ANTIOXIDANTS & REDOX SIGNALING
Volume 10, Number 9, 2008

DOI: 10.1089/ars.2008.2059

Forum Editorial

Peroxidase: A Term of Many Meanings

Leopold Flohé[1] and Fulvio Ursini[2]

Abstract

Peroxidase research has been instrumental in defining the principles of chemical catalysis. By now, enzymes termed peroxidases represent a heterogeneous group of distinct enzyme families that operate by different catalytic principles and fulfill diverse biological functions, detoxifying H_2O_2 being just one of many aspects. H_2O_2-dependent synthesis of secondary metabolites is the domain of heme peroxidases and related enzymes operating by transition metal catalysis, that often is mediated by free radical formation. Instead, the coenzyme-free glutathione peroxidases and peroxiredoxins only catalyze two-electron transitions and, thus, can reliably remove hydroperoxides without causing radical-mediated collateral damage. However, their ability to use hydroperoxides for the formation of specific disulfide bonds with and within particular proteins broadens their spectrum of biological activities to differentiation phenomena, redox regulation of metabolic processes, redox sensing, and signalling. The present Forum Editorial tries to guide the reader through the 190 years of equally bewildering and fascinating research on peroxidases up to the topical frontiers of the field that are addressed in this issue. *Antioxid. Redox Signal*. 10, 1485–1490.

Emerging Catalytic Principles

THE HISTORY OF PEROXIDASES starts in the early 19th century when the interpretation of biological phenomena was still dominated by the theory of vitalism. In 1818, the French baron Louis Jacques Thénard discovered *"eau oxygenée"* (H_2O_2) (75) and also observed that this novel compound is easily broken down by biological tissue. In contrast to the general thinking in those times, Thénard did *not* attribute the accelerated decomposition of H_2O_2 by biological material to a mysterious "vital force," but to the presence of a particular "substance," thus anticipating the concept of catalysis, which was later developed by Jöns Jacob Berzelius (3). The miraculous catalytic power was finally attributed to an "enzyme" that appeared to be ubiquitous in nature and, as a kind of prototype of a natural catalytic entity, was called "catalase" by Loew in 1900 (45) (now EC 1.11.1.6). A decomposition of H_2O_2, associated with the formation of colored compounds from polyphenols, was also detected in plants and animals already in the 19th century by Schönbein (69) and named "peroxidases" (now EC 1.11.1.7) by Linossier (44). During the first half of the last century, catalases and peroxidases were isolated, and their catalytic mechanism was recognized to be similar: In both cases, the "hematin" prosthetic group was shown to be oxidized to yield "compound I" by the strong oxidant H_2O_2 (E_0 for its reduction to water = 1.776 V) and then reduced again by H_2O_2 itself (E_0 for its oxidation to dioxygen = –0,682 V) in catalase, which thus catalyzes a dismutation of H_2O_2, or by an other reductant in the other peroxidases (36, 76). Due to the typical redox-sensitive spectral characteristics of their prosthetic group, the heme peroxidases soon became the favorite playground of spectroscopists, and fertilized many fields of biochemistry such as enzyme kinetics, the stopped-flow analysis of fast reactions (7, 68), and the biology of respiration and oxidative phosphorylation (9).

Up until the 1970s, it almost remained a dogma that peroxidases (EC 1.11.1.-) are heme proteins (8, 54). This fascination by heme catalysis hampered the appreciation of unrelated proteins sharing reaction specificity with the heme peroxidases. When Gordon C. Mills described a novel peroxidase that apparently was devoid of any colored prosthetic group (51), his discovery was accepted with great scepticism. In fact, the enzyme was declared not to exist at a meeting of the Federation of the American Societies of Experimental Biology in the early 1960s (Gerald Cohen, Mount Sinai School

[1]MOLISA GmbH, Magdeburg, Germany.
[2]Department of Biological Chemistry, University of Padova, Padova, Italy.

of Medicine, New York, 2001, personal communication). Mills' glutathione peroxidase (now known as GPx-1; EC1.11.1.9) had to wait for 16 years until it resurrected as the first selenoprotein to be discovered by Flohé (24) and Rotruck (66) in 1973 and, thus, marks the beginning of the still evolving field of selenium biochemistry (27, 43). In retrospect, selenium catalysis could explain the extraordinary rate constants for the reaction of glutathione peroxidases with hydroperoxides (46, 65) that had been shown to be equivalent to, or even higher than, those of heme peroxidases (26). GPx-type proteins have meanwhile grown up to a large and diversified family. Many of them turned out not to be specific for glutathione but react with protein thiols (*i.e.*, thioredoxin-type proteins), which renders their historic name obsolete (47). Their remaining characteristic feature is a redox-active (seleno)cysteine residue that is integrated into the protein chain and, in the 3-D structure (18), forms the center of a catalytic triad (Sec, Gln Trp) (18) or, more precisely, of a tetrad (Sec, Gln, Asn, Trp) (78). In most of the family members, the selenocysteine is actually replaced by cysteine, which usually results in lower specific activity. But rate constants near those observed with heme or selenoproteins may also be seen with some of the Cys homologues of GPxs (47), revealing that even a simple cysteine residue, if appropriately activated by its protein environment, can account for efficient peroxidatic activity. A prerequisite of the reactivity of C_P is a low pK_a of its thiol or selenol, respectively, since only the thiolate or selenolate forms, as strong nucleophiles, can readily attack the hydroperoxide to produce a sulfenic or selenenic acid derivative. However, the rate constants calculated for thiolates of several low molecular weight thiols are comparatively low, falling within the range of 18–26 M^{-1} sec^{-1} (84). Therefore, additional effects such as proton shuttling and polarization of the peroxide bond have to be considered to account for the enzymatic rate constants in the range of 10^5–10^7 (78) or $>10^8$ M^{-1} s^{-1} in the case of the seleno-enzymes (26). Similarly, low rate constants have been calculated for the oxidative inactivation of catalytic cysteine in tyrosine phosphatases (14), suggesting that unlikely only a low pK_a can account for the oxidation of a crucial thiol under physiological conditions.

Sulphur catalysis is also the hallmark of the most recently evolving peroxidase family, the peroxiredoxins (EC 1.11.1.15). Here the cysteine sulphur is activated by coordination with a threonine (or serine) residue and the positive charge of a nearby arginine (23). What now has become known under the term "peroxiredoxin" (Prx) was originally discovered by electron microscopy as the 10-membered ring-shaped macromolecule "torin" of erythrocytes far ahead of its functional characterization (30). More commonly, the discovery of peroxiredoxins is ascribed to Earl Stadtman and Sue Goo Rhee who described a "thiol-specific antioxidant protein" of yeast (37) that later turned out to be a thioredoxin peroxidase (64). Since, the peroxiredoxin family has grown up in the databases to more than 500 entries that comprise at least five distinct molecular clades (41). The common denominator is the above-mentioned catalytic triad in which the "peroxidatic cysteine" (C_P) is oxidized by a hydroperoxide to a sulfenic acid (61). Thus far, the mechanism is analogous to that of the glutathione peroxidases. As in glutathione peroxidases, also the regeneration of the ground-state enzyme is usually achieved by thiols but depends on the subfamilies. In a so-called "typical 2-cysteine peroxiredoxin" the proximal reductant of the sulfenic acid is a cysteine residue (C_R for "resolving cysteine") of a second subunit within the oligomeric protein; in "atypical 2-cysteine peroxiredoxins," the C_R is a cysteine of the same subunit. In both cases, the primarily formed inter- or intra-subunit disulfide bond is then typically reduced by "redoxins," that is, proteins with a thioredoxin fold and containing a Cys–X–X–Cys (or Cys–X–X–Sec) motif such as the flavin-based disulfide reductases of bacteria (59), the wide-spread thioredoxins (25), or the tryparedoxins of kinetoplasts (4, 55). In the 1-cysteine peroxiredoxins, the sulfenic acid form has to be directly reduced by substrate itself. For most of the 1-cysteine peroxiredoxins, the physiological reductant is still unknown; it may be glutathione (62) or ascorbate (53). Despite operating with sulphur catalysis, the rate constants for the oxidative and reductive part of peroxiredoxin catalysis may be impressive, the k'_{+1} and k'_{+2} values ranging from 2×10^3 to 4×10^7 $M^{-1}s^{-1}$ and 4×10^4 to 1×10^7 $M^{-1}s^{-1}$, respectively (79).

Starting from the 1950s, peroxidases using NADH (EC 1.11.1.1) or NADPH (EC 1.11.1.2) as reductant have been repeatedly described and characterized as flavoproteins (11, 52). To our best knowledge, however, direct involvement of a flavin cofactor in hydroperoxide reduction has never been demonstrated. Instead, the hydroperoxide acceptor appears to be a reactive cysteine again (60). Moreover, the metabolic links of GPx-type and Prx-type peroxidases to Cys–X–X–Cys and other cysteine-containing proteins, to GSH, other low molecular weight thiols and finally to NAD(P)H now provide a molecular basis for the majority of NAD(P)H-dependent peroxidase activities that were believed to represent enzymatic entities. Prominent examples are the alkylhydroperoxide reductases of enterobacteria (Ahp) being a two-component system of a disulfide reductase (AhpF) and a peroxiredoxin (AhpC) (6, 74), related thioredoxin-dependent Prxs of *Escherichia coli* (5), *Mycobacterium tuberculosis* (35), *Plasmodium* sp. (15), and others (16), and the "trypanothione peroxidase" activity of kinetoplasts that reflects a complex redox cascade comprising the NADPH-dependent trypanothione reductase, tryparedoxin, and tryparedoxin peroxidase (55), homologous mitochondrial isozymes, and an equally NADPH/ trypanothione/tryparedoxin-dependent GPx-type peroxidase (4, 42). In all these examples, the flavin is not directly involved in the peroxidatic function but belongs to the disulfide reductase of the system.

In short, nature does not rely on a single catalytic principle to reduce hydroperoxides. The catalysis of peroxidases may depend on iron (or manganese) porphyrin prosthetic groups, the highly reactive heteroatom selenium, an activated sulphur or, as not yet mentioned, exotic metal cofactors such as vanadate (81, 82).

Antioxidant, Pro-Oxidant, and Synthetic Roles of Peroxidases

The diversity of catalytic mechanisms of peroxidases is reflected in diverse, in part opposite, roles. The obvious reflex to consider peroxidases as antioxidant enzymes, because they eliminate H_2O_2, other hydroperoxides, or peroxynitrite, misleads in most cases.

It has for long been recognized that mammalian heme-

containing haloperoxidases (EC 1.11.1.8 and 1.11.1.10) are by no means "antioxidants." Clearly, myeloperoxidase, eosinophil peroxidase (39), salivary peroxidase (40), and lactoperoxidase (29) use H_2O_2 to generate more aggressive oxidants to fight intruding micro-organisms (38), and the bactericidal cocktail produced by these enzymes also accounts for oxidative tissue damage in infectious and inflammatory diseases (13). In fact, heme peroxidases in general, with the notable exception of catalases, rather tend to promote than to inhibit oxidative damage, because the reduction of the oxidized enzymes ("compound I") is often achieved by one-electron transitions, which implies formation of free radicals from the reducing substrates that may lead to chain reactions. In prokaryotes, heme proteins often display both catalase and peroxidase activity (86). Accordingly, these enzymes are pivotal to the elimination of H_2O_2, but may simultaneously promote oxidations, as is, for example, evident from the oxidative activation of isoniazid to the real bactericidal agent by KatG in *Mycobacterium tuberculosis* (72).

The oxidative potential of heme peroxidases, however, may also be used for specific syntheses. One of the best documented examples is the synthesis of thyroid hormones by iodide peroxidase (70, 71). The ability to halogenate organic compounds is, however, not restricted to heme peroxidases but is equally achieved by the vanadium enzymes (81). Another example of specific synthesis is the formation of prostaglandin H from arachidonate by the heme peroxidase component within the cyclo-oxygenase complex (56). Heme peroxidases of plants are also implicated in the synthesis of secondary metabolites (2), but it remains a challenge to work out the specific functions of the individual plant enzymes which *in toto* are presumed to have more functions than a Swiss army knife (57).

In contrast, peroxidases operating by selenium or sulphur catalysis tend to only catalyze two-electron transitions and, thus, allow a safer forecast of their metabolic role. They were, and still are, widely assumed to lower hydroperoxide levels, thereby counteracting oxidative stress without any complications due to unspecific by-products. In essence, this expectation could be corroborated. Free radical intermediates of GPx- or Prx-type peroxidases have never been detected and ample evidence accumulated reveals that, at least for GPx-1 in mammals (22), the Prx-dominated trypanothione system of kinetoplasts (4) and bacterial AhpC and thioredoxin peroxidases (16) are indeed important antioxidant devices. Nevertheless, the seemingly semantic question remains whether these enzymes are primarily used to reduce hydroperoxides or, as, for instance, iodide peroxidase, use hydroperoxides for purposes that are unrelated to antioxidant defence. GPx-4 (EC 1.11.1.12) may be quoted as striking example for the latter option: During mammalian spermiogenesis, GPx-4 behaves like a "moonlighting protein" in transforming itself, at the expense of hydroperoxides, from an active peroxidase into a constituent of the mitochondrial sheath (80), a process that is indispensable for appropriate sperm function (21). Mechanistically, the oxidized selenocysteine specifically selenylates a cysteine residue of GPx-4 itself, whereby its peroxidase activity is abrogated and GPx polymers are synthesized (49). A "pro-oxidant" role is also observed with the nuclear form of GPx-4: It promotes chromatin condensation by catalyzing protein disulfide formation (12). Similarly, the products of peroxiredoxin catalysis (*i.e.*, oxidized redoxins), may have a greater physiological impact than a small decline of the concomitant hydroperoxide tone (see below). Furthermore, peroxiredoxins tend to thiolate other proteins *via* their oxidized C_P (33), a phenomenon that is gaining increasing attention in the context of redox regulation.

Thiol-Dependent Peroxidases In Redox Regulation

The concept of protein phosphorylation/dephosphorylation (20) has long overshadowed the relevant redox processes in metabolic regulation. In the meantime, redox sensitivity of many phosphorylation cascades has been firmly established (19), and it thus has become evident that both regulatory principles are interrelated and complementary (10). Moreover, the upstream effectors such as insulin, other hormones, cytokines, and growth factors themselves proved to induce the formation of $\cdot O_2^-$, H_2O_2, or lipid hydroperoxides, which modulate their signaling cascades (1, 17, 28, 48, 50).

Interestingly, probably the first enzyme reported to be redox regulated was a phosphatase. Starting in 1965, Pontremoli and Horrecker reported on the activation of fructose diphosphatase and, in consequence, gluconeogenesis by physiological disulfides such as cystamine and oxidized CoA (58), and later hydroperoxide exposure was shown to induce gluconeogenesis in perfused rat liver (73). Also protein phosphatase activity proved to be modulated by redox events (31). Typically, these phosphatases are inhibited by H_2O_2 and, thus, protein kinase-mediated signaling is often enhanced under oxidizing conditions. This mode of oxidant-dependent modulation of protein phosphorylation has now become a generally accepted regulatory principle (63). Other modes of redox regulation of signaling cascades that primarily depend on phosphorylation are, for example, the inhibitory binding of reduced, but not oxidized thioredoxin to the apoptosis signal regulating kinase-1 (ASK1) (67), facilitating DNA binding of NFκB by reduced thioredoxin (32), and the oxidation of pivotal cysteines in Keap1 that results in nuclear translocation and activation of the transcription factor Nrf2 (34).

The molecular analysis of redox phenomena with regulatory impact still appears to be rudimentary. The ideas that redox regulation is just a consequence of the cellular "redox potential," "peroxide tone," "thiol/disulfide status," or "free radical generation" are unsatisfactory, since they do not meet two basic requirements of meaningful regulatory circuits: i) specificity of messengers, and ii) separate switches to control activation and termination. Clearly, aggressive radicals such as OH· or RO· that attack a large variety of biomolecular targets can be disregarded as specific effectors of regulatory processes. Related concern may also be valid for H_2O_2, peroxynitrite or lipid peroxidation products, but these less promiscuously reacting oxidants may be rendered more specific by peroxidases acting as peroxide sensors which, as oxidized forms, selectively interact with target proteins. Alternatively, the target protein itself might sense the oxidant by means of an exceptionally reactive thiol, as is proposed for some phosphatases. For regulatory and chemical reasons, such activation process can hardly be reversed by a direct backward reaction. A sulfenic acid, once formed by the reaction of a hydroperoxide with a protein target, is not re-

versed by lowering the peroxide tone, and a sulfinic acid requires enzymatic repair (85). Mixed disulfides between target proteins and GSH or other proteins are not readily reversed either when the thiol/disulfide status returns to normal, but are regenerated with the aid of glutaredoxin, thioredoxin, or others. A few examples of how thiol-dependent peroxidases contribute to redox regulation have been reported in recent years. Vivancos and coworkers (83) describe a yeast peroxiredoxin acting as H_2O_2 sensor in the activation of the transcription factor Pap1 which regulates antioxidant gene transcription in response to H_2O_2. Interestingly, the Pap1 activation occurs only at low H_2O_2 concentrations, which implies that a protective mechanism is triggered before the peroxide can cause unspecific damage. At high peroxide exposure, the peroxiredoxin is inactivated due to oxidation to a sufinic acid and instead triggers the mitogen-activated protein kinase Sty1 pathway, the latter event being specifically reversed by the sulfireductase Srx1 (83). A second example involves a GPx-type protein, Orp1, as H_2O_2 sensor. When the C_P of Orp1 is oxidized to the sulfenic acid form, it becomes disulfide-linked to the transcription factor Yap1 which thereby is activated; the shut-off part of the regulatory circuit (*i. e*., regeneration of reduced Orp1 and Yap 1 from the hetero-dimer), is then catalyzed by thioredoxin (77). In both cases, in analogy to the GPx4 polymerization mentioned above, the peroxidases themselves act as protein-thiylating agents, which might turn out to be regulatory principle that is by far more important than lowering the peroxide level.

Why a Forum Issue Now?

It may seem impossible to squeeze a diversified field such as the peroxidases into a small ARS issue. The attempt may also appear superfluous in view of the realm of published reviews. However, the area is at present developing so quickly that most of these compilations are already out-dated at the time of publication. More importantly, many aspects of the area have not yet reached the awareness of most researchers that were interested in "oxidative stress" and related problems. The focus has long been on oxidant-derived pathology, the long known benefits of the oxidative burst in the defence against infections has hardly become a priority issue of the various clubs dedicated to free radicals research, and the emerging interest in redox regulation still suffers from limited understanding of underlying mechanisms.

As it commonly happens in biochemistry, also in the peroxidase field mechanistic analyses in micro-organisms and small experimental animals are paving the way to medical application, although it is hard to forecast what of the emerging knowledge will turn out to be valuable. The intention of the guest editors, therefore, was to present some topical state-of-the-art reviews and stimulating papers in order to broaden the vision towards the following aspects: (a) the fundamental differences of peroxidase families and the diversity within the peroxidase families; (b) the evolutionary diversifications of antioxidant enzyme systems, particularly in pathogens, and their possible therapeutic implications; (c) unsuspected peroxidase functions that might help to unravel signalling specificity, complexity of redox regulation, and metabolic adaptation; and (d) molecular mechanisms of peroxidases which, beyond their stand-alone interest, may disclose general features of peroxide susceptibility of biomolecules.

We accompany this Forum issue with the hope of convincing the reader that one of the oldest subjects of biochemical sciences ever was, and still is, good for surprises and, in view of its theoretical and practical implications, deserves continuing attention.

References

1. Aslan M and Ozben T. Oxidants in receptor tyrosine kinase signal transduction pathways. *Antioxid Redox Signal* 5: 781–788, 2003.
2. Barceló a RA and Pomara F. Plant peroxidases: versatile catalysts in the synthesis of bioactive natural products. *Bioactive Natural Products* 27: 735–791, 2002.
3. Berzelius JJ. Einige Ideen über eine bei der Bildung organischer Verbindungen in der lebenden Natur wirksame, aber bisher nicht bemerkte Kraft (translated into German by F. Wöhler). *Jahres-Bericht über die Fortschritte der physischen Wissenschaften* 15: 237–245, 1836.
4. Castro H and Tomás AM. Peroxidases of trypanosomatids. *Antioxid Redox Signal* 10: 1593–1606, 2008.
5. Cha MK, Kim HK, and Kim IH. Thioredoxin-linked "thiol peroxidase" from periplasmic space of *Escherichia coli*. *J Biol Chem* 270: 28635–28641, 1995.
6. Chae HZ, Robison K, Poole LB, Church G, Storz G, and Rhee SG. Cloning and sequencing of thiol-specific antioxidant from mammalian brain: alkyl hydroperoxide reductase and thiol-specific antioxidant define a large family of antioxidant enzymes. *Proc Natl Acad Sci USA* 91: 7017–7021, 1994.
7. Chance B and Herbert D. The enzyme substrate compounds of bacterial catalase and peroxides. *Biochem J* 46: 402–414, 1950.
8. Chance B and Schonbaum GR. The nature of the primary complex of catalase. *J Biol Chem* 237: 2391–2395, 1962.
9. Chance B and Williams GR. The respiratory chain and oxidative phosphorylation. *Adv Enzymol Relat Areas Mol Biol* 17: 65–134, 1956.
10. Chiarugi P and Buricchi F. Protein tyrosine phosphorylation and reversible oxidation: two cross-talking posttranslation modifications. *Antioxid Redox Signal* 9: 1–24, 2007.
11. Conn EE, Kraemer LM, Liu PN, and Vennesland B. The aerobic oxidation of reduced triphosphopyridine nucleotide by a wheat germ enzyme system. *J Biol Chem* 194: 143–151, 1952.
12. Conrad M, Moreno SG, Sinowatz F, Ursini F, Kolle S, Roveri A, Brielmeier M, Wurst W, Maiorino M, and Bornkamm GW. The nuclear form of phospholipid hydroperoxide glutathione peroxidase is a protein thiol peroxidase contributing to sperm chromatin stability. *Mol Cell Biol* 25: 7637–7644, 2005.
13. Davies MJ, Hawkins CL, Pattison DI, and Rees MD. Mammalian heme peroxidases: from molecular mechanisms to health implications. *Antioxid Redox Signal* 10: 1199–1234, 2008.
14. Denu JM and Tanner KG. Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. *Biochemistry* 37: 5633–5642, 1998.
15. Deponte M, Rahlfs S, and Becker K. Peroxiredoxin systems of protozoal parasites. In: *Peroxiredoxin Systems. Structures and Functions. Subcellular Biochemistry 44*, edited by Flohé L and Harris JB. New York: Springer, 2007, pp 219–229.

16. Dubbs JM and Mongkolsuk S. Peroxiredoxins in bacterial antioxidant defense. In: *Peroxiredoxin Systems. Structures and Functions. Subcellular Biochemistry 44*, edited by Flohé L and Harris JR. New York: Springer, 2007, pp 143–193.
17. Eling TE and Glasgow WC. Cellular proliferation and lipid metabolism: importance of lipoxygenases in modulating epidermal growth factor-dependent mitogenesis. *Cancer Metastasis Rev* 13: 397–410, 1994.
18. Epp O, Ladenstein R, and Wendel A. The refined structure of the selenoenzyme glutathione peroxidase at 0.2-nm resolution. *Eur J Biochem* 133: 51–69, 1983.
19. Finkel T. Redox-dependent signal transduction. *FEBS Lett* 476: 52–54, 2000.
20. Fischer EH. Cellular regulation by protein phosphorylation: a historical overview. *Biofactors* 6: 367–374, 1997.
21. Flohé L. Selenium in mammalian spermiogenesis. *Biol Chem Hoppe Seyler* 388: 987–995, 2007.
22. Flohé L and Brigelius–Flohé R. Selenoproteins of the glutathione system In: *Selenium. Its Molecular Biology and Role in Human Health*, edited by Hatfield D, Berry MS, and Gladyshev VN. Heidelberg, New York: Springer, 2006, pp 161–172.
23. Flohé L, Budde H, Bruns K, Castro H, Clos J, Hofmann B, Kansal–Kalavar S, Krumme D, Menge U, Plank–Schumacher K, Sztajer H, Wissing J, Wylegalla C, and Hecht HJ. Tryparedoxin peroxidase of *Leishmania donovani*: molecular cloning, heterologous expression, specificity, and catalytic mechanism. *Arch Biochem Biophys* 397: 324–335, 2002.
24. Flohé L, Günzler WA, and Schock HH. Glutathione peroxidase: a selenoenzyme. *FEBS Lett* 32: 132–134, 1973.
25. Flohé L and Harris JR. (Eds). *Peroxiredoxin Systems. Structures and Functions. Subcellular Biochemistry 44*. New York, Springer, 2007, pp 1–389.
26. Flohé L, Loschen G, Günzler WA, and Eichele E. Glutathione peroxidase, V. The kinetic mechanism. *Hoppe Seylers Z Physiol Chem* 353: 987–999, 1972.
27. Fomenko DE, Xing W, Adair BM, Thomas DJ, and Gladyshev VN. High-throughput identification of catalytic redox-active cysteine residues. *Science* 315: 387–389, 2007.
28. Griendling KK and Ushio–Fukai M. Reactive oxygen species as mediators of angiotensin II signaling. *Regul Pept* 91: 21–27, 2000.
29. Hamon CB and Klebanoff SJ. A peroxidase-mediated, streptococcus mitis-dependent antimicrobial system in saliva. *J Exp Med* 137: 438–450, 1973.
30. Harris JR. Release of a macromolecular protein component from human erythrocyte ghosts. *Biochim Biophys Acta* 150: 534–537, 1968.
31. Heffetz D, Bushkin I, Dror R, and Zick Y. The insulinomimetic agents H_2O_2 and vanadate stimulate protein tyrosine phosphorylation in intact cells. *J Biol Chem* 265: 2896–2902, 1990.
32. Hirota K, Murata M, Sachi Y, Nakamura H, Takeuchi J, Mori K, and Yodoi J. Distinct roles of thioredoxin in the cytoplasm and in the nucleus. A two-step mechanism of redox regulation of transcription factor NF-kappaB. *J Biol Chem* 274: 27891–27897, 1999.
33. Hofmann B, Hecht HJ, and Flohé L. Peroxiredoxins. *Biol Chem Hoppe Seyler* 383: 347–364, 2002.
34. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, and Yamamoto M. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev* 13: 76–86, 1999.
35. Jaeger T, Budde H, Flohé L, Menge U, Singh M, Trujillo M, and Radi R. Multiple thioredoxin-mediated routes to detoxify hydroperoxides in *Mycobacterium tuberculosis*. *Arch Biochem Biophys* 423: 182–191, 2004.
36. Keilin D and Hartree EF. Purification of horseradish peroxidase and comparison of its properties with those of catalase and methemoglobin *Biochem J* 49: 88–104, 1951.
37. Kim K, Kim IH, Lee KY, Rhee SG, and Stadtman ER. The isolation and purification of a specific "protector" protein which inhibits enzyme inactivation by a thiol/Fe(III)/O_2 mixed-function oxidation system. *J Biol Chem* 263: 4704–4711, 1988.
38. Klebanoff SJ. Myeloperoxidase: friend and foe. *J Leukoc Biol* 77: 598–625, 2005.
39. Klebanoff SJ. Iodination of bacteria: a bactericidal mechanism. *J Exp Med* 126: 1063–1078, 1967.
40. Klebanoff SJ and Luebke RG. The antilactobacillus system of saliva. Role of salivary peroxidase. *Proc Soc Exp Biol Med* 118: 483–486, 1965.
41. Knoops B, Loumaye E, and van der Eecken V. Evolution of the peroxiredoxins. In: *Peroxiredoxin Systems. Structures and Functions, Subcellular Biochemistry 44*, edited by Flohé L and Harris JR. New York: Springer, 2007, pp 27–40.
42. Krauth–Siegel LR, Comini MA, and Schlecker T. The trypanothione system. In: *Peroxiredoxin Systems. Structures and Functions, Subcellular Biochemistry 44*, edited by Flohé L and Harris JR. New York: Springer, 2007, pp 231–265.
43. Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigo R, and Gladyshev VN. Characterization of mammalian selenoproteomes. *Science* 300 : 1439–1443, 2003.
44. Linossier G. Contribution a l'étude des ferments oxidants. Sür la peroxidase du pups. *C R Soc Biol, Paris* 50: 373–375, 1898.
45. Loew O. A new enzyme of general I in organisms. *Science* 279: 701–702, 1900.
46. Maiorino M, Aumann KD, Brigelius–Flohé R, Doria D, van den Heuvel J, McCarthy J, Roveri A, Ursini F, and Flohé L. Probing the presumed catalytic triad of selenium-containing peroxidases by mutational analysis of phospholipid hydroperoxide glutathione peroxidase (PHGPx). *Biol Chem Hoppe Seyler* 376: 651–660, 1995.
47. Maiorino M, Ursini F, Bosello V, Toppo S, Tosatto SC, Mauri P, Becker K, Roveri A, Bulato C, Benazzi L, De Palma A, and Flohé L. The thioredoxin specificity of Drosophila GPx: a paradigm for a peroxiredoxin-like mechanism of many glutathione peroxidases. *J Mol Biol* 365: 1033–1046, 2007.
48. Maulik N and Das DK. Redox signaling in vascular angiogenesis. *Free Radic Biol Med* 33: 1047–1060, 2002.
49. Mauri P, Benazzi L, Flohé L, Maiorino M, Pietta PG, Pilawa S, Roveri A, and Ursini F. Versatility of selenium catalysis in PHGPx unraveled by LC/ESI-MS/MS. *Biol Chem Hoppe Seyler* 384: 575–588, 2003.
50. May JM and de Haen C. Insulin-stimulated intracellular hydrogen peroxide production in rat epididymal fat cells. *J Biol Chem* 254: 2214–2220, 1979.
51. Mills GC. Hemoglobin catabolism. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. *J Biol Chem* 229: 189–197, 1957.
52. Mizushima S and Kitahara K. Purification and properties of DPNH peroxidase in Lactobacillus case. *J Gen Appl Microbiol* 8: 56–62, 1962.
53. Monteiro G, Horta BB, Pimenta DC, Augusto O, and Netto LE. Reduction of 1-Cys peroxiredoxins by ascorbate changes the thiol-specific antioxidant paradigm, revealing another function of vitamin C. *Proc Natl Acad Sci USA* 104: 4886–4891, 2007.

54. Morrison M and Schonbaum GR. Peroxide-catalysed halogenations. *Ann Rev Biochemistry* 45: 861–888, 1976.
55. Nogoceke E, Gommel DU, Kiess M, Kalisz HM, and Flohé L. A unique cascade of oxidoreductases catalyses trypanothione-mediated peroxide metabolism in *Crithidia fasciculata*. *Biol Chem Hoppe Seyler* 378: 827–836, 1997.
56. Ohki S, Ogino N, Yamamoto S, and Hayaishi O. Prostaglandin hydroperoxidase, an integral part of prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes. *J Biol Chem* 254: 829–836, 1979.
57. Passardi F, Cosio C, Penel C, and Dunand C. Peroxidases have more functions than a Swiss army knife. *Plant Cell Rep* 24: 255–265, 2005.
58. Pontremoli S, Luppis B, Traniello S, Rippa M, and Horecker BL. Fructose diphosphatase from rabbit liver.IV. Sulfhydryl groups and their relation to the catalytic activity. *Arch Biochem Biophys* 112: 1–15, 1965.
59. Poole LB. Bacterial defenses against oxidants: mechanistic features of cysteine-based peroxidases and their flavoprotein reductases. *Arch Biochem Biophys* 433: 240–254, 2005.
60. Poole LB and Claiborne A. The non-flavin redox center of the streptococcal NADH peroxidase. II. Evidence for a stabilized cysteine-sulfenic acid. *J Biol Chem* 264: 12330–12338, 1989.
61. Poole LB and Ellis HR. Identification of cysteine sulfenic acid in AhpC of alkyl hydroperoxide reductase. *Methods Enzymol* 348: 122–136, 2002.
62. Ralat LA, Manevich Y, Fisher AB, and Colman RF. Direct evidence for the formation of a complex between 1-cysteine peroxiredoxin and glutathione S-transferase pi with activity changes in both enzymes. *Biochemistry* 45: 360–372, 2006.
63. Rhee SG. Cell signaling. H2O2, a necessary evil for cell signaling. *Science* 312: 1882–1883, 2006.
64. Rhee SG, Chae HZ, and Kim K. Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radic Biol Med* 38: 1543–1552, 2005.
65. Rocher C, Lalanne JL, and Chaudière J. Purification and properties of a recombinant sulfur analog of murine selenium-glutathione peroxidase. *Eur J Biochem* 205: 955–960, 1992.
66. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, and Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. *Science* 179: 588–590, 1973.
67. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, and Ichijo H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* 17: 2596–2606, 1998.
68. Schonbaum GR and Chance B. Catalase. In: *The Enzymes*, edited by Boyer PD. New York: Academic Press 1976, pp 363–408.
69. Schönbein CF. Ueber die Katalytische Wirksamkeit organischer Materien und deren Vebreitung in der Pflanzen-und Thierwelt. *J Prakt Chem* 98: 323–344, 1863.
70. Schweizer U, Chiu J, and Köhrle J. Peroxides and peroxide-degrading enzymes in the thyroid. *Antioxid Redox Signal* 10: 1577–1592, 2008.
71. Serif GS and Kirkwood S. Enzyme systems concerned with the synthesis of monoiodotyrosine. II. Further properties of the soluble and mitochondrial systems. *J Biol Chem* 233: 109–115, 1958.
72. Sherman DR, Mdluli K, Hickey MJ, Arain TM, Morris SL, Barry CE 3rd, and Stover CK. Compensatory ahpC gene expression in isoniazid-resistant *Mycobacterium tuberculosis*. *Science* 272: 1641–1643, 1996.
73. Sies H, Gerstenecker C, Menzel H, and Flohé L. Oxidation in the NADP system and release of GSSG from hemoglobin-free perfused rat liver during peroxidatic oxidation of glutathione by hydroperoxides. *FEBS Lett* 27: 171–175, 1972.
74. Tartaglia LA, Storz G, Brodsky MH, Lai A, and Ames BN. Alkyl hydroperoxide reductase from *Salmonella typhimurium*. Sequence and homology to thioredoxin reductase and other flavoprotein disulfide oxidoreductases. *J Biol Chem* 265 : 10535–10540, 1990.
75. Thénard LJ. Observations sur des combinaisons nouvelles entre l'oxigène et divers acides. *Ann Chim Phys* 8: 306–313, 1818.
76. Theorell AHT. The nature and mode of action of oxidation enzymes. Nobel Lecture, December 12, 1955. In: *Nobel Lectures, Physiology or Medicine 1942-1962*, Amsterdam: Elsevier Publishing Company, 1964, pp 480–495.
77. Toledano MB, Delaunay A, Monceau L, and Tacnet F. Microbial H2O2 sensors as archetypical redox signaling modules. *Trends Biochem Sci* 29: 351–357, 2004.
78. Tosatto SCE, Bosello V, Fogolari F, Mauri P, Roveri A, Toppo S, Flohé L, Ursini F, and Maiorino M. The catalytic site of glutathione peroxidases. *Antioxid Redox Signal* 10: 1515–1525, 2008.
79. Trujillo M, Ferrer–Sueta G, Thomson L, Flohé L, and Radi R. Kinetics of peroxiredoxins and their role in the decomposition of peroxynitrite. In: *Peroxiredoxin Systems. Structures and Functions, Subcellular Biochemistry 44*, edited by Flohé L and Harris JR. New York: Springer, 2007, pp 83– 113.
80. Ursini F, Heim S, Kiess M, Maiorino M, Roveri A, Wissing J, and Flohé L. Dual function of the selenoprotein PHGPx during sperm maturation. *Science* 285: 1393–1396, 1999.
81. Vilter H. Vanadium-dependent peroxiodases. In: *Vanadium and Its Role in Life*, edited by Sigel H and Sigel A. New York: M. Dekker, 1995, pp 365–362.
82. Vilter H. Peroxidase from *Phaeophyceae*: a vanadium-dependent (V)-dependent peroxidase from *Ascophyllum nodosum*. *Phytochemistry* 23: 1387–1390 1984.
83. Vivancos AP, Castillo EA, Biteau B, Nicot C, Ayte J, Toledano MB, and Hidalgo E. A cysteine-sulfinic acid in peroxiredoxin regulates H2O2-sensing by the antioxidant Pap1 pathway. *Proc Natl Acad Sci USA* 102: 8875–8880, 2005.
84. Winterbourn CC and Metodiewa D. Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide. *Free Radic Biol Med* 27: 322–328, 1999.
85. Woo HA, Chae HZ, Hwang SC, Yang KS, Kang SW, Kim K, and Rhee SG. Reversing the inactivation of peroxiredoxins caused by cysteine sulfinic acid formation. *Science* 300: 653–656, 2003.
86. Zamocky M, Furtmüller PG, and Obinger C. Evolution of catalases from bacteria to humans. *Antioxid Redox Signal* 10: 1527–1547, 2008.

Address reprint requests to:
Fulvio Ursini
Department of Biological Chemistry
Viale G. Colombo, 3
I-35121 Padova, Italy

E-mail: fulvio.ursini@unipd.it

Date of first submission to ARS Central, February 28, 2008; date of acceptance, March 6, 2008.

This article has been cited by:

1. Leopold Flohé , Stefano Toppo , Giorgio Cozza , Fulvio Ursini . 2011. A Comparison of Thiol Peroxidase Mechanisms. *Antioxidants & Redox Signaling* **15**:3, 763-780. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
2. Saveria Pastore, Valentina Mariani, Daniela Lulli, Emanuela Gubinelli, Desanka Raskovic, Serena Mariani, Andrea Stancato, Chiara de Luca, Alessandra Pecorelli, Giuseppe Valacchi, Alla I. Potapovich, Vladimir A. Kostyuk, Liudmila G. Korkina. 2011. Glutathione peroxidase activity in the blood cells of psoriatic patients correlates with their responsiveness to Efalizumab. *Free Radical Research* **45**:5, 585-599. [CrossRef]
3. Fabio Di Lisa, Martina Semenzato, Andrea Carpi, Sara Menazza, Nina Kaludercic, Roberta Menabò, Marcella CantonMitochondrial Dysfunction in Cell Injury and Cardiotoxicity 1-23. [CrossRef]
4. G. FREDDIFunctionalisation of wool and silk fibres using non-proteolytic enzymes 193-234. [CrossRef]
5. Leopold Flohé. 2009. The labour pains of biochemical selenology: The history of selenoprotein biosynthesis. *Biochimica et Biophysica Acta (BBA) - General Subjects* **1790**:11, 1389-1403. [CrossRef]
6. Christine H. Foyer , Graham Noctor . 2009. Redox Regulation in Photosynthetic Organisms: Signaling, Acclimation, and Practical Implications. *Antioxidants & Redox Signaling* **11**:4, 861-905. [Abstract] [Full Text PDF] [Full Text PDF with Links]
7. Fabio Di Lisa, Nina Kaludercic, Andrea Carpi, Roberta Menabò, Marco Giorgio. 2009. Mitochondrial pathways for ROS formation and myocardial injury: the relevance of p66Shc and monoamine oxidase. *Basic Research in Cardiology* . [CrossRef]
8. D. Koua, L. Cerutti, L. Falquet, C. J. A. Sigrist, G. Theiler, N. Hulo, C. Dunand. 2009. PeroxiBase: a database with new tools for peroxidase family classification. *Nucleic Acids Research* **37**:Database, D261-D266. [CrossRef]