Fenton Chemistry of 1,3-Dimethyluracil

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Abstract: Hydroxyl radicals were generated in the Fenton reaction at pH 4 ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$, $k \approx 60$ L mol^{-1} s^{-1}) and by pulse radiolysis (for the determination of kinetic data). They react rapidly with 1,3-dimethyluracil, 1,3-DMU ($k = 6 \times 10^9$ L mol^{-1} s^{-1}). With H_2O_2 in excess and in the absence of O_2, 1,3-DMU consumption is 3.3 mol per mol Fe^{2+}. 1,3-DMUglycol is the major product (2.95 mol per mol Fe^{2+}). Dimers, prominent products of ${}^{\bullet}OH$-induced reactions in the absence of Fe^{2+}/Fe^{3+} (Al-Sheikhly, M.; von Sonntag, C. *Z. Naturforsch*. **1983**, *31b*, 1622) are not formed. Addition of ${}^{\bullet}OH$ to the C(5)−C(6) double bond of 1,3-DMU yields reducing C(6)-yl **1** and oxidizing C(5)-yl radicals **2** in a 4:1 ratio. The yield of reducing radicals was determined with tetranitromethane by following the buildup of nitroform anion. Reaction of **1** with Fe^{3+} that builds up during the reaction or with H_2O_2 gives rise to a short-chain reaction that is terminated by the reaction of Fe^{2+} with **2**, which re-forms 1,3-DMU. In the presence of O_2, 1.1 mol of 1,3-DMU and 0.6 mol of O_2 are consumed per mol Fe^{2+} while 0.16 mol of 1,3-DMU-glycol and 0.17 mol of organic hydroperoxides (besides further unidentified products) are formed. In the presence of O_2, **1** and **2** are rapidly converted into the corresponding peroxyl radicals ($k = 9.1 \times 10^8$ L mol^{-1} s^{-1}). Their bimolecular decay ($2k = 1.1 \times 10^9$ L mol^{-1} s^{-1}) yields ∼22% $HO_2^{\bullet}/O_2^{\bullet -}$ in the course of fragmentation reactions involving the C(5)−C(6) bond. Reduction of Fe^{3+} by $O_2^{\bullet -}$ leads to an increase in ${}^{\bullet}OH$ production that is partially offset by a consumption of Fe^{2+} in its reaction with the peroxyl radicals (formation of organic hydroperoxides, $k \approx 3 \times 10^5$ L mol^{-1} s^{-1}; value derived by computer simulation).

Introduction

In the Fenton reaction, a transition metal ion in its low oxidation state (e.g., Fe^{2+}) reacts with H_2O_2 yielding ${}^{\bullet}OH$ whereby the transition metal ion is oxidized to its higher oxidation state [e.g., Fe^{3+}, reaction 1, $k \approx 60$ L mol^{-1} s^{-1}].[1−4]

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \qquad (1)$$

In living cells, oxidative DNA damage can occur in Fenton-type reactions due to the presence of low concentrations of transition-metal ions and also low concentrations of H_2O_2 formed in autoxidation processes and as a side reaction in the respiratory chain. The presence of superoxide, $O_2^{\bullet -}$, that reduces the transition metal ions such as Fe^{3+} or Cu^{2+} to the lower oxidation state[5] may favor this process. In the food packaging industry, H_2O_2 is widely used for disinfection,[6−8] and Fenton reactions are thought to play an important role in the killing of bacteria and spores.[9]

The ${}^{\bullet}OH$ radical is also a major contributor in the damaging of DNA by ionizing radiation.[10] Here, it is due to the radiolysis of the water [reaction 2] that surrounds the DNA. In ex-vivo (model) studies, the yield of ${}^{\bullet}OH$ can be doubled by reacting the solvated electron with N_2O [reaction 3]. This renders a system that provides 90% ${}^{\bullet}OH$ and 10% $H^{\bullet}$.

$$H_2O \xrightarrow[\text{radiation}]{\text{ionizing}} e_{aq}^-, {}^{\bullet}OH, H^{\bullet}, H^+, H_2O_2, H_2 \qquad (2)$$

$$e_{aq}^- + N_2O + H_2O \rightarrow {}^{\bullet}OH + OH^- + N_2 \qquad (3)$$

From a study of the Fenton reaction with DNA,[11] it has been concluded that some of the DNA radicals formed must be capable of enhancing H_2O_2 consumption by reducing Fe^{3+} to Fe^{2+}. This effect may be related to the observation that there are noticeable differences in the product spectrum obtained with DNA constituents upon γ-radiolysis of their aqueous solutions and the Fenton reagent.[12−15] So far, the origin of these intriguing differences has not been studied in detail. A DNA model system

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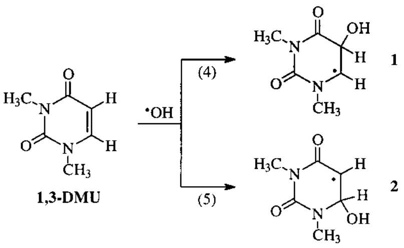
for which material balance has been obtained is 1,3-dimethyluracil (1,3-DMU).[16] Thus, 1,3-DMU has an advantage over other DNA models and may allow the study of the effect of Fe^{2+}, Fe^{3+}, and H_2O_2 on the products and their yields, and this system may be sufficiently simple to allow some generalizing conclusions.

Upon $^{\bullet}OH$ attack, 1,3-dimethyl-5-hydroxy-5,6-dihydrouracil-6-yl **1** and 1,3-dimethyl-6-hydroxy-5,6-dihydrouracil-5-yl **2** radicals are formed in a 4:1 ratio [reactions 4 and 5].[16] H-abstraction at the methyl groups is of little importance (see below).



The radical **1** has reducing, while radical **2** has oxidizing properties, and it will be shown in the present paper that this has a dramatic effect on the product distribution and their yields in the Fenton vs. radiolytic systems.

Experimental Section

1,3-Dimethyluracil (Fluka), hydrogen peroxide, ferrous ammonium sulfate (Merck), and tetranitromethane (Aldrich) were used as received. Catalase was obtained from Boehringer Mannheim. Solutions were prepared in Milli-Q-filtered (Millipore) water, and the pH was adjusted with either perchloric acid or sodium hydroxide. The solutions were deaerated by purging with argon (99.996%, Messer Griesheim) for 20 min or were saturated with O_2. The Fenton reaction was carried out at pH 4 by injecting an Fe^{2+} solution into a solution containing 1,3-DMU and H_2O_2. After completion of the Fenton reaction, i.e. a few minutes, the solution was neutralized and Fe^{3+} removed by filtration.

Analytical Procedures. For derivatization of the products formed in argon-purged solutions into volatile trimethylsilyl (TMS) ethers, the reaction mixture was rotary evaporated to dryness and trimethylsilylated with *N*,*N*-bis-trimethylsilyltrifluoroacetamide (BSTFA, Macherey-Nagel) in pyridine (dried over KOH). GC−MS analysis (Hewlett-Packard 5890 Series II) of the TMS ethers showed that there are two major products with identical mass spectra that are assigned to the TMS ethers of *cis*- and *trans*-5,6-dihydro-5,6-dihydroxy-1,3-dimethyluracil (1,3-DMU-glycol): [MW = 318] *m*/*z* 318 (3), 303 (15), 213 (8), 204 (7), 187 (40), 147 (40), 132 (30), 102 (21), 75 (25), 73 (100)] (cf. also data in ref 16). Gas chromatography with flame ionization detection shows that the two isomers are formed in a 6:1 ratio.

The decay of 1,3-DMU and the buildup of products at varying concentrations of Fe^{2+} were monitored by HPLC (25 cm Nucleosil C-18, methanol−water 1/10 v/v) coupled with a diode array detector (Merck-Hitachi L-3000). Under these conditions, the two 1,3-DMU-glycols elute together at 3.5 min and 1,3-DMU at 9 min. With water as eluent, *trans*- and *cis*-1,3-DMU-glycol nearly fully separate (retention times 6 and 6.5 min with peak areas of 1:6, as in gas chromatography). For quantification of 1,3-DMU-glycol, material was isolated by preparative HPLC. Here, it is also evident that the two glycols are formed in quite different yield (see above). The major fraction has been assigned by ^{1}H NMR (Bruker 250 MHz, measured in $DCCl_3$) as the cis isomer by comparing our data [δ_H ppm: 3.16 (3H, s, CH_3), 3.21 (3H, s, CH_3), 4.3 (1H, d, *J* 4 Hz, *H*-5) and 5.0 (1H, d, *J* 4 Hz, *H*-6)] with those reported in the literature.[17,18] The minor fraction [δ_H ppm: 3.08 (3H, s, CH_3), 3.21 (3H, s, CH_3), 4.15 (1H, d *J* 4 Hz, *H*-5) and 4.8 (1H, d, *J* 4 Hz, *H*-6)] is due to the trans isomer, cf. refs 17 and 18. In O_2-saturated solutions *cis*- and *trans*-1,3-DMU-glycols are formed in a 2:1 ratio. 1,3-Dimethylparabanic acid was detected as a minor product after rotary concentration and separation of the products by preparative HPLC. It has been characterized by ^{1}H NMR in D_2O [δ_H ppm: 3.09 (6H, s, CH_3)] in agreement with the value reported in the literature.[19] Its mass spectrum confirms the assignment: [MW 142] *m*/*z* 142 (70), 114 (13), 86 (6), 70 (18) 58 (100) 57 (54), 56 (64).

Formaldehyde was determined spectrophotometrically using the Hantzsch method.[20] Formic acid was determined by ion chromatography (Dionex DX-100) on an AS-9 column using 9×10^{-3} mol L^{-1} aqueous carbonate as eluent. The yield of organic hydroperoxides was determined with the help of molybdate-activated iodide[21] after destruction of H_2O_2 with catalase (10 μl per 10 mL solution). The O_2-uptake was measured with a Clark electrode (Wissenschaftliche Werkstätten, Weilheim).

γ-Radiolysis and Kinetic Studies. Because the radiolytic data reported earlier[16] are the basis of the present study, we have repeated these experiments. They proved to be reproducible (data not shown). γ-Radiolyses were carried out at a dose rate of 0.15 Gy s^{-1}; doses ranged between 100 and 400 Gy. For pulse radiolysis, an 11 MeV linear electron accelerator (Electronika 003, Thorium, Moscow) delivering 43 Gy pulses of 7 ns duration was used. For dosimetry, the thiocyanate system was used taking $G \times \epsilon = 5.2 \times 10^{-4}$ m^2 J^{-1} (at 472 nm) for the formation of $(SCN)_2^{\bullet -}$ in N_2O-saturated solutions.[22] Stopped-flow experiments were performed with a Biologic SF3 setup.

Kinetic simulations were carried out with the help of the Chemical Kinetics Simulator software, version 1.01 (from IBM, Almaden Research Center).

Results and Discussion

Kinetics of the Fenton Reaction. The kinetics of the formation of Fe(III) in the reaction of H_2O_2 with Fe^{2+} in the presence of 1,3-DMU was followed at 306 nm (Figure 1).

The buildup at 306 nm is biphasic. As is calculated from the data in Figure 1, the rate constant of the faster and major part, attributed to the reaction of Fe^{2+} with H_2O_2, has a rate constant of ~60 L mol^{-1} s^{-1} (for a compilation of rate constants determined in this study see Table 2). This value is in agreement with reported data.[1,34] This fast buildup at 306 nm is followed

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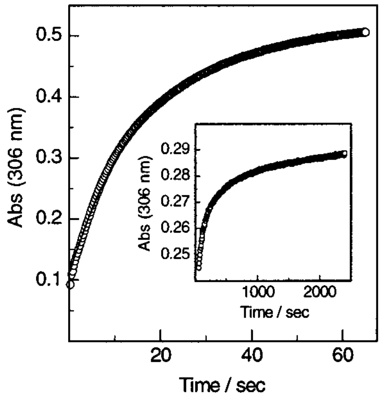


Figure 1. Kinetics of the reaction of H_2O_2 (1×10^{-3} mol L^{-1}) with Fe^{2+} (2×10^{-4} mol L^{-1}) in the presence of 1,3-DMU (1×10^{-3} mol L^{-1}) at pH 4 as followed at 306 nm using the stopped-flow technique for mixing H_2O_2/1,3-DMU with Fe^{2+}. Inset: slow increase of the absorbance at 306 nm (at 1×10^{-3} mol L^{-1} Fe^{2+}) followed in the UV spectrophotometer by time-drive.

Table 1. γ-Radiolysis of N_2O-Saturated Aqueous Solutions of 1,3-Dimethyluracil (1×10^{-3} mol L^{-1})

product	γ/pH 3 *G* value	γ/pH 6.5 *G* value	Fenton/Ar % of Fe^{2+}	Fenton/O_2 % of Fe^{2+}
5,6-dihydro-5-hydroxy-1,3-DMU	0.4	0.75	absent	
5,6-dihydro-6-hydroxy-1,3-DMU	<0.1	0.2	absent	
1,3-dimethylisobarbituric acid	0.15	0.1	absent	
5,6-dihydro-5,6-dihydroxy-1,3-DMU	1.5	0.85	295	16
dimers	1.8[a]	3.6[a]	absent	absent
formic acid			nd	10
formaldehyde			nd	4
organic hydroperoxides				17
1,3-dimethylparabanic acid			traces	observed
nitroform anion[b]			80	24
dioxygen consumption				62
1,3-DMU consumption	3.9	5.7	330	110

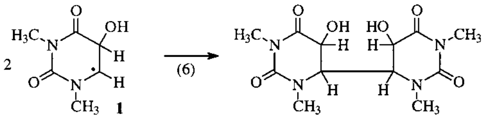
[a] In monomer units [b] In the presence of tetranitromethane (1×10^{-3} mol L^{-1} in argon-saturated and 1×10^{-4} mol L^{-1} in O_2-saturated solutions); nd = not determined. Products and their *G* values (in units of 10^{-7} mol J^{-1}),[16] compared with the yields (% of Fe^{2+} consumed) in the Fenton reaction (deaerated and O_2-saturated, [1,3-DMU] = 1×10^{-3} mol L^{-1}, [H_2O_2] = 1×10^{-3} mol L^{-1}, [Fe^{2+}] up to 3×10^{-4} mol L^{-1}, cf. Figure 2).

Table 2. Compilation of Rate Constants Determined in the Present Study

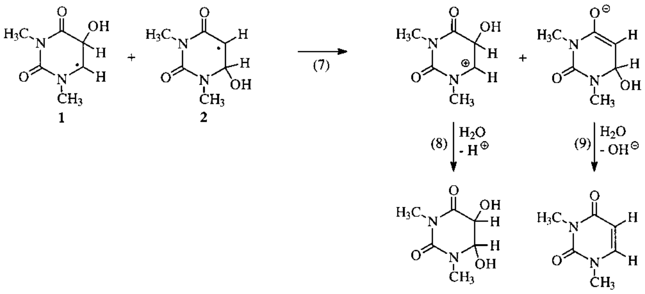
reaction	rate constant/L mol^{-1} s^{-1}
$Fe^{2+} + H_2O_2 \rightarrow {}^{\bullet}OH + Fe^{3+} + OH^-$	~60
${}^{\bullet}OH$ + DMU → **1**, **2**	6×10^9
1, **2** + **1**, **2** → products	8×10^8
1, **2** + O_2 → **1a**, **2a**	9.1×10^8
1a, **2a** + **1a**, **2a** → products	1.1×10^9

by a slower one (~6 s^{-1}, cf. inset in Figure 1). This change in absorbance is most likely not connected with the Fenton reaction, but since these experiments have been carried out at pH 4, the aqua-Fe(III) species formed is certainly not Fe^{3+}, i.e. a complex mixture of (still soluble) $Fe(OH)_n^{(3-n)+}$ species and oligomeric aggregates must be formed. Thus, this slow further increase at 306 nm may be connected with these hydrolytic and aggregation processes.

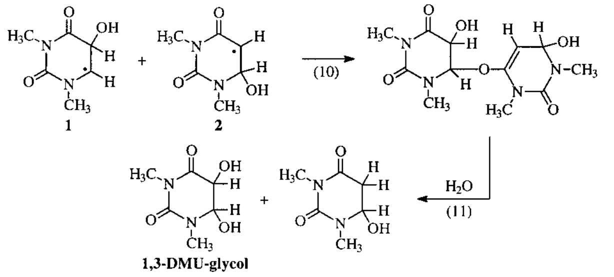
Deoxygenated Solutions. When 1,3-DMU is reacted with ${}^{\bullet}OH$ generated by ionizing radiation (in the absence of Fe^{2+}/Fe^{3+}), the primary ${}^{\bullet}OH$-adduct radicals **1** mainly dimerize (reaction 6) (cf. Table 1).



The cross-termination of the reducing radical **1** with the oxidizing radical **2** has been suggested to occur mainly by electron transfer (reactions 7−9) as the most likely route to the product second in importance, 1,3-DMU-glycol.



Acid-catalyzed rearrangement of radical **1** into the thermodynamically more stable radical **2** leads to an increase of the 1,3-DMU-glycol yield at the expense of that of the dimers (cf. Table 1). Concomitantly, the 1,3-DMU consumption is reduced due to its reformation upon one-electron reduction of radical **2** (reaction 9). A dimerization of **1** with **2** involving the mesomeric form of **2** with spin density at oxygen (for recent studies on this type of reaction see refs 23 and 24) and subsequent hydrolysis of the enol ether may also contribute to 1,3-DMU-glycol formation (reactions 10 and 11). Here, 5,6-dihydro-6-hydroxy-1,3-dimethyluracil should be the concomitant product. Its comparatively low yield (Table 1) indicates that this pathway should be minor.



Disproportionation reactions by H-transfer of radicals **1** and **2** must also occur, but the relatively low yields of 5,6-dihydro-5-hydroxy-DMU, 5,6-dihydro-6-hydroxy-DMU, and 1,3-dimethylisobarbituric acid and the fact that 1,3-dimethylbarbituric acid was not detected among the products indicate that in this system the disproportionation by H-transfer must be minor compared to dimerization and disproportionation by electron transfer.

When ${}^{\bullet}OH$ is generated in the Fenton reaction at room temperature (reaction 1), dimers are no longer formed, and the 1,3-DMU-glycols now dominate (Figure 2). In addition, small

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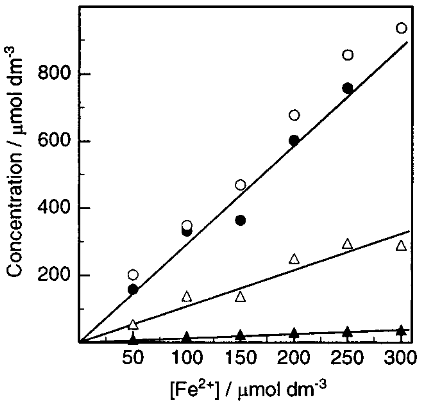


Figure 2. Reaction of Fe^{2+} with H_2O_2 (10^{-3} mol L^{-1}) in the presence of 1,3-DMU (10^{-3} mol L^{-1}), argon-saturated (○, ●) and O_2-saturated (△, ▲) at pH 4.0. Consumption of 1,3-DMU (○, △) and buildup of the sum of the 1,3-DMU-glycols (●, ▲) as a function of the Fe^{2+} concentration.

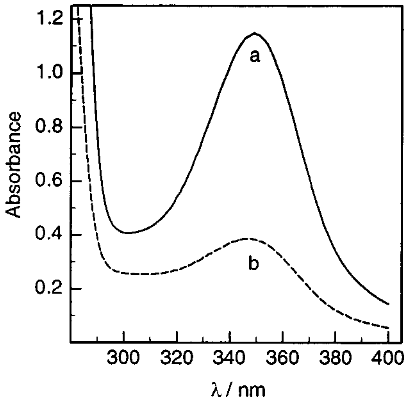
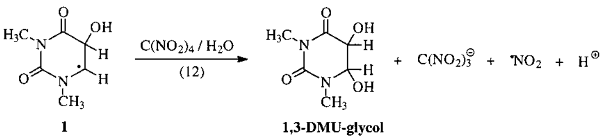


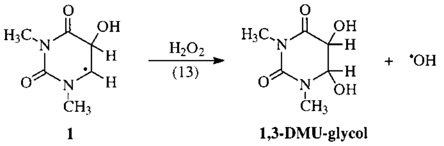
Figure 3. UV absorption spectra after the reaction of Fe^{2+} (1×10^{-4} mol L^{-1}) with H_2O_2 (1×10^{-3} mol L^{-1}) and 1,3-DMU (1×10^{-3} mol L^{-1}) in the presence of TNM: (a) argon saturated, [TNM] = 1×10^{-3} mol L^{-1}; (b) O_2-saturated, [TNM] = 1×10^{-4} mol L^{-1}. The absorption at 350 nm is due to the formation of nitroform anion and that at ~305 nm is largely due to Fe(III).

amounts of 1,3-dimethylparabanic acid (1,3-dimethylimidazolidine-2,4,5-trione) are formed as well.

As can be seen from these data (cf. also Table 1), the yield of 1,3-DMU-glycol is considerably higher than the primary •OH yield formed according to reaction 1. Thus, a short-chain reaction must take place. It has been mentioned above that radical **1** has reducing properties. The yield of the reducing radical **1** had been monitored by its rapid reaction with tetranitromethane, TNM [reaction 12; $k \approx 2 \times 10^9$ L mol^{-1} s^{-1}; cf. refs 25 and 26]. The product of this reaction is the nitroform anion, which is monitored by its strong absorption at 350 nm (ϵ = 15 000 L mol^{-1} cm^{-1}). This reaction has now also been carried out under the conditions of the Fenton reaction. As can be seen from the data shown in Figure 3, the yield of the nitroform anion is ~80% of the Fe^{2+} consumed, in good agreement with the earlier[16] determination by pulse radiolysis.



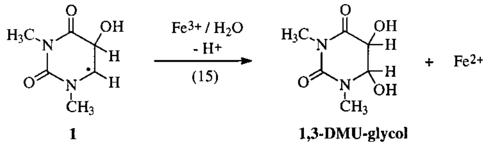
Radical **1**, having reducing properties, is expected to react with H_2O_2 at a noticeable rate according to reaction 13.



The corresponding reaction of the weakly reducing hydroxymethyl radical with H_2O_2 (reaction 14) has a rate constant of 6×10^4 L mol^{-1} s^{-1}.[27]

$$^{\bullet}CH_2OH + H_2O_2 \rightarrow CH_2{=}O + {}^{\bullet}OH + H_2O \qquad (14)$$

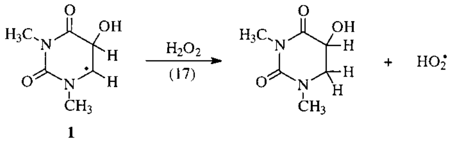
One must also consider an oxidation by the product of reaction 1, Fe^{3+} (reaction 15). In its effect, this reaction is equivalent to reaction 13, since Fe^{2+} formed in reaction 15 will also yield •OH (cf. reaction 1). Most "reducing" radicals react with $Fe(CN)_6{}^{3-}$ at close to diffusion-controlled rates ($k \approx 2 \times 10^9$ L mol^{-1} s^{-1}).[28] For the radicals derived from 5,6-dihydrouracil, a rate constant of 4×10^9 L mol^{-1} s^{-1} has been determined.[26] As aqua-Fe^{3+} may be slightly more reactive [$E°$ (Fe^{2+}/Fe^{3+}) = 0.77 V at pH 1, $E°$ ($Fe(CN)_6{}^{4-}/Fe(CN)_6{}^{3-}$) = 0.67 V at pH 1],[29] k_{15} is most likely not much slower than 10^8 L mol^{-1} s^{-1}.



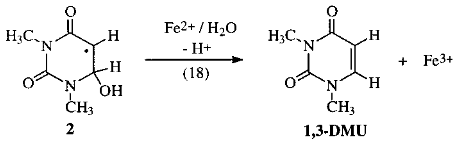
For the methanol system, it has been shown that the chain reaction is terminated by an H-abstraction from H_2O_2 (reaction 16) whose rate, however, is comparatively low (2.75×10^3 L mol^{-1} s^{-1}).[27]

$$^{\bullet}CH_2OH + H_2O_2 \rightarrow CH_3OH + HO_2{}^{\bullet} \qquad (16)$$

Thus, for the present system, one may write the equivalent reaction 17. Here, however, this reaction is not chain-terminating since $HO_2{}^{\bullet}/O_2{}^{\bullet-}$ are capable of reducing Fe^{3+} to Fe^{2+} which continues the chain (see below).



At room temperature, there is no experimental evidence for the formation of large amounts of 5,6-dihydro-5-hydroxy-1,3-DMU that should have been formed in reaction 17, and we hence favor reaction 18 as the major contributor to chain termination. In reaction 5, radical **2** is formed, and being an oxidizing radical it is expected to react fairly fast with Fe^{2+} (reaction 18). The reduction of uracil-derived radicals by Fe^{2+} has been envisaged before,[30] but from these data it is not possible to arrive at a rate constant.



The reaction of the oxidizing radical **2** with H_2O_2 can most likely be neglected. Here, only an H-abstraction could play a role. The reaction of the relatively reactive ${}^{\bullet}CH_3$ with H_2O_2 has (in water) a rate constant of only 2.7×10^4 L mol^{-1} s^{-1},[31] but radical **2**, although "oxidizing" (i.e., a good electron acceptor) may be a poor H-abstractor such as the related formylmethyl radical.[32,33] We therefore do not consider the reaction of **2** with H_2O_2 to play a significant role here.

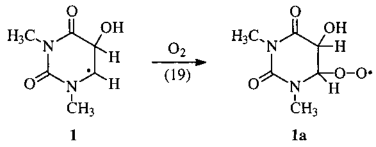
It has been mentioned above that small amounts of 1,3-dimethylparabanic acid were observed as well. No straightforward mechanism can be put forward at present to account for its formation under these conditions (note that the formation of 1,3-dimethylparabanic acid most likely requires more than two oxidation equivalents; i.e., it may be a secondary product or further H_2O_2 is involved in its formation).

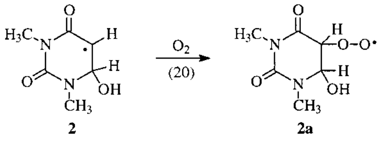
When experiments are carried out at 70 °C, 1,3-DMU consumption rises to 400% and the glycol yield drops to 150%. This indicates that additional processes must gain in importance. No details have been investigated.

Products in the Presence of Dioxygen. When the Fenton reaction is carried out in the presence of dioxygen, 1.1 mol of 1,3-DMU is consumed per mol Fe^{2+} reacted (Figure 2). The yield of 1,3-DMU-glycol is now only ~15% of 1,3-DMU consumed. The HPLC chromatogram shows a number of further products that are not observed in the absence of dioxygen. A detailed product study has not been undertaken, but the reactions of ${}^{\bullet}OH$ in the presence of dioxygen with the parent, uracil, may be a good guide. This system has been investigated in detail.[35] At pH 3, the uracil−glycol yield was $G = 1.1 \times 10^{-7}$ mol J^{-1}, i.e. ~19% of the ${}^{\bullet}OH$ yield, reasonably close to the 1,3-DMU-glycol yield found in the present study.

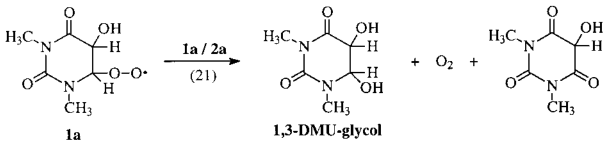
Attack of ${}^{\bullet}OH$ at the methyl groups is expected to yield mainly formaldehyde (for a review on peroxyl radicals in aqueous solutions see ref 36). This product is formed only in 4% yield; i.e., H-abstraction from the methyl groups is a minor process, as expected.

In the presence of dioxygen, the chain reaction is largely suppressed, as now the consumption of 1,3-DMU equals ~110% of Fe^{2+} reacted (cf. Table 1). The reason for this is the fast addition of dioxygen to radicals **1** and **2** (reactions 19 and 20). This rate constant has been determined at 1.9×10^9 L mol^{-1} s^{-1},[37] and we find a slightly lower value (9.1×10^8 L mol^{-1} s^{-1}, see below). A value around 10^9 L mol^{-1} s^{-1} is typical for the rate of O_2-addition to carbon-centered radicals.[36,38]

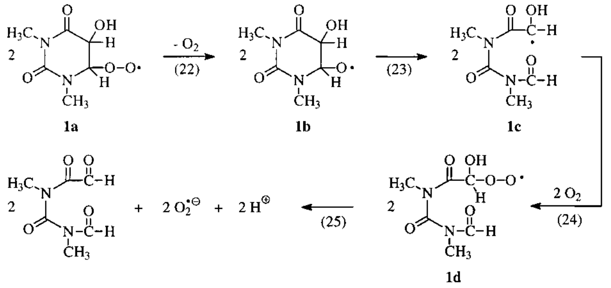




There will be a number of decay routes of the resulting peroxyl radicals **1a** and **2a** (cf. ref 36). Following the formation of a short-lived tetroxide, there will be various decay routes. The most likely route to 1,3-DMU-glycol, the so-called Russell mechanism, is depicted in reaction 21.



Since the yield of 1,3-DMU-glycol is only 16%, evidently further reactions have to be considered. From the products obtained with uracil,[35] one has to expect that reactions 22−25 must also occur in the present system.



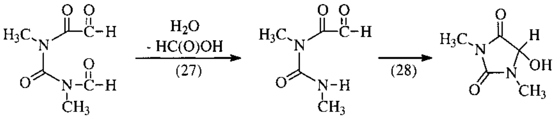
Upon the bimolecular decay of peroxyl radicals **1a** (a similar scheme can be written for **2a**), the oxyl radical **1b** is formed (reaction 22). This undergoes rapid β-fragmentation (reaction 23). The resulting radical **1c** adds dioxygen (reaction 24), and the peroxyl radical **1d** thus formed eliminates $HO_2{}^{\bullet}/O_2{}^{\bullet-}$ (reaction 25).

In the presence of TNM, $O_2{}^{\bullet-}$ is rapidly scavenged thereby forming the nitroform anion (reaction 26, $k = 2 \times 10^9$ L mol^{-1} s^{-1}).[5]

$$O_2{}^{\bullet-} + C(NO_2)_4 \rightarrow O_2 + C(NO_2)_3{}^- + {}^{\bullet}NO_2 \qquad (26)$$

According to the data shown in Figure 3, it is calculated that the reactions 22−25 contribute ~22% to the decay of the peroxyl radicals. This is supported by pulse-radiolytic experiments (see below).

We have recently shown that formyl compounds such as the one formed in reaction 25 are not very stable and eliminate formic acid (reaction 27).[39] The resulting product gives then rise to the corresponding hydroxyhydantoine (reaction 28) which is a typical product in the peroxyl radical chemistry of the uracil/thymine family.[10] Formate has been detected as one of the products (cf. Table 1).



The nitroform anion yield in the presence of TNM (cf. Figure 3) monitors the yield of $O_2{}^{\bullet-}$ formed in reaction 25, and the formate yield may be taken as an indicator of reactions 27/28. The nitroform anion yield has been found at ~24%. About 10% of this is formed in the reaction of **1** with TNM (reaction 12) in competition with its reaction with O_2. A yield of ~22% of $O_2{}^{\bullet-}$ released during the bimolecular decay of the peroxyl radical is also calculated from the pulse radiolysis data reported below.

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(35) Schuchmann, M. N.; von Sonntag, C. *J. Chem. Soc., Perkin Trans. 2* **1983**, 1525−1531.

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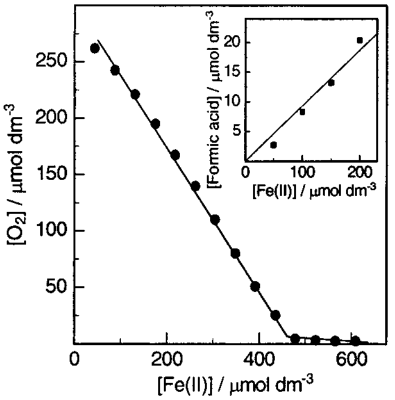
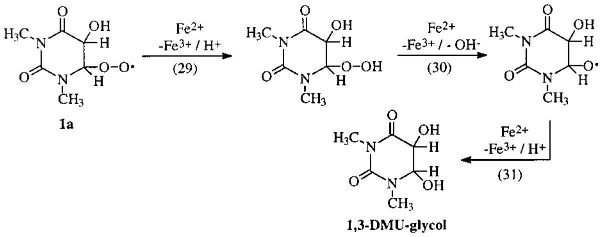


Figure 4. Consumption of O_2 in the reaction of H_2O_2 (1×10^{-3} mol L^{-1}) with Fe^{2+} in the presence of 1,3-DMU (1×10^{-3} mol L^{-1}) as a function of the Fe^{2+} concentration. Inset: formation of formic acid as a function of the Fe^{2+} concentration.

In competition with their bimolecular decay, the peroxyl radicals **1a** and **2a** can become reduced by Fe^{2+} (e.g., reaction 29).



The rate constant for the reaction of Fe^{2+} with $HO_2^{\bullet}$ at pH 1 is 1.2×10^6 L mol^{-1} s^{-1}.[5] An $Fe^{2+}/HO_2^{\bullet}$ adduct has been considered to be formed in the first step. Whether in our present system such an adduct is also as easily formed is not known, i.e., reaction 29 may proceed with a somewhat different rate constant. This type of reaction and subsequent reactions such reactions 30 and 31 are certainly induced (cf. ref 40) by the somewhat more powerful reductant Fe^{II}(EDTA). In the presence of an excess of H_2O_2 as in the present study, reactions 30 and 31 will be largely suppressed; i.e., the hydroperoxide formed in reaction 29 will be protected by the competing reaction 1 and thus largely survive. Indeed, an organic hydroperoxide is formed with ~17% yield (the yield vs. [Fe^{2+}] plot is linear, data not shown). We thus do not consider the potential reactions 30 and 31 to contribute to the formation of 1,3-DMU-glycol to a major extent.

As is seen from Table 1, a small amount of formaldehyde is formed in the presence of dioxygen. This product is due to reactions that follow an attack of $^{\bullet}OH$ at the methyl groups. Its yield being that low, this reaction is not taken into account any further.

A further measurable entity in this system is the consumption of dioxygen. As can be seen from Figure 4, 0.62 mol of O_2 are consumed per mol Fe^{2+} reacted. This low value indicates that excessive oxidative degradation does not take place in this system.

From the data shown in Figure 2, it is calculated that ~110% 1,3-DMU is consumed per Fe^{2+} consumed. This means that during the course of the reaction, Fe^{2+} must have been regenerated producing further $^{\bullet}OH$ via reaction 1. This regeneration of Fe^{2+} is caused by $O_2^{\bullet -}$ (reaction 32, $k = 1.5 \times 10^8$ L mol^{-1} s^{-1})[41] formed in reaction 25.

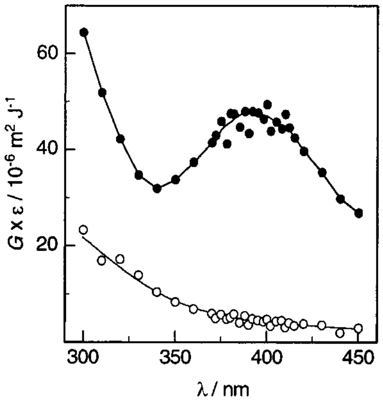


Figure 5. Pulse radiolysis of N_2O-saturated aqueous solutions of 1,3-DMU (1×10^{-3} mol L^{-1}) (●) and in N_2O/O_2 (4:1)-saturated solutions (○). Spectra taken 200 ns (●) and 1.4 µs (○) after a 43 Gy pulse of 7 ns duration.

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2 \qquad (32)$$

The yield of $O_2^{\bullet -}$ as determined by the TNM experiment is ~22%. On the other hand en route to the hydroperoxide two mol Fe^{2+} are consumed. This has to be taken into account when putting up a $^{\bullet}OH$ balance.

Pulse Radiolysis. For modeling the system by computer-assisted simulations, some rate constants are required which were determined using the pulse radiolysis technique. In the radiolysis of water (reactions 2 and 3), $^{\bullet}OH$ and $H^{\bullet}$ are formed with *G* values of 5.8×10^{-7} mol J^{-1} and 0.6×10^{-7} mol J^{-1}. When N_2O-saturated solutions containing 1×10^{-3} mol L^{-1} 1,3-DMU were pulse irradiated with a 43 Gy pulse a transient with $\lambda_{max} = 390$ nm (Figure 5) builds up with $k_{obs} = 6 \times 10^6$ s^{-1} (data not shown).

Thus, the rate constant of $^{\bullet}OH$ with 1,3-DMU is 6×10^9 L mol^{-1} s^{-1}, identical with the value reported[42] for its parent, uracil (for a compilation of rate constants determined in this study see Table 2). The decay of the transient follows second-order kinetics with $2k = 8 \times 10^8$ L mol^{-1} s^{-1} (data not shown).

In the presence of dioxygen, $H^{\bullet}$ (from reaction 1) and a small fraction of the solvated electrons are converted into $HO_2^{\bullet}/O_2^{\bullet -}$ (reaction 33, $k = 1.2 \times 10^{10}$ L mol^{-1} s^{-1})[42] while the OH-adduct radicals are converted into the corresponding peroxyl radicals (reactions 19 and 20).

$$H^{\bullet} + O_2 \rightarrow HO_2^{\bullet} \qquad (33)$$

These absorb only weakly in the accessible wavelength region (Figure 5), and the formation of the peroxyl radicals can be followed by monitoring the decay at, e.g., 400 nm (Figure 6). A plot of k_{obs} vs the dioxygen concentration yields a straight line with a slope of 9.1×10^8 L mol^{-1} s^{-1} and an intercept of $\sim 2.3 \times 10^5$ s^{-1} (cf. inset in Figure 6). The slope represents the rate of peroxyl radical formation. At the high dose per pulse used in these experiments, the bimolecular decay of the radicals involved in this system can no longer be neglected, and the observed intercept is due to this process. When experiments are carried out with low doses per pulse, such intercepts disappear (cf., e.g., refs 43 and 44), unless the reaction with

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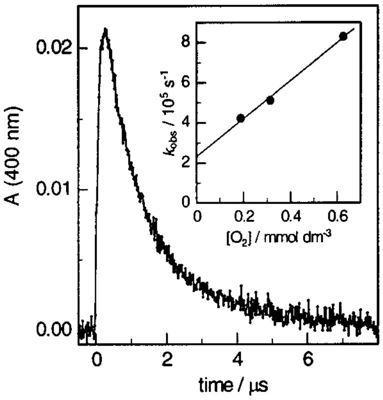

Figure 6. Pulse radiolysis of N_2O/O_2 (1:1)-saturated aqueous solutions of 1,3-DMU (1×10^{-3} mol L^{-1}). Decay of the OH-adduct radicals at 400 nm as a function of time; 43 Gy pulse of 7 ns duration. Inset: Plot of k_{obs} vs the dioxygen concentration.

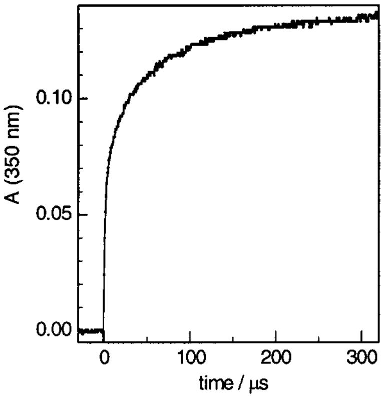

Figure 7. Pulse radiolysis of N_2O/O_2 (4:1)-saturated solutions of 1,3-DMU (1×10^{-3} mol L^{-1}) in the presence of 1×10^{-5} mol L^{-1} TNM; 43 Gy pulse. Formation of nitroform anion at 350 nm.

dioxygen is noticeably reversible.[45,46] In such a case, however, the precursor of the peroxyl radical is always in equilibrium with the peroxyl radical at short times, and its concentration depends on the dioxygen concentration. In the present system, this is not the case (note that the absorbance characterized by a maximum at 400 nm fully decays within 8 μs), and thus, reversibility as a cause of the intercept can be excluded.

In the presence of low concentrations of TNM, its reaction with $HO_2^{\bullet}/O_2^{\bullet -}$ [$pK_a(HO_2^{\bullet}) = 4.8$][5] from reaction 33 and with a small fraction of **1** (that are mainly converted into peroxyl radicals, see above) leads to a very rapid formation of nitroform anion (reaction 26).

In Figure 7, such an experiment is shown, and the first 44% of the signal are due to these reactions ($k_{obs} = 2 \times 10^5$ s^{-1}). The subsequent buildup of nitroform anion follows second-order kinetics.

The kinetics of the formation of nitroform anion in the second-order buildup can be used to calculate the rate of the bimolecular decay of the peroxyl radicals, because the rate of the reaction of $O_2^{\bullet -}$ with TNM is fast (2×10^9 L mol^{-1} s^{-1})[47] compared to the bimolecular decay (for earlier studies using this approach to determine the rate of the bimolecular decay of peroxyl radicals, see refs 48 and 49). From the data shown in Figure 7, a rate constant of $2k = 1.1 \times 10^9$ L mol^{-1} s^{-1} is calculated. This rate constant is very typical for the decay of primary and secondary peroxyl radicals in aqueous solutions.[36] When the decay of the dihydrouracil-derived peroxyl radicals had been studied,[50] the formation of $O_2^{\bullet -}$ during the bimolecular decay had not been realized. The buildup of the relatively strongly absorbing $O_2^{\bullet -}$ and its very slow decay had led to an estimate of $\sim 2 \times 10^7$ L mol^{-1} s^{-1} for the decay of these peroxyl radicals. In view of the present data, their decay must be much nearer to the value for the related peroxyl radicals studied here, i.e. 2 orders of magnitude higher.

The pulse radiolytic data of the TNM experiment yield another important information. From the yield of nitroform anion formed during the bimolecular decay, it is calculated that the $O_2^{\bullet -}$ yield formed during the bimolecular decay is 22% of the $^{\bullet}OH$ yield. This value is in agreement with the conclusions drawn from the TNM experiment in the Fenton reaction.

Simulations. The mechanism proposed in this paper has been modeled for the Chemical Kinetics Simulator. Simulations were run using the rate constants compiled in Table 2. Very important for the simulation is the ratio of reducing and oxidizing radicals **1** and **2**. Reducing radicals have been determined at ~82% and oxidizing radicals at ~18% of the total radical yield,[16] in agreement with our present data. Possibly, the small yield of radicals formed by H-abstraction from the *N*(1)-and *N*(3)-methyl group have been monitored together with the reducing radicals **1**. Being that minor, they were not included in the simulation. For the oxidation of **1** by Fe^{3+} and the reduction of **2** by Fe^{2+}, rate constants of 1×10^8 and 1×10^6 L mol^{-1} s^{-1} have been assumed. For the reactions of **1** with H_2O_2, the same rate constants as those found for the $^{\bullet}CH_2OH$ radical have been taken. With this set of rate constants, the experimental data in the absence of O_2 are well reproduced. With these assumptions, the simulation shows that the oxidation of **1** by H_2O_2 can be neglected in comparison with its oxidation by Fe^{3+} that is formed in the course of the reaction.

In the presence of O_2, the radicals **1** and **2** are converted into the corresponding peroxyl radicals **1a** and **2a**, and since the reaction is close to diffusion-controlled (cf. Table 2), Fe^{2+}/Fe^{3+} cannot effectively compete. There is, however, a competition between the bimolecular decay of **1a**/**2a** and their reduction by Fe^{2+} that will give rise to the formation of the organic hydroperoxide. Simulations can reproduce the hydroperoxide yield if the rate constant of this reaction is set at 3×10^5 L mol^{-1} s^{-1}. This value is not far from the reported value for the corresponding reaction of $HO_2^{\bullet}$ with Fe^{2+} ($k = 1.2 \times 10^6$ L mol^{-1} s^{-1}).[41]

Conclusions

It has been suggested that in the Fenton reaction with DNA in the absence of dioxygen reducing DNA radicals may contribute to an increased H_2O_2 consumption.[11] This view is supported by the present study. In DNA, this enhancement may be largely due to pyrimidine-derived radicals, since these form upon $^{\bullet}OH$ attack reducing radicals to a much larger extent (cf.

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the present system) than the purine-derived ones.[10] In the cellular environment, the rate of formation of DNA radicals by the Fenton reaction will be low (it has been estimated[51] that close to DNA there is only one transition-metal ion in its low oxidation state per ~10^3 base pairs). DNA is a polyanion, and thus the bimolecular decay of the DNA-derived radicals will be considerably slowed by electrostatic forces (cf., e.g., ref 52). Due to these two effects, the intrinsic lifetime of the DNA-derived radicals (with respect to their bimolecular decay) will be very long. At high H_2O_2 concentrations such as prevail in disinfection processes, there must now be a considerable chance of these radicals to react with H_2O_2 (for the reactions of radicals with H_2O_2 see, e.g., refs 27 and 31) rather than with a transition-metal ion as in this study. In other cases, e.g., when the Fenton reaction is triggered by metabolic processes (i.e., at low H_2O_2 concentrations) and dioxygen is present at a sufficient concentration to convert the DNA radicals into the corresponding peroxyl radicals, the latter may undergo intramolecular reactions that are not encountered with the much shorter-lived nucleobase- or nucleotide-derived peroxyl radicals. Indeed, in the case of DNA, $^{\bullet}$OH-induced dioxygen uptake is noticeably higher than that of an equivalent mixture of nucleotides.[53] To account for a regeneration of Fe^{2+} it has tentatively suggested that in the cellular environment and at 0 °C this may proceed via a slow reduction of Fe^{3+} by H_2O_2.[51] In the present system where we deal with aqua-Fe^{3+}, there is no indication for such a reaction. However in the cellular environment, Fe^{3+} is likely to be complexed, and its slow reduction by H_2O_2 cannot be excluded on the basis of our experiments.

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