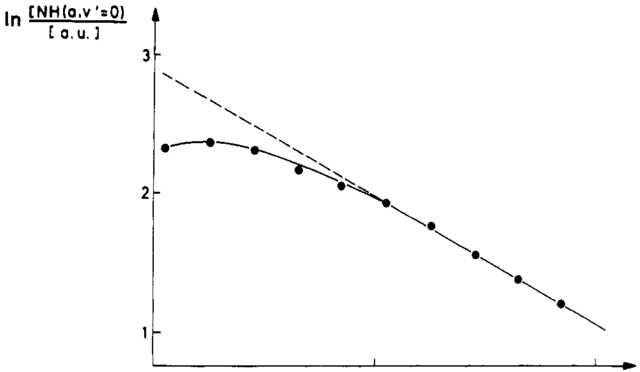

Figure 13. Semilogarithmic plot of NH(a,v''=0) concentration profile following photolysis of HN_3 at λ_L = 248 nm in the presence of N_2, as given in ref 14: $p(HN_3)$ = 1.7 μbar, $p(N_2)$ = 21 mbar.

assumed for all v''. Inserting these data in a computer simulation allows a corrected rate constant

$$k_1 = (1.7 \pm 0.2) \times 10^{11}\ \mathrm{cm^3/(mol\ s)}$$

to be obtained. This value is slightly lower if a two-quanta deactivation mechanism

$$\mathrm{NH}(a,v''{=}2) + N_2 \rightarrow \mathrm{NH}(a,v''{=}0) + N_2$$

is taken into consideration.

The quenching of NH(a,v''=2) by N_2

$$\mathrm{NH}(a,v''{=}2) + N_2 \rightarrow \mathrm{prod.} \qquad (2)$$

is shown in Figure 11 as a ln[NH(a,v''=2)] vs Δt plot. Plots of this kind were linear under all observed experimental conditions. The overall rate constant $k_2 = 1.5 \times 10^{11}$ cm^3/(mol s) can be evaluated from the plot k^I vs $p(N_2)$, as shown in Figure 12. This leads to the value

$$k_2 = (1.7 \pm 0.2) \times 10^{11}\ \mathrm{cm^3/(mol\ s)}$$

if we apply the same corrections for vibrational deactivation of higher states as described above.

In the quenching experiments of NH(a,v''=0) with N_2,[14] in which the NH(a) was produced from the HN_3 photolysis at λ_L = 248 nm, an increase was found in the NH(a,v''=0) profile for small reaction times (Figure 13). For probe laser delay times $\Delta t > 22$ μs a linear decrease of ln $I_F(\Delta t)$, i.e. of [NH(a,v''=0)](Δt), was observed. The straight line in Figure 13 is an extrapolation of the measured NH(a,v''=0) concentration toward Δt = 0. The deviation from the linear plot in Figure 13 can be understood as a formation of the rovibronic state NH(a,v''=0,J'') by a source different from the direct HN_3 photolysis. Rotational relaxation can be ignored, since it is completed within a significantly shorter time scale (ns). The reproduction of NH(a,v''=0) can be explained now by vibrational relaxation.

The quenching rate constants of NH(a,v'') by N_2 are all significantly smaller than gas kinetic collision numbers; thus, an activation barrier can be expected, which was determined directly for NH(a,v''=0)[23] and also recently for NH(a,v''=1)[24] when the temperature dependence of the collisional quenching of NH(a) was measured.

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The Reaction of Superoxide Radical Anion with Dithiothreitol: A Chain Process

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The radical-induced oxidation of dithiothreitol (DTT) by O_2 to 4,5-dihydroxy-1,2-dithiane (ox-DTT) in aqueous solutions of pH 8.7 proceeds via a chain reaction. Radicals such as OH generated by the radiolysis of N_2O/O_2-saturated aqueous solutions abstract an H atom from DTT. The resulting DTT(-H)$^\bullet$ radicals cyclize and deprotonate, yielding the disulfide radical anion ox-DTT$^{\bullet-}$. The latter reacts with O_2 ($k = 7.1 \times 10^8$ dm^3 mol^{-1} s^{-1}) giving rise to ox-DTT and $O_2^{\bullet-}$. The *G* value of ox-DTT formation increases with decreasing dose rate, displaying a linear yield vs (dose rate)$^{-1/2}$ relationship indicative of a chain reaction. The chain is inhibited by superoxide dismutase, showing that $O_2^{\bullet-}$ is a chain carrier. Hydrogen peroxide is not a chain product, from which it is concluded that $O_2^{\bullet-}$ does not abstract hydrogen from DTT but rather forms a short-lived complex with the DTT anion which then predominantly decomposes into an RSO$^\bullet$ radical and an OH$^-$ ion, (and to a lesser extent into an OH radical and an RSO$^-$ ion). In a second step, the RSO$^\bullet$ radical reacts with DTT (probably the anionic form DTT$^-$), thus regenerating DTT(-H)$^\bullet$. The resulting sulfenic acid eliminates water and yields the chain product ox-DTT. An overall propagation rate constant of 35 dm^3 mol^{-1} s^{-1} has been estimated.

Introduction

The superoxide radical anion participates in many chemical systems that undergo autoxidation by the action of O_2. In particular in aqueous solution, it is released from many peroxyl radicals.[1-3] Due to its slow self-termination reaction it can build up to rather high steady-state concentrations. In fact, self-termination of two $O_2^{\bullet-}$ radicals is very slow if it occurs at all ($k \leq 0.35$ dm^3 mol^{-1} s^{-1} at pH 13), while $O_2^{\bullet-}$ terminates readily ($k_{11} = 9.7 \times 10^7$ dm^3 mol^{-1} s^{-1}) with its conjugated acid $HO_2^\bullet$ (pK_a($HO_2^\bullet$) = 4.8).[4]

In the living cell where $O_2^{\bullet-}$ is an unavoidable byproduct of metabolism,[5-8] it can cause considerable damage (e.g. oxygen

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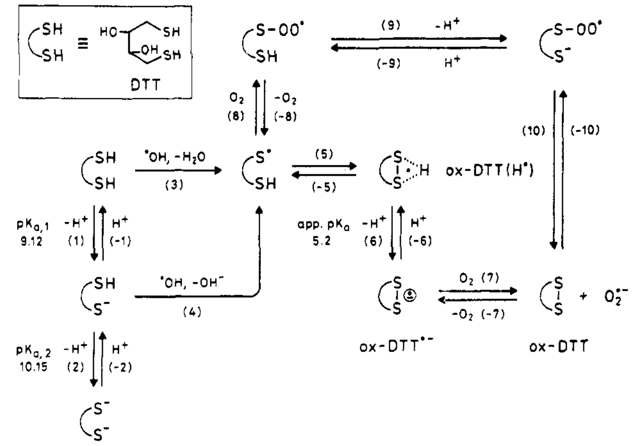
stress).[9] In order to cope with this problem, most living systems are provided with suitable enzymes, the superoxide dismutases (SODs), which destroy $O_2^{\bullet-}$. Although, generally speaking, $O_2^{\bullet-}$ is a rather inert radical (i.e., a very poor H abstractor.[10,11] While the O–H bond dissociation energy in H_2O_2 is 90 kcal mol^{-1}, it is less than 70 kcal mol^{-1} in HO_2^-: see ref 11.), in its reaction with transition-metal ions and their complexes it can ultimately lead to species that are much more reactive and most of its cell-damaging properties are attributed to such reactions.[1]

Regarding the reactivity of $O_2^{\bullet-}$ toward thiols, opinion is divided. For its reaction with cysteine a rate constant of <15 dm^3 mol^{-1} s^{-1} has been given,[12] in keeping with the well-known fact of the low H-abstractive power of $O_2^{\bullet-}$ (cf. refs 10–15). In contrast, for its reaction with dithiothreitol and other diols, including glutathione, rate constants that are larger by several orders of magnitude have been reported.[16-18] Powerful antioxidants such as pyrogallol and *n*-propyl gallate do in fact react quite readily with $O_2^{\bullet-}$ ($k \approx 3 \times 10^5$ dm^3 mol^{-1} s^{-1}),[19] but although these phenolic compounds contain relatively weakly bound hydrogen (O–H; cf. ref 20), $O_2^{\bullet-}$ reacts with these antioxidants by addition to the aromatic ring rather than by H abstraction.[19] This poses the question of whether the reaction of $O_2^{\bullet-}$ with thiols can indeed be as fast as has been reported,[18] and if such a reaction occurs, whether it really is an H-abstraction reaction. The answer to this question is of considerable interest, since in some cases, the cellular glutathione pool can be as high as 10^{-2} mol dm^{-3}. With a rate constant of about 10^6 dm^3 mol^{-1} s^{-1},[18] the reaction of $O_2^{\bullet-}$ with glutathione would be its main sink, much more important than its reaction with SOD (although SODs react very fast, $k \approx 2 \times 10^9$ dm^3 mol^{-1} s^{-1},[1] their concentrations are much lower than that of glutathione).

Having studied rather extensively the free-radical chemistry of 1,4-dithiothreitol (DTT) and its disulfide (ox-DTT),[21-26] we thought it both worthwhile and promising to take a closer look at this interesting question. The use of DTT as the thiol carries with it the advantage that superoxide yield enhancers such as formate ion are unnecessary, since it generates "its own" $O_2^{\bullet-}$ (see below). It will be seen that $O_2^{\bullet-}$ does indeed react with DTT, but with a rate constant that must be several orders of magnitude lower than those reported.[16,18] It will also be shown that its reaction with DTT is not by H abstraction.

SCHEME I



Experimental Section

1,4-Dithiothreitol (Merck) was used as received, 4,5-dihydroxy-1,2-dithiane was also obtained from Merck. Millipore Q filtered water was saturated with N_2O/O_2 (4:1 v/v) (Messer-Griesheim) for about half an hour. DTT was added to a concentration of 6×10^{-4} mol dm^{-3} and the pH of the solution adjusted to 8.7 with NaOH. The solution was then divided, part of it being irradiated with a ^{60}Co γ source at the desired dose rate, and the rest kept as the reference. Immediately after irradiation, both the irradiated solution and the reference were neutralized to block H_2O_2-induced and autoxidation reactions (see below). ox-DTT was determined by HPLC on a nucleosil 5C-18 column (15 cm × 5 mm) by optical detection at 285 nm, with aqueous $CH_3OH(2\%)/KH_2PO_4(0.005$ mol dm$^{-3})$ as eluent; in some cases it was also measured spectrophotometrically at 288 nm, taking $\epsilon(288) = 270$ dm^3 mol^{-1} cm^{-1}. The consumption of DTT was measured spectrophotometrically at 412 nm by the 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's reagent) method,[27] taking $\epsilon(412) = 13\,600$ dm^3 mol^{-1} cm^{-1}. Hydrogen peroxide was measured spectrophotometrically at 410 nm as its titanium sulfate complex, taking $\epsilon(410) = 700$ dm^3 mol^{-1} cm^{-1}.[28] The radiolysis results were corrected for the effects of an underlying autoxidation reaction (see below) by using a blank taken from the substrate solution before irradiation. Superoxide dismutase (bovine erythrocyte, Boehringer Mannheim) and catalase (beef liver, Boehringer Mannheim), when used, were present in concentrations of 20 mg dm^{-3} and 40 mg dm^{-3}, respectively. All experiments were carried out at 20 ±1 °C.

Pulse radiolysis was done with a Van de Graaff accelerator delivering 2.8-MeV electron pulses of 0.4-μs duration. The most recent additions to this setup have been reported in ref 29.

Results and Discussion

A precondition of studying any reaction of $O_2^{\bullet-}$ with other species, if it is relatively slow, is to make sure that $O_2^{\bullet-}$ is not drained away too fast by dismutation. This can be achieved by working at elevated pH where this reaction is slow.[4] On the other hand, it is known that autoxidative processes with thiols set in as the pH is raised. We found that at pH 8.7 autoxidative degradation of the DTT was still slow enough so as not to exceed the radiolytic effect, even at the lowest dose rates used, while at less alkaline pH the characteristics of the radiolytic process become indistinct.

Free radicals were generated by γ-radiolysis. When the ionizing radiation interacts with water, OH radicals, solvated electrons, and H atoms are formed by reaction i. In N_2O/O_2-saturated

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

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

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

solutions (4:1 v/v), the solvated electrons produce additional OH radicals (reaction ii, $k_{ii} = 9.1 \times 10^9$ dm^3 mol^{-1} s^{-1}).[30] The OH radicals and the H atoms react with the solute DTT (see Scheme I, reactions 3 and 4), the latter also with oxygen (reaction iii, $k_{iii} = 2.1 \times 10^{10}$ dm^3 mol^{-1} s^{-1}).

The reaction of the OH radical with DTT is fast (reaction 3, $k_3 = 1.5 \times 10^{10}$ dm^3 mol^{-1} s^{-1}),[22] and not much slower with the DTT monoanion (reaction 4). This reaction makes a contribution under the conditions of these experiments, which have been carried out mainly at pH 8.7, close to the first pK_a of DTT at 9.12 (equilibrium 1/−1; see Scheme I).

H atoms react with DTT, in a manner similar to reaction 3. We have determined k(H$^{\bullet}$ + DTT) by following the absorption buildup at 380 nm, λ_{max} of ox-DTT(H)$^{\bullet}$ (cf. ref 22) at pH 1 where all of the solvated electrons are converted into H atoms,[1] in the presence of 1 mol dm^{-3} *t*-butyl alcohol to scavenge the OH radicals.[1] At low DTT concentrations, the rate of buildup of the 380 nm absorption is determined by the reaction of H$^{\bullet}$ with DTT and k_{obsd} is proportional to [DTT], but at higher DTT concentrations k_{obsd} becomes independent of [DTT] and is determined solely by reactions 5 and −5. k_{obsd} is then found to be 1.5×10^6 s^{-1}, in agreement with earlier observations[22] (at pH 4, in N_2O-saturated solution, in the absence of *t*-butyl alcohol). From the low-concentration data (k_{obsd} vs [DTT]. Not shown: the shape of the plot is analogous to that shown in a diagram in ref 22.), we calculate k(H$^{\bullet}$ + DTT) = 3×10^9 dm^3 mol s^{-1}. For a compilation of the rate constants determined in this study, see Table I.

The open-chain thiyl radical is in near-equilibrium with its ring-closed (three-electron-bonded)[31] isomer ox-DTT(H)$^{\bullet}$ (reactions 5, −5). The latter deprotonates yielding the disulfide radical anion ox-DTT$^{\bullet-}$ (reactions 6, −6).[22,32,33] This system displays an apparent pK_a value of 5.2,[22] but since the equilibrium constant $K_{5,-5}$ is not exactly known, this value cannot be attributed to the pK_a value of equilibrium 6/−6.[26] Details of the somewhat complex situation are not relevant to the present study; it suffices to know that under the conditions of our experiments ox-DTT$^{\bullet-}$ is formed with a rise time of about 10 μs. The ox-DTT$^{\bullet-}$ intermediate is characterized by a strong absorption at 390 nm (ϵ = 5900 dm^3 mol^{-1} cm^{-1}).[22] It is well-known that disulfide radical anions such as ox-DTT$^{\bullet-}$ rapidly react with O_2, giving rise to the disulfide and $O_2^{\bullet-}$.[32,33] Using the pulse radiolysis technique (cf. ref 22), we have determined the rate constant for the reaction of ox-DTT$^{\bullet-}$ with O_2 (reaction 7) by following the decay of the absorption of ox-DTT$^{\bullet-}$ at 390 nm as a function of the oxygen concentration (data not shown). The value thus obtained for k_7 is 7.1×10^8 dm^3 mol^{-1} s^{-1}, slightly lower than that of 1.37×10^9 dm^3 mol^{-1} s^{-1} given earlier by Chan and Bielski.[33] Although reaction 7 is reversible, the equilibrium lies far to the right under the conditions of our experiments (pH 8.7, N_2O/O_2 (4:1 v/v) saturation). In competition with ring closure (reaction 5), the open-chain DTT radical can be scavenged by oxygen (reaction 8). Such reactions are known to be rapid as well as reversible; in the case of glutathione, k(GS$^{\bullet}$ + O_2) has been determined at 2×10^9 dm^3 mol^{-1} s^{-1} and k(GSOO$^{\bullet}$ → GS$^{\bullet}$ + O_2) at 10^6 s^{-1}.[34] We have repeated such experiments with 2-mercaptoethanol, which represents a subunit of DTT, and found similar results. Hence we believe that the same situation holds for DTT. Due to the fast reversibility of reaction 8, the intermediacy of a thioperoxyl radical merely delays the formation of ox-DTT and $O_2^{\bullet-}$. Even if reaction 9 proceeded fast enough to compete with reaction −8, the same products are expected due to reaction 10.

TABLE I: Compilation of Rate Constants Determined in This Study

reaction	k/dm^3 mol^{-1} s^{-1}
H$^{\bullet}$ + DTT	3×10^9
ox-DTT$^{\bullet-}$ + O_2	7.1×10^8
H_2O_2 + DTT monoanion	7
H_2O_2 + DTT dianion	15
$O_2^{\bullet-}$ + DTT monoanion	35[a]

[a] Chain propagation at pH 8.7, see text.

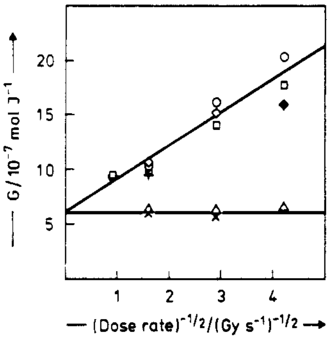

Figure 1. G values (unit: 1×10^{-7} mol J^{-1}) of disulfide production and substrate consumption in γ-irradiated DTT solution (6×10^{-4} mol dm^{-3}, N_2O/O_2-saturated (4:1 v/v)) at pH 8.7: in the presence of SOD, (△) G(DTT consumption) and (×) G(ox-DTT); in the presence of catalase, (+) G(DTT consumption) and (◇) G(ox-DTT); without any of these enzymes, (□) G(DTT consumption) and (○) G(ox-DTT). G values in the absence of catalase are uncorrected for the effect of reactions 13 and 14 during the time of irradiation. The samples were acidified immediately after irradiation to quench these reactions, as well as the autoxidation.

The ultimate fate of a DTT radical in the presence of oxygen at this pH is determined by the deprotonation reaction, reaction 6, and consists in the formation of ox-DTT and $O_2^{\bullet-}$. In the *absence* of any further reaction of $O_2^{\bullet-}$ with DTT, the yield of ox-DTT should be G($^{\bullet}$OH) = 5.8×10^{-7} mol J^{-1} plus a fraction of more than 70% of the H atoms (0.4×10^{-7} mol J^{-1}) scavenged by DTT (the rest disappear in reaction iii). Hence, in the absence of a chain reaction, the ox-DTT yield will be about 6.2×10^{-7} mol J^{-1}. But, as shown in Figure 1, the yield is dose-rate-dependent and much higher than this at lower dose rates. This means that there is a chain reaction that obviously depends on the presence of $O_2^{\bullet-}$ since SOD, which rapidly dismutates the $O_2^{\bullet-}$ radicals, acts as an inhibitor.

In the absence of SOD, the $O_2^{\bullet-}$ radicals dismutate only slowly according to reaction 11. The slowness of this reaction is largely due to the low equilibrium concentration of $HO_2^{\bullet}$ prevailing at pH 8.7 (pK_a($HO_2^{\bullet}$) = 4.8; $k_{11} = 9.7 \times 10^7$ dm^3 mol^{-1} s^{-1}).[4]

$$O_2^{\bullet-} + HO_2^{\bullet} \xrightarrow{H^+} O_2 + H_2O_2 \qquad (11)$$

Thus, at this pH $O_2^{\bullet-}$ can build up to rather high steady-state concentrations, and if it reacts with DTT by H abstraction (or by any reaction equivalent to this), the chain reaction sets in. In Figure 1, G(DTT consumption) and G(ox-DTT) are plotted against the inverse of the square root of the dose rate. A straight line with an intercept at about 6×10^{-7} mol J^{-1} is obtained. It is noteworthy that in contrast to SOD, catalase, which destroys the H_2O_2 as it is formed in the course of the irradiation, has only little if any effect on the chain length. No sulfur-containing product other than ox-DTT has been detected, and G(DTT consumption) = G(ox-DTT). This is compatible with reaction 11 being chain-terminating but appears incompatible with any termination reaction involving thioperoxyl radicals.

Chain reactions in the radiolytic oxidation of thiols in the presence of oxygen are apparently common (cf. refs 17, 35, 36). For the evaluation of the mechanism of such chain reactions,

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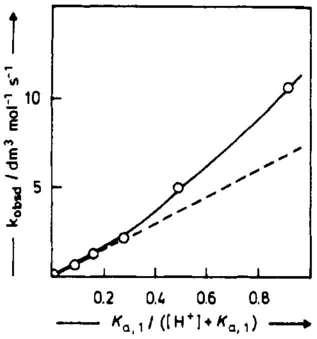

Figure 2. Rate constant of the reaction of hydrogen peroxide with DTT: (O) observed rate constant as a function of $K_{a,1}/([H^+ + K_{a,1})$; (---) extrapolation, representing the contribution of the reaction of H_2O_2 with DTT monoanion.

hydrogen peroxide is a key product. There are essentially three possibilities regarding the rank of H_2O_2: it could be a chain product or a termination product, or it might be restricted to the so-called molecular H_2O_2 yield (formed in reaction i). This will allow mechanistic inferences to be drawn (see below).

Reaction of H_2O_2 with DTT in Anionic Form. There is a complication with respect to the quantitative determination of $G(H_2O_2)$ in alkaline thiol solutions. It is known (cf. ref 37) that H_2O_2 oxidizes thiols, giving rise to the disulfide without the intervention of radiation: thiolate ion reacts with H_2O_2 by forming the corresponding sulfenic acid (reaction 12), which is then converted by further thiolate into the disulfide (reaction 13).

$$RS^- + H_2O_2 \rightarrow RSOH + OH^- \tag{12}$$

$$RSOH + RS^- \rightarrow RSSR + OH^- \tag{13}$$

We have found that reaction 12 is the rate-determining step in this sequence by following both the decay of H_2O_2 and the buildup of disulfide in a made up solution of DTT (6×10^{-4} mol dm^{-3}) and H_2O_2 (5×10^{-5} mol dm^{-3}). Both rate constants are identical. The observed rate constant (definition: rate of either H_2O_2 consumption or disulfide formation equal to $k_{obsd}[DTT_{total}][H_2O_2]$) can be plotted vs $K_{a,1}/([H^+] + K_{a,1})$[38] where $K_{a,1}$ is the equilibrium constant of the first dissociation of DTT ($pK_{a,1} = 9.12$). This plot (Figure 2) is a straight line as long as the pH is below about 8.7, but curves upward as the pH is increased beyond this value. This is proof that in this pH range, apart from reaction of the monoanion, the reaction of DTT dianion with hydrogen peroxide also makes a contribution. The second dissociation of DTT has a $pK_{a,2} = 10.15$.

From the slope of the straight part of the line in Figure 2, we calculate that the rate constant of reaction 12 is 7 dm^3 mol^{-1} s^{-1}, which comes out very close to the values for cysteamine and cysteine.[38] Moreover, from the difference between the experimental k_{obsd} and the value obtained by extrapolation to pH 10.2 (cf. dashed line in Figure 2), considering only the reaction of DTT monoanion with hydrogen peroxide, a rate constant of 15 dm^3 mol^{-1} s^{-1} is estimated for the reaction of DTT dianion with hydrogen peroxide. Thus, at a pH of 8.7 and a DTT concentration of 6×10^{-4} mol dm^{-3}, conditions under which the majority of the reported experiments have been done, the half-life of the reaction of hydrogen peroxide with DTT is 6.5 minutes. This is fast in the context of postirradiation product analysis, and accordingly the reaction has to be taken into account when assessing the radiolytic yield of ox-DTT.

In an experiment undertaken for the purpose of making sure that, apart from the complication introduced by its reactivity toward the thiolate ion, a quantitative determination of H_2O_2 in our alkaline solutions is feasible, an N_2O/O_2-saturated thiol-free formate solution was irradiated at pH 10.3. In this system, OH radicals from the radiolysis of water react with formate yielding $CO_2^{\bullet-}$ radicals, which react rapidly with oxygen and give rise to carbon dioxide and $O_2^{\bullet-}$ radicals. The latter disproportionate according to reaction 11. The observed value of $G(H_2O_2) = 3.75 \times 10^{-7}$ mol J^{-1} is in agreement with expectation. It includes the so-called molecular yield of about 0.8×10^{-7} mol J^{-1} from reaction i. It is hence ascertained that H_2O_2 can be adequately determined at this pH.

Formation of ox-DTT and H_2O_2 upon Radiolysis. For a proper determination of the radiolytic yield of H_2O_2 and ox-DTT, the reaction of H_2O_2 with the DTT anions should be quenched by the addition of acid immediately after cessation of irradiation. If this is done, then, after a dose of 126 Gy (dose rate 0.35 Gy s^{-1}), 3.1×10^{-5} mol dm^{-3} H_2O_2 is found which corresponds to an apparent G value of 2.4×10^{-7} mol J^{-1}. (This is lower than the expected value of 3.6×10^{-7} mol J^{-1} and reflects the fact that already while the irradiation is still going on, some of the H_2O_2 being produced disappears in reaction 12.) As Figure 1 shows, this effect is relatively minor beside the radiolytic one: the points "with catalase" lie only moderately below those without. The concomitant disulfide yield G(ox-DTT) is 10.6×10^{-7} mol J^{-1}. When the radiolyzed solutions are *not* acidified but left alkaline, $G(H_2O_2)$ drops with the expected half-life while G(ox-DTT) increases beyond the value of 10.6 (see Figure 3) by an equivalent amount.

Mechanistic Considerations. Since $G(H_2O_2)$ exceeds the molecular yield (reaction i) but is considerably lower than G(ox-DTT), we may rule out reactions 14 and 15 (Scheme II), where the rate-determining reaction would be H abstraction by $O_2^{\bullet-}$ radical from an S–H function of DTT (reaction 14).

SCHEME II

$$O_2^{\bullet-} + DTT \rightarrow H_2O_2 + \text{ox-DTT}^{\bullet-} \tag{14}$$

$$\text{ox-DTT}^{\bullet-} + O_2 \rightarrow \text{ox-DTT} + O_2^{\bullet-} \tag{15}$$

$$\text{net: } DTT + O_2 \rightarrow \text{ox-DTT} + H_2O_2 \tag{16}$$

This scheme requires that in the reaction chain (neglecting the slow oxidation of DTT by H_2O_2), *equal* amounts of ox-DTT and H_2O_2 should be formed (overall reaction 16). Indeed the total H_2O_2 yield should be even *higher*, by 0.8×10^{-7} mol J^{-1}, i.e. the molecular H_2O_2 formed in reaction i. As can be seen from Figure 3, this is far from the case, and it is easy to show that the loss of H_2O_2 through oxidation of DTT during the time of irradiation would not materially alter this picture. Thus Scheme 2 does not explain the results, *$O_2^{\bullet-}$ does not react with DTT by H abstraction*, and an alternative mechanism must operate, such as presented in Scheme III.

SCHEME III

$$O_2^{\bullet-} + DTT^- + H^+ \rightleftarrows (DTT\cdots O_2^{\bullet-}) \tag{17}$$

$$(DTT\cdots O_2^{\bullet-}) \rightarrow HS{\sim}SO^{\bullet} + OH^- \tag{18}$$

$$(DTT\cdots O_2^{\bullet-}) \rightarrow HS{\sim}SO^- + {}^{\bullet}OH \tag{19}$$

$$HS{\sim}SO^- + H^+ \rightarrow \text{ox-DTT} + H_2O \tag{20}$$

$$HS{\sim}SO^{\bullet} + DTT \rightarrow HS{\sim}SOH + \text{ox-DTT}^{\bullet-} + H^+ \tag{21}$$

$${}^{\bullet}OH + DTT \rightarrow H_2O + \text{ox-DTT}^{\bullet-} + H^+ \tag{3}$$

$$\text{ox-DTT}^{\bullet-} + O_2 \rightarrow \text{ox-DTT} + O_2^{\bullet-} \tag{7}$$

$$\text{net: } 2DTT + O_2 \rightarrow 2\text{ox-DTT} + 2H_2O \tag{22}$$

It can be shown that this scheme leads to the stoichiometry (22). It is based on the well-known fact that compounds of divalent sulfur have a pronounced tendency to form complexes with radicals (formation of three-electron bonds).[1,31,39] We postulate equilibrium 23/−23, drawing attention to an analogy between

$$RS^- + O_2^{\bullet-} \rightleftarrows RS(OO^-)^{\bullet-} \tag{23/−23}$$

$$RS(OO^-)^{\bullet-} + H_2O \rightleftarrows RS(OOH)^{\bullet-} + OH^- \tag{24/−24}$$

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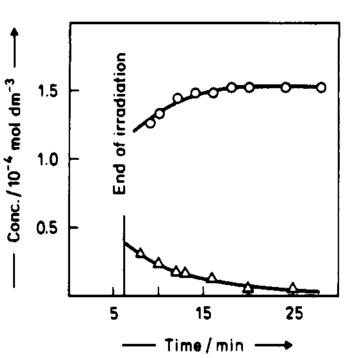

Figure 3. Postirradiation effect in DTT solution (6.3×10^{-4} mol dm^{-3}, N_2O/O_2-saturated (4:1 v/v)) at pH 8.7; radiolysis product concentration as a function of time: (○) ox-DTT: (△) hydrogen peroxide.

$RS(OO^-)^{\bullet -}$ and the well-documented $RSSR^{\bullet -}$. It has been reported that sulfur free-radical complexes exist where one of the sulfur atoms is formally replaced by nitrogen or even oxygen.[40-42] It is likely that the dioxygen function of this radical dianion is protonated, i.e. in the hydroperoxide form, except under very alkaline conditions (equilibrium 24/−24). It may be recalled that the pK_a of H_2O_2 is 10.9.

We may envisage β-cleavage of the intermediate $RS(OOH)^{\bullet -}$ (in Scheme III denoted as $(DTT\cdots O_2^{\bullet -})$, giving rise to either an $RSO^{\bullet}$ radical and an OH^- ion (reaction 18) or a sulfenate ion and an OH radical (reaction 19). Due to the much higher solvation energy of the hydroxide ion compared to the sulfenate ion, it is expected that reaction 18 is favored over reaction 19. A DTT-derived sulfinyl radical has recently been observed by ESR methods at low temperatures in a γ-irradiated methanol matrix upon annealing;[43] under these conditions (neutral pH), this radical is probably generated from the thiylperoxyl radical by oxygen transfer to a thiol group[44] (cf. also refs 45–47).

The question of the intermediacy of an OH radical can be tackled. Hydroxyl radicals react rapidly with primary and secondary alcohols by mainly (>80%) abstracting a hydrogen atom in α-position relative to the alcohol function (reaction 25). The

$$^{\bullet}OH + R_2CHOH \rightarrow H_2O + {}^{\bullet}C(R_2)OH \qquad (25)$$

$$^{\bullet}C(R_2)OH + O_2 \rightarrow {}^{\bullet}O_2C(R_2)OH \qquad (26)$$

$$^{\bullet}O_2C(R_2)OH \rightarrow HO_2^{\bullet} + R_2C{=}O \qquad (27)$$

$$^{\bullet}O_2C(R_2)OH + OH^- \rightarrow {}^{\bullet}O_2C(R_2)O^- + H_2O \qquad (28)$$

$$^{\bullet}O_2C(R_2)O^- \rightarrow O_2^{\bullet -} + R_2C{=}O \qquad (29)$$

α-hydroxyalkyl radicals thus formed combine rapidly with oxygen (reaction 26) and the resulting α-hydroxyalkylperoxyl radicals readily eliminate $HO_2^{\bullet}$, or in basic solutions, $O_2^{\bullet -}$ (reactions 27–29).[1,48]

With such alcohols, the $O_2^{\bullet -}$ radical itself cannot propagate a chain reaction,[14] but if in reactions 17 and 19 it is converted by DTT into a further OH radical, the ketone yield must increase beyond that in the absence of DTT. Experiments using 2-propanol and 2-butanol (3×10^{-2} mol dm^{-3}) to scavenge the OH radicals in the presence and absence of DTT (6×10^{-4} mol dm^{-3}) have been carried out at pH 8.7 and the yields of acetone and 2-butanone determined. It was found that the presence of DTT makes no significant difference. From this we conclude that reaction 19 is of minor importance and that the postulated complex mainly decays by reaction 18. This is in keeping with what we know about the decomposition of *t*-butylhydroperoxide by the solvated electron, where the formation of *t*-butoxyl radicals and hydroxide ions is also favored over the formation of *t*-butoxide ions and an OH radical.[49]

From the foregoing, we may further conclude that the $RSO^{\bullet}$ radicals must be rather poor H abstractors and must display a considerable preference of reacting with the thiol rather than the alcohol, the latter being present in 50-fold excess. In fact, an electron transfer from thiolate to $RSO^{\bullet}$ seems more likely than an H-abstraction reaction (reaction 30): this kind of behavior is exhibited by other moderately oxidizing radicals (cf. refs 24, 50, 51).

$$RSO^{\bullet} + RS^- \rightarrow RSO^- + RS^{\bullet} \qquad (30)$$

It is seen that the mechanism of Scheme III does not involve the formation of hydrogen peroxide as a chain product, in agreement with observation. Hydrogen peroxide results from the molecular yield (reaction i) and the bimolecular termination of $HO_2^{\bullet}/O_2^{\bullet -}$ (reaction 11).

We may attempt an estimate of an effective rate constant of the reaction of $O_2^{\bullet -}$ with DTT, neglecting path 19. Figure 1 shows what is interpreted as the diagrammatical representation of expression 31, which characterizes a chain reaction. As it stands,

$$G_{\text{ox-DTT}} = \frac{2k_{17}}{1 + \dfrac{k_{-17}}{k_{18}}}\left(\frac{G_{\text{init}}}{k_8}\right)^{1/2}\frac{[\text{DTT}]}{r^{1/2}} + G_{\text{init}} \qquad (31)$$

this expression (r, dose rate) is based on several assumptions: (i) the steady-state is maintained for free radicals; (ii) the terminating radical is $O_2^{\bullet -}$; (iii) initiation leads quantitatively to $O_2^{\bullet -}$ and ox-DTT, via a sequence culminating in reaction 7; (iv) $O_2^{\bullet -}$ is a chain carrier. (Hint: $^1/_2 v_{\text{ox-DTT,chain}} = v_{18} = v_{17} - v_{-17}$; $v_{\text{initiation}} = v_{\text{termination}}$.) The intercept represents the nonchain contribution and agrees with the expected value (*total* of water radicals). From the slope (by use of $k(O_2^{\bullet -}/HO_2^{\bullet}$ self-termination) = 1×10^4 dm^3 mol^{-1} s^{-1} at pH 8.7 from ref 4 and substitution of the value of 6×10^{-4} mol dm^{-3} for the total DTT concentration; the factor would be 2.8-fold larger if it referred to the concentration of the DTT monoanion $[\text{DTT}_{\text{anion}}] = K_a\,[\text{DTT}_{\text{total}}])[H^+] + K_a)$), we calculate $2k_{17}/(1 + k_{-17}/k_{18})$, the effective rate constant, at 70 dm^3 mol^{-1} s^{-1}. The factor of 2 arises because *two* molecules of ox-DTT are formed for each DTT^- entering into reaction 17, i.e. in one period of the chain. At low dose rates where the chain reaction is quite pronounced the addition of SOD reduces *G*(ox-DTT) to its nonchain value. This is a strong indication that $O_2^{\bullet -}$ radical is indeed a chain carrier. Our value of 70 cm^3 mol^{-1} sl^{-1} for $2k_{17}/(1 + k_{-17}/k_{18})$, which is also twice the propagation rate constant, is several orders of magnitude lower than the literature value of 1×10^6 dm^3 mol^{-1} s^{-1},[18] but not far from the 15 dm^3 mol^{-1} s^{-1} given by Bielski and Shiue for cysteine.[12] On the other hand, if the value taken from ref 18 were correct, for *G*(ox-DTT) one would calculate 74000×10^{-7} mol J^{-1} at a dose rate of 0.4 Gy s^{-1} ((dose rate)$^{-1/2}$ = 1.6 (Gy s^{-1})$^{-1/2}$). This discrepancy is far beyond the error limit of our experiments.

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Registry No. DTT, 3483-12-3; $O_2^{\bullet -}$, 11062-77-4.

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