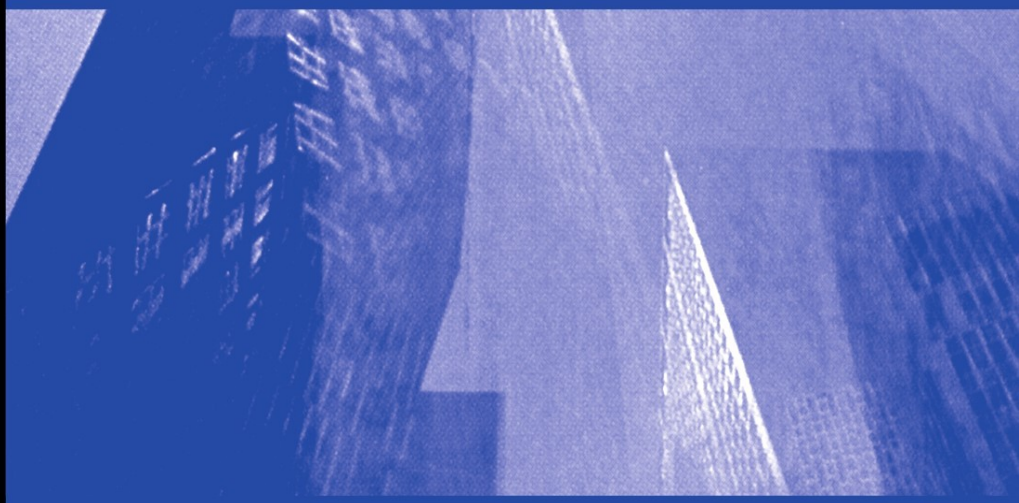
ADVANCED TOPICS IN SCIENCE AND TECHNOLOGY IN CHINA

Junqiu Liu • Guimin Luo • Ying Mu
Editors

Selenoproteins and Mimics



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Selenoproteins and Mimics

With 120 figures, 28 of them in color

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Foreword

The research area of selenoproteins has seen considerable progress in recent years. The initial identification of selenium as an essential trace element was followed by the characterization of glutathione peroxidases as selenoproteins, which subsequnetly resulted in a wealth of information on various other selenoproteins. Early on, organic selenium compounds were identified as mimics of glutathione peroxidase activities. The current state of knowledge in these two research fields is brought together in this volume.

Regarding selenoproteins, following their identification and the study of their structure and characterization of their function and regulating, there have been efforts to bring their role into perspective in terms of physiology and pathology. An early landmark was Keshan disease, and current aspects are presented here on various exciting topics.

Selenoprotein mimics have been associated with the functioning of compounds capable of carrying out the reduction of hydroperoxides, a domain of glutathione peroxidases. The present volume contains valuable information on current knowledge in this field, addressing chemically new types of compounds and their potential in therapeutic applications. As not all functions of selenoproteins are uncovered yet, further types of selenoprotein mimics can be expected in the future.

It is noteworthy that the editor, Professor Junqiu Liu, and his other colleagues from China have contributed to this research area with highly interesting work in recent years, so that one can truly formulate that there is a tradition in Chinese research in the field of selenium and selenoproteins, stemming from the initial observation of the role of the trace element in Keshan disease to the current research fronts in the 21st century. Congratulations to this and to a fine book which hopefully will lead to further projects and research excitement.

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Preface

The element selenium was first discovered in 1817 by Berzelius and was recognized as an essential nutrient in the late 1950s. However, the biochemical role of selenium was not established until 1973 with the discovery of the selenoprotein, glutathione peroxidase (GPx). In 1973, selenium was indentified as an essential component of the active site of selenoenzyme GPx. From this year the biochemical and biological role of selenium began to be established. One important advance in this area is the investigation of selenoproteins. Selenoproteins exist in all major forms of life, eukaryote, bacteria and others. They are proteins which includes selenocysteine residues. Selenoproteins are important constituents of a number of enzymes with a range of functions including antioxidant function, thyroid hormone metabolism, male fertility and immune mechanisms. Selenium occurs in selenoproteins as specifically incorporated selenocysteine, and selenocysteine is recognized as the 21st amino acid. There is a rather complicated pathway of selenocysteine biosynthesis and specific incorporation into selenoproteins. The biosynthesis of selenocysteine is regulated by four genes and begins with the aminoacylation of the amino acid serine by the enzyme serine synthetase to produce Ser-tRNASec. Research suggests that the mammalian genome encodes 25 selenoprotein genes, while more than 40 selenoprotein genes may exist in different tissues. Thus, the number of selenoproteins indentified has grown substantially in recent years although the functions of only about half of these selenoproteins are understood. Conventionally, iodothyronine deiodinases, thioredoxin reductases, selenophosphate synthetase, selenoprotein P, selenoprotein W and the well-known glutathione peroxidases represent important classes of selenoproteins, and recent indentified selenoproteins includes selenoproteins Sel15, SelH, SelI, SelK, SelM, SelN, SelO, SelR, SelS, SelT, SelU, SelV, SelX, and SelZ. Their functions may be less understood or even unknown.

It is well known that selenium associate with human health and disease. For selenium-related disease, typical example includes Keshan disease, a selenium deficiency disease, which was first described in the early 1930s in China. Observational studies show that selenium can be benefical for immune system, reducing the cardiovascular and cancer mortality. Recent studies indicate that selenium shows important influence on asthma, arthritis, male infertility and HIV/AIDS.

To explore the functional importance of selenium in selenoproteins, significant

efforts have directed toward the development of biomimetic chemistry of selenoproteins. In this regard, the main progress focus on the simulation of the behaviors of selenium in selenoenzyme GPx. Up to now, a number of organoselenium/tellurium compounds and artificial selenoproteins were designed to mimic the natural GPx. A typical example is ebselen (2-phenyl-1,2-benzisoselenazol- 3[2*H*]-one), this "small molecular selenoenzyme" has been widely investigated as an artificial GPx from abundant experiments to clinic trials. Important progress has been made recently for the design of selenoantibody and seleno/telluro-glutathione tansferases, and these artificial selenoenzmes show amazed catalytic behaviors rivaling natural ones!

In this book, we combine the introduction of the recent development of selenoproteins with the advance in their functional imitation. Thus the book associates crossed subjects including biology, chemistry and medical science. This book consists of two parts with 20 chapters. The first part which was titled "Selenoproteins" describes major aspects of the identified selenoproteins with identified functions, these selenoproteins include glutathione peroxidases, thyroid hormone deiodinase. Thioredoxin reductases, selenophosphate synthetase, selenoprotein P, selenoprotein W, deiodinase, thioredoxin and selenoprotein T. The biosynthesis mechanism of selenoproteins is also discussed in this Part. The introduction of the bioinformatics of selenoproteins will help us to obtain insights into selenium utilization, distribution and the discovery of new selenoproteins. The description of main diseases such as cancers, brain diseases and heart diseases, and the occurrence of different forms of selenium in foods will give us a rough picture of the relationship of selenoproteins with human health. The second part which was titled "Selenoprotein mimics" presents the recent progress of biomimetic chemistry of selenoprotein. This part provides an overview for the reasonable design and synthesis of artificial models with selenoenzyme activity. Thus, a series of ideas and approaches for the design of artificial selenoproteins including chemical and biological methods has been described.

We hope that the biomimetic chemistry of selenoproteins will enrich both pharmaceutical and academic aspect of selenium and selenoproteins and also hope that the two part of this book will facilitate each other. The progress of selenoproteins would facilitate the design and preparation of artificial selenoproteins, and at the same time the functional imitation of selenoproteins would increase the understanding for the structures and functions of selenoproteins, and also for their further application in human health. This book provides a new review of selenoproteins, their mimetic chemistry and their varied aspects of health. Research in these directions is in progress although there are still many unanswered questions. It provides a platform for the scientists, researchers and students in the field of selenoproteins, their mimetic chemistry and others. This book should be suitable for wide readers in chemistry, biology and medical science.

Junqiu Liu, Guimin Luo
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Glutathione Peroxidases

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The present state of knowledge on glutathione peroxidases (GPxs) is reviewed with particular emphasis on general catalytic principles and the biology of mammalian glutathione peroxidases. GPxs make up a ubiquitous family of proteins defined by sequence homology, the common functional denominator being their ability to reduce hydroperoxides by thiols. Catalysis is mediated by an active-site selenocysteine or cysteine. Eight distinct GPxs have been identified in mammals, five of them being selenoproteins in man. While glutathione specificity prevails in vertebrate GPxs, thioredoxins or related redoxins appear to be common substrates in plant, bacterial and protist GPxs. Specific reactions of GPxs with other protein thiols are also observed. The basic catalytic scheme allows the enzymes to adopt diversified biological roles ranging from defence against peroxide challenge, redox regulation of metabolic processes and transcription, apoptosis to cellular differentiation. The roles of the individual mammalian GPxs are discussed in the light of distinct substrate specificities, distribution, subcellular compartmentation, expression patterns and data from inverse genetics. It is outlined that the multiple coexisting GPxs and functionally related peroxiredoxins likely build up a system of enzymes that, with discrete functional overlap, complement each other in meeting specific biological tasks far beyond fighting oxidative stress.

1.1 Introduction

In 1973, glutathione peroxidase (GPx) was identified as a selenoprotein, in fact the

first one to be discovered in higher organisms [1, 2]. The enzyme, which is now known as GPx1 catalyzed the reduction of H_2O_2 and organic hydroperoxides by glutathione. Its selenoprotein nature finally explained why traces of selenium are essential for defense against an oxidative challenge in the vertebrate organism. The fact that the first mammalian selenoprotein was a peroxidase, however, also led to the misconception that the essential trace element selenium is simply a "biological antioxidant". The early history of glutathione peroxidase and selenium biochemistry with all its interdependencies, serendipities and surprises were the subject of a recent essay [3] and shall not be repeated here in detail. It may suffice to say that research on GPxs was pivotal to our present understanding of selenium's role in biology, its function as a catalytic entity in enzymes, as well as providing an understanding of a most complex mechanism in its co-translational incorporation into selenoproteins [4-8].

Over the past three decades, GPxs, defined as proteins with high sequence similarity, have been detected in almost every domain of life. The majority of these proteins, however, are neither selenoproteins nor glutathione peroxidases, if this term is to characterize their catalytic role. The selenium-containing glutathione peroxidases prevail in vertebrates and have only been sporadically detected in lower organisms such as platyhelminths (e.g. *Schistosoma mansoni*, *S. japonicum* and *Echinococcus granulosus*) [9, 10], Cnidaria (*Hydra vulgaris*) and protists [11], and exceptionally in insects (in the tick *Boophilus microplus*) [12] and bacteria [13]. In most of the invertebrate species, all yeasts and higher plants, the active site selenocysteine of the glutathione peroxidases is replaced by cysteine. Interestingly, this change in the redox-active moiety is often associated with a switch in substrate specificity: most of the non-Se glutathione peroxidases appear to hardly react with glutathione (GSH). Instead, these GPx homologues, like most of the peroxiredoxins, are preferentially or exclusively reduced by "redoxin"-type proteins such as thioredoxin [14] or tryparedoxin [15]. A yeast GPx homologue has also been described to specifically react with a particular SH group of a transcription factor and to thereby initiate the expression of protective enzymes [16]. However, the selenium-containing GPxs are not always specific for GSH either. In fact, a strict specificity for GSH has only been documented for the prototype which gave the name to the entire family, i.e. GPx1 [17], whereas e.g. GPx4 has been reported to react with a variety of protein thiols [18-20] including SH groups of GPx4 itself [21, 22].

The ramification that the GPx family experienced during evolution [11, 23] renders it obsolete to talk about "glutathione peroxidase" as a functionally well-defined enzymatic entity. Many of the family members might not at all share the basic biological role of GPx1, which is to reduce H_2O_2 or other hydroperoxides at the expense of GSH to cope with oxidative challenge. Moreover, the term "glutathione peroxidase" has been used to describe enzymes that may similarly catalyze the reduction of hydroperoxides by GSH, but are neither structurally nor phylogenetically related to the family, such as GSH-S-transferases [24], selenoprotein P [25] or human peroxiredoxin VI [26].

The growing complexity of thiol-dependent hydroperoxide metabolism has

been discussed in many topical reviews, each one focusing on particular aspects such as evolution [11, 23], specificities [20], kinetics [27, 28], catalytic mechanism [20, 27], regulation of enzyme expression [29, 30] and its involvement in redox regulation [30-32], male fertility [33, 34], apoptosis [35, 36], viral infections [37], thyroid [38] or brain function [39]. And the overlap between the glutathione- and thioredoxin-dependent hydroperoxide metabolizing systems may be distilled from respective monographs [40, 41]. By the end of June 2009, a PubMed search for the key word "glutathione peroxidase" yielded 10,928 entries, which reveals the impossibility of covering the entire field in this review with an allotted maximum length of 20 pages. This article will therefore be essentially confined to general aspects of GPx catalysis and the peculiarities of the mammalian selenium-containing peroxidases.

1.2 Glutathione Peroxidase Reaction

The GPx that gave its name to the entire family [3] catalyzes the reduction of H_2O_2 and soluble organic hydroperoxides at the expense of GSH. This first glutathione peroxidase, now called GPx1, is a tetrameric enzyme consisting of four identical subunits (Fig. 1.1). Having remained the only known GPx for more than two decades, it also served as a prototype for working out the kinetic mechanism, sequence and structure, specificity and the catalytic principle which involves oxidation of the active site selenium and step-wise reduction by GSH. The present mechanistic understanding of this enzyme, which is widely relevant to other types of GPx, is critically reviewed in the paragraphs below.

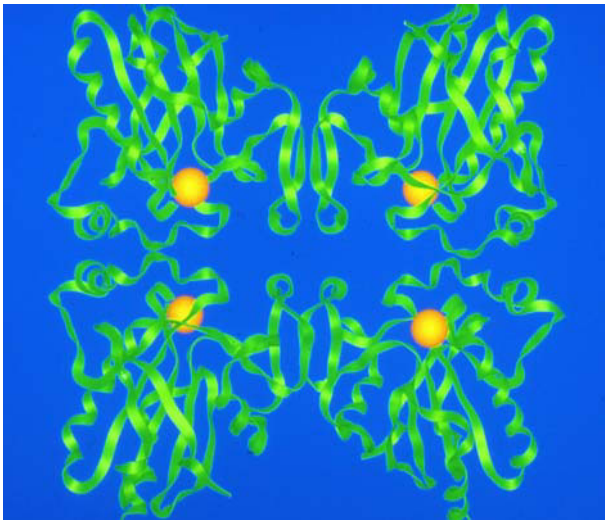

Fig. 1.1. Structure of GPx1. The representation shows the homo-tetrameric enzyme with its four selenium atoms as orange balls. Reproduced from the data set of Epp et al. [52] by K. D. Aumann, *Helmholtz-Zentrum für Infektionsforschung*, Braunschweig, Germany

1.2.1 Basic Catalytic Principle

Typically, glutathione peroxidases catalyze the reduction of H_2O_2 by GSH according to Eq.(1.1).

$$H_2O_2 + 2\ GSH \rightarrow GSSG + 2\ H_2O \quad (1.1)$$

Depending on the particular enzyme, a more or less broad scope of hydroperoxides may be reduced (Eq.(1.2)) ,

$$ROOH + 2\ GSH \rightarrow GSSG + ROH + H_2O \quad (1.2)$$

and the reductant GSH may be partially or fully replaced by other thiols (Eq.(1.3)),

$$H_2O_2 + GSH + RSH \rightarrow GSSR + 2\ H_2O \quad (1.3)$$

or

$$H_2O_2 + 2\ RSH \rightarrow RSSR + 2\ H_2O \quad (1.4)$$

All these reactions seem to be chemically trivial and indeed proceed spontaneously, provided the thiol groups are dissociated. In reality, however, these reactions (Eqs.(1.1) – (1.4)), each one involving three molecules, proceed according to a lower order of kinetics than anticipated, since they do not require any ternary collision of the three molecules but result from a sequence of two binary collisions (Eqs.(1.5) and (1.6)). The first thiol, which has to be present in its thiolate form, reacts with the hydroperoxide, whereby a sulfenic acid is formed.

$$RS^- + H_2O_2 \rightarrow RSOH + OH^- \quad (1.5)$$

The latter then dissociates and reacts with the second thiol to form the disulfide.

$$RSO^- + H^+ + RSH \rightarrow RSSR + H_2O \quad (1.6)$$

The enzymatic catalysis of hydroperoxide reduction by thiols mimics much of this simple chemistry but speeds up the reaction rate by orders of magnitude (Eqs.(1.7) and (1.8)). The enzymatic trick is that the hydroperoxide has not to directly attack a poorly reactive, since hardly dissociated, thiol such as GSH. Instead, a highly-reactive thiol or selenol within the enzyme, which is the "peroxidatic cysteine" (C_P) or "peroxidatic selenocysteine" (U_P), reduces the hydroperoxide.

$$E\text{-}S^- + H_2O_2 \rightarrow E\text{-}SOH + OH^- \quad (1.7)$$

$$E\text{-}Se^- + H_2O_2 \rightarrow E\text{-}SeOH + OH^- \quad (1.8)$$

The sulfenic or selenenic acid residue of the enzyme, in analogy to Eq.(1.6), readily reacts with the substrate thiol to form a mixed (selena) disulfide which, by thiol-disulfide exchange, is reduced by the second thiol (Eqs.(1.9) and (1.10)).

$$E\text{-}S(Se)O^- + H^+ + RSH \rightarrow E\text{-}S(Se)SR + H_2O \quad (1.9)$$

$$\text{E-S(Se)SR} + \text{RSH} \rightarrow \text{E-S(Se)}^- + \text{H}^+ + \text{RSSR} \qquad (1.10)$$

In essence this basic scheme is valid for the "real" glutathione peroxidases and, with some modifications, also for the GPx-type [14] and peroxiredoxin-type thioredoxin peroxidases [41]. In the latter two cases, the first reducing thiol is a cysteine residue of the enzyme itself (called the "resolving" cysteine, C_R), and the resulting disulfide form of the enzyme is then reduced by the CXXC motif of a redoxin-type protein.

1.2.2 Kinetics

The catalytic principle outlined above is best described by the German term "*Zwischenstoffkatalyse*" (catalysis by intermediate formation), as it was developed by the German chemist Wilhelm Ostwald in the beginning of the last century [42]. It means that the catalysis is achieved by a sequence of partial reactions of the reactants with the catalyst and modifications thereof, each being faster than the non-catalyzed overall reaction. In our example, the catalyst is oxidized by H_2O_2 (Eq.(1.8)), and the intermediate E-SeOH (*Zwischenstoff*) thus formed is stepwise reduced by GSH (Eqs.(1.9) and (1.10)). The correlation in enzymological terms is the "enzyme substitution mechanism", as defined by Dalziel in 1957 [43]. This catalytic principle, which is by no means uncommon for oxidoreductases, differs substantially from "central complex mechanisms", where two or more reactants are assembled at the enzyme's active site in a productive way to facilitate their interaction. This difference between mechanisms has to be stressed, because it has a major impact on kinetics and, in consequence, on the enzyme's function in a biological context. While enzymes with central complex mechanisms are best characterized by Michaelis constants and maximum velocities, these classical parameters adopt a completely different physical meaning in enzyme substitution mechanisms or, as in the case of the selenoperoxidases, may not be applicable at all: The K_m and V_{max} values of GPx1 [44] and all other selenium-containing GPxs investigated so far are infinite [27]. This seemingly odd behavior does not reflect a low affinity of substrates to GPx, but simply reveals a high reactivity of the substrates with the enzyme or its derivates, respectively. In contrast to the Michaelis-Menten theorem, it is not a reaction of substrates within an enzyme / substrate complex that is rate-limiting in the GPx reaction, but the speed of productive collisions of the ground-state enzyme with a hydroperoxide (Eq.(1.8)) or the formation of binary complexes between GSH and one of the oxidized enzyme forms (Eqs.(1.9) and (1.10)). Although the formation of such complexes (omitted in Eqs.(1.9) and (1.10)) is not evidenced by steady-state kinetics, they have to be inferred for GPx1 at least from its pronounced donor substrate specificity. In line with this interpretation, saturation kinetics are sometimes observed with GPx homologues working with the less reactive C_P [27] or with other thiol peroxidases relying on sulphur catalysis [28].

With the above consideration, the initial rate equation for GPx1 [44] and identically for GPx3 [45] and GPx4 [45, 46] becomes surprisingly simple (Eq.(1.11)):

$$[E_0] / v_0 = 1 / k'_{+1} \cdot [ROOH] + 1 / k'_{+2} \cdot [GSH] \quad (1.11)$$

Therein k'_{+1} is the apparent net forward rate constant for partial reaction (Eq.(1.8)) and, in view of the irreversibility of this step and lacking evidence for a specific enzyme/hydroperoxide complex, may be regarded as the bimolecular rate constant k_{+1} that characterizes the oxidation of the ground state enzyme with the hydroperoxide. k'_{+2} is less well defined. It is the net forward rate constant for the reductive part of the catalytic cycle and physically means the net forward rate constant for the association of GSH with the oxidized (Eq.(1.12)) or partially reduced enzyme (Eq.(1.13)), whichever is smaller, or a hybrid constant, if they are similar.

$$E\text{-}SeOH + GSH \rightarrow [E\text{-}SeOH \cdot GSH] \quad (1.12)$$

$$E\text{-}SeSG + GSH \rightarrow [E\text{-}SeSG \cdot GSH] \quad (1.13)$$

The complexes, however, never accumulate and therefore remain kinetically silent, since the reactions according to Eq.(1.9) and Eq.(1.10) proceed within these complexes with a non-rate-limiting, i.e. higher velocity. Despite its poorly-defined physical meaning, k'_{+2} is a useful constant to predict turnover rates under varying physiological conditions.

1.2.3 Physiological Consequences of Kinetic Mechanism

For all mammalian selenium-containing GPxs so far analyzed, a $k_{+1} > 10^7$ L/(mol·s) (for H_2O_2) was determined, whereas the k'_{+2} is two to three orders of magnitude smaller. As the oxidative step is so much faster than the reductive ones, the enzyme is almost 100% oxidized if its velocity is measured at similar substrate concentrations, as commonly done *in vitro*. Under such conditions the rate equation (Eq.(1.11)) simplifies to Eq.(1.14):

$$v_0 = k'_{+2} \cdot [GSH] \cdot [E_0] \quad (1.14)$$

which means that the turnover depends on the concentration of GSH and over a wide range is independent of the H_2O_2 concentrations. In fact, the enzyme seems always "saturated" with H_2O_2 and an apparent K_M is hard to measure. This observation has frequently led to the misconception that the enzymes similarly respond to variations in substrate concentrations *in vivo*. The opposite is correct: the general rate equation (Eq.(1.12)) yields that, at physiological substrate concentrations of 1 – 10 mmol/L GSH and an estimated maximum of 1 µmol/L H_2O_2 or other hydroperoxides, the enzyme is largely reduced, even if k_{+1} is two orders of magnitude larger than k'_{+2}. With $[E_0] = [E_{red}]$, however, the rate equation simplifies to Eq.(1.15).

$$v_0 = k'_{+1} \cdot [\mathrm{ROOH}]\,[\mathrm{E}_0] \tag{1.15}$$

This implies that *in vivo* the GPx turnover in most cells is independent of the concentration of GSH, unless it drops to less than 10^{-4} mol/L. This straightforward consequence of the kinetic parameters of the enzymes seemingly conflicts with observations relating impaired antioxidant defense to moderately-lowered GSH content in tissues. The solution of the enigma is provided by uneven GSH concentrations in cells and cellular compartments. A drop in GSH by, e.g. 20%, likely means that GSH is practically zero in 20% of the cells. Such GSH depletion is not reached before the rate of H_2O_2 production exceeds the rate of GSH regeneration by glutathione reductase or the NADPH supply systems, respectively. Exceptionally, this happens physiologically in special cells but commonly marks a transition point from physiology to pathophysiology.

1.2.4 Facts, Unknowns and Guesswork

While the basic principles of the glutathione peroxidase reaction, according to Eqs.(1.7) – (1.13), are generally accepted, many details still remain enigmatic.

1.2.4.1 Catalytic Relevance of U_P or C_P Dissociation

From alkylation studies and general chemical considerations, it appears obvious that the ground state enzyme presents its active site selenocysteine as a selenolate, and the extreme efficiency of the selenoperoxidases is usually explained by the comparatively low pK_a of selenocysteine (pK_a = 5.2) versus cysteine (pK_a = 8.3). Unfortunately, this reasoning, although repeated even in most recent publications [47], does not really lead to any satisfactory interpretation of experimental data:

i) Fully-dissociated low molecular weight thiols do not react with H_2O_2 faster than with bimolecular rate constants near 50 L/(mol·s) [48], while corresponding rate constants for cysteine residues in GPx- or Prx-type peroxidases ranging around 10^6 L/(mol·s) are by no means exceptional [27, 28].

ii) Within the architecture of the GPx active site C_P or U_P appear to be similarly dissociated, as has been suggested by pK_a calculations [20] and demonstrated by velocities of alkylation [49, 50], which equally requires the thiolate or selenolate form, respectively. The efficiencies of recombinant cysteine homologues of Se-GPxs, however, are typically three orders of magnitude smaller [49-51] and k_{+1} values near 10^8 L/(mol·s), as determined for natural GPx1 [44], have never been observed with any of the thiol peroxidases working with sulphur catalysis [27, 28].

iii) The electro-negativity of sulphur and selenium does not differ significantly enough to account for the substantial difference in catalytic efficiency either. Thus, in short, the dissociation of C_P or U_P, respectively, although being a prerequisite

for the enzyme's reaction with ROOH, neither explains the catalytic efficiency of GPxs in general nor the superiority of the selenium-containing ones.

1.2.4.2 Mechanism of U_P Activation

An activation of the U_P by neighboring residues had already been deduced from the first X-ray structure of a GPx, that of GPx1 [52]. Although the U_P in this structure was over-oxidized to a seleninic acid, it seemed plausible that in the ground state enzyme the selenium atom might be hydrogen-bonded to the amide nitrogen of a glutamine and the imino nitrogen of a tryptophan, whereby a catalytic triad consisting of Sec (or Cys), Gln and Trp is formed, which over the years became a characteristic signature of the entire family. In this triad the selenol function should be forced into dissociation and further polarized for a nucleophilic attack on the peroxy bond of the substrate. The catalytic relevance of these conserved residues could indeed be verified by site-directed mutagenesis of GPx4 [49] and others [15, 50]. More recently, the triad concept had to be amended, since a strictly conserved Asn that contacts the U_P or C_P from the core of the protein proved to have an even higher impact on activity than Gln and Trp so far implicated, whereby the catalytic triad grew up to a tetrad [20]. The residues were shown to facilitate dissociation of C_P (a U_P would be dissociated anyway) [20, 49, 50] and S-alkylation [49, 50] and thus contribute to the nucleophilicity of the active site chalcogen, which is a necessary, though not sufficient, condition for catalytic efficiency (see subsection 3.4.1). Recent re-calculations based on all known GPx structures, however, revealed that these residues are in an ideal position to form a stable hydrogen bond between each other but not so with the active site S or Se. It therefore might not be a direct hydrogen bonding but the generally high density of labile protons in the surrounding of U_P or C_P, respectively, that enforces its dissociation [27]. It is further tempting to speculate that the surface-exposed residues Gln and Trp are more important for the polarization of the peroxy bond than for the activation of C_P or U_P. Finally, a few exceptions from the canonical triad / tetrad concept have been reported: The canonical Gln is replaced by Glu in poplar GPx [53] and by Ser in human GPx8 [23] and could be replaced by Gly without loss of activity in the GPx of Chinese cabbage [54], which reveals a certain plasticity of the otherwise strictly conserved active site. Collectively, structural, genetic and functional investigations have unraveled some important features of (seleno) cysteine activation in GPx catalysis, but we are still far from a conclusive concept. The extreme efficiencies of the magic sulphur and/or selenium atoms still remain enigmatic.

1.2.4.3 Chemical Nature of Oxidized GPx

Another puzzle in GPx catalysis is the precise chemical nature of the oxidized

enzyme. In Eqs.(1.7) – (1.9) and (1.12), it is boldly shown as a sulfenic or selenenic acid derivative of C_P or U_P, respectively. In fact, this assumption is little else but a postulate based on the stoichiometry of the reaction of one (seleno) cysteine residue with one hydroperoxide molecule. Admittedly, the oxidation of cysteine residues to sulfenic acids in proteins is not uncommon and has been amply demonstrated to occur in the analogous peroxiredoxin catalysis [55]. With the selenoperoxidases, however, the situation is less clear. The postulated selenenic acid form has so far never been demonstrated experimentally. In X-ray crystallography the selenium of GPx1 was seen as seleninic acid [52]. Instead, by mass spectrometry oxidized GPx4 [20] and GPx1 [56] consistently showed a molecular mass that was lower than that of the reduced enzyme by two mass units. This finding would be compatible with elimination of H_2O from the postulated selenenic acid form and, in analogy to the catalysis of atypical 2-cysteine peroxiredoxins, might be interpreted as indicating the formation of an intramolecular selenyl-sulfide bond. There is, however, no cysteine residue in GPx1 or GPx4 that could serve as such C_R. Alternatively, an initially-formed selenenic acid could react with a nearby amino, imino or amido group in analogy to the redox cycle of the GPx mimic ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) [57]. An analogous sulfenamide bond has been identified in oxidized protein tyrosine phosphatases such as PTP1B [58, 59] and PTPα [60] by X-ray crystallography. Like the selenyl-amide bond on oxidized ebselen, the sulfenamide in the PTPs is readily reduced by GSH [58-60], and it is therefore tempting to speculate that the first intermediate of the GPx cycle is indeed a selenyl-amide formed between the active site selenium and one of the triad/tetrad components. However, none of the suspected Se-N bonds could so far be detected by systematic mass spectrometry investigations, nor were they revealed by X-ray studies. It therefore appears wise to address oxidized GPx as a “selenenic acid equivalent” until its chemical nature has been clarified.

1.2.4.4 Structures and Substrate Specificities

As mentioned above, GPx1 is highly specific to GSH. Its GSH specificity has been attributed to a lysine residue (K91′) and 4 arginine residues (R57, R103, R184 and R185 in bovine GPx1) which surround the active site selenium and serve to successively direct the two GSH molecules into an orientation that allows reaction of the GSH sulphur with the selenium [61]. This view has been corroborated by modeling and molecular dynamics calculations [62] but has so far not been verified by mutagenesis studies (Fig. 1.2). Therefore, the relative importance of the five basic residues must still be rated as uncertain. A contribution of these residues to GSH binding is, however, also supported by the circumstantial evidence that their deletion or replacement by non-equivalent residues, as is observed in members of GPx subfamilies other than GPx1, leads to gradual or complete loss of GSH specificity. The GPx2 subfamily has three of these residues