

# Radical-Induced Oxidation of Dithiothreitol in Acidic Oxygenated Aqueous Solution: A Chain Reaction

Manohar Lal,<sup>†</sup> Raghavendra Rao,<sup>‡</sup> Xingwang Fang,<sup>‡,§</sup>  
Heinz-Peter Schuchmann,<sup>‡</sup> and Clemens von Sonntag<sup>\*,‡</sup>

Contribution from the Chemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400085, India, Max-Planck-Institut für Strahlenchemie, Stiftstrasse 34-36, P.O. Box 101365, D-45470 Mülheim an der Ruhr, Germany, and Department of Technical Physics, Peking University, Beijing 100871, China

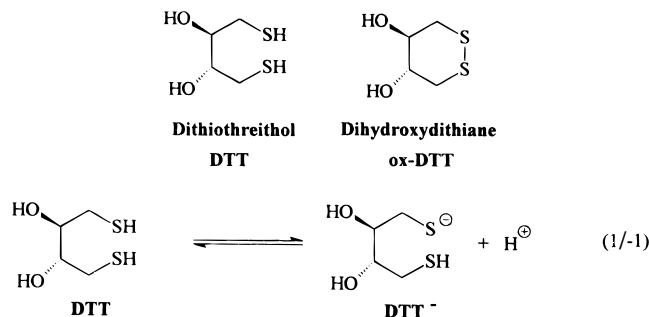
Received March 7, 1997<sup>⊗</sup>

**Abstract:** Sulfur-centered radicals derived from dithiothreitol (DTT) have been generated radiolytically in aqueous solution in the neutral to acid pH range. In the presence of O<sub>2</sub>, these are eventually transformed by a chain reaction into dihydroxydithiane (ox-DTT) and H<sub>2</sub>O<sub>2</sub>. In acid solution, the chain character of the reaction becomes more pronounced with decreasing pH. The radiolytic yield of the products (*G* value) also depends on the DTT concentration and the dose rate. The HO<sub>2</sub><sup>•</sup> radical carries the chain, abstracting an H atom from the DTT molecule (*k* = 120 dm<sup>3</sup> mol<sup>-1</sup> s<sup>-1</sup>), while its conjugated base, the O<sub>2</sub><sup>•-</sup> radical (p*K*<sub>a</sub>(HO<sub>2</sub><sup>•</sup>) = 4.8), does not react with DTT. The chain is continued when the DTT-derived radicals react with O<sub>2</sub>, yielding ox-DTT and HO<sub>2</sub><sup>•</sup> radicals. The self-termination of the HO<sub>2</sub><sup>•</sup>/O<sub>2</sub><sup>•-</sup> radicals breaks the chain.

## Introduction

The radiolysis of thiols in oxygenated aqueous solution proceeds with degradation yields that under many conditions are well above 6 × 10<sup>-7</sup> mol J<sup>-1</sup> and therefore indicative of a chain process. The oxidation of some monothiols in acidic and neutral solution has been studied and found to be a fairly complex process resulting in a variety of products, prominent among them the disulfide.<sup>1–6</sup> This process appears to involve the hydroperoxyl HO<sub>2</sub><sup>•</sup> and/or the thiylperoxyl RSOO<sup>•</sup> radicals; the involvement of the latter is indicated by the formation of sulfenic and sulfonic acids.

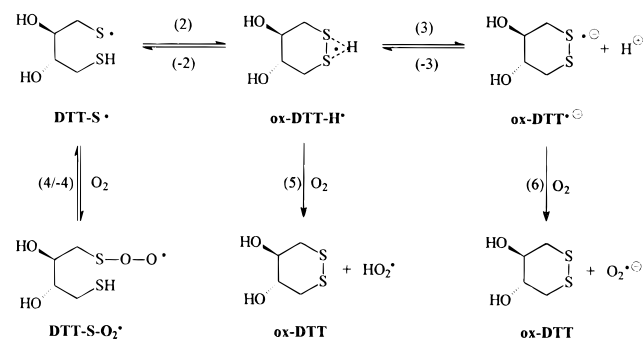
The behavior of the dithiol dithiothreitol (DTT) contrasts with that of the monothiols in that disulfide formation is strongly facilitated compared with the situation in the monothiols (intramolecular formation of the disulfide bond is favored over the intermolecular linkage); the probability of thiylperoxyl radical formation is therefore correspondingly reduced. DTT is known to undergo a chain oxidation reaction both in acidic solution (preliminary results have shown a stoichiometry of DTT + O<sub>2</sub> → ox-DTT + H<sub>2</sub>O<sub>2</sub>)<sup>5</sup> (ox-DTT = dihydroxydithiane) as well as in basic solution (p*K*<sub>a</sub>(DTT) = 9.12, *cf.* equilibrium 1;



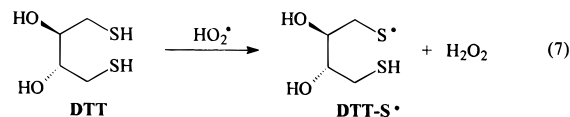
second p*K*<sub>a</sub> value at 10.15) with an overall stoichiometry of 2DTT + O<sub>2</sub> → 2ox-DTT + 2H<sub>2</sub>O.<sup>7</sup> This chain reaction is also

given by its stereoisomer dithioerythritol but not by their monothiolic subunit 2-mercaptoethanol.

Under these conditions, the free-radical oxidation of DTT unfolds as expressed by reactions 2–6. Below the p*K*<sub>a</sub> of ox-



DTT-H<sup>•</sup> which is near 5.2, DTT-S<sup>•</sup> and ox-DTT-H<sup>•</sup> exist side by side;<sup>8</sup> the exact ratio is not known, but on the basis of the data reported earlier,<sup>8,9</sup> it is reasonable to assume that it is close to unity. At pH above 6, the oxidation process is practically exclusively determined by the ox-DTT<sup>•-</sup> species which reacts rapidly with O<sub>2</sub>, thereby yielding ox-DTT and the superoxide radical, O<sub>2</sub><sup>•-</sup> (reaction 6; *k*<sub>6</sub> = 7.1 × 10<sup>8</sup> dm<sup>3</sup> mol<sup>-1</sup> s<sup>-1</sup>,<sup>7</sup> 1.37 × 10<sup>9</sup> dm<sup>3</sup> mol<sup>-1</sup> s<sup>-1</sup>,<sup>10</sup>). The superoxide radical is a very poor H-abstracter and hence cannot induce a chain reaction by abstracting an H-atom from DTT, in contrast to HO<sub>2</sub><sup>•</sup> where H<sub>2</sub>O<sub>2</sub> is produced (reaction 7). In fact, the stoichiometry of



the “basic” chain reaction shows that another mechanism must

(5) Lal, M. *Radiat. Phys. Chem.* **1994**, *43*, 595–611.

(6) Zhang, X.; Zhang, N.; Schuchmann, H.-P.; von Sonntag, C. *J. Phys. Chem.* **1994**, *98*, 6541–6547.

(7) Zhang, N.; Schuchmann, H.-P.; von Sonntag, C. *J. Phys. Chem.* **1991**, *95*, 4718–4722.

(8) Akhlaq, M. S.; von Sonntag, C. *Z. Naturforsch.* **1987**, *42c*, 134–140.

(9) von Sonntag, C. In *Sulfur-centered Reactive Intermediates in Chemistry and Biology*; Chatgililoglu, C., Asmus, K.-D., Eds.; Plenum: New York, 1990; pp 359–366.

<sup>†</sup> Bhabha Atomic Research Centre.

<sup>‡</sup> Max-Planck-Institut für Strahlenchemie.

<sup>§</sup> Peking University.

<sup>⊗</sup> Abstract published in *Advance ACS Abstracts*, June 1, 1997.

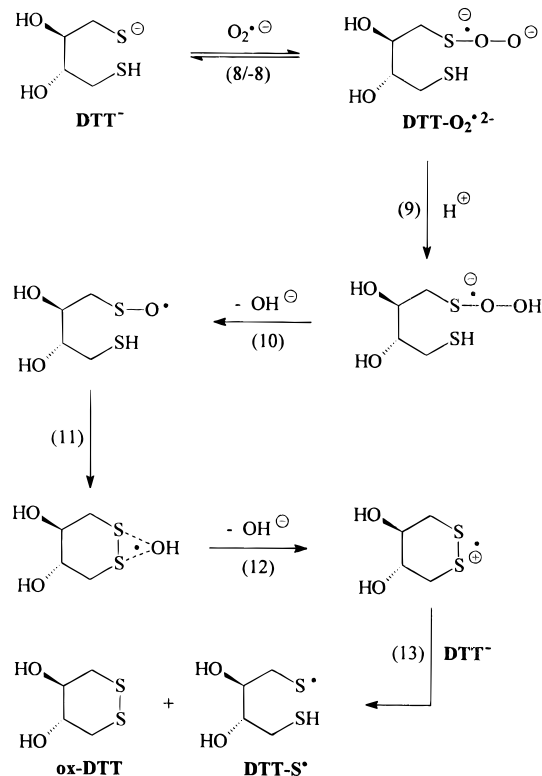
(1) Lal, M. *Radiat. Effects* **1974**, *22*, 237–242.

(2) Lal, M. *Radiat. Effects* **1974**, *22*, 49–52.

(3) Lal, M. *Can. J. Chem.* **1976**, *54*, 1092–1097.

(4) Lal, M. *Radiat. Phys. Chem.* **1982**, *19*, 427–434.

prevail, since  $\text{H}_2\text{O}_2$  is not a chain product. Moreover, the "basic" chain reaction materializes only when DTT is deprotonated. It has been suggested that the first chain step consists of the addition of the superoxide radical to the DTT-anion forming the three-electron bonded  $\text{DTT-O}_2^{\bullet 2-}$  intermediate (equilibrium 8; cf. ref 7). Such a chain reaction is not given



by 2-mercaptoethanol which is "half" of the DTT molecule. The reason for this probably is that the presence of a suitably-disposed additional thiol group is required within the molecule (only then might disulfide-bond formation be sufficiently fast) in order to allow the reaction sequence 11–13 to occur, which we now think to culminate in the propagation step, reaction 13. Disulfide radical cations are known to be fairly strong oxidants.<sup>11</sup> By considering the ensemble of reactions 2–13 as well as the  $\text{p}K_{\text{a}}$  of DTT ( $\text{p}K_{\text{a}1}(\text{DTT}) = 9.1$ ) and  $\text{HO}_2^\bullet$  ( $\text{p}K_{\text{a}}(\text{HO}_2^\bullet) = 4.8$ ),<sup>12</sup> one realizes that the conditions for the occurrence of a chain reaction are only fulfilled in two disjoint pH regions: in the basic pH region where  $\text{DTT}^-$  and  $\text{O}_2^{\bullet -}$  coexist and the acidic region where  $\text{DTT}$  and  $\text{HO}_2^\bullet$  coexist. In contrast, under near-neutral conditions, essentially  $\text{DTT}$  and  $\text{O}_2^{\bullet -}$  exist side by side and are mutually unreactive; thus no chain reaction can develop. The aim of the present paper is to characterize quantitatively the mechanism of the acidic chain reaction.

### Experimental Section

1,4-Dithiothreitol (DTT, Merck and Sigma) and 4,5-dihydroxy-1,2-dithiane (ox-DTT, Merck) were used as received. DTT solutions (concentrations up to  $1.5 \times 10^{-2} \text{ mol dm}^{-3}$ ) were freshly made up in Millipore-Q-filtered water saturated with air corresponding to an  $\text{O}_2$  concentration of  $2.4 \times 10^{-4} \text{ mol dm}^{-3}$ . The pH of the solution was adjusted to the desired value with  $\text{HClO}_4$ . Samples were irradiated with a  $^{60}\text{Co}$   $\gamma$ -source at the chosen dose rate. The products ox-DTT

(10) Chan, P. C.; Bielski, B. H. J. *J. Am. Chem. Soc.* **1973**, *95*, 5504–5508.

(11) Wardman, P. *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637–1755.

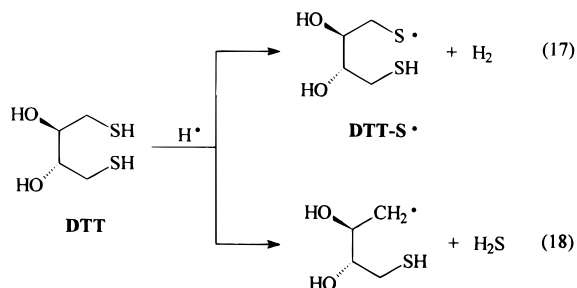
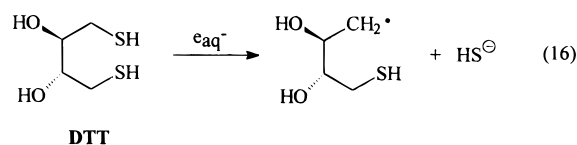
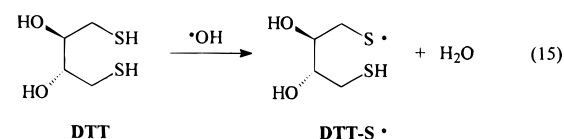
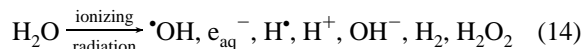
(12) Bielski, B. H. J.; Cabelli, D. E.; Arudi, R. L.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1985**, *14*, 1041–1100.

and  $\text{H}_2\text{O}_2$  were determined immediately after irradiation. ox-DTT was determined by directly measuring the absorbance of irradiated solutions at 283 nm and confirmed by HPLC on a Nucleosil 5C-18 column by optical detection at the same wavelength, with aqueous  $\text{CH}_3\text{OH}$  (2%)/ $\text{KH}_2\text{PO}_4$  ( $5 \times 10^{-3} \text{ mol dm}^{-3}$ ) as the eluent at a flow rate of  $1 \text{ mL min}^{-1}$ ; calibrations were done with authentic material.  $\text{H}_2\text{O}_2$  yields were estimated spectrophotometrically at 410 nm as the titanium sulfate complex, taking  $\epsilon(410) = 700 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ .<sup>13</sup>  $G$  values of ox-DTT and  $\text{H}_2\text{O}_2$  were obtained from the slopes of yield vs dose plots (cf. Figure 3).

Pulse radiolysis was done with a Van de Graaff accelerator delivering 2.8-MeV electron pulses of 0.4- $\mu\text{s}$  duration. The most recent additions to this setup have been reported.<sup>14</sup>

### Results and Discussion

**The Radical-Generating System.** Hydroxyl radicals, solvated electrons, and H atoms are generated in the radiolysis of dilute aqueous solutions (reaction 14). Their radiation-chemical yields are  $G(\bullet\text{OH}) \approx G(e_{\text{aq}}^-) = 2.9 \times 10^{-7} \text{ mol J}^{-1}$  and  $G(\text{H}) = 0.6 \times 10^{-7} \text{ mol J}^{-1}$ .<sup>15</sup> Hydroxyl radicals abstract a hydrogen atom from DTT (reaction 15). The solvated electrons undergo dissociative capture (reaction 16). The H atoms react both by hydrogen abstraction and by  $\text{H}_2\text{S}$  formation (reactions 17 and 18).



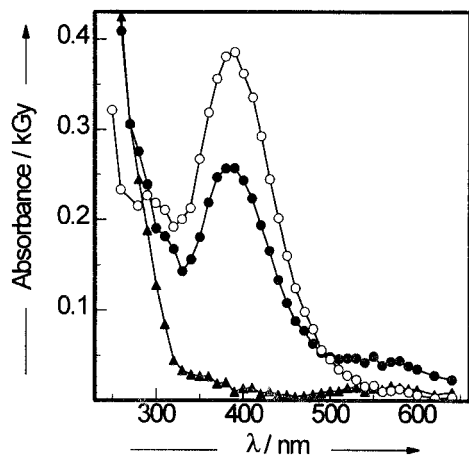
Some further reactions of  $e_{\text{aq}}^-$  and the H atom in this system have to be taken into account. At low pH ( $<2$ ), the solvated electron reacts mostly with  $\text{H}^+$  (reaction 19). H atoms and solvated electrons give rise directly to some  $\text{HO}_2^\bullet/\text{O}_2^{\bullet -}$  radicals (reactions 20 and 21;



$\text{p}K_{\text{a}}(\text{HO}_2^\bullet) = 4.8$ ).<sup>12</sup> Carbon-centered radicals are formed in the desulfuration reactions 16 and 18. At sufficiently high DTT

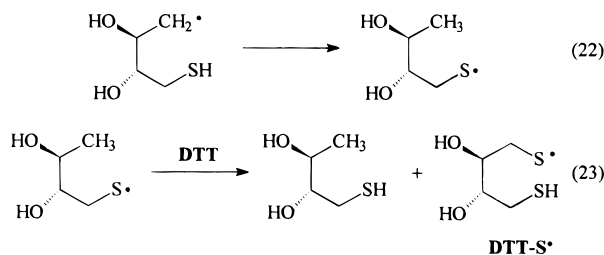
(13) Eisenberg, G. M. *Ind. Eng. Chem., Anal. Ed.* **1943**, *15*, 327–328.

(14) von Sonntag, C.; Schuchmann, H.-P. *Methods Enzymol.* **1994**, *233*, 3–20.



**Figure 1.** Pulse radiolysis of  $N_2O$ -saturated (○) and  $N_2O/O_2$  (4:1)-saturated aqueous solutions (pH 3.6) of DTT ( $10^{-4} \text{ mol dm}^{-3}$ ; ○, 5  $\mu\text{s}$ , ●, 1.5  $\mu\text{s}$ , and ▲, 7  $\mu\text{s}$  after a 6-Gy pulse). In the absence of  $O_2$ , the absorbance disappears very slowly by second-order kinetics and remains essentially unchanged within 10  $\mu\text{s}$  after the pulse.

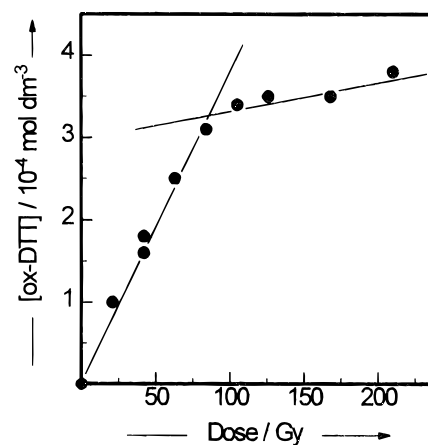
concentrations such as have been used in these experiments, these radicals are essentially quantitatively converted into DTT- $S^{\bullet}$  radicals via reactions 22 and 23.



The rate constant of hydrogen transfer from DTT to carbon-centered radicals is on the order of  $10^7$ – $10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ .<sup>15</sup> Thus the first-order rate constant of the intramolecular hydrogen transfer reaction 22 will be around  $10^8 \text{ s}^{-1}$  (assuming an “intramolecular thiol concentration” of  $10 \text{ mol dm}^{-3}$ ): the 2-mercaptoethanol-derived thiyl radical is reduced by DTT with a rate constant of  $10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ .<sup>8</sup> The loss of DTT- $S^{\bullet}$  via thiylperoxyl radical formation (reaction 4) is negligible because the reverse reaction is expected to be fast,<sup>6,16</sup> moreover any radical derived from DTT- $SOO^{\bullet}$  in an irreversible reaction (cf. ref 6) is expected eventually to be transformed into DTT- $S^{\bullet}$  by its reaction with DTT. Thus, at the typical DTT concentrations of  $10^{-2} \text{ mol dm}^{-3}$  used in our experiments, all primary radicals are converted into DTT- $S^{\bullet}$  radicals.

**Pulse Radiolysis.** A strong optical absorption near 390 nm is observed with DTT after OH-radical attack. This has been attributed to ox-DTT- $H^{\bullet}$ , which exists in equilibrium with its ring-opened isomer DTT- $S^{\bullet}$  (cf. equilibrium 2), since thiyl radicals show no absorption at this wavelength.<sup>8</sup> In the presence of  $O_2$  the 390 nm absorption decays very rapidly (Figure 1). The rate of this decay increases with the  $O_2$  concentration, and a value of  $2 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  is estimated for  $k_5$ .

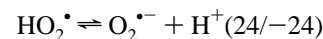
Thiylperoxyl radicals which are produced in the reaction of thiyl radicals with  $O_2$  (reaction 4) have a weak absorption in the 530–560 nm region.<sup>6,16,17</sup> This reaction is reversible (equilibrium 4).<sup>6,16</sup> In the present system, only a small absorption around 560 nm is observed during the decay of the 390-nm band. This is due to the rapid reverse reaction –4 ( $k_{-4}$



**Figure 2.**  $\gamma$ -Radiolysis of air-saturated solutions of DTT ( $10^{-2} \text{ mol dm}^{-3}$ ) at pH 2.3 at a dose rate of  $0.085 \text{ Gy s}^{-1}$ . Formation of ox-DTT (corrected for an ox-DTT impurity in the DTT sample) is given as a function of the dose; a break point can be defined which characterizes the dose near which  $O_2$  has been completely consumed.

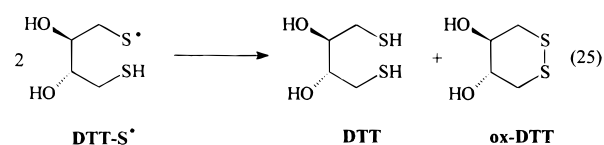
on the order of  $10^6 \text{ s}^{-1}$ , if the 2-mercaptoethanol system<sup>6</sup> is taken as a guide), as well as to the rapid attainment of the DTT- $S^{\bullet} = \text{ox-DTT-H}^{\bullet}$  equilibrium ( $k_3 = 1.5 \times 10^6 \text{ s}^{-1}$ ).<sup>8</sup>

The reaction of ox-DTT- $H^{\bullet}$  with  $O_2$  (reaction 5) is irreversible, and most likely an  $HO_2^{\bullet}$  radical is formed in a dismutation reaction where the weakly-bound H atom is detached by the  $O_2$  molecule. Thus in acid solutions the primary DTT- $S^{\bullet}$  radicals are rapidly converted into ox-DTT and  $HO_2^{\bullet}$  radicals. The latter are in equilibrium with their conjugated base,  $O_2^{\bullet-}$  ( $pK_a(\text{HO}_2^{\bullet}) = 4.8$ , equilibrium 24).



It has been noted above that  $O_2^{\bullet-}$  is not capable of abstracting an H atom from DTT. On the other hand,  $HO_2^{\bullet}$  shows a resemblance to alkylperoxyl radicals and can abstract H atoms as these do. The low-dose-rate experiments reported in the following will allow derivation of a rate constant for this reaction.

**Product Studies at Low Dose Rates.** Under chain-reaction conditions, i.e. at low pH,  $G(\text{ox-DTT})$  and  $G(\text{H}_2\text{O}_2)$  practically match. Moreover, the data indicate that there is no reaction of any significance which leads to a consumption of  $O_2$  beyond that taken up by the chain reaction leading to ox-DTT and  $\text{H}_2\text{O}_2$ . The consumption of  $O_2$  has been estimated by following the formation of ox-DTT as a function of dose (Figure 2). Its rate remains high as long as  $O_2$  is present to sustain the chain reaction but slows down to the rate in oxygen-free systems when the mechanism changes and ox-DTT is formed in the disproportionation reaction (eq 25). The data in Figure 2 give a value around  $40 \times 10^{-7} \text{ mol J}^{-1}$  for  $G(\text{ox-DTT})_{\text{oxic}}$  under these conditions;  $G(\text{ox-DTT})_{\text{anoxic}} \approx 3 \times 10^{-7} \text{ mol J}^{-1}$  conforms to expectation.

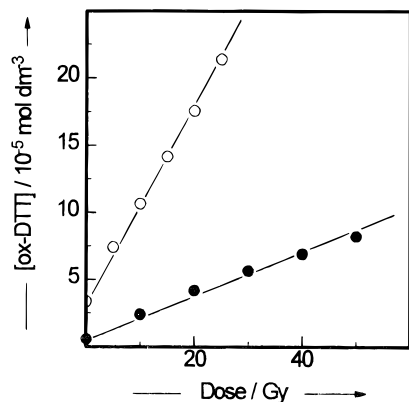


The linearity of the yield of ox-DTT with dose even as  $O_2$  is being consumed shows that the chain length does not depend

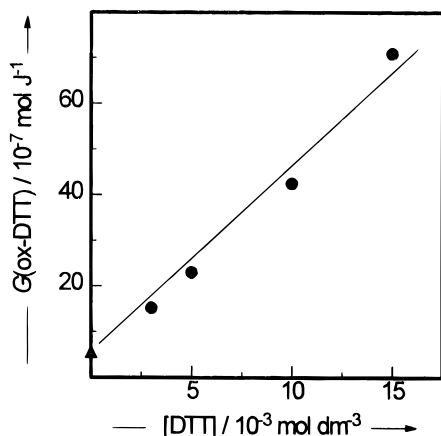
(15) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor and Francis: London, 1987.

(16) Tamba, M.; Simone, G.; Quintiliani, M. *Int. J. Radiat. Biol.* **1986**, *50*, 595–600.

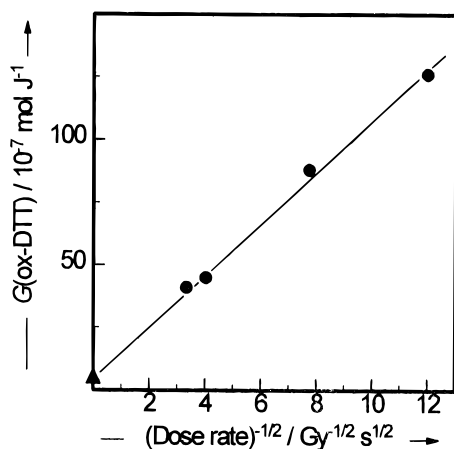
(17) Jayson, G. G.; Stirling, D. A.; Swallow, A. J. *Int. J. Radiat. Biol.* **1971**, *19*, 143–156.



**Figure 3.**  $\gamma$ -Radiolysis of air-saturated solutions of DTT (O,  $1.5 \times 10^{-2} \text{ mol dm}^{-3}$ ; ●,  $3 \times 10^{-3} \text{ mol dm}^{-3}$ ) at pH 2 at a dose rate of  $0.085 \text{ Gy s}^{-1}$ . The intercepts are due to an ox-DTT impurity in the DTT samples.



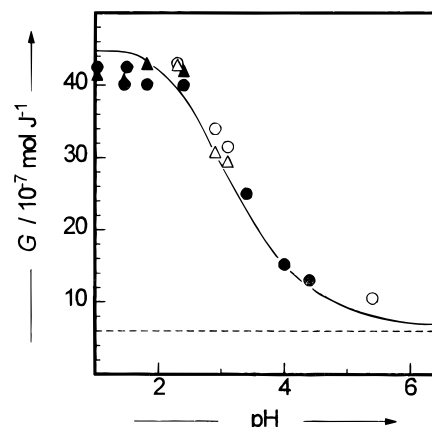
**Figure 4.**  $\gamma$ -Radiolysis of air-saturated solutions of DTT at pH 2 at a dose rate of  $0.085 \text{ Gy s}^{-1}$  (●; the value represented by ▲ is the nonchain yield of ox-DTT; see text).



**Figure 5.**  $\gamma$ -Radiolysis of air-saturated solutions of DTT ( $10^{-2} \text{ mol dm}^{-3}$ ) at pH 2.3.  $G(\text{ox-DTT})$  is shown as a function of the inverse of the square root of the dose rate (●; the value represented by ▲ is the nonchain yield of ox-DTT which is  $6 \times 10^7 \text{ mol J}^{-1}$ ).

on the  $\text{O}_2$  concentration (Figures 2 and 3). It does depend, however, on the DTT concentration (Figure 4).

This observation implies that the slow propagation step must involve DTT. As is the case with chain reactions, there is a pronounced dose rate effect. In Figure 5,  $G(\text{ox-DTT})$ , measured at pH 2.3 and a DTT concentration of  $10^{-2} \text{ mol dm}^{-3}$ , is plotted as a function of the inverse of the square root of the dose rate. The intercept reflects the ox-DTT yield in the absence of a chain



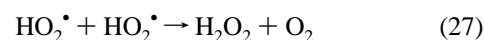
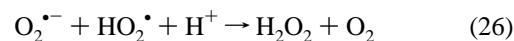
**Figure 6.**  $\gamma$ -Radiolysis of air-saturated solutions of DTT ( $10^{-2} \text{ mol dm}^{-3}$ ) as a function of pH at a dose rate of  $0.085 \text{ Gy s}^{-1}$ . Formation of ox-DTT (●, Mülheim data; ○, Trombay data) and  $\text{H}_2\text{O}_2$  (▲, Mülheim data; △, Trombay data). The dashed line indicates the nonchain contribution to  $G(\text{ox-DTT})$ . The solid line is computed on the basis of the overall rate constant (pH-dependent)<sup>12</sup> of the self-termination of  $\text{HO}_2^\bullet/\text{O}_2^{\bullet-}$  radicals and  $k_7 = 130 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ .

reaction, i.e. under conditions where the rate of reaction 7 is much smaller than the rate of  $\text{HO}_2^\bullet$  radical termination (reaction 27; see below). The slope is proportional to the chain propagation rate constant (see below).

The mechanistically most important feature is the strong pH dependence of the chain length. As shown in Figure 6, no chain reaction is observed in close to neutral solutions (the dotted line in Figure 6 represents the nonchain yield of ox-DTT). Upon lowering the pH,  $G(\text{ox-DTT})$  increases and levels out to a value of above 40 at about pH 2.

**Mechanistic Considerations.** The pulse radiolytic data prove that ox-DTT- $\text{H}^\bullet$  reacts rapidly with  $\text{O}_2$ , yielding  $\text{HO}_2^\bullet$  (reaction 5). The fast reversibility of the reaction of  $\text{O}_2$  with DTT- $\text{S}^\bullet$  radical (equilibrium 4) and the fast equilibrium between DTT- $\text{S}^\bullet$  and ox-DTT- $\text{H}^\bullet$  (equilibrium 2) do not allow the buildup of high steady-state concentrations of DTT- $\text{S}^\bullet$  radicals; essentially all ox-DTT- $\text{H}^\bullet$ /DTT- $\text{S}^\bullet$  is very rapidly converted by  $\text{O}_2$  into ox-DTT and  $\text{HO}_2^\bullet$ . This conclusion remains unaffected, should there exist a direct conversion of DTT- $\text{S}^\bullet$  into ox-DTT and  $\text{HO}_2^\bullet$  which is not ruled out. The apparent rate constant, taken from the decay of the absorption of ox-DTT- $\text{H}^\bullet$  at 390 nm, is  $2 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ . This high value indicates that none of the DTT-derived radicals reach sufficiently high steady-state concentrations to play a role in the termination reaction. Hence this is left to the  $\text{HO}_2^\bullet$  radical. In acid solution, the production of the chain-reaction products ox-DTT and  $\text{H}_2\text{O}_2$  is ensured by the sequence of the steps 5 and 7.

The  $\text{HO}_2^\bullet$  radical is in equilibrium with its conjugated base,  $\text{O}_2^{\bullet-}$  (equilibrium 24,  $\text{p}K_a = 4.8$ ). Its termination with  $\text{O}_2^{\bullet-}$  is faster (reaction 26,  $k_{26} = 9.7 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ ) than its self-termination (reaction 27;



$k_{27} = 8.3 \times 10^5 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ ).<sup>12</sup> This must be taken into account when calculating the steady-state concentrations of the  $\text{HO}_2^\bullet$  radical as a function of dose rate and pH.

In near to neutral solutions, no chain reaction is observed, in agreement with earlier observations.<sup>7</sup> The reason for this is the fact that superoxide  $\text{O}_2^{\bullet-}$  which dominates under these conditions is not capable of abstracting an H atom from DTT; it

eventually disappears through reaction 26. As the pH is lowered the ratio  $\text{HO}_2^*/\text{O}_2^{\bullet-}$  increases. At pH 4.8 it reaches unity; however at that pH the overall decay rate of  $\text{HO}_2^*/\text{O}_2^{\bullet-}$  according to reactions 26 and 27 is about 25 times faster than at pH 2 and below,<sup>12</sup> and it can be shown that the steady-state concentration of  $\text{HO}_2^*$ , and therefore the chain length, at pH 4.8 should not be one-half but only about one-tenth that observed at pH 2 and below. The data shown in Figure 6 bear this out.

The rate constant  $k_7$  for the propagating step 7 can be calculated from the slope of Figure 5, on the basis of eq 28.

$$G(\text{ox-DTT})_{\text{chain}} \dot{D} = \{k_7[\text{DTT}]\}[(G_{\text{init}}\dot{D})/k_{27}]^{1/2} \quad (28)$$

Chain termination is by reaction 27 ( $\dot{D}$ , dose rate;  $G_{\text{init}}$ , radiation-chemical yield of free radicals). Upon substitution, eq 28 yields  $k_7 = 110 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ . The fit of the data in Figure 6 was obtained using  $k_7 = 130 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ . A value of  $120 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  for  $k_7$  is therefore reported.

#### The Reaction $\text{HO}_2^* + \text{DTT}$ as a Model for $\text{RO}_2^* + \text{RSH}$ .

Hydroperoxyl  $\text{HO}_2^*$  may be considered as model of the type of peroxy radical  $\text{RO}_2^*$  that is devoid of electron-withdrawing groups. The present system allows to extract the rate constant  $k(\text{HO}_2^* + \text{RSH})$  (here,  $\text{RSH} = \text{DTT}$ ) under experimental conditions that preclude any interference by the thiolate ion form  $\text{RS}^-$ , which is known to react much faster with a given peroxy radical than its protonated form  $\text{RSH}$ .<sup>18</sup> Now it is by no means uncommon that rate constants are reported<sup>19</sup> for the reaction  $\text{RO}_2^* + \text{RSH}$  which have been determined under pH conditions that are much closer to the  $\text{p}K_{\text{a}}$  of the thiol than in the present case, e.g. peroxy radicals derived from polynucleotides and DNA are reported<sup>20</sup> to react with thiols with rate constants ranging from  $8 \times 10^3$  to  $1.5 \times 10^5 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  (however,

(18) Simic, M. G.; Hunter, E. P. L. *J. Free Radicals Biol. Med.* **1986**, *2*, 227–230.

(19) Neta, P.; Huie, R. E.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1990**, *19*, 413–513.

(20) Schulte-Frohlinde, D.; Behrens, G.; Oenal, A. *Int. J. Radiat. Biol.* **1986**, *50*, 103–110.

these values have recently been revised downward, and for the reaction of DNA with glutathione, a value of  $\leq 4 \times 10^2 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  has been determined).<sup>21</sup> In the light of the foregoing, this indicates that in cases where considerably higher rate constants than  $k_7$  are found this may be due to a contribution of the much more reactive thiolate form present in a low but significant proportion.

In the living cell, the reaction  $\text{RO}_2^* + \text{RSH}$  is of considerable importance. The thiol glutathione is present in high concentrations (on the order of  $5 \times 10^{-3} \text{ mol dm}^{-3}$ ),<sup>22</sup> and it plays a major role in the repair of radiation-induced DNA damage on the free-radical stage.<sup>23–25</sup> This repair process exists in competition with the addition of  $\text{O}_2$  to these (carbon-centered) DNA radicals (“fixation of damage”). *In-vitro* model studies, i.e. radiolysis of DNA in aqueous solution,<sup>15,26</sup> suggest that a major pathway for the disappearance of these DNA-peroxy radicals is by free-radical termination. Owing to the macromolecular nature of these radicals, these termination reactions are relatively slow.<sup>21,27</sup> In the light of the present study the question arises, especially with regard to the living cell, to what extent the DNA-peroxy radicals are reduced by glutathione. This reaction would lead to DNA hydroperoxides whose noxiousness is not yet known but may be considerable.

**Acknowledgment.** The work has been partially supported by the European Commission, Project F14P-CP95-0011.

JA970744L

(21) Hildenbrand, K.; Schulte-Frohlinde, D. *Int. J. Radiat. Biol.* **1997**, *71*, 377–385.

(22) Held, K. D. In *Oxygen radicals in Biology and Medicine*; Simic, M. G., Taylor, K. A., Ward, J. F., von Sonntag, C., Eds.; Plenum Press: New York, 1988; pp 441–448.

(23) Alper, T. *Int. J. Radiat. Biol.* **1983**, *44*, 313–314.

(24) von Sonntag, C.; Schuchmann, H.-P. In *Sulfur-centered Reactive Intermediates in Chemistry and Biology*; Chatgililoglu, C., Asmus, K.-D., Eds.; Plenum: New York, 1990; pp 409–414.

(25) Harrop, H. A.; Held, K. D.; Michael, B. D. *Int. J. Radiat. Biol.* **1991**, *59*, 1237–1251.

(26) Dizdaroglu, M. *Free Radicals Biol. Med.* **1991**, *10*, 225–242.

(27) Ulanski, P.; Bothe, E.; Hildenbrand, K.; von Sonntag, C.; Rosiak, J. M. *Nucleonika* **1997**, in press.