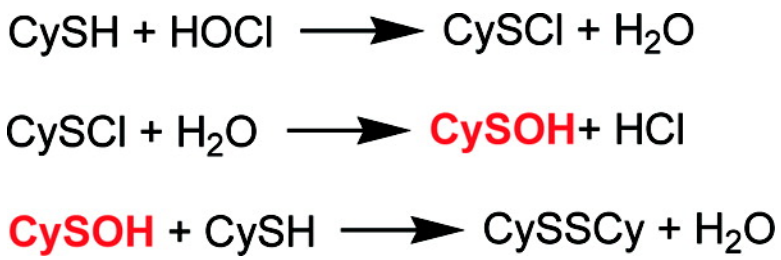
Article

Reactive Sulfur Species: Kinetics and Mechanisms of the Oxidation of Cysteine by Hypohalous Acid to Give Cysteine Sulfenic Acid

Pter Nagy, and Michael T. Ashby

J. Am. Chem. Soc., **2007**, 129 (45), 14082-14091• DOI: 10.1021/ja0737218 • Publication Date (Web): 16 October 2007



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 4 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 10/16/2007

Reactive Sulfur Species: Kinetics and Mechanisms of the Oxidation of Cysteine by Hypohalous Acid to Give Cysteine Sulfenic Acid

Péter Nagy and Michael T. Ashby*

Contribution from the Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019

Received May 23, 2007; E-mail: MAshby@ou.edu

Abstract: Cysteine sulfenic acid has been generated in alkaline aqueous solution by oxidation of cysteine with hypohalous acid (HOX, X = Cl or Br). The kinetics and mechanisms of the oxidation reaction and the subsequent reactions of cysteine sulfenic acid have been studied by stopped-flow spectrophotometry between pH 10 and 14. Two reaction pathways were observed: (1) below pH 12, the condensation of two sulfenic acids to give cysteine thiosulfinate ester followed by the nucleophilic attack of cysteinate on cysteine thiosulfinate ester and (2) above pH 10, a pH-dependent fast equilibrium protonation of cysteine sulfenate that is followed by rate-limiting comproportionation of cysteine sulfenic acid with cysteinate to give cystine. The observation of the first reaction suggests that the condensation of cysteine sulfenic acid to give cysteine thiosulfinate ester can be competitive with the reaction of cysteine sulfenic acid with cysteine.

Introduction

Cysteine (CySH)[1] is one of the least frequently employed amino acids in biosynthesis,[2] presumably due in part to its reactivity relative to the other amino acids, particularly with respect to oxidation. However, it is this reactivity that is exploited by many defensive mechanisms for oxidative stress. Thus, the cysteine-containing tripeptide glutathione is the predominant non-protein thiol in eukaryotes[3] and some prokaryotes,[4,5] and millimolar concentrations are found in some cells, wherein it plays a role in maintaining redox homeostasis. Cysteine itself is not used to control redox potentials *in vivo* for a variety of reasons that include the fact that under certain circumstances, ironically, CySH can act as a pro-oxidant (vis-à-vis Fenton chemistry).[6] In addition to serving as a redox buffer, the thiol-disulfide equilibrium of cysteine derivatives is known to play many other functions *in vivo*, including the covalent cross-linking of polypeptide chains in proteins (so as to impose tertiary structure), signal transduction, and the regulation of enzyme activity. Although it has been recognized for more than 30 years that cysteine residues in proteins can be "overoxidized" beyond the disulfide form by relatively mild oxidants,[7] it has been generally assumed that the one-electron couple between thiol and disulfide is the only redox chemistry of biological relevance. However, biochemical and structural evidences have been recently introduced for functional protein sulfenic acid moieties in native proteins, e.g., NADH peroxidase,[8] NADH oxidase,[9] nitrile hydratase,[10] and certain peroxiredoxins.[11−14] In addition, sulfenic acid derivatives have been implicated in the redox status regulation of certain cellular functions. While still not viewed as pedestrian, advancements in our understanding of protein derivatives of sulfenic acids during the past decade have begun to clarify their niche in biochemistry. However, in contrast to the relatively stable sulfenic derivatives of cysteine that have been identified in proteins, the parent compound remains elusive. In fact, of the common redox derivatives of CySH (Scheme 1), only cysteine sulfenic acid (CySOH) has not been isolated. Furthermore, previous reports of the characterization of the reaction mechanisms of CySOH *in situ* [for example the rate of its comproportionation with CySH to give cystine (CySSCy)[15,16]] have been discredited.[17,18] With the exception

(1) References to compounds without charges are inclusive of all acid/base derivatives. Specific proton states are referenced in the Discussion section and some of the schemes.

(2) Pe'er, I.; Felder, C. E.; Man, O.; Silman, I.; Sussman, J. L.; Beckmann, J. S. *Proteins: Struct., Funct., Bioinf.* **2003**, *54*, 20−40.
(3) Meister, A. *J. Biol. Chem.* **1988**, *263*, 17205−17208.
(4) Smirnova, G. V.; Oktyabrsky, O. N. *Biochemistry (Moscow)* **2005**, *70*, 1199−1211.
(5) Fahey, R. C.; Brown, W. C.; Adams, W. B.; Worsham, M. B. *J. Bacteriol.* **1978**, *133*, 1126−9.
(6) Nagy, P.; Becker, J. D.; Mallo, R. C.; Ashby, M. T. The Jekyll and Hyde Roles of Cysteine Derivatives During Oxidative Stress. In *New Biocides Development: The Combined Approach of Chemistry and Microbiology*; Zhu, P., Ed.; American Chemical Society: Washington, DC., 2007, in press.
(7) Allison, W. S. *Acc. Chem. Res.* **1976**, *9*, 293−299.
(8) Yeh, J. I.; Claiborne, A. *Methods Enzymol.* **2002**, *353*, 44−54.
(9) Mallett, T. C.; Parsonage, D.; Claiborne, A. *Biochemistry* **1999**, *38*, 3000−3011.
(10) Endo, I.; Nojiri, M.; Tsujimura, M.; Nakasako, M.; Nagashima, S.; Yohda, M.; Odaka, M. *J. Inorg. Biochem.* **2001**, *83*, 247−253.
(11) Claiborne, A.; Mallett, T. C.; Yeh, J. I.; Luba, J.; Parsonage, D. *Adv. Protein Chem.* **2001**, *58*, 215−276.
(12) Claiborne, A.; Yeh, J. I.; Mallett, T. C.; Luba, J.; Crane, E. J., III; Charrier, V.; Parsonage, D. *Biochemistry* **1999**, *38*, 15407−15416.
(13) Claiborne, A.; Miller, H.; Parsonage, D.; Ross, R. P. *FASEB J.* **1993**, *7*, 1483−1490.
(14) Van den Broek, L. A. G. M.; Delbressine, L. P. C.; Ottenheijm, H. C. J. Biochemistry and metabolic pathways of sulfenic acids and their derivatives. In *The Chemistry of Sulphenic Acids and Their Derivatives*; Patai, S., Ed.; John Wiley: New York, 1990; pp 701−721.
(15) Luo, D.; Smith, S. W.; Anderson, B. D. *J. Pharm. Sci.* **2005**, *94*, 304−316.
(16) Anderson, B. D.; Luo, D. *J. Pharm. Sci.* **2005**, *95*, 19−24.
(17) Ashby, M. T.; Nagy, P. *J. Pharm. Sci.* **2005**, *95*, 15−18.
(18) Ashby, M. T.; Nagy, P. *Int. J. Chem. Kinet.* **2006**, *39*, 32−38.

10.1021/ja0737218 CCC: $37.00

Scheme 1. Nomenclature and Oxidation States of Common Derivatives of Cysteine

	CySH	CySOH	$CySO_2H$	$CySO_3H$
ox state	-2	0	+2	+4
name	thiol	sulfenic acid	sulfinic acid	sulfonic acid

	CySSCy	CySSCy (=O)	CySSCy (=O, =O)
ox state	-1/-1	+1/-1	+3/-1
name	disulfide	thiosulfinate ester	thiosulfonate ester

of a few derivatives that are stabilized through steric hindrance, H-bonding, or conjugation,[19−24] sulfenic acids are generally considered to be transient species.[25] We describe herein the facile generation of CySOH under alkaline conditions (wherein it exists principally as the sulfenate anion)[26] by the reaction of CySH with HOX (X = Cl or Br). Of note, HOX is produced during inflammatory response by defensive human peroxidases, and one of its principal targets *in vivo* is CySH.[27−31] Thus, the redox cascade that begins with the oxidation of CySH by HOX is also of physiological relevance.[6,32−36]

Results

Products of the Reaction of CySH with HOX (X = Cl or Br). When HOCl is reacted with excess CySH, the only cysteine-derived product that is observed by ^{1}H NMR (within 5 min of mixing) is CySSCy. Furthermore, when turbulent (stopped-flow) mixing is employed, lower ratios of CySH:HOCl (down to a ratio of 2:1) also produce essentially only CySSCy (and sometimes a small amount of $CySO_2^-$) at pH 11.3. We have observed that when turbulent conditions are employed to mix a 1:1 ratio of CySH with HOCl at pH 11.3 (stopped-flow or a hand-mixer consisting of two Hamilton syringes and a T-mixer), a 3.3:1:1.1 mixture of CySSCy, cysteine sulfinic acid ($CySO_2H$), and cysteine sulfonic acid ($CySO_3H$) is observed by ^{1}H NMR. However, if less vigorous methods are employed to initiate the reaction between CySH and HOCl, such as the use of pipettes, a 1:1 mixture CySSCy and *N*,*N*′-dichlorocystine (NDC = $[\text{-SCH}_2\text{CH(NHCl)(CO}_2\text{H)}]_2$) is produced.[37,38] While CySSCy was the main stable product that was observed for the reaction of HOX (X = Cl or Br) with excess CySH between pH 10 and 14, at very high pH, under some circumstances, $CySO_2H$ was a minor product of the reaction of CySH and HOX via a mechanism that cannot be attributed to the hydrolysis of CySSCy (because the latter reaction is not kinetically competent).[39] We will later attribute the production of $CySO_2H$ during the oxidation of CySH by HOX to overoxidation (the reaction of multiple equivalents of HOX).

Kinetics of the Reaction of CySH with HOX (X = Cl or Br). The kinetics of the reaction of CySH with HOCl have been previously investigated by Armesto et al.[40] We have repeated some of these measurements (data not shown), and we have confirmed their rate constants: $k(\text{HOCl}) = 1.2 \times 10^9\ \text{M}^{-1}\ \text{s}^{-1}$ and $k(\text{OCl}^-) = 1.9 \times 10^5\ \text{M}^{-1}\ \text{s}^{-1}$. However, we disagree with the conclusion that cysteine sulfenyl chloride (CySCl) has transitory stability (*vide infra*). We have also carried out preliminary measurements for the reaction of CySH with HOBr. Even under very alkaline conditions, the reaction tests the limits of the stopped-flow method. For most of the stopped-flow experiments that are described herein, the oxidation of CySH by HOX (X = Cl or Br) occurred within the time of mixing. Following these oxidation reactions, two subsequent reactions are observed when the pH is between 10 and 12 (Figure 1). One of the reactions is essentially pH independent between pH 10 and 12 (although it becomes pH dependent below pH 10; data not shown).[39] The other reaction exhibits a complex pH dependency, whereby nonlinear $[H^+]$ dependency was observed between pH 10 and 12 (Figure 6), and linear $[H^+]$ dependency was observed between pH 12 and 14 (Figures 2 and 3). The effective second-order rate constants of the pH-dependent reaction between pH 12 and 14 (which were computed by dividing the observed pseudo-first-order rate constants by the concentration of the reactant in excess, i.e., $k_{\text{eff}} = k_{\text{obs}}/[\text{CySH}]_0$) can be modeled with a linear relationship of k_{eff} versus $1/[\text{OH}^-]$ that passes through the origin (Figure 2 and squares and solid line of Figure 3). Above pH 10, the pH-dependent reaction also exhibits a linear dependence on $[\text{CySH}]_0$ that is the same regardless of whether HOCl or HOBr is employed as the oxidant (e.g., Figure 4). We note that the reaction that exhibits pH dependency cannot be monitored below pH 10 (*vide supra*). Thus, the pH-dependent reaction exhibits the same rate for X = Cl and X = Br and overall third-order kinetics above pH 12: first-order each in [oxidized cysteine intermediate], [CySH], and $[H^+]$. The reaction, that is essentially pH independent, also exhibits the same rates for X = Cl and Br (Figure 1) between pH 10 and 12, and overall second-order kinetics: first-order each in [oxidized cysteine intermediate] and [CySH]. In addition, the pH-independent reaction was also observed as a minor reaction at pH > 12 (data not shown). The pH-independent reaction will be attributed later to the reaction of cysteine

(19) Goto, K.; Shimada, K.; Furukawa, S.; Miyasaka, S.; Takahashi, Y.; Kawashima, T. *Chem. Lett.* **2006**, *35*, 862−863.
(20) Goto, K.; Holler, M.; Okazaki, R. *J. Am. Chem. Soc.* **1997**, *119*, 1460−1461.
(21) Ishii, A.; Komiya, K.; Nakayama, J. *J. Am. Chem. Soc.* **1996**, *118*, 12836−12837.
(22) Tripolt, R.; Belaj, F.; Nachbaur, E. *Z. Naturforsch., B: Chem. Sci.* **1993**, *48*, 1212−1222.
(23) Yoshimura, T.; Tsukurimichi, E.; Yamazaki, S.; Soga, S.; Shimasaki, C.; Hasegawa, K. *Chem. Commun.* **1992**, 1337−1338.
(24) Nakamura, N. *J. Am. Chem. Soc.* **1983**, *105*, 7172−7173.
(25) Patai, S. *The Chemistry of Sulfenic Acids and Their Derivatives*; John Wiley: New York, 1990.
(26) O'Donnell, J.; Schwan, A. *J. Sulfur Chem.* **2004**, *25*, 183−211.
(27) Davies, K. J. A. NATO Advanced Study Institute Series A296; 1998, pp 253−266.
(28) Pattison, D. I.; Davies, M. J. *Chem. Res. Toxicol.* **2001**, *14*, 1453−1464.
(29) Pattison, D. I.; Davies, M. J. *Biochemistry* **2004**, *43*, 4799−4809.
(30) Pattison, D. I.; Hawkins, C. L.; Davies, M. J. *Chem. Res. Toxicol.* **2003**, *16*, 439−449.
(31) Hawkins, C. L.; Pattison, D. I.; Davies, M. J. *Amino Acids* **2003**, *25*, 259−274.
(32) Giles, G. I.; Tasker, K. M.; Collins, C.; Giles, N. M.; O'Rourke, E.; Jacob, C. *Biochem. J.* **2002**, *364*, 579−585.
(33) Giles, G. I.; Tasker, K. M.; Jacob, C. *Gen. Physiol. Biophys.* **2002**, *21*, 65−72.
(34) Giles, G. I.; Tasker, K. M.; Jacob, C. *Free Radical Biol. Med.* **2001**, *31*, 1279−1283.
(35) Giles, G. I.; Jacob, C. *Biol. Chem.* **2002**, *383*, 375−388.
(36) Jacob, C.; Lancaster, J. R.; Giles, G. I. *Biol. Soc. Trans.* **2004**, *32*, 1015−1017.
(37) Nagy, P.; Ashby, M. T. *Chem. Res. Toxicol.* **2005**, *18*, 919−923.
(38) Nagy, P.; Ashby, M. T. *Chem. Res. Toxicol.* **2007**, *20*, 79−87.
(39) Nagy, P.; Lemma, K.; Ashby, M. T. *J. Org. Chem.* **2007**. In press.
(40) Armesto, X. L.; Canle L, M.; Fernandez, M. I.; Garcia, M. V.; Santaballa, J. A. *Tetrahedron* **2000**, *56*, 1103−1109.

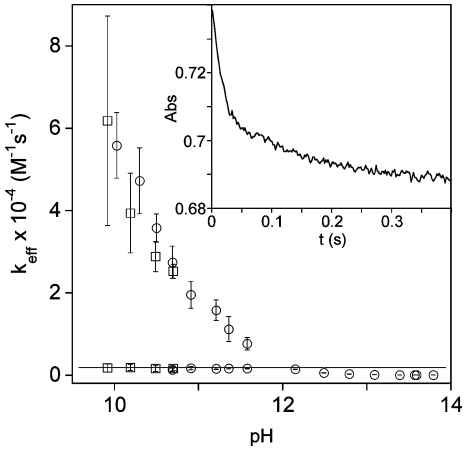


Figure 1. Dependency on pH of the two reactions that follow the reaction of CySH with HOX; X = Cl (circles) and X = Br (squares). The pH-dependent reaction, which can be observed for the entire pH range of 10−14, is assigned to: CySOH + CySH → CySSCy + H_2O (reaction **2** → **3** of Table 1). The pH-independent reaction, which is only observed below pH 12, is assigned to the reaction: CyS(═O)SCy + CySH → CySSCy + CySOH (reaction **8** → **9** of Table 1). Kinetic traces were collected at λ = 268 nm. Conditions: combination of two sets of data: (1) For pH < 12: $[HOX]_0$ = 500 μM, $[CySH]_0$ = 5.0 mM, pH = 9.92−12.15, [iP] = 0.02 M, I = 0.1 ± 0.02 M (iP), T = 18 °C. (2) For pH > 12: $[HOX]_0$ = 50 μM, $[CySH]_0$ = 500 μM, $[OH^-]$ = 0.05−1.0 M, I = 1.0 M (NaOH + $NaClO_4$), T = 18 °C. The line draws attention to the fact that the data points for the slower reaction that lie below pH 12 are not in line with the pH-independent reaction above pH 12 (cf. Figures 2 and 3). (Inset) Kinetic trace at pH = 11.21, as monitored at λ = 268 nm, that illustrates the faster (pH-dependent) reaction and the slower (pH-independent) reaction.

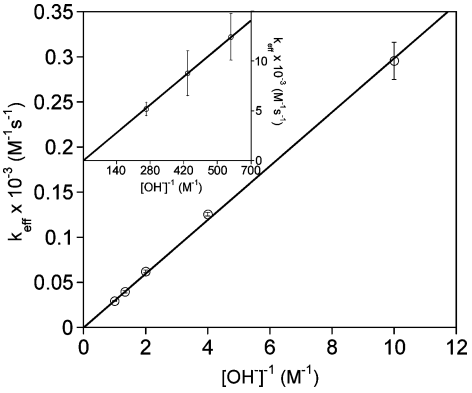


Figure 2. $[OH^-]^{-1}$ dependency of the reaction of CySOH with CySH, where CySOH was generated *in situ* by the oxidation of CySH with HOCl. These data are for pH > 12 and 11.2 < pH < 11.6 (inset). The rate constants exhibit a nonlinear relationship with respect to $[OH^-]^{-1}$ at pH < 11.2. The solid lines, which pass through the origins, represent least-squares fits of the data. Conditions: $[CySH]_0$ = 25.0 mM, $[HOCl]_0$ = 2.5 mM, $[OH^-]$ = 0.1−1.0 M, I = 1.2 M (NaOH + $NaClO_4$), λ = 300 nm, T = 18 °C. (Inset) $[CySH]_0$ = 5.0 mM, $[HOCl]_0$ = 500 μM, pH = 11.2−11.6, iP = 0.02 M, I = 0.1 ± 0.02 M (iP), T = 18 °C, λ = 268 nm.

thiosulfinate ester (CyS(═O)SCy) with CySH. The origin of the minor amounts of CyS(═O)SCy that are sometimes observed will be addressed in the Discussion section.

Quantification of [OH⁻] That Is Released After the Reaction of CySH with HOCl. The reactions that follow the oxidation of CySH by HOCl were followed in the presence of a pH indicator to quantify the amount of OH^- that is released during these reactions. Tropeolin O (4-[(2,4-dihydroxyphenyl)-azo]benzenesulfonate), which has a pK_a of 11.8 and is colorimetric in the pH range of 11.0 < pH <12.7, proved to be a suitable indicator. Control experiments were carried out to show

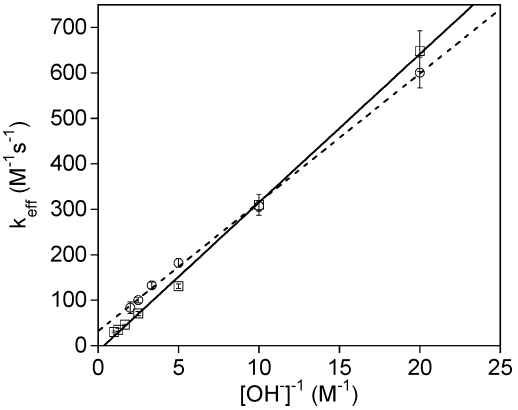


Figure 3. $[OH^-]^{-1}$ dependency of the reaction of CySOH with CySH, where CySOH was generated *in situ* by the reaction of CyS(═O)SCy with CySH (circles) or the reaction of HOX (X = Cl or Br) with CySH (squares). Kinetic traces were collected at λ = 268 nm. The dashed line (slope = 28.3(5) s^{-1} and int = 32(5) M^{-1} s^{-1}) and solid line (slope = 32.6(7) s^{-1} and int = −12(6) M^{-1} s^{-1}) represent least-squares fits of the data for the circle and square data, respectively. Conditions: circles: $[CyS(═O)SCy]_0$ = 125 μM, $[CySH]_0$ = 2.5 mM, $[OH^-]$ = 0.05−0.5 M, I = 1.0 M ($NaClO_4$ + NaOH), T = 18 °C; squares: $[HOCl]_0$ = 50 μM, $[CySH]_0$ = 0.5 mM, $[OH^-]$ = 0.05−1 M, I = 1.0 M ($NaClO_4$ + NaOH), T = 18 °C.

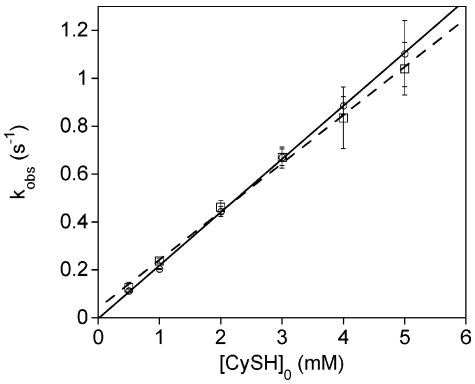


Figure 4. Relationship between $[CySH]_0$ and the kinetics of the pH-dependent reaction that follows the oxidation of CySH by HOX for X = Cl (circles) and X = Br (squares). The lines represent least-squares fits for X = Cl (solid line) and X = Br (dashed line). Conditions: $[CySH]_0$ = 0.5−5 mM, $[HOX]_0$ = 50 μM, pH = 13, I = 1.0 M (NaOH + $NaClO_4$), T = 18 °C, λ = 268 nm.

that the rate of the reaction of HOCl with Tropeolin O is negligible compared to the rate of HOCl with CySH under the conditions of our experiments (see Experimental Section). CySOH was generated *in situ* in a single-mixing stopped-flow experiment by the reaction of 10 mM CySH with 0.5 or 1 mM HOCl at pH 11.3 (in the absence of a pH buffer) within the mixing time of the instrument, and its subsequent reactions with the remaining excess of CySH were monitored. The pseudo-first-order rate constants that were determined from the absorbance changes at 500 nm (the absorbance maximum of the deprotonated Tropeoline O) were identical to the rate constants that were determined in control experiments at 268 nm in the absence of the indicator. A ΔAbs vs $[OH^-]$ calibration curve was established by titration of the indicator with a standardized NaOH solution in the presence of CySH inside the observation cell of the stopped-flow apparatus (data not shown). Double-exponential kinetic traces were observed (at 500 nm) when 1 mM HOCl was reacted with 10 mM CySH to generate CySOH (Figure 5). The rate constants of the two processes correspond to the slower reaction of CySH with CyS(═O)SCy (the reaction

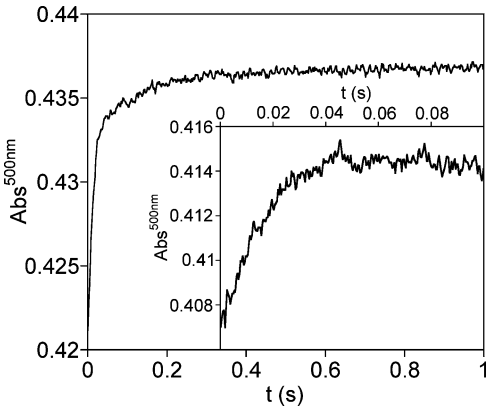


Figure 5. Single-mixing stopped-flow experiment for the quantification of [OH^-] that is released upon the reaction of CySOH and CySH using the acid−base indicator Tropeolin O. Kinetic traces were recorded at $\lambda = 500$ nm. Conditions: $[CySH]_0 = 10$ mM, $[HOCl]_0 = 1$ mM or 0.5 mM for the inset, [Tropeolin O] = 60 μM, pH = 11.3, [buffer] = 0 M, $T = 18$ °C.

that is essentially pH independent above pH 10) and the faster reaction of CySH with CySOH (the pH-dependent reaction), *vide supra*. In contrast, using 0.5 mM HOCl and the same amount of CySH (10 mM) resulted in a single-exponential kinetic trace with a rate constant that was identical to the one measured for the pH-dependent reaction under these conditions (Figure 5, inset). The absorbance change in this later case corresponds to the release of 1 mM OH^-, which is two molar equivalents with respect to $[HOCl]_0$. The net absorbance change of the pH-dependent and pH-independent reactions in the former case correspond to the release of two molar equivalents of OH^-, based on the $[HOCl]_0$. The contribution of the slower pathway (pH-independent reaction) to the net absorbance change in this case was less than 15%, indicating that it is only a minor reaction.

Discussion

Products of the Reaction of CySH with HOX (X = Cl or Br). We have shown that the reaction of a 1:1 molar ratio of CySH with HOCl at pH 11.3 results in a 3.3:1:1.1 mixture of CySSCy, $CySO_2H$, and $CySO_3H$ when turbulent mixing conditions are employed. Our observations contrast with those of Armesto et al., who suggested that the sulfenyl chloride (CySCl) exhibits transient stability.[40] The idea that CySCl exhibits some stability in aqueous solution has been propagated in the subsequent literature.[41−46] The principal evidence for the existence of CySCl was the observation by Armesto et al. of the release of Cl^- over a period of 20 min when 1 mM of CySH was treated with 1 mM HOCl between pH 7 and 12, as determined by a Cl^--selective electrode.[40] We have previously shown that CySSCy reacts with HOCl to give *N*,*N*′-dichlorocystine (NDC).[37] When a 1:1 molar ratio of CySSCy is reacted with HOCl, a 1:1 molar ratio of CySSCy to NDC is observed as the products.[37] Thus, when a 1:1 ratio of CySH and HOCl is reacted under conditions of inefficient mixing, the following reaction sequence is expected:

$$2\ CySH + HOCl \rightarrow CySSCy + H_2O + HCl \quad (1)$$

$$CySSCy + HOCl \rightarrow 1/2\ CySSCy + 1/2\ NDC + H_2O \quad (2)$$

$$2\ CySH + 2\ HOCl \rightarrow 1/2\ CySSCy + 1/2\ NDC + 2\ H_2O + HCl \quad (3)$$

Transient ^1H NMR spectra that are observed at pH 7.5 reveal that the decomposition of NDC occurs via an unsymmetrical species that we have assigned to *N*-chlorocystine (NCC). The transient spectrum of NCC is subsequently observed to disappear with the formation of the spectrum of a mixture of CySSCy and $CySO_3H$ at pH 7.5, and CySSCy and $CySO_2H$ at pH 11.3. Since we have observed that the rate of the decomposition of NDC[37] is on the same time scale as the formation of Cl^- that was reported by Armesto et al.,[40] we conclude that they may not have employed sufficiently efficient mixing methods and that the Cl^- they observed was probably produced by NDG as it decomposed.

Kinetics and Mechanisms of the Oxidation of CySH by Hypohalous Acids. Hypohalites are known to react with nucleophiles via very fast reactions that sometimes approach the diffusion-controlled limit.[28−31,47,48] Such reactions can be thought of as a nucleophilic attack on the electrophilic hypohalous acids (HOX). The conjugate base OX^- is generally 3−5 orders of magnitude less reactive than the corresponding conjugate acids HOX.[29,47] Since $pK_a^{HOCl} = 7.47$ and $pK_a^{HOBr} = 8.59$, OX^- does not generally participate as an oxidant except under the most alkaline conditions.[47] A large number of mechanistic studies of the reactions of HOX have shown that they generally take place with the transfer of X^+ to the nucleophile.[49,50] In an aqueous environment, the halogenated intermediate usually undergoes rapid hydrolysis via reactions that can sometimes be observed, but are oftentimes too fast. One might expect such hydrolysis reactions to be particularly facile under the alkaline conditions of the present study. However, the kinetics of the reaction of CySH with HOCl have been previously investigated by Armesto et al. ,and they have reached the surprising conclusion that cysteine sulfenyl chloride (CySCl) has a significant lifetime.[40] As explained in the previous section, we have repeated some of these measurements, and we agree with their rate constants (Scheme 2, $k_1 = 1.2 \times 10^9$ M^{-1} s^{-1} and $k_2 = 1.9 \times 10^5$ M^{-1} s^{-1}), but we disagree that CySCl exhibits a significant lifetime. On the basis of evidence that will be discussed shortly, we conclude instead that the hydrolysis of CySCl occurs during the mixing of our stopped-flow measurements (Scheme 2, k_3). Thus, the oxidation of CySH with HOX provides a very rapid method of generating CySOH on the stopped-flow time scale.

Possible Elementary Redox Reactions of CySH and its Redox Derivatives. Since no $CySO_2H$ or $CySO_3H$ was observed

(41) Canle L, M.; Maskill, H.; Santaballa, J. A. Experimental methods for investigating kinetics. In *Investigation of Organic Reactions and Their Mechanisms*; Maskill, H., Ed.; Blackwell: Oxford, 2006; pp 46−78.
(42) Pattison, D. I.; Davies, M. J. *Curr. Med. Chem.* **2006**, *13*, 3271−3290.
(43) Ison, A.; Odeh, I. N.; Margerum, D. W. *Inorg. Chem.* **2006**, *45*, 8768−8775.
(44) Harwood, D. T.; Kettle, A. J.; Winterbourn, C. C. *Biochem. J.* **2006**, *399*, 161−168.
(45) Pattison, D. I.; Davies, M. J. *Biochemistry* **2006**, *45*, 8152−8162.
(46) Davies, M. J.; Hawkins, C. L. *Free Radical Res.* **2000**, *33*, 719−729.
(47) Nagy, P.; Beal, J. L.; Ashby, M. T. *Chem. Res. Toxicol.* **2006**, *19*, 587−593.
(48) Ashby, M. T.; Carlson, A. C.; Scott, M. J. *J. Am. Chem. Soc.* **2004**, *126*, 15976−15977.
(49) Jia, Z.; Margerum, D. W.; Francisco, J. S. *Inorg. Chem.* **2000**, *39*, 2614−2620.
(50) Kumar, K.; Day, R. A.; Margerum, D. W. *Inorg. Chem.* **1986**, *25*, 4344−4350.

Scheme 2. Proposed Mechanism, Equilibrium, and Rate Constants (at 18 °C) for the Reaction of CySH and HOCl at 10 < pH < 14

$$HOCl \rightleftharpoons H^+ + OCl^- \qquad pK_a^{HOCl} = 7.5$$

$$CySH^0 \rightleftharpoons CyS^- + H^+ \qquad pK_{a2s}^{CySH} = 8.5$$

$$CySH^- \rightleftharpoons CyS^{2-} + H^+ \qquad pK_{a3s}^{CySH} = 10.0$$

$$CySOH^- \rightleftharpoons CySO^{2-} + H^+ \qquad pK_a^{CySOH} < 10$$

$$CyS^{2-} + HOCl \rightarrow CySCl^- + OH^- \qquad k_1 = 1.2\times10^9\ M^{-1}s^{-1}$$

$$CyS^{2-} + OCl^- + H_2O \rightarrow CySCl^- + 2\ OH^- \qquad k_2 = 1.9\times10^5\ M^{-1}s^{-1}$$

$$CySCl^- + OH^- \rightarrow CySO^{2-} + H^+ + Cl^- \qquad k_3 = \text{fast}$$

2 → 3 $CySOH^- + CyS^- \rightarrow CySSCy^{2-} + H_2O \qquad k_{23} = 10^5\text{-}10^8\ M^{-1}s^{-1}$

2 → 3' $CySOH^- + CyS^{2-} \rightarrow CySSCy^{2-} + OH^- \qquad k_{23'} = 1.840(3)\times10^{15}\ M^{-2}s^{-1}\times K_a^{CySOH}\ M$

4 → 5 $CySOH^- + CySO^{2-} \rightarrow CyS(=O)SCy^{2-} + OH^- \qquad k_{45} = \text{competitive with } k_{23} \text{ for pH<12}$

8 → 9' $CyS(=O)SCy^{2-} + CyS^- \rightarrow CySSCy^{2-} + CySO^{2-} + H^+ \qquad k_{89'} = 3.6(7)\times10^3\ M^{-1}s^{-1}$

8 → 9" $CyS(=O)SCy^{2-} + CyS^{2-} \rightarrow CySSCy^{2-} + CySO^{2-} \qquad k_{89''} = 4.6(3)\times10^3\ M^{-1}s^{-1}$

as reaction intermediates or products under the reaction conditions that were employed in this study, we will focus our attention here on the remaining five derivatives of CySH that are given in Scheme 1 (those within the box). Furthermore, since termolecular reactions are improbable, we only consider here the pairwise reactions of these five compounds. We can compute the number of pairwise combinations of $n = 5$ objects, where the order is unimportant and allow each object to pair with itself, using the formula:

$$\frac{n!}{2 \times (n-2)!} + n = \frac{5!}{2 \times 3!} + 5 = 15 \qquad (4)$$

In considering the redox products of these elementary reactions, the number of redox-active sulfur centers (#S, which is related to the mass balance) and the sum of the oxidation states ($\sum$ON, which is related to the total redox balance) must be conserved. Some of the resulting elementary reactions are expected to be degenerate (the reactants are indistinguishable from the products), others will be redundant (the products of one reaction are the reactants of another reaction, and *vice versa*), and many will not be kinetically competent with respect to alternative reaction pathways. Furthermore, more than one reaction pathway may be possible for each bimolecular reaction. In addition to the 15 pairwise combinations, we must also consider CySSCy, CyS(=O)SCy, and CyS(=O)$_2$SCy (cysteine thiosulfonate ester) as possible reactants and products (i.e., hydrolysis, where the reaction partner is non-redox-active water). Thus, Table 1 summarizes the 18 possible elementary reactions for the five CySH derivatives of Scheme 1 that are under consideration. The tie-lines that are illustrated in Table 1 represent the only possible nondegenerate redox reactions (e.g., the abbreviation **2 → 3** refers to the nondegenerate reaction of CySH and CySOH that can only yield CySSCy). Importantly, when we refer to these reactions by number (e.g., **2 → 3**, **4 → 5**, **8 → 9**, **10 → 11**, and **15 → 16**) throughout the remainder of this discussion, we are not referencing any particular acid/base state of the reactants and products (as many of the reactions are expected to exhibit multiple proton states that may affect the kinetics of a given reaction). The proton states will be specifically referenced when the mechanisms are discussed later.

Relevant Elementary Redox Reactions. This study is focused on reaction **2 → 3** and the subsequent reaction **8 → 9** (*vide infra*). However, it is important to address the relevance of the other possible elementary reactions of redox derivatives of CySH. As mentioned before, $CySO_2H$ and $CySO_3H$ were not observed as products under the conditions of our experiments. Furthermore, since $CySO_2H$ and $CySO_3H$ were found to be relatively inert toward the five CySH derivatives that are considered herein (data not shown), they have not been included as possible intermediates in the reactions that are the subject of this study. We have shown in a previous study[51] that both the hydrolysis of CyS(=O)$_2$SCy and its reaction with CySH result in the formation of $CySO_2H$ (and that CyS(=O)$_2$SCy does not react with CySSCy). Thus, the possible elementary reactions **10 → 11** and **16 → 15** can be eliminated from our models. In the same study, we have reported the detailed kinetics of reaction **5 → 4**.[51] Reaction **5 → 4** is kinetically incompetent, since it is apparent that reaction **8 → 9** is at least 100 times faster than reaction **5 → 4** under all the experimental conditions used. With respect to reaction **9 → 8**, the effect of [CySSCy] on the reactions of CySH with CyS(=O)SCy and CySOH was studied by double-mixing stopped-flow spectroscopy (data not shown). The rates of these reactions were found to be independent of [CySSCy], even at relatively high CySSCy concentrations,

(51) Nagy, P.; Ashby, M. T. *Chem. Res. Toxicol.* **2007**, *20*, 1364−1372.

Table 1. Reactants and Products of the Possible Elementary Redox Reactions of CySH, CySOH, CySSCy, CyS(=O)SCy, and $CyS(=O)_2SCy$

		#S	ΣON
1	2 CySH	2	-4
2	CySH + CySOH	2	-2
3	CySSCy	2	-2
4	2 CySOH	2	0
5	CyS(=O)SCy	2	0
6	$CyS(=O)_2SCy$	2	+2
7	CySH + CySSCy	3	-4
8	CySH + CyS(=O)SCy	3	-2
9	CySOH + CySSCy	3	-2
10	CySH +$CyS(=O)_2SCy$	3	0
11	CySOH + CyS(=O)SCy	3	0
12	CySOH + $CyS(=O)_2SCy$	3	+2
13	2 CySSCy	4	-4
14	CySSCy + CyS(=O)SCy	4	-2
15	$CyS(=O)_2SCy$ + CySSCy	4	0
16	2 CyS(=O)SCy	4	0
17	$CyS(=O)_2SCy$ + CyS(=O)SCy	4	+2
18	2 $CyS(=O)_2SCy$	4	+4

which suggests that reaction **9** → **8** is not relevant to this study. In connection with our recent study of the reactions of CyS(=O)SCy, we investigated the possible relevance of reaction **4** → **5**. We concluded that reaction **4** → **5** is not competitive with the reaction of CySH and CySOH (**2** → **3**) at pH 13 and reaction **4** → **5** is only a minor reaction pathway at pH 14. However, we believe that this is the reaction that produces the intermediate CyS(=O)SCy that was observed at pH < 12 (*vide infra*). A detailed discussion of the mechanism of the reaction of CySOH with itself (i.e., condensation and/or disproportionation) is outside the scope of the present study.

Kinetics and Mechanisms of the Reaction of CySH and CySOH. The kinetics data we have obtained for the reactions that follow the oxidation of CySH by HOX (X = Cl or Br) (Figure 1) and our mechanistic interpretation of those data (Scheme 2) are self-consistent with our previous studies of the mechanisms of the reactions of CyS(=O)SCy with OH^- and CySH (albeit without the inclusion of the k_{23} term of Scheme 2, which is irrelevant above pH 12).[39,51] Following the oxidation of CySH by HOX, two slower reactions are observed (as explained in the Results section). The reaction that is essentially pH independent, which is observed between pH 10 and 12 (Figure 1), yields a rate constant that is approximately a factor of 3 times smaller than the value of $k_{89'}$ (Scheme 2) that we previously measured for the reaction of CyS(=O)SCy and CySH in this pH regime.[39] We note that, while the ionic strength was set to be 1 M in all of the experiments that have been discussed thus far, due to experimental limitations (see Experimental Section), the ionic strength was 0.1 ± 0.02 M for the experiments that were carried out to obtain the data of Figure 1 for pH < 12. We attribute the 3-fold difference in the rate constants of the pH-independent reaction to the difference in the ionic strength. The fact that the pH-independent reaction could be observed requires the formation of CyS(=O)SCy as an intermediate. While we have concluded that the condensation of two CySOH (reaction **4** → **5**) is relatively slow at pH > 12, we believe that this is the reaction that produces the transient CyS(=O)SCy at pH < 12. The following observations are consistent with this hypothesis: (1) When the $[CySH]_0$ dependency of reaction **2** → **3** was studied at pH = 10.7, the absorbance change that is associated with the pH-independent reaction (**8** → **9**) decreased with $[CySH]_0$. At $[CySH]_0$:$[CySOH]_0$ ≥ 16:1, the pH-independent reaction (**8** → **9**) could not be detected. (2) The same phenomenon was observed when the reaction was followed at pH = 11.3 and λ = 500 nm, using Tropeolin O as a pH indicator to monitor the amount of OH^- that was released during the course of the reactions. At $[CySH]_0$:$[CySOH]_0$ = 10:1, reactions **8** → **9** and **2** → **3** were both observed, but at $[CySH]_0$:$[CySOH]_0$ = 20:1, only the pH-dependent reaction (**2** → **3**) was observed. Hence, the condensation reaction (**4** → **5**) is competitive with the reaction of CySH and CySOH (reaction **2** → **3**) at pH < 12 under the conditions of Figure 1. Quantification of the contribution of the pH-independent (**8** → **9**) pathway by following the release of OH^- using a pH indicator revealed that it is only a minor reaction (<15%). This reaction was not observed (or only observed as a minor reaction, *vide infra*) above pH 12, which is an observation that is consistent with the reaction mechanism of Scheme 2. The pH-dependent (**2** → **3**) reaction that is observed above pH 12 (Figures 1−3) corresponds to the pH-dependent (**2** → **3**) reaction that occurs above pH 12 when an authentic sample of CyS(=O)SCy is reacted with CySH (the rate constants for the two reactions as a function of $1/[OH^-]$ are compared side-by-side in Figure 3). This latter reaction was attributed to reaction **2** → **3**, the comproportionation of CySH with CySOH, in our previous study of the reaction of CySH with CyS(=O)SCy.[39] The rate law for reaction **2** → **3** is given by eq 5 (which is identical to eq 8 in reference 39):[39]

$$\text{rate} = \frac{-\mathrm{d}[\mathrm{CySOH}^-]}{\mathrm{d}t} = k_{23'}[\mathrm{CyS}^{2-}][\mathrm{CySOH}^-] = \frac{k_{23'}}{K_a^{\mathrm{CySOH}}}[\mathrm{H}^+][\mathrm{CySH}]_T[\mathrm{CySOH}]_T \quad (5)$$

On the basis of the fit in Figures 2 and 3 (solid line), the following values were obtained for $k_{23'}/K_a^{\mathrm{CySOH}}$: Figure 2, 1.840(3) × 10^{15} M^{-2} s^{-1}; Figure 2 inset, 1.238(5) × 10^{15} M^{-2} s^{-1}; and Figure 3, 2.01(4) × 10^{15} M^{-2} s^{-1}. The values derived from Figures 2 and 3 are comparable to one another, but $k_{23'}/K_a^{\mathrm{CySOH}}$ obtained from the fit of the data in the inset of Figure 2 is a factor of 1.5 smaller. We explain this difference by the fact that the ionic strength in the latter experiment was significantly different.

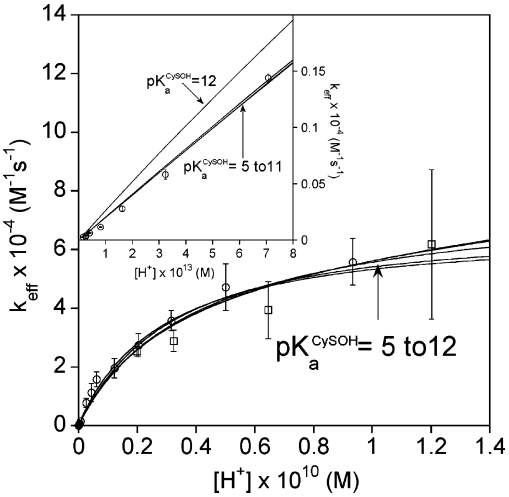


Figure 6. Relationship of $[H^+]$ and k_{eff}, measured for the reaction of CySOH with CySH, at pH < 12. CySOH was generated in situ by the reaction of CySH and HOX, where X = Cl (circles) or Br (squares). The solid line represents the least-squares fit of the data to eq 6. Kinetic traces were recorded at $\lambda = 268$ nm. Conditions: $[CySH] = 5.0$ mM, $[HOCl]_0 = 500$ μM, pH = 9.92−12.15, iP = 0.02 M, $I = 0.1 \pm 0.02$ M (iP), $T = 18$ °C. The lines shown are the fits to eq 6, using various fixed values of $5 < K_a^{CySOH} < 12$.

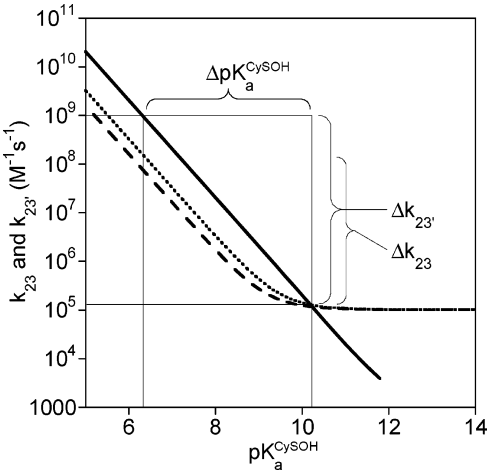


Figure 7. Relationship between the least-squares fit of the k_{eff} data for the reaction of CySH with CySOH using eq 6 as a function of fixed values of K_a^{CySOH}. The computed values of k_{23} using a floating value for $k_{23'}$ (long dashes), and the corresponding values for $k_{23'}$ (solid line) are illustrated. The fitted values of k_{23} (short dashes) when $k_{23'}/K_a^{CySOH}$ was fixed to the value that was determined at high pH ($1.840(3) \times 10^{15}$ M^{-2} s^{-1}) are also shown. The estimated error of $k_{23'}$ exceeded one standard deviation when K_a^{CySOH} was larger than 12 (i.e., only one rate constant was required to fit the data).

The reaction of CySH and CySOH could not be monitored below pH 12 when CySOH was generated by the nucleophilic attack of CySH on CyS(═O)SCy because the production of CySOH became rate limiting.[39] In contrast, CySOH is produced much more rapidly during the reaction of CySH by HOX. Thus, the pH-dependent reaction of Figure 1 that is observed below pH 12 is a continuation of the pH-dependent reaction that is observed above pH 12. However, in contrast to the data above pH 12 that exhibit a linear dependency on $1/[OH^-]$ (or $[H^+]$), the pH-dependent data of Figure 1 that lie below pH 12 exhibit a nonlinear dependency on $[H^+]$ (Figure 6). We explain this observation by the mechanism that is depicted in Scheme 2. Using the nomenclature we previously employed to identify the microscopic protonation states and their corresponding proton dissociation constants,[39] the following rate equation can be derived on the basis of the mechanism of Scheme 2:

$$\text{rate} = \frac{-\text{d[CySOH]}}{\text{d}t} = \frac{K_{a2S}^{CySH}[H^+]k_{23} + K_{a2n}^{CySH}K_{a3s}^{CySH}k_{23'}}{[H^+]^2 + K_{a2n}^{CySH}[H^+] + K_{a2s}^{CySH}[H^+] + K_{a2n}^{CySH}K_{a3s}^{CySH}} \times \frac{[H^+]}{K_a^{CySOH} + [H^+]}[CySH]_T[CySOH]_T \quad (6)$$

This derivation assumes that the thiolate forms of CySH (CyS^- and CyS^{2-}) and the species that is produced upon the addition of one proton to $CySO^{2-}$ (presumably the sulfenic acid $CySOH^-$) are the reactive species. Note that it is apparently not necessary to add two protons to $CySO^{2-}$ to protonate the sulfenyl moiety, because a first-order dependency on $[H^+]$ is observed for the high-pH data of Figure 3 (reaction **2** → **3′**). Note that eq 6 simplifies to eq 5 when pH > 12 (because $[H^+]^2 < K_{a2n}^{CySH}[H^+] < K_{a2s}^{CySH}[H^+] \ll K_{a2n}^{CySH}K_{a3s}^{CySH}$ and $K_a^{CySOH} > [H^+]$). Thus, the deviation from linearity below pH 12 of the plot of the rate constants vs $[H^+]$ (Figure 6) can be explained by the protonation of $CySO^{2-}$ (which should eventually result in a plateau as the reactive $CySOH^-$ becomes the dominant species). Alternatively, the deviation from linearity can be explained by the protonation of CyS^{2-} (which would result in the formation of the less reactive CyS^- and $CySH^-$ with the depletion of CyS^{2-}). Figure 7 was produced by fixing K_{a2n}^{CySH}, K_{a2s}^{CySH}, and K_{a3s}^{CySH} to their known values; by fixing K_a^{CySOH} to incremental values between pH 5 and 14; and by computing the values of k_{23} and $k_{23'}$ using eq 6 and nonlinear least-squares methods. The estimated errors for k_{23} and $k_{23'}$ were approximately 10% for all fixed values of K_a^{CySOH} between pH 5−12. The model of eq 6 fails when K_a^{CySOH} is fixed to a value greater than 12, as indicated by the estimated error for $k_{23'}$ that exceeds its value, which indicates that only a single rate constant is required to fit the data when $K_a^{CySOH} > 12$ and the $K_{a2n}^{CySH}K_{a3s}^{CySH}k_{23'}$ term of eq 6 becomes insignificant. The rate data we have collected at high pH, those data that conform to eq 5, provide a definitive value of $k_{23'}/K_a^{CySOH} = 1.840(3) \times 10^{15}$ M^{-2} s^{-1}. When this value is inserted into eq 6 as a fixed value and the value of k_{23} is computed, the dotted line of Figure 7 is produced. This result demonstrates that it is not possible to compute K_a^{CySOH} from the available data. Nonetheless, assuming that $k_{23} \leq k_{23'}$ and $k_{23'}$ does not exceed the diffusion limit, Figure 7 places restrictions on the possible values of the variables: $10^5 < k_{23} < 10^8$ M^{-1} s^{-1}, $10^5 < k_{23'} < 10^9$ M^{-1} s^{-1}, and $6 < pK_a^{CySOH} < 10$. We note that these results are consistent with our previous study of the oxidation of CySH with H_2O_2.[18]

To further validate our interpretation of the rate data of Figure 6 and the boundary values of pK_a^{CySOH}, we have measured the number of molar equivalents of OH^- that are released during the reaction we attribute to **2** → **3**. Specifically, we wished to distinguish between the alternative mechanisms depicted in Scheme 3 (where ↑ represents an increase in molar equivalents of OH^- and ↔ represents no change in the $[OH^-]$). When an excess of CySH (10 mM) was reacted with HOCl (500 μM) at pH 11.3 (above the known value of K_{a3s}^{CySH}) in the presence of

Scheme 3

Mechanism 1: pH

$RS^- + OCl^- + H_2O \longrightarrow RSCl + 2\ OH^-$ 2↑ fast

$RSCl + RS^- \longrightarrow RSSR + Cl^-$ ↔ rds

Mechanism 2: (pK_a^{CySOH} << 11.3)

$RS^- + OCl^- \longrightarrow RSO^- + Cl^-$ ↔ fast

$RSO^- + RS^- + H_2O \longrightarrow RSSR + 2\ OH^-$ 2↑ rds

Mechanism 3: (pK_a^{CySOH} >> 11.3)

$RS^- + OCl^- + H_2O \longrightarrow RSOH + Cl^- + OH^-$ ↑ fast

$RSOH + RS^- \longrightarrow RSSR + OH^-$ ↑ rds

Net Reaction:

$2\ RS^- + OCl^- + H_2O \longrightarrow RSSR + Cl^- + 2\ OH^-$ 2↑

a pH indicator (Tropeolin O), two molar equivalents of OH^- were released during the rate-determining step (rds), which was indicated by the increase in the absorbance at 500 nm (the absorption maximum of the deprotonated form of Tropeolin O) throughout the reaction. This observation is inconsistent with Mechanisms 1 and 3, but it is consistent with Mechanism 2 of Scheme 3. This experiment further demonstrates that CySOH is the reacting species, not CySX (X = Cl or Br), and that $pK_a^{CySOH} \ll 11.3$ (which is consistent with the analysis of Figure 7, that suggests that $pK_a^{CySOH} < 10$). As a final comment regarding the value of pK_a^{CySOH}, we note that the change in absorption that is observed in the stopped-flow experiments that produced the data of Figure 6 decreases with increasing $[H^+]$ (as indicated by the larger error bars that are associated with the low pH data of Figure 6). This suggests that the conjugate base we are monitoring during these reactions exhibits a smaller molar absorptivity than the corresponding conjugate acid. Since we are presumably monitoring $CySO^{2-}$ in these experiments, the decreased change in absorption indicates we are near pK_a^{CySOH} at the lower pH limit of our measurements. Since we have previously observed that the rate constants for the reactions of CyS^- and CyS^{2-} toward $CyS(=O)SCy^{2-}$ are essentially the same[39] and the graph of Figure 7 produces a value of $pK_a^{CySOH} = 10.3$ when $k_{23} = k_{23'}$, it is likely that pK_a^{CySOH} is near this value. The only way of knowing for certain is to observe a plateau below pH 10. Although the reaction between CySH and CySOH presumably occurs within the time frame of the stopped-flow experiment at lower pH (at least above pH 9), the small change in absorption that accompanies the reaction precludes making the measurement.

Other Observations. We occasionally observed (by ^1H NMR) $CySO_2H$ as a minor product in the reaction of HOX (X = Cl or Br) and CySH when pH > 12. $CySO_2H$ could be the result of several different reactions, such as overoxidation of CySH by HOX, disproportionation of CySZ (Z = Cl, Br or OH), reaction of CyS(=O)SCy with CySZ (Z = Cl, Br or OH), or the formation of $CyS(=O)_2SCy$ as an intermediate followed by its subsequent reaction with CySH or its hydrolysis. We have carried out several experiments to determine the origin of the $CySO_2H$: (1) The CySSCy:$CySO_2H$ ratio decreases with pH. At pH = 11.3, the only observable product (by ^1H NMR) is CySSCy (even at 1:2 = HOCl:CySH concentrations, $CySO_2H$ is only a minor product when the pH = 11.3). (2) The CySSCy:$CySO_2H$ ratio increases (by ^1H NMR) with $[CySH]_0$. (3) The calculated yield of CySOH (based on the ΔAbs in the reaction as determined by stopped-flow UV experiments) decreases with $[HOX]_0$ at constant $[CySH]_0$ and is significantly different for X = Br and Cl. (4) Significantly more $CySO_2H$ (by ^1H NMR) was generated when HOBr was used (as compared when HOCl was used) as the primary oxidant. We note once more that HOBr reacts with CySH more rapidly than HOCl. These observations suggest the production of $CySO_2H$ (which is once more only a minor product in the reaction of CySH with HOX) is a consequence of overoxidation of CySH by HOX (X = Cl or Br).

Sometimes the pH-independent reaction of $CyS(=O)SCy$ with CySH was also observed as a minor reaction above pH 12. The maximum amount of the $CyS(=O)SCy$ intermediate never exceeded 10% in the reaction of CySH with HOX (based on the ΔAbs; for calibration curves see related paper).[39] $CyS(=O)SCy$ could be formed in several different reactions that include the condensation of CySZ (Z = Cl, Br, or OH), the reaction of CySZ with CySSCy, the reaction of $CyS(=O)_2SCy$ with CySSCy, or the reaction of $CyS(=O)_2SCy$ with CySH. The following observations were made: (1) The reaction of $CyS(=O)_2SCy$ with CySH results in the formation of $CySO_2H$ and CySSCy, not CySOH or $CyS(=O)SCy$ (at low pH). (2) $CyS(=O)_2SCy$ does not react with CySSCy on the time scale of our experiments. (3) The amount of $CyS(=O)SCy$ as an intermediate increases with [HOX] (X = Cl or Br) at constant $[CySH]_0$. (4) The amount of $CyS(=O)SCy$ as an intermediate decreases with pH. At pH 14 the reaction of $CyS(=O)SCy$ with CySH could not be observed. (5) When HOBr was used as the oxidant, significantly more $CyS(=O)SCy$ was formed as an intermediate than when HOCl was the reaction partner of CySH. (6) CySZ (X = Cl, Br, or OH) does not react with CySSCy on the time scale of our experiments. These observations suggest that the main pathway that leads to the formation of $CyS(=O)SCy$ (which once more is only a minor intermediate in the reaction of CySH with OX^-) is the condensation of CySX (X = Cl or Br) with CySZ (Z = Cl, Br, or OH).

Related Mechanistic Studies. The results we have obtained for the redox derivatives of CySH contrast with previous studies of aromatic analogues in several ways: (1) The hydrolysis of aryl disulfides to produce the corresponding thiols and sulfenic acids is fast compared to hydrolysis of CySSCy (reaction **3** → **2**).[52,53] (2) Aryl derivatives of sulfenic acids are relatively stable under alkaline conditions as compared to CySOH.[52,54] (3) Aryl sulfenyl halides undergo facile hydrolysis,[55,56] but under some conditions other reactions can be competitive.[56] As we have noted previously,[51] aromatic redox derivatives of thiols exhibit mechanisms that appear to be very different from those of aliphatic derivatives. We suspect conjugative effects of aromatic substituents play an important role in orchestrating these differences.

A Comparison of the Different Methods of Generating CySOH. There are four possible elementary bimolecular reactions of CySOH with the five redox derivatives that we have

(52) Blakeley, R. L.; Riddles, P. W.; Zerner, B. *Phosphorus Sulfur Relat. Elem.* **1980**, *9*, 127−135.
(53) Donovan, J. W.; White, T. M. *Biochemistry* **1971**, *10*, 32−38.
(54) Riddles, P. W.; Blakeley, R. L.; Zerner, B. *Anal. Biochem.* **1979**, *94*, 75−81.
(55) Hogg, D. R.; Stewart, J. *J. Chem. Soc., Perkin Trans. 2* **1974**, 43−47.
(56) Kice, J. J.; Cleveland, J. P. *J. Am. Chem. Soc.* **1970**, *92*, 4757−378.

considered in this contribution (i.e., **2** → **3**, **4** → **5**, **9** → **8**, and **11** → **10** of Table 1). The opposite reactions can potentially offer methods of generating CySOH (i.e., **3** → **2**, **5** → **4**, **8** → **9**, and **10** → **11**). We have shown in a previous study that hydrolysis of CyS(═O)SCy (**5** → **4)** is too slow to permit the study of CySOH[51] and the hydrolysis of CySSCy (**3** → **2**) is even slower. The reaction of CyS(═O)$_2$SCy with CySH (**10** → **11**) remains unexplored. However, we have recently undertaken a detailed mechanistic study of the reaction of CyS(═O)SCy with CySH (**8** → **9**) to generate CySOH.[39] Unfortunately, the latter reaction is only competitive with the subsequent reactions of CySOH above pH 12. As evidenced herein, an alternative approach to generating CySOH *in situ* is the oxidation of CySH by reactions that are capable of delivering an oxygen atom. Sluggish oxidants such as hydrogen peroxide are ineffective for producing CySOH at a sufficient rate for studying the subsequent reactions.[17,18] However, in the present study we demonstrate CySOH can be generated by the facile reaction of CySH with hypohalous acids (HOX, X = Cl or Br).

Conclusions

The oxidation of CySH by HOX (X = Cl, Br) produces CySOH. Above pH 10, this reaction is sufficiently facile so that the subsequent comproportionation of CySOH and CySH to produce CySSCy can be observed. The self-condensation of CySOH to produce CyS(═O)SCy can become competitive for pH < 12. The results we have obtained for both reactions are consistent with our study of the reaction of CyS(═O)SCy with CySH to produce CySSCy and CySOH.[39] However, the method of producing CySOH by oxidation of CySH with HOX has allowed us to extend the lower pH limit for exploring the chemistry of CySOH. This study establishes that pK_a^{CySOH} for the sulfenyl group of CySOH is between 6 and 10, and it places a lower limit on the rate constant for the reaction of CySH and CySOH of 10^5 M^{-1} s^{-1}.

Experimental Section

Reagents. All chemicals were A.C.S. certified grade or better. Water was doubly distilled in glass. Solutions of NaOH, mostly free of CO_2 contamination, were quantified by titration with potassium hydrogen phthalate or standardized HCl or $HClO_4$ solutions using phenolphthalein as an indicator. HCl and $HClO_4$ were standardized against bicarbonate. The buffer solutions were prepared from the solids K_3PO_4, $NaH_2PO_4 \cdot H_2O$, Na_2HPO_4, and $Na_3PO_4 \cdot 12\ H_2O$, the ionic strength was adjusted with $NaClO_4$, and the pH/pD was adjusted with NaOH, NaOD, $HClO_4$, or DCl. L-Cystine, L-cysteine, L-cysteic acid monohydrate, L-cysteinesulfinic acid monohydrate, peracetic acid, deuterium chloride (35 wt % solution in D_2O), NaOD (40 wt % solution in D_2O), $NaClO_4$, and K_3PO_4 were used as received from Sigma-Aldrich. $NaH_2PO_4 \cdot H_2O$, Na_2HPO_4, and $Na_3PO_4 \cdot 12H_2O$ were used as received from Mallinckrodt. Deuterium oxide (99.9%) was obtained from Cambridge Isotope Laboratories. The monosodium salt of 4-[(2,4-dihydroxyphenyl)azo]-benzenesulfonic acid (Tropeolin O) was received from Allied Chemical and Dye Corporation (New York, U.S.A.). Stock solutions of NaOCl were prepared by sparging Cl_2 into a 0.3 M solution of NaOH. The sparging was stopped when the $[OCl^-]$ achieved ca. 100 mM, as determined spectrophotometrically ($\epsilon\ (OCl^-)_{292\ nm} = 350\ M^{-1}\ cm^{-1}$). Solutions of NaOBr were prepared by adding Br_2 to ice cold solutions of NaOH.[57] Solutions of OBr^- were standardized spectrophotometrically at 329 nm ($\epsilon_{329} = 332\ M^{-1}\ cm^{-1}$).[57] The solutions of OBr^- were used within 2 h of the preparations to minimize errors due to decomposition.

pH/pD Measurements. The $[OH^-]$ for the unbuffered solutions were determined by acid−base titration against standardized HCl or standardized $HClO_4$ solutions. The $[H^+]$ of the buffered solutions was determined with an Orion Ion Analyzer EA920 using an Ag/AgCl combination pH electrode. The ionic strength was kept constant at 1.0 M for most solutions ($NaClO_4$ + NaOH/iP), *vide supra*. To obtain the $[H^+]$ or $[OH^-]$ of the buffered solutions from the measured pH values, all pH measurements were corrected for the "Irving factor"[58] and the ionic product of water (pK_w) that were measured by titration of a 1.0 M $NaClO_4$ solution by a standardized 0.1 M NaOH (in 1.0 M $NaClO_4$) solution. pD measurements in D_2O were made using the same pH electrode by adding 0.4 units to the measurement.[59]

NMR Studies. ^{1}H NMR spectra were recorded with a Varian XL-300 spectrometer at 20 (±0.5) °C. Deuterated buffers were prepared from D_2O solutions of anhydrous K_3PO_4 by adding DCl, by dilution of a 40 wt % NaOD solution with D_2O or by dilution of a 35 wt % DCl solution with D_2O. The chemical shifts (ppm) were referenced to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS, $\delta = 0.015$ ppm). Turbulent mixing of the reagents was necessary to ensure homogeneity of the reaction mixtures in the time frame of the chemical reaction, and this was achieved for the NMR studies by employing a hand mixer comprising two Hamilton syringes and a T-mixer. Failure to quickly mix solutions of HOX and CySH produced different (generally unreproducible) results.

UV/Vis Spectroscopy. Electronic spectra were measured using a HP 8452A diode array spectrophotometer using quartz cells with calibrated 1 mm, 2 mm, and 1 cm path lengths at 20 °C, or the monochromator of the HI-TECH SF-61 DX2 stopped-flow instrument with a Xe arc lamp at 18 °C.

General Description of the Stopped-Flow Studies. Kinetic measurements were made with a HI-TECH SF-61 DX2 stopped-flow spectrophotometer using a Xe arc lamp and a PMT detector. A double-mixing mode was used for some of the experiments. All the stopped-flow measurements were made at a temperature of 18 °C maintained in the observation cell with a Lauda RC-20 circulator. The adiabatic temperature increases that were associated with the pH-jump experiments were determined experimentally to be less than 1 °C.

Reaction of CySH with HOX (X = Cl or Br). The pH and $[CySH]_0$ dependencies of the reaction rates were investigated under pseudo-first-order conditions with at least a 10-fold excess of $[CySH]_0$ over [HOX] in single-mixing stopped-flow experiments. In most cases, the reaction between HOX and CySH was over during the mixing, and only the subsequent reactions were observed. The ionic strength of the reaction mixtures were kept constant at $I = 1.0$ M in most cases (iP/NaOH + $NaClO_4$). The ionic strength was determined by the 0.02 M iP (i.e., 0.1 ± 0.02 M), and no extra $NaClO_4$ was added at pH < 12 when the pH dependency was studied. The reason was to avoid possible contaminations from the $NaClO_4$ (that could be significant due to the low concentrations of the reactants) and to eliminate the salt effect, to be able to follow the reaction at relatively low pHs using the stopped-flow method at $T = 18$ °C.

Quantification of $[OH^-]$ That Is Released in the Subsequent Reaction of CySH with HOCl. The amount of OH^- that is released in the subsequent reaction of CySH and HOCl was measured in a single-mixing stopped-flow experiment at pH = 11.3 in the presence of a pH indicator (Tropeolin O). The indicator was added to the CySH solution. No reaction was observed between CySH and the indicator during the experiment. The reaction of Tropeolin O with HOCl under these conditions was found to be negligibly slow compared to the reaction of CySH with HOCl. The pH of each solution was set using a 0.1 M NaOH solution and a calibrated pH electrode in the absence of a pH buffer.

(57) Troy, R. C.; Margerum, D. W. *Inorg. Chem.* **1991**, *30*, 3538−3543.
(58) Irving, H. M. N. H.; Miles, M. G.; Pettit, L. D. *Anal. Chim. Acta* **1967**, *38*, 475−488.
(59) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188−190.

Kinetic Data Analysis. The monochromatic kinetic traces were fit with HI-TECH KinetAsyst 3.14 software (Hi-Tech, UK). Polychromatic data were analyzed using SPECFIT/32 (Spectrum Software Associates), a multivariate data analysis program. The concentration dependencies of the pseudo-first-order rate constants were obtained by linear least-squares fits of the data with KaleidaGraph 3.6 (Synergy Software).

Acknowledgment. We appreciate the financial support we have received from the National Science Foundation (CHE-0503984), the American Heart Association (0555677Z), the Petroleum Research Fund (42850-AC4), and the National Institutes of Health (1 R21 DE016889-01A2).

JA0737218