REACTION OF HYDROXYL RADICALS WITH THE FERROUS AND FERRIC IRON CHELATES OF DIETHYLENETRIAMINE-N,N,N′,N″,N″-PENTAACETATE

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Reactions of the hydroxyl radical with ferrous and ferric diethylenetriamine-N,N,N′,N″,N″-pentaacetate (DTPA) complexes in neutral aqueous solution were studied using a ^{60}Co-γ radiolysis source for radical production. Hydroxyl radicals oxidize the ferrous chelate of DTPA to the ferric chelate, as determined by changes in the optical absorbance. In the presence of equimolar concentrations of ferric and ferrous chelates, HO· is "trapped" by Fe(III)DTPA²⁻ and yields of Fe(III)DTPA²⁻ are substantially reduced. Both *tert*-butyl alcohol and methanol react efficiently with hydroxyl radicals. *tert*-Butyl alcohol produces unreactive radicals, and is thus an effective scavenger. However, the reducing hydroxymethyl radical produced upon reaction of HO· with methanol effectively reduces Fe(III)DTPA²⁻ present in the system, so that solutions initially containing the more absorbing Fe(III)DTPA²⁻ are bleached. These results with alcoholic hydroxyl radical scavengers illustrate possible complications from scavengers such as methanol which produce reactive products which may themselves stimulate further reaction.

KEY WORDS: Hydroxyl radicals, hydroxyl radical scavengers, *tert*-butyl alcohol, methanol, iron chelates, diethylenetriamine-N,N,N′,N″,N″-pentaacetic acid (DTPA).

INTRODUCTION

Chain reactions involving low-molecular weight chelates of iron and oxygen radicals are believed to play an important role in reperfusion tissue damage following ischemia.[1,2] It has been found that victims of cardiac arrest experience a second phase of neural- and cardiac-tissue damage following re-establishment of blood flow: a contributing mechanism appears to be lipid peroxidation via an oxygen radical mechanism requiring metal ions. Biological mechanisms for the generation during ischemia of low molecular weight chelates of iron, and for the production of hydrogen peroxide, have been found and point to Fenton-type reactions as mediators of the lipid peroxidation. A major point of interest is the nature of the reactive species produced in these Fenton reactions at neutral pH: is the hydroxyl radical produced as in acid pH, or does some other species result? As early as 1932, Bray and Gorin[3] proposed the heterolysis of the peroxy bond leading to a ferryl ion species:

$$Fe^{II} + H_2O_2 \rightarrow (Fe^{IV}O)^{2+} + H_2O \tag{1}$$
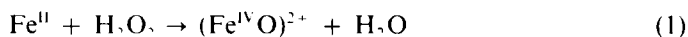
Walling and Amarnath[4] have proposed formation of an Fe^{IV} intermediate, $(Fe^{IV}OH)^{3+}$, which can dissociate in simple acid aquoiron solution giving hydroxyl

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radical, but which could be sufficiently stabilized by suitable ligands in non-acid solution to become the principal oxidant:

$$Fe^{II} + H_2O_2 \xrightarrow{-HO^-} (Fe^{IV}OH)^{3+} \rightleftarrows Fe^{III} + HO\cdot \qquad (2)$$
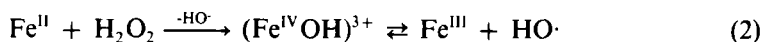
Recent studies of reactions of hydrogen peroxide with polyaminocarboxylate chelates of ferrous iron by Koppenol and Rush,[5,6,7] Winterbourn and Sutton,[8,9,10] and by Richter and Rahhal[11,12] have produced abundant evidence that these Fenton-type reactions in neutral solution produce iron-oxo species, rather than free hydroxyl radical.

To correctly interpret results of model studies involving iron chelates, knowledge of reactions of the chelates themselves with oxygen radicals is needed. Butler and Halliwell[13] and Buettner, Doherty, and Patterson[14] have shown that Fe(III)DT-PA^{2-}(DTPA: diethylenetriamine-N,N,N′,N″,N″-pentaacetate) reacts slowly, if at all, with the superoxide radical ($O_2^-\cdot$). On the other hand, Fe(III) EDTA$^1-$ (EDTA: ethylenediamine-N,N,N′,N′-tetraacetate) and Fe(III)HEDTA (HEDTA: N-hydroxyethylenediamine-N,N′,N′-triacetate) react rapidly[14] with O_2^-. Fe(II)DTPA^{3-} reacts with $O_2^-\cdot$ with a bimolecular rate constant[13] of $2 \times 10^7 M^{-1} s^{-1}$; however, a complex probably is formed as has been observed for EDTA.[13,15,16] The rate of the oxidation of the ferrous DTPA complex by hydroxyl radical has been studied by pulse radiolysis.[17] The degradation of the ligand in Fe(III)EDTA$^1-$ via HO$\cdot$ attack has been examined by ^{60}Co-γ radiolysis of neutral solutions.[18]

This paper reports studies of the reaction of the hydroxyl radical with ferrous and ferric chelates of diethylenetriamine-N,N,N′,N″,N″-pentaacetate (Fe(II)DTPA^{3-} and Fe(III)DTPA^{2-}) using ^{60}Co-γ radiation to produce the hydroxyl radicals and optical absorption spectrophotometry to follow the oxidation of Fe(II)DTPA^{3-} to Fe(III)DTPA^{2-}. Solutions containing ferrous or ferrous *and* ferric chelates of DTPA were studied.

EXPERIMENTAL MATERIALS AND METHODS

Ferrous ion was added as ferrous ammonium sulfate (Fe(NH$_4$)(SO$_4$)$_2 \cdot 6H_2O$), and ferric ion as ferric ammonium sulfate (Fe(NH$_4$)(SO$_4$)$_2 \cdot 12H_2O$)). Iron salts were reagent grade from Baker. Diethylenetriamine-N,N,N′,N″,N″-pentaacetic acid was reagent grade from Fluka. All other compounds were reagent grade and used as received. Nitrous oxide was atomic absorption grade from Linde. Water used in preparing reaction solutions was first glass-distilled and then passed through a Millipore Milli-Q reagent grade water system.

Samples were irradiated in gas-tight syringes with Teflon seals (25 mL, Spectrum Medical Industries) in a Gamma-Cell ^{60}Co-γ source whose activity was determined using the Fricke dosimeter.[19] At the measured dose rate, a species with G = 6.0 would be produced at the rate of $7.99 \times 10^{-8} M s^{-1}$. All ferrous solutions were prepared shortly before use and extensive precautions were taken to minimize exposure to oxygen of the extremely air-sensitive complex. Following irradiation, the sample absorbance at 200 nm was read by flushing the solution through a micro flow cell (NSG Precision Cells, Inc., Type 72) in a Cary 17 UV-VIS spectrophotometer. Absorbance of the unirradiated solution was determined for comparison. The ferric chelate has a larger molar absorbtivity at 260 nm ($\varepsilon_3 = 9250 M^{-1} cm^{-1}$), and $\Delta\varepsilon = \varepsilon_3 - \varepsilon_2 = 8890 M^{-1} cm^{-1}$ (260 nm is the λ for maximum $\Delta\varepsilon$).
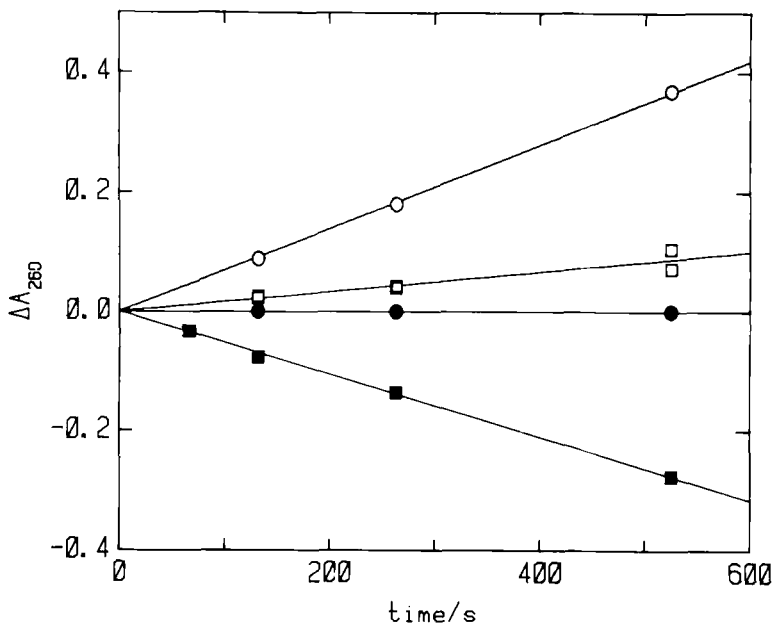
FIGURE 1   Dependence of the yield of Fe(III)DTPA^2 on total concentration of hydroxyl radical produced in ^{60}Co-γ irradiation experiments. All solutions contained 5 mM phosphate buffer at pH 7.0, and were saturated with N_2O. Iron ions and ligand were present in equal concentrations. (○) [Fe(II)DT-PA^{3-}]$_0$ = 500 μM, [MeOH] = 0; (●) [Fe(II)DTPA^3]$_0$ = 500 μM, [MeOH] = 0.6 M; (□) [Fe(II)DT-PA^{3-}]$_0$ = [Fe(III)DTPA^2]$_0$ = 100 μM,   [MeOH] = 0;   (■)   [Fe(II)DTPA^3]$_0$ = [Fe(III)DT-PA^2]$_0$ = 100 μM, [MeOH] = 0.6 M.

RESULTS AND DISCUSSION

The reactions of the hydroxyl radical with ferrous and ferric DTPA complexes in aqueous solution were studied by producing HO· radiolytically in a ^{60}Co-γ source. Two types of experiments were done: solutions contained either Fe(II)DTPA^3 or Fe(II)DTPA^{3-} and Fe(III)DTPA^2. The effects of the hydroxyl radical scavengers tert-butyl alcohol and methanol on the reaction of HO· with the chelates were determined. Solutions containing the iron chelate (or chelates) and phosphate buffer at pH 7.0 were saturated with N_2O and irradiated. Sample absorbances were determined before and after irradiation to determine the concentration of Fe(II)DTPA^{2-} formed in the irradiation. In all experiments, the yield of Fe(III)DTPA^{2-} was a linear function of irradiation time, Figure 1.

Reaction of Hydroxyl Radical with the Ferrous Chelate of DTPA

Irradiation of solutions of Fe(II)DTPA^3 saturated with N_2O should give the reactions in Scheme 1. The production of hydroxyl radicals and solvated electrons from water radiolysis and the subsequent conversion of the solvated electron to
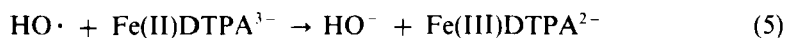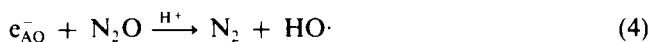
SCHEME 1 $H_2O \rightarrow HO·, e_{AQ}^-, H$ (3)

$$e_{AQ}^- + N_2O \xrightarrow{H^+} N_2 + HO·$$ (4)

$$HO· + Fe(II)DTPA^{3-} \rightarrow HO^- + Fe(III)DTPA^{2-}$$ (5)

TABLE I

G-values for production of Fe(III)DTPA^{2-} in ^{60}Co-γ radiolysis experiments.[a]

[Fe(II)DTPA^{3-}]$_0$ (μM)	[Fe(III)DTPA^{2-}]$_0$ (μM)	[ROH] (M)	G(Fe(III)DTPA^{2-})[b]
500	0	0	5.90
100	100	0	1.42
500	0	0.3[c]	0.23
500	0	0.6[d]	0.00
100	100	0.6[d]	−4.45

[a] Other experimental conditions are given in the legend of Figure 1.
[b] G-Values obtained from the slopes of lines in Figure 1, except experiment with *tert*-butyl alcohol.
[c] *tert*-Butyl alcohol
[d] Methanol

hydroxyl radical are rapid, well-known reactions.[20] Lati and Meyerstein[17] have shown that reaction of Fe(II)EDTA^{2-} with HO$\cdot$ proceeds via oxidation of the ferrous iron to Fe(III), rather than oxidation of the ligand. Presumably, the reaction with Fe(II)DTPA^{3-} would follow the same path, so that the observed product of reaction 5 would be Fe(III)DTPA^{2-}. Considering the high reactivity of the hydroxyl radical with ligands such as DTPA,[17] it is to be expected that the initial product of reaction 5 is a chelate with oxidized ligand (Fe(II)DTPA^{2-}) and that rapid intramolecular electron transfer gives the observed ferric chelate.

The difference in the absorbances of the irradiated (A$_1$) and unirradiated (A$_U$) samples were determined. Since

$$A_U = \varepsilon_2[Fe(II)DTPA^{3-}]_0$$

and

$$A_I = \varepsilon_2([Fe(II)DTPA^{3-}]_0 - [Fe(III)DTPA^{2-}]) + \varepsilon_3[Fe(III)DTPA^{2-}]$$

it follows that

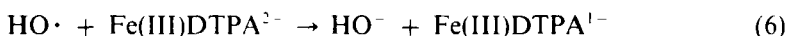$$\Delta A = (\varepsilon_3 - \varepsilon_2)[Fe(III)DTPA^{2-}].$$

Using the above expression for ΔA, the Fricke dosimeter result, and $\Delta\varepsilon$ for the ferric/ferrous species, one obtains G(Fe(III)DTPA^{2-}) = 5.90, where the G-value is the number of radicals formed per 100 eV of energy absorbed, Table I. G-values of hydroxyl radical, solvated electron, and hydrogen atom from the radiolysis of dilute aqueous solutions are 2.7, 2.6, and 0.6, respectively.[20] In nitrous oxide solution, where e$_{AQ}^-$ is converted to HO$\cdot$, one obtains G(HO$\cdot$) = 5.3. The G(Fe(III)DTPA^{2-}) obtained in these experiments is only slightly larger than the value of 5.3 expected when all HO$\cdot$ oxidize ferrous chelate and H atom does not affect the yield, in agreement with the mechanism in Scheme 1.
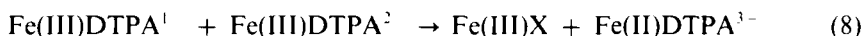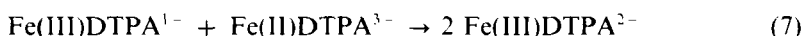
Reactions of the Hydroxyl Radical with Ferrous and Ferric Chelates of DTPA

In the experiments of the previous section, the maximum ratio of Fe(II)DTPA^{3-} to Fe(III)DTPA^{2-} was 11:1, so that reactions of HO$\cdot$ with Fe(III)DTPA^{2-} were minimized. However, in a variety of systems of interest, concentrations of ferric and ferrous chelates are comparable. Thus, experiments were done in which Fe(III)DTPA^{2-} was added initially in equimolar concentration with Fe(II)DTPA^{3-}. Irradiation of these solutions should give the reactions in Scheme 1 and reaction 6. Scavenging of e$_{AQ}^-$ by Fe(III)DTPA^{2-} (competing with N$_2$O, reaction 4) is not included since it

accounts for only 1.6% of e_{AQ}^- at the highest [Fe(III)DTPA^{2-}] used. The bimolecular rate constant[20] for reaction 4 is 9.1 $\times$ 10^9 M^{-1} s^{-1}, while k_{BI} for solvated electron[15] plus Fe(III)EDTA^{1-} is 3 $\times$ 10^{10} M^{-1} s^{-1}; however, [N$_2$O] is 21 mM at 25°C while the maximum [Fe(III)DTPA^{2-}] was 0.10 mM. Fe(III)DTPA^1 represents the radical produced by HO · attack on the ligand of the ferric complex, the expected reaction.[17,18] Possible fates for Fe(III)DTPA^{1-} are: reduction by

$$HO\cdot\ +\ Fe(III)DTPA^{2-}\ \rightarrow\ HO^-\ +\ Fe(III)DTPA^{1-} \tag{6}$$

Fe(II)DTPA^{3-} to give two Fe(III)DTPA^2, reaction 7; oxidation by Fe(III)DTPA^{2-} to yield a ferric ion chelated with oxidized DTPA, reaction 8 (X represents the product of two electron oxidation of DTPA); and intramolecular electron transfer to yield a ferrous ion chelated with oxidized DTPA, reaction 9. If reaction 6 were followed by

$$Fe(III)DTPA^{1-}\ +\ Fe(II)DTPA^{3-}\ \rightarrow\ 2\ Fe(III)DTPA^{2-} \tag{7}$$

$$Fe(III)DTPA^1\ +\ Fe(III)DTPA^2\ \rightarrow\ Fe(III)X\ +\ Fe(II)DTPA^{3-} \tag{8}$$

$$Fe(III)DTPA^{1-}\ \rightarrow\ Fe(II)X \tag{9}$$

reaction 7, the effect of reaction 6 would be neutralized and ΔA, and hence G(Fe(III)DTPA^{2-}), would be the same whether or not Fe(III)DTPA^{2-} were initially added, i.e., each HO · would yield one Fe(III)DTPA^{2-}. If reaction 6 were followed by reaction 8, ΔA would be decreased by addition of [Fe(III)DTPA^{2-}], provided the spectrum of Fe(III)X is not too different from that of Fe(III)DTPA^{2-}: consumption of HO · via reactions 6 and 8 results in reduction of a Fe(III)DTPA^{2-}, instead of the oxidation of a Fe(II)DTPA^{3-} which occurs when HO · is consumed via reaction 5, giving a net loss of two Fe(III)DTPA^2 which would have been present if Fe(III)DTPA^{2-} had not been initially added. The result of reaction 6 followed by reaction 9 is essentially the same as 6 followed by 8 (assuming that the molar absorbtivity of Fe(II)X is not too different from that of Fe(II)DTPA^{3-}): addition of Fe(III)DTPA^2 to the initial solution would reduce ΔA since a potential Fe(III)DTPA^{2-} is not produced, and one Fe(III)DTPA^2 is reduced.

The experimental observation was that ΔA *was reduced* to 24% of the value seen when no Fe(III)DTPA^{2-} was initially added, Figure 1, giving a G(Fe(III)DTPA^{2-}) of only 1.42. Thus, it is clear that the Fe(III)DTPA^1 radicals produced in reaction 6 are not reduced by Fe(II)DTPA^3, reaction 7. In summary, HO · radicals are "trapped" by Fe(III)DTPA^{2-} and the yield of oxidation of Fe(II)DTPA^{3-} is reduced substantially. In the presence of initially added Fe(III)DTPA^{2-}, the mechanism for reaction of hydroxyl radical with ferrous and ferric chelates of DTPA is Scheme 1 plus reactions 6 and 8 (or 9).

The relative rates of reaction of HO · with Fe(II)DTPA^3 and Fe(III)DTPA^{2-} can be estimated from these data. If the percent of HO · reacting with Fe(II)DTPA^{2-} is denoted by x, then G(Fe(III)DTPA^2)) in the presence of equimolar ferrous and ferric chelates will be (100–2x) percent of the value observed in the absence of added ferric chelate, since one potential Fe(III)DTPA^2 is lost and one Fe(III)DTPA^2 is reduced (via reaction 6 followed by 8) each time HO · reacts with the ferrous chelate. Since this value is 24%, we obtain the result that 62% of the HO · react with Fe(II)DTPA^{3-}, while 38% react with Fe(III)DTPA^2. This 2:1 ratio is in reasonable agreement with the relative rates of reaction[17,18] of HO · with Fe(II)EDTA^{2-} and Fe(III)EDTA^{1-}, i.e., a ratio of 5:1. This estimate is limited by the assumption that the iron chelates with X (two-electron oxidized DTPA) have molar absorbtivities the same as the chelates with DTPA.
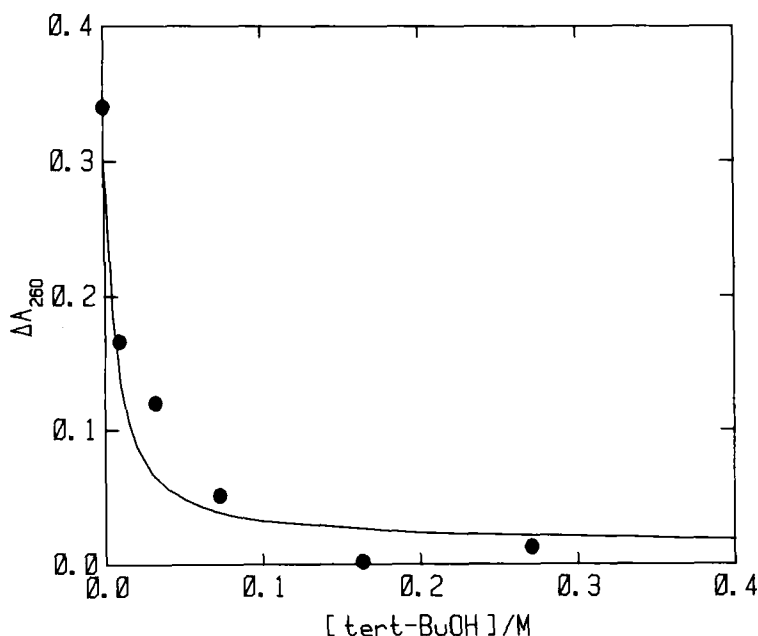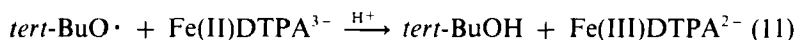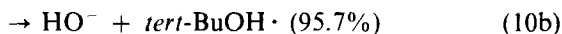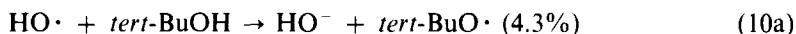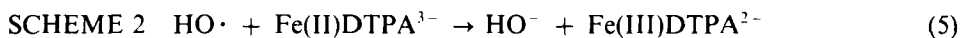
FIGURE 2 Protection of Fe(II)DTPA^{3-} from oxidation via hydroxyl radical by the addition of *tert*-BuOH, as determined in ^{60}Co-γ radiolysis experiments. The solid line is the calculated ΔA_{260} based on the rate constants given in the text. [Fe(II)DTPA^{3-}]$_0$ = 500 μM. The data are corrected for a small ΔA (0.02) which resulted from oxygen contamination of the samples during manipulation and irradiation.

Scavenging of Hydroxyl Radicals by tert-Butyl Alcohol

tert-Butyl alcohol is a celebrated scavenger of HO$\cdot$, and is used routinely in radiation chemistry to convert this reactive radical to unreactive *tert*-BuOH radicals. The effectiveness of *tert*-butyl alcohol in scavenging HO$\cdot$ in the presence of iron chelates was determined by measuring the decrease in the yield of Fe(III)DTPA^{2-} with increasing [*tert*-BuOH] in the irradiation of Fe(II)DTPA^{3-} solutions, Figure 2. The expected mechanism includes reactions 3 and 4, followed by Scheme 2.
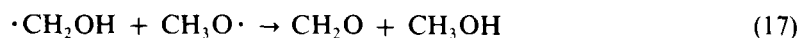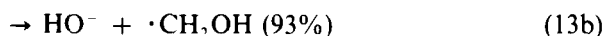
$$\text{SCHEME 2} \quad \text{HO}\cdot + \text{Fe(II)DTPA}^{3-} \rightarrow \text{HO}^- + \text{Fe(III)DTPA}^{2-} \tag{5}$$

$$\text{HO}\cdot + \textit{tert}\text{-BuOH} \rightarrow \text{HO}^- + \textit{tert}\text{-BuO}\cdot \ (4.3\%) \tag{10a}$$

$$\rightarrow \text{HO}^- + \textit{tert}\text{-BuOH}\cdot \ (95.7\%) \tag{10b}$$

$$\textit{tert}\text{-BuO}\cdot + \text{Fe(II)DTPA}^{3-} \xrightarrow{\text{H}^+} \textit{tert}\text{-BuOH} + \text{Fe(III)DTPA}^{2-} \tag{11}$$

$$\textit{tert}\text{-BuOH}\cdot \rightarrow \text{products} \tag{12}$$

Fe(II)DTPA^{3-} and *tert*-BuOH compete for HO$\cdot$ in reactions 5 and 10, with rate constants of 5.0 $\times$ 10^9 (for Fe(II)EDTA) and 6.0 $\times$ 10^8 M^{-1} s^{-1}, respectively.[17,20] When *tert*-BuOH is oxidized by the hydroxyl radical, 4.3% of the product radicals are peroxy radicals which can oxidize the Fe(II)DTPA^{3-}, reaction 11. The remaining carbon-centered radicals (95.7% of the total) are highly stable and are unlikely to oxidize the Fe(II)DTPA^{3-}.[21] The observed dramatic decrease in the yield

of Fe(III)DTPA^{2-} with increasing alcohol concentration agrees quantitatively with the predicted mechanism, as seen by the agreement between the data points and the calculated curve in Figure 2. Thus, *tert*-BuOH is an effective inhibitor of reaction 5, and will strongly inhibit the production of Fe(III)DTPA^{2-} via oxidation of Fe(II)DTPA^{3-} by HO˙

Eberhardt[22] has reported that the carbon-centered *tert*-butyl alcohol radical reacts with Fe(II)EDTA^{2-} in *acidic solution* by reductive elimination to yield olefins. Clearly, this reaction does not occur with Fe(II)DTPA^3 in *neutral solution* since G(Fe(III)DTPA^{2-}) *decreases dramatically* when *tert*-BuOH is added, in quantitative agreement with Scheme 2. Whether this arises from the different protonation of the chelates in acidic vs. neutral solution, or from differences in the EDTA and DTPA chelates is not clear.

Scavenging of Hydroxyl Radicals by Methanol

Methanol is known to react rapidly with the hydroxyl radical ($k_{Bi} = 9.7 \times 10^8$ M^{-1} s^{-1})[20] yielding a reducing radical (˙CH$_2$OH, 93%) and an oxidizing radical (CH$_3$O˙, 7%).[21] Thus, methanol and *tert*-butyl alcohol react with HO˙ with equal efficiency; however, the radicals produced in the reaction of HO˙ with methanol are much more reactive than the major radical produced from *tert*-BuOH. To study the effects of scavenging of HO˙ by MeOH in the presence of iron ions chelated with DTPA, two types of ^{60}Co-γ irradiation experiments were done: solutions containing only the ferrous chelate, and solutions containing both the ferrous and ferric chelates were irradiated. With sufficient [MeOH], HO˙ reacts with the alcohol instead of the ferrous iron and reactions 3 and 4 followed by Scheme 3 are anticipated. Fe(III)DTPA^{2-}

SCHEME 3 $\quad$ HO˙ + MeOH → HO$^-$ + CH$_3$O˙ (7%) $\hspace{2cm}$ (13a)

$$\to \text{HO}^- + \cdot\text{CH}_2\text{OH (93\%)} \hspace{2cm} (13\text{b})$$

$$\text{CH}_3\text{O}\cdot + \text{Fe(II)DTPA}^{3-} \xrightarrow{\text{H}^+} \text{CH}_3\text{OH} + \text{Fe(III)DTPA}^{2-} \hspace{0.5cm} (14)$$

$$\cdot\text{CH}_2\text{OH} + \text{Fe(III)DTPA}^{2-} \to \text{CH}_2\text{O} + \text{Fe(II)DTPA}^{3-} + \text{H}^+ \hspace{0.3cm} (15)$$

$$\cdot\text{CH}_2\text{OH} + \cdot\text{CH}_2\text{OH} \to (\text{CH}_2\text{OH})_2 \hspace{2cm} (16)$$

$$\cdot\text{CH}_2\text{OH} + \text{CH}_3\text{O}\cdot \to \text{CH}_2\text{O} + \text{CH}_3\text{OH} \hspace{2cm} (17)$$
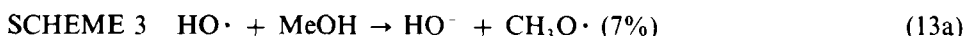
produced via oxidation of Fe(II)DTPA^{3-} by the methoxy radical, reaction 14, could be reduced by the hydroxymethyl radical, reaction 15, so that no absorbance change would be seen. This expectation was confirmed in the irradiations of the solutions containing only the ferrous chelate: there was no absorbance change when methanol was added to the solutions, Figure 1. When both Fe(II)DTPA^{3-} and Fe(III)DTPA^{2-} are initially present, the predominant radical reaction with iron complexes is expected to be reduction of Fe(III)DTPA^{2-} by ˙CH$_2$OH, reaction 15. The rate constant for this reaction in acidic pH, with ligands other than DTPA, has been reported to be about 1×10^8 M^{-1} s^{-1}.[23,24] Methanol prevents formation of Fe(III)DTPA^{2-} via reaction 5, and the hydroxymethyl radical produced in reaction 13 reduces Fe(III)DTPA^{2-} which was initially present. Thus, a net loss of Fe(III)DTPA^{2-} would result from scavenging of HO˙ by MeOH. Since essentially all the HO˙ radicals react with MeOH, the G-values for the hydroxymethyl and methoxy radicals will be 4.93 and

0.37, respectively. Thus, if all methanol radicals react via reactions 14 and 15, $G(Fe(III)DTPA^{2-}) = -4.56$ would be expected. Experimentally, bleaching of the sample (negative values of ΔA_{260}) was observed, with $G(Fe(III)DTPA^{2-}) = -4.45$. Thus, most of the methanol radicals react via reactions 14 and 15, dramatically reducing the concentration of $Fe(III)DTPA^{2-}$.

In conclusion, in experiments where hydroxyl radical is produced, methanol efficiently scavenges $HO\cdot$ preventing formation of new $Fe(III)DTPA^{2-}$, and a major portion of the product hydroxymethyl radicals reduces $Fe(III)DTPA^{2-}$ present in the system. In all cases, addition of methanol to a reaction system will reduce the yield of $Fe(III)DTPA^{2-}$ when it is produced from the oxidation of $Fe(II)DTPA^{3-}$ by the hydroxyl radical. In general, the radicals produced from methanol are highly reactive. For example, methanol radicals play a crucial role in chain reactions which lead to the radiation induced oxidation of methanol by hydrogen peroxide.[25] In the oxidation of ferrous chelates of DTPA by hydrogen peroxide, methanol strongly stimulates the reaction in $Fe(II)DTPA^{3-}$ excess experiments, probably through a chain mechanism.[16]

SUMMARY

Hydroxyl radicals oxidize the ferrous chelate of DTPA to the ferric chelate, as shown by the essentially stoichiometric conversion of Fe(II)DTPA to Fe(III)DTPA in ^{60}Co-γ radiolysis experiments. In the presence of equimolar concentrations of ferric and ferrous chelates, $HO\cdot$ is "trapped" by $Fe(III)DTPA^{2-}$ and yields of $Fe(III)DTPA^{2-}$ are substantially reduced. Results indicate that the ligand radical formed by attack of $HO\cdot$ upon $Fe(III)DTPA^{2-}$ is oxidized by $Fe(III)DTPA^{2-}$ producing a ferric ion chelated with a two-electron oxidized DTPA molecule, reaction 8.

Both *tert*-butyl alcohol and methanol scavenge hydroxyl radicals effectively. Radicals produced from *tert*-butyl alcohol do not oxidize any additional ferrous chelates, and thus simply protect $Fe(II)DTPA^{3-}$ from oxidation. The reducing hydroxymethyl radical produced upon reaction of $HO\cdot$ with methanol (93% of the product radicals) effectively reduce $Fe(III)DTPA^{2-}$ present in the system, so that solutions initially containing the more absorbing $Fe(III)DTPA^{2-}$ are bleached. These results with alcoholic hydroxyl radical scavengers point out possible complications from use of scavengers which produce reactive products when scavenging $HO\cdot$. The reactive products may themselves stimulate further reaction and invalidate attempts to interpret experimental results. Thus, *tert*-butyl alcohol is in general a suitable scavenger for hydroxyl radicals, while methanol may lead to system complications.

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