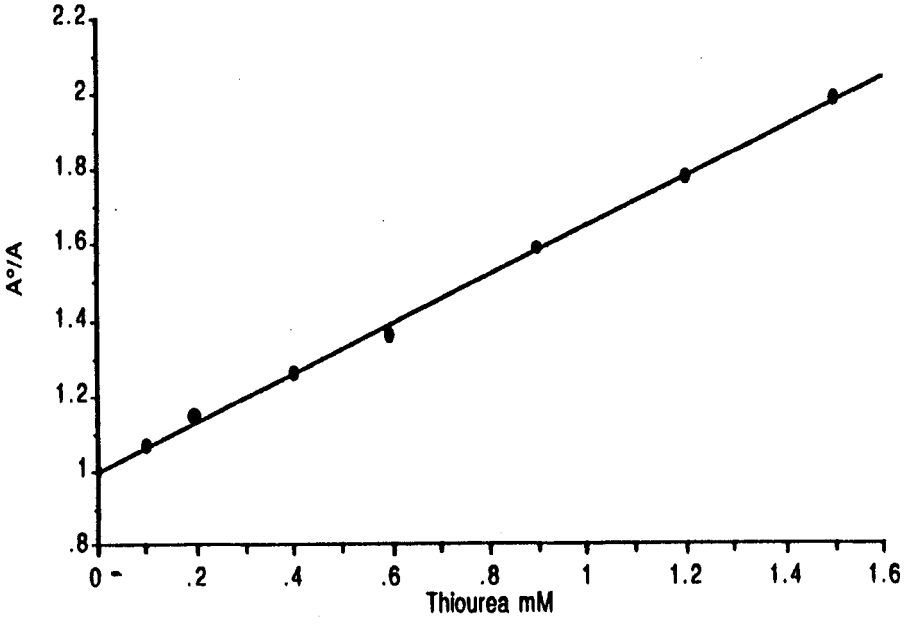

FIGURE 11 Relationship between A°/A and thiourea concentration. [D] = 3 mM, [AA] = 1.5 mM, [H_2O_2] = 0.85 mM, [Fe^{2+}] = 0.03 mM and [EDTA] = 0.045 mM. The reaction was carried out in phosphate buffer pH 7.4 at 37°C for 15 min.

reagents. The rate constant obtained for thiourea under these conditions is 7.6×10^9 $M^{-1}s^{-1}$. Although a rate constant of 3.9×10^9 $M^{-1}s^{-1}$ was reported in literature,[20] determined by pulse radiolysis techniques, it was remarked that this value could be 2 or 3 times larger depending on the experimental conditions. Our value is within the expected range.

Linear plots were also obtained in many similar experiments with other scavengers. Table 3 summarizes the results obtained. It should be noted that in the absence of ascorbic acid, the plots of A°/A vs scavenger concentrations did not give straight lines for the scavengers noted in Table 3, except mannitol, alanine, methionine and N-acetylmethionine (results not shown). For those scavengers whose solubility in water is low, it was preferential to use a low concentration of deoxyribose, in order to make possible the competition for ·OH radicals between deoxyribose and the scavengers. Moreover, a relatively high concentration of ascorbic acid should be used when the scavenger significantly influences the rate of ·OH radical production in the Fenton system, which may be revealed in a nonlinear competition plot. Otherwise, the concentrations of deoxyribose and ascorbic acid were chosen at random. Table 3 shows that all the rate constants obtained for the reactions of ·OH radicals with a wide range of different substances, are very similar to those obtained by pulse radiolysis techniques. Moreover, the rate constants obtained in this study are very close to those obtained in our previous study[12] (in the absence of ascorbic acid) for mannitol (1.8×10^9 $M^{-1}s^{-1}$), methionine (5.1×10^9 $M^{-1}s^{-1}$) and alanin (1.2×10^8 $M^{-1}s^{-1}$). These results as well as those obtained for mercaptoethanol, cysteine and thiourea indicate that the presence of ascorbic acid in the Fenton system effectively suppresses the interference between some scavengers and the Fenton reagents, and consequently simplifies the kinetic competition.

CONCLUSION

The ·OH radical scavengers which react with either Fe^{2+} or H_2O_2 modify the rate of the OH radical generation in the Fenton reaction, resulting in a nonlinear competition

TABLE 3
Rate constants for reactions of substances with hydroxyl radicals: a comparison of results obtained by the deoxyribose assay and by pulse radiolysis.

Experimental Conditions	Substances	$k \times 10^{-9}$ ($M^{-1}s^{-1}$)	
		This Work	Pulse Radiolysis
(a)* [AA] = 0.6 mM [D] = 0.6 mM	p-aminobenzoic acid	8.8	9.9[24]
	dimethylsulfoxide	6.6	7.0[20]
	ethanol	1.9	2.2[20]
	benzamide	3.8	4.6[20]
	4-hydroxybenzoic acid	8.7	9.0[20]
(a)* [AA] = 1.5 mM [D] = 3 mM	mannitol	1.6	1.8[25]
	methanol	0.72	0.78[20]
	alanin	0.26	0.12[23]
	methionine	6.6	6.5[26]
	N-acetylmethionine	6.9	6.7[20]
	benzoate	5.6	5.7[20]

(a)*: The reaction system consisted of H_2O_2 (0.85 mM), Fe^{2+} (0.03 mM), EDTA (0.045 mM) and different concentrations of the substance studied. The reaction was carried out in phosphate buffer, pH 7.4, at 37°C for 15 min.

plot. Owing to its reducing properties, ascorbic acid added in the Fenton system strongly increases the rate of ·OH radical production by regenerating Fe^{2+} from Fe^{3+}. Thus, by regulating the ascorbic acid concentration, it is possible to minimize the interaction observed between some scavengers and the Fenton reagents, to the point that a linear competition plot of A°/A vs scavenger concentrations is obtained with an offset equal to unity. As a result, the Fenton system, in the presence of ascorbic acid, and deoxyribose can be used to measure the rate constants for the reactions of ·OH radicals with scavengers which react with either Fe^{2+} or H_2O_2, such as cysteine, thiourea etc. Moreover, owing to the k_x term presented in the kinetic competition equation, the experimental conditions do not influence the rate constant determination. Consequently, a low concentration of deoxyribose can be used and this renders it possible to determine the rate constants for the reactions of ·OH radicals with those scavengers whose solubility in water is low.

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