Kinetics of the Reactions between Sulfide Radical Cation Complexes, $[S\therefore S]^+$ and $[S\therefore N]^+$, and Superoxide or Carbon Dioxide Radical Anions

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The reaction of superoxide radical anions with sulfide radical cation complexes represents an important sulfoxide-forming process. Here, absolute rate constants for the reaction of sulfur−sulfur $[>S\therefore S<]^+$ and sulfur−nitrogen ($[>S\therefore NH_2R]^+$) three-electron bonded sulfide radical cation complexes with superoxide and, for comparison, carbon dioxide radical anion have been measured by pulse radiolysis. For two different sulfur−sulfur bonded species, the *intermolecular* complex from dimethyl sulfide and the *intramolecular* complex from 1,5-dithia-3-hydroxycyclooctane, the rate constants for the reaction with superoxide are on the order of $1.6 \times 10^{10}\ M^{-1}\ s^{-1}$ and with the carbon dioxide radical anion on the order of $(6.5 \pm 0.2) \times 10^9\ M^{-1}\ s^{-1}$. The fact that the stronger reducing carbon dioxide radical anion shows the lower rate constant can be rationalized by the higher internal reorganization energy of $^{\bullet}CO_2^-$ as compared to $O_2^{\bullet -}$. The rate constant for the reaction of superoxide with the sulfur−nitrogen bonded radical cation of Met-Gly, $k = 5.3 \times 10^9\ M^{-1}\ s^{-1}$, is 3-fold lower as compared to that of the reaction with the sulfur−sulfur bonded radical cation complexes.

Oxidation reactions of organic sulfides are of great importance in biological systems,[1,2] in the atmosphere,[3] and on surfaces.[4] Often such oxidations are carried out by hydroxyl radicals, generated through a variety of processes such as Fenton type reactions,[5−7] the homolytic decomposition of peroxynitrous acid,[8,9] the photolysis on semiconductor surfaces (e.g., CdS or TiO_2),[4] or the effects of ionizing radiation on water.[10]

In aqueous solution, the reaction of hydroxyl radicals with sulfides predominantly yields sulfide radical cations, $>S^{\bullet +}$ (reactions 1 and 2; representatively shown for dimethyl sulfide), which stabilize through association with an additional nonoxidized sulfide molecule (reaction 3).[11,12] Alternatively, other nucleophiles, X, such as hydroxyl, carboxyl, ester, or amino functions can substitute for the nonoxidized sulfide to yield a variety of sulfide radical cation−nucleophile complexes (reaction 4).[13]

$$HO^{\bullet} + S< \rightarrow >S^{\bullet}{-}OH\ (\mathbf{1}) \tag{1}$$

$$\mathbf{1} \rightarrow HO^- + >S^{\bullet +} \tag{2}$$

$$>S^{\bullet +} + S< \rightleftharpoons [>S\therefore S<]^+\ (\mathbf{2a}) \tag{3}$$

$$>S^{\bullet +} + X^{n-} \rightleftharpoons [>S^{\bullet}{-}X]^{(1-n)+}\ (\mathbf{2b}) \tag{4}$$

Electronically, sulfur−sulfur[14] and sulfur−nitrogen[15] bonded complexes are best described as $2\sigma/1\sigma^*$ three-electron bonded structures with one electron localized in the antibonding σ^* orbital of the sulfur−sulfur or sulfur−nitrogen bond. In contrast, for sulfur−oxygen bonded complexes, σ, σ^*, or π radical structures have been described.[16] Usually, sulfide radical cations and/or their respective complexes react via (i) deprotonation in the α position to yield α-(alkylthio)alkyl radicals, (ii) disproportionation, or (iii) one-electron oxidation of appropriate electron donors, yielding sulfide and the oxidized electron donor.[11,12] However, recently we have quantified additional reaction channels of aliphatic organic and peptide sulfide radical cation complexes which directly lead to the respective two-electron oxidation products, the sulfoxides, through (a) a hydroxide-dependent reaction with molecular oxygen (reaction 5)[17] or (b) the reaction with superoxide radical anion (reactions 6 and 7).[18,19]

$$\mathbf{2a} + O_2 \xrightarrow{HO^-} >S{=}O + >S + H^+/O_2^{\bullet -} \tag{5}$$

$$\mathbf{2a} + O_2^{\bullet -} \rightarrow [>S^{(+)}{-}O{-}O^{(-)} + S<] \tag{6}$$

$$[>S^{(+)}{-}O{-}O^{(-)} + S<] \rightarrow 2\ >S{=}O \tag{7}$$

Reactions 6 and 7 represent an important sulfoxide-forming mechanism when sulfides are oxidized under conditions where hydroxyl radicals and superoxide are generated simultaneously. This is the case for some biological conditions of oxidative stress, i.e., respiratory burst,[20] and certainly when sulfides are subjected to photolysis on the surface of semiconductors such as, e.g., TiO_2 in aqueous suspensions.[4] In the latter system, photolytic charge-separation results in the simultaneous formation of surface-bound hydroxyl radicals and superoxide (through the reduction of surface-bound oxygen by conduction-band electrons).

To fully describe the reactions of sulfide radical cation complexes with superoxide, absolute rate constants are necessary. Here, we report on time-resolved pulse radiolysis studies to obtain absolute rate constants for the reactions of various sulfide radical cation complexes with superoxide, $O_2^{\bullet -}$, and, for comparison, carbon dioxide radical anion, $^{\bullet}CO_2^-$.

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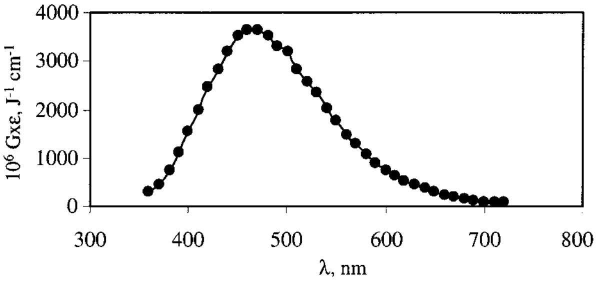


Figure 1. Optical spectrum recorded 3 μs after pulse irradiation of an N_2O-saturated aqueous solution, pH 7, containing 1×10^{-2} M DMS.

Experimental Section

Materials. Dimethyl sulfide (DMS) and 1,5-dithia-3-hydroxycyclooctane (DTHCO) were from Aldrich Chemical Co. (Milwaukee, WI), and the peptide Met-Gly was from Bachem (King of Prussia, PA). All chemicals were of p.a. quality and used as received. Solutions were made up with deionized water provided by a Millipore Milli-Q system. The pH values were adjusted by the addition of either NaOH or $HClO_4$.

Pulse Radiolysis. Pulse radiolysis experiments were performed with the Notre Dame Titan 8 MeV Beta model TBS-8/16-1S linear electron accelerator with typical pulse lengths of 10 ns. Absorbed doses per pulse were on the order of 1−14 Gy (1 Gy = 1 J/kg). Dosimetry was based on N_2O-saturated solutions containing 10^{-2} M KSCN, taking a radiation chemical yield of $G = 0.635$ μM J^{-1} and a molar extinction coefficient of 7580 M^{-1} cm^{-1} at 472 nm for the $(SCN)_2^{\bullet -}$ radical. Here the G value denotes the concentration of species produced/converted per 1.0 J absorbed energy. A description of the pulse radiolysis setup, data collection, and processing and details of the chemical dosimeter can be found elsewhere.[21] Experiments were performed with continuously flowing solutions at room temperature (~23 °C). Experimental error limits are ±10% unless specifically noted.

Results

Radiolysis of Water. Pulse irradiation of water leads to the formation of the primary reactive species shown in reaction 8.[10] In N_2O-saturated solutions, the hydrated electrons, e_{aq}^-, are converted into $HO^\bullet$ radicals according to reaction 9.

$$H_2O \rightarrow e_{aq}^-, HO^\bullet, H^\bullet \qquad (8)$$

$$e_{aq}^- + N_2O \rightarrow HO^\bullet + HO^- + N_2 \qquad (9)$$

The effective radiation chemical yields, G, of the primary species available for the reaction with a substrate depend on the concentration of the added substrate. For N_2O-saturated solutions, the effective radiation chemical yield of $HO^\bullet$, $G^{N_2O}(HO^\bullet)$, converting a given substrate S into substrate radicals $S^\bullet$ can be calculated according to eq I (where k_s represents the rate constant for the reaction of $HO^\bullet$ with S).[22] For air-saturated solutions, a first approximation is based on $G(H^\bullet) = 0.06$ μM J^{-1}, $G(e_{aq}^-) = 0.29$ μM J^{-1}, and $G^{air}(HO^\bullet) \approx 0.5\ G^{N_2O}(HO^\bullet)$, where $G^{N_2O}(HO^\bullet)$ is calculated according to eq I.[22]

$$G^{N_2O}(HO^\bullet) = 0.54 \times 10^{-6} + 0.31 \times 10^{-6} \frac{k_s[S]/(4.7 \times 10^8))^{1/2}}{1 + (k_s[S]/(4.7 \times 10^8))^{1/2}} \qquad (I)$$

Dimethyl Sulfide (DMS). *Formation and Decay of* $[(DMS)_2]^{\bullet +}$ *(2a).* Figure 1 displays the optical spectrum recorded 3 μs after pulse irradiation of an N_2O-saturated aqueous solution, pH 7, containing 1×10^{-2} M DMS. The absorbance with $\lambda_{max} = 465$ nm is characteristic for the radical cation complex **2a**, formed according to reactions 1−3 (at 10^{-2} M DMS, equilibrium 3 is located nearly completely on the side of complex **2a**).[11,12] Division of the radiation chemical yield, expressed as $G\epsilon_{465} = 3.63 \times 10^{-3}$ J^{-1} cm^{-1} by the known extinction coefficient of **2a** at 465 nm,[11] $\epsilon_{2a,465} = 6200$ M^{-1} cm^{-1}, yields $G(\mathbf{2a}) = 0.58$ μM J^{-1}. For the specific conditions employed, eq I predicts $G^{N_2O}(HO^\bullet) = 0.64$ μM J^{-1}. Thus, the fraction of $HO^\bullet$ converting into **2a** amounts to $G(\mathbf{2a})/G(HO^\bullet) = 0.91$. The residual fraction (ca. 0.1) of $HO^\bullet$ yields α-(alkylthio)alkyl radicals **3**, likely via direct hydrogen abstraction from DMS (reaction 10). The latter absorb with $\lambda_{max} = 285 \pm 5$ nm and do not interfere with the optical spectrum of **2a** in the visible region.[11,12]

$$HO^\bullet + DMS \rightarrow H_2O + {}^\bullet CH_2{-}S{-}CH_3\ (\mathbf{3}) \qquad (10)$$

When the same experiment is performed in the additional presence of either $(2.0{-}8.7) \times 10^{-3}$ M phosphate buffer, 2×10^{-1} M $NaClO_4$, or 2×10^{-2} M $NaHCO_2$, there is no significant change in the spectral characteristics of **2a**. However, the initial radiation chemical yields slightly drop from $G\epsilon_{465} = 3.63 \times 10^{-3}$ J^{-1} cm^{-1} (no buffer) to 3.43×10^{-3} J^{-1} cm^{-1} (2×10^{-3} M phosphate) or 3.29×10^{-3} J^{-1} cm^{-1} (8.7×10^{-3} M phosphate). At the same time, with increasing phosphate concentrations, a slight acceleration of the overall formation of **2a** is observed. Since phosphate does not react with $HO^\bullet$, we believe that phosphate reacts both as general acid, accelerating reaction 2, and as general base promoting the decomposition of **2a** (reaction 12; see below).

The radiation chemical yields $G\epsilon_{465}$ are, of course, significantly reduced in the presence of 2×10^{-2} M HCO_2^-, exclusively rationalized by a competitive reaction of $HO^\bullet$ with HCO_2^- (see below).

For high concentrations of DMS, e.g., $\geq 2 \times 10^{-2}$ M, the decay of **2a** shows pure second-order kinetics over an applied dose range of 1.9−14.5 Gy, corresponding to initial concentrations of $[\mathbf{2a}]_i = (1.1{-}8.5) \times 10^{-6}$ M, yielding $2k_{11} = 1.2 \times 10^9$ M^{-1} s^{-1}.

$$2\ \mathbf{2a} \rightarrow \text{products} \qquad (11)$$

At lower concentrations of DMS, the decay of **2a** contains an additional first-order component due to the deprotonation of $>S^{\bullet +}$ (reaction 12), shifting equilibrium 3 toward the side of the monomeric radical cation.[11]

$$>S^{\bullet +} \rightarrow H^+ + {}^\bullet CH_2{-}S{-}CH_3 \qquad (12)$$

*Reaction of **2a** with Superoxide (Reaction 6).* The reaction between **2a** and superoxide (reaction 6) was investigated in air-saturated aqueous solutions, pH 7.0, containing $(0.1{-}2.0) \times 10^{-2}$ M DMS and $(0.1{-}2.0) \times 10^{-1}$ M $NaHCO_2$ at a constant ratio of $[HCO_2^-]:[DMS] = 10$.

$$\mathbf{2a} + O_2^{\bullet -} \rightarrow [>S^{(+)}{-}O{-}O^{(-)} + S<] \qquad (6)$$

Under these conditions, hydrated electrons, e_{aq}^- ($G = 0.29$ μM J^{-1}), directly react with O_2 to yield superoxide (reaction 13; $k_{13} = 1.9 \times 10^{10}$ M^{-1} s^{-1} [23]).

$$e_{aq}^- + O_2 \rightarrow O_2^{\bullet -} \qquad (13)$$

Hydrogen atoms, $H^\bullet$ ($G = 0.06$ μM J^{-1}), yield superoxide either via direct reaction with O_2 (reaction 14, $k_{14} = 2.1 \times 10^{10}$ M^{-1}

s^{-1} [23]) or via reaction with formate (reaction 15, $k_{15} = 2.1 \times 10^8$ M^{-1} s^{-1} [23]), where the resulting carbon dioxide radical anion rapidly reduces oxygen (reaction 16, $k_{16} = 4 \times 10^9$ M^{-1} s^{-1} [24]) (the actual ratio between reactions 14 and 15 varies with the employed concentration of formate in the individual experiments).

$$H^{\bullet} + O_2 \rightarrow H^+ + O_2^{\bullet-} \tag{14}$$

$$H^{\bullet} + HCO_2^- \rightarrow H_2 + {}^{\bullet}CO_2^- \tag{15}$$

$${}^{\bullet}CO_2^- + O_2 \rightarrow CO_2 + O_2^{\bullet-} \tag{16}$$

The hydroxyl radicals branch between reaction 1 and hydrogen transfer from formate to yield ${}^{\bullet}CO_2^-$ (reaction 17; $k_{17} = 3.2 \times 10^9$ M^{-1} s^{-1} [23]).

$$HO^{\bullet} + HCO_2^- \rightarrow H_2O + {}^{\bullet}CO_2^- \tag{17}$$

On the basis of $k_1 \approx 1.0 \times 10^{10}$ M^{-1} s^{-1} we expect that at a constant ratio of $[HCO_2^-]/[DMS] = 10$, ca. 24% of the initially available $HO^{\bullet}$ react with DMS yielding hydroxysulfuranyl radicals **1** (reaction 1). (Initially, it was reported that $k_1 = 1.9 \times 10^{10}$ M^{-1} s^{-1}.[11] However, in view of our recent data with other small sulfides[12] we believe that k_1 is closer to 1.0×10^{10} $M^{-1}s^{-1}$. This lower rate constant leads also to a much better agreement between experimentally measured and theoretically predicted yields of sulfide radical cations in the presence of formate.)

Pulse irradiation of an air-saturated aqueous solution containing 1×10^{-2} M DMS and 1×10^{-1} M HCO_2^- yields $G\epsilon_{465} = (0.39 \pm 0.03) \times 10^{-3}$ J^{-1} cm^{-1}, corresponding to $G(\mathbf{2a}) = 0.063 \pm 0.005$ μM J^{-1} at 1μs after the pulse, in fair accordance with the expected 0.075 μM J^{-1}. The radiation chemical yield of superoxide is then derived as

$$G(O_2^{\bullet-}) = G(H^{\bullet}) + G(e_{aq}^-) + G^{air}(HO^{\bullet}) - G(\mathbf{1}) = 0.06 + 0.29 + 0.34 - 0.063/0.9 = 0.62\ \mu M\ J^{-1}$$

where $G(\mathbf{1}) = G(\mathbf{2a})/0.9$.

Similar calculations hold for all applied experimental conditions varying the DMS concentration in the range $(0.1-2.0) \times 10^{-2}$ M and the formate concentration in the range $(0.1-2.0) \times 10^{-1}$ M. This ensured the ratio of the initial reactant concentrations being $[O_2^{\bullet-}]_i/[\mathbf{2a}]_i \approx 10$ and, therefore, pseudo-first-order kinetics for the reaction of **2a** with $O_2^{\bullet-}$. For illustration, a first-order computer fit of the decay of species **2a** at 465 nm is presented in Figure 2.

Irradiation doses between 1.6 and 13.2 Gy were applied to air-saturated aqueous solution, pH 7.0, containing 1×10^{-2} M DMS and 1×10^{-1} M HCO_2^-, generating $(1.0-8.3) \times 10^{-7}$ M **2a** and $(1.0-8.3) \times 10^{-6}$ M $O_2^{\bullet-}$. The pseudo-first-order rate constants, k_{obs}, for the respective absorption−time traces of **2a** were plotted as a function of $[O_2^{\bullet-}]$ as displayed in Figure 3. From the slope of the straight line we calculate $k_6 = 7.4 \times 10^9$ M^{-1} s^{-1} irrespective of the absence or presence of 2×10^{-3} M phosphate buffer.

Linear correlations between k_{obs} and $[O_2^{\bullet-}]$ were also obtained for air-saturated aqueous solutions, pH 7.0, containing the combinations 1×10^{-3} M DMS/1×10^{-2} M HCO_2^- and 2×10^{-2} M DMS/2×10^{-1} M HCO_2^-; from the slopes we obtain $k_6 = 1.0 \times 10^{10}$ and 9.6×10^9 M^{-1} s^{-1} respectively.

Effect of Ionic Strength. Because of the different applied formate concentrations, the above rate constants were determined at different ionic strengths, μ. As reaction 6 represents a reaction between two ions of opposite charge, the kinetic salt effect needs to be taken into account. Hence, the effect of ionic strength was experimentally determined in air-saturated aqueous solutions, pH 7.0, containing 5×10^{-3} M DMS, 5×10^{-2} M HCO_2^-, and various additional concentrations of $NaClO_4$ between 0 and 1.52×10^{-1} M. The pseudo-first-order rate constants k_{obs} for the decay of **2a** were determined for various doses and k_6 calculated as $k_{obs}/[O_2^{\bullet-}]$. The respective second-order rate constants and ionic strengths are related through the Brönstedt−Bjerrum eq II where $\alpha = 0.509$ in water at 25 °C.[25]

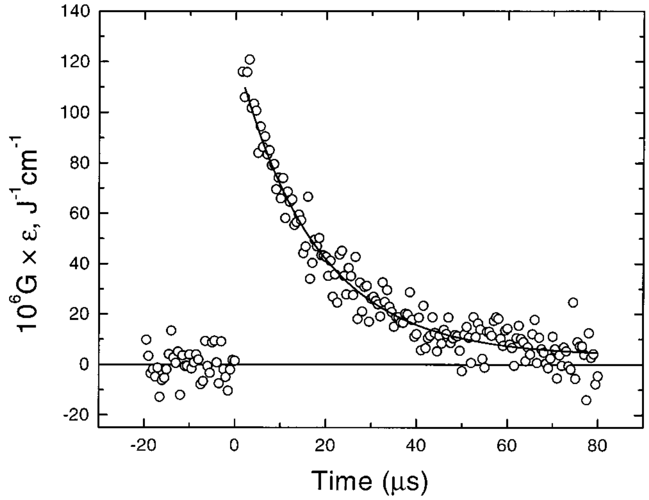


Figure 2. Absorption vs time profile for **2a**, monitored at $\lambda = 465$ nm, following pulse irradiation of an air-saturated aqueous solution, pH 7, containing 5×10^{-3} M DMS, 5×10^{-2} M $NaHCO_2$, and 1.78×10^{-2} M $NaClO_4$.

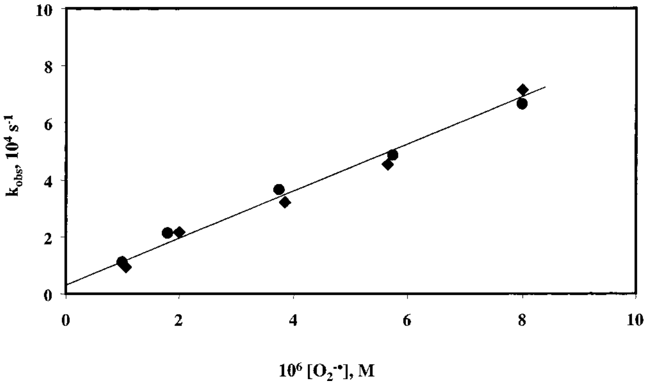


Figure 3. Plot of k_{obs} for the first-order decay of **2a** as a function of superoxide radical anion concentration. Conditions: pulse irradiation of air-saturated aqueous solutions, pH 7, containing 10 mM DMS, 0.1 M $NaHCO_2$ and either no (●) or 2×10^{-3} M (◆) phosphate.

$$\log k_6 = \log k_{6,0} + \frac{2z_A z_B \alpha \sqrt{\mu}}{1 + \sqrt{\mu}} \tag{II}$$

Figure 4 displays a plot of log k_6 vs $1.02\mu^{1/2}/(1 + \mu^{1/2})$ which yields the expected straight line with a slope of $z_A z_B = -1$. Extrapolation to $\mu = 0$ yields $k_{6,0} = 1.6 \times 10^{10}$ M^{-1} s^{-1}. All experimentally obtained rate constants have, therefore, been corrected to $\mu = 0$ according to eq II and are summarized in Table 1.

*Reaction of **2a** with ${}^{\bullet}CO_2^-$ (Reaction 18).* Reaction 18 of **2a** with ${}^{\bullet}CO_2^-$ was investigated in oxygen-free, N_2O-saturated

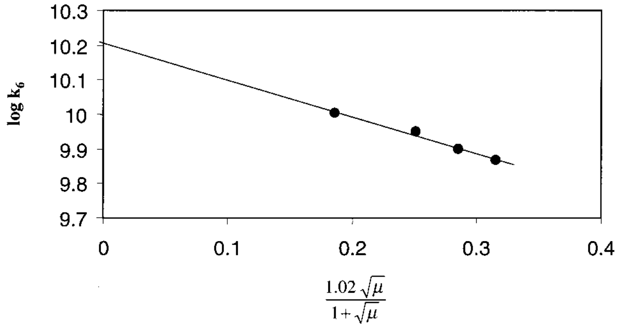


Figure 4. Dependence of log k_6 on the ionic strength.

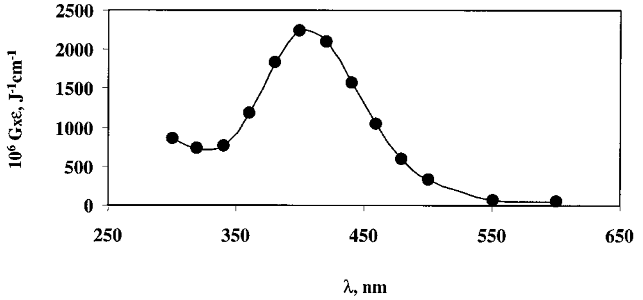


Figure 5. Optical spectrum recorded 4 μs after pulse irradiation of an N_2O-saturated aqueous solution, pH 7, containing 2×10^{-4} M HDTCO (**4**) and 0.2 M $NaClO_4$.

TABLE 1: Summary of Rate Constants, Extrapolated to Ionic Strength $\mu = 0$

	rate constant, $M^{-1} s^{-1}$	
species	$O_2^{-\bullet}$	${}^\bullet CO_2^-$
$(DMS)_2^{+\bullet}$ **2a**	$k_{6,0} = 1.60 \times 10^{10}$	$k_{16,0} = 6.7 \times 10^9$
$(HDTCO)^{+\bullet}$ **5**	$k_{22,0} = 1.63 \times 10^{10}$	$k_{21,0} = 6.3 \times 10^9$
$[(S\therefore N)$Met-Gly$]$ **7**	$k_{24,0} = 5.30 \times 10^9$	nd

aqueous solutions, pH 7.0, containing $(1-20) \times 10^{-3}$ M DMS and $(1-20) \times 10^{-2}$ M HCO_2^- at the constant ratio of $[HCO_2^-]/[DMS] = 10$.

$$\mathbf{2a} + {}^\bullet CO_2^- \rightarrow \text{products} \qquad (18)$$

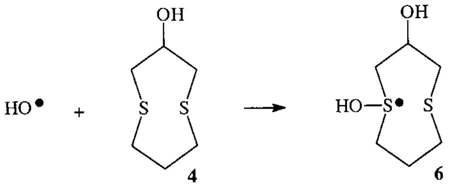
Under these conditions, we obtained $G({}^\bullet CO_2^-)/G(\mathbf{2a}) \approx 5-6$, where ${}^\bullet CO_2^-$ was generated via reactions 15 and 17. This ensured conditions of pseudo-first-order kinetics for the reaction of **2a** with ${}^\bullet CO_2^-$. Doses per pulse were varied between 1 and 13 Gy similar to the conditions described for reaction 6.

Computer fits of the decay of **2a** (absorption vs time traces at 465 nm) gave first-order fits for all doses, yielding k_{obs}, which were plotted as a function of $[{}^\bullet CO_2^-]$ in order to derive k_{18} at the various ionic strengths. Subsequently, log k_{18} was corrected according to eq II to yield $k_{18,0} = 6.7 \times 10^9$ $M^{-1} s^{-1}$ for $\mu = 0$.

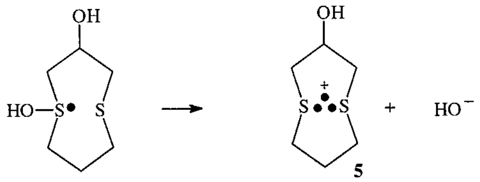
3-Hydroxy-1,5-dithiacyclooctane (HDTCO; 4). The oxidation of the cyclic dithiane HDTCO (**4**) yields the intramolecularly sulfur−sulfur bonded radical cation complex **5** where complex formation is independent of substrate concentration. Thus, three-electron bonded sulfide radical cation complexes of **4** can be generated at submillimolar substrate concentrations, permitting higher ratios of $[HCO_2^-]$/[sulfide].

Formation and Decay of the Radical Cation $[HDTCO]^{+\bullet}$ (5). Figure 5 displays the optical spectrum obtained 4 μs after pulse irradiation of an N_2O-saturated aqueous solution, pH 7, containing 2×10^{-4} M HDTCO and 2×10^{-1} M $NaClO_4$. It is characterized by an absorption maximum at $\lambda_{max} = 400$ nm and a radiation chemical yield of $G\epsilon_{400} = (2.3 \pm 0.12) \times 10^{-3}$ $J^{-1} cm^{-1}$.

By analogy to the oxidation of 1,5-dithiocyclooctane (1,5-DTCO) to $[1,5\text{-DTCO}]^{+\bullet}$ ($\lambda_{max} = 400$ nm, $\epsilon_{400} = 5800$ $M^{-1} cm^{-1}$ [13]) by $HO^\bullet$, the spectrum is assigned to the intramolecularly sulfur−sulfur three-electron bonded radical cation of HDTCO, $[HDTCO]^{+\bullet}$ (**5**), formed according to reactions 19 and 20. Using eq I, the effective radiation chemical yield of $HO^\bullet$ reacting with HDTCO (to yield hydroxysulfuranyl radical **6**) is calculated as $G = 0.56$ $\mu M J^{-1}$ (taking $k_{19} \approx 1.0 \times 10^{10}$ $M^{-1} s^{-1}$, by analogy to the reaction of $HO^\bullet$ with other sulfides[12]). Assuming that **6** nearly stoichiometrically converts into **5**, the extinction coefficient is calculated as $\epsilon_{5,400} = 4100$ $M^{-1} cm^{-1}$.



(19)



(20)

Radical cation **5** decomposes via mixed-order kinetics as the respective traces of $G\epsilon_{400}$ vs time could be fitted neither to second-order nor to first-order kinetics. We note that, in addition to deprotonation and/or disproportionation, **5** may suffer a kinetically first-order fragmentation of the $C_\alpha - C_\beta$ bond as such a process has been observed for the (hydroxyethyl)methyl sulfide radical cation generated from 2-(methylthio)ethanol, $CH_3-S^{(+\bullet)}-CH_2CH_2-OH$.[26] However, the radical cation **5** is sufficiently stable for investigating its reaction with superoxide and ${}^\bullet CO_2^-$; over the whole employed dose range of 2.2−8.8 Gy, corresponding to an initial concentration of **5** of $(1.0-3.9) \times 10^{-6}$ M, the first half-lives for the decomposition of **5** varied between 538 and 185 μs.

*Reaction of **5** with ${}^\bullet CO_2^-$ (Reaction 21).* The reaction of **5** with ${}^\bullet CO_2^-$ (reaction 21) was investigated in N_2O-saturated aqueous solution, pH 7.0, containing 2×10^{-4} M HDTCO and 5×10^{-3} M HCO_2^-.

$$\mathbf{5} + {}^\bullet CO_2^- \rightarrow \text{products} \qquad (21)$$

Under these conditions, the decay at 400 nm is clearly biphasic; a rapid initial decay with $t_{1/2} < 35$ μs, dependent on $[{}^\bullet CO_2^-]$, is followed by a significantly slower decay with $t_{1/2} > 800$ μs (Figure 6). This slower decaying species has an absorption spectrum with $\lambda_{max} \approx 400$ nm (see insert in Figure 6). Evidence for its formation is not only obtained in N_2O but also air-saturated solutions where **5** reacted with $O_2^{\bullet -}$ (see below). Hence, we believe that this residual absorption represents the product of a side reaction between $HO^\bullet$ and HDTCO rather than a product from the reaction of **5** with ${}^\bullet CO_2^-$. From the difference between the initial absorbance at 400 nm and the residual absorbance observed after the complete decay of **5**, we obtain $G(\mathbf{5}) = 0.044$ $\mu M J^{-1}$ and $G({}^\bullet CO_2^-) = 0.61$ $\mu M J^{-1}$, i.e., $[{}^\bullet CO_2^-]/[\mathbf{5}] \approx 14$. Values for k_{obs} are calculated from first-order

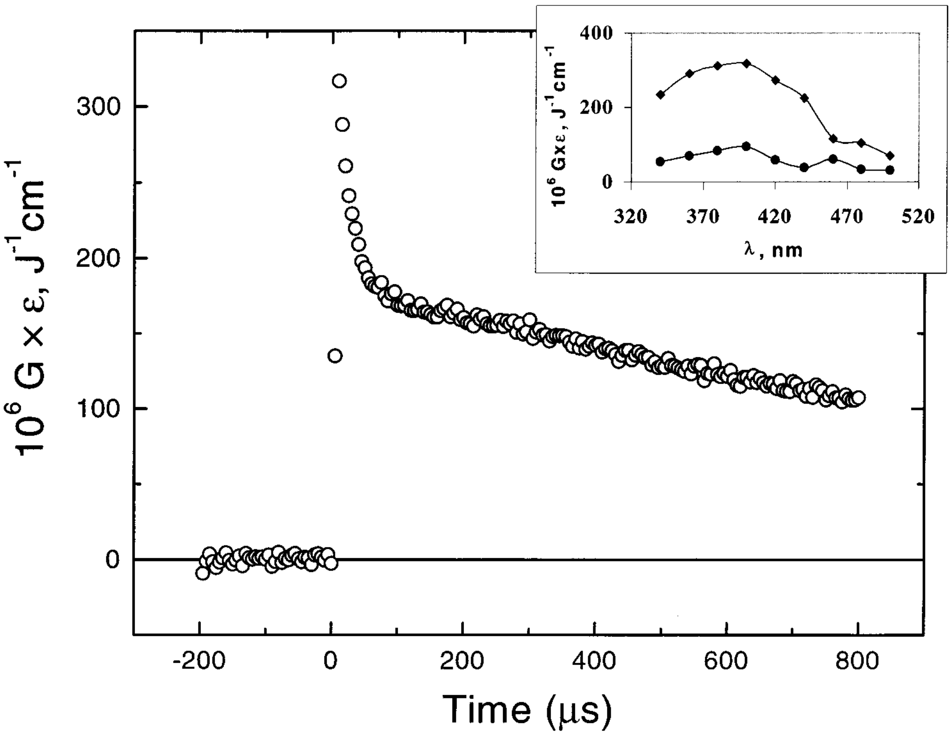


Figure 6. Absorption vs time profile at $\lambda = 400$ nm following pulse irradiation of an N_2O-saturated aqueous solution, pH 7, containing 2×10^{-4} M HDTCO and 5×10^{-3} M $NaHCO_2$. Insert: (upper trace) spectrum 7.5 μs after the pulse; (lower trace) spectrum at ca. 800 μs after the pulse.

computer fits of the decay of the initial 400 nm absorbance. A plot of the respective k_{obs} as a function of [$^{\bullet}CO_2^-$] gave a straight line with a slope corresponding to $k_{21} = 5.4 \times 10^9$ M^{-1} s^{-1} at $\mu = 5 \times 10^{-3}$ M. By application of eq II, we obtain $k_{21,0} = 6.3 \times 10^9$ M^{-1} s^{-1} at $\mu = 0$ (see Table 1).

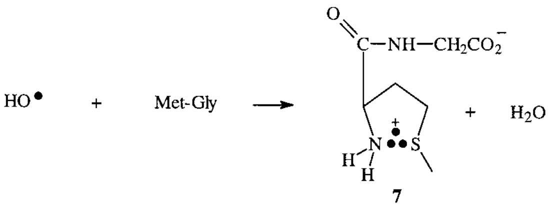
*Reaction of **5** with Superoxide (Reaction 22).* The reaction between **5** and superoxide was investigated in air-saturated aqueous solutions, pH 7, containing (1.0−2.0) $\times 10^{-4}$ M HDTCO and 5.0×10^{-3} M $NaHCO_2$.

$$\mathbf{5} + O_2^{-\bullet} \rightarrow \text{products} \qquad (22)$$

Under these conditions, we obtain $G(\mathbf{5}) = 0.025 \pm 0.02$ μM J^{-1}. The combined yields of superoxide then amount to $G(O_2^{\bullet-}) = G(H^{\bullet}) + G(e_{aq}^-) + G^{air}(HO^{\bullet}) - G(\mathbf{5}) = 0.06 + 0.29 + 0.29 - 0.025 = 0.62$ μM J^{-1}, and the ratio $[O_2^{\bullet-}]/[\mathbf{5}] = 24.8$. The absolute concentration of superoxide was varied in the range of (3.9−10.6) $\times 10^{-6}$ M by changing the radiation dose between 6.5 and 17 Gy.

The initial decay of **5**, measured at 400 nm is accelerated by increasing concentrations of superoxide. However, under these conditions also the residual 400 nm absorbance decayed faster as compared to deoxygenated solutions, resulting in overall biphasic kinetics at 400 nm. Hence, the traces were well fit by biexponential computer fits yielding two first-order rate constants where the higher one was ascribed to reaction 22 and the slower to a presently uncharacterized reaction of the residual 400 nm absorbance. From the plot k_{obs} vs $[O_2^{\bullet-}]$ we obtained $k_{22} = 1.39 \times 10^{10}$ M^{-1} s^{-1} for $\mu = 5 \times 10^{-3}$ M. Application of eq II yields $k_{22,0} = 1.63 \times 10^{10}$ M^{-1} s^{-1} for $\mu = 0$, in excellent agreement with $k_{6,0}$ (see Table 1).

Methionylglycine. The reaction of methionylglycine with hydroxyl radicals yields the sulfur−nitrogen bonded radical cation complex [(S∴N)Met-Gly] (**7**) (reaction 23), characterized through an absorption spectrum with $\lambda_{max} = 385$ nm.[27] In the absence of superoxide, **7** is relatively stable and decays with first half-lives on the order of 200−400 μs.



7

(23)

The reaction of **7** with superoxide (reaction 24) was examined through pulse irradiation of air-saturated aqueous solutions, pH 7.0, containing 2×10^{-4} M Met-Gly and 2×10^{-3} M $NaHCO_2$ at various doses (where the ratio of the initial yields of superoxide and **7** was $[O_2^{\bullet-}]/[\mathbf{7}] = 9.3$).

$$\mathbf{7} + O_2^{\bullet-} \rightarrow \text{products} \qquad (24)$$

Under these conditions, species **7** decayed according to pseudo-first-order kinetics, monitored at 385 nm, with k_{obs} linearly dependent on the initial concentration of superoxide. A plot of k_{obs} vs $[O_2^{\bullet-}]$ yields $k_{24} = 4.8 \times 10^9$ M^{-1} s^{-1} at $\mu = 2 \times 10^{-3}$ and, extrapolated to $\mu = 0$, $k_{24,0} = 5.3 \times 10^9$ M^{-1} s^{-1} (see Table 1).

Discussion

The reaction of sulfide radical cation−nucleophile complexes with superoxide represents an efficient sulfoxide-forming process under conditions where significant amounts of sulfide radical cation complexes and superoxide are formed simultaneously.[18,19] On the basis of the comparison of relative product yields in H_2O and D_2O, we concluded that freely diffusing singlet oxygen, $^1O_2$, potentially formed according to the one-electron-transfer reaction 25, does not contribute to sulfoxide formation[18,19] (the lifetime of $^1O_2$ is ca. 10−16 times higher in D_2O as compared to H_2O,[28,29] but sulfoxide yields did not increase accordingly[18,19]).

$$\mathbf{2a/2b} + O_2^{\bullet -} \rightarrow \text{products} + {}^1O_2 \qquad (25)$$

Instead, we proposed that superoxide directly adds to the sulfide radical cation complex to yield a persulfoxide species without intermediate singlet oxygen (represented in reaction 6). The persulfoxide (and/or its hydrated form, the hydroperoxysulfurane[18,19]) reacts with a second non-oxidized sulfide to yield 2 equiv of sulfoxide.

Additional examples confirming the tendency of superoxide to react via addition rather than electron transfer with oxidizing radicals have been provided, e.g., for the reaction of superoxide with tyrosyl[30] and tryptophan[31] radicals.

Table 1 shows that for two different sulfur−sulfur bonded radical cation complexes the reaction with superoxide is consistently higher by a factor of 2.4−2.6 relative to the reaction with $^\bullet CO_2^-$. The $^\bullet CO_2^-$ radical is characterized by the more negative reduction potential [$E(CO_2/^\bullet CO_2^-) = -1.9$ V,[32] $E(O_2/O_2^{\bullet -}) = -0.16$ V[33] vs NHE]. However, several theoretical calculations predict the smaller internal reorganization energy for the couple $O_2/O_2^{\bullet -}$ ($\lambda_i = 88.6$ kJ/mol[34]) as compared to $CO_2/^\bullet CO_2^-$ ($\lambda_i = 447$ kJ/mol;[35] a comparison of different theoretical approaches to obtain λ_i for $CO_2/^\bullet CO_2^-$ has been provided by Bennett and Warlop[36]), whereas the solvent reorganization energies of both couples appear to be rather similar.[36] Hence, the trend of the observed reactivity is in line with the calculated reorganization energies. On the basis of the small size of both anion radicals, it can be assumed[34] that they react with the radical cation complexes via inner-sphere mechanisms. We note that oxygen-centered radicals (such as hydroxyl and peroxyl radicals) often react via inner-sphere mechanisms[37,38] (see, for example, reaction 1). Whereas the reaction of superoxide with the radical cation complexes is expected to yield persulfoxide,[18,19] the intermediates in the reactions of sulfide radical cation complexes with $^\bullet CO_2^-$ remain to be characterized.

The reaction of superoxide with the sulfur−nitrogen bonded complex from methionylglycine is ca. 3-fold slower as compared to the reaction with the sulfur−sulfur bonded complexes. This drop in reactivity may, in part, reflect the lower probability of superoxide to encounter the sulfur in the sulfur−nitrogen bonded complex as compared to the symmetrical sulfur−sulfur bonded complex. However, other factors such as the spin density and electron density on the sulfur may play an additional role and an evaluation of these parameters must await more detailed calculations[15] of the electronic structure of sulfur−nitrogen bonded radical cation complexes.

From a biological point of view, it is important to note that the reaction of superoxide with the sulfide radical cation−nucleophile complexes studied here proceeds faster than the reaction of superoxide with superoxide dismutase ($k \approx 2 \times 10^9$ M^{-1} s^{-1} [39]). Thus, sulfide radical cation−superoxide reactions represent a potential source for sulfoxide formation in biological systems (e.g., at methionine residues in proteins) when tissue is exposed to high concentrations of reactive oxygen species.

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