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The sulfinic acid switch in proteins†

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Recent studies on the redox behaviour of cysteine residues in peptides and proteins have dramatically changed our perspective of the amino acid's role in biocatalysis, intracellular redox sensing and cell signalling. Cysteine sulfinic acid formation in proteins, for example, has long been viewed as an irreversible 'overoxidation' process that might lead to loss of activity, especially under conditions of oxidative stress. Within the last year, several research groups have independently shown that sulfinic acids can be reduced to thiols *in vivo*. An enzyme with sulfinic acid reductase activity, called sulfiredoxin, has been isolated from yeast and a gene encoding a human analogue has been identified in the human genome. Reversibility of sulfinic acid formation opens the door to a range of yet unexplored redox cycles, cell signalling processes and reduction mechanisms. These cysteine-based redox processes will be of enormous interest to chemists, biochemists, biologists and the medical community alike.

Introduction

The last couple of years have seen a rapidly growing interest in the redox behaviour of cysteine residues in peptides and proteins. Sulfur has the ability to occur in many different oxidation states in biological systems, where it partakes in a range of diverse redox reactions (Table 1).[1,2] The resulting post-translational cysteine modifications in proteins include, for example, thiyl radicals, disulfides, sulfenic, sulfinic and sulfonic acids and disulfide-*S*-oxides (Fig. 1). Several of these modifications, such as sulfenic and sulfinic acids, have long been ignored by biochemists, but recent methodological advances have allowed their identification in a surprisingly large number of proteins. In addition, emerging concepts, such as the notion of Reactive Sulfur Species (RSS) and cysteine-based redox signalling, have provided more defined roles for these 'unusual' sulfur oxidation states.[3–5]

† Electronic supplementary information (ESI) available: *Frontispiece:* The sulfinic acid switch in Prx responds to elevated hydrogen peroxide concentrations, turning antioxidant catalysis into a signal for cell death. The recently discovered Srx rescue pathway provides a cellular mechanism to flip the switch back to 'catalysis'. The centrepiece is an X-ray crystal structure of the decameric form of human (2-Cys) peroxiredoxin at 1.7 Å resolution.[9] The figure of the protein was produced using BOBSCRIPT.[24] See http://www.rsc.org/suppdata/ob/b4/b406180b/

Claus Jacob received a BSc(Hons) in Chemistry from the University of Leicester in 1993 and a doctorate in Bioinorganic Chemistry from the University of Oxford in 1997. He moved to Harvard Medical School in 1996 to undertake postdoctoral work on zinc homeostasis with Bert Vallee as a fellow of the Alexander von Humboldt Foundation. In 1999, Claus joined the University of Exeter where he is currently a senior lecturer in inorganic chemistry. His research is centred on the biological chemistry of oxygen, sulfur and selenium, for which he has just been awarded an EPSRC Advanced Research Fellowship. Claus is also Chairman of Exeter Antioxidant Therapeutics Ltd.

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Table 1 Cysteine-based redox couples observed in enzymes. While the thiol/disulfide couple is commonly observed, the other redox couples are less frequently found. Together, they provide a range of sulfur oxidation states and redox mechanisms for very different biological purposes. This list is currently expanding and should therefore not be seen as complete

Redox couple	Functional group	Sulfur oxidation states (R = +1)	Redox mechanism	Examples of occurrence in proteins
Thiol/disulfide	RSH/RSSR	−2/−1	Thiol disulfide exchange Two-electron transfer	Commonly observed, *e.g.* Glutathione reductase
Thiol/thiyl radical	RSH/RS·	−2/−1	One-electron transfer Hydrogen atom transfer	Ribonucleotide reductase Pyruvate formate lyase Benzylsuccinate synthase
Thiol/sulfenic acid	RSH/RSOH	−2/0	Oxygen atom transfer with subsequent thiol/disulfide exchange or hydride transfer	Peroxiredoxins Bacterial NADH oxidases and peroxidases

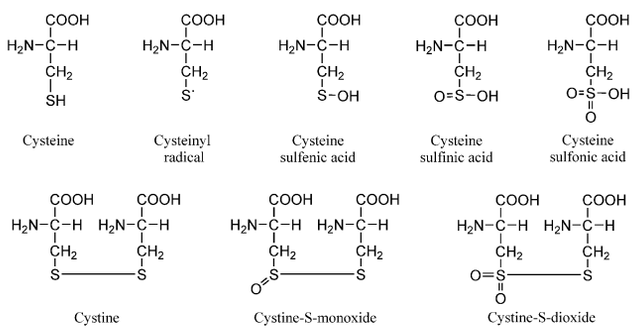


Fig. 1 Post-translational modifications of cysteine that play a significant role in proteins *in vivo*.[2]

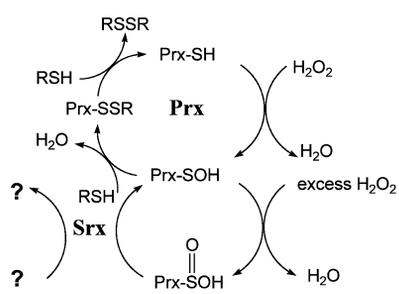
Many of these discoveries challenge existing biochemical paradigms, as illustrated by the breathtaking developments in the field of sulfinic acid biochemistry during the last twelve months. Firstly, sulfinic acids found in proteins, with a few notable exceptions (such as iron-containing nitrile hydratases),[2] have long been considered as isolated 'accidents' or artefacts, caused by random cysteine oxidation during protein purification. It has recently become apparent, however, that sulfinic acids might be formed in proteins *in vivo* to a considerably larger extent, and much more controlled, than previously thought. Aebersold's group, for example, has used a proteomics approach to show that sulfinic acids are present in a wide range of proteins *in vivo*.[6] Similarly, a sulfinic acid has been consistently identified at the active site of the human antioxidant protein peroxiredoxin-II (Prx-II).[7–9]

Secondly, there has been a general consensus that a sulfinic acid could not be reduced back to the sulfenic acid or thiol under *in vivo* conditions. This notion has also been challenged. In April 2003, several papers were published in *Science* showing for the first time that Prx enzymes are *reversibly* inactivated by 'overoxidation' of the catalytic cysteine residue to a sulfinic acid and compelling evidence was presented that formation of cysteine sulfinic acids in proteins might be a reversible process *in vivo*.[7,8] At the time, however, the biological component reducing the sulfinic acid moiety was still unknown. In October 2003, Biteau *et al.* published a paper in *Nature* where they identified a protein, sulfiredoxin (Srx1), that was able to reduce cysteine sulfinic acid to cysteine in *S. cervisiae*.[10]

Considered together, these discoveries have opened the door to a wide range of yet unexplored, cellular control and signalling mechanisms that rely on cysteine. It is now possible, and even necessary, to study redox-transformations involving thiols, sulfenic and sulfinic acids. Such investigations provide a fertile ground for multidisciplinary studies involving synthetic and analytical chemists, biochemists, cell biologists and the medical community. We will discuss here some of these just emerging facts, possibilities and opportunities in the field of biological sulfur chemistry. As is appropriate for a glance at an 'emerging area', we will limit the discussion to the most recent, perhaps 'hottest' topics in this field, and refer to related areas in passing.

Sulfinic acid formation in peroxiredoxin enzymes

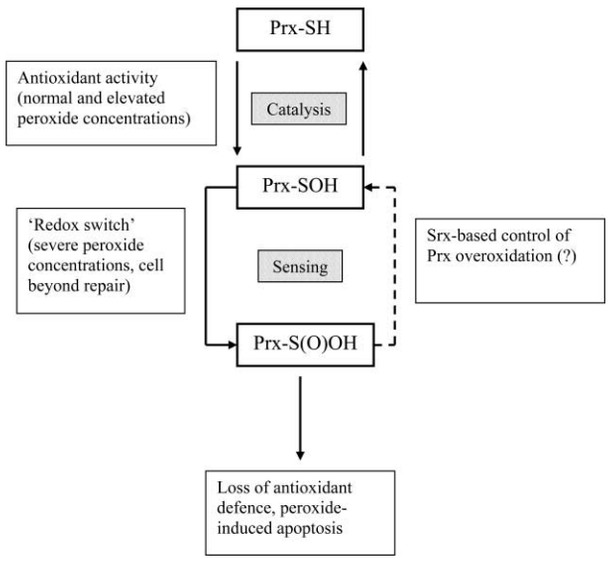
We will start with the peroxiredoxins (Prx), a class of long neglected, yet important human antioxidant enzymes that occur in high concentrations in human cells such as erythrocytes (where Prx-II is the third most abundant protein), and compose 0.1–0.8% of the soluble protein in other mammalian cells.[11] Six different classes of Prx enzymes are known to date (Prx-I to Prx-VI), and they are found throughout cell organelles and in the cell membrane. From a biochemist's perspective, the catalytic mechanism of these peroxidase enzymes is as simple as it is effective (Scheme 1): Prx enzymes rely solely on one (1-Cys Prx) or two (2-Cys Prx) cysteine residues and use a thiol/sulfenic acid redox couple to rapidly reduce peroxide in the presence of an external thiol. Redox catalysis is provided by 'just' one (or two) amino acid side chains, no metal ions or organic co-factors are required!



Scheme 1 The cysteine redox cycle in peroxiredoxins. While the normal redox cycle involves a thiol and sulfenic acid, overoxidation leads to the formation of a sulfinic acid that can be reduced by Srx. This mechanistic cycle applies to both, 1-Cys and 2-Cys Prx. The latter form an intermediate, intramolecular disulfide that is then reduced by thiols (RSH can, for example, be thioredoxin).

The simplicity of the catalytic mechanism is, however, only half the story. Unlike most metal ions, the cysteine residue can take on many different oxidation states *in vivo* (a recent count of sulfur oxidation states that occur *in vivo* stands just short of ten[2]). Hydrogen peroxide, the natural substrate of Prx, is able to rapidly and effectively oxidise sulfenic to sulfinic acids, and such an 'overoxidation' of the sulfenic to a sulfinic acid with subsequent loss of enzyme activity has been observed in Prx enzymes.[7,9] This transformation has dramatic biochemical implications, the whole extent of which is only slowly becoming apparent.[4]

As illustrated in Scheme 2, substrate-induced enzyme inhibition makes sense from a sensing point of view. Under normal conditions, Prx detoxifies hydrogen peroxide, using the thiol/sulfenic acid redox cycle. In the presence of extreme oxidative stress (OS), however, the concentration of peroxide exceeds the capacity of the enzyme and leads to sulfinic acid formation to form an inactive enzyme.[9] Prx 'switches off' to allow further build-up of peroxide. The cysteine residue in Prx can therefore be seen as a 'sensor' for intracellular hydrogen peroxide concen-



Scheme 2 Postulated sensing and feedback mechanism for peroxiredoxins. Sensing is provided by the active site amino acid cysteine, whose thiol group is able to alternate between the thiol, sulfenic and sulfinic acid oxidation states. While the former two oxidation states are used for catalytic activity, the latter two provide the 'redox switch'.

trations (and OS), with the sulfenic acid providing a 'switch' from catalytic antioxidant defence in the presence of elevated amounts of peroxide to apoptosis once the peroxide concentration has reached limits that can no longer be tolerated by the cell.[4,12]

As a consequence, oxidation of thiols (and sulfenic acids) to sulfinic acids might endow proteins with a simple, internal sensor for OS (primarily peroxide) and allow them to 'respond' accordingly (*i.e.* by changing their activity). Interestingly, sulfinic acids can be seen as an almost ideal sensor for OS, since they are formed in the presence of a range of reactive oxygen and nitrogen species, such as peroxides, superoxide and peroxynitrite.[13] Furthermore, sulfinic acids provide the endpoint of many sulfur based redox cascades, such as the disulfide-*S*-dioxide pathway recently discussed in the context of Reactive Sulfur Species.[3,14,15] The extent of sulfinic acid formation would therefore be a good measure of 'total' OS in the cell.

The status of sulfinic acids in proteins, primarily Prx, has therefore changed dramatically over the last few years from 'unfortunate accident' to 'almost ideal redox switch'. Aebersold's proteomics studies, where a large number of proteins containing sulfinic acid modifications were found,[6] combined with mounting evidence that a range of other proteins, such as the transcription factors AP-1 (Jun) and Fos,[16] protein tyrosine phosphatases B (PTPBs)[17] and the insulin receptor,[18] are also controlled by similar, cysteine-based redox processes, hints at a much more widespread *in vivo* occurrence and function of sulfinic acids than previously thought.

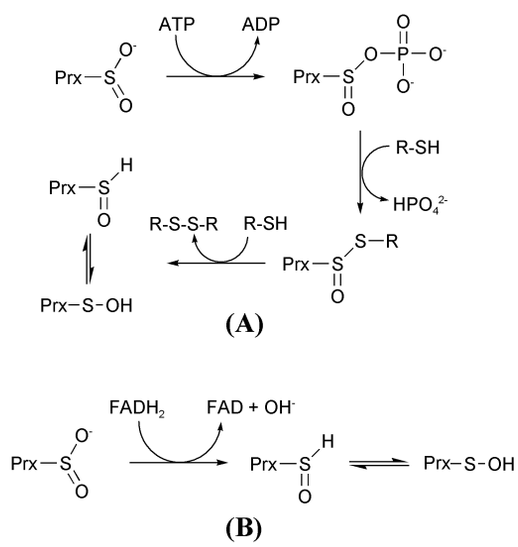
Nevertheless, *irreversible* sulfinic acid formation in Prx seems to be highly uneconomical: the sensor, once it has responded to OS, would become useless, and the protein would be wasted. At this point, the sulfinic acid story takes an unexpected, yet amazing twist with the appearance of a new player on the stage, called sulfiredoxin.

Sulfiredoxin and sulfinic acid reduction

From a physiological perspective, the notion of a sulfenic/sulfinic acid 'switch' might entail a cellular rescue pathway for reinstating the enzyme's antioxidant activity once OS has subsided. This would, however, require a partner for Prx with the exceptional ability to reduce a sulfinic acid under physiological conditions. The possibility of sulfinic acid reduction was first conclusively described in *Science* in April 2003.[7] Woo *et al.* used mammalian cell lines to show that 'overoxidized' Prx enzymes were reactivated *in vivo*, presumably by a system able to reduce sulfinic acid to thiol.[7] Although the authors were unable to identify such a protein at the time, there was clear evidence of sulfinic acid reductase activity in their system. Details about the isolation of such a protein from yeast, its substrate peroxiredoxin Tsa1, and its possible catalytic mechanism were published in October 2003.[10] This protein was named sulfiredoxin (Srx1), and an analysis of the human genome has confirmed the presence of a similar gene in humans.[10] It is therefore likely that sulfinic acid formation is also reversible in human Prx enzymes, and possibly other overoxidized proteins. Rather than being an irreversible, 'dead end' process, sulfinic acid formation might be controlled by a combination of oxidative stressors, Prx and Srx to provide a novel way of sensing, regulating and signalling redox changes within the cell.

From a chemist's point of view, one of the most interesting questions resulting from the discovery of sulfiredoxin is its redox mechanism. Under physiological conditions *in vitro*, sulfinic acids are notoriously difficult to reduce to thiols.[19] For example, cysteine sulfinic acid cannot be reduced by glutathione at pH 7, even if a large excess of reducing agent is used. This distinguishes it from cysteine sulfenic acid that is readily reduced by thiols in a reaction involving nucleophilic attack of the thiol(ate) at sulfur and subsequent substitution of OH^-. In sulfinic acids, however, the hydroxyl group of the acid is fully deprotonated at neutral pH and cannot serve as leaving group (pK_a less than 2 for cysteine sulfinic acid,[20] compared to 8.5 for cysteine thiol and 6.1 for cysteine sulfenic acid[21]).

It has therefore been suggested that the sulfinic acid is phosphorylated first to generate a good leaving group that can then be replaced by a thiol(ate) to form phosphate and a disulfide-*S*-monoxide (thiosulfinate, RS(O)SR) in the process (Scheme 3).[10] The latter is known to react readily with thiols (R′SH) and, after oxidizing four thiol equivalents, forms itself two fully reduced thiols (RSH). Such a combination of a phosphorylation reaction and a set of thiol-based nucleophilic substitution reactions provides an elegant solution to the sulfinic acid reduction problem. While there is little chemical evidence for phosphorylated sulfinic acid species in the literature to date, other sulfinic acid modifications might also form leaving groups. From a chemist's perspective, sulfinic acid



Scheme 3 Srx1-catalysed sulfinic acid reduction as postulated by Biteau *et al.* (**A**).[10] For comparison, a reaction modelled on bacterial NADH peroxidase is shown in (**B**).

reduction has hardly been studied recently and, with the growing biochemical interest, might now experience a renaissance.

Although there are few similarities between sulfenic and sulfinic acids as far as their respective reactivities are concerned, considering the mechanistic pathways for sulfenic reduction in proteins is rather instructive. While nucleophilic substitution reactions play a role in the Prx enzymes, hydride transfer from NADH to sulfenic acid *via* FAD is used by bacterial NADH oxidases and NADH peroxidases (Scheme 3).[2]

Although speculative at this time, a similar mechanism for sulfinic acid reduction would be very effective: Initial hydride transfer to a (phosphorylated) sulfinic acid, resulting in a sulfenic acid and the leaving group, would be followed by a second hydride transfer step, straightaway leading to the formation of thiol and OH^-. While the nucleophilic exchange mechanism to reduce a (phosphorylated) sulfinic acid to thiol requires a total of four nucleophilic substitutions, the hydride transfer pathway is much simpler and only requires two subsequent hydride transfers from $FADH_2$ (Scheme 3). In addition, it avoids the formation of reactive intermediates, such as disulfide-*S*-oxides that can undergo uncontrolled reactions in the presence of cysteine residues (a specific disulfide-*S*-oxide reductase has not been identified to date).

Outlook and future directions

The discovery of Srx1 opens the door to exciting, yet hardly understood biological redox-transformations. Very recent publications, such as a paper by Budanov *et al.* in the April 2004 issue of *Science*, not only support the new idea of sulfinic acid redox switches in proteins, but also underline the enormous excitement this important area of biological redox chemistry has started to generate.[22] The evaluation of the structure, substrate specificity, redox properties, mechanism and biological role of the Srx proteins will, no doubt, be an important step towards a better understanding of sulfinic acids, their reduction and their role *in vivo*. These studies will bring together research (and researchers) from organic chemistry, spectroscopy, biochemistry, cell biology and physiology. While the work by Biteau *et al.* should be seen as a first, major breakthrough, one can safely expect more excitement in this field to come. This is particularly true for sulfinic acid based redox sensing and signalling, an area only just emerging. In addition, its is not inconceivable that sulfinic acids could also partake in redox catalysis, *e.g.* as part of a sulfenic/sulfinic acid pair catalysing oxygen transfer reactions (Scheme 1).

While cysteine sulfinic acid illustrates best the recent excitement in biological cysteine oxidation, other modifications, such as cysteine sulfenic acid and cysteine-centred radicals receive growing attention since they can now be better identified in proteins thanks to progress in spectroscopic techniques. Similarly, disulfide-*S*-oxides (thiosulfinates and thiosulfonates) are just emerging as part of the redox transformations of cysteine; some of these chemical species are enzymatically formed from sulfoxides in truly stunning pieces of catalytic (bio)chemistry.[23] Considering that the amino acid cysteine is present in almost all proteins, such modifications are likely to be more widespread—and considerably more important in Biology than previously thought.

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References

1 N. M. Giles, A. B. Watts, G. I. Giles, F. H. Fry, J. A. Littlechild and C. Jacob, *Chem. Biol.*, 2003, **10**, 677–693.
2 C. Jacob, G. I. Giles, N. M. Giles and H. Sies, *Angew. Chem., Int. Ed. Engl.*, 2003, **42**, 4742–4258.
3 G. I. Giles, K. M. Tasker and C. Jacob, *Free Radical Biol. Med.*, 2001, **31**, 1279–1283.
4 L. B. Poole, P. A. Karplus and A. Claiborne, *Annu. Rev. Pharmacol. Toxicol.*, 2004, **44**, 325–347.
5 T. Finkel, *FEBS Lett.*, 2000, **476**, 52–54.
6 T. Rabilloud, M. Heller, F. Gasnier, S. Luche, C. Rey, R. Aebersold, M. Benahmed, P. Louisot and J. Lunardi, *J. Biol. Chem.*, 2002, **277**, 19396–19401.
7 H. A. Woo, H. Z. Chae, S. C. Hwang, K. S. Yang, S. W. Kang, K. Kim and S. G. Rhee, *Science*, 2003, **300**, 653–656.
8 Z. A. Wood, L. B. Poole and P. A. Karplus, *Science*, 2003, **300**, 650–653.
9 E. Schröder, J. A. Littlechild, A. A. Lebedev, N. Errington, A. A. Vagin and M. N. Isupov, *Structure*, 2000, **8**, 605–615.
10 B. Biteau, J. Labarre and M. B. Toledano, *Nature*, 2003, **425**, 980–984.
11 H. Z. Chae, H. J. Kim, S. W. Kang and S. G. Rhee, *Diabetes Res. Clin. Pract.*, 1999, **45**, 101–112.
12 J. R. Stone, *Arch. Biochem. Biophys.*, 2004, **422**, 119–124.
13 R. I. Viner, T. D. Williams and C. Schoneich, *Biochemistry*, 1999, **38**, 12408–12415.
14 G. I. Giles, K. M. Tasker, C. Collins, N. M. Giles, E. O'Rourke and C. Jacob, *Biochem. J.*, 2002, **364**, 579–585.
15 G. I. Giles and C. Jacob, *Biol. Chem.*, 2002, **383**, 375–388.
16 A. Claiborne, J. I. Yeh, T. C. Mallett, J. Luba, E. J. Crane, V. Charrier and D. Parsonage, *Biochemistry*, 1999, **38**, 15407–15416.
17 R. L. van Montfort, M. Congreve, D. Tisi, R. Carr and H. Jhoti, *Nature*, 2003, **423**, 773–777.
18 E. Schmid, A. Hotz-Wagenblatt, V. Hack and W. Droge, *FASEB J.*, 1999, **13**, 1491–1500.
19 K. Brocklehurst, F. Willenbrock and E. Salih, in *Hydrolytic Enzymes*, ed. A. Neuberger and K. Brocklehurst, Elsevier, Amsterdam, 1987, p. 39–158.
20 C. M. Holman and W. F. Benisek, *Biochemistry*, 1994, **33**, 2672–2681.
21 H. J. Forman, M. Torres and J. Fukuto, *Mol. Cell. Biochem.*, 2002, **234**, 49–62.
22 A. V. Budanov, A. A. Sablina, E. Feinstein, E. V. Koonin and P. M. Chumakov, *Science*, 2004, **304**, 596–600.
23 K. C. Agarwal, *Med. Res. Rev.*, 1996, **Vol. 16**, 111–124.
24 P. J. Kraulis, *J. Appl. Crystallogr.*, 1991, **24**, 946–950.