

Kinetics of One-Electron Oxidation of Thiols and Hydrogen Abstraction by Thiyl Radicals from α -Amino C–H Bonds

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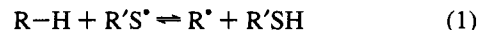
Abstract: One-electron oxidation of cysteine, homocysteine, and glutathione by azide radical in alkaline solution (pH 10.5), where both the amino and the SH groups are deprotonated, has been investigated by pulse radiolysis. Reducing α -aminoalkyl radicals ($^{\bullet}\text{CR}$), which are formed via intramolecular rearrangement of thiyl radicals, were detected using methylviologen as oxidant in the kinetic analysis. The general scheme of the reactions is sketched. Thiyl radicals either equilibrate with $\text{RSSR}^{\bullet-}$ in reaction 5, $\text{RS}^{\bullet} + \text{RS}^- = \text{RSSR}^{\bullet-}$, or undergo intramolecular transformation via equilibrium 6, $\text{RS}^{\bullet} = ^{\bullet}\text{CR}$. At pH 10.5, equilibrium 6 is completely shifted to the right, resulting in α -aminoalkyl radical formation. The rate constants in the reaction scheme for cysteine, homocysteine, and glutathione were measured. With the rate constants obtained, the decay kinetics of $\text{RSSR}^{\bullet-}$ into $^{\bullet}\text{CR}$ was simulated, and it agreed with that measured at 420 nm. At pH 10.5, the first-order rate constants for the transformation (k_6) were determined to be 2.5×10^4 , 1.8×10^5 and $2.2 \times 10^5 \text{ s}^{-1}$ for cysteine, homocysteine, and glutathione, respectively. The rate constants for intermolecular hydrogen abstraction by thiyl radicals from α -amino C–H bonds of alanine and glycine were determined at the same pH to be 7.7×10^5 and $3.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively. Thermodynamic estimation places the reduction potential $E^\circ(\text{H}_2\text{NC}(\text{CO}_2^-)\text{CH}_3^{\bullet}, \text{H}^+/\text{H}_2\text{NCH}(\text{CO}_2^-)\text{CH}_3)$ at ca. 1.22 V, which implies a rather weak tertiary C–H bond in the anion of α -amino acids. Thus, an intramolecular hydrogen abstraction mechanism for the transformation of thiyl radical to α -amino carbon-centered radical is postulated. Molecular geometry plays an important part in deciding the transformation rates (k_6) of different thiyl radicals.

Introduction

Thiyl radicals are important species in chemical as well as biological redox processes.¹ They are known to be the intermediates in the electron or hydrogen donation reaction, the so called “repair” reaction of thiols toward the targeted carbon-centered radicals.¹ Among the thiols that act as reducing agents in biological systems, glutathione is the most abundant.^{2,3} The free radical chemistry of glutathione has been studied for a long time. A general scheme for the one-electron oxidation of glutathione in neutral and alkaline solutions was proposed, based on investigations of the oxidation of glutathione by the OH radical and the one-electron oxidants N_3^{\bullet} , and $\text{CO}_3^{\bullet-}$.^{4–6} During OH radical-induced oxidation of glutathione, two strong reducing radicals were observed in addition to the thiyl radical. One of the reducing radicals is believed to form upon addition of the OH radical to the amino nitrogen in the glutamyl unit. It has a G value of 0.5 and decarboxylates. The other was suggested to be an α -aminoalkyl radical formed via hydrogen abstraction by OH $^{\bullet}$ from the α -amino carbon atom. The pH dependence of formation of the α -amino radical was found to correspond to the $\text{p}K_a$ value of the amino group in the glutamic

acid residue. Neta and Fessenden⁷ in their early paper reported ESR evidence indicating the formation of an α -aminoalkyl radical after OH $^{\bullet}$ oxidation of cysteine in alkaline solution. More recent pulse radiolytic ESR work by Grierson et al.⁸ verified the existence of an α -amino carbon-centred radical following OH $^{\bullet}$ oxidation of glutathione. However, the radical was concluded to be the result of an intramolecular transformation of the glutathione thiyl radical. The rate constant of $1.2 \times 10^5 \text{ s}^{-1}$ was measured for this reaction around pH 10.5. In near-neutral pH, the rate of this reaction was found to increase with OH $^-$ concentration, and a rate constant of $5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ was given for the OH $^-$ -induced transformation reaction. This rate constant was explained as the rate constant of deprotonation of the NH_3^+ group. The formation of an α -aminoalkyl radical following $\text{Br}_2^{\bullet-}$ oxidation of glutathione at pH 9 was reported recently by Prütz et al.⁹

Since C–H bonds, in general, are stronger than S–H bonds, thiyl radicals are usually regarded as unreactive with respect to hydrogen abstraction. Thus equilibrium 1 is usually shifted to the left unless an exceptionally weak C–H bond in the RH species is present. Thiyls do, however, abstract hydrogen from



C–H bonds in alcohols, ethers, or polyunsaturated fatty acids.¹⁰ The α -amino C–H bonds in an amino acid become substantially weaker when the electron-withdrawing ammonium group is converted into the electron-donating amino group by depro-

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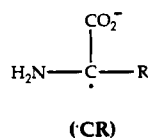
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Chart 1



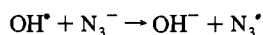
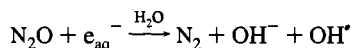
nation at high pH. It is thus interesting to test the kinetics of hydrogen abstraction from α -amino C—H bonds by different thyl radicals. α -Aminoalkyl radicals are known to be strong reductants¹¹ (reduction potential, $E^\circ(\text{NH}_2=\text{CHR}^+/\text{NH}_2\text{CHR}^\bullet)$ ca. -1.9 V vs NHE).¹² Methylviologen (MV^{2+}), which is a moderate electron acceptor (reduction potential, $E_{\text{red}} = -0.448$ V),¹³ can serve as a good probe for reducing radicals in pulse radiolysis experiments.¹¹

In the present work, glutathione, cysteine, and homocysteine were chosen as substrates since they all possess thiol groups and α -amino C—H bonds. At pH 10.5, the thiol and amino groups of these compounds, which have $\text{p}K_a$ values around 9.5,¹⁴ are fully deprotonated. The azide radical (N_3^\bullet) employed is known as a pure one-electron oxidant; hence, the complications caused by using OH radical as oxidant are avoided. The rate constants for the one-electron oxidation of cysteine, homocysteine, and glutathione by N_3^\bullet and the subsequent reactions were examined by pulse radiolysis of alkaline solutions. Intramolecular transformation of the thyl radical to an α -aminoalkyl radical ($\bullet\text{CR}$ is shown in Chart 1) was found to be the prevalent reaction route in the system. The rate constants of intermolecular hydrogen abstraction from alanine and glycine by the cysteine thyl radical were also examined. Estimation of reaction energetics supports the hydrogen abstraction mechanism postulated.

Experimental Section

Glutathione (GSH) and its oxidized form (GSSG), cysteine (CysSH), cystine (CysSSCys), homocysteine (HCysSSH), alanine (Ala), and glycine (Gly) were purchased from Sigma. Homocysteine (HCysSH), N,N,N',N' -tetramethyl-1,4-phenylenediamine (TMPD), methylviologen hydrate (MV^{2+}), NaHCO_2 , and NaN_3 were obtained from Aldrich. All chemicals of highest purity commercially available were used as received.

All solutions were prepared using Millipore-deionized water. One-electron oxidation of thiols was achieved with azide radicals, generated by irradiation of N_2O -saturated 0.1 M NaN_3 solutions. In N_2O saturated solution, hydrated electrons are quantitatively converted into OH radicals.



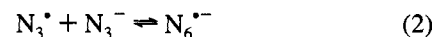
One-electron reduction of the oxidized form of thiols (RSSR) was attained with the hydrated electron (e_{aq}^-) in Ar saturated solution. *tert*-Butyl alcohol (1 M) was added to scavenge OH^\bullet radicals. The pH of the solution was adjusted by adding sodium hydroxide. All the experiments were carried out at room temperature.

Pulse radiolysis was performed at room temperature using doses of 7–12 Gy/pulse, corresponding to 4.3×10^{-6} to 7.4×10^{-6} M radical solutions. In this dose range, the extent of second-order radical combination reactions can be considered minimal. The 3-MeV linear accelerator used has a pulse length of 6 ns. The computerized optical

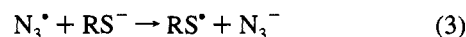
detection system has been described elsewhere.¹⁵ Dosimetry was performed with a N_2O -saturated 10^{-2} M KSCN solution taking $G\epsilon = 4.42 \times 10^{-3} \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$ at 500 nm. The yield of $\text{MV}^{\bullet+}$ was measured at 625 nm, using a N_2O -saturated 10^{-2} M sodium formate and 5×10^{-4} M MV^{2+} solution with $G(\text{MV}^{\bullet+}) = 6.22 \times 10^{-7} \text{ mol J}^{-1}$ as standard.

Results

1. Azide Radical Attack on Thiolate. It was shown by Butler et al.¹⁶ that N_3^\bullet equilibrates with $\text{N}_6^{\bullet-}$ with an equilibrium constant $K_2 = 0.33 \text{ dm}^3/\text{mol}^{-1}$. The rate constants of N_3^\bullet



reacting with thiolates (k_3) were thus obtained from direct measurements of the decay of the $\text{N}_6^{\bullet-}$ radical. At 1.0 M N_3^- ,



equilibrium 2 was established within less than 1 μs . Radical $\text{N}_6^{\bullet-}$ has an absorption maximum at 645 nm with $\epsilon = 6600 \text{ M}^{-1} \text{ cm}^{-1}$. The decay of $\text{N}_6^{\bullet-}$ was titrated versus thiolate concentration at a high (1.0 M) sodium azide concentration at pH 10.5. The rate constant k_3 was deduced from the slope of the linear fit of the experimental points and was corrected for equilibrium 2 under the assumption that $\text{N}_6^{\bullet-}$ itself reacts with RS^- much slower than does N_3^\bullet . The rate constants k_3 at pH 10.5 were calculated to be 4.4×10^9 , 4.1×10^9 , and $4.7 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ for CysSH, HCysSH, and GSH, respectively.

2. e_{aq}^- Reaction with RSSR. The hydrated electron reduces disulfide to the disulfide radical anion (reaction 4), which subsequently dissociates into the corresponding thyl radical and thiolate as shown in equilibrium 5. The rate constant k_4 was



determined by measuring the decay of e_{aq}^- at 650 nm in the presence of 1 M *t*-BuOH and 5×10^{-4} M RSSR in Ar-purged solution. The rate constants k_4 were found to be 4.2×10^9 , 4.5×10^9 , and $2.0 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ for cystine, homocysteine, and GSSG, respectively, at pH 10.5. In the absence of RS^- , the first-order rate constant for the back-reaction (k_{-5}) of equilibrium 5 was obtained by measuring the decay rate of $\text{RSSR}^{\bullet-}$ at 420 nm in the presence of 2×10^{-3} M RSSR. To make sure that the reformation of $\text{RSSR}^{\bullet-}$ through reaction 5 was impossible, 2×10^{-3} M TMPD was added to the solution to capture RS^\bullet ($k_{\text{TMPD}+\text{RS}^\bullet} = 2.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$),¹⁷ thus driving the reverse reaction 5 to completion. The rate constants k_{-5} for the dissociation of the dimeric radical anions at pH 10.5 were found to be 1.7×10^6 , 9×10^5 , and $2 \times 10^5 \text{ s}^{-1}$ for CysSH, HCysSH, and GSH, respectively.

3. Reactions with Methylviologen. After N_3^\bullet oxidation in solutions containing varying concentrations of RS^- and MV^{2+} at pH 10.5, constant yields of $G(\text{MV}^{\bullet+}) = (5.8 \pm 0.3) \times 10^{-7} \text{ mol J}^{-1}$ were obtained when $[\text{MV}^{2+}]$ was in the range 5×10^{-4} to 3×10^{-3} M. This yield corresponds to an efficiency of 93% of the primary radicals, based on N_2O -saturated 10^{-2} M sodium formate solution containing 5×10^{-4} M MV^{2+} as standard with

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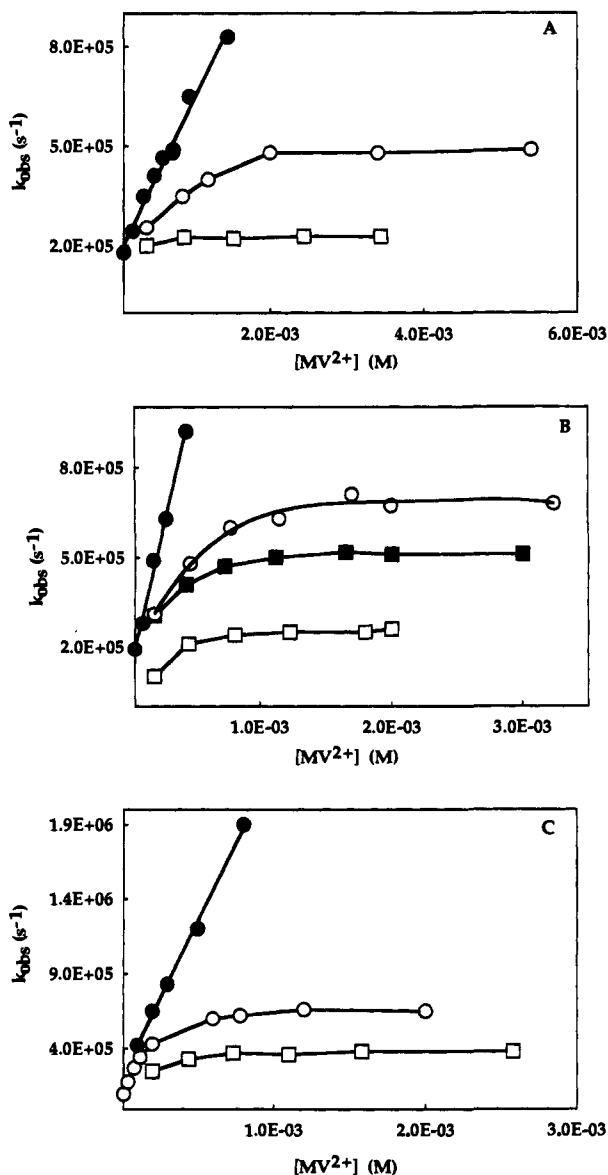


Figure 1. Buildup rates of MV^{2+} at 625 nm as a function of $[MV^{2+}]$ at (A) $[CysS^{-}]$ of (●) 1×10^{-3} M, (○) 5×10^{-4} M, (□) 2×10^{-4} M; (B) $[HCysS^{-}]$ of (●) 1×10^{-2} M, (○) 1×10^{-3} M, (■) 6×10^{-4} M, (□) 4×10^{-4} M; and (C) $[GS^{-}]$ of (●) 1×10^{-2} M, (○) 1×10^{-3} M, (□) 5×10^{-4} M at pH 10.5.

$G(MV^{+}) = 6.22 \times 10^{-7}$ mol J $^{-1}$. With $[MV^{2+}]$ below 5×10^{-4} M, $G(MV^{+})$ decreases because radical combination reactions gain in importance.

The buildup of MV^{+} at 625 nm as a function of MV^{2+} concentration was also measured using varying concentrations of CysSH, HCysSH, and GSH at pH 10.5 (Figure 1).

As can be seen from Figure 1, the k_{obs} versus $[MV^{2+}]$ plots show a proportionate increase at lower $[MV^{2+}]$ and reached a maximum plateau value at higher $[MV^{2+}]$. The concentration of MV^{2+} to reach the plateau and the maximum value of k_{obs} increased with increasing $[RS^{-}]$ in the system. This maximum k_{obs} indicates a fast capture of reducing radicals by MV^{2+} so that the rate-determining step becomes the reaction producing the reducing radical. With 2×10^{-3} M MV^{2+} , the maximum k_{obs} was observed at $[RS^{-}]$ ranging from 1×10^{-5} M to 3×10^{-4} M for CysSH, 1×10^{-4} M to 1×10^{-3} M for HCysSH, and 1×10^{-4} M to 2×10^{-3} M for GSH. Plots of the buildup rate of MV^{+} versus $[RS^{-}]$ in the above range were linear, with distinct intercepts as shown in Figure 2. These intercepts clearly

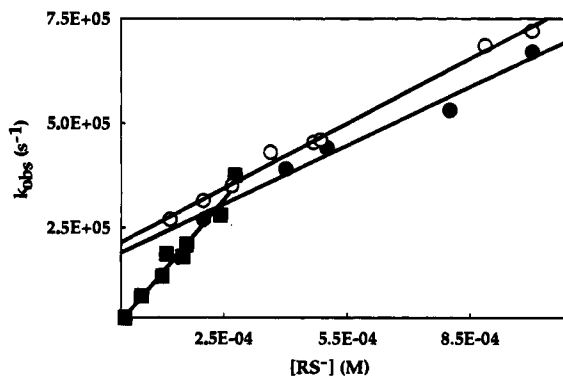
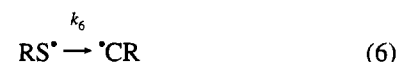


Figure 2. Buildup rates of MV^{2+} at 625 nm as a function of (■) $[CysS^{-}]$, (○) $[HCysS^{-}]$, and (●) $[GS^{-}]$ in the presence of 2×10^{-3} M MV^{2+} at pH 10.5.

indicated that a second reducing radical was formed concomitantly with $RSSR^{*-}$. A reasonable reaction is the intramolecular transformation of thiyl radical to the α -amino carbon-centered radical (*CR) via reaction 6. Both $RSSR^{*-}$ and α -amino carbon-



centered radicals reduce MV^{2+} through reactions 7 and 8. In

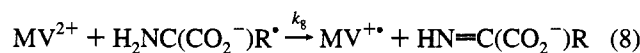


Figure 2, as explained above, the buildup rates of MV^{+} were determined using eq 9. Thus the slopes and intercepts in Figure

$$k_{obs} = k_6 + k_5[RS^{-}] \quad (9)$$

2 yield the corresponding second-order rate constants of reaction 5 (k_5) and the first-order rate constants of intramolecular transformation of the thiyl radical (k_6), respectively. The rate constants k_5 were found to be 1.2×10^9 , 5.4×10^8 , and 4.5×10^8 M $^{-1}$ s $^{-1}$ for CysSH, HCysSH, and GSH, respectively. The rate constants (k_6) of intramolecular transformation for the respective three species at pH 10.5 were measured to be 2.5×10^4 , 2.2×10^5 , and 1.8×10^5 s $^{-1}$. Based on the rate constants k_5 and k_{-5} obtained, the equilibrium constants K_5 were calculated to be 706, 600, and 2250 M $^{-1}$ for CysSH, HCysSH, and GSH, respectively.

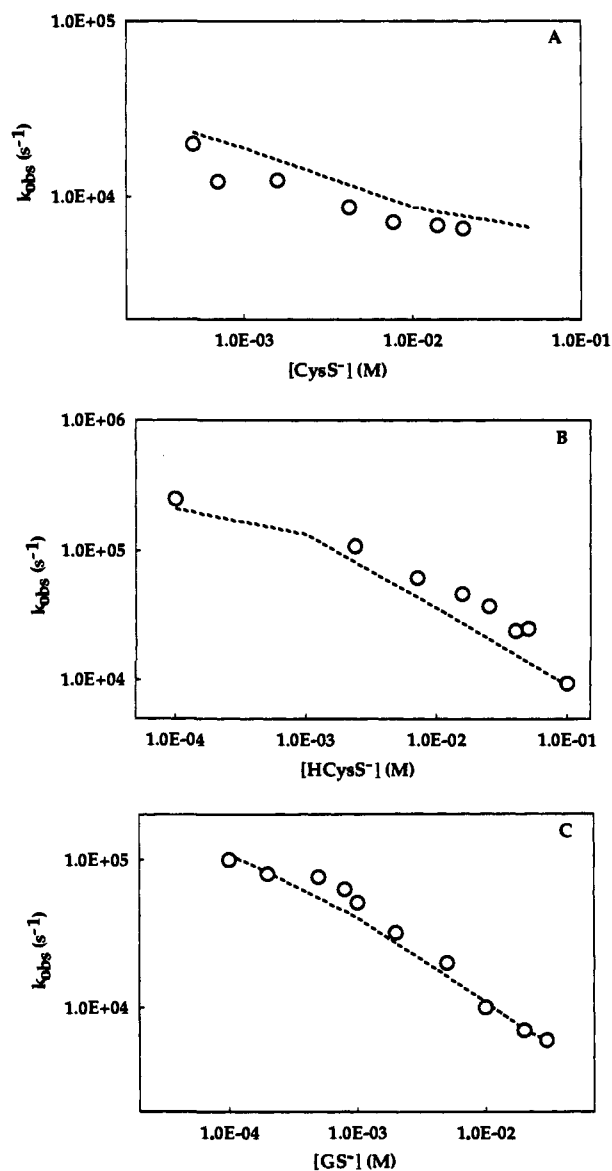
When 0.001 M CysS $^{-}$, 0.01 M HCysS $^{-}$, and 0.01 M GS $^{-}$ were used in the reactions as shown in Figure 1, there were linear increases in the buildup rates of MV^{+} versus $[MV^{2+}]$. In the plots, the slope of the line represented the rate constant of $RSSR^{*-}$ reducing MV^{2+} (k_7), and it was found to be 1.5×10^9 , 1.9×10^9 , and 2.2×10^9 M $^{-1}$ s $^{-1}$ for fully deprotonated CysSH, HCysSH, and GSH, respectively.

Table 1 compiles the rate constants measured in this work.

4. Decay Kinetics of $RSSR^{*-}$. During one-electron oxidation of thiols, the decay kinetics of the dimeric radical anion $RSSR^{*-}$ was found to depend on the RS^{-} concentration.^{4,18} The first-order decay rates of $RSSR^{*-}$ at varying concentrations of RS^{-} were measured at 420 nm after N_3^* oxidation of CysS $^{-}$, HCysS $^{-}$, and GS $^{-}$ at pH 10.5. A computer simulation program (AECL MAKSIMA-CHEMIST) was used to simulate the decay kinetics of $RSSR^{*-}$ using the rate constants obtained in this work

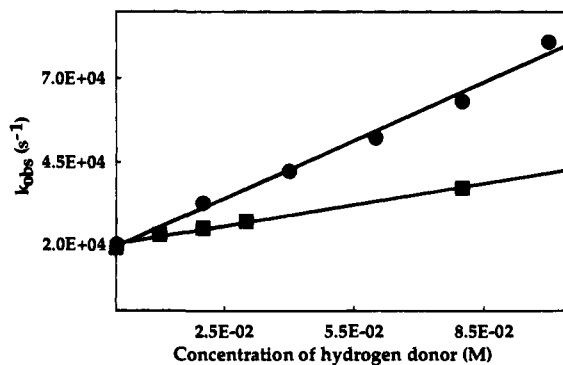
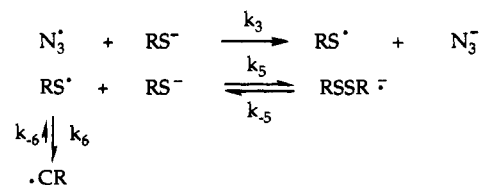
Table 1. Compilation of the Rate Constants Measured in This Work

reaction	CysSH	HCysSH	GSH
$k_3 (N_3^* + RS^-) (M^{-1} s^{-1})$	4.4×10^9	4.1×10^9	4.7×10^9
$k_4 (e_{aq}^- + RSSR^-) (M^{-1} s^{-1})$	4.2×10^9	4.5×10^9	2.0×10^9
$k_5 (RS^* + RS^-) (M^{-1} s^{-1})$	1.2×10^9	5.4×10^8	4.5×10^8
$k_{-5} (RSSR^{2-} \rightarrow RS^* + RS^-) (s^{-1})$	1.7×10^6	9.0×10^5	2.0×10^5
$k_6 (RS^* \rightarrow \cdot CS) (s^{-1})$	2.5×10^4	2.2×10^5	1.8×10^5
$k_7 (RSSR^{2-} + MV^{2+}) (M^{-1} s^{-1})$	1.5×10^9	1.9×10^9	2.2×10^9
K_5	706	600	2250

**Figure 3.** Plots of the observed decay rates of corresponding disulfide radical anion $RSSR^{2-}$ at 420 nm (○) and a simulated one (dotted lines) versus (A) $[CysS^-]$, (B) $[HCysS^-]$, and (C) $[GS^-]$ at pH 10.5.

and assuming the radical combination reactions to have rate constants of $1 \times 10^9 M^{-1} s^{-1}$. As shown in Figure 3, the simulated results agreed exactly with the experimental ones in the case of GSH, but agreement was not as good in the CysSH and HCysSH cases.

5. Hydrogen Abstraction from Ala and Gly by Cysteine Thyl Radical. The hydrogen abstraction by cysteine thyl radicals from the two amino acids alanine and glycine were studied by using high concentrations of Ala and Gly as hydrogen donors. For CysSH at pH 10.5, the intramolecular transformation rate was slow ($2.5 \times 10^4 s^{-1}$), and the equilibrium 5 was

**Figure 4.** Plots of the observed decay rates of cysteine disulfide radical anion $CysSSCys^{2-}$ at 420 nm versus (●) [Ala] and (■) [Gly] in the presence of $5 \times 10^{-4} M$ CysSH at pH 10.5.**Scheme 1**

rapidly established. As a result, the decay kinetics of $CysSSCys^{2-}$ followed eq 10,¹⁹ where K_5 is the equilibrium constant of

$$k_{obs} = (k_6 - k_r)/(1 + K_5[CysS^-]) + k_r \quad (10)$$

reaction 5 and k_r is the pseudo-first-order radical combination rate during the first half-life. Considering the radical concentration in the system, k_r was calculated to be $5 \times 10^3 s^{-1}$. In the presence of an intermolecular hydrogen donor, eq 10 is modified to eq 11, where k_d is the pseudo-first-order rate constant of a

$$k_{obs}' = k_d[D]/(1 + K_5[CysS^-]) + k_{obs} \quad (11)$$

hydrogen donor D reacting with $CysS^*$, and k_{obs} is the rate without D. It was found that the observed decay rate, k_{obs}' of the cysteine radical anion in the presence of Ala and Gly increased linearly with increasing [Ala] and [Gly], as shown in Figure 4. From eq 11, rate constants $k_d(\text{Ala}) = 7.7 \times 10^5 M^{-1} s^{-1}$ and $k_d(\text{Gly}) = 3.2 \times 10^5 M^{-1} s^{-1}$ can be derived from the slopes of the lines, after taking $K_5 = 706 M^{-1}$ as the equilibrium constant for CysSH.

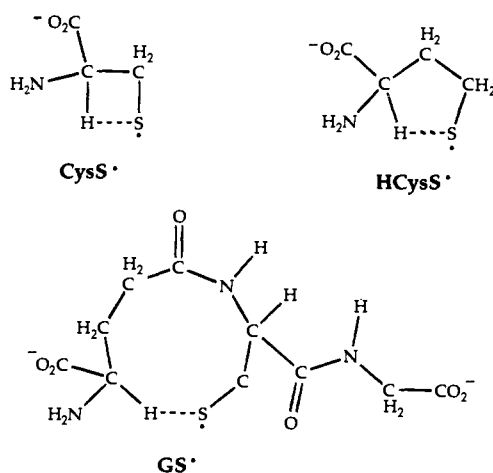
Discussion

Based on our kinetic findings, the general reaction (Scheme 1) for the one-electron oxidation of CysSH, HCysSH, and GSH can be drawn. Reaction 3 and equilibrium 5 with different thiols are well established in the literature.¹ Equilibrium 6 was first suggested by Grierson et al.⁸ The main contribution of this work is the demonstration of the general role of equilibrium 6 in the reaction of thyl radicals with α -amino acids in alkaline solution.

The reaction of N_3^* with $CysS^-$, $HCysS^-$, and GS^- was shown to be a fast step at pH 10.5. Basically, $k_3 > k_5$ is a prerequisite for measuring the rate constants k_5 and k_6 . The rate constant k_3 for GS^- in alkaline solution was found to be much higher than previously reported data at neutral pH.²⁰ The

(19) In high $[CysS^-]$, equilibrium 5 was rapidly established. Thus $C = [CysS^*] + [CysSSCys^{2-}]$, $K_5 = k_5/k_{-5} = [CysSSCys^{2-}]/[CysS^*][CysS^-]$, and $[CysS^*] = C/(1 + K_5[CysS^-])$. $-d[CysSSCys^{2-}]/dt = -dC/dt = k_{obs}C = k_d[CysS^*] + k_r[CysSSCys^{2-}] = \{(k_6 - k_r)/(1 + K_5[CysS^-]) + k_r\}C$. So, $k_{obs} = (k_6 - k_r)/(1 + K_5[CysS^-]) + k_r$.

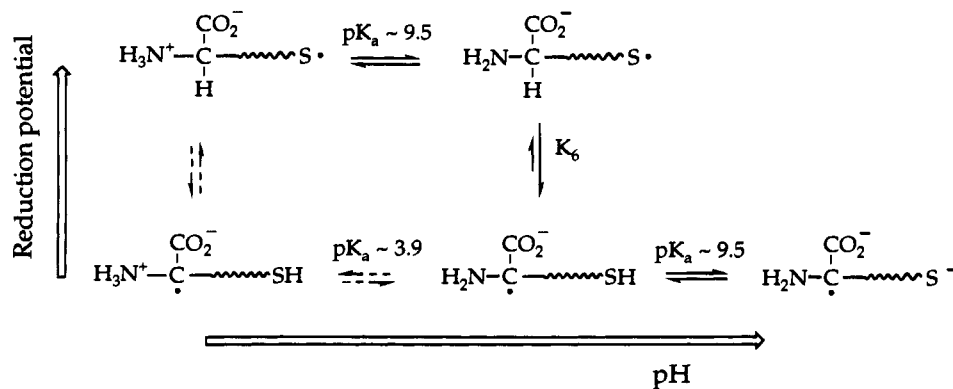
Chart 2



rate constant $4.5 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ for k_5 with GSH is close to the published value of $6 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$,¹⁸ obtained by measurement of the buildup of $\text{GSSG}^{\cdot-}$ at 420 nm. The equilibrium constant $K_5 = 2.25 \times 10^3 \text{ M}^{-1}$ for GSH is slightly lower than a recently reported value of $3.5 \times 10^3 \text{ M}^{-1}$ by Prütz et al.⁹ The magnitudes of both k_5 and k_{-5} for the three thiols were found to be in the order $\text{GSH} < \text{HCysSH} < \text{CysSH}$.

In our work, the intramolecular hydrogen abstraction rate constant k_6 for GSH at pH 10.5 was found to be $1.8 \times 10^5 \text{ s}^{-1}$, close to the one measured directly as the buildup rate at 280 nm by Grierson et al. ($1.2 \times 10^5 \text{ s}^{-1}$).⁸ Using MV^{2+} to capture the reducing radical, k_6 for the three thiols were found to be in the order, $\text{CysSH} \ll \text{GSH} \approx \text{HCysSH}$. The significant difference in k_6 for HCysSH ($2.2 \times 10^5 \text{ s}^{-1}$) and CysSH ($2.5 \times 10^4 \text{ s}^{-1}$) supports the intramolecular hydrogen abstraction reaction mechanism postulated. The possible structures for the intermediates involved in the hydrogen abstraction mechanism were drawn as in Chart 2. HCysS• can abstract an α -amino hydrogen via the sterically favorable five-membered ring configuration. However, the essentially identical values for k_6 of GSH and HCysSH are somewhat surprising, as a nine-membered transition state should not be as favorable as a five-membered one. From the decay kinetics, as shown in Figure 3, simulated results agreed exactly with the experimental one in the case of GSH but resulted in certain mismatch in the HCysSH and CysSH cases. This deviation indicates that the actual k_6 for HCysSH could be even larger. Bear in mind that the measurement of k_6 was limited by the rate of reaction 3 in the system. An estimation on the actual k_6 through computer simulation of the decay kinetics was around $(3-4) \times 10^5 \text{ s}^{-1}$.

Scheme 2



In contrast, the deviation for CysSH occurred in the opposite direction, indicating the actual k_6 for CysSH to be lower than $2.5 \times 10^4 \text{ s}^{-1}$. This is expected because of the difficulty to form a four-membered transition state demanded by the hydrogen abstraction reaction in the CysSH system.

Thiols and thiolates are known as hydrogen or electron donors in biological "repair" reactions with rate constants in the order of $10^6-10^8 \text{ M}^{-1} \text{ s}^{-1}$.^{21,22} However, the thiyl radical itself, which is formed in the "repair" reaction, is a strong oxidant. Hydrogen abstraction from 2-propanol by penS• with a rate constant of $1.4 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ was reported by Schöneich et al.²³ The authors have discussed the possible role of thiyl radical-induced biological damage. Akhlaq et al.²¹ also reported that dithiothreitol thiyl radicals abstract an H atom in α position to the ether function of 2,5-dimethyltetrahydrofuran with a rate constant of $5 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$. The planar ether radical thus formed was repaired by thiol with k_{-1} around $6 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$. Thus the general equilibrium 1 is usually more to the left side of the reaction.¹⁰ For the α -amino thiol, the equilibrium ratio between CR and RS^{\cdot} varies with pH. The pertinent pK_a values are depicted in Scheme 2. In the scheme, the $pK_a \sim 3.9$ is taken from the paper by Hiller and Asmus.¹¹ Grierson et al. observed the transformation of glutathione thiyl radical into CR with full yield even at pH 7.5.⁸ If we take "full yield" to mean larger than 90%, we derive $K_6 \geq 10^3$, taking into account the $pK_a = 9.5$ for the amino group in the glutamyl unit of the GSH.

From the thermochemical point of view, the formation of α -amino carbon-centered radicals are also favored in equilibrium 6. The reduction potential reported by Armstrong et al.¹² for $\text{H}_2\text{NCH}_2\text{CH}_3$ in aqueous solution is $E^\circ(\text{H}_2\text{NCH}_2\text{CH}_3^{\cdot}, \text{H}^+/\text{H}_2\text{NCH}_2\text{CH}_3) = 1.42 \text{ V}$. For the amino acid thiol species, the α -carboxyl effect on the C-H bond energy can be estimated from our recent investigation on the hydrogen abstraction reactions of bromine atom from several hydrogen donors.²⁴ In that work, the free energy of homolysis of the α C-H bond in $\text{CH}_3\text{CH}_2\text{OH}$ was estimated to be higher by ca. 5 kcal/mol as compared with the one in $\text{CH}_3\text{CH}(\text{OH})\text{CO}_2^-$. Assuming a similar difference to hold between $\text{CH}_3\text{CH}_2\text{NH}_2$ and $\text{CH}_3\text{CH}(\text{CO}_2^-)\text{NH}_2$, we arrive at an $E^\circ(\text{H}_2\text{NC}(\text{CO}_2^-)\text{CH}_3^{\cdot}, \text{H}^+/\text{H}_2\text{NCH}(\text{CO}_2^-)\text{CH}_3) \approx 1.22 \text{ V}$ according to Benson's additivity rules for molecular properties.²⁵ This reduction potential would thus correspond to a C-H bond energy of 84.6 kcal/mol under the reasonable assumption that the $\Delta G^\circ(\text{aq-g})$ values are similar for $\text{H}_2\text{NC}(\text{CO}_2^-)\text{CH}_3^{\cdot}$ and $\text{H}_2\text{NCH}(\text{CO}_2^-)\text{CH}_3$. The reduction potential $E^\circ(\text{RS}^{\cdot}, \text{H}^+/\text{RSH}) = 1.33 \pm 0.02 \text{ V}$ was reported by Surdhar and Armstrong²⁶ for the alkyl thiyl radicals. A value of $E^\circ(\text{RS}^{\cdot}, \text{H}^+/\text{RSH}) = 1.36 \pm 0.02 \text{ V}$ can be obtained after correction for the phenol reference as reported earlier.²⁷ From these data, an estimation of the equilibrium constant of reaction

6 could be made, based on the relation $(RT/F) \ln K = \Delta E_i$, where ΔE_i is the difference in the reduction potential of the two couples. With $\Delta E_i = 0.14$ V in this case, $K_6 = 200$ was derived. This estimation agrees with the experimental one made by Grierson et al. at least semiquantitatively.⁸

From the above evidences, a significant fraction of thiyl radicals formed through repair processes may go to form α -amino carbon-centered radicals even at a physiological pH of 7.5. In aerobic conditions, α -amino carbon-centered radicals are known to convert into corresponding peroxy radicals and

subsequently form α -imino acids and superoxide radical anion ($O_2^{\bullet-}$).²⁸⁻³⁰ Further attempts should be made at direct measurement of equilibrium 6, as it is presumably of biological importance.

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Registry Numbers supplied by the authors: CysSH, 52-90-4; HCysSH, 870-93-9; GSH, 70-18-8; CysS*, 35772-84-0; GS*, 40055-99-0; Gly, 56-40-6; Ala, 56-41-7; MV²⁺, 4685-14-7.

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