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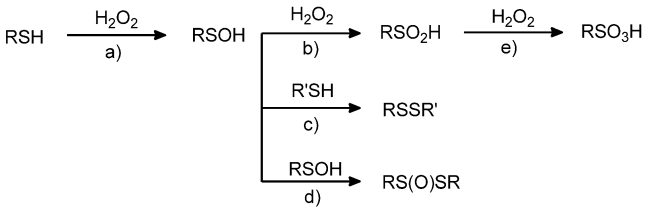
On the Reactions of Thiols, Sulfenic Acids, and Sulfinic Acids with Hydrogen Peroxide

*Jean-Philippe R. Chauvin and Derek A. Pratt**

Abstract: *The reaction of thiols with H_2O_2 is central to many processes essential to life, from protein folding to redox signaling. The initial products are assumed to be sulfenic acids, but their observation, and the kinetic and mechanistic characterization of their subsequent reactions, has proven challenging. The introduction of a 9-fluorotriptycene substituent enabled the use of ^{19}F NMR to directly monitor the reaction of a thiol with H_2O_2 to yield a sulfenic acid, and its subsequent oxidation to sulfinic and sulfonic acids. The oxidations are specific base catalyzed, as revealed by the lack of isotope effects and the dependence of the kinetics on pH but not buffer concentration.*

Thiol oxidation is among the most ubiquitous reactions in biology, playing key roles in protein folding,[1] redox homeostasis,[2] signal transduction,[3] and the regulation of gene expression.[4] Of particular interest are oxidations by H_2O_2, the pervasive "reactive oxygen species" that is believed to reflect the global redox state of the cell.[5] The reaction of cysteine and other thiols with H_2O_2 has been extensively studied.[6] Despite the common assumption that the immediate product is a sulfenic acid (Scheme 1 a), to our knowledge,



Scheme 1. Competing fates of putative sulfenic acid intermediates formed upon the oxidation of thiols with H_2O_2.

there are no examples in which this has been directly demonstrated. Instead, sulfenic acid intermediates are indirectly identified following derivatization.[7] As such, kinetic and mechanistic studies of thiol oxidation have generally been carried out by monitoring the loss of the thiol in the presence of excess H_2O_2—reaction profiles that could not be corroborated by monitoring concomitant formation of the corresponding sulfenic acid.[8] The persistence of sulfenic acids is limited by their oxidation to sulfinic and sulfonic acids (Scheme 1 b,e), reaction with thiols to yield disulfides (Scheme 1 c), and self-condensation to yield thiosulfinates (Scheme 1 d).[9]

Sulfenic acids can be rendered persistent with bulky substituents that prevent access for nucleophiles to its low-lying $\sigma_{S\text{-}O}^*$ orbital.[10] We recently used Nakamura's 9-triptycenesulfenic acid[10a,d] to characterize the radical chemistry of the sulfenic acid moiety,[11] as well as that of the corresponding selenenic acid.[12] Emboldened by this success, we wondered whether the triptycene backbone would enable direct monitoring of the oxidation of a thiol to a sulfenic acid, and subsequent oxidations to the sulfinic and sulfonic acids, thereby permitting rigorous kinetic and mechanistic characterization of each of these reactions.

Early efforts to monitor the oxidation of 9-triptycenethiol to 9-triptycenesulfenic, sulfinic, and sulfonic acids were complicated by difficulties with their reproducible separation and quantitation. We surmised that the introduction of a fluorine atom at the other bridgehead position would enable direct monitoring of the reaction since it was likely that the ^{19}F chemical shifts would depend on the oxidation state of the transannular sulfur atom. Indeed, when the fluorinated 9-triptycenethiol (**1**) was treated with H_2O_2 in buffered methanol, four signals were clearly visible in the ^{19}F NMR spectra (Figure 1). Moreover, these signals evolved in a predictable way, with the thiol giving way to two successively formed intermediates (the presumed sulfenic and sulfinic acids) that eventually gave way to a single product (the presumed sulfonic acid).

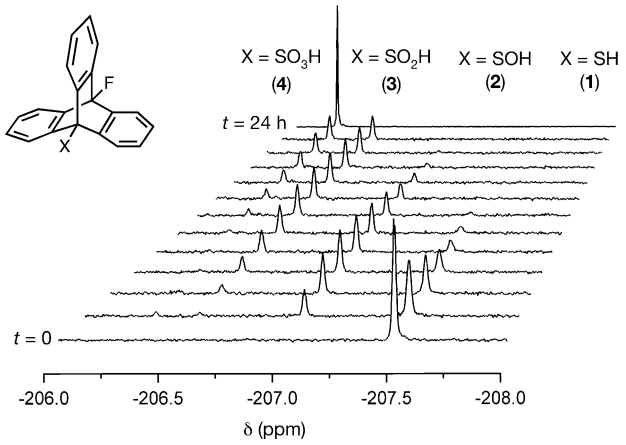


Figure 1. Representative ^{19}F NMR spectra recorded during the reaction of 9-fluoro-10-triptycenethiol (5 mM) with H_2O_2 (50 mM) in buffered methanol at $^{s}_{s}$pH 12.1 (20 mM 2,2,6,6-tetramethylpiperidine) at 20 °C.

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A representative reaction profile obtained over 1 h at 20°C is shown in Figure 2. This data could be fit to a simple kinetic model for successive bimolecular reactions with H_2O_2, from which rate constants of 0.033, 0.013 and 0.00050 $M^{-1}s^{-1}$ were derived for the reactions of thiol, sulfenic acid, and sulfinic acid, respectively. These results suggest that the thiol is most easily oxidized, followed by sulfenic acid and sulfinic acid.

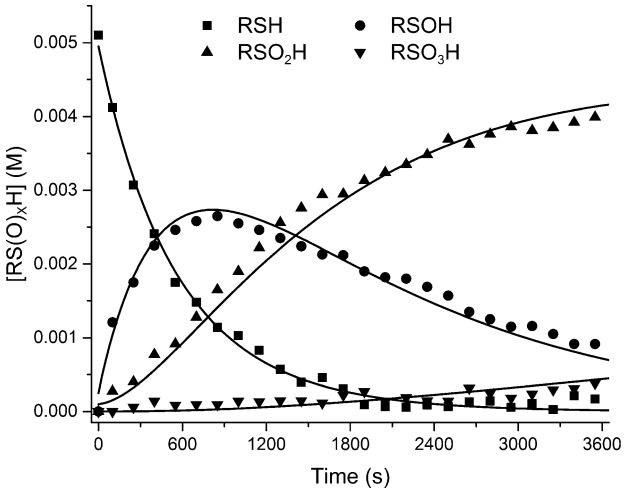
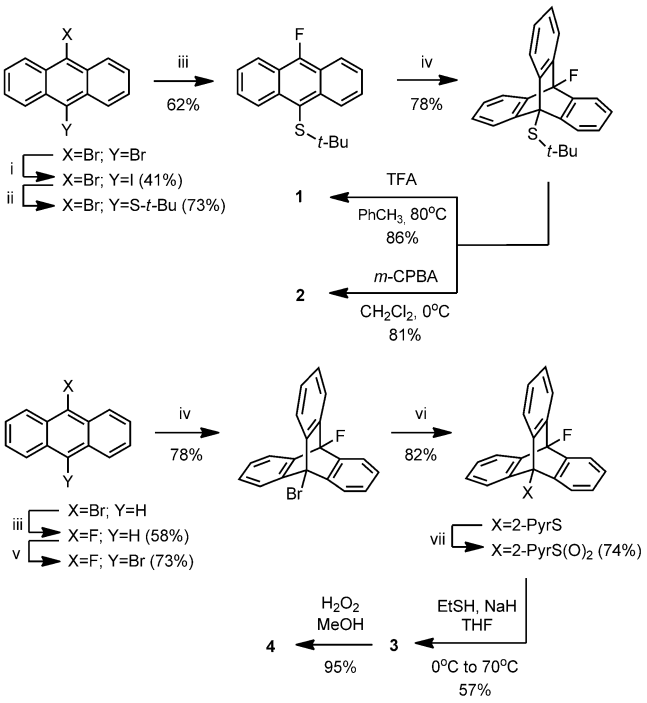


Figure 2. Representative reaction profile for the oxidation of 9-fluoro-10-triptycenethiol (5 mM) by H_2O_2 (50 mM) in buffered methanol at ^s_spH 11.5 (20 mM 2,2,6,6-tetramethylpiperidine) at 20°C. Concentrations were determined directly by ^{19}F NMR relative to an internal standard ($PhCF_3$). Solid lines are from numerical fitting of the data to a simple kinetic scheme for consecutive bimolecular reactions of the thiol, sulfenic acid, and sulfinic acid with H_2O_2.



Scheme 2. Synthesis of fluorinated 9-triptycenethiol (**1**), sulfenic acid (**2**), sulfinic acid (**3**), and sulfonic acid (**4**). i) BuLi, THF, −78°C, then I_2. ii) *t*BuSH, CuI, *t*BuOK, 1,10-phen, 110°C, $PhCH_3$. iii) BuLi, THF, −78°C, then NFSI. iv) Anthralinic acid, isoamylnitrite, TCA, THF. v) NBS, DMF, 0°C. vi) BuLi, PhH/MTBE, −20°C, then $(2\text{-PyrS})_2$. vii) $RuCl_3$, $NaIO_4$, H_2O/EtOAc. THF = tetrahydrofuran, phen = 1,10-phenanthroline, TCA = trichloroacetic acid, NBS = *N*-bromosuccinimide, DMF = *N*,*N*-dimethylformamide, TFA = trifluoroacetic acid, *m*CPBA = *m*-chloroperbenzoic acid, Pyr = pyridine, NFSI = *N*-fluorobenzenesulfonimide, MTBE = methyl *tert*-butyl ether.

Given these results, we independently synthesized the fluorinated 9-triptycenesulfenic (**2**), sulfinic (**3**),[13] and sulfonic (**4**) acids (Scheme 2) and confirmed that they correspond to the two intermediates and final product, respectively, observed in the oxidations (see Figure 1).

With the authentic reaction intermediates in hand, we also determined the rate constants for each reaction step independently (Figure 3), obtaining essentially indistinguishable results from those obtained by fitting the data in Figure 2, that is, $k_{obs} = 0.029$, 0.014, and 0.00058 $M^{-1}s^{-1}$ for the oxidation of the thiol, sulfenic acid, and sulfinic acid, respectively.

The role of a thiol/thiolate pre-equilibrium in the oxidation of thiols by H_2O_2 has been demonstrated.[6h] We sought to both confirm that this was also the case for the current system, and also investigate its role in the reactions of the sulfenic and sulfinic acids with H_2O_2. Results of reactions carried out in buffered methanol at ^s_spH[14] 6.6 to 13.5 are shown in Figure 4.

The observed rate constants for thiol and sulfenic acid oxidation were dependent on the pH of the medium, reaching a maximum at around ^s_spH ≈ 12 and 13, respectively, which roughly correspond to the pK_a values of the thiol and sulfenic acids (determined independently to be 11.6 and 12.8 under the same conditions, see the Supporting Information).[15] The plateau at ^s_spH values above the pK_a indicate that the inherent reactivity of the sulfenate anion is greater than of the thiolate.

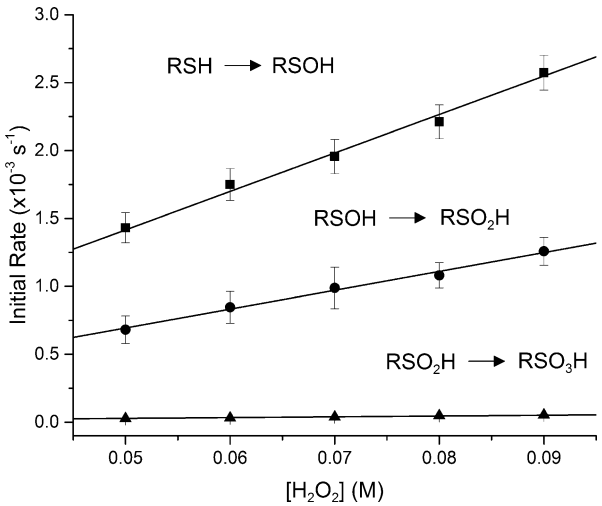


Figure 3. Initial rates of reaction of 9-fluoro-10-triptycenethiol (■), 9-fluoro-10-triptycenesulfenic acid (●), and 9-fluoro-10-triptycenesulfinic acid (▲) as a function of H_2O_2 concentration in buffered methanol at ^s_spH 11.5 (20 mM 2,2,6,6-tetramethylpiperidine) at 20°C. Reaction progress was determined directly by ^{19}F NMR on 5 mM of substrate.

The rate of sulfinic acid oxidation was independent of pH in the range that was studied, which is expected since it exists as

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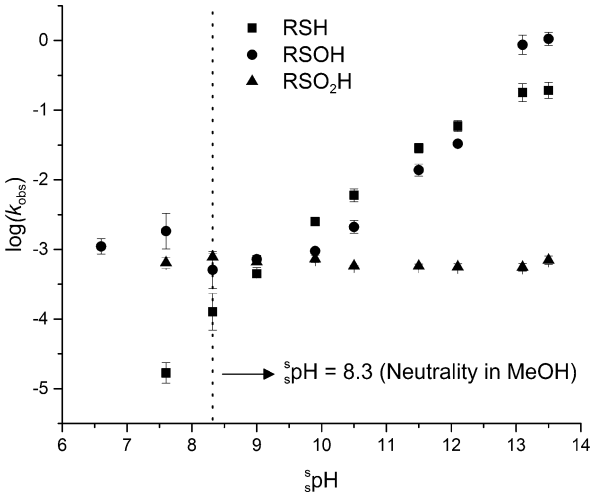
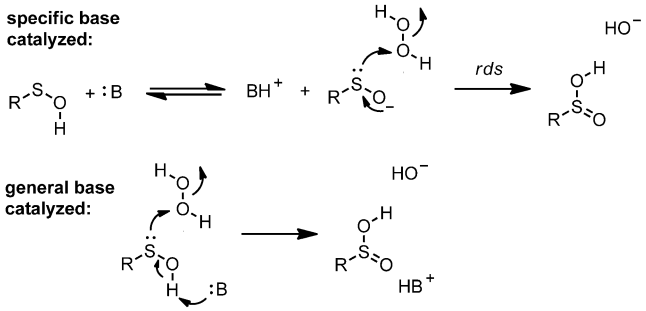


Figure 4. pH-Dependence of the kinetics of oxidation of 9-fluoro-10-triptycenethiol (ν), 9-fluoro-10-triptycenesulfenic, acid (λ), and 9-fluoro-10-triptycenesulfinic acid (π) by H_2O_2 in methanol at 20 °C.

the sulfinate throughout this pH range ($pK_a = 4.3$, see the Supporting Information). Interestingly, the rates of oxidation of the thiol, sulfenic acid, and sulfinic acid converge around neutral pH; in fact, the reactivity increases slightly as the oxidation state of the sulfur increases. At neutral pH, the sulfinic acid is fully ionized and the sulfinate anion is evidently more reactive than the sulfenic acid, which is not ionized at neutral pH. Moreover, it would appear that the sulfenic acid is more reactive than the thiol, which is consistent with the computed reactivities of simple thiols and sulfenic acids with H_2O_2.[16]

These results establish that base catalysis enhances the reactivity of both thiols and sulfenic acids to oxidation. To learn whether the mechanism is specific or general base catalysis (illustrated for the reaction of the sulfenic acid in Scheme 3), we carried out corresponding reactions in methanol-*d* (at $^{s}_{s}pH = 9.9$). These yielded negligible kinetic isotope effects of 1.1 ± 0.1 and 1.2 ± 0.2 for oxidations of the thiol and sulfenic acid, respectively (corresponding experiments with the sulfinic acid yielded $k_H/k_D = 1.1 \pm 0.2$), thus implying that no acidic proton is transferred in the rate-determining step of the reaction. This rules out a general base catalyzed reaction, as well as concerted transfer of the thiol proton to the



Scheme 3. Base catalysis in the oxidation of a sulfenic acid by H_2O_2.

departing hydroxide ion during the substitution—a suggestion that has been made based on computations.[17]

A specific base catalysis mechanism was corroborated by experiments carried out at constant pH and varying buffer concentration, which yielded small changes in rate for reactions of thiol and essentially no change in rate for reactions of either sulfenic or sulfinic acids (see the Supporting Information). The weak dependence of the rate of reaction of thiol on buffer concentration is proposed to arise from H-bonding between the conjugate acid of the buffer and H_2O_2 (which increases its electrophilicity), since switching the buffer from amine to borate yielded rates that were invariant with buffer concentration.

The reactivity of the thiol towards alkyl hydroperoxides was also assessed using this approach (Figure 5). One each of a primary (benzyl), secondary (tetralyl), and tertiary (cumyl) hydroperoxide were examined. The reactions cleanly yielded sulfenic, then sulfinic, and finally, sulfonic acids. The relative rates decreased with increasing substitution, as expected owing to both electronic and steric factors, but the differences were actually quite small (i.e., $k_{obs} = 0.0031$, 0.0019, and $0.0012\,M^{-1}s^{-1}$ for benzyl, tetralyl, and cumyl hydroperoxides, respectively). Associated KIE values and pH/buffer dependence suggest that the mechanism of thiol oxidation by hydroperoxides is also specific base catalyzed (see the Supporting Information).

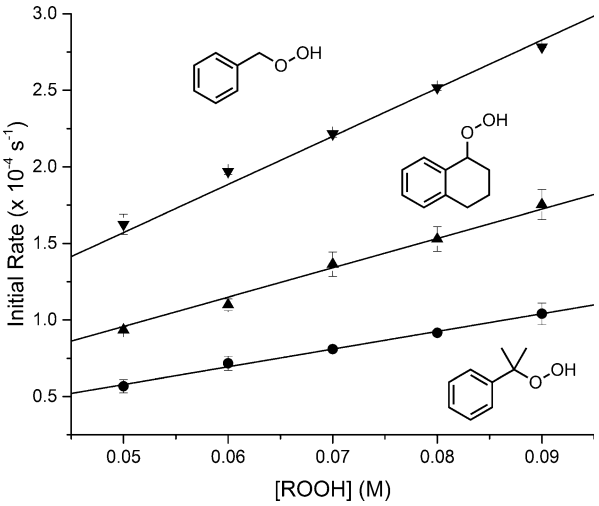


Figure 5. Initial rates of reaction of 9-fluoro-10-triptycenethiol (5 mM) with benzyl hydroperoxide (▼), tetralin hydroperoxide (▲), and cumyl hydroperoxide (●) in buffered methanol of $^{s}_{s}pH$ 9.9 (20 mM *N*-methylpiperidine) at 20 °C. Reaction progress was determined directly by ^{19}F NMR.

It is well known that the reactivity of a protein thiol can be modulated significantly by the immediate environment of the cysteine residue. It has long been recognized that proximal basic residues lead to significant rate enhancements for reactions with H_2O_2.[18] Moreover, work on peroxiredoxins, thiol-containing enzymes responsible for the detoxification (reduction) of H_2O_2 and alkyl hydroperoxides, has clearly shown the role of activating the peroxide to nucleophilic

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attack by the thiol.[19] Our work provides a baseline for comparison of the inherent chemical reactivity of thiol, sulfenic acid, and sulfinic acid toward hydrogen peroxide (and hydroperoxides), which may aid in the interpretation of trends that are observed in peroxiredoxins and other protein thiols, in general. Indeed, it would appear that the thiol microenvironment must be very carefully controlled and nucleophiles carefully juxtaposed to avoid overoxidation of the intermediate sulfenic acid to the sulfinic acid, given that outside the vicinity of pH neutrality, the sulfenic acid and sulfenate anion are more reactive than the thiol and thiolate anion, respectively.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: hydrogen peroxide · oxidation · sulfenic acid · sulfinic acid · thiols

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Communications

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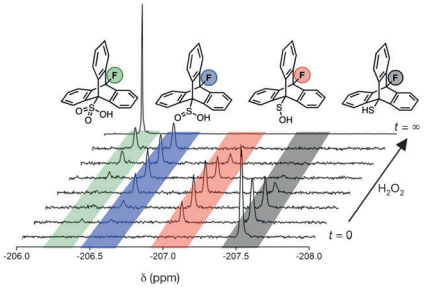

SO, SOO, SOOO: The initial products of the reaction of thiols with H_2O_2 are assumed to be sulfenic acids, but their observation, and the kinetic and mechanistic characterization of their subsequent reactions, has proven challenging. The introduction of a 9-fluorotriptycene substituent enabled the use of ^{19}F NMR to directly monitor the reaction of a thiol with H_2O_2 to yield a sulfenic acid, and its subsequent oxidation to sulfinic and sulfonic acids.

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