Chapter 17
Basics and News on Glutathione Peroxidases

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Abstract The catalytic mechanism of glutathione peroxidases and its variations in the subfamilies are reviewed and biological roles of the individual enzymes are compiled. The oxidative part of the catalytic cycle involves a water-mediated charge separation in the reaction center leading to dissociation of the selenocysteine (or cysteine) residue and binding of the delocalized proton in a highly energized position. In this environment, a suitably bound H_2O_2 is cleaved without any energy barrier in a concerted reaction yielding water and a selenenic (sulfenic) acid. Depending on family subtype and physiological conditions, the unstable oxidized enzymes form intramolecular disulfide or selenenylamide bonds. The reductive part of the cycle involves the reaction of selenenic (sulfenic) acid with a thiol and (selena) disulfide exchange. Trivial in principle, the reduction steps are most variable within the family, which explains its diversified specificities ranging from GSH to thioredoxin, disulfide isomerases, and particular SH groups of other proteins. The versatility in substrate and co-substrate use predestines these proteins for redox regulation, either as competitors for hydroperoxide utilization by other regulatory proteins or as sensor(s)/transducer(s) in hydroperoxide-initiated signaling cascades.

Keywords Apoptosis • Carcinogenesis • Diversity • Glutathione peroxidases • Mechanism • Oxidative protein folding • Redox regulation • Spermiogenesis • Specificity

17.1 Introduction

Cellular glutathione peroxidase, now known as GPx1, was the first and for a decade the only mammalian protein that had been identified as a selenoprotein. Its functional, chemical, and structural characterization was instrumental to our present understanding of selenoprotein biosynthesis and enzymatic selenium catalysis and also helped to clarify some aspects of selenium deficiency syndromes. Early studies

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on this enzyme and their implications for the entire field of selenium biochemistry have recently been compiled in an amply-referenced retrospective [1] and will not be reiterated herein. GPx1 is only one member of a large peroxidase family present in all kingdoms of life. Only a minority of these proteins carry out selenium catalysis (SecGPx). In most of them, the active site selenocysteine (Sec) is replaced by cysteine (CysGPx). CysGPxs prevail in plants, archaea, bacteria, protists, and non-vertebrate species in general, while in vertebrates, four or five of the eight GPxs are selenoproteins [2]. The number of exceptions to this rule, however, appears to be growing. SecGPxs have been discovered in remote species such as the platyhelminths *Schistosoma mansoni* [3], *S. japonica* [4], *Echinococcus granulosus* [5], the lung fluke *Paragonimus westermani* [6], and probably other worms [7], the green alga *Chlamydomonas rheinhardtii* [8], the fresh water polyp *Hydra vulgaris* [7], the myriapod *Strigamia maritima* [9], different crab and shrimp species [10–12], the arachnidal tick *Rhipicephalus* (*Boophilus*) *microplus* [7], but rarely in bacteria [13] or in insects with the blood-sucking *Rhodnius prolixus* being the only exception thus far [14]. Members of the GPx family, as defined by sequence homology and the highly conserved catalytic tetrad comprising Sec (or Cys), Gln, Asn and Trp residues [15], have adopted diverse functions during evolution. The 2-Cys-GPx subfamily common in bacteria, plants, protists, and insects primarily uses thioredoxin (Trx) instead of glutathione (GSH) as a reductant [16, 17], and the glutathione peroxidase activity of GPx1 appears not to be the predominant role of all vertebrate GPxs either. The scenario is further complicated by the glutathione peroxidase activities of structurally unrelated enzymes of the GSH-*S*-transferase and peroxiredoxin families [18].

The scope of this chapter is to outline general mechanistic features of the canonical GPxs, to describe the diversifications and to discuss their established and potential biological functions. For more detailed information, we refer to the chapters of this book focusing on individual GPxs (see Chaps. 18, 38, 43 and 49) and recent reviews [19–21].

17.2 The Glutathione Peroxidase Reaction

In general, GPxs catalyze the reduction of hydroperoxides by thiols. In the mammalian SecGPxs1-4, the reductant primarily used is GSH as shown in Equation 17.1 (Eq. 17.1).

$$\mathrm{ROOH} + 2\mathrm{GSH} \rightarrow \mathrm{ROH} + \mathrm{H_2O} + \mathrm{GSSG} \qquad (17.1)$$

The specificity for GSH appears to gradually decline from GPx1 to GPx4 in line with the loss of Arg residues implicated in GSH binding [22, 23], and in the CysGPxs7 and 8 and many non-mammalian CysGPxs, the substrates most widely used are dithiol proteins such as protein disulfide isomerase (PDI) or Trx, respectively (Eq. 17.2).

$$ROOH + Prot(SH)_2 \rightarrow ROH + H_2O + ProtS_2 \quad (17.2)$$

With all SecGPxs so far investigated, and with many of the CysGPxs, a kinetic pattern is observed that does not comply with the Michaelis-Menten theory: a ping-pong pattern with infinite V_{max} and K_M values [24]. Such kinetics, however, are not unusual for oxidoreductases and were, in fact, initially anticipated in the systematics of multi-substrate enzymatic reactions in 1957 [25], when also the first GPx was discovered [26]. They simply reveal that: i) we have to consider the GPx reaction as a sequence of independent bimolecular reactions; and ii) typical enzyme-substrate complexes are either not formed or the monomolecular downstream reactions within the complexes are faster than the formation of these complexes. The latter option is more realistic in view of the weak, but obvious substrate specificities of the GPxs.

A recent re-investigation of the GPx reaction using the Density Functional Theory (DFT) corroborates this interpretation of the kinetic pattern [27] and simultaneously explains the extreme reaction rates of GPxs [28]. By employing DFT, an active site model can be calculated from seven amino acids comprising the reacting Sec (Cys) in the conserved NVAxxU/C motif, the Trp and Asn of the conserved WNF motif, and the essential Gln [15, 29]. The tetrad residues therein are connected by multiple hydrogen bonds, which facilitate delocalization or shuttling of protons [24, 27]. If this active site model is complemented by a single water molecule, the proton of the Sec selenol migrates via water to the imino nitrogen of the Trp. By this charge separation, two prerequisites for a fast oxidation of Sec are met: i) the selenol function is fully dissociated; and ii), more importantly, the delocalized proton is bound in a highly unstable and reactive position. The hydroperoxo group of the substrate is accommodated between the selenium and Trp. Within this complex, the delocalized proton is prevented from shuttling back to selenium. When it proceeds along the electrochemical gradient, it hits the peroxo bond, and, in a concerted reaction, H_2O (or ROH, respectively) is formed as an ideal leaving group, while Sec becomes oxidized to a selenenic acid, as shown in Eq. 17.3.

$$[E-Se^- \rightarrow {}^{H}O-O^{H} \leftarrow H-OH \leftarrow H^+-Trp] \rightarrow [E-Se-OH \bullet 2H_2O \bullet Trp] \quad (17.3)$$

As a corollary, the calculations revealed that this reaction within the unstable GPx-H_2O_2 complex proceeds without any energy barrier. The rate-limiting step in the oxidative part of the GPx cycle is not the decay of the enzyme-substrate complex, as presumed in the Michaelis-Menten theory, but the binding of the hydroperoxide to the active site, which is reflected in the measurable rate constant, k_{+1}. Interestingly, DFT-calculations qualitatively yielded the same solution for CysGPx, which complies with surprisingly high k_{+1} values observed with natural CysGPxs [28]. Similar conditions prevail in the reductive part of the SecGPx reaction. To bind a flexible and multiply charged molecule, such as GSH, in a way that its SH function is oriented towards the Se-O^- function of the oxidized enzyme [23] requires time. Yet once this has been achieved, the reaction leading to the Se-glutathionylated intermediate (E-Se-SG in Eq. 17.4) will proceed fast enough to prevent accumulation of

the E_{ox}-GSH complex. Similarly, generation of the symmetric disulfide GSSG is likely faster than a productive binding of GSH to the intermediate (Eq. 17.5).

$$[E-Se-O^{-}\bullet H^{+}\bullet GSH] \rightarrow E-Se-SG+H_2O \tag{17.4}$$

$$[E-Se-SG\bullet GSH] \rightarrow [E-Se^{-}\bullet H^{+}]+GSSG \tag{17.5}$$

Thus, in all these individual steps of the catalytic cycle, the complexes do not accumulate, which leads to the unusual lack of saturation kinetics. This peculiarity of SecGPxs is, however, not shared by all CysGPxs. In particular, 2-Cys-GPxs often display typical Michaelis-Menten-type saturation kinetics (for examples, see [28]). This GPx subfamily, like the atypical 2-Cys-peroxiredoxins, have a second conserved Cys in a flexible loop. After the peroxidatic Cys (C_P; the one homologous to Sec in SecGPx) has been oxidized by H_2O_2 to a sulfenic acid, it has to build an intramolecular disulfide bridge with a second Cys, before the reductive portion of the catalytic cycle is initiated. The second conserved Cys is therefore called the 'resolving cysteine', C_R. Like in peroxiredoxins, the disulfide form of 2-Cys-GPxs is typically reduced by a redoxin such as Trx or tryparedoxin. Several factors may contribute to the change in kinetic behavior of 2-Cys-GPxs: i) the catalytic efficiency of the C_P is lower than that of the peroxidatic Sec in SecGPxs (by analogy called U_P), which might critically slow down the reaction within the complexes; ii) a rigid molecule such as a redoxin may be bound faster than the flexible GSH; and iii) bridging of C_P to C_R, which, as with peroxiredoxins, requires a substantial conformational change [17, 24, 30, 31] might become the rate limiting step.

In short, the oxidative part of GPx catalysis is similar for all subtypes of the family and appears to be only quantitatively affected by an exchange of Sec by Cys. In contrast, the reductive steps of the catalytic cycle are surprising with perplexing variations [24, 28]. However, apart from these diversifications, a fundamental difference between Sec- and CysGPxs exists in the manner, in which the enzymes evade destruction by their aggressive substrates, the hydroperoxides. The C_P of CysGPxs has a stronger tendency to become over-oxidized than the homologous Sec. In 2-Cys-GPxs, this over-oxidation is likely prevented by the reaction of oxidized C_P with C_R, which simultaneously creates the interaction site for redoxins [17]. In this respect, these CysGPxs mimic 2-Cys-peroxiredoxins, for which the protective role of C_R has been convincingly demonstrated [24]. C_P may also form an intramolecular disulfide with a Cys that is not a C_R in the sense that it is required for the reduction by specific substrates, as is postulated for mammalian GPx7 [32]. Alternatively, intermolecular disulfide bridges may be formed, as is evident from massive polymerization of Cys mutants in mammalian SecGPx4 [27, 33]. Simultaneously, oxidation of C_P to sulfinic and sulfonic acid is also observed [27]. While homologous Se-thiylation appears to be common also in SecGPxs [22, 27, 34], over-oxidation of Sec to a seleninic or selenonic acid ($R\text{-}SeO_2^-$ or $R\text{-}SeO_3^-$, respectively) has never been detected. Instead, SecGPxs, when exposed to hydroperoxide in the absence of any thiol substrate, form a selenenylamide bond with the nitrogen of a peptide bond downstream of Sec (Eq. 17.6). This surprising reaction

has been verified for mammalian SecGPx1 and SecGPx4 [27] and may be assumed to be common to all SecGPxs. Intriguingly, the homologous reaction with CysGPxs was neither suggested by DFT-calculations nor detected by MS analysis [27].

$$E-Se-O^{-}+H^{+}+R_2NH \rightarrow R_2N-Se-E+H_2O \qquad (17.6)$$

The ground state enzyme can be regenerated from this alternate oxidized form, which stores the oxidation equivalents of the labile selenenic acid as a selenenylamide that is stable within the architecture of the active site, but is readily transformed into a dehydro-alanine residue, if the enzyme is denatured [27].

17.3 Diversification Within the GPx Family

17.3.1 Structure and Substrate Specificity

The catalytic tetrad comprising Sec46 (Cys46), Gln81, Trp136 and Asn137 in rats is conserved in all but two of the hundreds known GPx sequences. In mammalian GPx8, the Gln residue is replaced by Ser and in a poplar GPx (GPx5 of *Populus trichocarpa*; *Pt*GPx5) by Glu. In agreement with site-directed mutagenesis results [29, 35], these modifications at the active site indicate that individual tetrad residues, with exception of Sec (Cys), are not absolutely essential for peroxidase activity and may in part complement each other. However, the tetrad Gln has been implicated in productive hydroperoxide binding and, interestingly, a back-mutation of Glu to Gln in *Pt*GPx5 dramatically improved affinity and reactivity with *t*-butyl hydroperoxide [36], which reveals that the canonical tetrad structure is indeed the optimal one for the oxidative portion of GPx catalysis. Apart from the tetrad structure, the quaternary structure may also affect hydroperoxide specificity of a GPx. In the tetrameric mammalian SecGPxs (GPx1, 2 and 3), selenium is localized in a flat valley near the subunit interface, but is freely exposed to the surface in the monomeric GPx4. This is likely one of the reasons, why GPx4-type enzymes are peculiar in efficiently reducing hydroperoxides of complex lipids. Furthermore, GPx4 displays a large, positively charged surface surrounding the reaction center, which predestines it for interaction with the negative polar heads of phospholipids in bio-membranes [28, 37].

While the typical tetrad structure determines the reaction of GPxs with ROOH, the reductive part of the catalytic cycle appears not to depend on the retention of this architectural peculiarity [24, 31, 36]. Extensive Molecular Dynamics calculations [23] indicated that the specificity of the prototype SecGPx1 for GSH is primarily achieved by binding of the carboxyl groups of GSH to the guanidine groups of four Arg residues surrounding the reaction center (for graphical illustrations see [19, 23, 24, 38]). In GPx2, three of the Arg residues are still conserved, and the donor substrate specificity is presumed to be similar to that of GPx1 [39]. In the extracellular

GPx3, only two of the Arg residues are left, and the enzyme accepts Trx and glutaredoxin apart from GSH [40]. Finally, GPx4 has none of the conserved Arg residues, but still is preferentially reduced by GSH, which in this case is achieved by binding GSH to Lys residues [24]. GPx4, however, appears to be the least specific GPx with respect to donor substrates. Apart from a variety of low molecular mass thiols, it accepts protein thiols, including those of GPx4 itself [34], and peptides containing Cys-Cys doublets, as are present in keratin-associated proteins [41]. The mammalian CysGPxs7 has been discussed to bind GSH via hydrophobic interaction [42], but lacks any of the basic residues implicated in specific binding of GSH to GPx1-4 [42–44]. Accordingly, the substrate binding site of GPx7 proved to be versatile: GPx7 is reduced by protein disulfide isomerase (PDI) with a rate constant that is two orders of magnitude higher than that of GSH [32]. GPx7 also interacts with glucose-regulated protein 78 (GRP78) [45]. Furthermore, PDI-specificity is inferred for GPx8 [43]. The donor substrate specificities of the remaining vertebrate GPxs have not yet been systematically investigated. Redoxin-specificity is the domain of the non-vertebrate 2-Cys-GPxs. In these proteins, the reaction with the CxxC motif of the redoxins depends on disulfide formation between the C_P and a C_R, the latter being located in a remote domain. The conformational change required for disulfide formation creates a completely new substrate interaction site, which predisposes the oxidized enzymes for the reaction with alternate substrates.

17.3.2 Diversified Biological Roles

Originally, all GPxs were considered antioxidant enzymes able to ameliorate hydroperoxide challenges, and the individual members were believed to back up or complement each other with their particular specificities. A common denominator indeed appears to be an anti-apoptotic and anti-inflammatory action of these enzymes. Otherwise, this view has to be rated as an over-simplification.

- GPx1 is still considered to be responsible for detoxification of H_2O_2 and other soluble hydroperoxides. Knockout studies reveal that GPx1 is not of vital importance. However, *Gpx1*$^{-/-}$ mice are highly susceptible to oxidative challenges such as exposure to bacterial lipopolysaccharide, macrophage activation, or viral infections [38]. Accordingly, GPx1-deficiency is being widely discussed in the context of diseases linked to oxidative stress [46]. However, over-expression of GPx1 also yielded mice with symptoms of type-II-diabetes or metabolic syndrome [47]. An unexplained finding remains why *Gpx1*$^{-/-}$ hepatocytes are more resistant to peroxynitrite exposure than wild type cells, although GPx1 is able to reduce peroxynitrite [48].
- GPx2, the gastrointestinal form, is believed to sustain the delicate balance of proliferation and apoptosis of the gut epithelium [49]. *Gpx2*$^{-/-}$ mice show enhanced apoptosis in gastrointestinal epithelia and are more susceptible to inflammation-mediated carcinogenesis than wild type mice, yet transplanted

tumors grow faster, if GPx2 was normal [50]. In tumor prevention, GPx1 and GPx2 apparently act synergistically. In double knockout mice, colitis and intestinal tumors develop without any inflammatory challenge apart from gut colonization with non-pathogenic bacteria [51]. Collectively, the results reveal that GPx1 cannot fully compensate for the loss of GPx2 (for more details, see Chap. 38, [20] and [52]).

- Knockout of the extracellular GPx3 did not display an obvious phenotype. Recently, however, accelerated platelet aggregation and multiple thrombi have been observed in *Gpx3*$^{-/-}$ mice [53]. Also, these mice, like *Gpx2*$^{-/-}$ mice, proved to be more susceptible to azoxymethane/dextran sodium sulfate used to model inflammation-mediated colon carcinogenesis [54].
- The *Gpx4* gene is expressed in three distinct ways: all three forms are selenoproteins, all can catalyze the same reactions, but each plays a different role. The nuclear form, nGPx4, has a different N-terminus, since its expression uses a start codon within the first intron. Knockout of *nGpx4* yields a mild phenotype, with impaired chromatin compaction and morphological alterations in sperm as the only established disturbances [55]. For expression of the mitochondrial form, mGPx4, transcription initiates from an upstream start codon. mGPx4 is identical with the cytosolic cGPx4 after cleavage of the mitochondrial import sequence. Knockout of *mGpx4* causes complete loss of male fertility of otherwise healthy mice [56]. Thus, mGPx4 is the expression form that 'moonlights for fertility' in the late phase of spermiogenesis, where it forms the mitochondrial sheath by polymerization and co-polymerization with Cys-rich proteins [34, 41]. It should be stressed that this function of mGPx4 is the opposite of an antioxidant one. Rather, it is an anabolic process: the use of ROOH to build a complex structure that is indispensable for sperm function [34, 57]. Deletion of the complete *Gpx4* gene as well as selective knockout of *cGpx4* leads to embryonic death around day 7 p.c. (reviewed by Conrad [58]). This essentiality of cGPx4 probably reflects a very special 'antioxidant' function, which is its ability to efficiently reduce hydroperoxides within membranes. The still hypothetical explanations for the essentiality of cGPx4 are interrelated: i) cGPx4 can remove all traces of peroxidized lipids, thereby preventing activation of lipoxygenases [21], including 12,15-lipoxygenase [59], which has been implicated in membrane destruction during tissue remodeling [21]; ii) it reduces the products of 12,15-lipoxygenase, which can cause an alternate way of programmed cell death, designated AIF-mediated apoptosis [60]; and iii) it interferes with another type of programmed cell death, ferroptosis, which like AIF-mediated apoptosis involves 12,15-lipoxygenase products, yet leads to LOOH/iron-mediated cell death via initiation of free radical chain reactions [61, 62]. The common denominator of these hypotheses is cell destruction by unbalanced 12,15-lipoxygenase activity, when cGPx4 is missing. Accordingly, one would expect that inactivation of 12,15-lipoxygenase should rescue *Gpx4* knockout mice, which unfortunately remains controversial: pharmacological inhibition of lipoxygenases rescued the mice [60], while disruption of the 12,15-lipoxygenase gene failed to do so [63].

GPx4 also antagonizes RIP3-mediated necroptosis of erythroid precursor cells, which again is driven by oxidation [64] (See also Chaps. 18 and 43).

- GPx5 is an extracellular CysGPx. Its location in the epididymis suggests a role in sperm physiology [65]. Its precise function, however, remains elusive.
- GPx6 is a SecGPx in man, but a CysGPx in rodents and other animals [66]. It is found in the olfactory bulb, and since its discovery, has been suspected to complement the olfactory sensory system [67]. Its precise role remains to be elucidated.
- GPx7 (also known as NPGPx) is a CysGPx with clear preference for PDI as a reducing substrate [32]. It is located at the luminal site of the endoplasmic reticulum (ER) [43, 68]. It is presumed to use the excess H_2O_2 produced by Ero1α (ER oxidoreductin 1α) for oxidative protein folding via oxidation of PDI family members [20]. Moreover, GPx7 has been reported to oxidize GRP78, thereby enhancing the chaperone activity of the latter [45]. Deficiency of GPx7 causes obesity in mice and apparently also in humans [69]. Knockout of *Gpx7* is associated with multiple organ dysfunctions, increased risk of carcinogenesis, and shortened life span [68].
- GPx8 is also a CysGPx and, like GPx7, is located in the ER and implicated in oxidative protein folding [43, 68]. Although its reaction center is similar to that of GPx7, discrete differences in specificities might serve distinct roles of the thiol peroxidases of the ER (GPx7, GPx8 and peroxiredoxin IV) in the complex scenario of protein folding [21, 68, 70, 71]. Moreover, GPx8 appears to be a negative regulator of fibroblast growth factor and insulin signaling [72].

The involvement of GPxs in metabolic regulation is a topical focus of interest. Like the peroxiredoxins, GPxs also can interfere with signaling cascades in multiple ways and, intriguingly, in opposing directions [21]. They can simply remove hydroperoxides that are positive regulators of signaling cascades, as is, for example, discussed for impaired NF-kB activation due to over-expression of GPx1 [73] and GPx4 [74], and for blunted insulin response in GPx1-over-expressing mice [47]. Inversely, peroxidases, because of their extreme reactivity with hydroperoxides, are predestined to act as sensors for H_2O_2 or other ROOH. 'Peroxide sensing', in this case, refers to reacting with hydroperoxide. The oxidized peroxidase then can transduce the oxidant signal by oxidizing a downstream component of the signaling cascade [75]. This regulatory principle has been verified for different types of thiol peroxidases [21], the first example being the direct interaction of a GPx with a transcription factor in yeast: H_2O_2 sensing by a Trx-specific 2-Cys-GPx Orp1, and activation of the transcription factor Yap1 via thiol oxidation and subsequent expression of protective genes [76]. Analogous reaction schemes were found with peroxiredoxins in yeast [77], and more recently, in a mammalian system, wherein Prx2 senses peroxide and transduces the signal by oxidizing the transcription factor STAT3 [78]. The sequence of reaction steps is essentially the same for both types of thiol peroxidases, GPxs and peroxiredoxins: oxidation of C_P and, instead of immediate reduction by Trx or GSH, disulfide formation by reaction of sulfenic acid with an SH function of a target protein as alternate substrate followed by disulfide

exchange reactions. A first example of a GPx as a H_2O_2 sensor in higher animals is the interaction of GPx7 with GRP78 as noted above. Oxidized C_P (Cys57) forms an internal disulfide with Cys86, resulting in an intermolecular disulfide of Cys86 with Cys41or Cys420 of GRP78, and disulfide reshuffling creates the activated chaperone with a Cys41-Cys420 disulfide bond [45]. The reaction scheme is believed to sense excess H_2O_2 by GPx7 and, via GRP78 activation, prevent accumulation of misfolded proteins due to unspecific thiol oxidation by H_2O_2 in the ER [45].

A homologous sensing mechanism has not been described thus far for SecGPxs. However, the metamorphosis of GPx4 in the late phase of sperm maturation from an active peroxidase to an enzymatically inactive structural protein makes use of homologous chemistry: in shortage of the preferred substrate GSH, the oxidized enzyme uses protein thiols as alternate substrates. Instead of activating a regulatory protein, the oxidized GPx4 in spermiogenesis ends up in dead-end intermediates with alternate substrates that, although enzymatically inactive, fulfill a vital function [34, 57]. Therefore, in theory, there is no reason why SecGPxs should not be involved in alternate substrate-mediated redox regulation. In fact, the recent discovery of selenenylamide formation in oxidized SecGPxs [27] adds a new perspective, i.e., these alternate intermediates of the catalytic cycle are only formed under pronounced deficiency of reducing substrate and they display an active site architecture distinct from the regular one and, thus, could be suspected to be specifically designed for alternate substrate reactions.

17.4 Concluding Remarks

Research on glutathione peroxidases has experienced many unexpected turns. The discovery of an erythrocyte enzyme, "which protects hemoglobin from oxidative breakdown" [26], did not predict that it marked the beginning of a dramatic development. Whereas early studies were limited to oxidative damage, GPx research appears to have influenced the entire field of selenium biochemistry [1], resulting in a more nuanced comprehension of redox biochemistry comprising the obvious hazards of aerobic life, but equally the pivotal role of peroxides in host defense, redox regulation and cell differentiation [79, 80]. Over the last few decades, GPx family members kept surprising us with new biological roles such as the diabetogenic potential of GPx1, the involvement of GPx4 in spermiogenesis and special types of programmed cell death, cross-talk of GPxs with the Trx system, the contribution of GPx7 and 8 to oxidative protein folding and the sensor function of GPx7. Despite the 16,096 PubMed hits on "glutathione peroxidase" (by January 1, 2016), the still steadily increasing knowledge involving this fascinating class of proteins suggests that the summit of related discoveries in the seemingly old GPx family has yet to be reached.

Our current understanding of the catalytic cycle of an archetypal SecGPx in mammals is summarized in Fig. 17.1.

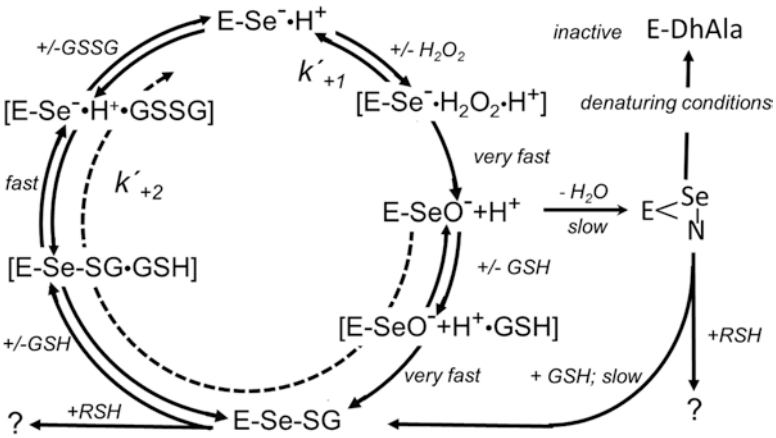

Fig. 17.1 Update of the catalytic cycle of a typical mammalian SecGPx. Reduction of H_2O_2 by GSH is chosen as example (*left* site of the scheme). The apparent rate constants k'_{+1} and k'_{+2} are those that are easily assessed by steady state kinetics. k'_{+1} is the net forward rate constant for the formation of the complex between the ground state enzyme and H_2O_2 ($>10^7$ $M^{-1}s^{-1}$), k'_{+2} is the net forward rate constant for the entire reductive part of the cycle (*dashed line*; $>10^5$ $M^{-1}s^{-1}$). "very fast" means that the partial reaction within a complex (shown in *square brackets*) is faster than the formation of the latter. Alternate substrate reactions with suitable thiols (RSH) are possible starting from each of the catalytic intermediates (*no brackets*). DhAla designates dehydroalanine in irreversibly inactivated enzyme that results from β-cleavage of Sec under denaturing conditions

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