Nature Reviews Chemistry | Published online 25 Jan 2017; doi:10.1038/s41570-016-0013

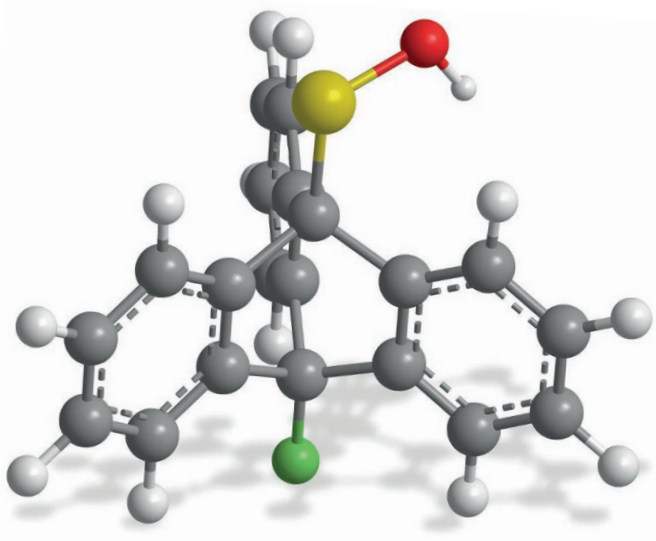
THIOL OXIDATION

A slippery slope

> These are common reactions, but have historically been very difficult to study

The oxidation of thiols — molecules of the form RSH — can afford many products. From least to most oxidized, these include disulfides (RSSR), as well as sulfenic (RSOH), sulfinic (RSO_2H) and sulfonic (RSO_3H) acids. Such chemistry is pervasive in nature, in which disulfide bonds between cysteine residues stabilize protein structures, and where thiols and thiolates often undergo oxidation by H_2O_2 or O_2 in order to protect important biological structures from damage. Among the oxidation products, sulfenic acids are particularly reactive and their existence is often only putative. However, Jean-Philippe Chauvin and Derek Pratt report in *Angewandte Chemie International Edition* the direct observation of a sulfenic acid as the initial product in thiol oxidation.

Oxidizing a thiol with H_2O_2 — the quintessential reactive oxygen species in both laboratories and in living systems — is simple to perform but difficult to understand because many products can form. Indeed, RSH oxidation may proceed too quickly for intermediates like RSOH to be spotted and may also afford intractable mixtures. Addressing the first problem, Chauvin and Pratt slowed the reactions down by using "very sterically bulky thiols, whose corresponding sulfenic acids were known to be isolable but were yet to be thoroughly explored in terms of reactivity". The second problem was tackled by modifying the model system, 9-mercaptotriptycene, by including a fluorine substituent to serve as a spectroscopic handle. The bulky 9-mercapto-10-fluorotriptycene — featuring thiol and fluoro groups at the sp^3-hybridized bridgehead carbons — is readily prepared from the *tert*-butyl thioether, which in turn is formed by the Diels–Alder reaction of 9-*tert*-butylthio-10-fluoroanthracene with benzyne.



Rachael Tremlett/Macmillan Publishers Limited

What of the oxidation of 9-mercapto-10-fluorotriptycene? Treatment with H_2O_2 in buffered methanol sequentially converts the thiol to the corresponding sulfenic, sulfinic and sulfonic acids. Each species gives rise to a well-resolved ^{19}F NMR resonance, with more oxidized species appearing at successively lower field. Monitoring the reaction mixture allows determination of a rate constant for each of the three successive oxidations, which are first order in both the sulfur compound and H_2O_2. The kinetics agree with rates observed when H_2O_2 is added to authentic samples of thiol, sulfenic and sulfinic acid.

The observed reactivities also mesh well with the p*K*a values of the thiol, sulfenic and sulfinic acid (11.6, 12.8 and 4.3, respectively). The reaction rate for each of the oxidations increases with pH up to a plateau at which pH exceeds p*K*a and the oxidizable conjugate base predominates. Here, the maximum rate constants indicate the order of reactivity: $RSO^- > RS^- >> RSO_2^-$. When the reactions are carried out in methanol-d_1, the obtained kinetic isotope effect values (k_H/k_D) are all in the range 1.1–1.2, indicating that no acidic proton is transferred in the rate-determining step. Rather, the oxidations involve a specific base-catalysed mechanism wherein an acid–base equilibrium precedes the rate-determining nucleophilic attack of RS^-, RSO^- or RSO_2^- on H_2O_2.

If the environment deviates even slightly from being pH neutral, H_2O_2 (and alkylhydroperoxides, which are mimics of lipid peroxides) will react with the sulfenic acid/sulfenate faster than the thiol/thiolate, reflecting the slippery slope that is thiol oxidation. For biology to use sulfenic acids it must overcome this bias to curb overoxidation during protein folding, cell signalling or peroxide detoxification. The results of Chauvin and Pratt point to biomolecules having finely tuned regions — including residues affecting hydrogen bonding and local pH — proximal to thiols that can in some cases reverse the innate reactivity bias. Pratt now plans to investigate "if sulfenic acids are involved in other oxidations of thiols, including those induced by ozone, as well as singlet and triplet oxygen. These are common reactions, but have historically been very difficult to study". Yet, history has long smiled on fundamental research of this ilk, which places us closer to uncovering the many secrets sulfur still keeps.

David Schilter

ORIGINAL ARTICLE Chauvin, J.-P. R. & Pratt, D. A. On the reactions of thiols, sulfenic acids, and sulfinic acids with hydrogen peroxide. *Angew. Chem. Int. Ed.* http://dx.doi.org/10.1002/anie.201610402 (2016)