extracted into 15 mL of 1:1 phenol–chloroform. Ether (150 mL) was added to precipitate the organocobalamin. The pink solid was recovered by centrifugation, washed repeatedly with ether, and dried under vacuum. Yield, 0.15 g (0.11 mmol, 56% based on B_{12_a}). UV–visible, λ_{max} ($10^{-3}\epsilon_{max}$): pH 6.8, 520 (8.41), 368 (10.80), 355 (13.67).

((Tetrahydro-2H-pyryl)methyl)cobalamin. B_{12_a} (0.25 g, 0.19 mmol) was dissolved in 10 mL of methanol. The resulting cherry red solution was saturated with N_2, and 0.05 g of $NaBH_4$ (0.13 mmol) was added to produce a dark green solution of B_{12_s}. Addition of 0.03 mL of 2-(bromomethyl)tetrahydro-2H-pyran resulted in an immediate color change to pinkish red. After 1 h of stirring under N_2 to complete the reaction, the solution was mixed with 100 mL of water, and the organocobalamin was extracted into 15 mL of 1:1 phenol–chloroform. Ether (150 mL) was added to the phenol–chloroform layer to precipitate the organocobalamin. The pink-red solid was recovered by centrifugation and washed with ether. The product was dried in vacuo overnight. UV–visible, λ_{max} ($10^{-3}\epsilon_{max}$): pH 6.8, 521 (6.76), 372 (9.02), 340 (11.72).

The ^1H NMR spectra of the cobalamins are detailed in Table V. Chemical shift assignments are based on comparisons with the published spectra of ethylcobalamin[27] and (5'-deoxyadenosyl)cobalamin[28] and on the pH dependencies of the resonances.

Organobis(dimethylglyoximato)cobalt(III) Complexes ([R-Co(DH)$_2$]). Dimethyl sulfide adducts of neopentyl-Co(DH)$_2$ and c-$C_5H_9CH_2$-Co-(DH)$_2$ were prepared by literature procedures.[29] The dimethyl sulfide ligands were removed by dissolving the adducts in N_2-saturated water and evaporating to dryness under vacuum.

Solvents and Buffers. Buffer solutions (0.1 M) for UV–visible spectral studies were prepared by using CH_3COOH–CH_3COONa for pH 3–5 and K_2HPO_4–KH_2PO_4 for pH 6–8 in deionized distilled water that was previously boiled and stored under N_2. Buffer solutions (0.1 M) for ^1H NMR spectral studies were prepared with CD_3COOD–CD_3COONa for pD 3–5 and K_2HPO_4–KH_2PO_4 for pD 6–8 in 99.8% D_2O. Ethylene glycol was purified by a literature procedure.[30]

Identification and Quantification of Thermolysis Products by ^1H NMR. Samples for ^1H NMR analysis were prepared in a flask equipped with a high-vacuum valve and a side arm. After the desired quantity of the solid compound was weighed and dissolved in N_2-saturated D_2O buffer solution, the valve was closed and the flask placed in a thermostated oil bath (±0.5 °C). The reaction mixture was cooled and sampled periodically under a N_2 atmosphere. Subsequently, the sample was oxidized by air, and a known concentration of internal reference (TSP-d_4) was

added. The thermolysis products were identified by comparison with the ^1H NMR spectra of authentic compounds and quantified by integration and comparison with the internal reference.

Kinetics Measurements. Samples for kinetic studies were prepared in a 1-cm Pyrex glass cell equipped with a high-vacuum valve and a side arm. The desired quantity of the solid compound was dissolved in the N_2-saturated aqueous buffer solution or ethylene glycol in the cell. The cell was closed and placed in the thermostated cell compartment of the UV–visible spectrophotometer or (with concordant results) in a thermostated oil bath. In the former case, the disappearance of the alkylcobalamins and appearance of B_{12_r} were continuously monitored by measuring the absorbance at 650 nm. (No decomposition was observed when organocobalamin solutions were irradiated at this wavelength for 24 h at 25 °C.) In the latter case, the cell was removed from the oil bath periodically, cooled immediately, and placed in the thermostated cell compartment (25 °C), and the absorbance at 650 nm was recorded. These operations were performed in the dark.

The kinetic measurements were performed under pseudo-first-order conditions and the reactions followed for at least 3 half-lives. First-order rate constants were computed by linear least-squares regression.

Base On–Base Off Equilibrium Constants. Alkylcobalamin solutions (1×10^{-4} to 2×10^{-4} M) were prepared as for the kinetic measurements. Spectra were recorded over the pH range 2–7 and pK_a values, defined by eq 9, were determined by fitting spectral titration curves at ca. 522 nm. Values of $\Delta H'$ and $\Delta S'$ listed in Table I were determined by linear least-squares regression fitting of the temperature dependence of the absorbance (A_{obsd}) at 522.5 nm (520 nm in the case of ethylene glycol) to the equation

$$\ln\left[(A_a - A_{obsd})/(A_{obsd} - A_0)\right] = -\Delta H'/RT + \Delta S'/R \qquad (22)$$

The absorbance of the base-off form (A_0) was assumed to be that of the limiting protonated form, i.e., $[\overline{Co\ NH^+}]$, at low pH. The absorbance of the base-on form (A_a) was determined from the fitting procedure. The measured absorbances were corrected for thermal expansion of the solutions. Values of $(k_1)_{corr}$ were computed according to eq 11.

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Reduction of Hydrogen Peroxide by the Ferrous Iron Chelate of Diethylenetriamine-*N,N,N',N'',N''*-pentaacetate

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Abstract: Ferrous iron complexed with diethylenetriamine-*N,N',N'',N''*-pentaacetate (DTPA) reacts with hydrogen peroxide in neutral aqueous solution at room temperature to yield an oxidizing species that is not scavenged by *tert*-butyl alcohol, under conditions where >90% of hydroxyl radical would be scavenged. Further, the oxidizing species reacts with $Fe^{II}DTPA^{3-}$ and H_2O_2 at virtually the same rate, in contrast to the known behavior of the hydroxyl radical. These observations, the effects of methanol on the reaction, and the kinetic and stoichiometric results of the experiments clearly establish that *the oxidizing species produced is not the hydroxyl radical,* but an iron–oxo species such as the ferryl ion. The oxidizing species is formed in an apparent first-order reaction, when either hydrogen peroxide or $Fe^{II}DTPA^{3-}$ is in kinetic excess. The bimolecular reaction rate constant is $(1.37 \pm 0.07) \times 10^3\ M^{-1}\ s^{-1}$.

The reduction of hydrogen peroxide by iron chelates is a well-known reaction.[1] In acid solution, it appears that the reduction produces the hydroxyl radical (HO•) (eq 1). This is the

$$Fe(II) + H_2O_2 = Fe(III) + HO^- + HO^\bullet \qquad (1)$$
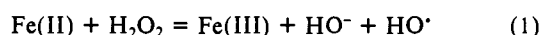
key reaction in the production of hydroxylated products by Fenton's reagent in strongly acid solutions. The rate in acid solution has been measured.[2-8] Acid solution values have been

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quoted on many occasions for reactions in neutral solution, since neutral values were unavailable. This is clearly unsatisfactory since in acid solution the iron is coordinated with water molecules, while in neutral solution other ligands are required to prevent precipitation of ferric hydroxide.

There is currently a great deal of interest in the reduction of H_2O_2 by iron complexes in neutral solution, especially in view of recent discoveries on post-reflow tissue damage following ischemia.[9] It has been found that victims of cardiac arrest experience a second phase of neural- and cardiac-tissue damage following reestablishment 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. Two points are of interest: (1) what are the rates of reduction by different iron chelates—ligands that produce low rates of reduction are possible therapeutic agents in post-ischemic treatments—and (2) what is the species produced when the peroxide is reduced—is it hydroxyl radical, the ferryl ion, or some other species?

Limited work has been done on the direct measurements of the reaction between H_2O_2 and ferrous iron in neutral or near-neutral solution.[10-12] The participation of the reaction in the catalytic decomposition of H_2O_2 in neutral solution has been studied extensively.[1,13,14] Oakes and co-workers have examined in detail the decomposition of H_2O_2 catalyzed by *ferric* chelates of ethylenediamine-N,N,N',N'-tetraacetate (EDTA).[15] The identity of the product in neutral solution has been the subject of much debate—biological and chemical evidence points in several directions.[16] Some results indicate that $HO^{\bullet}$ is produced,[13-15,17-20] while others say that a species other than $HO^{\bullet}$ is produced.[21-23] Walling and co-workers[13,14] have discussed the production and reaction of "caged" hydroxyl radicals, trapped by the metal chelate immediately after formation. Recent work of Stubbe and co-
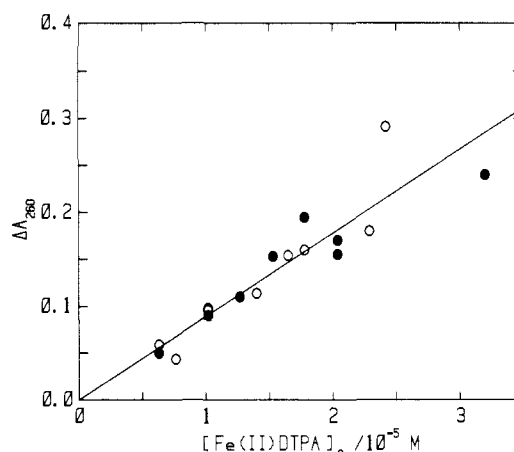

Figure 1. Dependence of the yields of $Fe^{III}DTPA^{2-}$ on $[Fe^{II}DTPA^{3-}]_0$ in the H_2O_2-excess experiments. (●) [t-BuOH] = 0.6–1.4 M; (O) t-BuOH absent. The solid line is the linear least-squares fit of the data to the equation $y = bx$; i.e., the y intercept is set at the origin.

workers[24] has shown site-specific cleavage of DNA by iron chelates of bleomycin (BLM), via Fe(III)–H_2O_2–BLM or Fe(II)–O_2–BLM: the cleavage is accomplished by "activated" BLM and does not involve hydroxyl radical. In contrast, Dervan and co-workers[25] observed site-specific cleavage of DNA with methidium propyl-EDTA–iron(II) in the presence of O_2, enhanced by reducing agents: it was concluded that the most likely active species was the hydroxyl radical.

High-valence iron species have been proposed in several instances.[26] The ferryl ion has been proposed as the reactive intermediate in peroxidases and cytochromes P-450. Iron–oxo species with the iron in high oxidation states have been characterized spectroscopically. Balch and co-workers[27] have obtained nuclear magnetic resonance spectra of transient synthetic ferryl porphyrin complexes at low temperatures. Groves and co-workers[28] have demonstrated the formation of an oxoiron(IV) porphyrin cation radical, related to compound I of horseradish peroxidases. Ferryl myoglobin has been characterized by ESR,[29] Mössbauer,[30] and resonance Raman spectroscopy.[31]

In this paper we report an investigation of the rates of reduction of H_2O_2 by ferrous ion complexed with diethylenetriamine-N,N',N'',N''-pentaacetate (DTPA) and present evidence that an iron–oxo species such as $Fe^{IV}(DTPA)O^{3-}$, and not the hydroxyl radical, is produced in the reaction between the ferrous iron chelate and H_2O_2.

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Experimental Section

Reagents. Ferrous ion was added as ferrous ammonium sulfate (Fe-(NH$_4$)(SO$_4$)$_2$·6H$_2$O). Ferric ion was added as ferric ammonium sulfate (Fe(NH$_4$)(SO$_4$)$_2$·12H$_2$O)). Iron salts were reagent grade from Baker. Diethylenetriamine-$N,N,',N'',N''$-pentaacetic acid was reagent grade from Fluka. All other compounds were reagent grade and used as received. Nitrogen used for purging solutions of O$_2$ was ultrahigh purity from Linde (>99.999% N$_2$). Water used in preparing reaction solutions was first glass-distilled and then passed through a Millipore Milli-Q reagent grade water system.

Stopped-Flow Experiments. A modified Cary 17 UV–visible spectrophotometer was used for the stopped-flow experiments. The observation cell was a micro flow cell from NSG Precision Cells, Inc. (Type 72). The reaction solutions were placed in gas–liquid syringes with Teflon seals which efficiently exclude air from the samples (Spectrum Medical Industries). Solutions were driven through the cell by a custom-made, high-speed motorized syringe driver (Richter and Co., Pittsburgh, PA) which gave flows of 0.9 mL s^{-1}. The dead time of the mixing procedure was 0.3 s, based on the fraction of reaction that had occurred prior to the stopping of the flow. The time response of the system was determined by the oxidation of ferrous phenanthroline complexes by Ce(IV).[32] All experiments were carried out at room temperature. Absorbance versus time data were fit with a first-order kinetics equation using a linear least-squares fitting routine. When appropriate, a least-squares fit to the equation $y = bx$ was used; i.e., the y intercept was set at the origin.

Sample absorbance was measured at 260 nm, the wavelength of maximum $\Delta\epsilon$ for the Fe^{II}DTPA^{3-}/Fe^{III}DTPA^{2-} pair. ϵ was 9250 M^{-1} cm^{-1} for Fe^{III}DTPA^{2-}, and $\Delta\epsilon_{23}$ was 8890 M^{-1} cm^{-1}.

Determination of Hydrogen Peroxide. Hydrogen peroxide concentrations were determined by using the Ghormley iodometric method.[33]

Results

Buffered aqueous solutions containing equimolar concentrations of ferrous ammonium sulfate and diethylenetriamine-N,N,N',N'',N''-pentaacetic acid (DTPA) were mixed with buffered solutions of hydrogen peroxide in a stopped-flow spectrophotometer. In some experiments, the solutions contained *tert*-butyl alcohol (*t*-BuOH) or methanol. The ferrous-DTPA complex was oxidized to the ferric-DTPA complex, as indicated by spectral changes. Reactions between Fe^{II}DTPA^{3-} and H$_2$O$_2$ were carried out with excess concentrations of either Fe^{II}DTPA^{3-} or H$_2$O$_2$. In each type of experiment, the concentration of the excess reagent was at least an order of magnitude greater than that of the other reactant.[34] The absorbance changes in the mixed solutions were recorded as a function of time.[35] Total absorbance changes were obtained and the kinetics of the oxidation of the ferrous-DTPA complex were determined. In H$_2$O$_2$-excess experiments, the loss of [H$_2$O$_2$]

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(34) For the general bimolecular reaction A + B → C, the extent of reaction (x) in mol/dm^3 is described by

$$([A]_0 - [B]_0)^{-1} \ln \{([B]_0/[A]_0)([A]_0 - x)/([B]_0 - x)\} = k_{BI}t$$

When [A]$_0 \gg$ [B]$_0$, this reduces to

$$\ln ([B]_0/([B]_0 - x)) = (k_{BI}[A]_0)t$$

where $k_{BI}[A]_0$ will be the observed first-order rate constant. If [A]$_0 \geq$ 10[B]$_0$, plots of ln ([B]$_0$/([B]$_0 - x$)) versus time will appear linear and give excellent correlation coefficients. On the other hand, if [A]$_0 <$ 10[B]$_0$, such a plot will exhibit curvature: there will appear to be a fast initial reaction followed by a first-order process, i.e., an apparent biphasic reaction. This comment bears on a recent paper by Rush and Koppenol[11] on the reaction between hydrogen peroxide and ferrous EDTA. The authors state that the reaction is biphasic; however, in the illustrative figure, [H$_2$O$_2$]$_0$/[Fe^{II}EDTA^{2-}]$_0$ = 2.75, so that the data would appear to be biphasic even when there were simply a straightforward bimolecular reaction.

(35) Since both Fe^{II}DTPA^{3-} and Fe^{III}DTPA^{2-} have nonzero absorbances at 260 nm, the observed absorbance is

$$A = \epsilon_2[Fe^{II}DTPA^{3-}] + \epsilon_3[Fe^{III}DTPA^{2-}]$$

where ϵ_2 and ϵ_3 are the molar absorptivities of Fe^{II}DTPA^{3-} and Fe^{III}DTPA^{2-}, respectively. Mass-balance considerations yield

$$\Delta A = A_\infty - A = (\epsilon_3 - \epsilon_2)([Fe^{II}DTPA^{3-}] - [Fe^{II}DTPA^{3-}]_\infty).$$

Since $\epsilon_3 > \epsilon_2$, $(A_\infty - A)$ will be positive.

Figure 2. Dependence of the yield of Fe^{III}DTPA^{2-} on [H$_2$O$_2$]$_0$ in the Fe^{II}DTPA^{3-}-excess experiments. (●) [*t*-BuOH] = 0.6–1.4 M; (O) *t*-BuOH absent. The line is the fit of the data to $y = bx$.
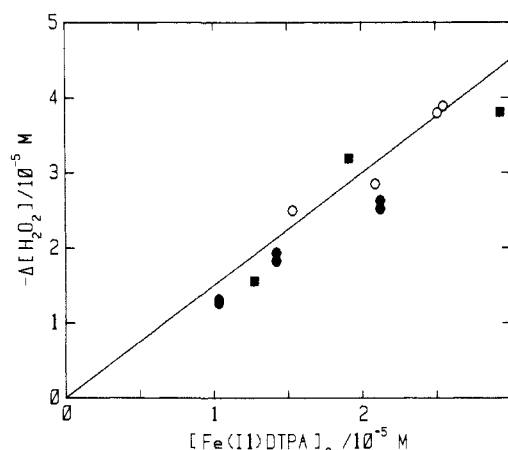
Figure 3. Consumption of H$_2$O$_2$ in the H$_2$O$_2$-excess experiments. In all experiments, [*t*-BuOH] = 0 and [H$_2$O$_2$]$_0$/[Fe^{II}DTPA^{3-}]$_0$ = 10.00 ± 0.15. (O, ■) [MeOH] = 0; (●) [MeOH] = 0.6 M; (O, ●) [Fe^{III}DTPA^{2-}]$_0$ = 0; (■) [Fe^{III}DTPA^{2-}]$_0$/[Fe^{II}DTPA^{3-}]$_0$ = 1.00 ± 0.05. The solid line is the fit to $y = bx$ of data with no MeOH and no Fe^{III}DTPA^{2-} added.

was determined. In experiments examining the effect of alcoholic hydroxyl radical scavengers, the alcohol concentration was 0.6–1.4 M. All experiments were done at pH 7.0, using a 5 mM phosphate buffer. All solutions were rigorously purified of oxygen by purging with nitrogen.

Stoichiometry of the Oxidation. The total absorbance changes at 260 nm (the λ with the maximum $\Delta\epsilon$) were determined in both the H$_2$O$_2$- and the Fe^{II}DTPA^{3-}-excess experiments. In the H$_2$O$_2$-excess experiments, all the Fe^{II}DTPA^{3-} is oxidized to Fe^{III}DTPA^{2-}, and ΔA increases linearly with [Fe^{II}DTPA^{3-}]$_0$ (Figure 1). The slope of the line, (8.9 ± 1.0) × 10^3 M^{-1} cm^{-1}, agrees with the $\Delta\epsilon$ determined for the two iron chelates; i.e., $\Delta\epsilon_{23}$ = 8890 M^{-1} cm^{-1}. The ΔA were the same in the presence and absence of *t*-BuOH.[36] In the Fe^{II}DTPA^{3-}-excess experiments, all the H$_2$O$_2$ was decomposed and ΔA increased linearly with [H$_2$O$_2$]$_0$ (Figure 2). The slope of the ΔA versus [H$_2$O$_2$]$_0$ plot was (1.50 ± 0.21) × 10^4 M^{-1} cm^{-1}, indicating 0.59 ± 0.07 H$_2$O$_2$ decomposed per Fe^{II}DTPA^{3-} oxidized. The ΔA were the same in the presence or abscence of *t*-BuOH.[36]

The dependence of the consumption of H$_2$O$_2$ on [Fe^{II}DTPA^{3-}]$_0$ in the H$_2$O$_2$-excess experiments was determined (Figure 3). The peroxide consumed increased linearly with [Fe^{II}DTPA^{3-}]$_0$, giving Δ[H$_2$O$_2$]/[Fe^{II}DTPA^{3-}]$_0$ = 1.50 ± 0.31. The effect of the

(36) In Figures 1, 2, 5, and 6, data are presented for measurements in the presence and absence of *tert*-butyl alcohol. In each case, the slopes of the lines obtained when the alcohol-containing and alcohol-free data were analyzed separately agreed within experimental uncertainty. Thus, the slopes reported for each figure are obtained by fitting *all* the data.

Table I. Effect of Methanol[a] on the Reaction between H_2O_2 and $Fe^{II}DTPA^{3-}$

[H_2O_2], 10^{-5} M	[Fe^{II}DTPA^{3-}], 10^{-5} M	k_{obsd}, s^{-1}		k_+/k_-[b]	ΔA		ΔA_+/ΔA_-[c]
		no MeOH	+MeOH		no MeOH	+MeOH	
10.2	1.03	0.14	0.14	1.0	0.10	0.10	1.0
14.5	1.40	0.26	0.26	1.0	0.12	0.12	1.0
21.0	2.11	0.26	0.26	1.0	0.18	0.18	1.0
0.92	12.2	0.11	0.44	3.9	0.22	0.16	0.73
1.38	15.3	0.13	0.61	4.6	0.30	0.12	0.40
1.84	19.6	0.30	0.67	2.2	0.22	0.13	0.59
2.40	24.1	0.35	0.49	1.4	0.39	0.23	0.59

[a] All experiments used [MeOH] = 0.6 M. [b] k_+ and k_- are values of k_{obsd} with and without methanol, respectively. [c] $ΔA_+$ and $ΔA_-$ are values of $ΔA$ with and without methanol, respectively.
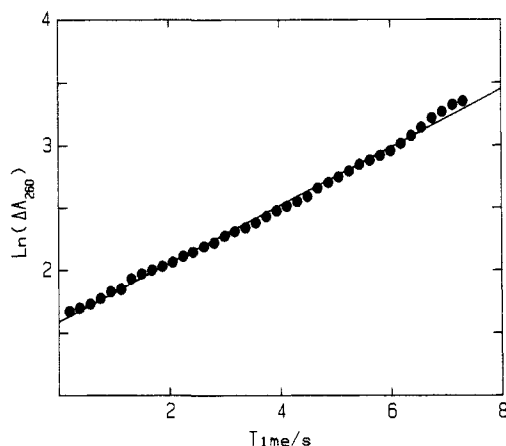
Figure 4. Production of $Fe^{III}DTPA^{2-}$ via oxidation of $Fe^{II}DTPA^{3-}$ by H_2O_2 as seen in a first-order plot of $ΔA$ data for an $Fe^{II}DTPA^{3-}$-excess experiment. The reaction conditions were [t-BuOH] = 0, [Fe^{II}DTPA^{3-}]$_0$ = 2.60 × 10^{-4} M, [H$_2$O$_2$]$_0$ = 2.28 × 10^{-5} M. The line is a linear least-squares fit of the data to the equation $y = a + bx$. Zero time is set at the time when the flow was stopped.

presence of $Fe^{III}DTPA^{2-}$ on the yield was determined in experiments where equimolar concentrations of $Fe^{III}DTPA^{2-}$ and $Fe^{II}DTPA^{3-}$ were added to reaction solutions, with [H$_2$O$_2$]$_0$/[Fe^{II}DTPA^{3-}]$_0$ kept at 10.00 ± 0.15. The presence of the $Fe^{III}DTPA^{2-}$ had no effect on the decomposition of the H_2O_2 (Figure 3).

Reaction Order. Reactions between $Fe^{II}DTPA^{3-}$ and H_2O_2 were carried out with excess concentrations of either $Fe^{II}DTPA^{3-}$ or H_2O_2, with the concentration of the excess reagent at least an order of magnitude greater than that of the other reactant. *Under all conditions*, the reaction was observed to be pseudo-first-order over the entire reaction (Figure 4). In no case was a biphasic reaction observed.[34] The sample absorbance increased to a plateau, which was stable for at least several minutes. In the H_2O_2-excess experiments the reaction was first order with respect to [Fe^{II}DT-PA^{3-}], and in the $Fe^{II}DTPA^{3-}$-excess experiments the reaction was first order with respect to [H$_2$O$_2$], as indicated by the linearity of the ln ($ΔA$) versus time plots.[37]

(37) In general,[35] $ΔA = (\epsilon_3 - \epsilon_2)([Fe^{II}DTPA^{3-}] - [Fe^{II}DTPA^{3-}]_\infty)$. In the H_2O_2-excess experiments, [Fe^{II}DTPA^{3-}]$_\infty$ = 0 and thus $ΔA = (\epsilon_3 - \epsilon_2)$[Fe^{II}DTPA^{3-}].
In the $Fe^{II}DTPA^{3-}$-excess experiments, the value of $ΔA$ depends on the stoichiometry of the reaction. Represent the reaction by

$$nFe^{II}DTPA^{3-} + H_2O_2 = nFe^{III}DTPA^{2-} + 2HO^-$$
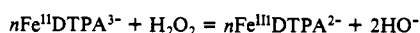
Mass- and electron-balance considerations then give

$$[Fe^{II}DTPA^{3-}]_\infty = [Fe^{II}DTPA^{3-}]_0 - n[H_2O_2]_0$$

and

$$[Fe^{III}DTPA^{2-}] = n([H_2O_2]_0 - [H_2O_2])$$

Thus, for the $Fe^{II}DTPA^{3-}$-excess experiments one obtains

$$ΔA = n(\epsilon_3 - \epsilon_2)[H_2O_2]$$
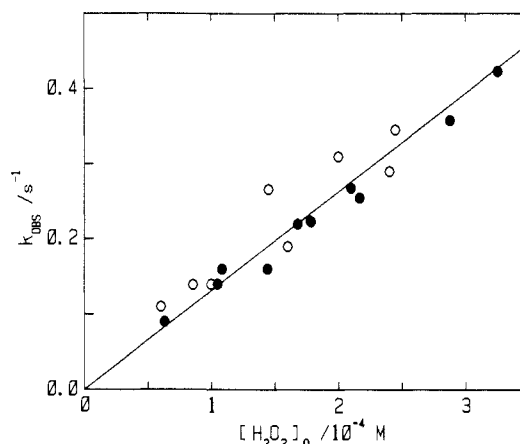
Figure 5. Effect of [H$_2$O$_2$]$_0$ on k_{obsd} in the H_2O_2-excess experiments. (O) [t-BuOH] = 0; (●) [t-BuOH] = 0.6–1.4 M. [H$_2$O$_2$]$_0$/[Fe^{II}DTPA^{3-}]$_0$ ≥ 10 in all cases. The line is a fit of the data to $y = bx$.
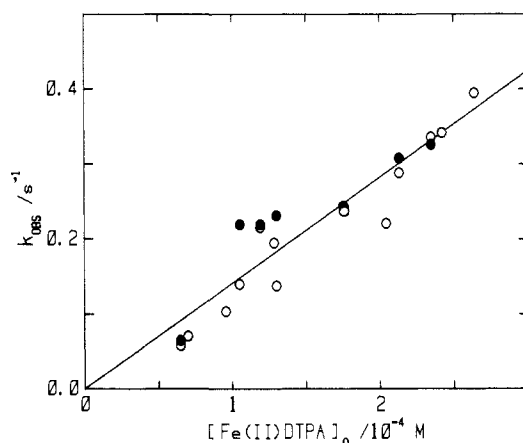
Figure 6. Effect of [Fe^{II}DTPA^{3-}]$_0$ on k_{obsd} in the $Fe^{II}DTPA^{3-}$-excess experiments. (O) [t-BuOH] = 0; (●) [t-BuOH] = 0.6–1.4 M. [H$_2$O$_2$]$_0$/[Fe^{II}DTPA^{3-}]$_0$ ≥ 10 in all cases. The line is a fit of the data to $y = bx$.

The observed first-order rate constants in the H_2O_2-excess experiments were linearly dependent on [H$_2$O$_2$]$_0$ (Figure 5) and were the same in the presence and absence of t-BuOH.[36] k_{obsd} was independent of [Fe^{II}DTPA^{3-}]$_0$. The bimolecular rate constant was (1.32 ± 0.09) × 10^3 M^{-1} s^{-1}.

The observed first-order rate constants in the $Fe^{II}DTPA^{3-}$-excess experiments were linearly dependent on [Fe^{II}DTPA^{3-}]$_0$ (Figure 6). k_{obsd} was the same in the presence or absence of t-BuOH[36] and was independent of [H$_2$O$_2$]$_0$.[38] The bimolecular rate constant obtained was (1.41 ± 0.11) × 10^3 M^{-1} s^{-1}. The ratio of the k_{BI}

(38) In some initial $Fe^{II}DTPA^{3-}$-excess experiments, the ratio of DTPA to iron was greater than 1.00. In these cases, it was seen that k_{obsd} increased as [H$_2$O$_2$] was increased (the ratio [Fe^{II}DTPA^{3-}]$_0$/[H$_2$O$_2$]$_0$ was >10 in all cases).

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obtained in the $Fe^{II}DTPA^{3-}$-excess experiments to the value from the H_2O_2-excess experiments is 1.07 ± 0.16 so that the bimolecular rate constant was the same, within experimental uncertainty, whichever reactant was in excess: when all the k_{obsd} data were combined (k_{obsd} plotted versus [excess reagent]), the bimolecular rate constant was $(1.37 \pm 0.07) \times 10^3$ M^{-1} s^{-1}. The rate constant was dependent on pH, k_{obsd} decreasing with increasing pH.

Effects of Methanol on the H_2O_2 Decomposition. The effect of the hydroxyl radical scavenger methanol on the reaction between H_2O_2 and $Fe^{II}DTPA^{3-}$ was determined in H_2O_2- and $Fe^{II}DTPA^{3-}$-excess experiments (Table I and Figure 3). In the H_2O_2-excess experiments, the observed rate constants were unaffected by the addition of methanol. The observed ΔA were unaffected by MeOH, indicating that all the $Fe^{II}DTPA^{3-}$ is oxidized to $Fe^{III}DTPA^{2-}$, as in the absence of MeOH. There was little effect, if any, on the amount of H_2O_2 decomposed per $Fe^{II}DTPA^{3-}$ oxidized when MeOH was added (Figure 3).
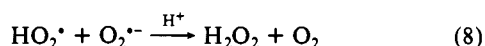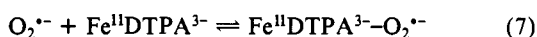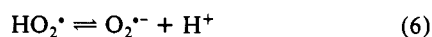
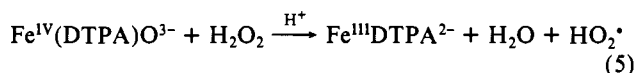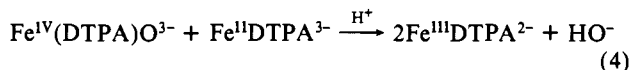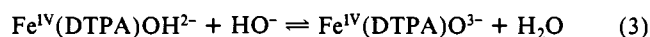When the excess reagent was $Fe^{II}DTPA^{3-}$, the effect of methanol was pronounced: the rate of H_2O_2-decomposition was increased by a factor of 2–4, and the production of $Fe^{III}DTPA^{2-}$ was reduced to 40–70% of the methanol-free value. Thus, instead of 0.59 molecule of H_2O_2 decomposed per $Fe^{II}DTPA^{3-}$ oxidized, 0.8–1.5 H_2O_2 were decomposed, i.e., an approximate 1:1 ratio instead of a 1:2 ratio.

Discussion

The principal experimental observations on the reaction between hydrogen peroxide and ferrous iron chelated with DTPA are as follows: (1) in the H_2O_2-excess experiments, 1.50 ± 0.31 molecules of H_2O_2 are consumed per $Fe^{II}DTPA^{3-}$ oxidized, and the presence of high concentrations of $Fe^{III}DTPA^{2-}$ had no effect on this value; (2) in the $Fe^{II}DTPA^{3-}$-excess experiments, 0.59 ± 0.07 H_2O_2 is consumed per $Fe^{II}DTPA^{3-}$ oxidized; (3) when H_2O_2 is present in excess, the reaction is pseudo first order with respect to $Fe^{II}DTPA^{3-}$, with a k_{BI} of $(1.32 \pm 0.09) \times 10^3$ M^{-1} s^{-1}; (4) when $Fe^{II}DTPA^{3-}$ is present in excess, the reaction is pseudo first order with respect to H_2O_2, with a k_{BI} of $(1.41 \pm 0.11) \times 10^3$ M^{-1} s^{-1}, so that the ratio of k_{BI} obtained in the $Fe^{II}DTPA^{3-}$- and H_2O_2-excess cases, respectively, is 1.07 ± 0.16; (5) in both types of experiments, the reaction was first order over the entire decomposition—there was no initial faster decomposition; (6) *tert*-butyl alcohol had no effect on rate constants or reaction stoichiometries, even though t-BuOH is an efficient quencher of the oxidation of $Fe^{II}DTPA^{3-}$ by the hydroxyl radical (HO$^\bullet$) under the present reaction conditions; and (7) methanol had essentially no effect on the reaction when H_2O_2 was in excess.

These observations support a mechanism in which an iron–oxo species such as $Fe^{IV}(DTPA)OH^{2-}$ is produced in the initial reaction (Scheme I). Reaction 2 is the rate-determining step, with all subsequent reactions occurring rapidly.
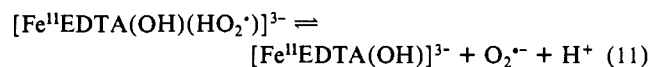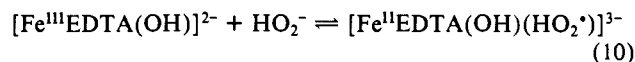
Scheme I

$$Fe^{II}DTPA^{3-} + H_2O_2 \rightarrow Fe^{IV}(DTPA)OH^{2-} + HO^- \text{ (slow)} \quad (2)$$

$$Fe^{IV}(DTPA)OH^{2-} + HO^- \rightleftharpoons Fe^{IV}(DTPA)O^{3-} + H_2O \quad (3)$$

$$Fe^{IV}(DTPA)O^{3-} + Fe^{II}DTPA^{3-} \xrightarrow{H^+} 2Fe^{III}DTPA^{2-} + HO^- \quad (4)$$

$$Fe^{IV}(DTPA)O^{3-} + H_2O_2 \xrightarrow{H^+} Fe^{III}DTPA^{2-} + H_2O + HO_2^\bullet \quad (5)$$

$$HO_2^\bullet \rightleftharpoons O_2^{\bullet-} + H^+ \quad (6)$$

$$O_2^{\bullet-} + Fe^{II}DTPA^{3-} \rightleftharpoons Fe^{II}DTPA^{3-}-O_2^{\bullet-} \quad (7)$$

$$HO_2^\bullet + O_2^{\bullet-} \xrightarrow{H^+} H_2O_2 + O_2 \quad (8)$$

The equilibrium between $Fe^{IV}(DTPA)O^{3-}$ and $Fe^{IV}(DTPA)OH^{2-}$, reaction 3, is included because of evidence that the hydroxyl radical is produced when $Fe^{II}DTPA^{3-}$ is oxidized by H_2O_2 under mildly acidic pH conditions.[18] In mildly acid solution, formation of $Fe^{IV}(DTPA)OH^{2-}$ would be favored and the possibility of

dissociation of the $Fe^{IV}(DTPA)OH^{2-}$ to give HO$^\bullet$ would be increased (reaction 9). Reaction 9 was proposed by Walling and

$$Fe^{IV}(DTPA)OH^{2-} \rightleftharpoons Fe^{III}DTPA^{2-} + HO^\bullet \quad (9)$$
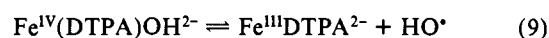
Amarnath[14] to possibly account for the apparent production of free hydroxyl radical in simple acid aquoiron systems, but not in biochemical systems involving iron-containing enzymes.

Butler and Halliwell[17] and Buettner, Doherty, and Patterson[39] have shown that $Fe^{III}DTPA^{2-}$ reacts slowly, if at all, with the superoxide radical ($O_2^{\bullet-}$). On the other hand, $Fe^{II}DTPA^{3-}$ reacts rapidly with $O_2^{\bullet-}$, with a bimolecular rate constant[17] of 2×10^7 M^{-1} s^{-1}. Probably, a complex is formed as has been observed for EDTA.[15,17,40] The complex is equivalent to the long-lived complex formed between deprotonated H_2O_2 and $Fe^{III}EDTA^-$ in alkaline solution[15] (equilibrium 10). Francis, Cummins, and Oakes[15]

$$[Fe^{III}EDTA(OH)]^{2-} + HO_2^- \rightleftharpoons [Fe^{II}EDTA(OH)(HO_2^\bullet)]^{3-} \quad (10)$$

$$[Fe^{II}EDTA(OH)(HO_2^\bullet)]^{3-} \rightleftharpoons [Fe^{II}EDTA(OH)]^{3-} + O_2^{\bullet-} + H^+ \quad (11)$$

postulate the formation of free radicals in the $Fe^{III}EDTA^-$-catalyzed decomposition of H_2O_2 in alkaline solution via release of superoxide from the $[Fe^{II}EDTA(OH)(HO_2^\bullet)]^{3-}$ complex (equilibrium 11). Thus, formation of the complex between $O_2^{\bullet-}$ and $Fe^{II}DTPA^{3-}$ (equilibrium 7) serves only to slow the uncatalyzed disproportionation[41] of $O_2^{\bullet-}$ and $HO_2^\bullet$. As a result of the disproportionation, only 0.5 H_2O_2 would be decomposed with each occurrence of reaction 5.

Product of the Initial Reaction. The paramount feature of Scheme I is that the interaction of $Fe^{II}DTPA^{3-}$ and H_2O_2 *does not produce "free" hydroxyl radicals*, under the experimental conditions used. This is clear from (i) kinetic and stoichiometric results, (ii) the effect of the hydroxyl radical scavenger *tert*-butyl alcohol, (iii) the effects of the hydroxyl radical scavenger methanol, and (iv) the relative rates of reaction of the product of reaction 2 with H_2O_2 and $Fe^{II}DTPA^{3-}$ (k_4 and k_5). Points i, ii, and iii are discussed in the sections immediately following; point iv is covered in a latter section. The results expected if the hydroxyl radical were produced in reaction 12 are outlined and the discrepancies of this model with the observed results are discussed.

Hydroxyl Radicals in the $Fe^{II}DTPA^{3-}$-Excess Experiments. If HO$^\bullet$ were the product of the reaction between $Fe^{II}DTPA^{3-}$ and H_2O_2, the reaction mechanism in the experiments with excess $Fe^{II}DTPA^{3-}$ would be given by Scheme II. Reaction 13 is rapid compared with reaction 12. No reaction between HO$^\bullet$ and H_2O_2 is shown since the rate constant for HO$^\bullet$ reaction with the iron chelate is 200 times than that for reaction with H_2O_2,[42,43] and less than 1% of the HO$^\bullet$ would react with H_2O_2. No reaction between HO$^\bullet$ and $Fe^{III}DTPA^{2-}$ is shown, since at the completion of the reaction (the time when [$Fe^{III}DTPA^{2-}$] is the largest) only 4% of the HO$^\bullet$ would be reacting with $Fe^{III}DTPA^{2-}$.[44,45] Thus, Scheme II covers all reactions to be expected in the $Fe^{II}DTPA^{3-}$-excess experiments.

(39) Buettner, G. R.; Doherty, T. P.; Patterson, L. K. *FEBS Lett.* **1983**, *158*, 143–146.

(40) Ilan, Y. A.; Czapski, G. *Biochim. Biophys. Acta* **1977**, *498*, 386–394.

(41) Bielski, B. H. J.; Allen, A. O. *J. Phys. Chem.* **1977**, *81*, 1048–1050.
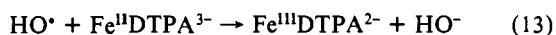
(42) Lati, J.; Meyerstein, D. *J. Chem. Soc., Dalton Trans.* **1978**, 1105–1118. $k(HO^\bullet + Fe^{II}EDTA^{2-}) = 5.0 \times 10^9$ M^{-1} s^{-1}; the rate constant for reaction with $Fe^{II}DTPA^{3-}$ would be expected to be the same.

(43) Christensen, H.; Sehested, K.; Corfitzen, H. *J. Phys. Chem.* **1982**, *86*, 1588–1590. $k(HO^\bullet + H_2O_2) = 2.7 \times 10^7$ M^{-1} s^{-1}.

(44) Kundu, K. P.; Matsuura, N. *Int. J. Radiat. Phys. Chem.* **1975**, *7*, 565–571. $k(HO^\bullet + Fe^{III}EDTA^-) = 1.1 \times 10^9$ M^{-1} s^{-1}; the value for the DTPA complex should be comparable.

(45) The ratio of $Fe^{II}DTPA^{3-}$ to H_2O_2 is 10:1, while 0.59 H_2O_2 is decomposed per $Fe^{II}DTPA^{3-}$ oxidized. Thus, when all the H_2O_2 is consumed, there will be 0.20 $Fe^{III}DTPA^{2-}$ for each $Fe^{II}DTPA^{3-}$. Since the ratio of rate constants for the reaction of HO$^\bullet$ with $Fe^{II}DTPA^{3-}$ and $Fe^{III}DTPA^{2-}$ respectively is 5:1, 3.9% of HO$^\bullet$ would be reacting with $Fe^{III}DTPA^{2-}$ when all H_2O_2 is consumed.

Scheme II

$$Fe^{II}DTPA^{3-} + H_2O_2 \rightarrow Fe^{III}DTPA^{2-} + HO^{\bullet} + HO^{-} \qquad (12)$$

$$HO^{\bullet} + Fe^{II}DTPA^{3-} \rightarrow Fe^{III}DTPA^{2-} + HO^{-} \qquad (13)$$

Lati and Meyerstein[42] have shown that oxidation of ferrous chelates of EDTA by $HO^{\bullet}$ 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 ferric-DTPA chelate is produced in reaction 13. Considering the high reactivity of the hydroxyl radical with ligands such as DTPA,[42] the initial product of reaction 13 probably is a chelate with oxidized ligand ($Fe^{II}DTPA^{2-}$); however, this is followed by rapid intramolecular electron transfer to give the observed ferric chelate.

The stoichiometry predicted by Scheme II is 0.5 H_2O_2 consumed per $Fe^{II}DTPA^{3-}$ oxidized. Experimentally this value was observed with $Fe^{II}DTPA^{3-}$ in excess; i.e., $[H_2O_2]_0/\Delta[Fe^{II}DTPA]$ = 0.59 ± 0.07. Thus, Scheme II is compatible with the stoichiometric result observed in the $Fe^{II}DTPA$-excess experiments.

The rate law for the disappearance of H_2O_2 predicted by Scheme II is given by eq 14, when reaction 12 is the rate-con-
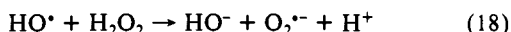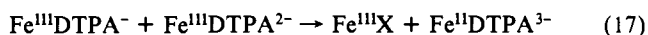
$$-d[H_2O_2]/dt = k_{12}[Fe^{II}DTPA^{3-}][H_2O_2] \qquad (14)$$

trolling step. In the $Fe^{II}DTPA^{3-}$-excess experiments, $[Fe^{II}DTPA^{3-}]$ is virtually constant and the rate of loss of H_2O_2 is given by eq 15. When $Fe^{II}DTPA^{3-}$ is in excess $\Delta A \propto [H_2O_2]$,[37] and plots

$$-d[H_2O_2]/dt = k_{12}[Fe^{II}DTPA^{3-}]_0[H_2O_2] \qquad (15)$$

of ln (ΔA) versus time would be linear with a slope of k_{12}-$[Fe^{II}DTPA^{3-}]_0$. Such linear plots were obtained. In addition, plots of k_{obsd} versus $[Fe^{II}DTPA^{3-}]_0$ would be linear and would have a slope equal to $1k_{12}$. Such a linear plot was obtained (Figure 6). Thus, the hydroxyl radical mechanism in Scheme II is consistent with the stoichiometric and kinetic results in the $Fe^{II}DTPA$-excess experiments (without alcoholic scavengers), and discrepancies must come from other experimental results.
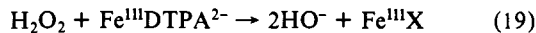
Hydroxyl Radicals in the H_2O_2-Excess Experiments. In the H_2O_2-excess experiments, the predicted hydroxyl radical mechanism is more complicated than Scheme II. As reaction proceeds and $[Fe^{III}DTPA^{2-}]/[Fe^{II}DTPA^{3-}]$ increases, $HO^{\bullet}$ will attack $Fe^{III}DTPA^{2-}$ and oxidize the ligand to give $Fe^{III}DTPA^{-}$ (reaction 16). $Fe^{III}DTPA^{-}$ is then oxidized by $Fe^{III}DTPA^{2-}$ (reaction 17),

$$HO^{\bullet} + Fe^{III}DTPA^{2-} \rightarrow HO^{-} + Fe^{III}DTPA^{-} \qquad (16)$$

$$Fe^{III}DTPA^{-} + Fe^{III}DTPA^{2-} \rightarrow Fe^{III}X + Fe^{III}DTPA^{3-} \qquad (17)$$

$$HO^{\bullet} + H_2O_2 \rightarrow HO^{-} + O_2^{\bullet-} + H^{+} \qquad (18)$$

as determined by Rahhal and Richter[46,47] in studies of the reaction of $HO^{\bullet}$ with iron chelates of DTPA. X in $Fe^{III}X$ represents a two-electron oxidation product of DTPA. Thus, in addition to Scheme II, H_2O_2-excess experiments also require reactions 16 and 17. Reaction 18 now must be included since $Fe^{III}DTPA^{2-}$ reacts more slowly with $HO^{\bullet}$ than does $Fe^{II}DTPA^{3-}$, so that near the end of the oxidation of the $Fe^{II}DTPA^{3-}$ 21% of the $HO^{\bullet}$ would be reacting with H_2O_2.[43,44]

In the absence of reaction 16, only the reactions in Scheme II would pertain. Thus, early in the reaction between H_2O_2 and

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(46) Rahhal, S.; Richter, H. W., submitted for publication. A study of the reactions of hydroxyl radical with ferric and ferrous chelates of DTPA was done using conditions identical with those used for the iron chelate/hydrogen peroxide reactions of this paper, except that H_2O_2 was not present.

(47) The ^{60}Co-irradiation experiments[46] do not differentiate between oxidation of $Fe^{III}DTPA^{-}$ by $Fe^{III}DTPA^{2-}$, reaction 17, and intramolecular electron transfer of $Fe^{III}DTPA^{-}$ to give $Fe^{II}X$: $Fe^{III}DTPA^{-} \rightarrow Fe^{II}X$. If the reaction sequence when $Fe^{III}DTPA^{2-}$ accumulates is reactions 12, 16, and intramolecular electron transfer, the summary reaction is

$$H_2O_2 + Fe^{II}DTPA^{3-} \rightarrow 2HO^{-} + Fe^{II}X$$

Presumably, $Fe^{II}X$ could function as $Fe^{II}DTPA^{3-}$ in reaction 12 to reduce H_2O_2 and propagate the chain.

$Fe^{II}DTPA^{3-}$ the observed decomposition of H_2O_2 should be 0.5 H_2O_2 per $Fe^{II}DTPA^{3-}$, just as predicted for the $Fe^{II}DTPA^{3-}$-excess experiments. As reaction proceeds and $Fe^{III}DTPA^{2-}$ accumulates, reactions 12, 16, and 17 will occur in sequence, giving the summary reaction 19. Thus, *a peroxide molecule is decomposed without*

$$H_2O_2 + Fe^{III}DTPA^{2-} \rightarrow 2HO^{-} + Fe^{III}X \qquad (19)$$

the oxidation of a ferrous chelate,[48] and this sequence of reactions constitutes a *catalytic chain decomposition* of H_2O_2 in which $Fe^{II}DTPA^{3-}$ reduces H_2O_2 (reaction 12) and is then regenerated (reaction 17). Because of this chain decomposition, one $Fe^{II}DTPA^{3-}$ can decompose many H_2O_2, once the $[Fe^{III}DTPA^{2-}]$ has increased sufficiently so that reaction 16 can occur. In consequence, as reaction proceeds, $\Delta[H_2O_2]/[Fe^{II}DTPA^{3-}]_0$ will increase from 0.5 to some higher value, giving some average value as the final result when all the $Fe^{II}DTPA^{3-}$ has been oxidized. The observed value of $\Delta[H_2O_2]/[Fe^{II}DTPA^{3-}]_0$ with H_2O_2 in excess was 1.50. This seems to be somewhat in agreement with the expectation from the hydroxyl radical mechanism; however, experiments were done in which $Fe^{III}DTPA^{2-}$ was added in equimolar concentrations with $Fe^{II}DTPA^{3-}$ to H_2O_2-excess experiments. If Scheme II plus reactions 16, 17, and 18 describes the mechanism for H_2O_2-excess experiments, the addition of $Fe^{III}DTPA^{2-}$ would initiate immediately the chain H_2O_2 decomposition and would *greatly increase* $\Delta[H_2O_2]/[Fe^{II}DTPA^{3-}]_0$. In actuality, *there was no effect*; addition of $Fe^{III}DTPA^{2-}$ to the initial reaction mixture did not change $\Delta[H_2O_2]/[Fe^{II}DTPA^{3-}]_0$ (Figure 3). Thus, the stoichiometric results in the H_2O_2-excess experiments establish that hydroxyl radicals *are not produced* in reduction of H_2O_2 by $Fe^{II}DTPA^{3-}$, as given in reaction 12.

The kinetic results in the H_2O_2-excess experiments also assert that the hydroxyl radical is not the product of reaction between H_2O_2 and $Fe^{II}DTPA^{3-}$. Assuming Scheme II plus reactions 16, 17, and 18 as the mechanism and making steady-state assumptions for $HO^{\bullet}$ and $Fe^{III}DTPA^{-}$ give eq 20 for the rate loss of Fe^{II}-

$$-d[Fe^{II}DTPA^{3-}]/dt = k_{12}[H_2O_2]_0[Fe^{II}DTPA^{3-}] +$$
$$\{(k_{13}[Fe^{II}DTPA^{3-}] -$$
$$k_{16}[Fe^{III}DTPA^{2-}])(k_{12}[H_2O_2]_0[Fe^{II}DTPA^{3-}])\}/$$
$$\{k_{13}[Fe^{II}DTPA^{3-}] + k_{16}[Fe^{III}DTPA^{2-}] + k_{18}[H_2O_2]_0\} \qquad (20)$$

$DTPA^{3-}$. At the start of the reaction when $k_{13}[Fe^{II}DTPA^{3-}] \gg k_{16}[Fe^{III}DTPA^{2-}]$, it is also true that $k_{13}[Fe^{II}DTPA^{3-}] \gg k_{18}$-$[H_2O_2]$ and the rate of disappearance of $Fe^{II}DTPA^{3-}$ would be

$$-d[Fe^{II}DTPA^{3-}]/dt = 2k_{12}[H_2O_2]_0[Fe^{II}DTPA^{3-}] \qquad (21)$$

As the reaction progresses, a point is reached (when 80% of the $Fe^{II}DTPA^{3-}$ has been oxidized)[49] where $k_{13}[Fe^{II}DTPA^{3-}]$ = $k_{16}[Fe^{III}DTPA^{2-}]$. At this time, the rate of disappearance of $Fe^{II}DTPA^{3-}$ would be

$$-d[Fe^{II}DTPA^{3-}]/dt = k_{12}[H_2O_2]_0[Fe^{II}DTPA^{3-}] \qquad (22)$$

When H_2O_2 is in excess, $\Delta A \propto [Fe^{II}DTPA^{3-}]$.[37] Thus, early in the reaction between H_2O_2 and $Fe^{II}DTPA^{3-}$ plots of ln (ΔA) versus time would be linear with a slope of $2k_{12}[H_2O_2]_0$. Near the end of the experiments, the same plot of data would have a slope of $1k_{12}[H_2O_2]_0$; i.e., the slope of the ln (ΔA) versus time plots *would show curvature*. This was not observed: the absorbance decays gave excellent first-order plots over the whole of the reaction.
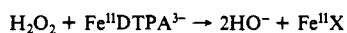
Furthermore, the k_{BI} obtained from the k_{obsd} (initial part of reaction) versus $[H_2O_2]_0$ plot would be $2k_{12}$. Thus, the two different excess-reagent cases should give k_{BI} that differ by a factor

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(48) If H_2O_2 is decomposed by a $HO^{\bullet}$ radical mechanism and $Fe^{III}X$ indeed is produced, then the molar absorptivities of $Fe^{III}DTPA^{2-}$ and $Fe^{III}X$ apparently are very little different: in these experiments where $Fe^{II}DTPA^{3-}$ is oxidized with excess H_2O_2 (so that all $Fe^{II}DTPA^{3-}$ is oxidized) the observed ΔA of the solutions is equal to the value calculated from the difference in molar absorptivities of $Fe^{III}DTPA^{2-}$ and $Fe^{II}DTPA^{3-}$.

(49) k_{13} is about 5 times[42,44] k_{16}. Thus, $k_{13}[Fe^{II}DTPA^{3-}]$ = $k_{16}[Fe^{III}DTPA^{2-}]$ when $[Fe^{III}DTPA^{2-}]_0$ = $5[Fe^{II}DTPA^{3-}]_0$, or one of every six $Fe^{II}DTPA^{3-}$ has been oxidized.

of 2. The actual observation was that the rate constants are very nearly equal: $k_{Bi}(H_2O_2$ excess$)/(k_{Bi}(Fe^{II}DTPA^{3-}$ excess$)) = 0.94 \pm 0.14$. In summary, the stoichiometric and kinetic behaviors of the reaction make it very clear that hydroxyl radical is not the product of the reaction between $Fe^{II}DTPA^{3-}$ and H_2O_2.

Effect of *tert*-Butyl Alcohol on Kinetics and Stoichiometry. The results of ^{60}Co γ radiolysis studies[46] of $Fe^{II}DTPA^{3-}$ and $Fe^{III}DTPA^{2-}$ solutions under conditions identical with those in this study establish that if H_2O_2 were reduced by $Fe^{II}DTPA^{3-}$ to yield free hydroxyl radical (reaction 12), then the addition of *t*-BuOH to the reaction mixtures would reduce the yield of $Fe^{III}DTPA^{2-}$ in the $Fe^{II}DTPA^{3-}$-excess experiments by blocking reaction 13. A reduction in $\Delta[Fe^{III}DTPA^{3-}]/[H_2O_2]_0$ by a factor of 2 would be produced; however, *no reduction was observed*.
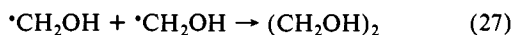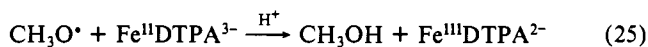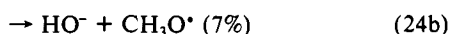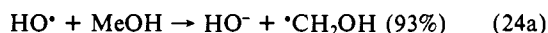
In the H_2O_2-excess experiments, the addition of *t*-BuOH would block reaction 16 and would give eq 23 for consumption of

$$-d[Fe^{II}DTPA^{3-}]/dt = 1k_{12}[H_2O_2]_0[Fe^{II}DTPA^{3-}] \quad (23)$$

$Fe^{II}DTPA^{3-}$. Thus, the k_{obsd} in the presence of *t*-BuOH would be smaller by a factor of 2 when compared with the values obtained in the absence of *t*-BuOH (during the early part of the reaction, eq 21). *No such reduction of k_{obsd} was seen*. Indeed, *t*-BuOH had no effect on the reaction stoichiometries or rate constants obtained under any of the conditions examined, indicating that the species produced is completely unreactive with *t*-BuOH, and thus cannot be the hydroxyl radical.

Effects of Methanol on Kinetics and Stoichiometry. If hydroxyl radicals were formed in the reduction of H_2O_2 by $Fe^{II}DTPA^{3-}$, the reactions expected to occur in the presence of methanol are given by Scheme III. In ^{60}Co γ radiolysis experiments[46] using solutions identical with those used here except that H_2O_2 was absent, addition of methanol *completely prevented oxidation of $Fe^{II}DTPA^{3-}$ by hydroxyl radical*. In solutions where both $Fe^{II}DTPA^{3-}$ and $Fe^{III}DTPA^{2-}$ were initially present, addition of methanol resulted in bleaching of the solutions upon reaction with hydroxyl radical; i.e., the hydroxymethyl radicals produced in reaction 24a reduced the initially added $Fe^{III}DTPA^{2-}$.

Scheme III

$$Fe^{II}DTPA^{3-} + H_2O_2 \rightarrow Fe^{III}DTPA^{2-} + HO^{\bullet} + HO^{-} \quad (12)$$

$$HO^{\bullet} + MeOH \rightarrow HO^{-} + {}^{\bullet}CH_2OH \ (93\%) \quad (24a)$$

$$\rightarrow HO^{-} + CH_3O^{\bullet} \ (7\%) \quad (24b)$$

$$CH_3O^{\bullet} + Fe^{II}DTPA^{3-} \xrightarrow{H^+} CH_3OH + Fe^{III}DTPA^{2-} \quad (25)$$

$${}^{\bullet}CH_2OH + Fe^{III}DTPA^{2-} \rightarrow CH_2O + Fe^{II}DTPA^{3-} + H^+ \quad (26)$$

$${}^{\bullet}CH_2OH + {}^{\bullet}CH_2OH \rightarrow (CH_2OH)_2 \quad (27)$$

$${}^{\bullet}CH_2OH + CH_3O^{\bullet} \rightarrow CH_2O + CH_3OH \quad (28)$$

In the H_2O_2 experiments, $Fe^{III}DTPA^{2-}$ formed in reaction 25 would be reduced in reaction 26 by the hydroxymethyl radical (which is produced in 13-fold excess relative to the oxidizing methoxy radical), as seen in the ^{60}Co γ radiolysis experiments. In contrast to the radiolysis experiments, not all $Fe^{III}DTPA^{2-}$ would be reduced since the $Fe^{III}DTPA^{2-}$ produced via reaction 12 exceeds the yield of hydroxymethyl radical.

In the H_2O_2-excess experiments, a large portion of the $Fe^{III}DTPA^{2-}$ produced in reaction 12 would be recycled to $Fe^{II}DTPA^{3-}$ via reaction 26 so that $\Delta[H_2O_2]/[Fe^{II}DTPA^{3-}]_0$ *should increase sharply* upon addition of MeOH. The experimental observation was that $\Delta[H_2O_2]/[Fe^{II}DTPA^{3-}]_0$ perhaps *decreased slightly* upon addition of methanol (Figure 3), in clear disagreement with the prediction of the hydroxyl radical mechanism. Also, the kinetics of the reaction were unaffected by the presence of methanol (Table I).

In the $Fe^{II}DTPA^{3-}$-excess experiments, the effect of the addition of methanol would be to greatly reduce the number of $Fe^{II}DTPA^{3-}$ needed to reduce the H_2O_2 present; i.e., the number of molecules of H_2O_2 decomposed per $Fe^{II}DTPA^{3-}$ oxidized would greatly increase. A doubling in the value was observed, consistent with

the prediction (Table I). The rate of peroxide decomposition was increased by a factor of 2–4. This increase in rate cannot be reconciled with Scheme III since the only reaction involving H_2O_2 is reaction 12, which would be unaffected by the regeneration of the $Fe^{II}DTPA^{3-}$: [$Fe^{II}DTPA^{3-}$] is already a virtual constant. The increase in rate cannot be explained by attack of ${}^{\bullet}CH_2OH$ on H_2O_2 (reaction 29), since this reaction is slow relative to reaction 26.[50,51]

$${}^{\bullet}CH_2OH + H_2O_2 \rightarrow CH_2O + H_2O + HO^{\bullet} \quad (29)$$
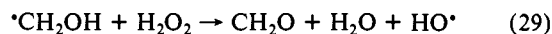
In summary, the effects of addition of methanol in both H_2O_2- and $Fe^{II}DTPA^{3-}$-excess experiments preclude the production of hydroxyl radical in the reaction between $Fe^{II}DTPA^{3-}$ and H_2O_2.

Reactions with H_2O_2 in Excess. The experimental evidence supports the mechanism in Scheme I, where reaction 2 is the rate-controlling step. In the context of Scheme I, if reaction 5 were substantially faster than reaction 4, 100% of the $Fe^{IV}(DTPA)O^{3-}$ would react with H_2O_2 and the rate of loss of $Fe^{II}DTPA^{3-}$ would be

$$-d[Fe^{II}DTPA^{3-}]/dt = k_2[H_2O_2]_0[Fe^{II}DTPA^{3-}] \quad (30)$$

Since $\Delta A \propto [Fe^{II}DTPA^{3-}]$ in the H_2O_2-excess experiments,[37] plots of ln (ΔA) versus time would be linear with a slope equal to k_{obsd}, where $k_{obsd} = k_2[H_2O_2]_0$. Such linear plots were indeed observed (Figure 4). Plots of k_{obsd} versus $[H_2O_2]_0$ would be linear and the k_{Bi} determined from the slope of the plot would equal k_2. Such linear plots were observed experimentally (Figure 5).

At the other extreme, if reaction 4 were much faster than reaction 5, 100% of the $Fe^{IV}(DTPA)O^{3-}$ would react with $Fe^{II}DTPA^{3-}$; then the rate of loss of $Fe^{II}DTPA^{3-}$ would be

$$-d[Fe^{II}DTPA^{3-}]/dt = 2k_2[H_2O_2]_0[Fe^{II}DTPA^{3-}] \quad (31)$$

Plots of ln (ΔA) versus time would be linear, with $k_{obsd} = 2k_2[H_2O_2]_0$. The k_{Bi} from the slope of the k_{obsd} versus $[H_2O_2]_0$ plot would equal $2k_2$. The resolution of the value of k_{Bi} will become clear shortly.

If reaction 5 were much faster than reaction 4, the reaction mechanism would consist essentially of reactions 2, 3, and 5–8, so that 1.5 H_2O_2 would be consumed per $Fe^{II}DTPA^{3-}$ oxidized. This agrees with the observed value of 1.50 ± 0.31. If reaction 4 were much faster than reaction 5, the mechanism would consist essentially of reactions 2, 3, and 4. In this case, two $Fe^{II}DTPA^{3-}$ would be oxidized for each H_2O_2 decomposed; i.e., $\Delta[H_2O_2]/[Fe^{II}DTPA^{3-}]_0$ would be 0.5: this clearly disagrees with the experimental value. Thus, the stoichiometry clearly shows that reaction 5 is much faster than reaction 4 in the H_2O_2-excess experiments, so that $k_5[H_2O_2]_0 \gg k_4[Fe^{II}DTPA^{3-}]_0$. Since $[H_2O_2]_0 \geq 10[Fe^{II}DTPA^{3-}]_0$ in these experiments, we can conclude that $k_5 \geq k_4$. In addition, we may conclude that the k_{Bi} obtained from the k_{obsd} versus $[H_2O_2]_0$ plot is k_2.

Reactions with $Fe^{II}DTPA^{3-}$ in Excess. In the $Fe^{II}DTPA^{3-}$-excess experiments, the oxidation of the $Fe^{II}DTPA^{3-}$ was pseudo first order with respect to H_2O_2. In the context of Scheme I with reaction 2 as the rate-controlling step, if the rate of reaction 4 were much faster than the rate of reaction 5, 100% of the $Fe^{IV}(DTPA)O^{3-}$ would react with $Fe^{II}DTPA^{3-}$ and the rate of loss of H_2O_2 would be

$$-d[H_2O_2]/dt = k_2[Fe^{II}DTPA^{3-}]_0[H_2O_2] \quad (32)$$

Since $\Delta A \propto [H_2O_2]$ in the $Fe^{II}DTPA^{3-}$-excess experiments,[37] plots of ln (ΔA) versus time would be linear with a slope equal to k_{obsd}, where $k_{obsd} = k_2[Fe^{II}DTPA^{3-}]_0$. Such linear plots were observed (Figure 4). Plots of k_{obsd} versus $[Fe^{II}DTPA^{3-}]_0$ would be linear, giving a k_{Bi} equal to $1k_2$. Such a linear plot was seen experimentally (Figure 6). If reaction 5 were much faster than reaction

(50) Gilbert, B. C.; Norman, R. O. C.; Sealy, R. C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 824–829. $k_{Bi} = 2.3 \times 10^4$ M^{-1} s^{-1}.

(51) Berdnikov, V. M.; Zhuravleva, O. S.; Terent'eva, L. a. *Bull. Acad. Sci. USSR, Div. Chem. Sci.* **1977**, *26*, 2050–2056: $k_{Bi} = 1 \times 10^8$ M^{-1} s^{-1} for aquated Fe(III) ions in pH 1 solution. Adams, G. E.; Willson, R. L. *Trans. Faraday Soc.* **1969**, *65*, 2981–2987: $k_{Bi} = 4.0 \times 10^9$ M^{-1} s^{-1} for ferricyanide ion in pH 7 solution.

4, the rate of loss of H_2O_2 would be

$$-d[H_2O_2]/dt = 1.5k_2[Fe^{II}DTPA^{3-}]_0[H_2O_2] \qquad (33)$$

since 0.5 H_2O_2 is consumed each time reaction 5 (followed by reactions 6 and 8) occurs. The k_{B1} from the k_{obsd} versus $[Fe^{II}DTPA^{3-}]_0$ plot would equal $1.5k_2$. If one assumes that the k_{B1} obtained in the H_2O_2-excess experiments is equal to k_2, as discussed above, then the k_{B1} obtained in the $Fe^{II}DTPA^{3-}$-excess experiments is $(1.07 \pm 0.16)k_2$. Since this value can be equated with 1.0 but not with 1.5 (within experimental uncertainty), the kinetic results establish that reaction 4 is much faster than reaction 5 in the $Fe^{II}DTPA^{3-}$-excess experiments.

The stoichiometric considerations are the same as in the H_2O_2-excess case: dominance of reaction 5 would give 1.5 H_2O_2 decomposed per $Fe^{II}DTPA^{3-}$ oxidized, while dominance of reaction 4 would give 0.5 H_2O_2 decomposed per $Fe^{II}DTPA^{3-}$ oxidized. The observed value of 0.54 ± 0.07 indicates that nearly 100% of the $Fe^{IV}(DTPA)O^{3-}$ reacts with $Fe^{II}DTPA^{3-}$ in the $Fe^{II}DTPA^{3-}$-excess case, so that reaction 4 is much faster than reaction 5, in agreement with the kinetic result. Thus, $k_4[Fe^{II}DTPA^{3-}]_0 \gg k_5[H_2O_2]_0$, and since $[Fe^{II}DTPA^{3-}]_0 \geq 10[H_2O_2]_0$ in the $Fe^{II}DTPA^{3-}$-excess experiments, it is concluded that $k_4 \geq k_5$.

Reaction of Iron–Oxo Species with H_2O_2 and $Fe^{II}DTPA^{3-}$. Stoichiometric and kinetic results from the H_2O_2- and $Fe^{II}DTPA^{3-}$-excess experiments establish that $k_5 \geq k_4$ and that $k_4 \geq k_5$, respectively. The only condition that satisfies both results is that k_4 and k_4 are *very nearly equal*. Since k_4 and k_5 are virtually equal, *it is clear that the product of the oxidation of $Fe^{II}DTPA^{3-}$ by H_2O_2 cannot be the hydroxyl radical*: the corresponding rate constants for the hydroxyl radical[42,43] differ by a factor of 200. Thus, the $Fe^{IV}(DTPA)OH^{2-}$ formed in reaction 2 does not dissociate to give $HO^\bullet$ (reaction 9). The near equality of k_4 and k_5 reinforces the conclusion from the stoichiometric and kinetic results and from the t-BuOH experiments, namely that the product of the interaction of H_2O_2 and $Fe^{II}DTPA^{3-}$ is not the hydroxyl radical, but another reactive species such as $Fe^{IV}(DTPA)O^{3-}$.

Reaction of the Iron–Oxo Species with Methanol. Methanol had essentially no effect on the H_2O_2–$Fe^{II}DTPA^{3-}$ reaction when $[H_2O_2]_0 \geq 10[Fe^{II}DTPA^{3-}]_0$. In contrast, when $Fe^{II}DTPA^{3-}$ was the excess reagent, methanol had a strong effect: the rate of H_2O_2 loss was increased by a factor of 2–4, and $[H_2O_2]_0/\Delta[Fe^{II}DTPA^{3-}]$ increased from 0.5 to 1. The increase in the rate of H_2O_2 loss by a factor of 2–4 probably indicates the initiation of a chain mechanism for H_2O_2 decomposition via the reaction of $Fe^{IV}(DTPA)O^{3-}$ with methanol. The approximate 1:1 ratio for H_2O_2 decomposition:$Fe^{II}DTPA^{3-}$ oxidation means that both H_2O_2 and $Fe^{II}DTPA^{3-}$ participate in the chain mechanism. Absence of change in the H_2O_2-excess experiments indicates that reaction of a chain carrier with peroxide terminates the chain. Chain reactions involving methanol radicals and H_2O_2 are well-known;[52] however, a chain carrier in these reactions is $^\bullet CH_2OH$ so that the chain would be quenched by the $Fe^{III}DTPA^{2-}$ product (reaction 26). The mechanism of the effect of methanol in the $Fe^{II}DTPA^{3-}$-excess experiments is not clear from reactions that might be expected to occur and certainly not from a hypothesis in which $HO^\bullet$ is formed as in reaction 12.

Summary. $Fe^{II}DTPA^{3-}$ is oxidized to $Fe^{III}DTPA^{2-}$ by H_2O_2 in neutral aqueous solutions at room temperature. The initial

reaction between $Fe^{II}DTPA^{3-}$ and H_2O_2 produces an oxidizing intermediate such as the ferryl ion, $Fe^{IV}(DTPA)O^{3-}$. The intermediate *is not the hydroxyl radical*. This conclusion is demonstrated by the following: (i) the stoichiometry and kinetic behavior of H_2O_2- and $Fe^{II}DTPA^{3-}$-excess experiments clearly contradict the behavior predicted from the mechanism (Scheme II) in which hydroxyl radical is the product of the initial interaction; (ii) changes expected in k_{obsd} and in stoichiometry upon addition of *tert*-butyl alcohol when hydroxyl radical is the product of the initial interaction (Scheme II) did not occur—t-BuOH had no effect on reaction rates or stoichiometries under any conditions tested, while it was shown in ^{60}Co γ radiolysis experiments that the alcohol efficiently prevents oxidation of $Fe^{II}DTPA^{3-}$ by $HO^\bullet$; (iii) changes expected in reaction stoichiometry upon addition of methanol did not occur—methanol had no effect in H_2O_2-excess experiments, while its effect in the $Fe^{II}DTPA^{3-}$-excess experiments was inconsistent with a hydroxyl radical mechanism; and (iv) the rate constants for reaction of the intermediate with H_2O_2 and $Fe^{II}DTPA^{3-}$ were very nearly equal, while the corresponding rate constants for reaction with $HO^\bullet$ differ by a factor of 200—the nearly equal values of the rate constants k_4 and k_5 were indicated by the stoichiometries and the k_{B1} obtained in the two limiting cases, where either H_2O_2 or $Fe^{II}DTPA^{3-}$ was in kinetic excess.

The initial reaction between $Fe^{II}DTPA^{3-}$ and H_2O_2 is a straightforward bimolecular reaction, with $k_{B1} = (1.37 \pm 0.07) \times 10^3$ M^{-1} s^{-1}. No biphasic behavior was observed when appropriate pseudo-first-order reaction conditions were used. The sequence of reactions in Scheme I accounts completely for the observed kinetic, stoichiometric, and hydroxyl radical scavenger results. Outstanding features include the production of an oxidizing intermediate (possibly a ferryl ion) which reacts with H_2O_2 and $Fe^{II}DTPA^{3-}$ with very nearly equal rate constants, which is unreactive with *tert*-butyl alcohol, and which reacts with methanol. The present experiments cannot differentiate between high oxidation state iron–oxo species such as the ferryl ion and the "caged" hydroxyl radicals (an "untrappable intermediate") supported by Walling and co-workers;[13,14] however, it is clearly established that "free" hydroxyl radicals is not produced.

It is of interest to compare the rate constants[7] for reaction of $Fe^{II}(H_2O)_6^{2+}$ and $Fe^{II}DTPA^{3-}$ with H_2O_2, which are 57.8 and 1370 M^{-1} s^{-1}, respectively. The oxidation–reduction potential of the $Fe(III)/Fe(II)$ pair in acid solution[53] is $+0.771$ V, while the potential of the DTPA chelated pair[54] is $+0.165$ V. Thus, $Fe^{II}DTPA^{3-}$ is the stronger reducing agent and gives the faster rate in a reduction reaction, which is the trend expected from Marcus theory. With other polyamino carboxylates, the rates of the initial reaction also decrease with increasing redox potential of the $Fe(III)/Fe(II)$ pair, even when subsequent reaction mechanisms vary, as has been discussed by Rahhal and Richter.[12]

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