Structure – Activity Correlation between Natural Glutathione Peroxidase (GPx) and Mimics: A Biomimetic Concept for the Design and Synthesis of More Efficient GPx Mimics

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Abstract: Among the organoselenium compounds that mimic the action of the natural enzyme glutathione peroxidase (GPx), there are certain basic differences in the activity, substrate specificity and mechanism. These differences arise mainly from the nature of the substituents near the reaction center, and stability and reactivity of the intermediates. As an attempt to draw some general concepts for the development of new mimics, a structure – activity correlation between natural GPx and some existing mimics is described.

Keywords: enzyme models · glutathione peroxidase · selenium · selenoenzymes · structure – activity relationships

Introduction

Selenium is an essential trace element that has provoked considerable interest owing to the recent identification of prokaryotic and eukaryotic enzymes containing the 21st amino acid, selenocysteine.[1] Because of the specific redox properties of selenium, the presence of a selenol group in the active site of an enzyme instead of a thiol confers a dramatic catalytic advantage. The critical role played by selenium is illustrated by mutational analyses of mammalian glutathione peroxidase (GPx), type I deiodinase (ID-1) and bacterial formate dehydrogenase H.[1–3] In each case, replacement of the native active site selenocysteine by a cysteine residue greatly reduced catalytic activity. Interestingly, replacement of the catalytically essential cysteine residue in glyceraldehyde 3-phosphate dehydrogenase (GAPDH) with selenocysteine converted the phosphate dehydrogenase into a peroxidase. The engineered selenoenzyme, selenoGAPDH, efficiently catalyzes the reduction of hydroperoxides with thiols and thus mimics the action of GPx.[4] It is known that not only the nucleophilic reactivity but also the electrophilic as well as radical scavenging properties of selenium contribute to the biological activity of selenium compounds. In this article we compare the structure and activity of native GPx with known mimics in order to derive some basic concepts for the development of new mimics. In general, organoselenium compounds that show biological activity have been synthesized under the aspect of isosteric replacement of oxygen or sulfur with selenium based on the known structure and pharmacological activity of oxygen- or sulfur-containing compounds.[5] GPx mimics, however, represent a unique class of compounds that have been developed by recognizing the structural and functional features of the native enzyme.

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Glutathione Peroxidase

Glutathione peroxidase is a mammalian antioxidant selenoenzyme which protects biomembranes and other cellular components from oxidative damage by catalyzing the reduction of a variety of hydroperoxides (ROOH), using Glutathione (GSH) as the reducing substrate.[6] Detailed kinetic studies and modeling of enzyme-substrate complexes have led to the suggestion of the reaction mechanism detailed in Figure 1. In this catalytic cycle, the selenol (EnzSeH) form of the enzyme reacts with peroxides to form selenenic acid (EnzSeOH). The nucleophilic attack of GSH at EnzSeOH

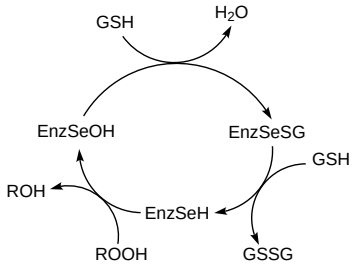

Figure 1. Catalytic cycle of GPx.

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produces the selenenyl sulfide (EnzSeSG) which further reacts with second equivalent of GSH to regenerate the selenol.

Redox Behavior of Selenium

Easy changes of the oxidization state is a characteristic property of selenium. Studies on organoselenium compounds indicate that selenium in comparison to sulfur can be more easily oxidized and reduced between valence state (II) and (IV).[7] Therefore, the lower redox potential of selenocysteine compared with cysteine is catalytically favorable for the EnzSeH →EnzSeOH conversion in the GPx cycle. The redox behavior of selenium also plays an important role during enzyme purification. It is known that the natural GPx and its sulfur mutant are overoxidized to seleninic acid ($EnzSeO_2H$) and sulfinic acid ($EnzSO_2H$), respectively, upon storage.[2, 3] However, only the selenium species $EnzSeO_2H$ can be reduced to EnzSeH state by treating the enzyme with an excess amount of GSH.[3] Owing to the above-mentioned properties, various selenium compounds were developed for the thiol-mediated destruction of hydroperoxides. While Ebselen (**1**, a well known GPx mimic) is known to be an efficient catalyst, the sulfur analogue **2** is void of any catalytic activity in the reduction of peroxides.[8] The higher efficiency of **1** compared with that of **2** in the prevention of lipid peroxidation initiated via radicals,[9] on the thiol reduction of ferric cytochrome c[10] and the reduction of peroxynitrite[11] are other significant examples where the redox properties of selenium play important roles. The redox behavior of selenium is particularly important when the selenium undergoes oxidation – reduction reaction similar to the process shown in Figure 2.

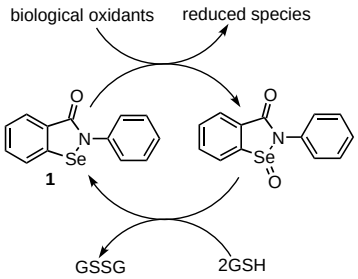
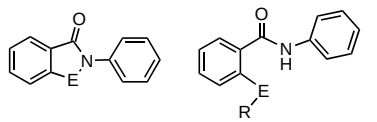


Figure 2. Redox shuttling between Se^{II} and Se^{IV} during the reduction of biological oxidants.

In addition to the above-mentioned redox properties, the higher efficiency of selenium compounds as compared with their sulfur analogues against oxidative stress correlates also with their catalytic effect in the deactivation of singlet molecular oxygen. The bimolecular rate constants (k) of quenching of molecular singlet oxygen $^1O_2$ ($^1O_2 \rightarrow {}^3O_2$) by some GPx mimics (**1**, **3**, **5**) and their sulfur analogues (**2**, **4**, **6**) are listed in Table 1. This Table shows that the rate constants of quenching of $^1O_2$ by Se-containing compounds are approximately one order of magnitude higher than that of the sulfur analogues.[12]



1: E = Se
2: E = S

3: E = Se; R = Me
4: E = S; R = Me
5: E = Se; R = CH_2Ph
6: E = S; R = CH_2Ph

Table 1. The rate constants (k) for the deactivation of singlet oxygen by **1**–**6**.

Compound	k [$dm^3 mol^{-1} s^{-1}$]
1	2.5×10^6
2	2.3×10^5
3	9.2×10^6
4	7.8×10^5
5	5.2×10^6
6	3.4×10^5

Stabilization and Activation of the Selenol Function

It is evident from various studies that two amino acid residues, that is tryptophan and glutamine, appear in identical positions in all known members of the GPx family.[6] According to the three-dimensional structure established for bovine cGPx, these residues could constitute a catalytic triad in which the selenol group of the selenocysteine is both stabilized and activated by hydrogen bonding with the imino group of the tryptophan residue and with the amido group of the glutamine residue (Figure 3).[13]

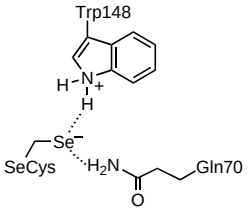
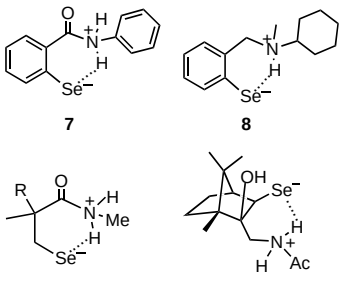


Figure 3. Interaction of the selenol group with amino acid residues in GPx.

This concept led to the development of Se-containing GPx mimics with basic amino/imino groups in close proximity to selenium. The selenol **7** derived from Ebselen (**1**) could be stabilized and activated by the secondary amino group. It has been proved that the proximate nitrogen base in certain compounds such as **8** activates the selenol into the kinetically much more reactive selenolate anion by an intramolecular $N^+H\cdots Se^-$ hydrogen bond.[14] The selenols **9**[15] and **10**[16] which are derived from other GPx mimics could also be activated by nearby heteroatoms.

The reduced form of selenosubtilisin, a semisynthetic selenoenzyme that also acts as a GPx mimic, is strongly



7 **8**

9 **10**

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stabilized by nearby histidine residues.[17] Because the enzyme-bound selenol is deprotonated by His64 to form a selenolate-imidazolium ion pair at all accessible pH values, it is expected to be highly reactive and therefore susceptible to oxidation by hydroperoxides. Another amino acid residue, Asn155, is also expected to play an important role in the stabilization of the selenolate (Figure 4). Similarly, the active site selenol (SeCys149) in selenoGAPDH interacts with a histidine residue (His176) to form an efficient selenolate–imidazolium ion pair.[4]

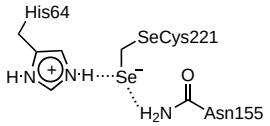


Figure 4. Stabilization of the selenolate form of selenosubtilisin.

Stability and Reactivity of the Selenenic Acid Function

The stability of EnzSeOH against further oxidation and its fast reaction with GSH are the two major factors in the second step of the catalytic cycle. Molecular modeling studies have shown that these requirements may be met in the following way.[18] The sulfur of GSH is "pressed" firmly against the selenium atom and forms hydrogen bonds with strong electrostatic attractions to the oxygen atom of the selenenic acid group and to the Arg184. The carboxylic group of the glycyl residue of GSH forms an ideal electrostatic bond with Arg57, and the carboxylic group of the γ-glutamyl residue of GSH is drawn into a positively charged cleft that is formed by Arg103 and the lysine residue 91 from the adjacent submit (Cys91B) (Figure 5).

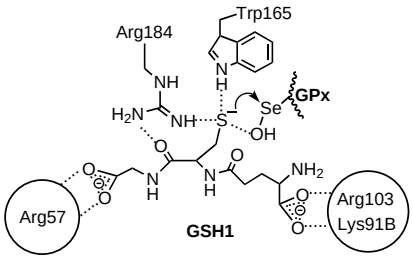
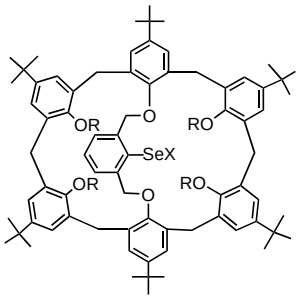


Figure 5. Significant binding interactions of the selenenic acid of GPx with GSH.[18]

Beyond these major interactions, Arg184 is engaged in hydrogen bonding with the cysteyl carbonyl of GSH. The EnzSeOH·GSH complex is, therefore, stabilized by a total of 16 bonds, and these bonds force the sulfur in GSH into a position where it is optimally positioned to form a selenenyl sulfide bridge by reaction with the selenenic acid. The crystal structure of human plasma GPx exhibits hydrogen bonding interactions between the oxygen atoms of the seleninic acid and the amino acid residues Trp153, Asn154, Gly46 and Leu47.[19] The selenenic acid function in selenosubtilisin also exhibits interactions with the nearby amino acid residues that are similar to the interactions shown for the corresponding selenolate (Figure 4). In the case of selenosubtilisin, the two oxygen atoms of the seleninic acid group form energetically favorable hydrogen bonds with the side chain amide of Asn155 and His64 (2.7 and 2.8 Å, respectively).[20]

In synthetic selenenic acids, the reactivity of the selenenic acid depends solely upon the electrophilic reactivity of selenium. The introduction of a basic amino/imino/ether group in close proximity to selenium is one approach to increase the electrophilic reactivity, since the divalent selenium is known to interact with nearby heteroatom(s) (O, N etc.) forming internally chelated species.[21] Such internal chelation contributes greatly to the electrophilic reactivity of the selenium in model compounds in the selenenic acid state. Okazaki et al. reported the synthesis and reactivity of a isolable selenenic acid **11** stabilized by *p-tert*-butylcalix[6]-arene macrocycle.[22] Despite its stability, compound **11** reacts

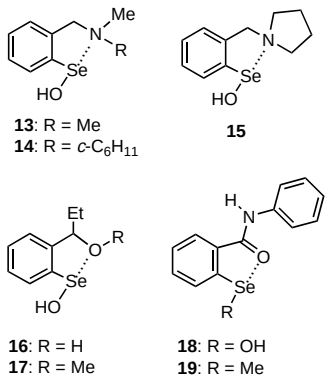


11: X = OH
12: X = S*n*Bu
(R = CH_2Ph)

with 1-butanethiol to form selenenyl sulfide **12**. Although the stability of **11** has been reported to be caused by steric protection, the internal chelation between selenium and one of the two oxygen atoms in the *ortho*-position may contribute to the reactivity of **11** towards 1-butanethiol. This assumption arises from the crystal structure of **11**, which indicates the existence of an attractive interaction between selenium and one of the oxygen atoms (Se···O distance: 2.64 Å). Because of this interaction, the O···Se–O moiety adopts a nearly linear arrangement.[22]

Compounds **13**, **14**, and **15**, which exhibit strong Se···N interactions indicated by ^{77}Se NMR studies, are known to react with thiols much faster than compounds that do not contain basic amino groups.[14] Similarly, the Se···O interactions in **16** and **17** may also contribute to the electrophilic reactivity of selenium.[23] The secondary amino group of Ebselen derivatives, which is involved in the stabilization of selenol, may not play a crucial role in the activation of selenenic acid. Instead, the carbonyl oxygen is expected to interact with the selenium as shown in **18**, although the Se···O interactions are normally weaker than the Se···N interactions. This assumption is supported by the crystal structure of **19** in which the oxygen atom is involved in a strong interaction with selenium.[24]

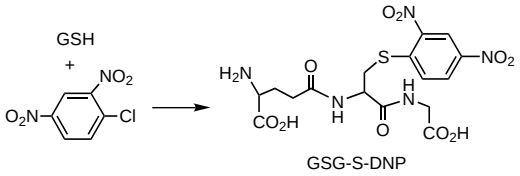
In the catalytic cycle, the internal chelation facilitates the attack of nucleophilic sulfur at the electrophilic selenium atom, thereby producing the selenenyl sulfide. The crystal structure of human plasma GPx supports this concept, as the selenium atom itself is involved in weak interactions with



Gln79 and Trp153 in the seleninic acid state (Se···N distances: 3.5 and 3.6 Å, respectively) in addition to the normal hydrogen-bonding interactions of other amino acid residues which are located in close proximity to selenium.[19]

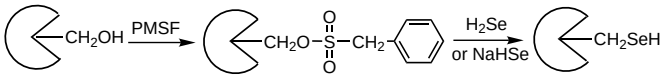
GSH Binding Sites

Apart from the Se···N or Se···O interactions, the generation of GSH binding sites within the model compounds is an alternative approach to increase GPx-like activity. The ability of the mimics for GSH recognition could be modified either by developing monoclonal antibodies[25] or by molecular imprinting.[26] In the first case, the highly reactive thiol group of GSH is protected by a 2,4-dinitrophenyl group to obtain the hapten GSH-S-DNP (Scheme 1). This hapten is cross-linked



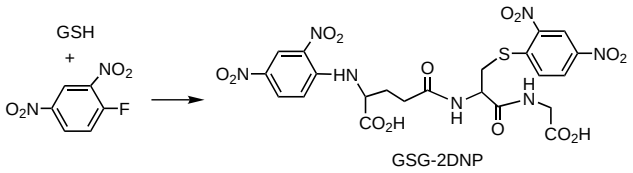
Scheme 1. Synthesis of the hapten GSH-S-DNP.

to BSA by using glutaraldehyde and the resulting antigen is used for developing monoclonal antibodies. The catalytic group selenocysteine is then incorporated into the antibodies combining sites by using chemical mutation (Scheme 2). The mutant (abzyme), which is capable of binding GSH-S-DNP, recognizes GSH for binding during the GPx cycle.[25]



Scheme 2. Chemical mutation of Ser residue on antibody into SeCys.

In the latter case, the GPx mimic is generated with GSH binding sites by using bioimprinting and chemical mutation. The GSH derivative (GSH-2DNP), used as the imprinting molecule, is synthesized by protecting the unstable thiol and amino groups by 2,4-dinitrophenyl groups (Scheme 3). The



Scheme 3. Synthesis of the hapten GSH-2DNP.

bioimprinting of albumin with GSH-2DNP binding sites followed by chemical mutation (Scheme 2) leads to the generation of cavities for GSH binding.[26] The GPx activities of the abzyme and the printed protein are 1100 times and 820 times, respectively, higher than that of Ebselen (Table 2).

Table 2. Comparison of the activity between some mimics and native GPx.[25, 26]

Compound	Activity [u μmol]$^{-1}$
selenocysteine	0.05
Ebselen (**1**)	0.99
abzyme	1097
printed protein	817
native GPx	5780

The lower activity of the printed protein as compared with that of abzyme is probably due to the presence of nonspecific binding sites for the carboxylic group of the glycyl residue of GSH, since these sites are produced by using a sterically bulky protecting group.

Reactivity of the Selenenyl Sulfide

The reaction of EnzSeSG with a second equivalent of GSH is found to be the crucial step for the regeneration of the active site selenolate. The molecular modeling shows that the major binding forces in EnzSeSG are the hydrogen-bonding interactions between the two carboxylic functions of GSH and Arg57, Arg103 and Lys91B.[18] During the binding of a second GSH molecule (GSH2) with EnzSeSG, some of the hydrogen bonds between the amino acid residues in GPx and GSH are broken in order to make the −Se−S− bond freely accessible for the attack by GSH2, which is obligatory to continue the catalytic cycle. In particular, the bond between N(Trp165) and S(GSH) (Figure 5) is broken and N(Trp165) now involves in a hydrogen-bonding interaction with the carboxyl group of GSH2, thus bringing the substrate closer to the −Se−S− bridge (Figure 6). The most striking feature in the structural changes during the binding of GSH2 is the coordination of amido nitrogen of Thr54 to the sulfur (Figure 6). This interaction would certainly contribute to the polarization of the −Se−S− bond.

When considering the model compounds, the enhancement in the reactivity of the selenenyl sulfides towards thiols must be considered as more significant than the stability of the selenenyl sulfide species. The Se···N and Se···O interactions

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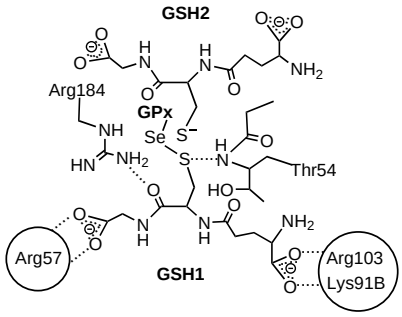
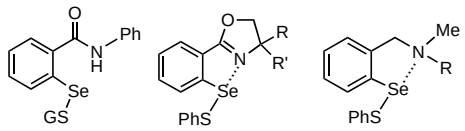


Figure 6. Significant binding interactions in the selenenyl sulfide form of GPx.[18]

or steric protection are not essential for this stability since the unsubstituted derivatives such as PhSeSPh are found to be reasonably stable. Since no amino acid residues near the active site of the GPx or in the mutants have been shown to interact with selenium, it is quite unlikely that the Se···N or Se···O interactions in selenenyl sulfides contribute to the regeneration of selenol by reaction with thiols. It is still not clear whether there is any significant Se···N or Se···O interaction in the selenenyl sulfide **20** derived from Ebselen. However, the secondary amino substituents might deprotonate the second GSH to provide nucleophilic thiolate (GS^-) for the attack on sulfur or might interact with the unbound GSH molecules to bring them closer to the reaction center.

This concept is supported by the properties of various other areneselenenyl sulfides that contain tertiary amino substituents in the *ortho*-position. The selenenyl sulfides with strong Se···N interactions (**21**, **22**) do not undergo facile reactions with thiols,[27] whereas the selenenyl sulfides with weak or no Se···N interactions (**23**, **24**) effectively regenerate the selenol by reaction with thiols.[14] When the Se···N interaction is very strong, the selenium atom becomes more electrophilic than sulfur and this leads to a nucleophilic attack of thiol at selenium rather than an attack at sulfur. This thiol interchange would hamper the regeneration of the active site selenol or selenolate.



20 **21**: R = R' = Me; **22**: R = Et, R' = H; **23**: R = Me; **24**: R = *c*-C_6H_{11}

It would be of a particular interest in future to find out whether any substituents having amino/imino/ether groups could be placed in such a way that the heteroatoms (N or O) interact with selenium in the selenenic acid state and with sulfur in the selenenyl sulfide state as observed in the case of natural GPx. As pointed out already, the S···N interactions between S(GSH) and N(Thr54) residue in GPx would increase the possibility of a nucleophilic attack of negatively charged thiolate at sulfur atom in the −Se−S− bridge. Similarly, the S···N or S···O interactions in the related synthetic compounds would increase the nucleophilic attack of thiols at sulfur rather than at selenium and therefore prevent the thiol interchange which is observed in the case of **21** and **22** with thiols.

Conclusion and Outlook

In summary, by comparing the structural features of the GPx model compounds developed so far with that of natural enzymes, several prerequisites for "efficient mimics" have been identified. According to these concepts, the organoselenium model compounds must be designed to have i) hydrogen bonding between selenol and nearby amino/imino/ether groups to force the selenol into full dissociation, ii) strong Se···N or Se···O interactions in the selenenic acid to increase the electrophilic reactivity of selenium and hence to enhance the reaction with thiols before the possible conversion to "overoxidized" selenium species, iii) amino/imino/ether groups that can interact with sulfur in the selenenyl sulfide intermediate, and iv) some specific sites for GSH binding. Unfortunately, none of the organoselenium models reported to date fulfill all of these prerequisites. The insights gained from the previous model studies, however, provide a solid basis not only for the development of more efficient GPx mimics but also for the design and synthesis of organoselenium compounds that could mimic the action of other selenoenzymes such as iodothyronine deiodinase for which successful synthetic mimics have not yet been developed.

Acknowledgement

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