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REDOX REGULATION

PARSING PROTEIN SULFINIC ACID SWITCHES

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The proteome-wide application of a probe that selectively labels cysteine residues oxidized to the sulfinic acid form reveals the mammalian *S*-sulfinylome and uncovers novel substrates of the sulfinyl reductase sulfiredoxin, opening yet unexplored realms of cysteine-based redox regulation.

The sulfur atom of the thiol side chain of cysteine (Cys) endows this amino acid with a rich redox chemistry that is exploited in multiple cellular functions. Sulfur chemical reactivity also renders cysteine vulnerable to oxidation to the sulfinic (R-SO₂H) and sulfonic (R-SO₃H) acid forms, which were historically considered to be irreversible oxidative protein damage. The advent of mass spectrometry (MS) allowed the identification of cysteine sulfinic acids in a handful of proteins, in particular the antioxidant peroxiredoxin family (Prx)¹, and then showed that, unexpectedly, the R-SO₂H moiety can be reduced by sulfiredoxin (Srx)². Still, the biological scope of cysteine residue sulfinylation has remained totally unexplored due to a lack of tools for its identification. In a new study, Akter and Fu et al. developed a chemical tool, named DiaAlk, for selectively trapping and tagging sulfinic acids³. Combined with click chemistry and quantitative proteomics, DiaAlk made it possible to reveal the native *S*-sulfinylome of human cell lines and, remarkably, to identify new Srx substrates.

Recognition of the extraordinary protein functionality endowed by cysteine residues has followed the slow pace of biochemical and molecular tool development (Fig. 1). For decades, cysteine has been known as a residue that provides conformational stability to proteins by forming covalent disulfide bonds (R-SS-R') or by coordinating metals, or that serves as catalytic center in various classes of enzymes. Thioredoxin and thioredoxin reductase, which reduce disulfide bonds through thiol-disulfide exchange, were discovered as the hydrogen donor system for ribonucleotide reductase (RNR)⁴. Later discoveries included the regulation of protein function by disulfide bond formation in response to H₂O₂ (ref. 5), by *S*-nitrosylation (R-SNO) in response to nitric oxide⁶, and the regulated cellular production of H₂O₂ by NADPH oxidases (Noxs) during growth factor signaling⁷. These observations, together with the advent of MS, led to the new concept of cysteine-based redox sensing and signaling⁸.

The R-SOH form of the cysteine residue is central in redox sensing and signaling, occurring on multiple proteins in cells stimulated by growth factors, as shown by shotgun redox proteomics. R-SOH, which is formed by the reaction of a nucleophilic cysteine residue with H₂O₂, or by R-SNO hydrolysis, is intrinsically instable, as it readily forms a disulfide by condensation with a proximal thiolate, a sulfenylamide by reaction with a proximal nitrogen, or a sulfinic (R-SO₂H) and then a sulfonic acid (R-SO₃H) by further oxidation. The cysteine sulfinic acid cannot be reduced by common thiol reductases, and its initial identification in very few enzymes meant it was viewed as an exotic modification until its identification in Prxs. Prxs are antioxidants that reduce H₂O₂, thereby forming a catalytic R-SOH, which either condenses into a disulfide reduced by thioredoxin, or is further oxidized to the R-SO₂H form when levels of H₂O₂ are high, inactivating enzyme antioxidant function¹. The finding that sulfinylation is a genetically encoded attribute, occurring in eukaryotes but not prokaryotes⁹, and is reversed by enzymatic reduction by Srx² changed the prevailing view of this modification from an irreversible form of protein damage to an evolutionarily acquired regulatory switch. The *S*-sulfinic acid switch regulates several cellular functions by its effect on cellular H₂O₂ levels¹⁰, and also converts Prxs into a potent protein chaperone during peroxide stress and aging¹¹. Whether the *S*-sulfinic acid switch regulates other H₂O₂ or NO-responding proteins could not be addressed, until the development of DiaAlk.

Using DiaAlk labeling coupled to shotgun MS, Akter and Fu et al. identified a total of 387 S-sulfinylated sites in 296 proteins common to the two cell lines analyzed. They also found that 40% of the S-sulfinylated sites are also present in the S-sulfenylome, consistent with the lineage of these modifications. Interestingly, the S-sulfenylome is much more responsive to exogenous H₂O₂ compared to the S-sulfinylome, consistent with the more facile attack of H₂O₂ on a thiol than on R-SOH. Lastly, by comparing the turnover of R-SO₂H sites between wild type mouse fibroblasts and those lacking Srx, they identified 55 new potential substrates of Srx.

These data provide a robust tool to explore a new realm of redox biology, the sulfinylation of cysteine residues at the individual protein and proteome-wide levels. DiaAlk will be invaluable to decipher the role of cysteine sulfinic acid formation as a regulatory switch or as an irreversibly oxidized end product. The low responsiveness of S-sulfinylation to H₂O₂ suggests that these events accumulate during proteins' lifetime, and thus should serve as good markers of chronic oxidative stress. Reduction of S-sulfinylated cysteine residues by sulfiredoxin could be a repair mechanism, or alternatively, indicate the presence of a regulatory switch. S-sulfinylation is probably non-enzymatic, requiring relatively high levels of H₂O₂, other peroxides, or other electrophiles. Its occurrence as a regulatory switch might thus be seen under oxidative stress, or within subcellular compartments accumulating H₂O₂ as is the case for one of the newly identified Srx substrates, the protein tyrosine phosphatase PTPN12. To elucidate the role of the sulfinic acid switches of other new sulfiredoxin substrates such as Parkinson disease protein DJ-1 and actin regulator cofilin, we will have to consider that oxidative stress is part of normal physiology. The newly identified Srx substrates also raise exciting questions about the slow kinetics and specificity of Srx catalysis.¹²

Figure 1. Milestone discoveries in redox biology. The progression of available tools and approaches has facilitated the expansion of known modifications and functions of cysteine residues.

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