Rate Constant Determination for the Reaction of Hydroxyl and Glutathione Thiyl Radicals with Glutathione in Aqueous Solution†

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Received: November 28, 1995; In Final Form: February 15, 1996⊗

The techniques of pulse radiolysis, laser photolysis, and absorption spectroscopy have been used to investigate the glutathione disulfide radical anion formation over the pH range 7.0−13.0 in aqueous solution. Photolysis of the disulfide anion formed from the one-electron oxidation of reduced glutathione perturbs the disulfide anion/thiyl radical equilibrium, allowing the rate constant for thiyl radical reaction with glutathione to be uniquely determined from the transient absorption bleach and subsequent pseudo-first-order recovery. These pH-dependent values were combined with measured disulfide equilibrium constants to calculate glutathione radical anion dissociation rate constants. From computer modeling of established mechanisms of the observed disulfide radical anion growths, pH-dependent rate constants for the reaction of hydroxyl radicals with glutathione to produce the thiyl radical were obtained. Utilizing literature ionization constants, values for hydroxyl and thiyl radical reactions with individual glutathione species were determined. The similarity of the measured values over the pH range 10−13 suggests that the rate constants for both the hydroxyl and oxide radical reaction with glutathione are essentially the same. These hydroxyl radical rate constants are contrasted with previously reported values determined using competition kinetics.

Introduction

The role of sulfhydryl (RSH) and disulfide (RSSR) compounds in radiation protection has been known for many years.[1,2] The in-situ discovery of thiyl ($RS^{\bullet}$) radicals in biological material[3,4] and the establishment of their versatile chemistry as intermediates in the repair reactions of carbon-centered radicals[5] have resulted in increased attention being paid to the reactions of such sulfur-centered radical species. Within cells glutathione is the most abundant non-protein-bound sulfhydryl that can act in this manner;[6,7] consequently, the investigation of its thiyl radical formation by primary water radiolysis radicals has been intensively pursued.[8] Although a general scheme for the one-electron oxidation of glutathione by the hydroxyl radical in neutral and alkaline solution has been established,[9] there is little, and inconsistent, rate constant data available for this reaction.[8]

In neutral or basic aqueous solutions, the production of the $RS^{\bullet}$ radicals from free sulfhydryls results in the formation of the reducing radical disulfide anion, $RSSR^{\bullet-}$. A large number of these radical anions have been shown to be formed by the mechanism[10]

$$^{\bullet}OH + RSH \rightarrow H_2O + RS^{\bullet} \quad (1)$$

$$RSH + {}^{-}OH \rightleftharpoons H_2O + RS^{-} \quad (2)$$

$$^{\bullet}OH + RS^{-} \rightarrow OH^{-} + RS^{\bullet} \quad (3)$$

$$RS^{\bullet} + RS^{-}\,(RSH) \rightleftharpoons RSSR^{\bullet-}\,(+H^{+}) \quad (4,-4)$$

The elucidation of individual rate constants from only the temporal behavior of the disulfide transient is complicated, as previous kinetic investigations[8] have shown that the rate constants for reactions 1, 3, and 4 are all similar in aqueous solution and also because the observed growths represent an approach to an equilibrium. This difficulty is reflected in the scatter of the available literature data for sulfhydryls.[8]

In a recent investigation,[11] absolute rate constants for reaction 4 were determined for the sulfhydryls cysteine and cysteamine, using a combined LINAC radiolysis−LASER photolysis technique. These experiments used electron pulse radiolysis to generate the radical disulfide anion via reactions 1−4 and then photolyzed the anion using the laser pulse. The photolysis of this transient causes a decrease in the $RSSR^{\bullet-}$ absorption, which subsequently recovers via first-order kinetics. As the bleach and subsequent recovery of the $RSSR^{\bullet-}$ absorption were demonstrated to be due only to the perturbation of the $RS^{\bullet}$/$RSSR^{\bullet-}$ equilibrium of reaction 4, the exponential recovery gives a direct and unique measurement of the (pseudo-first-order) rate constant for the disulfide anion formation reaction.

In a subsequent study[12] these rate constants were combined with measured cysteine disulfide radical anion equilibrium constants to allow calculation of rate constants for the disulfide dissociation reaction over a wide range of pH's. Knowing both component rate constants in reaction 4, the observed growth profiles of the cysteine $RSSR^{\bullet-}$ transient could then be used to accurately determine rate constants for hydroxyl and oxide radical reaction with this sulfhydryl.

This study utilizes these techniques in the determination of rate constants for the reaction of hydroxyl radicals with glutathione in aqueous solution, over the pH range 7.0−13.0 at room temperature. Using literature ionization constants, the constituent rate constants for hydroxyl radical reaction with individual glutathione species have also been calculated. These values are compared to the results of previous determinations.

Experimental Section

The setup used for these experiments has been described in detail in several previous publications;[13−15] hence only a brief description shall be given here. The pulse radiolysis system

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† The research described herein was supported by the Office of Basic Energy Sciences of the Department of Energy. This is contribution No. NDRL 3864 from the Notre Dame Radiation Laboratory.

⊗ Abstract published in *Advance ACS Abstracts,* April 15, 1996.

S0022-3654(95)03555-6 CCC: $12.00

consists of an 8 MeV LINAC, which delivered 10 ns electron pulses to the sample cell. An XeCl* excimer laser (Lumonics Hyper EX-400) was synchronized to photolyze the irradiated solution after a fixed time period. The pulse radiolysis traces were normalized for small fluctuations in radiolysis dose; the laser intensity did not vary significantly in these experiments.

Glutathione (Aldrich, 98% reduced) was used as received. Care was taken to minimize air oxidation of the glutathione as well as decomposition at higher pH. All solutions were prepared immediately before irradiation, by dissolving weighed amounts of glutathione in N_2O-saturated Millipore Milli-Q filtered water, which had been buffered to the appropriate pH using Baker Analyzed monobasic phosphate or borax at a concentration of 2.0×10^{-2} mol dm^{-3}. Exact pH values were obtained by addition of small amounts of NaOH (Fisher, ACS) or $HClO_4$ (Aldrich, ACS 70%) and using a pH meter. In N_2O-saturated solution, quantitative conversion of hydrated electrons to hydroxyl radicals occurs via

$$e_{aq}^- + N_2O + H_2O \rightarrow {}^{\bullet}OH + {}^-OH + N_2 \qquad (5)$$

Pulse radiolysis experiments were performed at room temperature (22 ± 1 °C) using initial hydroxyl radical concentrations of $(2-4) \times 10^{-6}$ mol dm^{-3}. Solutions were flowed through the irradiation cell at a rate sufficient to ensure that a fresh sample was irradiated each time, with typically about 80 pulses being averaged to obtain a single trace. Absolute dosimetry was carried out using N_2O-saturated SCN^- solutions[16] (10^{-2} mol dm^{-3}, $\lambda = 472$ nm, $G\epsilon = 4.92 \times 10^4$).

Results and Discussion

Photolysis Measurements. The glutathione radical disulfide anion, $GSSG^{\bullet -}$, has a characteristic absorbance with a maximum at 420−430 nm.[17] At lower pH's, this transient is replaced by another weak absorbance due to $GS^{\bullet}$, which has a peak at 330 nm and extends to 450 nm. Under the experimental conditions of this study, the best signals were obtained at 410 nm; at this wavelength the interference from the $GS^{\bullet}$ radical absorption was negligibly small (<2%).

The optical transient obtained from only the pulse radiolysis of a solution of glutathione, 2.57×10^{-3} mol dm^{-3} at pH 9.0, is shown in Figure 1a. The additional photolysis of this transient causes a bleach in this absorbance, as observed for cysteine previously.[11] By subtracting this bleach from only the pulse radiolysis absorption, the recovery trace was obtained, as shown in Figure 1b. The subtracted trace exhibited first-order kinetics and corresponds to the forward "averaged" rate constant, k_6, for glutathione thiyl radical reaction with both protonated and deprotonated glutathione to form the disulfide anion,

$$GS^{\bullet} + GS \rightleftharpoons \text{glutathione disulfide radical anion} \quad (6,-6)$$

However, it has previously been demonstrated[9,18−21] that an additional decomposition pathway exists, where the glutathione thiyl radical undergoes an intramolecular rearrangement to form the carbon-centered α-amino radical, written as the first-order reaction

$$GS^{\bullet} \rightarrow GC^{\bullet} \qquad (7)$$

Individual rate constants for reaction 7 have been previously determined over the pH range 8−11;[19] these values are shown in Figure 2. These rate constants were determined from extrapolated zero-dose, half-life measurements of the glutathione disulfide radical anion decay as well as from the growth kinetics of the carbon-centered radical. There was a marked glutathione

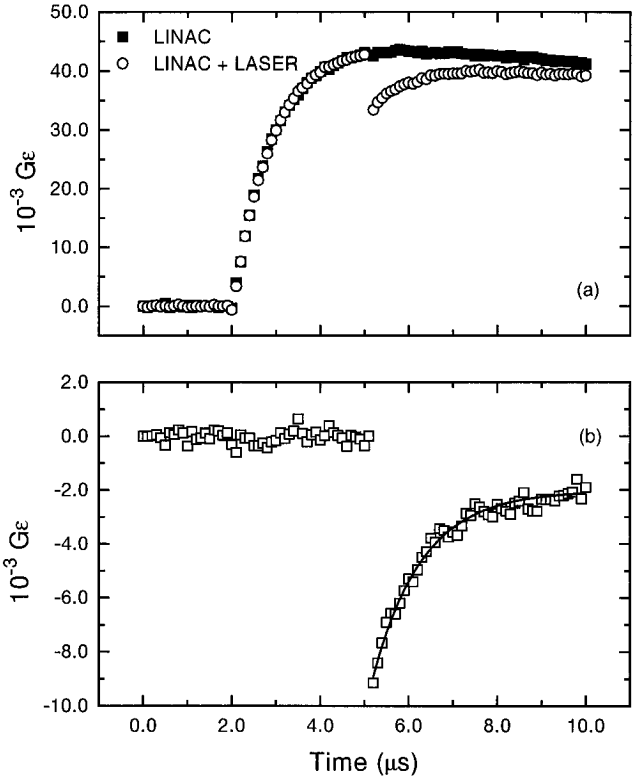

Figure 1. (a) Transient absorption change at 410 nm observed in the pulse radiolysis (■) of N_2O-saturated glutathione (2.57×10^{-3} mol dm^{-3}) solution at pH 9.0. Second trace (○) shows this system which has also been photolyzed by a laser pulse at 308 nm (135 mJ), resulting in a reduction of absorption (bleach) followed by recovery. (b) Difference between these two absorption profiles. Solid line is the predicted values of eq 8 in text, using the rate parameters $k_7 = 8.1 \times 10^3$ s^{-1} and $k_6[GS] = (9.94 \pm 0.83) \times 10^5$ s^{-1}.

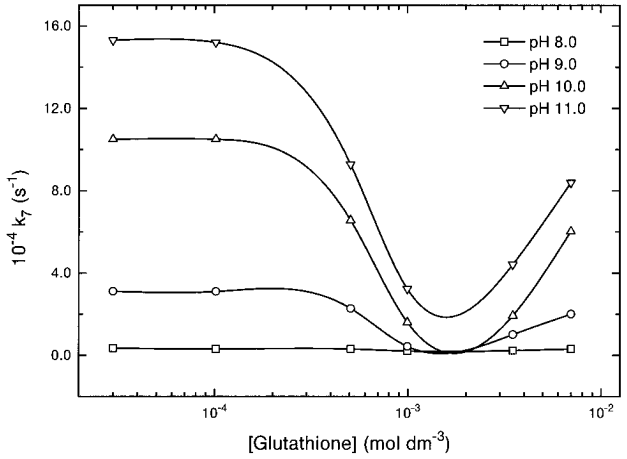

Figure 2. Measured pH and glutathione concentration dependence of the rate constants for glutathione thiyl radical intramolecular rearrangement to form the carbon-centered α-amino radical (reaction 7 in text), as taken from ref 19.

concentration and pH dependence observed for these values, as shown in Figure 2, with an upper rate constant for reaction 7 of ca. 1.5×10^5 s^{-1} determined at all glutathione concentrations and pH's. This limiting rate constant is in reasonable agreement with a subsequent determination at pH 10.5,[18] which calculated k_7 to be 2.2×10^5 s^{-1}.

There was also a significant dose dependence seen for reaction 7, with the observed rate constant doubling for a ca. 5 Gy increase in dose. However, the magnitude of the experimental photolytic-induced bleach measured in this study (typically 10−15% of the glutathione disulfide anion intensity) corresponds

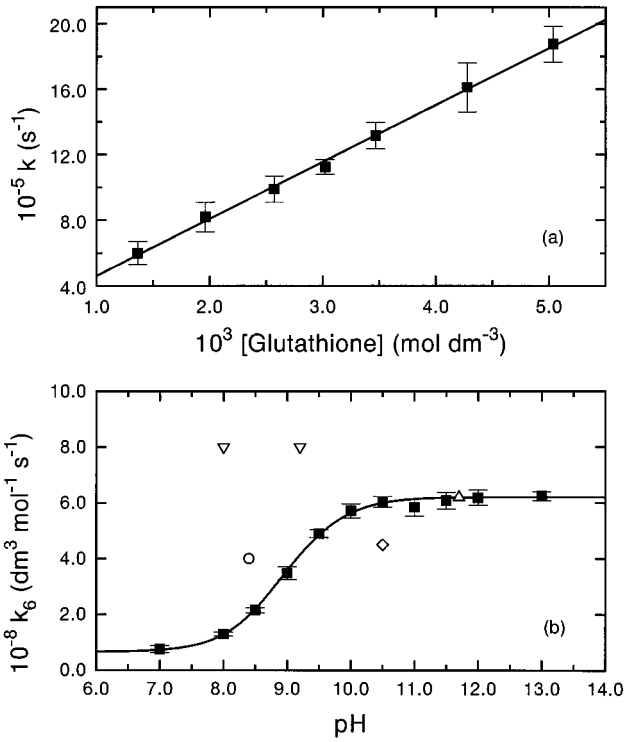

Figure 3. (a) Total glutathione concentration dependence of measured rate constants for radical disulfide anion formation in N_2O-saturated solutions at pH 9.0. Error bars are one standard deviation obtained from the fitting of eq 8 in text. Solid line corresponds to fitted second-order rate constant, with value $k_6 = (3.48 \pm 0.23) \times 10^9$ dm^3 mol^{-1} s^{-1}. (b) pH dependence of measured radical disulfide anion formation rate constants of this study, in comparison to previous literature determinations: (△) ref 10, (◇) ref 18, (▽) ref 22, and (△) ref 23. Solid line corresponds to predicted values using eq 16 given in text with limiting thiyl radical reaction rate constants as given in Table 2. Individual values are summarized in Table 1.

to the production of an initial glutathione thiyl radical concentration equivalent to a dose of ca. 1 Gy, which is low enough that the values shown in Figure 2[19] can be used without significant error.

Therefore, the observed recovery of the photolyzed transient incorporates both pathways for the formed GS$^\bullet$ radical, reactions 6 and 7, as given by the competition-kinetics expression

$$[\mathrm{GSSG}^{\bullet-}]_t = \frac{[\mathrm{GS}^\bullet]_0 k_6[\mathrm{GS}]}{k_6[\mathrm{GS}] + k_7}\{1 - \mathrm{e}^{-(k_6[\mathrm{GS}]+k_7)t}\} \qquad (8)$$

Fitting the recovery of Figure 1b to this equation, with a fixed value of $k_7 = 8.1 \times 10^3$ s^{-1}, a pseudo-first-order rate constant (k_6[GS]) of $(9.94 \pm 0.83) \times 10^5$ s^{-1} was obtained (see Figure 1b).

There are other potential pathways for GS$^\bullet$ radical reaction, notably the radical combination reactions

$$\mathrm{GS}^\bullet + \mathrm{GS}^\bullet \rightarrow \mathrm{GSSG} \qquad (9)$$

$$\mathrm{GS}^\bullet + \mathrm{GSSG}^{\bullet-} \rightarrow \text{products} \qquad (10)$$

Although the extent of these two reactions is believed to be small under the experimental conditions employed, their possible interferences were further minimized by performing the radiolysis−photolysis experiments over a range of total glutathione, GS, concentrations, to give scavenging plots as shown in Figure 3a. The weighted straight line fit to this data at pH 9.0 gives the second-order rate constant for reaction 6 as $k_6 = (3.48 \pm 0.23) \times 10^8$ dm^3 mol^{-1} s^{-1}.

TABLE 1: Summary of the Kinetic Parameters Used in the Determination of Hydroxyl Radical Reaction Rate Constants with Glutathione in Aqueous Solution

pH	10^{-8} k_6 (dm^3 mol^{-1} s^{-1})	K_6 (dm^3 mol^{-1})	10^{-5} k_{-6} (s^{-1})	10^{-9} k_{18} (dm^3 mol^{-1} s^{-1})
7.0	0.75 ± 0.12	338 ± 242	2.22 ± 1.62	3.34 ± 0.14
8.0	1.38 ± 0.07	478 ± 265	2.70 ± 1.50	2.87 ± 0.18
8.5	2.15 ± 0.09	1595 ± 167	1.35 ± 0.15	2.34 ± 0.09
9.0	3.48 ± 0.23	2798 ± 242	1.24 ± 0.14	1.41 ± 0.05
9.5	4.90 ± 0.14	3063 ± 390	1.60 ± 0.21	1.06 ± 0.06
10.0	5.79 ± 0.25	2205 ± 269	2.59 ± 0.34	0.79 ± 0.04
10.5	6.04 ± 0.19	2226 ± 109	2.71 ± 0.16	0.79 ± 0.03
11.0	5.85 ± 0.31	2079 ± 465	2.81 ± 0.64	0.80 ± 0.06
11.5	6.09 ± 0.30	2016 ± 134	3.02 ± 0.25	0.77 ± 0.02
12.0	5.98 ± 0.27	2023 ± 337	3.06 ± 0.53	0.75 ± 0.03
13.0	6.25 ± 0.16	1990 ± 144	3.14 ± 0.24	0.81 ± 0.03

Analogous experiments were performed over the pH range 7.0−13.0 to obtain the rate constants shown in Figure 3b and individually listed in Table 1. Values for k_7 at these pH's, over the experimental glutathione concentration range $(1-5) \times 10^{-3}$ mol dm^{-3}, were estimated by linear extrapolation/interpolation of the pH 8−11 rate constants given in Figure 2. Also shown are several previous determinations of this rate constant;[10,18,22,23] the results of this study are seen to be in excellent agreement with a "direct" measurement from the pseudo-first-order formation of GSSG$^{\bullet-}$ of 6.2×10^8 dm^3 mol^{-1} s^{-1} at pH 11.7,[10] but to differ significantly from values obtained at lower pH's.

Over the pH range of this study, there are three different glutathione species, designated GSH, GS$^-$, and GS^{2-}, all in equilibrium,[24]

$$\mathrm{GSH} \rightleftharpoons \mathrm{H}^+ + \mathrm{GS}^- \qquad \mathrm{p}K_{11} = 8.75 \qquad (11)$$

$$\mathrm{GS}^- \rightleftharpoons \mathrm{H}^+ + \mathrm{GS}^{2-} \qquad \mathrm{p}K_{12} = 9.65 \qquad (12)$$

and therefore the measured rate constants in Figure 3b are combinations of the limiting reactions

$$\mathrm{GS}^\bullet + \mathrm{GSH} \rightarrow \mathrm{GSSG}^{\bullet-} + \mathrm{H}^+ \qquad (13)$$

$$\mathrm{GS}^\bullet + \mathrm{GS}^- \rightarrow \mathrm{GSSG}^{\bullet-} \qquad (14)$$

$$\mathrm{GS}^\bullet + \mathrm{GS}^{2-} \rightarrow \mathrm{GSSG}^{\bullet 2-} \qquad (15)$$

It was assumed that the effects of the GS$^\bullet$ radical deprotonation at higher pH's were small. Transient absorption spectra of the disulfide radical anion measured at pH 9.0 (mostly GSSG$^{\bullet-}$) and pH 11.0 (mostly GSSG$^{\bullet 2-}$) were the same within experimental error; thus, on the basis of these three reactions, a general expression for the overall measured rate constant is given by[25]

$$k_6 = \frac{k_{13}[\mathrm{H}^+]^2 + k_{14}K_{11}[\mathrm{H}^+] + k_{15}K_{11}K_{12}}{K_{11}K_{12} + K_{11}[\mathrm{H}^+] + [\mathrm{H}^+]^2} \qquad (16)$$

Fitting this equation to the data shown in Figure 3b, with K_{11} and K_{12} fixed at their known pK_a values, gives the limiting rate constants $k_{13} = (6.62 \pm 0.76) \times 10^7$, $k_{14} = (4.64 \pm 0.19) \times 10^8$, and $k_{15} = (6.21 \pm 0.10) \times 10^8$ dm^3 mol^{-1} s^{-1} (Table 2). The predicted rate constants using these calculated values in eq 16 are shown as the solid line in Figure 3b and are seen to be in excellent agreement with the experimental data.

Equilibrium Constant Determinations. The overall equilibrium constants for glutathione radical disulfide anion formation, K_6, were calculated from only the radiolysis transient growth kinetics at 420 nm, as performed for cysteine previ-

TABLE 2: Calculated Rate Constants Obtained in This Study for Glutathione Thiyl and Hydroxyl Radical Reaction with Glutathione Species in Aqueous Solution

reaction	rate constant (dm^3 mol^{-1} s^{-1})
$GS^{\bullet} + GSH \rightarrow GSSG^{\bullet -} + H^+$	$(6.62 \pm 0.76) \times 10^7$
$GS^{\bullet} + GS^- \rightarrow GSSG^{\bullet -}$	$(4.64 \pm 0.19) \times 10^8$
$GS^{\bullet} + GS^{2-} \rightarrow GSSG^{\bullet 2-}$	$(6.21 \pm 0.10) \times 10^8$
$^{\bullet}OH + GSH \rightarrow H_2O + GS^{\bullet}$	$(3.48 \pm 0.11) \times 10^9$
$^{\bullet}OH + GS^- \rightarrow OH^- + GS^{\bullet}$	$(9.00 \pm 0.74) \times 10^8$
$^{\bullet}OH + GS^{2-} \rightarrow OH^- + GS^{\bullet -}$	$(7.68 \pm 0.13) \times 10^8$

ously.[12] The temporal behavior of the disulfide anion absorption was fitted to a combined first-order growth and decay exponential model, the second term being used to approximate the small amount of decay that occurred for some of these transients on the experimental time scales. By plotting the total glutathione concentration dependence on the fitted growth rate constants, a linear relationship was obtained, with the equilibrium constant given by K_6 = slope/intercept. The obtained data for pH 9.0 is shown in Figure 4a, with a calculated slope value of $(3.47 \pm 0.03) \times 10^8$ dm^3 mol^{-1} s^{-1} and an intercept of $(1.16 \pm 0.10) \times 10^5$ s^{-1}, corresponding to an equilibrium constant of $K_6 = 2798 \pm 242$ dm^3 mol^{-1}. This value is in very good agreement with, and more precise than, the equilibrium constant determined from the slope and intercept values of the second-order plots obtained from the radiolysis−photolysis experiments (Figure 3a), $K_6 = 2892 \pm 738$ dm^3 mol^{-1}.

The 420 nm measurements were repeated over the pH range 7.0−13.0, with the individual equilibrium constants obtained summarized in Table 1 and shown in Figure 4b. The pH dependence of these values is similar to that determined for cysteine previously;[12] increasing from ca. 300 to >3000 dm^3 mol^{-1} from pH 7.0 to 9.5 and then decreasing to a limiting value at higher pH's.

From the pH-dependent k_6 rate and K_6 equilibrium constants, the rate constants for the dissociation of the glutathione radical disulfide anion can readily be calculated; these values are shown in Figure 5 and listed in Table 1. It is important to note that this calculation only gives the k_{-6} value; however these rate constants are in excellent agreement with the total $GSSG^{\bullet -}$ decay rate constants obtained from the intercept values of the photolysis plots (Figure 3a). This means that the dissociation of $GSSG^{\bullet -}$ to again form the glutathione thiyl radical is the dominant pathway under these conditions, which implies that the direct conversion of the disulfide anion to the carbon-centered radical

$$GSSG^{\bullet -} \rightarrow GC^{\bullet} \qquad (17)$$

previously proposed[19] does not occur to any significant extent under the present conditions.

Despite the large errors associated with these dissociation rate constants at lower pH's, there is a discernible trend in the obtained pH behavior, consisting of a minimum value around pH 9.0 and an indication of a rise with increasing pH. An increase to a limiting value at higher pH's is also seen. This pH dependence is consistent with previous measurements for cystamine and cystine,[26] where the decay of the disulfide radical anion was directly observed following the reaction of the hydrated electron with the disulfide. For these two compounds, the increase at lower pH was attributed to protonation of the disulfide anion to give the short-lived sulfenium ($RSSRH^{\bullet}$) radical, which would dissociate to give $RS^{\bullet}$ and RSH, and the rise at higher pH was shown to exactly follow the pK_a for deprotonation of the disulfide amino group. Although in qualitative agreement, ascertaining whether glutathione also exhibits this behavior at higher pH's is not possible in this study, due to the large errors and because there are two pK_a values in this pH regime (8.75 and 9.65).[24]

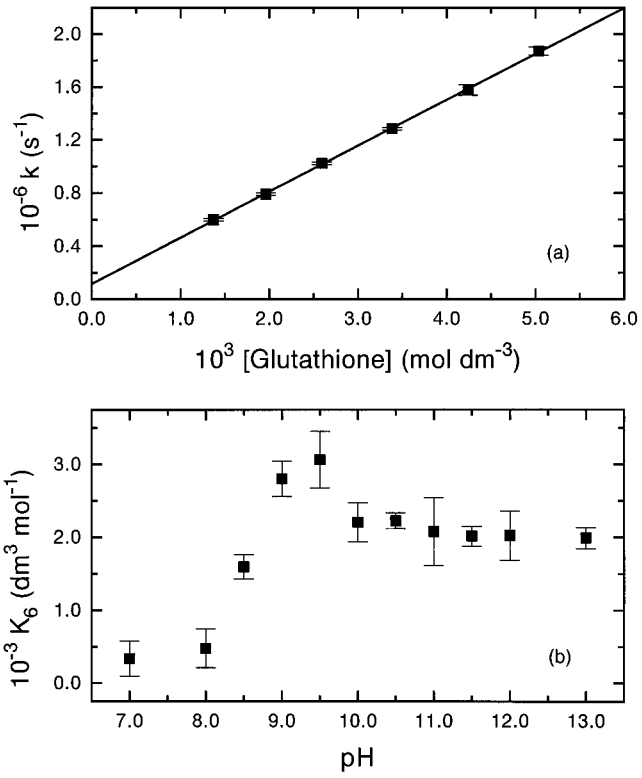

Figure 4. (a) Equilibrium constant determination for glutathione radical disulfide anion formation in N_2O-saturated solutions at pH 9.0 from 420 nm growth kinetics. From fitted linear slope and intercept values (see text), an equilibrium constant of $K_6 = 2798 \pm 242$ dm^3 mol^{-1} is calculated. (b) pH dependence of the calculated equilibrium constants for glutathione radical disulfide anion formation. Individual values are given in Table 1.

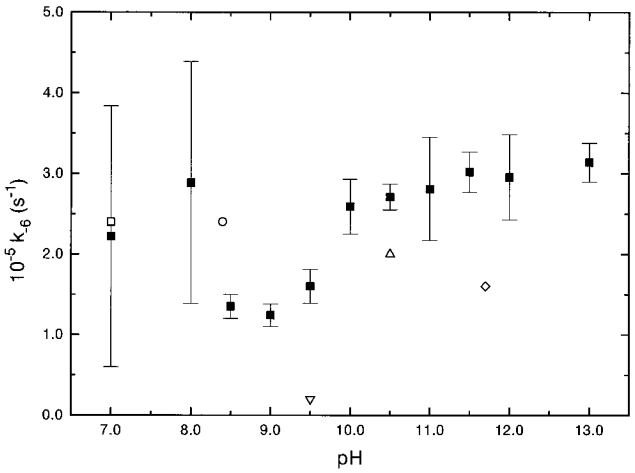

Figure 5. pH dependence of calculated rate constants for reaction -6, determined using measured disulfide radical equilibrium constants (Figure 4b) and scavenging rate constants (Figure 3b), in comparison to previous literature determinations: (◇) ref 10, (△) ref 18, (▽) ref 21, (□) ref 22, and (○) ref 23. Error bars correspond to one standard deviation as calculated from the uncertainty in these two measured values. Individual values are given in Table 1.

There have been several previous direct measurements for the $GSSG^{\bullet -}$ decay rate constant,[17,18,22,23,26] and these values are also shown in Figure 5. Although no pH dependence has been performed in any other single study, these values are seen to be in good agreement with the calculated rate constants of this study.

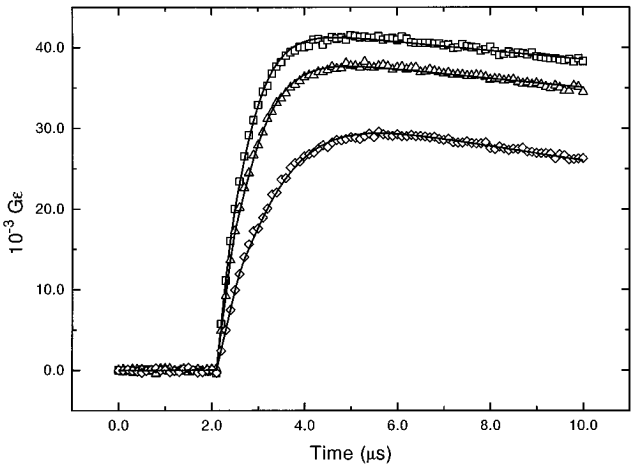

Figure 6. Typical fits obtained in the determination of pseudo-first-order rate constants for hydroxyl radical reaction with glutathione at pH 10.0. Data shown corresponds to glutathione concentrations of 3.51 $\times$ 10^{-3} (□), 2.89 $\times$ 10^{-3} (△), and 1.64 $\times$ 10^{-3} (◇) mol dm^{-3}. Fitted lines correspond to calculated values, using determined rate constants for reactions 6 and -6 (Table 1) and hydroxyl radical reaction rate constants of k_{18}[GS] = 2.59 $\times$ 10^6, 2.17 $\times$ 10^6, and 1.27 $\times$ 10^6 s^{-1}, respectively.

Hydroxyl Radical Reactions. Knowing the pH-dependent rate constants for reactions 6 and -6, individual rate constants for the reaction of hydroxyl radicals with glutathione,

$$^{\bullet}OH + GS \rightarrow GS^{\bullet} \qquad (18)$$

can then be determined. Using the three-reaction mechanism, consisting of reactions 6, -6, and 18, each transient glutathione disulfide radical anion growth was modeled using the numerical differential equation solving code FACSIMILE[27] to optimize the best pseudo-first-order rate constant for reaction 18. Typical fits obtained for three glutathione concentrations at pH 10.0 are shown in Figure 6.

The calculated first-order rate constants were then plotted against total glutathione concentration to give scavenging curves, as shown in Figure 7a. At pH 10.0, the overall rate constant is $k_{18} = (7.90 \pm 0.40) \times 10^8$ dm^3 mol^{-1} s^{-1}. These determinations were repeated over the pH range 7.0−13.0, with the values obtained listed in Table 1 and shown in Figure 7b.

There have been several previous measurements of hydroxyl radical reaction rate constants with glutathione,[9,21,22] and these values are also shown in Figure 7b. The rate constants obtained in this study are significantly different from all the previous competition-kinetics determinations, being lower in magnitude and showing the opposite pH behavior of decreasing with increasing pH. The observed pH behavior in this study is consistent with previous measurements of hydroxyl and oxide radical reaction with cysteine.[12]

Most of the competition-kinetics determinations have used hydroxyl radical reaction with SCN$^-$ as a reference reaction,[9,22] which introduces the additional complication that the two intermediates formed, GSSG$^{\bullet-}$ and $(SCN)_2^{\bullet-}$, have overlapping spectra. To accurately determine the hydroxyl radical reaction rate constant under these conditions requires accurate absorption coefficients for the glutathione radical species; however, the anion peak values have been reported over the range 6300−8300 dm^3 mol^{-1} cm^{-1}.[9,17] Moreover, it has been proposed that there may be an interfering reaction of the formed GS$^{\bullet}$ radicals with SCN$^-$, to give a product that absorbs in the same region.[28] There has only been one other reference compound used, tetramethylurea,[28] which gave a glutathione reaction rate constant that was much lower than all of the SCN$^-$ determinations. Based on these potential complications, the previously reported competition-kinetics reaction rate constants must be treated with some caution.

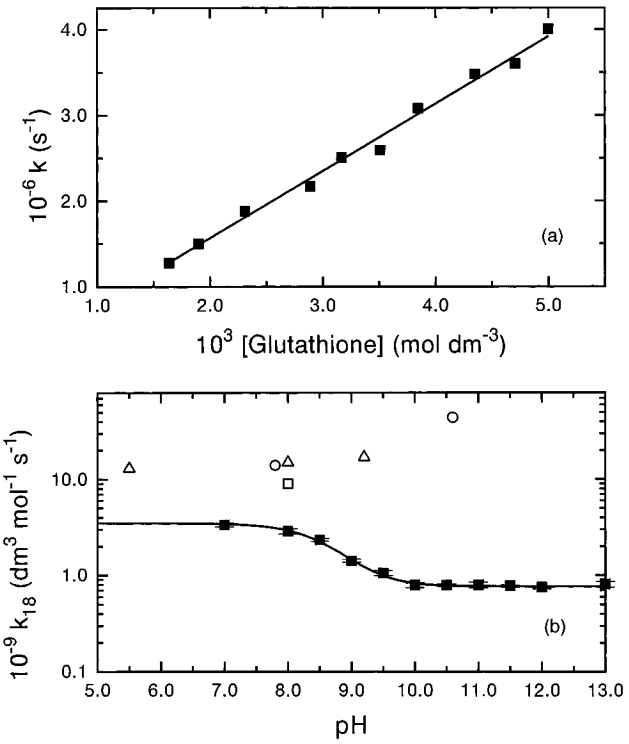

Figure 7. (a) Total glutathione concentration dependence of calculated rate constants for hydroxyl radical reaction in N_2O-saturated solutions at pH 10.0. Solid line corresponds to fitted second-order rate constant, with value $k_{18} = (7.90 \pm 0.42) \times 10^8$ dm^3 mol^{-1} s^{-1}. (b) pH dependence of rate constants for the reaction of hydroxyl and oxide radicals with glutathione in aqueous solution in comparison to previous literature data: (○) ref 9, (△) ref 22, and (□) ref 28. Individual values are given in Table 1. Error bars correspond to one standard deviation obtained from second-order rate constant determinations. Fitted solid line corresponds to the best fit of eq 16 in text, with limiting hydroxyl radical reaction rate constants as given in Table 2.

At higher pH's, deprotonation of the hydroxyl radical also occurs,

$$^{\bullet}OH \rightleftharpoons H^+ + {}^{\bullet}O^- \qquad (19)$$

with a measured pK_a value of 11.9.[29] In principle this means that there are six separate reactions that can occur, with both the hydroxyl and oxide radicals reacting with GSH, GS$^-$, and GS^{2-}. However, as the overall measured rate constant is essentially the same over the pH range 10.0−13.0, this implies that the rate constants for oxide and hydroxide reaction with GS$^-$ and GS^{2-} are effectively the same. Given that oxide radical reaction with GSH will only be a very minor reaction, the rate constants shown in Figure 7b are thus well described by the three limiting reactions

$$^{\bullet}OH + GSH \rightarrow GS^{\bullet} + H_2O \qquad (20)$$

$$^{\bullet}OH + GS^- \rightarrow GS^{\bullet} + OH^- \qquad (21)$$

$$^{\bullet}OH + GS^{2-} \rightarrow GS^{\bullet-} + OH^- \qquad (22)$$

By analogy to reactions 13−15, eq 16 can again be used to determine individual rate constants for these reactions. By fixing the two pK_a values of glutathione at their known values,[24] individual rate constants of $k_{20} = (3.48 \pm 0.11) \times 10^9$, $k_{21} = (9.00 \pm 0.74) \times 10^8$, and $k_{22} = (7.68 \pm 0.13) \times 10^8$ dm^3 mol^{-1}

s^{-1} were obtained (Table 2). The predicted rate constants using these values in eq 16 are shown as the solid line in Figure 7b and are seen to be in excellent agreement with the experimental data.

Conclusion

By combining the techniques of electron pulse radiolysis and laser photolysis, individual rate constants for the reactions

$$GS^{\bullet} + GSH \rightarrow GSSG^{\bullet -} + H^{+} \quad (13)$$

$$GS^{\bullet} + GS^{-} \rightarrow GSSG^{\bullet -} \quad (14)$$

$$GS^{\bullet} + GS^{2-} \rightarrow GSSG^{\bullet 2-} \quad (15)$$

have been elucidated from pH-dependent rate constants of radical disulfide anion formation as $k_{13} = (6.62 \pm 0.76) \times 10^7$, $k_{14} = (4.64 \pm 0.19) \times 10^8$, and $k_{15} = (6.21 \pm 0.10) \times 10^8$ dm^3 mol^{-1} s^{-1}. These values were combined with measured, pH-dependent, glutathione disulfide radical anion equilibrium constants to allow computation of rate constants for the hydroxyl radical reactions

$$^{\bullet}OH + GSH \rightarrow GS^{\bullet} + H_2O \quad (20)$$

$$^{\bullet}OH + GS^{-} \rightarrow GS^{\bullet} + OH^{-} \quad (21)$$

$$^{\bullet}OH + GS^{2-} \rightarrow GS^{\bullet -} + OH^{-} \quad (22)$$

as $k_{20} = (3.48 \pm 0.11) \times 10^9$, $k_{21} = (9.00 \pm 0.74) \times 10^8$, and $k_{22} = (7.68 \pm 0.13) \times 10^8$ dm^3 mol^{-1} s^{-1}. These rate constants are much lower and show the opposite pH dependence of previously reported competition kinetics values.

Acknowledgment. I wish to thank Dr. David Armstrong for his insightful comments on an earlier version of the manuscript.

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JP9535553