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Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

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The Chemistry of Sulfenic Acids

by

Jeffrey D. Kirsch

B-180 Medical Laboratories
Free Radical and Radiation Biology Program
The University of Iowa
Iowa City, IA 52242-1181

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Abbreviations

Å	angstrom
Cys	cysteine
ESR	electron spin resonance
FTIR,	fourier transform infrared
GC	gas chromatography
H^+	proton
H_2O_2	hydrogen peroxide
IRP	iron regulatory protein
KOH	potassium hydroxide
LC	liquid chromatography
MS	mass spectroscopy
NBD-Cl	7-chloro-4-nitrobenzo-2-oxa-1,3-diazole
NMR	nuclear magnetic resonance
$^{\bullet}NO$	nitric oxide
Npx	NADH peroxidase
RSH	sulfhydryl
RSOH	sulfenic acid
RSO_2H	sulfinic acid
RSO_3H	sulfonic acid

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Abstract

The understanding of redox chemistry is becoming increasingly important to an overall understanding of cell biology. Sulfenic acids represent an important class of redox-labile chemical moieties, of which evidence is mounting to support a regulatory role for protein structure and function. This review examines the structural and chemical evidence for this proposed role.

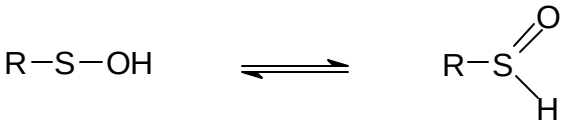
Introduction

A growing awareness of the important role that the cellular redox state plays in regulating physiology has brought with it a need to elucidate the mechanism whereby redox state effects such regulation. Several chemical moieties have been proposed as potential "sensors" of cellular redox status, most notably iron-sulfur clusters, such as those found in the mRNA-binding protein IRP, and the free sulfhydryls of cysteine residues in the DNA-binding domain of many transcription factors. The latter chemical moiety, RSH, can undergo a number of redox-induced modifications under physiologically relevant conditions, the most well appreciated being the formation of disulfide bonds with other RSH groups, and S-nitrosylation by reaction with $^{\bullet}NO$.

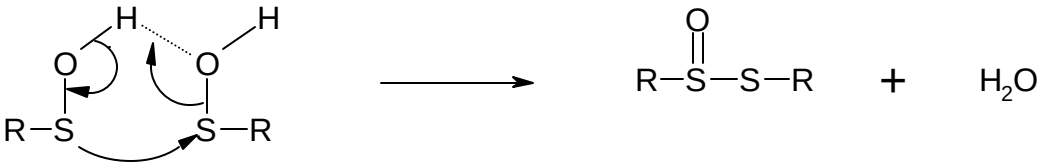
Oxidative modifications of RSH groups other than disulfide formation can, and do, occur, namely the formation of sulfenic (RSOH), sulfinic (RSO_2H), and sulfonic (RSO_3H) acids. Each addition of an oxygen atom to the sulfur in the series $RSH \rightarrow RSOH \rightarrow RSO_2H \rightarrow RSO_3H$ is accompanied by the loss of two electrons. Since the oxidation state of the sulfhydryl sulfur is –2, the oxidation states of the sulfur atoms in RSOH, RSO_2H, and RSO_3H are 0, +2, and +4, respectively [9]. Oxidation of RSH groups to sulfinic and sulfonic acids is considered to be an irreversible reaction, under physiologic conditions, while sulfenic acids have long been thought of as too reactive to exist as anything but transient intermediates [6, 9]. More recently, however, evidence has emerged that the formation of relatively stable protein sulfenates does occur, and can play a role in the regulation of cellular physiology [3, 4, 10]. With this biological relevance in mind, it becomes necessary to understand the chemistry of sulfenic acids, which is the goal of this review.

Background

Sulfenic acids are organosulfur oxyacids of the form RSOH, where R is an organic moiety. FTIR spectroscopic studies have shown evidence for sulfenic acids existing as two tautomers, of which the R-S-OH form predominates [1, 6, 7].



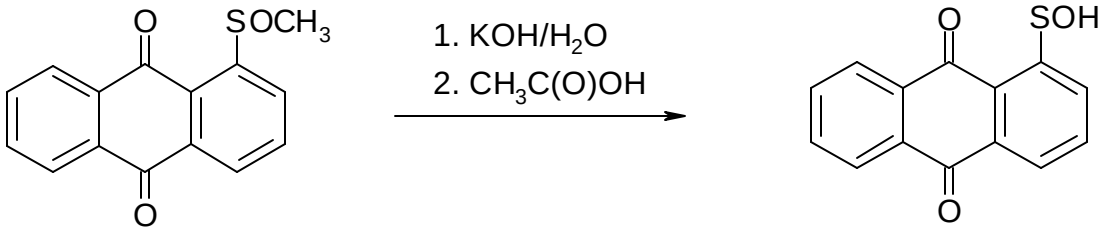
Unlike the more highly oxidized sulfinic and sulfonic acids, which are relatively stable, sulfenic acids are generally unstable and highly reactive compounds [6, 9]. In large part this is due to the high nucleophilicity of the sulfur atom. When considered with the electrophilicity of the organic R group, this nucleophilicity accounts for the tendency of sulfenic acids to undergo self-condensation to form the thiosulfinate [1, 6].



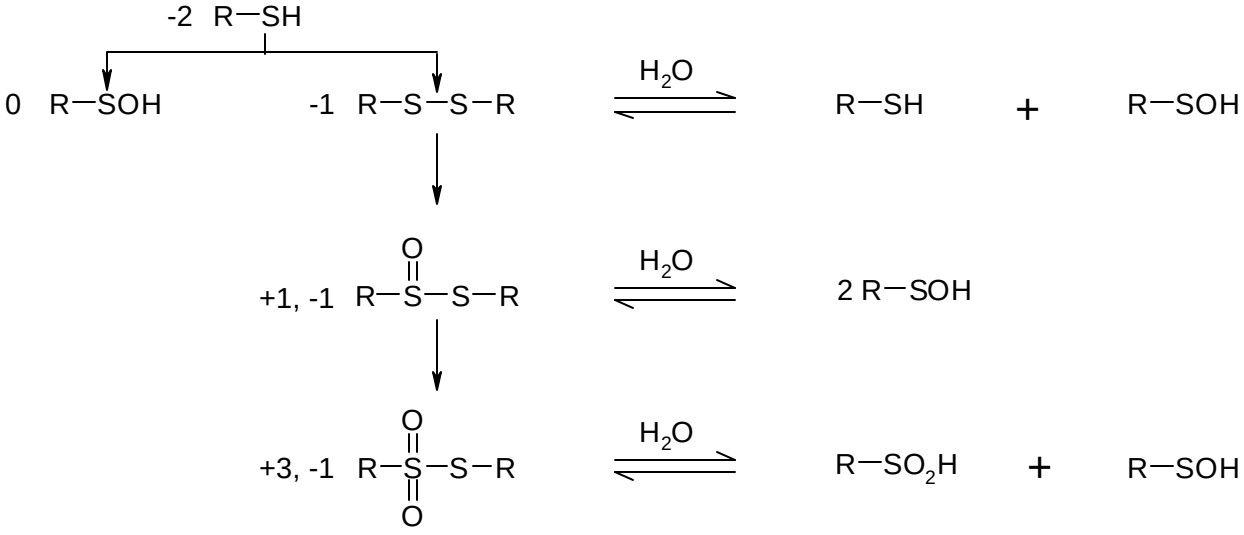
Numerous studies have pointed to a requirement for hydrogen bonding between the hydrogen atom of one sulfenic acid to the oxygen atom of another, which helps to explain the unusual stability of some sulfenic acids [1]. Consistent with the foregoing is the observation that for many of the stable sulfenic acids studied to date such hydrogen bonding is disfavored, either because of steric hindrance or energetic considerations [1].

Synthesis

The first stable sulfenic acid synthesis reported was that of anthraquinone-1-sulfenic acid, from the methyl sulfenate. Anthroquinone-1-methyl sulfenate, treated sequentially with a strong solution of KOH, followed by acetic acid, yields the sulfenate, as below [6].



Of perhaps greater interest, from a biological perspective, is the following scheme of sequential oxidations and hydrolyses. It should be noted that the two pathways illustrated are not mutually exclusive, and crossover can be expected to occur [9].

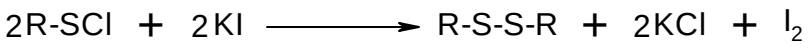


The oxidation states of the sulfur atoms are indicated immediately to the left of each structure. Where there are two sulfurs, the numbers correspond to the atoms reading left to right.

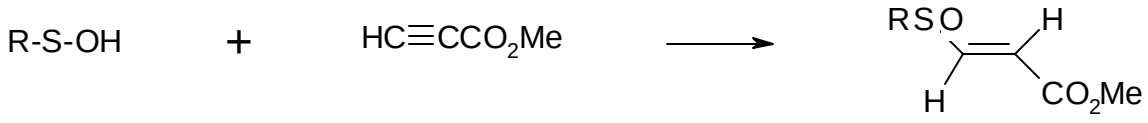
Oxidation to higher order products occurs, but is not depicted in the previous figure owing to its not contributing to the formation of RSOH.

Analytical Methods

A variety of analytical methods have been used to investigate the function and structure, as well as quantitation, of sulfenic acids. FTIR and ESR/NMR spectroscopy have all been used to investigate the structure of sulfenic acids and their derivatives, although in many cases reactive intermediates must first be trapped by interaction with other reagents [7, 12]. The simplest quantitative method commonly used is that of gas chromatography (GC), either alone or in combination with mass spectroscopy (MS), but this method can only be used with stable sulfenic acids or derivatives thereof [12]. Direct determination of sulfenyl halides is possible through the use of the following reaction [12].



The iodine liberated in the foregoing reaction may be directly titrated, although it should be noted that the presence of other oxidizers in the sample solution could interfere with the assay. One derivatizing reagent that has been reported to be useful in the determination of unstable sulfenic acids is methyl acetylenecarboxylate, as shown in the reaction below [12].



Following the above derivatization, the product can be analyzed by GC.

Biochemistry

There is a growing body of literature that suggests that sulfenic acids, as the Cys-SOH moiety, are important intermediates in many biochemical pathways [3, 4]. This has been somewhat difficult to demonstrate unambiguously, given the general highly reactive nature, and thus transitory lifetime, of sulfenic acids, but some progress has been made in this regard recently. Specifically, the crystal structure of a number of proteins with active site Cys residues have been determined, in the oxidized state, and found to possess Cys-SOH at those sites. Examples of the foregoing include the flavoprotein NADH peroxidase (Npx), wherein Yeh *et al.* determined the 2.8 Å crystal structure of oxidized Npx, and found the active site Cys to be in the form of a sulfenic acid [11]. In that work, it was proposed that stabilization of the active site Cys-SOH stemmed from electronic interaction with the flavin moiety, coupled with limited solvent access and weak interactions with other active site proximal residues. Of perhaps greater interest, from the standpoint of human biochemistry, is the example of hORF6 peroxiredoxin [2]. In that protein, we again see the motif of a solvent-inaccessible active site pocket, with crystallographic data supporting a stable active site Cys-SOH. Although the primary enzymatic function attributed to both of the above examples is the removal of H_2O_2, peroxiredoxins appear to be associated with modulation of signal transduction in mammalian cells, which makes them all the more intriguing [8].

Other proteins involved in cellular signal transduction are known or suspected to involve sulfenic acid reaction intermediates. Protein tyrosine phosphatases, PTP-1B being the best-studied example, are known to be inhibited by exposure to relatively low concentrations of H_2O_2, and it has been proposed that this inhibition is due to the formation of Cys-SOH at the catalytic Cys residue [5]. Sulfenic acid formation at the active site catalytic Cys was demonstrated by derivatizing the H_2O_2 treated PTP-1B with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl), a reagent that reacts with sulfhydryls and sulfenic acids to form a thioether and sulfoxide

derivative, respectively. These adducts were then resolved by tandem LC/MS/MS, which showed that the oxidized active site Cys was primarily in the form of the Cys-SOH. Furthermore, this Cys-SOH could be shown *in vitro* to be subject to glutathionylation, which protected the Cys from further oxidation to sulfinic or sulfonic acid. The authors propose that glutathionylation, which was reversible by glutaredoxin (thioltransferase), may represent a component of the oxidant-responsive signal transduction mechanism leading from oxidant production to net protein phosphorylation.

Summary

Redox chemistry is increasingly appreciated as playing an important, perhaps fundamental, role in the regulation of a wide range of cellular processes. Several potential intermediaries can be proposed as the mediators or targets of the cellular redox state, among them a product of sulfhydryl oxidation, sulfenic acid. Sulfenic acid possesses unique redox characteristics that lend it to this proposed regulatory role, such as its intermediate oxidation state, lying between the reversibly oxidized disulfide RSSR, and the irreversibly oxidized sulfinic and sulfonic acids. Recent evidence for relatively stable sulfenic acid intermediates supports this proposed role, and argues for further investigation of this functional group.

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