One-Electron Reduction of the Disulfide Linkage in Aqueous Solution. Formation, Protonation, and Decay Kinetics of the $RSSR^-$ Radical

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Abstract: The rupture of disulfide linkages by the one-electron transfer agents e_{aq}^- and H atoms in aqueous solution has been studied using the fast-reaction technique of pulse radiolysis and kinetic absorption spectrophotometry. Dithiodiacetic acid, dithiodipropionic acid, cystine, cystamine, cystine dimethyl ester, penicillamine disulfide, glutathione disulfide, and lipoic acid were investigated. The reaction rate constants of e_{aq}^- with these disulfide compounds (RSSR) were determined and were found to be markedly dependent upon the state of protonation of the amino groups (for the amino acid disulfides), with the rate decreasing on deprotonation of the NH_3^+ groups. The absorption maxima, extinction coefficients, and decay kinetics of the disulfide radical anions, $RSSR^-$, produced on reaction with e_{aq}^- were determined. For most compounds, the maxima were in the range 400–420 nm, with extinction coefficients $\sim$7–15 $\times 10^3$ M^{-1} cm^{-1}, and the decays followed a first-order process, except for lipoate which decayed by second-order kinetics. At pH >7, the decay rate of the $RSSR^-$ radicals of dithiodiacetic, dithiodipropionic, and lipoic acids and of glutathione were independent of pH, whereas all the other disulfides showed an increase in the rate with increase in pH. This increase was shown to follow the pK_a of the amino groups. At pH <7, the decay rate was found to increase again. This increase in rate was found to be dependent upon $[H^+]$, and the kinetics of protonation of the disulfide radical anions were measured. Values ranged from $6.0 \pm 1.5 \times 10^8$ to $7.0 \pm 1.5 \times 10^{10}$ M^{-1} sec^{-1} for cystine dimethyl ester and glutathione disulfide, respectively. The protonation of $RSSR^-$ probably produces the short-lived sulfenium radical which decomposes to form thiyl RS· radicals (λ_{max} $\sim$330 nm) and RSH. Thiyl radicals were produced by an independent reaction and the transient spectra observed with λ_{max} $\sim$330 nm were shown to be identical with the corresponding radicals formed on protonation of $RSSR^-$ radicals. The reaction of H atoms with these disulfides in acidic solutions was found to produce the same intermediates as those observed from the protonation of $RSSR^-$ radicals, with absorption maxima at $\sim$330 nm and low extinction coefficients indicating a similar rupture of S–S linkages. Decay rates of thiyl radicals ranging from $\sim 3.4 \times 10^9$ to 1.4×10^{10} M^{-1} sec^{-1} were determined. These results are discussed on the basis of the physicochemical properties of the disulfide linkage. The reaction of the radicals produced from lipoic acid differ from those of the other disulfides studied and are discussed separately.

The role and importance of disulfide bridges in enzymatic reactions are well known. The inactivation of enzymes as a consequence of the disruption of specific cystine residues can be brought about thermally (denaturation) and photochemically[2] and by various other physical and chemical perturbations. Rupture of the disulfide linkage (RSSR) can also occur by reduction with one-electron transfer agents. The simplest one-electron reducing agents are hydrated electrons, e_{aq}^-, and hydrogen atoms.

In the course of studying[3] the nature of the interaction of e_{aq}^- and H atoms with various enzymes in aqueous solution, it became apparent that the existing[4–7]

$$e_{aq}^- + RSSR \longrightarrow (RSSR)^- \quad (1)$$

$$(RSSR)^- \rightleftharpoons RS\cdot + RS^- \quad (2)$$

$$H + RSSR \longrightarrow [RSS(H)R] \quad (3)$$

information and understanding of the reactions 1 to 3 for simple disulfides were incomplete. Hence it was found necessary to study systematically these reactions for a number of disulfide compounds in which the S–S linkage is attached to different α- and β-substituted groups.

The $RSSR^-$ radical anion has been observed in the pulse radiolysis of aqueous solutions of cysteamine–cystamine,[4] cysteine–cystine,[5] hydrogen sulfide, and mercaptans.[6] These radical anions have characteristic broad absorption maxima at $\sim$410 $\pm$ 10 nm and high extinction coefficients. In addition, a transient absorption spectrum with λ_{max} $\sim$330 nm and a relatively low extinction coefficient have been reported[7] from the reaction of H atoms with glutathione disulfide.

This work examines in detail the reactions of e_{aq}^- and H atom, produced in the pulse radiolysis of aqueous solutions, with disulfide linkages present in a number of different RSSR compounds: dithiodiacetic acid, β,β'-dithiodipropionic acid, cystine, cystamine, cystine dimethyl ester, glutathione disulfide, penicillamine disulfide, and lipoic acid. The dependence of the reaction rate constants of e_{aq}^- upon the acid–base properties of these compounds has been determined. The decay kinetics of the $RSSR^-$ radicals have also been examined as a function of pH and the rates of protonation have been determined. The nature of the intermediates produced from the protonation of $RSSR^-$ radicals are compared with the intermediates

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Table I. Reaction Rate Constants of e_{aq}^- with Various Disulfide Compounds in Aqueous Solution

Disulfide	Structure	pK_a[a]	pH	$k(e_{aq}^- + S)$, M^{-1} sec^{-1} [b]
Dithiodiacetic acid	$(-SCH_2COOH)_2$	3.1, 4.2	10.8	$4.3 \pm 0.2 \times 10^9$
β,β'-Dithiodipropionic acid	$(-SCH_2CH_2COOH)_2$	~4–5	6.4	$4.4 \pm 0.3 \times 10^9$
			10.8	$4.3 \pm 0.3 \times 10^9$
Cystine	$[-SCH_2CH(NH_3^+)COOH]_2$	1.65, 7.85	6.2	$1.5 \pm 0.2 \times 10^{10}$ (1.3×10^{10})
			12.1	$5.0 \pm 0.5 \times 10^9$ (3.4×10^9)
Cystamine	$(-SCH_2CH_2NH_3^+)_2$	8.82, 9.16	6.7	$4.2 \pm 0.4 \times 10^{10}$
			11.1	$1.8 \pm 0.2 \times 10^{10}$
Cystine dimethyl ester	$[-SCH_2CH(NH_3^+)COOCH_3]_2$	6.9	6.3	$5.1 \pm 0.5 \times 10^{10}$ [c]
			9.2	$2.1 \pm 0.2 \times 10^{10}$
Penicillamine	$-SC(CH_3)_2CH(NH_3^+)COOH$		7.0	$1.2 \pm 0.1 \times 10^{10}$
			12.4	$3.5 \pm 0.4 \times 10^9$
Glutathione	$[HOOCCH(NH_3^+)(CH_2)_2\text{-}CONHCHCONHCH_2COOH$ (CH$_2$—S–)$]_2$	3.15, 4.03, 8.57, 9.54	6.8	$3.4 \pm 0.3 \times 10^9$
			12.6	$2.1 \pm 0.2 \times 10^9$ (4.6×10^9) [f]
Lipoic acid	$CH_2CH_2S—SCH(CH_2)_4COOH$	4.7[d]	7.0	$1.5 \pm 0.2 \times 10^{10}$ (1.5×10^{10})[e]

[a] pK_a values taken from "Handbook of Biochemistry," Chemical Rubber Co., Cleveland, Ohio, 1968. [b] Average of at least three runs. Values in parentheses from ref 9. [c] Value corrected taking $pK_a = 6.9$. [d] Reference 18. [e] Reference 10. [f] Reference 9, determined at pH 8.2.

produced from the reaction of H atoms with RSSR compounds.

Experimental Section

Single pulses of 2.3-MeV electrons and ~30-nsec duration were provided by the Febetron 705 machine (Field Emission Corp.). Experimental details of the pulse radiolysis set-up used have been described elsewhere.[8]

The water used was purified by triple distillation, radiolysis, and photolysis. All solutions were prepared just prior to use. The pH was adjusted after deaerating the solutions and a few minutes before exposing the solution to the electron pulse. Perchloric acid, phosphate (~1–3 mM), tetraborate (1–2 mM), and potassium hydroxide were used as buffers. In order to minimize direct photolysis of the disulfide compounds by the monitoring light source, a synchronized shutter was used which was opened for a total time duration of ~2–5 msec. A fresh solution was used for each pulse. Dosimetry was carried out[8] using KCNS solutions, and the extinction coefficients were derived taking $G(e_{aq}^-) = G(OH) = 2.8$, and $G(H) = 0.55$.

Dithiodiacetic acid and β,β'-dithiodipropionic acid were obtained from Aldrich and were recrystallized twice from mildly alkaline aqueous solution upon the addition of acid.

Cystine, cystamine, and glutathione disulfide were obtained from Calbiochem, cystine dimethyl ester from Cyclochemicals, pencillamine disulfide from Aldrich, and lipoic acid from Nutritional Biochemicals; all of these were used as received.

Results

The one-electron reduction of disulfide linkages in aqueous solutions was brought about by reaction with solvated electrons (e_{aq}^-) and hydrogen atoms. The short-lived intermediates formed were observed using the technique of pulse radiolysis and kinetic absorption spectrophotometry. The hydroxyl radicals produced from the radiolysis of water

$$H_2O \xrightarrow{\sim\sim} e_{aq}^-, H, OH$$

were scavenged by *tert*-butyl alcohol. The *t*-BuOH radical produced is known[8] to absorb below ~280 nm with a relatively low extinction coefficient. Whenever necessary, a correction for its absorption was applied. It was also established that the *t*-BuOH radical did not react with the disulfide compounds examined, under the experimental conditions used, to produce the observed intermediates since these disappeared in the presence of an electron scavenger like N_2O (1 atm).

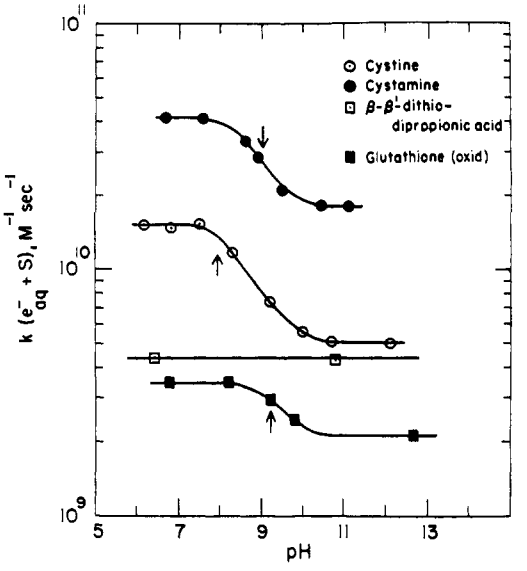

Figure 1. Dependence upon pH of the reaction rate constants of e_{aq}^- with some disulfides in aqueous solution: cystine, cystamine, β,β'-dithiodipropionic acid and glutathione disulfide. Arrows indicate dissociation constant of the amino groups in these compounds.

Reaction Rate Constants of e_{aq}^- with Disulfides

In order to ensure complete scavenging of e_{aq}^- by the disulfide compounds used, the rate constants of reaction 1 had to be determined at different pH values. These rates were determined in the presence of ~0.1 M *t*-BuOH and were monitored at 700 nm. Table I and Figure 1 present these results. Previously published rate constants[9,10] are also given in Table I.

A marked pH dependence can be noted for the rates of reaction 1 (see Figure 1). This was found to cor-

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(10) (a) R. L. Willson, *Chem. Commun.*, 1425 (1970); (b) J. W. Purdie, H. A. Gillis, and N. V. Klassen, *ibid.*, 1163 (1971).

Table II. Absorption Maxima, Extinction Coefficients, and Decay Kinetics of $(RSSR)^-$ and RS· Radicals of Various Disulfides in Aqueous Solution

Disulfide	Radical	pH	λ_{max}, nm	ϵ_{max}, M^{-1} [a]	Decay kinetics[b]
$(-SCH_2COOH)_2$	$RSSR^-$	8.6	400	9.5×10^3	2.4×10^6 sec^{-1}
	RS·	1.0	330	300	
$(-SCH_2CH_2COOH)_2$	$RSSR^-$	7.3	420	1.5×10^4	2.7×10^6 sec^{-1}
	RS·	1.0	330	430	1.4×10^{10} M^{-1} sec^{-1}
Cystine	$RSSR^-$	7.7	420	$\geq 8.8 \times 10^3$	2.9×10^5 sec^{-1}
	RS·	1.0	330	290	1.0×10^{10} M^{-1} sec^{-1}
Cystamine	$RSSR^-$	7.7	415	9.0×10^3	3.5×10^5 sec^{-1}
	RS·	1.0	330	315	1.4×10^{10} M^{-1} sec^{-1}
Cystine dimethyl ester	$RSSR^-$	5.3	420	8.3×10^3	1.9×10^5 sec^{-1}
	RS·	1.0	330	305	1.4×10^{10} M^{-1} sec^{-1}
Penicillamine	$RSSR^-$ [c]	7.5	450	7.3×10^3	1.3×10^6 sec^{-1}
Glutathione	$RSSR^-$	7.8	420	8.0×10^3	1.5×10^5 sec^{-1}
	RS·	3.9	325	580	3.4×10^9 M^{-1} sec^{-1}
	RS·	1.0	325	580	3.0×10^9 M^{-1} sec^{-1}
Lipoic acid	$RSSR^-$	7.8	410	9.2×10^3	1.4×10^8 M^{-1} sec^{-1}
	RSS(H)R[d]	1.0	385	6.9×10^3	4.5×10^9 M^{-1} sec^{-1}

[a] Values to ±10%, obtained from extrapolation of the decay kinetics to "zero" time. [b] Rates to ±15%. The $RSSR^-$ species have decay rates which are strongly dependent upon pH (see text). [c] At pH 1.0, the transient produced from the reaction with H atoms is very weak and does not appear to have a maximum at ~330 nm. [d] See discussion on nature of this intermediate.

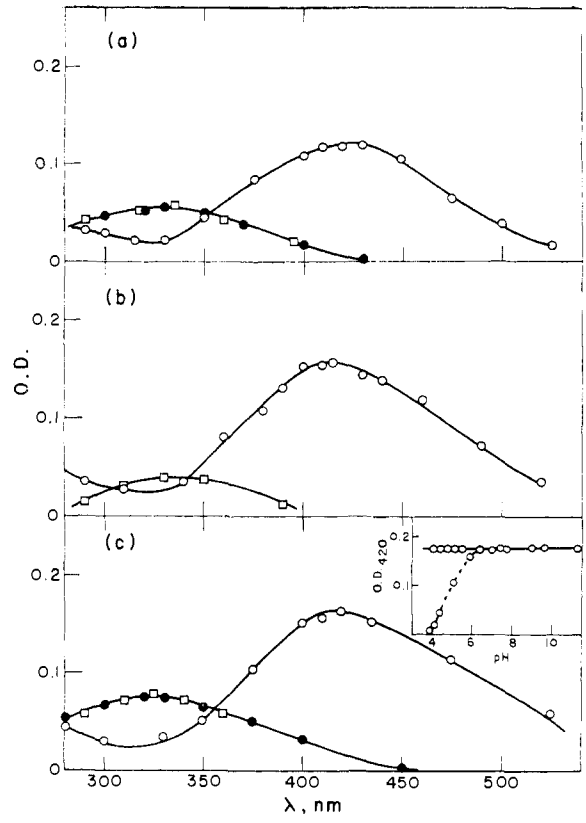

Figure 2. Transient absorption spectra produced by the reaction of e_{aq}^- and H atoms with disulfides in air-free aqueous solution: (a) 5 mM β,β'-dithiodipropionic acid at pH 7.3 (⊙), pH 4.2 (●), and pH 1.2 (⊡); (b) 10 mM cystamine at pH 6.7 (⊙) and pH 1.0 (⊡); (c) 5 mM glutathione disulfide at pH 7.8 (⊙), pH 3.9 (●), and pH 1.0 (⊡). All solutions contained 1.5 M *tert*-butyl alcohol, total dose ~4 krads/pulse at pH 6.7–7.8 and ~19 krads/pulse at pH <4.2. Spectra at λ <300 nm not corrected for the loss of substrate. Insert: Absorbance at 420 nm *vs.* pH. OD extrapolated to zero time (⊙) and read at 0.5 μsec (dotted line) after a 30-nsec pulse.

respond to the pK_a of the amino groups present in the disulfide compounds studied. Dithiodiacetic and dithiodipropionic acids do not have amino groups, and the rate constants of e_{aq}^- with these compounds are independent of pH in the range ~6–11. Furthermore, these latter compounds have values of $k(e_{aq}^- + SSRR)$ which are lower and close to that of the corresponding compound cystine at pH ~11, *i.e.*, beyond the pK_a = 7.85. Further discussions on the role of the amino groups in affecting the rate of formation and decay of $RSSR^-$ radical anions will be given in the Discussion section.

The rates of reaction of H atoms at pH 1 with cystine ($k = 8 \times 10^9$ M^{-1} sec^{-1}) and dithiodiacetic acid ($k = 1.0 \times 10^{10}$ M^{-1} sec^{-1}) have been reported[11] recently. Similar high rate constants for reactions 3 are expected for the other disulfides studied in this work.

Intermediates Produced from Reaction with e_{aq}^- and H Atoms

The interaction of e_{aq}^- with various disulfides in air-free aqueous solutions containing up to 1.5 M *t*-BuOH gave rise to transient optical absorption spectra with maxima in the region of 400–420 nm, depending on the nature of the disulfide compound (see Table II). The spectra of the $RSSR^-$ radical anions of β,β'-dithiodipropionic acid, cystamine, and glutathione disulfide are shown in Figure 2. The extinction coefficients of $RSSR^-$ appear to show small differences even though they all lie close to $\sim 1.0 \times 10^4$ M^{-1} cm^{-1}. These results cannot exclude the possibility that a few per cent of the electrons can lead to deamination of the amino acid disulfides.

When the pH of the solution is decreased under conditions such that all the e_{aq}^- reacts with RSSR and essentially none with H^+

$$e_{aq}^- + H^+ \longrightarrow H \qquad (4)$$

i.e., $k_1[RSSR]/k_4[H^+] \geq 20$, where $k_4 = 2.3 \times 10^{10}$ M^{-1} sec^{-1} (ref 9), the optical absorption band with a maximum at ~420 nm disappeared and a new weak absorption band with λ_{max} ~330 nm appeared (see Figure 2). The pH at which this conversion occurred was dependent upon the nature of the disulfide molecule and the rate of protonation of $RSSR^-$ (see below); for example, for β,β'-dithiodipropionic acid and glutathione disulfide the new band at 330 nm was observed at pH ~4.0 (see Figure 2).

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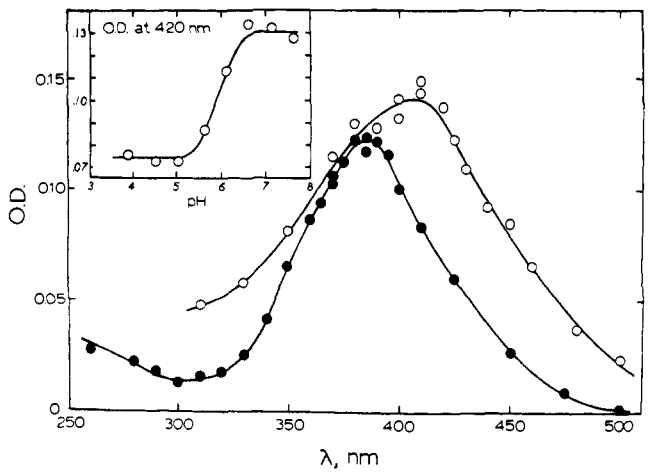
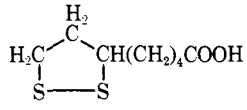


Figure 3. Optical absorption spectra of electron and H-atom adducts to 2.5 mM lipoic acid in oxygen-free aqueous solution at pH 7.0 (○) and pH 1.0 (●). Solutions contained 1.5 M t-BuOH, total dose ~2.4 krads/pulse. Insert: Change in absorbance extrapolated to zero time at 420 nm as a function of pH.

Upon reaction of these disulfides with H atoms at pH 1.0 (under these conditions all the e_{aq}^- are converted to H atoms *via* reaction 4, and the presence of ~1.5 M t-BuOH scavenges the OH radicals), transient absorptions were obtained for all the compounds studied. In all cases, except for lipoic acid, maxima at ~330 nm were obtained. Both the absorption maxima and the extinction coefficients (Table II and Figure 2) were identical with those derived from the reaction of e_{aq}^- with RSSR followed by protonation of the $RSSR^-$ radical anion.

The reaction of e_{aq}^- with the cyclic disulfide lipoic acid



produces the same characteristic band for $RSSR^-$ with λ_{max} 410 nm and ϵ_{410} 9.2×10^3 M^{-1} cm^{-1}. However, upon protonation of the $RSSR^-$ radical, or on reaction of H atoms with lipoic acid at pH 1.0, the intermediate produced has an absorption maximum at 385 nm and ϵ_{385} 6.9×10^3 M^{-1} cm^{-1} (see Figure 3). The interconversion of the two spectra occurs at pH 5.8, as shown in the insert to Figure 3.

Decay Kinetics

The $RSSR^-$ radical anion produced from the one-electron reduction of the disulfide linkage by e_{aq}^- in neutral solutions decays in all cases, except for lipoic acid, by a perfect first-order process. The rates obtained are given in Table II. Inasmuch as the pK_a values of most sulfhydryl compounds RSH are greater than 8, the position of the equilibrium represented by reaction 2 is largely to the right at pH <8, at low concentrations of $RSSR^-$, and in the absence of added RSH. In the presence of initially added RSH, the decay of $RSSR^-$ is considerably slower and the kinetics are of mixed order. This was shown, for example, on irradiation of a solution containing 10 mM cystamine, 0.5 mM cysteamine, 1.5 M t-BuOH, pH 10.25, in the presence of Ar (1 atm). Under these conditions all

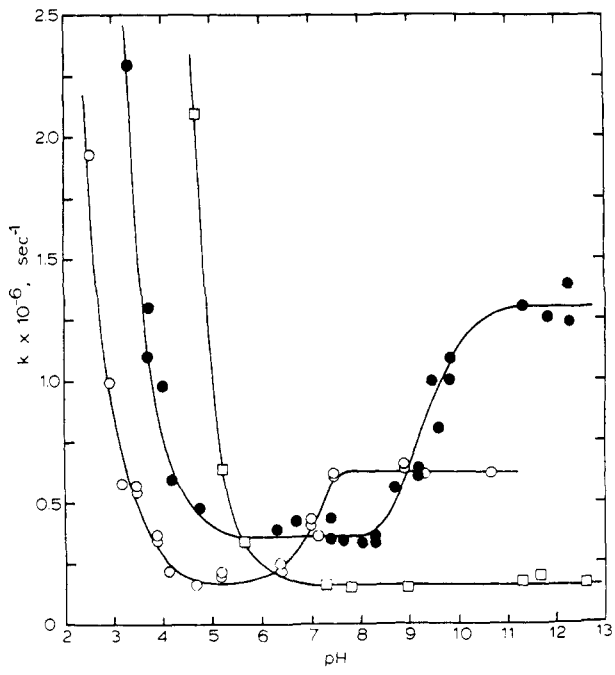


Figure 4. Dependence upon pH of the first-order decay rate constant of $RSSR^-$ radical anions in aqueous solution. Decay rates for glutathione disulfide (□), cystamine (●), and cystine dimethyl ester (○).

the e_{aq}^- reacted with cystamine and all the OH radicals with t-BuOH. The existence of equilibrium 2 was shown by the increased lifetime (a factor of ~20 under the experimental conditions used) of the $RSSR^-$ radical.

From Table II, it can be seen that k_2 is ~2–3 × 10^6 sec^{-1} for $(-SCH_2COO^-)_2^-$ and $(-SCH_2CH_2COO^-)_2^-$, and considerably slower for the disulfide derivatives of amino acids at pH ~7.0, where rates of k_2 ~1.5–6.0 × 10^5 sec^{-1} were determined for cystine, cystamine, cystine dimethyl ester, and glutathione. Note, however, that k_2(pencillamine) is ~1.5 × 10^6 sec^{-1}. $RSSR^-$ from lipoate was found to decay by a second-order process with $2k$ = 1.4 × 10^8 M^{-1} sec^{-1}. Further support for this second-order decay was obtained from the observed dependence of the half-life of the transient upon the concentration of $(lipoate)^-$.

In the pH range ~6–12, the decay kinetics of the $RSSR^-$ radical from dithiodiacetic and dithiodipropionic acids and glutathione disulfide were found to be independent of pH, while the other disulfides showed a marked dependence upon pH (see Figure 4). The first-order decay rates of $RSSR^-$ of cystamine and cystine dimethyl ester both increase with increase in pH. Presumably the decay of $RSSR^-$ from cystine would show a similar pH dependence. However, because of the low solubility of the parent compound, this point could not be pursued. It should be noted that k_2(cystine) = 2.9 × 10^5 sec^{-1} at pH 7.7 which is within the range of values shown for cystamine and cystine dimethyl ester. Penicillamine disulfide, $RSSR^-$, decays with pH-dependent kinetics with k_2 = 1.2 × 10^6, 1.4 × 10^6, and 2.9 × 10^6 sec^{-1} at pH 7.7, 8.6, and 11.7, respectively.

At pH values below ~6.0, the $RSSR^-$ decays much faster than in neutral solutions. Figure 4 shows these decays for cystamine, cystine dimethyl ester, and

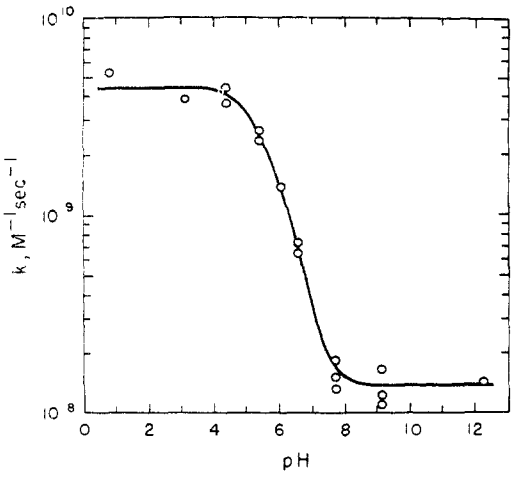


Figure 5. Dependence upon pH of the second-order decay rate of the e_{aq}^- and H atom adducts to lipoic acid. Solutions contained 2.5–5 m*M* lipoic acid and 1.5 *M* *t*-BuOH. Decays were monitored at 420, 380, and 460 nm.

glutathione. Values for penicillamine disulfide are 1.4×10^6, 2.3×10^6, and 2.8×10^6 sec^{-1} at pH 4.4, 3.4, and 3.3, respectively. The decays for dithiodiacetic and dithiodipropionic acids are already too fast and could not be followed at lower pH values.

For lipoic acid, the second-order decay of the RSSR$^-$ was found to be markedly dependent upon pH, with values ranging from $2k = 1.4 \times 10^8$ M^{-1} sec^{-1} at pH 8–12 and $2k = 4.5 \times 10^9$ M^{-1} sec^{-1} at pH 0.8–4.4 (see Figure 5).

The decay kinetics of the 330-nm band produced from the protonation of the RSSR$^-$ radical anion and/or directly from the reaction of H atoms with disulfides are in all cases second order; see Table II. This transient absorption has been assigned to the thiyl radical, RS·

$$2RS\cdot \longrightarrow RSSR \qquad (5)$$

Values of k_5 range from $\sim 3.4 \times 10^9$ to $\sim 1.4 \times 10^{10}$ M^{-1} sec^{-1} (Table II).

Discussion

The reactivity and direction of attack of free radicals with disulfides can be discussed initially on the basis of the physical and chemical properties[12–15] of the disulfide linkage. The S–S bond in disulfides is appreciably stronger (~70–75 kcal) than the O–O bond in peroxides (~40–45 kcal). This could account, in part, for the greater *relative* stability of electron adducts to RSSR compared to ROOR compounds. While the electron affinity of disulfides and many peroxides are comparable (e_{aq}^- reaction rate constants are $\sim 10^{10}$ M^{-1} sec^{-1}), only disulfides form RSSR$^-$ radical anions which in aqueous solutions have lifetimes longer than microseconds at room temperature. The ROOR$^-$ radical anion has not been observed (presumably has a lifetime $<10^{-8}$ sec) and rapidly dissociates to give RO· and RO$^-$; *e.g.*, OH radicals from H_2O_2 and $SO_4\cdot^-$ radicals from $S_2O_8^{2-}$ have been observed.

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(14) W. A. Pryor and K. Smith, *J. Amer. Chem. Soc.*, **92**, 2731 (1970).
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The covalent radius of the sulfur atom is also appreciably larger than that of the oxygen atom or carbon atom; the bond lengths[12] are C–C 1.53 Å, C–O 1.43 Å, and C–S 1.82 Å. The bond lengths involving bicovalent sulfur (S–S is 1.8–2.1 Å) are about 0.3–0.5 Å greater than C–O or C–C bonds. Steric accessibility of bicovalent sulfur to radical attack should therefore be quite favorable. Bond angles in bicovalent sulfur are also near the tetrahedral 109° angle. Furthermore sulfur can accommodate more than eight outer-valence electrons by utilizing d orbitals. Thus the addition of an electron to the disulfide linkage leads to an expansion of the sulfur octet and a delocalization of electrons from adjacent centers of electron density.

The reaction of solvated electrons with the disulfides studied in this work (and other aspects described below) can, in part, be interpreted on the basis of the properties of sulfur given above. The dependence of k_1 on the pK_a of the amino groups present in the disulfide compounds could be due to (a) the close proximity of the amino groups to the disulfide linkage in the three-dimensional structure of these compounds, (b) the accommodation of the negative charge in the d orbital of sulfur causing expansion of the sphere of interaction of the S–S group with neighboring functional groups, and (c) the change in the net overall charge of the disulfide compound. The charge in cystamine and cystine dimethyl ester changes from $2+ \rightarrow 0$ upon deprotonation of the amino group, and the electron rate drops by a factor of ~2. These two compounds have the highest rates of reaction with e_{aq}^- (Table I). Similar behavior is shown by cystine ($0 \rightarrow 2-$) and glutathione disulfide ($2- \rightarrow 4-$). The absence of an NH_3^+ group in β,β'-dithiodipropionic acid (charge $2-$) results in a value for k_1 which is independent of pH in the range 6–11.0, and with a value 4.4×10^9 M^{-1} sec^{-1}, close to that for cystine at pH 12.1 (charge $2-$) of 5.0×10^9 M^{-1} sec^{-1}. Similarly, the rate for cystine at pH 6 (net charge 0) is very close to that of cystamine at pH 11 (charge 0).

Examination of the e_{aq}^- rates in Table I and Figure 1 shows that the dependence of k_1 on pH cannot be explained simply by option c above. The Debye relationship[16] for the dependence of diffusion-controlled rate constants on the charges of the reacting species predicts a diminution of the rate by a factor of ~6 when e_{aq}^- reacts with a species with a change in charge of $2+ \rightarrow 0$ or $0 \rightarrow 2-$. Similarly, the factor of ~2 shown by glutathione disulfide is much too small for a $2- \rightarrow 4-$ change in the charge. It must be concluded that the rate of e_{aq}^- reaction is governed in large part by the nature of the *site* of attack. This is particularly illustrated in the case of glutathione disulfide which has the two amino groups removed from the vicinity of the S–S linkage and also has four peptide groups in the molecule. The peptide groups are known[17] to have a strong affinity for e_{aq}^-. Yet glutathione disulfide at pH 7 (charge $2-$) has a rate that is similar to $(-SCH_2-CH_2COO^-)_2$, despite the fact that the simple acid is a considerably smaller molecule and is lacking any amino groups. It must be concluded that the value of k_1 is dictated by e_{aq}^- attack at the disulfide group and

(16) P. Debye, *Trans. Electrochem. Soc.*, **82**, 265 (1942).
(17) M. Simic and E. Hayon, *Radiat. Res.*, 48, 244 (1971).

only to a relatively small extent on the net charge on the molecule.

It is interesting to note that although lipoic acid can be reduced to $RSSR^-$ through the interaction with the one-electron transfer agents CO_2^- and $(CH_3)_2\dot{C}OH$[10a] (these results have been confirmed), no transient absorbing at 420 nm is produced upon the action of these radicals with glutathione disulfide. Specifically, mixtures of 1.5 m*M* glutathione disulfide and 0.1 *M* formate or 2-propanol at pH 7 in the presence of N_2O (1 atm) were irradiated. Under these conditions e_{aq}^- is converted to OH which reacts with the formate or 2-propanol to give the reducing radical. Failure to observe an intermediate implies that the k value for reaction of CO_2^- or $(CH_3)_2\dot{C}OH$ radical with glutathione disulfide is $\ll 1 \times 10^7\ M^{-1}\ sec^{-1}$.

Decay Kinetics of $RSSR^-$ Radicals

The decay of the $RSSR^-$ radical anions of all the disulfide compounds studied (lipoic acid will be discussed separately below) followed first-order kinetics, producing the corresponding thiyl radicals according to reaction 2.

The observed increase in the value of k_2 for cystamine and cystine dimethyl ester in alkaline solutions was found to follow exactly the pK_a for deprotonation of the amino groups of these disulfides (see Figure 4). The behavior of penicillamine disulfide in this alkaline pH region follows this same pattern. On the other hand, the decay of $RSSR^-$ from dithiodiacetic and dithiodipropionic acids is very rapid and independent of pH in the range 7–11 ($k \sim 2.5 \times 10^6\ sec^{-1}$). It is clear that the $RSSR^-$ radical, with a high electron density in the expanded sulfur orbital, is unstable and that the environment around the $S\text{–}S^-$ group has a profound effect on the rate at which this reaction occurs. The three-dimensional model of the disulfide compounds showed that one configuration of the molecule placed the amino groups of cystamine, cystine, and cystine dimethyl ester extremely close to the sulfur atoms. In the case of the radicals, the increased locus of interaction of the expanded sulfur orbital could establish a preferential structure in which the NH_2 or NH_3^+ groups are more strongly attracted to the $S\text{–}S^-$ linkage. Such interaction would reduce the electron density at the sulfur atoms and cause a diminution in the rate of S–S bond scission. Thus, for cystamine and cystine dimethyl ester, k_2 at pH values where the amino group is deprotonated is less than that for the non-amino dithio acids. Protonation of the amino groups decreases the rate of decay, in accordance with the strong electron-attracting ability of the NH_3^+ groups. An intramolecular proton transfer with a fast relaxation time might be occurring. The NH_2 group in the cystine dimethyl ester apparently exhibits a stronger interaction with $S\text{–}S^-$ than does the same group in cystamine as evidenced by the limiting k_2 values in alkaline solution. This implies that the NH_2 group is a weaker base in the ester than in cystamine due to the strong inductive effect of the CO_2CH_3 groups. This is consistent with the pK_a values for deprotonation of the amino groups of these two compounds in their ground state (see Table I).

It is interesting to note that the value of k_2 for penicillamine disulfide shows the effect of NH_3^+ deprotonation (but to a rather small extent), with a decay rate that is comparable to those for the non-amino dithio acids. Because of the $(CH_3)_2C<$ unit between the amino and disulfide groups, steric hindrance probably prevents the NH_2 and NH_3^+ groups from making close contact with $S\text{–}S^-$. Apparently the interaction is sufficient for a pH dependence to be seen but not sufficient to cause a marked decrease in the value of k_2. In the case of glutathione disulfide, the amino groups are far removed from the sphere of interaction of the $S\text{–}S^-$ group and no effect on the value of k_2 is seen upon protonation or deprotonation of the amino groups. It is not clear, however, what effect the size of the molecule and the interaction of other groups (such as the peptide units) with $S\text{–}S^-$ would have on the rate of decay of the $RSSR^-$ radical.

At pH below ~6.0, the decay rate of $RSSR^-$ radical increases again (Figure 4) and eventually becomes too fast to follow with the available instrumental time resolution. This rapid decay gives rise to another transient absorption with maxima at ~330 nm and relatively low extinction coefficients (see Figure 2 and Table II). Identical spectra and extinction coefficients were obtained from the reaction of H atoms with these disulfides at pH 1.0.

Based on the following observations and arguments, it is suggested that the intermediate with λ_{max} ~330 nm produced either from the protonation of $RSSR^-$ radicals or from the reaction of H atoms with disulfide is the thiyl radical, RS.

$$RSSR^- + H^+ \longrightarrow RS\cdot + RSH \quad (6)$$

$$H + RSSR \longrightarrow RS\cdot + RSH \quad (7)$$

(a) Identical spectra, extinction coefficients, and decay kinetics were found for the radicals produced by dehydrogenation of RSH compounds by OH radicals and by H atom reaction with the corresponding disulfide compound, reactions 7 and 8. Figure 6 shows

$$OH + RSH \longrightarrow RS\cdot + H_2O \quad (8)$$

the transient spectra obtained from thiopropionic and dithiodipropionic acids.

$$OH + HSCH_2CH_2COO^- \longrightarrow \cdot SCH_2CH_2COO^- + H_2O \quad (9)$$

$$H + (\text{–}SCH_2CH_2COOH)_2 \longrightarrow \cdot SCH_2CH_2COOH + HSCH_2CH_2COOH \quad (10)$$

Similar results are shown in Figure 6 for the corresponding reaction with reduced and oxidized glutathione.

(b) While the decay rate of $RSSR^-$ in acidic solutions increases rapidly, the initial absorbance (*i.e.*, the spectrum of the primary intermediate) extrapolated to "zero time" remains unchanged, as shown for glutathione in Figure 2.

(c) Following from (b) above, it was found that the decay rate of $RSSR^-$ was pseudo first order dependent directly upon the hydrogen ion concentration. The observed first-order rates for glutathione, cystine dimethyl ester, and cystamine as a function of $[H^+]$ are shown in Figure 7. These linear plots support reaction 6 for the formation of $RS\cdot$ radicals in acidic solution, when the rate of reaction 6 exceeds that of reaction 2. Electrophilic attack on sulfur by H^+ leading to a cleavage of the S–S bond is well established.[13] Presumably the sulfenium radical intermediate ($R\dot{S}SHR$) produced in reactions 6 and 7 has a lifetime $\ll 10^{-7}$ sec.

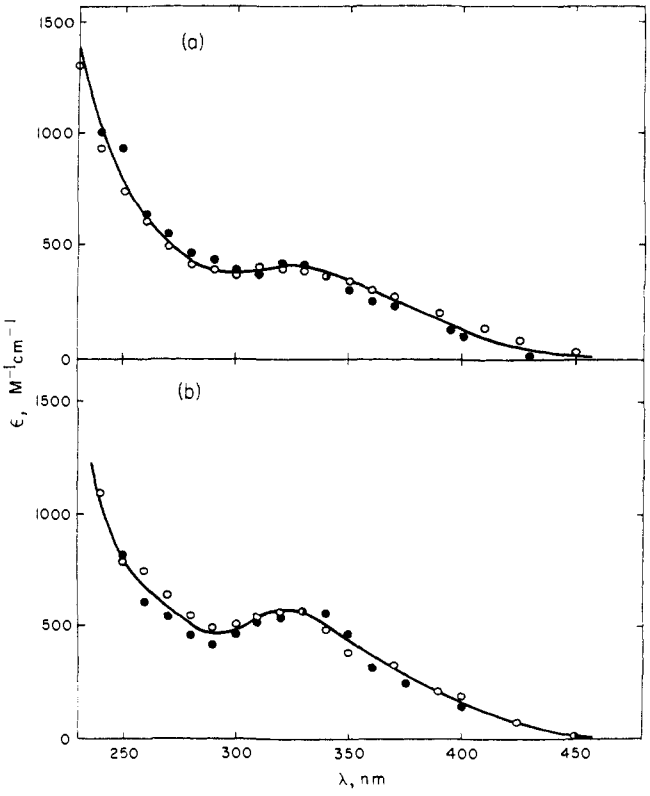


Figure 6. Optical absorption spectra of RS· radicals in aqueous solution: (a) 0.5 mM $HSCH_2CH_2COO^-$ at pH 6.0, N_2O (1 atm) (○), and 0.5 mM $(-SCH_2CH_2COOH)_2$ at pH 1.0, 1.0 M t-BuOH, N_2 (1 atm) (●); (b) 0.5 mM glutathione (red.) at pH 4.3, N_2O (atm) (○), and 0.5 mM glutathione disulfide, pH 1.0, 1.0 M t-BuOH, N_2 (1 atm) (●). Total dose ~19 krads/pulse. All spectra were corrected below 300 nm for loss of substrate (see text).

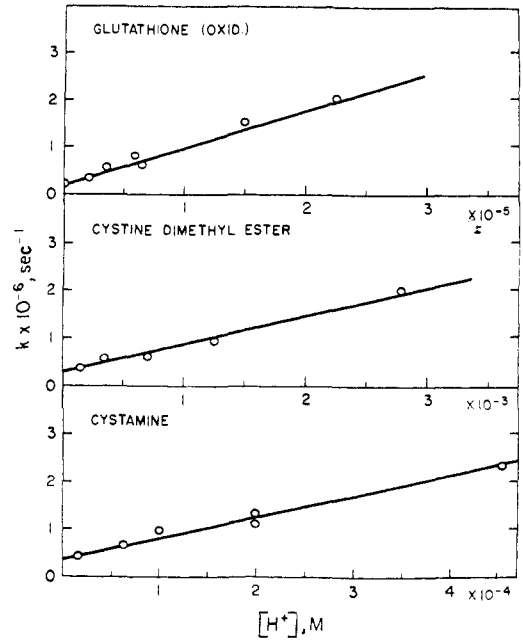


Figure 7. Plot of pseudo-first-order decay rate of $RSSR^-$ radicals as a function of $[H^+]$.

The results and conclusions reached here disagree with those postulated previously.[7] In the earlier work,[7] the similarity in the spectrum of the radical produced *via* reactions 7 and 8 was overlooked, and no correction was made to account for the decomposition of RSSR, which starts absorbing at wavelengths below ~310 nm. It is concluded, therefore, that except for lipoic acid (see below) the sulfenium radical is too short-lived to be observed with our available time resolution, $\sim 10^{-7}$ sec.

Kinetics of Protonation of $RSSR^-$ Radicals. Table III presents the rates of reaction 6 for the protonation of the $RSSR^-$ radicals of cystine dimethyl ester, cystamine, penicillamine disulfide, glutathione, and lipoic acid. A marked difference in the rates of protonation can be noted. For glutathione and lipoic acid, these rates are diffusion controlled, $k_6 \sim 5\text{–}7 \times 10^{10}$ M^{-1} sec^{-1}. In both cases, the rates of protonation are not affected by interaction with protonated amino groups (lipoic acid has none, and in glutathione the amino groups are far removed from the –S–S– linkage). With cystamine, overlap of the d orbital with the NH_3^+ group diminishes the electron density on the S–S– group and causes the decrease of k_6 to $\sim 4 \times 10^9$ M^{-1} sec^{-1}. The rate constant for protonation of $RSSR^-$ from penicillamine is about the same as for cystamine, which is consistent with the general similarities in their structures. The further decrease in the rate of k_6 for cystine dimethyl ester can be rationalized on the basis of the strong inductive effect of the CO_2CH_3 groups on the NH_3^+ groups. The pK_a of the latter compound is 6.9 compared to 7.85 for cystine.

Table III. Kinetics of Protonation of Disulfide Radical Anions in Aqueous Solution

Disulfide	$k(H^+ + RSSR^- \rightarrow RS\cdot + RSH)$, M^{-1} sec^{-1}
Cystine dimethyl ester	$6.0 \pm 1.5 \times 10^8$
Cystamine	$4.2 \pm 1.0 \times 10^9$
Penicillamine disulfide	$3.3 \pm 1.0 \times 10^9$
Glutathione (disulfide)	$7.0 \pm 1.5 \times 10^{10}$
Lipoic acid	$5.5 \pm 1.0 \times 10^{10}$ [a]

[a] Value based on only three hydrogen ion concentrations.

Reduction of Lipoic Acid. The chemistry and biological function of lipoic acid, a coenzyme for the oxidative decarboxylation of pyruvic acid, has been described in some detail.[18] This five-membered ring exhibits some thermochemical strain in the ring and a dihedral angle of only ~27°. In many respects this cyclic disulfide behaves differently from the other open-chain disulfides studied. In particular, the $RSSR^-$ produced decays *via* second-order kinetics as does the species produced by H atom attack at pH 1. Furthermore, both H atom attack and protonation of $RSSR^-$ gives a transient spectrum slightly shifted to the blue compared to $RSSR^-$, with λ_{max} at 385 nm and ϵ_{385} 6.9×10^3 M^{-1} cm^{-1}. Such a shift in the spectrum is consistent with the absorption spectra of the acidic and basic forms of a number of free radicals previously examined.[19] This 385-nm band is attributed to the sulfenium RSS(H)R radical which, in the case of the lipoic acid, is stabilized so that its spectrum and decay kinetics can be evaluated. From the data in Figure 3, pK_a for the dissociation of

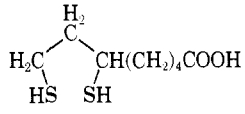
(18) L. J. Reed in "Comprehensive Biochemistry," Vol. 14, M. Florkin and E. H. Stotz, Ed., Elsevier, Amsterdam, 1966, p 99.
(19) See, *e.g.*, P. Neta, M. Simic, and E. Hayon, *J. Phys. Chem.*, 73, 4207, 4214 (1969).

this radical

$$RSS(H)R \rightleftharpoons RSSR^- + H^+ \quad (11)$$

is 5.85 ± 0.1. This acid–base effect is also seen in the second-order decay of protonation of $RSSR^-$, $k_{-11} = 5.5 \times 10^{10}\ M^{-1}\ sec^{-1}$, and is nearly diffusion controlled, as discussed in a previous section, because of the absence of amino groups.

The second-order decay of $RSSR^-$ requires that the bimolecular disappearance of the radical be competitive with its first-order intramolecular decay. Thus, under the conditions of these experiments, where $[RSSR^-] \sim 3 \times 10^{-5}\ M$ and $2k = 1.4 \times 10^8\ M^{-1}\ sec^{-1}$ for the decay of the radical, any first-order component for the disappearance of this radical must have a k less than $\sim 3 \times 10^3\ sec^{-1}$. Because of the close proximity of the sulfur atoms in this cyclic disulfide, the fragments produced upon S–S bond scission cannot diffuse away into the bulk of the solution, and thus intramolecular recombination predominates. The second-order decay of the $RSSR^-$ (and $R\dot{S}SHR$) probably leads to disproportionation, with the formation of the known[18] product dihydrolipoic acid.



The effect of pH on the decay rates of $RSSR^-$ and $R\dot{S}S(H)R$ of lipoic acid, Figure 5, may lie in the differences in their electronic structures: the $RSSR^-$ radical with its large, diffuse, electron density delocalized within the disulfide orbital system, and the sulfenium $R\dot{S}S(H)R$ radical in which the H atom is localized on one sulfur atom and the unpaired electron on the other.

Conclusion

The above results show that all the disulfide linkages have a high reactivity toward solvated electrons, and that the reaction rate constants are dependent upon the acid–base properties of the disulfide compounds. The decay rates of the disulfide radical anions $RSSR^-$ are markedly dependent upon pH. In the neutral to alkaline range, the first-order decay is dependent on the dissociation constants of the amino groups when present in a α position to the –S–S– group. In slightly acidic solutions, the $RSSR^-$ radicals decay by reaction with H^+ to form the corresponding thiyl radicals RS· (presumably *via* the sulfenium radical as intermediate). The kinetics of protonation range from 6.0×10^8 to $7.0 \times 10^{10}\ M^{-1}\ sec^{-1}$ for cystine dimethyl ester to glutathione disulfide, respectively. The reaction of H atoms with disulfide is shown to produce thiyl radicals.

Protonation of Macrocyclic Polyethers. Complexes with Hydrogen Bromide and Hydrogen Tribromide in Chloroform

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Abstract: Interaction of polydentate ethers with hydrogen bromide in chloroform solutions has been investigated conductometrically and spectrophotometrically. Complexation of molecular bromine with bromide ions served as a convenient probe for spectroscopic exploration of investigated systems. Polydentate ethers act as efficient ionizing agents, while hydrogen bonding is predominant in the case of monofunctional and certain bifunctional ethers. The macrocyclic polyether dicyclohexyl-18-crown-6 (DCC) was shown to act as an especially powerful proton solvating agent, its very high complexing power being apparently due to a combination of a relatively high basicity with the low entropy of complexation. At 25° the equilibrium constant, K_p, of the reaction DCC + HBr $\rightleftarrows$ $DCCH^+Br^-$ was found to be about $10^6\ M^{-1}$. For the linear polydentate glyme-2 and glyme-3, $K_p = 0.17$ and $0.20\ M^{-1}$ respectively, while for the aromatic macrocyclic polyethers DBC and Br-DBC, the values are 210 and $20\ M^{-1}$. Pertinent values of the entropies and of the enthalpies of complexation have also been determined for glyme-2 and for Br-DBC. In the case of THF and of glyme-1, the equilibrium constants, $K_1^{25°}$, of the hydrogen bonding interaction $R_2O + HBr \rightleftarrows R_2O\cdots HBr$, were found to be 50 and 13 M^{-1}, respectively, while the equilibrium constants of the formation of the ionic complexes were found to be about $0.6 \times 10^{-4}\ M^{-1}$. Study of the complexation reaction $Br_2 + DCCH^+,Br^- \rightleftarrows DCCH^+,Br_3^-$ in chloroform yields $K_3^{25°} = 3.7 \times 10^4\ M^{-1}$, $\Delta H_3 = -8.2$ kcal, and $\Delta S_3 = -6.6$ eu.

The tendency of ethers to form charge-transfer complexes with halogens[1,2] and their ability to form oxonium ions with halogen acids[3,4] and to bind hydrogen halides by strong dipole–dipole interactions,[5,6] as well as to act as effective solvating agents for cations,[7,8] are well established.[9]

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