

Intermolecular H-Abstraction of Thiyl Radicals from Thiols and the Intramolecular Complexing of the Thiyl Radical with the Thiol Group in 1,4-Dithiothreitol. A Pulse Radiolysis Study

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Z. Naturforsch. **42c**, 134–140 (1987); received September 18, 1986

Thiyl Radicals, Pulse Radiolysis, 1,4-Dithiothreitol, 2-Mercaptoethanol, Radiation Biology

Hydroxyl radicals were generated by pulse radiolysis of N_2O -saturated aqueous solutions. The OH radicals react with thiols such as 1,4-dithiothreitol by abstracting S-bound H-atoms ($k = 1.5 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$). The 1,4-dithiothreitol radical $HSCH_2(CHOH)_2CH_2S^\bullet$ (**2**) closes the ring ($k = 1.5 \times 10^6 \text{ s}^{-1}$) thereby forming the three-electron-bonded species $C_4H_7(CHOH)_2CH_2S-S^\bullet H$ (**3**) in equilibrium (**2**, **3**: $\epsilon(380 \text{ nm}) = 450 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$) which has a reported pK_a value of 5.2. At pH 7.2 **3** deprotonates ($k = 10^5 \text{ s}^{-1}$) yielding the ring-closed radical anion **4** which is characterized by a strong absorption at 390 nm ($\epsilon = 5900 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$). The deprotonation of **3** is catalyzed by HPO_4^{2-} ($k = 6.8 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$). The ring-closed radical anion **4** has also been generated by reacting *trans*-4,5-dihydroxy-1,2-dithian with the solvated electron and the H atom in Ar-saturated solution. Its protonation by protons yielding **3** occurs with $k = 1.8 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, with $H_2PO_4^-$ with $k = 3.8 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$.

In its reaction towards tetranitromethane **3** differs from its non-complexed analogue $HOCH_2-CH_2S^\bullet$ (**1**) in that **3** is rapidly oxidized ($k = 2 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) while **1** is not. Isoenergetic H transfer from 1,4-dithiothreitol to **1** occurs with a rate constant of $k = 1.7 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$.

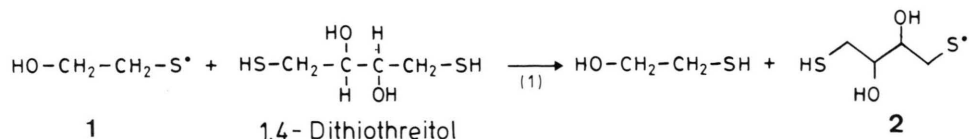
1,4-Dithiothreitol has pK_a values at 9.12 and 10.15. At pH 9.1 thiolate ions compete with ring-closure by complexing with **2** thereby forming an open-chain disulphide radical anion **6** which has very similar spectral properties as its ring-closed analogue **4**.

Introduction

It is well known that thiols are good H-donors and this H-donating property is considered to be one of the cells defense mechanism against free-radical damage of DNA, e.g. damage by ionizing radiation. Experiments to this point have also been done with model systems, nucleic acids in aqueous solution [1, 2]. Because 1,4-dithiothreitol is much more slowly oxidized by air than other thiols [3], this compound has been widely used in such studies. Rate constants for the H-donation reaction have been published so far only for the reaction of carbon-centered radicals (for a review see Ref. [4]). In the course of our study

on the free-radical-induced elimination of H_2S from 1,4-dithiothreitol, which is a chain reaction [5], the question arose how fast the (isoenergetic) H-transfer from a thiol to a thiyl radical may be. For measuring the rate constant of this reaction the thiyl radical derived from 2-mercaptoethanol **1** was reacted with 1,4-dithiothreitol forming the 1,4-dithiothreitol radical **2** (reaction (1)).

The 1,4-dithiothreitol radical **2** then complexes to a ring-closed species **3** (reaction (2)) which has been shown [6, 7] to absorb at longer wavelength than the thiyl radicals (cf. Fig. 1) and hence can be used as a monitoring system. The ring-closed form of the 1,4-



Reprint requests to Prof. Dr. C. v. Sonntag.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
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dithiothreitol radical **3** must have the open form **2** as a precursor. In separate experiments we measured the rate constant for the conversion of **2** into **3** and its deprotonation into **4** (reaction (4)).

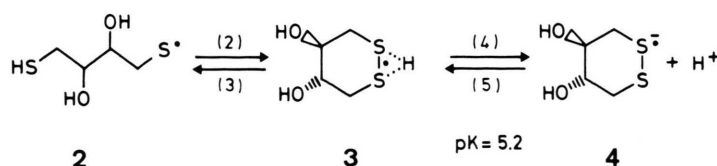


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The structurally related thiyl radicals **1** and **2** must have different chemical properties than the complexed radical **3** which could act as reducing agent. Experiments to this point will also be reported. The rate constants obtained in this study are compiled in Table I.

Table I. Compilation of rate constants measured for the 1,4-dithiothreitol system.

Rate constant	Reference
$k(1) = 5 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$	this work
$k(2) = 1.5 \times 10^6 \text{ s}^{-1}$	this work
$k(4) = 1.1 \times 10^5 \text{ s}^{-1}$	this work
$k(4_{\text{HPO}_4^{2-}}) = 6.8 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$	this work
$k(5) = 1.8 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$	this work
$k(5_{\text{H}_2\text{PO}_4^-}) = 3.8 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$	this work
$k(8) = 1.5 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$	[7]
$1.3 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1a}$	[7]
$1.5 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$	this work
$k(11) = 2 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$	this work
$k(12) = 2 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$	this work
$k(13) = 2 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$	this work

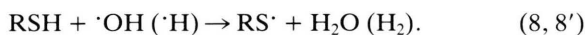
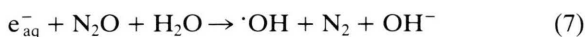
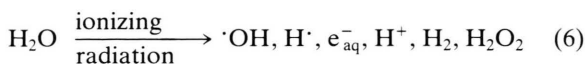
^a From competition studies.

Results and Discussion

Generation of the thiyl radicals

Radiation techniques have been used to generate the thiyl radical. 3 MeV electron pulses of 0.4 μs duration from a Van-de-Graaff electron accelerator have been delivered to an N_2O -saturated aqueous solution containing the thiol. The ionizing radiation produces as reactive intermediates OH radicals, H-atoms and solvated electrons (reaction (6)), and the latter is rapidly converted with N_2O into further OH radicals (reaction (7), $k(7) = 9.6 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$). At the prevailing N_2O concentration ($2 \times 10^{-2} \text{ mol dm}^{-3}$) $t_{1/2}(7) \approx 0.04 \mu\text{s}$; thus the solvated electrons are converted into OH within pulse duration. Hydroxyl radicals and H-atoms rapidly react with the thiols (RSH) thereby forming thiyl radicals (reaction

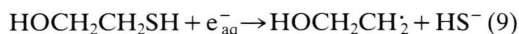
(8); $k(8) \approx 1.5 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ [8], $k(8') \approx 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ [9]).



The free-radical system described by reactions (6) and (7) consists of 90% OH radicals and 10% H-atoms. It is therefore mainly the OH radical which determines the rate of thiyl radical formation (reaction (8)).

H-Abstraction of the thiyl radical from thiol

To study the H-abstraction of a thiyl radical from a thiol 2-mercaptoethanol was used in excess ($0.1\text{--}1 \text{ mol dm}^{-3}$) over the 1,4-dithiothreitol concentration ($1 \times 10^{-3} \text{ mol dm}^{-3}$), thus in reaction (8) practically only 2-mercaptoethanol-derived thiyl radicals **1** are formed. At these high 2-mercaptoethanol concentrations there is also a contribution of 2-hydroxyethyl radicals by the reaction of the solvated electron with 2-mercaptoethanol (reaction (9)), but these radicals are also rapidly converted into 2-mercaptoethanol-derived thiyl radicals **1** by reaction (10) ($k(10) = 4.7 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ [10]).



The thiyl radicals **1** can react with 1,4-dithiothreitol (reaction (1)) thereby forming the 1,4-dithiothreitol radical **2** which is rapidly ($k(2) = 1.5 \times 10^6 \text{ s}^{-1}$, see below) converted into its ring-closed isomer **3**. As can be seen from Fig. 1 radical **3** differs in its absorption spectrum considerably from radical **1**. Hence, the rate of reaction (1) which is rate-determining can be measured by the pseudo-first-order buildup of the absorption at 380 nm (**2, 3**: $\epsilon(380) = 450 \pm 50 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$). From a series of such measurements thereby varying the 1,4-dithiothreitol concentration

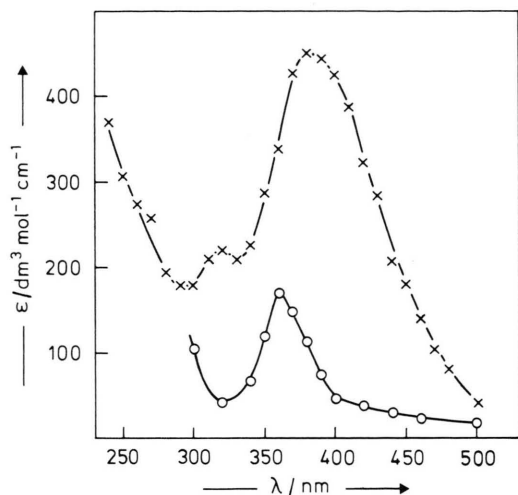


Fig. 1. UV absorption spectra recorded 5 μ s after the pulse of the thiyl radical derived from mercaptoethanol (**1**) (○) and of the ring-closed complexed thiyl radical derived from 1,4-dithiothreitol (**3**) (×) the assumption being made for the calculation of the extinction coefficient that a spectral contribution by **2** is minor. Pulse radiolysis of 10^{-3} mol dm^{-3} N_2O -saturated solutions at pH 4 of 2-mercaptoethanol and 1,4-dithiothreitol, respectively.

$k(1) = 1.7 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ has been measured. This value is lower than the corresponding rate constants of the H-abstraction by α -hydroxyalkyl radicals but some alkyl radicals also show similar or even lower rate constants [11]. The dependence of the rate constants of H-transfer reactions of thiols on the structure of the H-accepting radical are poorly understood at present [4] and it is therefore not possible to discuss reaction (1) in more detail.

*Intramolecular complexation of the 1,4-dithiothreitol-derived thiyl radical **2** with the thiol group leading to the ring-closed radical **3***

The reported rate constant of the OH radical with 1,4-dithiothreitol is $k(8) = 1.3 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ [7]. This rate constant has been obtained by competition using phenylalanine as competitor. One also can follow this reaction by the buildup of **3** at low 1,4-dithiothreitol concentration (*cf.* also [7]) where reaction (2) is fast compared to reaction (8). If one increases the 1,4-dithiothreitol concentration the rate of reaction (8) will increase, and $k(8) \times [1,4\text{-dithiothreitol}]$ will eventually approach or even become larger than $k(2)$. At high 1,4-dithiothreitol concentrations the rate-limiting step will no longer be reaction (8) but reaction (2). Fig. 2 shows a plot of $k(\text{obs})$

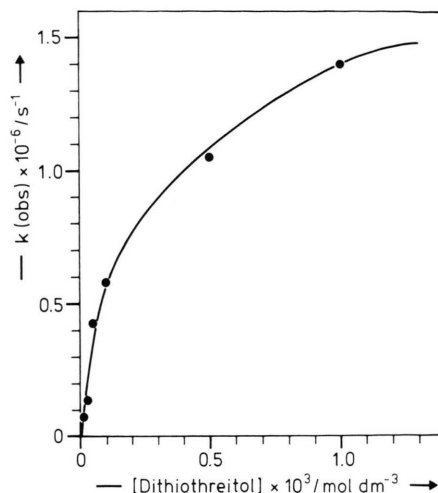
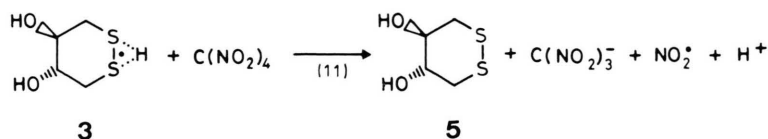


Fig. 2. Observed first order rate constant of the buildup of the 380 nm absorption attributed to the ring-closed complexed thiyl radical derived from 1,4-dithiothreitol (**3**) as a function of the 1,4-dithiothreitol concentration. Pulse radiolysis of N_2O -saturated solutions at pH 4.

of the 380 nm buildup (formation of **3**) as a function of the 1,4-dithiothreitol concentration at pH 4.

From the slope of the first part of the curve $k(8)$ can be calculated. The value of $k(8) = 1.5 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ thus obtained very well agrees with the value determined using the competition method [7]. At high 1,4-dithiothreitol concentration the curve flattens to approach a value of $k(2) = 1.5 \times 10^6 \text{ s}^{-1}$. This value is attributed to the rate constant for the complexation reaction (2). In reaction (2) a symmetrical form of **3** has been written in close analogy to other three-electron-bonded sulfur radicals [12]. Quantum-mechanical calculations at our institute are in progress to possibly decide whether such a species is symmetric or rather asymmetric.

Radicals **2** and **3** must be in equilibrium with one another. An estimate of the equilibrium constant cannot be given at present. However, in this context it is noted that in the lipoic acid [13] and in the present system the absorption spectra of the radical anions are very close. Their protonated forms are slightly hypsochrome shifted, but while in the lipoic acid system the extinction coefficients of the radical anion and its protonated form are very similar, that of the protonated form of the 1,4-dithiothreitol radical is one order of magnitude lower than that of its



anion. This could well be due to the fact that in the equilibrium radical **2**, *i.e.* the open-chain form is present at high percentage.

Oxidation of the ring-closed 1,4-dithiothreitol-derived thiol radical 3 by tetranitromethane

Thiyl radicals such as **1** are not oxidized by low concentrations ($< 10^{-3} \text{ mol dm}^{-3}$) of tetranitromethane at the time scale of a pulse radiolysis experiment ($< 1 \text{ ms}$).^{*} Hence it is expected that the analogous 1,4-dithiothreitol radical **2** should also not undergo a reaction with tetranitromethane. The fast buildup of the nitroform anion ($\epsilon(350 \text{ nm}) = 15000 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ [14]) formed in N_2O -saturated solution containing 1,4-dithiothreitol ($10^{-2} \text{ mol dm}^{-3}$) and tetranitromethane ($10^{-5} - 10^{-4} \text{ mol dm}^{-3}$) is attributed to reaction (11).

From the pseudo-first-order buildup kinetics the bimolecular rate constant $k(11) = 2 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ has been calculated.

The acid/base equilibrium of 1,4-dithiothreitol radicals 3 and 4

Radical **3** is a weak acid with a pK_a value of 5.2 [7]. Higher values of 5.3 [15] and of 5.5 have also been reported [6]. The disulphide radical anion **4** is characterized by a strong absorption at 390 nm ($\epsilon = 5900 \pm 500 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$). Lower values of $\epsilon(390 \text{ nm}) = 5000 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ [7] and $\epsilon(405 \text{ nm}) = 4500 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ [6] have been reported previously. At $\text{pH} \approx 7$ the rate of deprotonation of **3** (reaction (4)) is slow compared to ring closure (reaction (2)). Hence its rate constant can be determined with some accuracy by following the buildup of the 390 nm absorption as a function of time. The measured first-order rate constant is $k(4) = 1 \times 10^5 \text{ s}^{-1}$. As expected the deprotonation is base catalyzed. By varying the phosphate concentration at $\text{pH} 6.7$ $k(4_{\text{phosphate}}) = 1.7 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ was found. At $\text{pH} 6.7$ the deprotonating agent,

HPO_4^{2-} , amounts to 25% of the $\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$ couple and hence $k(4_{\text{HPO}_4^{2-}}) = 6.8 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$.

The rate constant for the protonation of the radical anion **4**, $k(5)$, can be measured by reacting the disulphide *trans*-4,5-dihydroxy-1,2-dithian with the solvated electron and the H atom (conditions: Ar-saturated solutions containing $10^{-3} \text{ mol dm}^{-3}$ *trans*-dihydroxy-1,2-dithian and $10^{-1} \text{ mol dm}^{-3}$ *t*-butanol, the latter to scavenge the OH radicals). At $\text{pH} 5.2$ the radical anion thus formed is protonated by the low concentrations of protons. The reaction can be monitored by the decay of absorbance at 390 nm where **4** absorbs more strongly than the mixture of **2** and **3**. With increasing phosphate concentration the decay becomes faster due to the protonation by H_2PO_4^- . From such measurements $k(5) = 1.8 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ and $k(5_{\text{H}_2\text{PO}_4^-}) = 3.8 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ have been calculated. From the ratio of $k(4)/k(5)$ a pK_a of 5.2 is calculated, in good agreement with the reported values (see above). A pK_a value of 5.2 is also found from the inflection point if in such an experiment the absorbance 30 μs after the pulse is plotted versus the pH (Fig. 3).

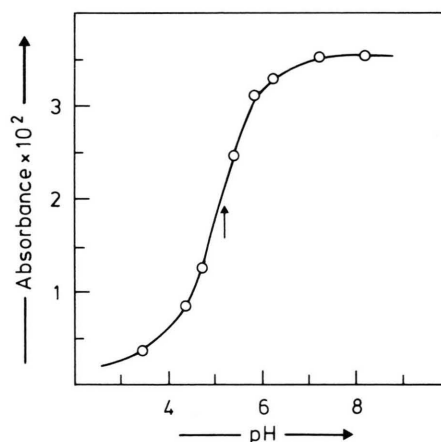


Fig. 3. Pulse radiolysis of Ar-saturated solutions of *trans*-4,5-dihydroxy-1,2-dithian $10^{-3} \text{ mol dm}^{-3}$ in the presence of 0.5 mol dm^{-3} *t*-butanol. Absorbance per Gy and cm at 390 nm 30 μs after a 2.5 Gy pulse vs. pH (phosphate buffered). The arrow indicates the inflection point at $\text{pH} 5.2$.

^{*} Note added in proof: For a recent determination of its redox potential see P. S. Surdhar and D. A. Armstrong, J. Phys. Chem. **90**, 5915 (1986).

Taking $G(e_{aq}^- + H^\bullet) = 3.4$ the extinction coefficient at 390 nm of **4** is calculated to be $6600 \pm 500 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ (measured at pH 8) in agreement with the value determined by oxidizing 1,4-dithiothreitol with OH radicals at pH 7.2 (see above).

Intermolecular reaction of radical 2 with the anions of 1,4-dithiothreitol

1,4-Dithiothreitol has pK_a values of thiol deprotonation of 9.12 and 10.15. Upon studying the formation of the 390 nm absorption as a function of the 1,4-dithiothreitol concentration at pH 9.1 it is observed that at low 1,4-dithiothreitol concentrations its buildup is much slower than at high 1,4-dithiothreitol concentrations (Fig. 4).

At low 1,4-dithiothreitol concentration the kinetics can be described by the formation of **2** followed by ring closure (reaction (2)) and subsequent spontaneous and OH-induced deprotonation (reactions (4) and (12)).

At a 1,4-dithiothreitol concentration of 10^{-4} mol dm^3 and at pH 9.2 $k(\text{obs}) = 4.1 \times 10^5 \text{ s}^{-1}$ has been found. Since $k(\text{obs}) = k(4) + k(12) \times [\text{OH}^-]$ and $k(4) = 10^5 \text{ s}^{-1}$, $k(12)$ is calculated to be $2 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. This is a very reasonable value for such OH^- -induced deprotonation reactions. The resulting species must be **4**. With increasing 1,4-dithiothreitol concentrations the buildup of the 390 nm absorption becomes biphasic. Concomitantly the contribution of the slow buildup to the overall reaction decreases while that of the fast one increases and also becomes faster. This observation is compatible with a competition of reactions (2) and (13). At pH 9.1 about half of the 1,4-dithiothreitol is present as mono anion ($pK_a = 9.12$ for the first deprotonation and $pK_a = 10.15$ for the second [16]). Thiolate ions are known to react

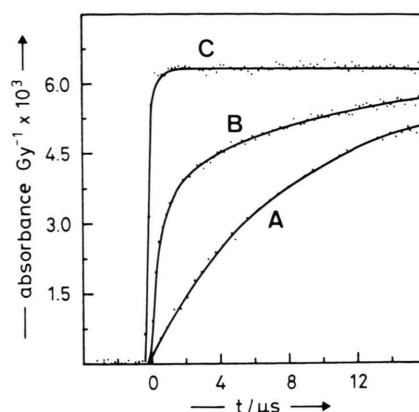
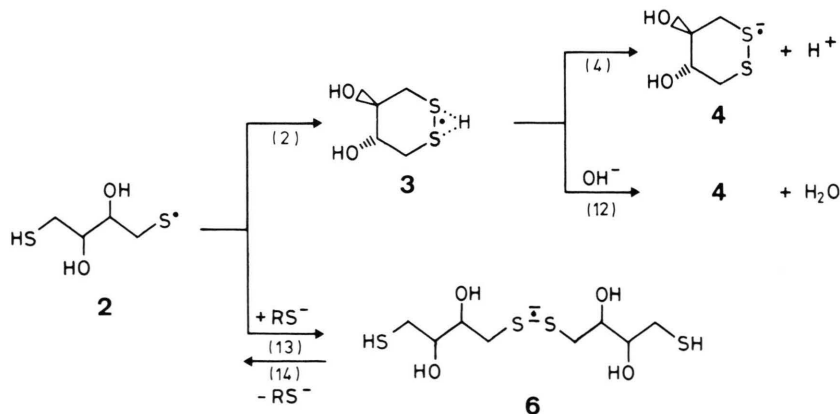
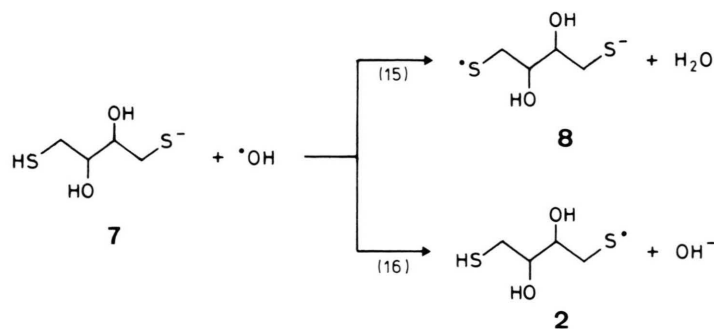


Fig. 4. Pulse radiolysis of N_2O -saturated solutions of 1,4-dithiothreitol at pH 9.1. Buildup of the 390 nm absorption as a function of time. **A** [1,4-dithiothreitol] = $5 \times 10^{-5} \text{ mol dm}^{-3}$; **B** [1,4-dithiothreitol] = $5 \times 10^{-4} \text{ mol dm}^{-3}$; **C** [1,4-dithiothreitol] = $5 \times 10^{-3} \text{ mol dm}^{-3}$.

rapidly ($k \approx 5 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ [17]) with thiyl radicals thereby forming disulphide radical anions which all absorb strongly around 400 nm. Thus at thiolate concentrations of $1.5 \times 10^{-3} \text{ mol dm}^{-3}$ 1,4-dithiothreitol anions successfully compete (reaction (13)) with the not all too fast ring closure (reaction (2)).

As can be seen from the scheme this competition does affect the yield but not the rate of the formation of **4** via reactions (4) and (12), but with increasing thiolate concentration reaction (13) becomes faster and hence the fast component both increases in yield and rate. The dimeric disulphide radical anion **6** appears to have the same absorption spectrum and also about the same extinction coefficient as its monomeric equivalent **4**. There is some indication that **6** might have a slightly higher extinction coefficient





than **4** (cf. also data reported in Ref. [7]), but these differences are close to the experimental error ($\leq 10\%$). Considering the backreaction (reaction (14)) which is likely to have a rate constant of around $5 \times 10^5 \text{ s}^{-1}$ (cf. [16, 18]) it is concluded that the dimeric disulphide radical anion **6** is not a stable entity in this system but is rapidly converted into the monomer **4**, at least at reasonably low thiolate concentrations ($\leq 10^{-2} \text{ mol dm}^{-3}$). The small difference in the extinction coefficients of **6** and **4** does not allow to measure this conversions.

At pH 9.15, the first pK_a of 1,4-dithiothreitol, one half of the 1,4-dithiothreitol molecules are deprotonated. Since they carry two SH functions one quarter of all the SH groups is deprotonated at this pH. With

the neutral 1,4-dithiothreitol OH radicals will give only radical **2** (reaction (8)). With the monoanion **7** there are two choices, H abstraction (reaction (15)) and electron transfer (reaction (16)). The latter yields again **2** while the former gives rise to its anion **8** which is the open chain form of **4**. It is expected that ring closure of **8** into **4** is as least as fast as reaction (2) and certainly faster than reaction (2) followed by the much slower reactions (4) and (12). However, at low 1,4-dithiothreitol concentrations at pH 9.1 there is no fast component detectable. Since thiolate ions react equally fast with OH as do thiols the yield of the fast component should have been as much as one quarter and hence easily detected (Fig. 5).

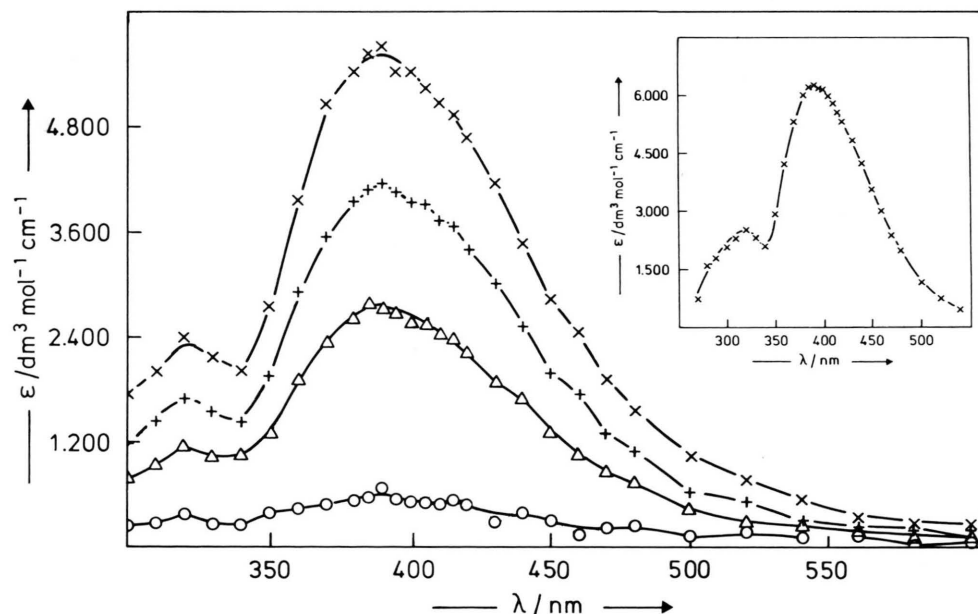


Fig. 5. Pulse radiolysis of N_2O -saturated solutions of 1,4-dithiothreitol at pH 9.1. Development of spectral changes at different times ($\circ = 1 \mu\text{s}$; $\triangle = 5 \mu\text{s}$; $+$ = $10 \mu\text{s}$; $\times = 30 \mu\text{s}$ after a 2.5 Gy pulse of $0.4 \mu\text{s}$ duration) at a 1,4-dithiothreitol concentration of $5 \times 10^{-5} \text{ mol dm}^{-3}$. Inset: $[1,4\text{-dithiothreitol}] = 5 \times 10^{-3} \text{ mol dm}^{-3}$, $1 \mu\text{s}$ after the pulse under otherwise identical conditions.

We therefore conclude that if the OH radical happens to react with the 1,4-dithiothreitol monoanion **7** it must preferentially undergo the electron transfer reaction (reaction (16)). This does not come as a surprise because the OH radical is strongly electrophilic and in its diffusion-controlled reactions highly position selective [19–21]. Reaction (16) might not be a true electron transfer reaction but might be preceded by a short-lived adduct. Such adducts are well known in sulfur free-radical chemistry.

Experimental

2-Mercaptoethanol (Merck) was redistilled prior to use, 1,4-dithiothreitol and *trans*-4,5-dihydroxy-

1,2-dithian (Merck) and tetranitromethane (Aldrich) were used as supplied. The pulse radiolysis setup has been described before [22]. In most of the experiments the dose range used was 2.5–6 Gy/pulse. The extinction coefficient of **3** is only low, therefore up to thirty five signals were averaged (CAT), for the more strongly absorbing species **4** and **6** average of ten signals was sufficient to yield a good signal to noise ratio.

1,4-Dithiothreitol very rapidly reacts with tetranitromethane. For this reason a N₂O-saturated solution containing 1,4-dithiothreitol and another one containing tetranitromethane had to be mixed just before the pulse.

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