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Sulfenic Acids: An Overview

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Abbreviations

BPV-1	bovine papillomavirus type 1
Cys	cysteine
ESI-MS	electrospray interface-mass spectrometry
FAD	flavin-adenine dinucleotide
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
NBD-Cl	7-chloro-4-nitrobenzo-2-oxa-1,3-diazole
NMR	nuclear magnetic resonance
ROS	reactive oxygen species
RSH	sulfhydryl
RSOH	sulfenic acid
RSO_2H	sulfinic acid
RSO_3H	sulfonic acid

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Abstract

Sulfenic acids are most commonly thought of as a transient intermediate product of thiol oxidation. Early studies suggested that sulfenic acids were simply intermediates due to the high reactivity of thiols forming sulfinic acid, sulfonic acid, or disulfide bonds. However, recent studies have provided evidence for functional sulfenic acids following the oxidation of sulfhydryl groups on cysteine residues. In the formation of cysteine-sulfenic acids, unique chemical properties allow sulfenic acids to accumulate and stabilize. This review will discuss the chemistry and detection methods of sulfenic acids. In addition, the biochemistry and importance of protein-sulfenic acids in biology will be addressed.

Introduction

The concept of oxidation-reduction reactions or redox cycles playing a role in the regulation of normal cellular mechanisms has recently become appreciated. Redox signaling plays an important role in various cellular functions including modulation of enzyme activity and activation/deactivation of transcription factors. The regulation of cellular signaling by redox cycles is thought to involve the modification of specific protein targets including those containing free sulfhydryl groups (RSH) of cysteine residues (Cys-SH) [1]. The most common result of RSH oxidation in biology is the formation of a disulfide bonds with other RSH; however, recent studies have identified sulfenic acids (RSOH) as critical intermediates in RSH oxidation. It is postulated that RSOH play a critical role in particular redox-regulated cell-signaling pathways. Two possible tautomeric structures for RSOH have been proposed (Figure 1); however, using microwave spectroscopic analysis it has been shown that the divalent sulfur tautomer R-S-O-H is the most prominent [2]. The reversibility of the RSH ↔ RSOH cycle makes sulfhydryls a potent target in redox-regulated cellular mechanisms [3]. Thus, the goal of this review is to summarize the chemistry and biochemistry of sulfenic acids while addressing the importance of RSOH in redox signaling.

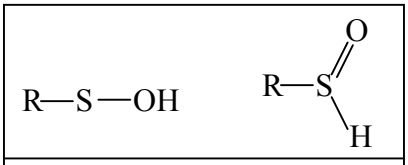


Figure 1. Proposed structures of sulfenic acid; however, spectroscopic analysis shows the structure on the left in the most prominent in biology.

Sulfenic Acid Chemistry

Sulfenic acids, the simplest ogranosulfur oxyacids, are formed by oxidation reactions of RSH groups [2]. In solution, the most common reaction of sulfenic acid is the formation of thiosulfinate (Figure 2), which occurs through the formation of a hydrogen-bonded sulfenic acid dimer that lowers the free energy of activation, thus contributing to the instability of RSOH [4]. This reaction is believed to be catalyzed by the formation of an intermolecular hydrogen bond

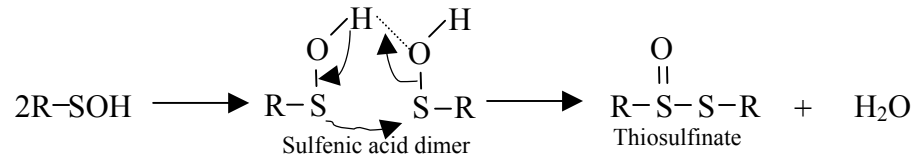
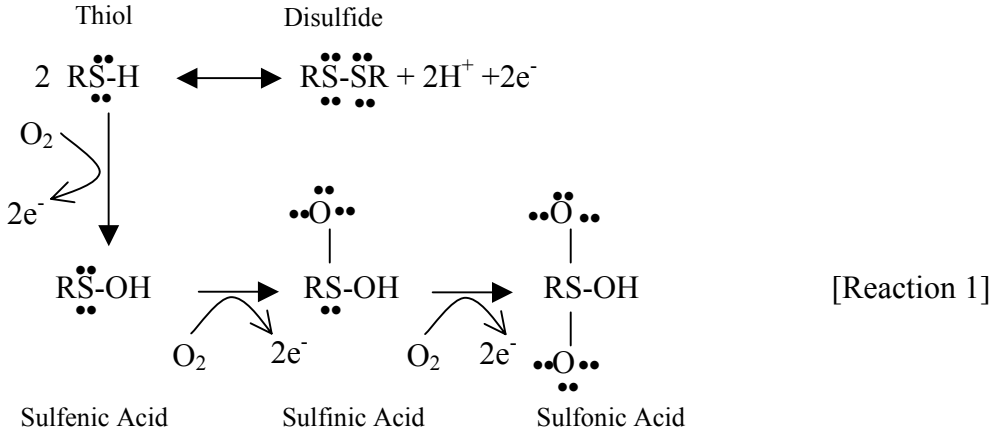


Figure 2. The self-condensation reaction of sulfenic acid yields thiosulfinate. The formation of an intermolecular hydrogen bond between two sulfenic acids leads to the instability of sulfenic acids.

between the hydrogen atom of one sulfenic acid group and the oxygen atom of a second (Figure 2). In biology, sulfenic acids are commonly thought to be transient intermediates in the oxidation of thiols to sulfinic and sulfonic acids and in the formation of disulfides from free thiols (Reaction 1). In this oxidation reaction, the sulfhydryl sulfur, with an oxidative state of –2, loses two electrons with the addition of oxygen; therefore, forming RSOH with an oxidative state



of 0 [4]. Subsequently, RSOH is further oxidized to sulfinic acid (RSO_2H) and sulfonic acid (RSO_3H) as oxygen is added at each step and two electrons are lost leaving RSO_2H and RSO_3H with oxidative states of +2 and +4, respectively (Reaction 1) [4]. Although RSOH were first described as transient intermediates, steric properties and unique electronic and intramolecular hydrogen-bonding effects have been implicated in the stabilization of sulfenic acids [5]. For example, steric hindrance shelters the accessibility of the –SOH group of 9-triptycene-sulfenic

acid (Figure 3A) for attack by other –SOH groups and thus prevents formation of the hydrogen-bonded dimer [5]. The presence of electron-withdrawing substituents diminishes the neuclophilicity of the sulfur and stablizes aromatic sulfenic acids. In addition, sulfenic acid is stabilized as a result of the intramolecular hydrogen bonding between the hydrogen atom of RSOH and a hydrogen bond acceptor as seen with anthraquinone-1-sulphenic acids (Figure 3B). The intramolecular hydrogen bond makes the compound unable to participate in the intermolecular hydrogen bonding at the expense of the energy loss associated with breaking the intramolecular bond [4].

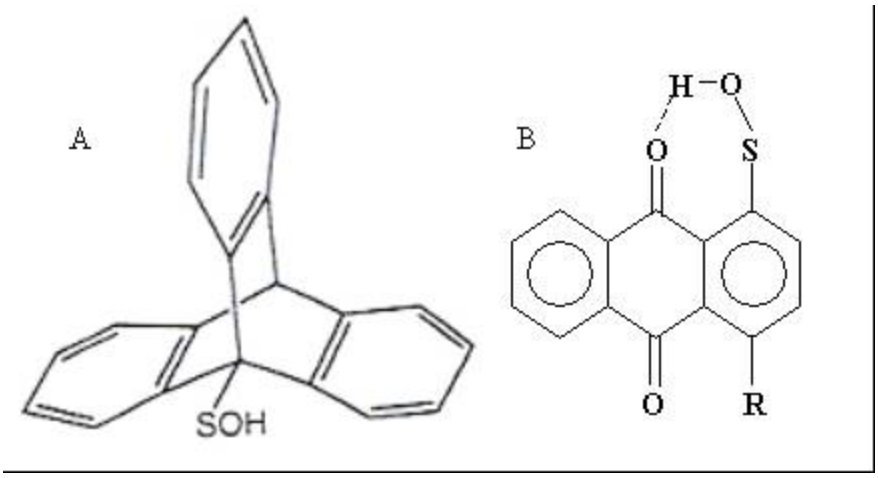
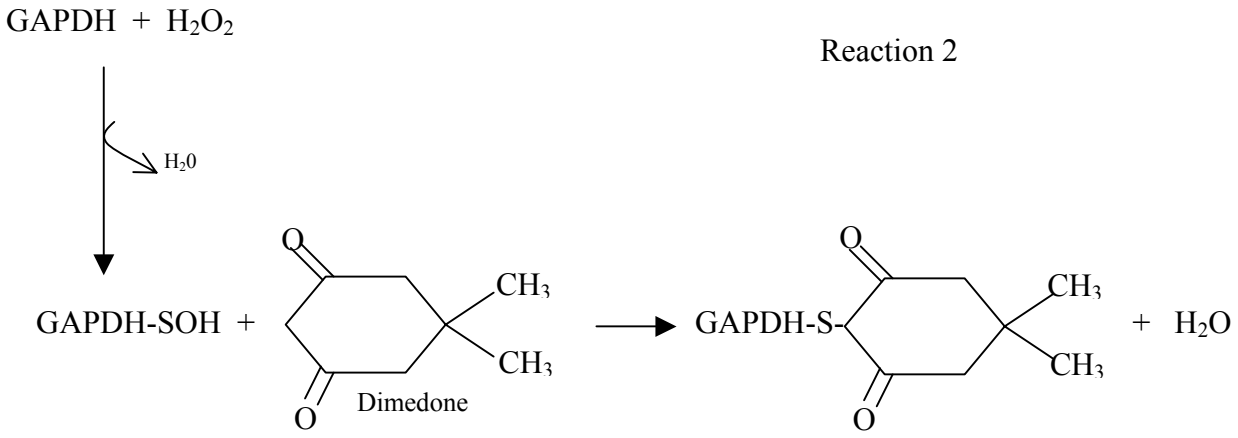


Figure 3. Stable protein-sulfenic acids. The steric hindrance of 9-triptycene-sulfenic acid (A) and the intramolecular hydrogen bonding property of anthraquinone-1-sulphenic acid (B) stabilizes sulfenic acid in these proteins.

Sulfenic Acid Detection Methods

The first evidence supporting the existence of stabilized protein-sulfenic acids in biology was in studies on the active site Cys-SH of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The stoichiometric oxidation of a single Cys-SH by hydrogen peroxide led to the inactivation of GAPDH activity that could be reversed by thiols [6]. In addition to the loss of GAPDH activity upon Cys-SH oxidation, there was an appearance of an acyl phosphatase

activity. Therefore, the initial claim of a stable Cys-SOH, as inferred through the analysis of the three-dimensional structure of GAPDH, could be confirmed by using the nucleophile, [^{14}C]dimedone to trap the Cys-SOH (Reaction 2) and allow for direct sequence analysis [6]. In



addition to dimedone, the Cys-SOH form of GAPDH can be trapped using alkenes because in solution RSOH react readily with alkenes. These techniques led to the identification of Cys149-SH as the active site for the oxidation to Cys-SOH in GAPDH [6].

Recently, Ellis and Poole have developed a technique to analyze Cys-SOH that takes advantage of the intrinsic nucleophilicity of Cys-SO$^-$ anion. In these studies, the amine and thiol

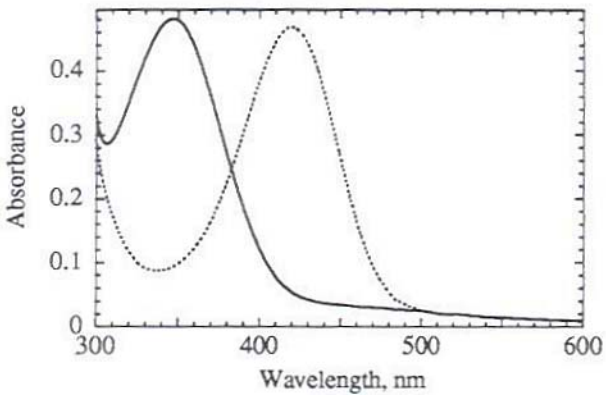


Figure 4. Reduced and oxidized forms of NBD adducts exhibit different UV-visible spectra. After treatment of hydrogen peroxide an oxidized Cys-S(O)-NBD (—) product has a shift in the UV-spectrum as compared to the reduced Cys-S-NBD (------) product; therefore, this technique can be used to identify the presence of Cys-SOH. This figure was taken from Ellis, H.R., and Poole, L.B. 1997. Novel application of 7-chloro-4-nitrobenzo-2-oxa-1, 3 diazole to identify cysteine sulfenic acid in the AhpC component of alkyl hydrogenperoxide reductase. *Biochemistry*. **36**:15013-15018.

reagent NBD-Cl was used in conjunction with UV-visible, fluorescence, and electrospray interface-mass spectrometric (ESI-MS) data [7]. Thus, if a protein is treated with NBD-Cl prior to hydrogen peroxide and a Cys-SH is oxidized to Cys-SOH the protein will exhibit different a

UV-visible spectrum (Figure 4), fluorescent characteristics, and ESI-MS values [7]. Continued progress is underway to identify and detect functional Cys-SOH using high-resolution crystal structures as well as ^{13}C NMR techniques.

Biochemistry: Protein-Sulfenic Acids

The stabilizing properties of sulfenic acids observed in solution established the foundation for the concept of stable protein-sulfenic acids. In addition to the stabilizing features discussed earlier, two additional criteria were established. 1) Protein-sulfenic acids must not be in the presence of other sulfhydryl groups because of the high reactivity of RSOH with thiols and the subsequent formation of intramolecular disulfide bonds. 2) Protein-sulfenic acids must have limited solvent access and be associated with apolar elements of protein structure [4]. When these criteria are met, it is thought that a sulfenic acid is in the ideal environment to be stable and accumulate during the reaction of a RSH with an oxidant. Indeed, using the methods described earlier, a handful of proteins have been identified that possess a functionally relevant sulfenic acid. These proteins have been grouped into the following categories: 1) FAD-dependent peroxide and disulfide reductases, 2) peroxiredoxins, phosphatases, and signal transduction proteins, and 3) transcription factors. In light of the our laboratory's interest, the remainder of this review will focus on sulfenic acids identified in transcription factors. However, other examples of protein-sulfenic acids have been discussed elsewhere [8-13].

Recently is has become well accepted that several transcription factors are redox sensitive. Particular studies have provided evidence for Cys-SOH involvement in protooncogenes c-Fos and c-Jun, E2 polypeptides encoded by bovine papillomavirus type 1 (BPV-1), and in regulatory protein OxyR [14-16]. c-Fos and c-Jun encode nuclear transcription factors that bind AP-1 sites of target genes and are involved in numerous signal transduction

pathways. Within the protein structure of Fos and Jun, a particular cysteine residue is conserved within the DNA-binding region (Cys153 of Fos, Cys272 of Jun) [14]. Abate et al. established the importance of these conserved cysteine residues through chemical modification with thiol reagents [17]. Cys152 and Cys272 account for DNA binding of Fos and Jun, respectively, as determined with thiol modification of Cys152Ser and Cys272Ser mutants [17]. The mutant proteins, in contrast to the wild-type proteins, did not require high concentrations of reducing agents to promote binding. In addition, DNA binding activity of the mutant proteins was not sensitive to cysteine alkylation or oxidation [17]; therefore, suggesting a role for Cys-SOH in Fos and Jun activity.

The E2 DNA-binding protein of BPV-1, like Fos and Jun, loses activity in the presence of thiol reagents or after chemical oxidation [15]. The E2 DNA binding domain has three cysteines, one of which (Cys340) is highly conserved among papillomavirus E2 proteins [15]. When Cys340 is mutated it becomes insensitive to chemical oxidation and following similar studies as those performed in the analysis of Fos and Jun [15], it is suggested that a Cys-SOH structure plays a role in inhibiting DNA binding of an oxidized E2 protein.

In our opinion, the most intriguing transcription factor that is altered by the presence of a Cys-SOH is the bacterial regulatory protein OxyR because the oxidation signal via hydrogen peroxide does not cause the binding of or dissociation from promoter sites; instead oxidation causes a conformational change in the already bound OxyR [16]. Activation of OxyR leads to the transcriptional activation of nine genes in the bacterial response to hydrogen peroxide-mediated oxidative stress in *E. coli* and *Salmonella typhimurium* [16]. Studies have demonstrated that high concentrations of reducing agents were required to prevent OxyR activation, thus, suggesting a role for a redox regulation in OxyR transcription activity. In

addition, mutagenesis of Cys199 resulted in a non-inducible OxyR protein [16], leading to the hypothesis that a Cys-SOH is a target for the oxidative stress signal mediated by hydrogen peroxide.

Summary and Conclusions

A large collection of work in recent years has provided substantial evidence that oxidation/reduction reactions and reactive oxygen species (ROS) play an important role in normal cellular function. The targets of redox cycling within a cell remains a topic that requires more investigation; however, as addressed in this review, the sulfhydryl group on cysteine residues, when presented in the proper environment, may represent such a target. The redox modulation of RSH leads to the production of sulfenic acid, a reactive intermediate of disulfide bond formation. The work reviewed here provides evidence of functionally relevant and stable cysteine-sulfenic acid proteins. The concept of ROS oxidizing a Cys-SH of a protein bound to DNA to cause a conformational change and subsequently alter the function of the protein (i.e. OxyR) should open the doors for investigation into the targets of ROS in mammalian cells. In particular, our group is currently investigating the target of superoxide radical in neuronal activation after stimulation with angiotensin II, the effector protein of the renin-angiotensin system. Interestingly, a convenient target would be a yet unidentified ion channel protein that caused the opening or closing of the channel as a result of a conformational change after an oxidizing reaction. In conclusion, the examples of functional protein-sulfenic acids reviewed here probably represent only a small fraction of Cys-SOH relevant proteins, as the Cys-SH $\leftrightarrow$ Cys-SOH redox cycle may be involved in an assortment of biological functions. Continued investigations are required using the newly developed elegant detection methods of sulfenic acids to better understand the role of stable protein-sulfenic acids in normal cellular functions.

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