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Sulfenic Acids

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Abbreviations

Cys-SOH	cysteine-sulfenic acid
ESI-MS	electrospray interface-mass spectrometry
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GSH	glutathione
NBD-Cl	7-chloro-4-nitrobenzo-2-oxa-1, 3 diazole
NMR	nuclear magnetic resonance
PTK	protein tyrosine kinase
PTP	protein tyrosine phosphatase
R-SH	sulfhydryl
R-SOH	sulfenic acid
$R\text{-}SO_2H$	sulfinic acid
$R\text{-}SO_3H$	sulfonic acid
RS-SR	disulfide
Trx	thioredoxin

Outline

Abstract

Sulfenic acids (R-SOH) usually result from mild oxidations of thiols. Due to their highly reactive properties in solution, R-SOHs have long been considered to only exist as transient reaction intermediates. However, in recent years, stable R-SOHs have been obtained and factors that contribute to their stabilization have been investigated. Additionally, cysteine-sulfenic acid (Cys-SOH) is now known to be essential for redox control of many protein functions, such as enzymatic catalysis and signal transduction. In this review, I will discuss the chemistry, biochemistry, and detection of sulfenic acids.

Introduction

The importance of cysteine residues in proteins that function in enzyme catalysis and/or redox signaling has been broadly recognized [1]. It is known now that the stable sulfenic acid derivatives of cysteine, the cysteine-sulfenic acid (Cys-SOH), is critical for the regulation of redox-sensitive enzymes and transcription factors [2, 3]. Sulfenic acids (R-SOH) are generally considered direct products of mild oxidations of sulfhydryl groups (R-SH). Being the simplest organosulfur oxyacids, R-SOHs have long been known as important transient reaction intermediates in organic and bioorganic chemistry of sulfur [4, 5]. However, in the past two decades, dramatic progress has been achieved in designing and synthesizing stable R-SOH derivatives. This article reviews the chemistry and biochemistry of R-SOHs, and also addresses the issues of the stabilization as well as the significance of Cys-SOH in protein function.

Sulfenic Acids Chemistry: Transient versus Stable

Theoretically, two possible tautomeric structures can be considered for sulfenic acids (**Figure 1**). Using microwave spectroscopic analysis, it has been shown that the *O*-protonated form (**Figure 1a**) is the predominant structure over the *S*-protonated "sulfoxide"(**Figure 1b**) [5].

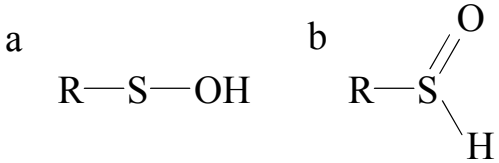

Figure 1. A sulfenic acid has two possible tautometic structures. The *O*-protonated form (panel a) has been shown to be the predominant structure over the *S*-protonated (panel b) [5].

R-SOHs have nucleophilic characteristics, which lead to their highly reactive properties. In solution, the most common reaction of a sulfenic acid is self-condensation to form thiosulphinate (**Figure 2**) [5]. This reaction proceeds through the formation of a sulfenic acid dimer linked

through a hydrogen bond, which has been demonstrated by IR analysis. The free energy of activation for thiosulphinate formation is reduced by this intermolecular hydrogen bond. In the next step, a nucleophilic attack at one R-SOH sulfur by the second finishes the reaction. Both the potential of forming intermolecular hydrogen bonds and the nucleophilicity of R-SOHs results in their instability in solutions [6].

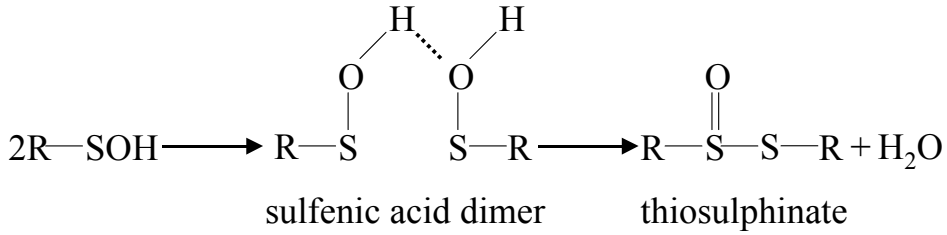

Figure 2. The self-condensation reaction of sulfenic acids. The reaction proceeds through the forming of an intermolecular hydrogen bond and results in thiosulphinate and water.

In addition to the self-condensation reaction, the R-SOHs are also known as the intermediates in the oxidation reactions of thiols into disulfides (RS-SR) and higher organosulfur oxyacids (*i.e.* sulfinic (R-SO_2H) and sulfonic (R-SO_3H) acids). The sulfur in R-SH loses two electrons upon oxidation to form R-SOH, which then undergoes further reactions to form reversibly (*e.g.* RS-SR) or irreversibly (*e.g.* R-SO_3H) modified groups (**Figure 3**) [2].

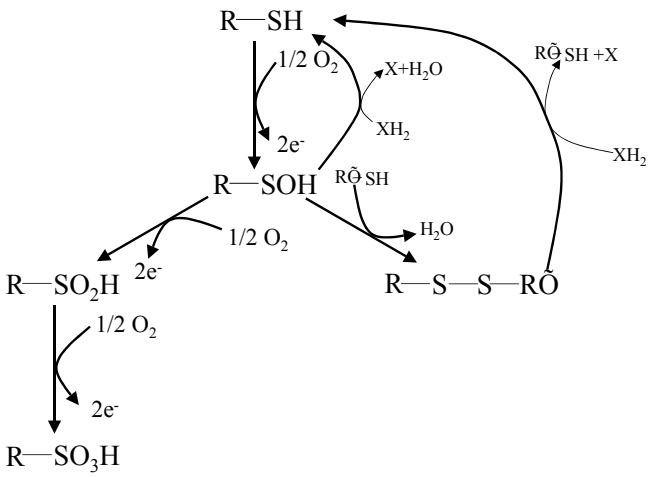

Figure 3 The involvement of sulfenic acids in the oxidation reactions of thiols. Upon oxidation, the sulfur in R-SH (oxidative state: -2) loses two electrons and forms R-SOH (oxidative state: 0). Depending on the environment, R-SOH has different fate: In the presence of reductant (XH_2), it can be reduced back to R-SH; when there is excessive amount of R'-SH (R' can be equal to R), R-SOH can react with thiols and form R-S-S-R', which can also be reduced back to R-SH by XH_2; in a oxidative environment, R-SOH will be further oxidized to form R-SO_2H (oxidative status: +2) and R-SO_3H (oxidative status: +4), which are not reversible [2].

Because of the high reactivity, for a long period, R-SOHs have been considered to be unstable and only exist as transient intermediates under normal conditions [4, 5]. However, in recent years there has been a dramatic progress in designing and synthesizing stable R-SOHs. This progress is attributed to a better understanding of several hydrogen bonding, steric, and electronic factors that can stablize R-SOHs [3, 6]. For instance, in anthraquinone-1-SOH (**Figure 4a**), an intramolecular hydrogen bond is formed between the hydrogen atom of the –SOH and the 9-carbonyl group. This intramolecular hydrogen bond prevents the formation of intermolecular hydrogen bond between two R-SOHs. In 9-triptycene-SOH (**Figure 4b**), a steric barrier is an unfavorable factor for the dimerization process. In addition, some electron-withdrawing substituents interfere with the nucleophilicity of certain aromatic R-SOHs. Generally, all stabilizing factors are about interfering with the self-condensation reaction.

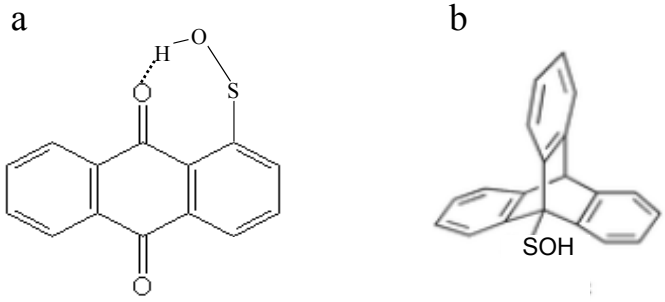

Figure 4. Two examples of stable sulfenic acids. The intramolecular hydrogen bond of anthraquinone-1-SOH (panel a) and the steric hindrance of 9-triptycene-SOH (panel b) prevent the self-condensation reaction, thus stabilize these two sulfenic acids. From [3].

Sulfenic Acids Biochemistry: Protein Sulfenic Acids

Protein sulfenic acids can be obtained by mild oxidation of the cysteine residues to form Cys-SOHs. The earliest supporting evidence for stable Cys-SOH came from studies in the 1970s on the Cys-SHs in the active centers of glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

and papain [7]. In addition to the stabilizing factors described above, the absence of nearby cysteine thiols (Cys-SH) is considered to be the primary factor for the stabilization of the Cys-SOHs [7].

The reversible reaction Cys-SH ↔ Cys-SOH provides the basis for the redox regulation of many protein activities. The Cys-SOHs have been accepted to be important for the redox control of enzymes and transcription factors from both bacterial and eukaryotic sources [2, 3]. In this review, I will discuss two examples: inactivation of phosphatase during signal transduction and activation of OxyR in oxidative stress. Hydrogen peroxide is the oxidant for Cys-SH in both cases.

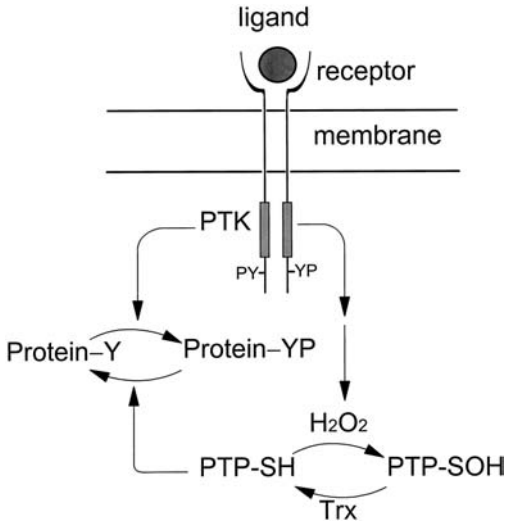

Figure 5. Conversion of Cys-SH into Cys-SOH inactivates protein tyrosine phosphatases (PTPs) in signal transductions. After receptor binding of cytokines or growth factors, H_2O_2 is produced by the cell to inhibit the PTPs functions. This reversible inhibition is realized by oxidation of Cys-SH on PTPs into Cys-SOH. From [8].

Hydrogen peroxide has been implicated in cellular responses to environmental stimuli, such as cytokines and growth factors. Many of these responses start from a ligand-receptor-mediated activation of protein tyrosine kinase (PTK). This activated PTK will then activate downstream signal cascades by phosphorylation of tyrosine residues on its substrates. In rest states, cells have

protein tyrosine phosphatases (PTPs) to dephosphorylate the substrates and inhibit or downregulate this activation. However, when a receptor binds to its ligand, the cell produces H_2O_2 to inactivate PTPs so that the signal cascade gets turned on. The whole process is illustrated by **Figure 5**. The mechanism of H_2O_2-mediated inactivation of PTP1B, one type of PTP, has been well studied [8]. This reversible inactivation is realized by an oxidation of the critical Cys215-SH residue at the active site into a stable Cys215-SOH derivative. The stability of Cys215-SOH in inactivated PTP1B has been supported by the crystal structure of its 37 kDa form [9]. In this crystal structure resolved in 1994, no other Cys-SH residues were observed within 9 Å of the Cys215 in the active site, so there would be no intracellular disulfide formation upon oxidation. Meanwhile, the presence of an intracellular hydrogen bond or an ion pair is suggested by the fact that the S_γ atom on Cys215 is very close (within 3 Å) to the $N_{\eta 1}$ atom on the Arg221 residue. All this evidence meet perfectly the criteria of stabilizing factors for the Cys-SOHs as described above. Quantitative analysis has shown that following stimulation of growth factors, about 30% of the total cellular PTP1B undergoes a reversible conversion from Cys215-SH into Cys215-SOH and loses activity [8]. The activity of PTP1B can then be restored by cellular thiols such as glutathione (GSH) or thioredoxin (Trx) as shown in **Figure 5**.

OxyR is an important transcription factor in the bacterial response to H_2O_2-mediated oxidative stress. The reduced form of OxyR binds to the promoter region of multiple anti-oxidative genes but does not activate transcription. Oxidation activates OxyR and turns on the transcription of those genes to protect from oxidative stress. Although there is still no consensus about what kind of oxidized form of OxyR activates transcription [10, 11], there is agreement that the sulfenic acid form is critical [2]. This sulfenic acid form was detected [11] by NBD-reaction analysis as discussed below.

Detection of Protein-SOHs

As the importance of Cys-SOH in protein functions becomes broadly accepted, it is becoming necessary for scientists to detect the presence of stable Cys-SOH residues. In a 1976 study of active-site Cys149-SH of GAPDH, Allison *et al.* provided the first evidence for the presence of stable Cys-SOH using an indirect approach [7]. Their approach involved using [C^{14}] dimedone, a nucleophile, to trap the Cys-SOH to form a stable C(2)-thioether adduct. The modification of Cys149 was then confirmed by sequence analysis.

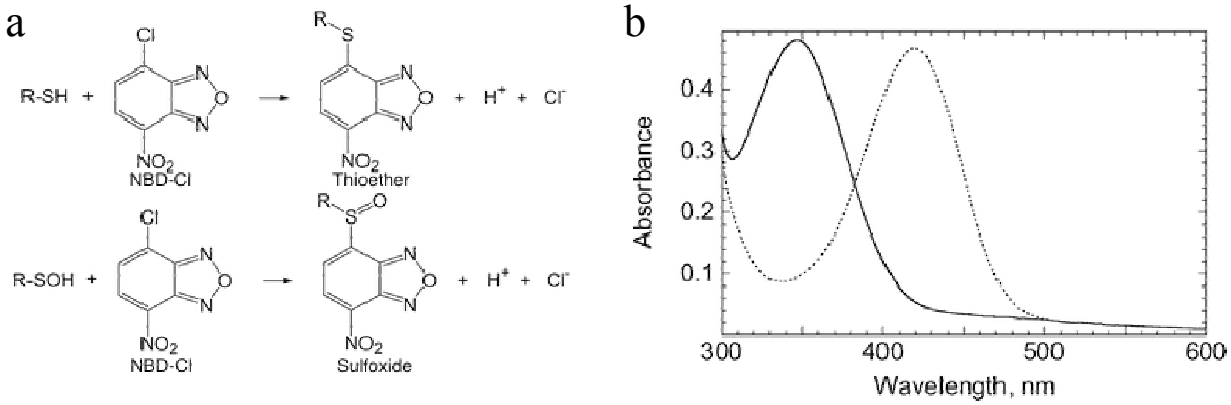

Figure 6. Detection of protein-SOH using NBD-Cl. NBD-Cl is able to react with both protein-SH and protein-SOH, resulting in different adducts (panel a). The UV-visible spectra for the NBD adduct with protein-SOH (panel b, solid line) is different from the NBD adduct with protein-SH (panel b, dotted line). Modified from [12].

Recently, Ellis and Poole developed a direct approach to detect the Cys-SOH modification [12], taking advantage of the nucleophilic property of the Cys-SO^- anion. In their approach, they used an amine and thiol reagent NBD-Cl (7-chloro-4-nitrobenzo-2-oxa-1, 3 diazole) to analyze the oxidation status of Cys46-SH in the active site of the bacterial peroxiredoxin AhpC. NBD-Cl is able to react with Cys-SH and Cys-SOH and forms two different adducts, thioether and sulfoxide, respectively (**Figure 6a**). On UV-visible spectra, these two adducts have different λ_{max} (**Figure 6b**). In addition, these two products also have different mass and fluorescent property,

which makes it possible to distinguish them from each other by using electrospray interface-mass spectrometric (ESI-MS) and fluorescence spectra data, respectively [12]. The NBD method is current broadly used to study the oxidation status of cysteine residues in proteins [2, 10].

Summary and Conclusions

The identification of stable protein-SOHs is accompanied by the progress in understanding the chemistry and stabilization factors of sulfenic acids. The importance of the Cys-SH ↔ Cys-SOH redox cycles in the regulation of protein functions, such as enzyme catalysis and signal transduction, has been widely appreciated. With recent technological advances such as high-resolution crystal structure analysis and ^{13}C nuclear magnetic resonance (NMR) techniques, we can develop protocols with combinations of different approaches to identify the presence of Cys-SOH and analyze its significance for the functions of the protein.

References

1. Jacob C, Giles GI, Giles NM, Sies H. (2003) Sulfur and selenium: the role of oxidation state in protein structure and function. *Angew Chem Int Ed Engl.* **42:**4742-4758.

2. Poole LB, Karplus PA, Claiborne A. (2004) Protein sulfenic acids in redox signaling. *Ann Rev Pharmacol Toxicol.* **44:**325-347.

3. Claiborne A, Mallett TC, Yeh JI, Luba J, Parsonage D. (2001) Structural, redox, and mechanistic parameters for cysteine-sulfenic acid function in catalysis and regulation. *Adv Protein Chem.* **58:**215-276.

4. Kice J. (1980) Mechanism and reactivity in reactions of organic oxyacids of sulfur and their anhydrids. *Adv Phys Org Chem.* **17:**65-181.

5. Hogg D. (1990) Chemistry of sulphenic acids and esters. In: Patai S, ed. *The Chemistry of Sulphenic Acids and Their Derivatives.* Chichester: John Wiley and Sons; pp 361-402.

6. Claiborne A, Miller H, Parsonage D, Ross RP. (1993) Protein-sulfenic acid stabilization and function in enzyme catalysis and gene regulation. *FASEB J.* **7:**1483-1490.

7. Allison W. (1976) Formation and reactions of sulfenic acids in proteins. *Acc Chem Res.* **9:**293-299.

8. Lee SR, Kwon KS, Kim SR, Rhee SG. (1998) Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor. *J Biol Chem.* **273:**15366-15372.

9. Barford D, Flint AJ, Tonks NK. (1994) Crystal structure of human protein tyrosine phosphatase 1B. *Science.* **263:**1397-1404.

10. Zheng M, Aslund F, Storz G. (1998) Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science.* **279:**1718-1721.

11. Kim SO, Merchant K, Nudelman R, Beyer WF, Keng T, DeAngelo J, Hausladen A, Stamler JS. (2002) OxyR: a molecular code for redox-related signaling. *Cell.* **109:**383-396.

12. Ellis HR, Poole LB. (1997) Novel application of 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole to identify cysteine sulfenic acid in the AhpC component of alkyl hydroperoxide reductase. *Biochemistry.* **36:**15013-15018.