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Chapter 9

Inhibitors and Activators of SOD, GSH-Px, and CAT

Xianyong Ma, Dun Deng and Weidong Chen

Additional information is available at the end of the chapter

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Abstract

Reactive oxygen species (ROS) is harmful to our health, and SOD, CAT, and GPX are the major antioxidant enzymes that defend us from effects of ROS. In medicine, food, and dairy industries, antioxidant enzymes often surround complex environments. For better utilization of these enzymes, the inhibitors (including competitive inhibitors and noncompetitive inhibitors) and activators of SOD, CAT, and GPX are descripted in detail in this chapter. Also, the structure and catalytic mechanism of these antioxidants are summarized.

Keywords: reactive oxygen species, superoxide dismutase, catalase, glutathione peroxidase

1. Introduction

Reactive oxygen species (ROS) such as superoxide ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) are highly reactive oxidant species, which are by-products of intracellular metabolic processes, causing macromolecular damage. Reactive oxygen species are harmful to health and cause damage to crucial substances such as DNA, RNA, proteins, and lipids. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) are three major antioxidant enzymes in animals. Superoxide dismutase (SOD), the first defense line of against oxygen-derived free radicals, catalyzes the dismutation of the superoxide anion ($O_2^{-\cdot}$) into hydrogen peroxide (H_2O_2), which isthen transformed into H_2O and O_2 by catalase. Glutathione peroxidase (GSH-PX or GPX) is a selenoprotein, which also protects the organism from oxidative damage; the main biochemical function of GPX is to reduce lipidic or nonlipidic hydroperoxides as well as H_2O_2 through oxidization of glutathione [1]. Although both enzymes can reduce H_2O_2, there are some differences between CAT and GPX. CAT, one of the enzyme with highest

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turnover rates,is the main enzyme involved in reduction of H_2O_2 via the Fenton reaction. This enzyme is almost exclusively expressed in peroxisomes [2]. GPX is specific for its hydrogen donor, but nonspecific for H_2O_2, and it degrades H_2O_2 using reduced glutathione in a powerful manner [3].

From the perspective of protection, improving antioxidase activity would be helpful for organisms to survive under various stresses, but from another respect, the antioxidase activity should be inhibited. Some anticancer agents, such as xenobiotics and radiation, act by producing ROS to kill tumor cells. Cells with high levels of antioxidant enzymes are resistant to these anticancer agents. Therefore, the levels of cellular antioxidant enzymes will influence the sensitivity of tumor cells to anticancer therapies [4]. Thus, more detailed information about an activator and inhibitor of SOD, CAT, and GPX should be known, for better utilization of these enzymes.

2. SODs

2.1. The structure and function of SOD

Superoxide dismutases (SODs, EC 1.15.1.1) are ubiquitous and one important class of antioxidant metalloenzymes against the harmful effects of superoxide free radicals. The main function of SODs was to decompose superoxide radicals into molecular oxygen and hydrogen peroxide inside cells, which reaction is as follows:

$$2O_2^{-\cdot} + 2H^+ \rightarrow O_2 + H_2O_2 \quad (1)$$

Based on the functional metal cofactors located at the active sites, four distinct classes of SODs have been found (**Figure 1**). SODs of Class I specifically require manganese or iron ion for catalytic activity (MnSOD and FeSOD) and enzymes that function with either of the two metal ions so-called cambialistic SODs. Class II is copper- and zinc-dependent enzymes (CuZn-SODs). Members of the two classes are found in both prokaryotes and eukaryotes. Nickel-containing SODs are mainly identified from marine actinomycetes and cyanobacteria [5, 6]. Enzymatic reactions of SODs depending on different metals indicate that SODs are developed by cells to offset the harmful effects of reactive oxygen species and match its surroundings.

Due to their antioxidative effects, SODs exhibited dramatic potential in medicine, cosmetic, food, agriculture, and chemistry industry. For example, considerable clinical experiments have shown that SODs could prevent oncogenesis and reduce the cytotoxic effects of anticancer drugs. Recently, SODs have found to prevent many diverse diseases such as cardiovascular diseases, diabetes, asthma, infertility, neurological disorders, and transplant rejection. SODs have also been successfully utilized as a major component in cosmetics for skin protection. In animal husbandry, SODs are considered to be one kind of strong antioxidative enzymes, which can reduce the oxidative stress of animal and prevent the oxidation of animal products, improve the quality of animal products such as meat, egg, or milk. In order to make better use

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of SODs, their characteristics, the most suitable environment and influencing factors, etc., should be known especially the information of inhibitor and activator.

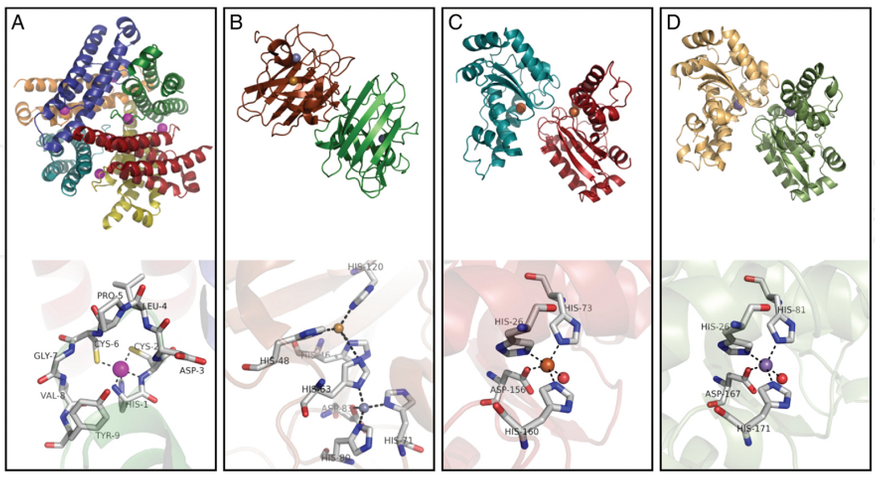

Figure 1. A comparison of the enzyme structures and active sites for the four SODs, (a) streptomyces coelicolor NiSOD (PDB, 1t6u), (b) human Cu/ZnSOD (PDB, 1pu0), (c) *E. coli* FeSOD (PDB, 1isa), and (d) MnSOD (PDB, 1vew). (adapted from reference [7]).

2.2. Inhibitors of SODs

2.2.1. Different inhibitors on sensitivity of SODs

Cu/Zn-SODs, Fe-SODs, and Mn-SODs are sensitive to different inhibitors, and we could distinguish these metal-specific SODs based on different inhibitory reactions (**Table 1**). The activity of Cu/Zn-SODs was inhibited by KCN, but Fe-SODs and Mn-SODs were not sensitive to KCN. Whereas, completely different phenomenon was observed when incubated in chloroform-ethanol (1:3, V/V) component solvent, as a result Fe-SODs and Mn-SODs activity were almost lost, while Cu/Zn-SOD was not sensitive to chloroform-ethanol. Fe-SODs and Mn-SODs are highly homologous and exhibit structural similarity, it is assumed that they originate from the same ancestry [8]. Fe-SODs and Mn-SODs can be distinguished by their different sensitivities to H_2O_2, because Mn-SOD activity was not sensitive to H_2O_2. In addition, NaN_3 also is used to detect the type of MnSOD, when the SOD was inhibited by neither KCN nor H_2O_2 [9].

SODs	Inhibitors			
	KCN	Chloroform-ethanol	H_2O_2	NaN_3
Cu/Zn-SOD	+	-	+	+
Fe-SOD	-	+	+	+
Mn-SOD	-	+	-	+

"+" indicate reaction and "-" indicate no reaction.

Table 1. Effect of inhibitors on different cofactors of SODs activity.

2.2.2. Effects of metal ions on SOD activity

Cu^{2+}, Zn^{2+}, Fe^{2+}, and Mn^{2+} are cofactors of superoxide dismutase, and they are vital to enzymatic activity. But heterogeneous expressed SODs were often existed in terms of apoenzyme or combined other metals present in a culture medium [10], thus might result in partial or total loss of activity. So that reconstituted metal ion is necessary to recovery activity. Apoenzyme was prepared by a metal removal procedure according reference [11] and simplified as follow:

1. Purified SOD was dialyzed against denature buffer: 20 mM 8-hydroxyquinoline, 2.5 mM guanidinium chloride, 5 mM Tris, 0.1 mM EDTA, pH 3.8, at 4°C for 18 h.
2. Purified SOD was dialyzed against 5 mM Tris buffer (pH 7.8), containing 1 mM metal ions for 18 h.
3. Excess metal ions were removed by dialysis against 5 mM Tris buffer (pH 7.8).

To be attention, only specific metal ions combined to SOD, the activity could be recovered. But for Fe-SOD and Mn-SOD, because they share highly homologous, Fe-substituted Mn-SODs also are active, but the activity is lower than Mn-reconstituted SOD.

SODs are metalloenzyme, and they will be inhibited by chelators [12, 13], such as EDTA and cuprizone (a copper-specific chelator). They should be avoided losing metal cofactors caused by chelators in the experiment. But a Mn-SOD from *Mycobacterium* sp. JC1 DSM 3803 was not sensitive to 10 mM EDTA [8], which may be due to the highly tightness of metal cofactors binding to enzymes.

Co^{2+}, Hg^{2+}, K^{+} and, Al^{3+} and other metal ions also show their inhibition effects on SOD activity. A Mn-SOD from deep-sea thermophile *Geobacillus* sp. EPT3 was activated only by Mn^{2+} among nine tested metal ions [12]. A Cu-/Zn-SOD from black soybean was also activated only by Cu^{2+} [13] and a similar result was shown in the report of Liu et al. [14]. But there are a few exceptions, for example, a manganese-containing superoxide dismutase was activated by Cu^{2+}, Zn^{2+}, and Al^{3+}.

2.2.3. Singlet molecular oxygen inactivation of superoxide dismutase

High reactive singlet molecular oxygen ($^1O_2$) is one kind of short-lived intermediate from oxidation reaction which oxidizes a variety of biological molecules easily, including lipids, nucleic acids, and proteins, and it also promotes deleterious processes such as lipid peroxidation, membrane damage, and cell death [15]. The biochemical production of singlet oxygen has been proposed to contribute to the destructive effects on a number of biological processes [16]. *In vivo*, singlet molecular oxygen is produced under normal and pathophysiological conditions. It is known to be particularly reaction with histidine, which often located at the active sites of SODs, so singlet molecular oxygen may result in prevention of the activities of those enzymes including CATs and GPXs. *In vitro*, singlet molecular oxygen could be produced by photoactived dyes, such as methylene blue or rose bengal [17], so inactivation of SODs, CATs, and GPXs should be avoid in application.

2.3. Different activator on activity of SODs

2.3.1. Effect of carbohydrates on activities of superoxide dismutase

Carbohydrates, such as maltose, sucrose, lactose, trehalose, glucose, D-fructose, D-trehalose, D-xylose, and so on could stabilize an enzyme structure. For example, trehalose plays a strong promotive effect on superoxide dismutase [18]. Trehalose is a kind of polyol compound with many hydroxyl groups, which has strong hydration ability and can change the free energy to the favorable direction in solution. The multihydroxyl structure of trehalose can connected with both the surface of the enzyme protein and the external water through hydrogen bonding, so that the structure of the enzyme is stable, and the enzyme activity was protected [19].

2.3.2. Polyethylene glycol modified SOD to improve its efficiency

Polyethylene glycols (PEGs) are considered to be a safety and nonimmunogenic materials. They also have multihydroxyl compounds and can be activated by many activators, such as cyanuric, dicycolhexylcarbodiimide, N-hydroxysucciniimide, and 1,1'-carbonyldiimidazole, then activated polyethylene glycol was conjugated with the ε-NH_2 group of SOD. PEG conjugated with SOD not only enhance the stability of the enzyme, but also avoid enzymatic immunogenicity. For example, Beckman et al. [20] reported that a superoxide dismutase conjugated with polyethylene glycol greatly increased endothelial the cell oxidant resistance and half-life of the enzyme.

2.3.3. Cyclodextrin modified SOD to enhance its stability

Cyclodextrins (CD), cyclic oligosaccharides containing six (α-CD), seven (β-CD) or eight (γ-CD) α-1-4-linked D-glucopyranose units have been used to stabilize enzymes in order to increase their activities and favor immobilization. On the one hand, superoxide dismutase modified by β-cyclodextrin could improve its performance. A superoxide dismutase was glycosylated by cyclodextrin-branched carboxymethylcellulose and its plasma half-life time was prolonged from 4.8 min to 7.2 h, its anti-inflammatory activity also increased by 2.2 times [21]. On the other hand, cyclodextrin and its derivative could synthesize SOD mimics. Puglisi et al. [22] reported a 6A,6B-Dideoxy-6A,6B-di[(N-salicylidene)amino]-β-cyclodextrin conjugated with a manganese(III) complex showed a SOD-like activity and a good solubility that favor its application.

3. CAT

3.1. The structure and function of CAT

CAT(EC 1.11.1.6) catalyzes the decomposition of hydrogen peroxide to water and oxygen, widely exists in animals, plants, and microorganisms. Its uniqueness lies in the enzymatic prosthetic group(ferriprotoporphyrin IX) that could catalyze the same reaction as the holoenzyme.

$$2H_2O_2 \rightarrow O_2 + 2H_2O \quad (2)$$

According to the significant catalytic activity, CATs can be divided into three distinct subgroups: typical catalases, a typical catalases and catalase-peroxidases [23]. Two subgroups, typical catalases and catalase-peroxidases, contain heme, but the third group has no heme, namely manganese catalases. Most of catalases belong to typical catalases, except catalases in the domain of Archaea. Although there are differences in the primary structure among these typical catalases, but the three-dimensional structure appears well conserved. Most of these hydroperoxidases are homotetramers with four prosthetic heme groups (**Figure 2(a)**, PDB, 1E93) [24].

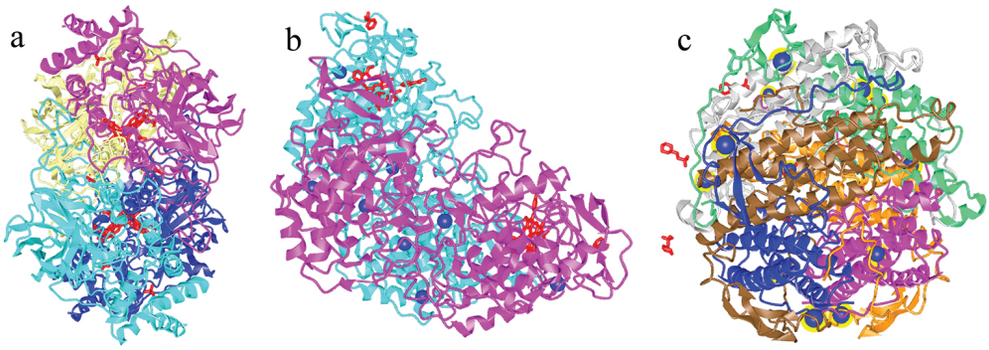

Figure 2. The three-dimensional structure of three types of catalases. (a) a typical catalase (depleted in iron) from *Proteus mirabilis*, (b) a catalase-peroxidase from *Synechococcus elongatus* PCC7942, (c) a manganese catalase from *Lactobacillus plantarum*. Color scheme: blue ball—Fe or Mn, red—heme group, others colors indicate different subunit.

Catalase-peroxidases may originate from ancestral, a relatively large momomeric unit comprising more than 700 amino acids, indicating that they probably from duplication of an ancestral gene [25]. And catalase-peroxidases show much higher sequence homology with heme peroxidases than with typical catalases. Recently, a three-dimensional structure of catalase-peroxidases has been obtained and is shown in **Figure 2(b)** (PDB, 3WXO) [26].

There is little structural information about manganese catalases up to now. Subunits with molecular weights around 30 kDa are recognized as tetramers or hexamers, and are remarkably stable at high temperatures [23, 27]. A crystal structure of a manganese catalase from *Lactobacillus plantarum* at 1.33a resolution is established (**Figure 2(c)**, PDB, 1O9I) [28].

3.2. Inhibitor of catalase

3.2.1. Noncompetitive inhibitor

3.2.1.1. Sodium azide, amine, and cyanide

Sodium azide (NaN_3), amine, and cyanide are nonspecific inhibitors of CAT. Catalase-peroxidases are very sensitive to NaN_3, a lower concentration of NaN_3 could lead to the enzyme

lose its activity by 50%. The inhibitory efficiency order was sodium azide>amine>cyanide>3-amino-1,2,4-triazole(**Table 2**) [29]. A similar result was found in the other typical monofunctional catalases [30, 31].

Inhibitors	Inhibitor concentration (mM) required for 50% inhibition	
	Catalase	Peroxidase
3-Amino-1,2,4-triazole	6	30
Hydroxylamine	0.02	1.5
Sodium azide	0.025	0.15
Potassium cyanide	1	1

Table 2. Effect of inhibitors on the catalase and peroxidase activity of the catalase-peroxidase of *Archaeoglobus fulgidus* [29].

3.2.1.2. 3-Amino-1,2,4-triazole

3-Amino-1,2,4-triazole (aminotriazole, ATZ) as a noncompetitive catalase-specific inhibitor is used to study on physiological changes in organisms [32]. Aminotriazole could combine catalase-H_2O_2 compound I, thus results in loss of enzymatic activity. In alcohol-induced liver injury, catalase plays a dual role. On the one hand, catalase could scavenge hydrogen peroxide originated from alcohol to water, but on the other hand, catalase decomposes alcohol that might be harmful to liver, some research studies [33] show that catalase is inhibited by ATZ, which attenuated alcohol-induced acute liver injury.

3.2.1.3. Salicylic acid

Salicylic acid acts as an electron donor for the peroxidative cycle of catalase, it is a noncompetitive inhibitor of catalases. It is interesting to note that different CAT salicylic acid exhibits different inhibitory property. CAT1 and CAT2 are two isoenzymes from maize (*Zea mays* L.). The Lineweaver-Burk plot of SA inhibition of CAT1 and CAT2 shows that CAT1 is noncompetitive manner, while CAT2 is inhibited in a competitive manner [34]. SA has a dual function on catalase, which means SA can both inhibit and activate its activity. Durner and Klessig [35] examined the effects of SA on the formation of the various redox states or reaction intermediates of catalase (**Figure 3**). The absorption spectrum of compounds I, II and III was different, thus various redox states or reaction intermediates of catalase can be distinguished spectroscopically by their absorption spectra in the Soret (near UV) region. Through the difference at the absorption spectra of intermediates, SA was confirmed acting as a one-electron donor that siphons compound I from the extremely fast catalytic cycle into the relatively slow peroxidative cycle (~1000 times slower) by promoting the formation of compound II [36, 37].

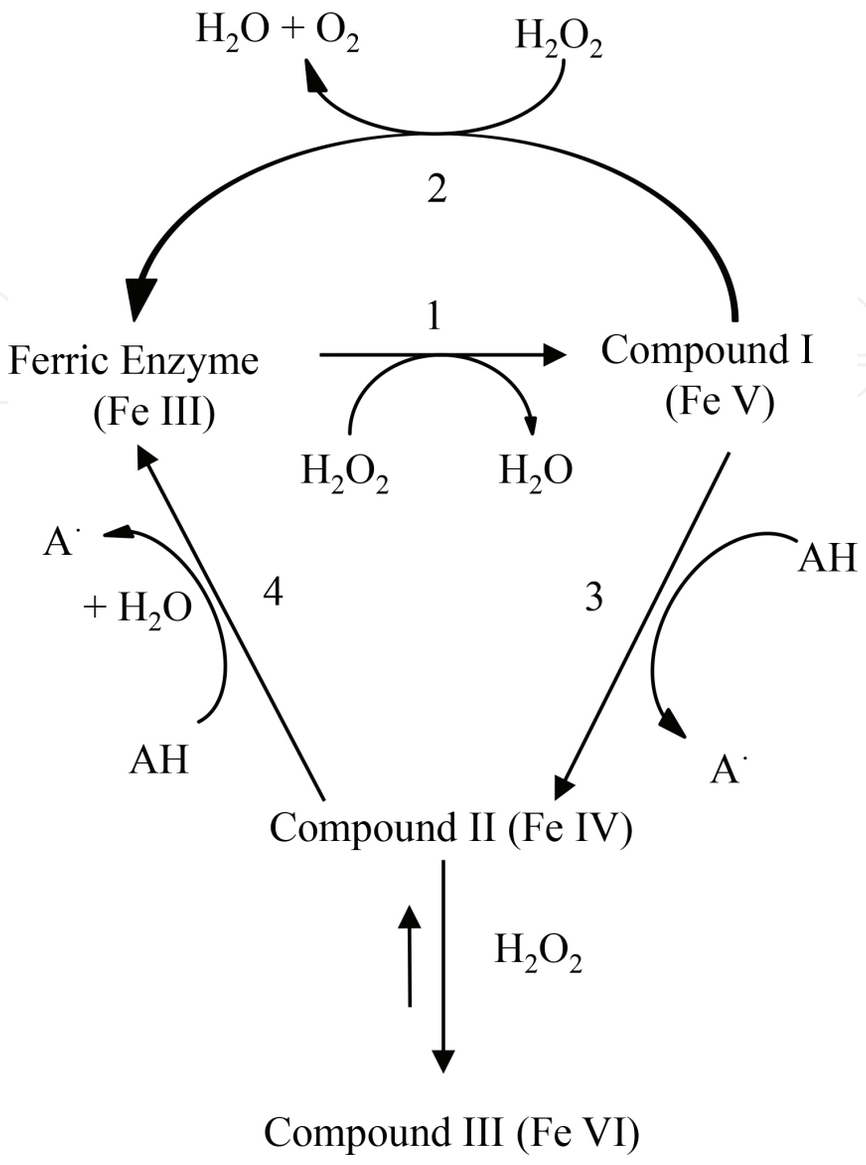

Figure 3. The reaction cycles of catalase [35].

3.2.2. Competitive inhibitor

3.2.2.1. Metal ions

Catalase mainly used in industrial sectors such as textiles, pulp, and paper, their work environment often with high concentration of metal ions. Previous studies have elaborated that catalase can be inhibited by certain metal ions (including Cu^{2+}, Zn^{2+}, and Ag^{+}), a process depends on the metal, concentration, the tissue, and species [38]. Lee et al. [39] compared several divalent metal ions on catalase-peroxidase (KatG) activity, only the manganese ion revealed some inhibitory effects on the recombinant KatG activity, and EDTA could relieve partly inhibited activity. This implies that manganese may competitively bind to near the heme group and be involved in the enzyme reaction.

3.2.2.2. ρ-Hydroxybenzoic acid is a competitive inhibitor of catalases from maize

Phenolic compounds, such as salicylic acid, aspirin, benzoic acid, o-coumaric acid, and ϱ-hydroxybenzoic acid play a role in the induction of abiotic stress resistance. But only ϱ-hydroxybenzoic acid showed the inhibitory effect on two catalases from maize in a competitive manner, the other compounds were in noncompetitive manner. Weak inhibition by ϱ-hydroxybenzoic acid was also found in both isozymes, only 15 and 9% activity was inhibited, respectively [34].

3.3. Activator of catalases

Metformin is a commonly used antidiabetic drug with AMP-activated protein kinase (AMPK)-dependent hypoglycemic activities. A recent study [40] shows that metformin can significantly enhance the activity of catalase. Although metformin bound to CAT byinteracting with hydrogen bonds…., metformin did not affect the expression level of catalase, just affecting its activities, such as Lys449, Val450, and Glu455 residues in murine CAT. The preliminary study indicated that metformin might be a new drug to alleviate oxidative injury and enhance the defense ability of antioxidants.

4. GPX

4.1. The structure and function of GPX

GPX is an important selenium-containing enzyme which protects cells from lipid peroxide damage and H_2O_2. GSH-Px widely existed in the body, there are eight family members: GPX1 is the most abundant selenoperoxidase and is ubiquitously expressed in almost all tissues; GPX2 expression is most prominent in the gastrointestinal tract cytoplasm; GPX3 is greatest expressed in the kidney, and also in various tissues, and is secreted into extracellular fluids as a glycoprotein; GPX4 is the only GPX enzyme that reduces phospholipid hydroperoxides, different with other members, it is not a tetramer, but a monomer; GPX6 was identified as a selenoprotein in the human genome by homology search. GPX1-4 and GPX6 are selenium-containing protein, but GPX5 does not contain selenocysteine or Se in active site [41, 42]. More recently, GPX7 (NPGPx) and GPX8 were discovered, but detailed information about these two kinds of enzymes is little up to now [43].

Molecular weight of GSH-PX in human red blood cell was 95,000 Da and that in bovine red blood cell was 83,000 Da, and they are all tetrameric selenoenzyme. A typical crystal structure of human glutathione peroxidase (2I3Y) is shown in **Figure 4**.

GSH-PX contains one selenocysteine per subunit and selenocysteine plays an important role in catalyst degradation of lipid peroxide, a widely accepted mechanism was proposed as follow:

$$E-CysSe^{-}+H^{+}+ROOH \rightarrow E-CysSeOH+ROH \quad (3)$$

$$E-CysSeOH+GSH \rightleftharpoons E-CysSe-SG+H_2O \quad (4)$$

$$E-CysSe-SG+GSH \rightleftharpoons E-CysSe^{-}+GSSH+H^{+} \quad (5)$$

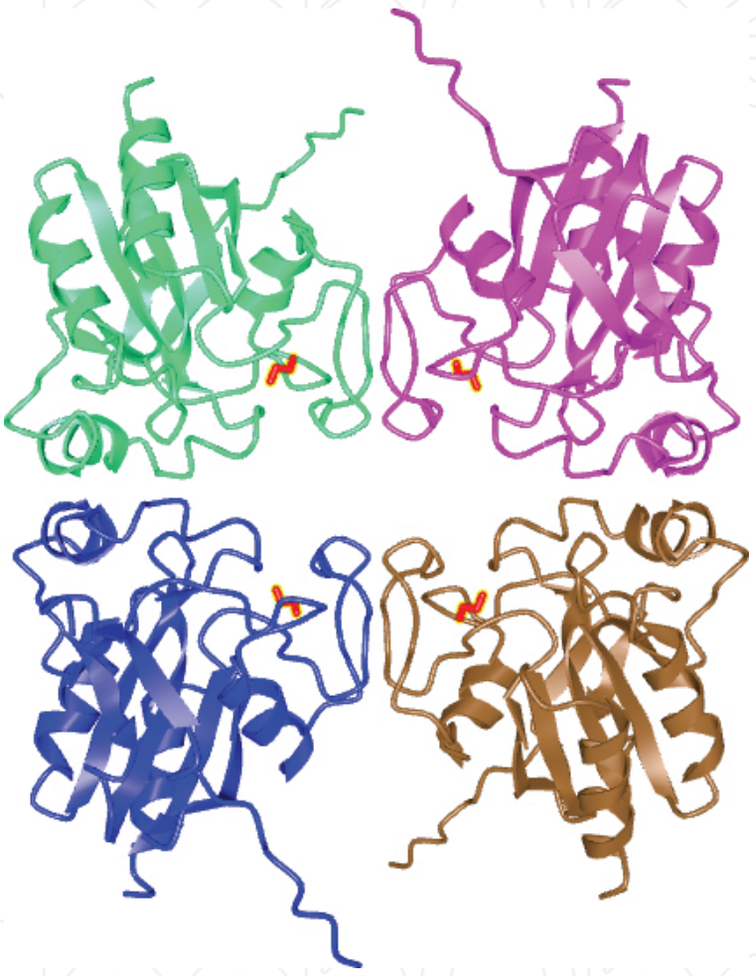

Figure 4. Three-dimensional structure of human glutathione peroxidase.

4.2. The effect of selenium on GPX activity

There are approximate 25 selenoproteins in humans, and selenium is an essential cofactor for these proteins, including the glutathione peroxidases. There is a complex relationship among utilization of selenium, GPX activity, and methylation. GPX synthesis utilizes selenium via selenocysteine and homocysteine is derived from *S*-adenosylhomocysteine, and the latter was formed as a result of methylation reactions including methylation of selenium [44]. From the view of selenium forms, the inorganic forms (such as sodium selenate and sodium selenite) of

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selenium were more effective on increasing the GPX activity compared with selenomethionine [44]. To be attention, different members of GPXs response differently to selenium deficiency, a phenomenon called the "hierarchy of selenoproteins." This means that upon selenium deprivation, some proteins decline fast, whereas others remain synthesized until selenium becomes severely deficient [45]. The expression of GPX1 mRNA, protein, and its activity in tissues is more sensitive than other selenoperoxidases or selenoproteins [46].

4.3. The effect of vitamin E on GPXs activity

DL-Alpha-tocopherol (vitamin E) also is an antioxidant, but it is different from selenium acting on GPX directly, vitamin E plays its antioxidant function through combining free radical, named "chain-breaking reaction." Vitamin E was considered to be the first line of defense against lipid peroxidation and free oxygen radicals that might suppress the enzymes, such as GPX. It seems that the sensitivity of GPXs was various. In vitamin E-deficient rat brain microsomes, phospholipid hydroperoxide glutathione peroxidase activity was significantly decreased but GPX activity was not affected. And in liver homogenate, phospholipid hydroperoxide glutathione peroxidase activity was approximately 20 times lower than that of GPX [47].

4.4. Inhibitors of GPXs

4.4.1. Competitive inhibitors

4.4.1.1. Misonidazole

GPX can combine to an electrophilic compound that might result in loss of its activity. More and more evidence shows that upregulation of the GPX system may serve to protect cancer cells from oxidative stress caused by anticancer drugs, thus block GPX that may help to treat cancer disease. A number of inhibitors of GPXs have been reported to use as therapeutics, such as thiol-containing inhibitors that bind covalently to a selenium atom in the active site [48], nonthiol inhibitors misonidazole [49]. However, thiols tend to combine ubiquitous multivalent metal ions and are easily oxidized, thus leading to nonspecific interactions with proteins. Recently, acylhydrazones have been reported as potential inhibitors of bovine glutathione peroxidase [50]. These inhibitors overcome the disadvantages of thiol-containing inhibitors, but the efficiency needs to be further improved.

4.4.1.2. Penicillamine and its analogues

D-Penicillamine is a drug to chelate metals in tissue and promotes its excretion in the urine. D-Penicillamine hydrochloride could competitively inhibit GSH-PX, that means the concentration of hydrogen peroxide and reduced glutathione were inversely proportion [51].

L-penicillamine hydantoin is an analogue of glutathione, but the acting configuration is different from D-penicillamine hydrochloride. After treated with L-penicillamine hydantoin, GPX activity was inhibited whatever the peroxide (H_2O_2, terl-butyl hydroperoxide orcumene

hydroperoxide) used as substrate of the reaction. In the presence of 100 μM L-penicillamine hydantoin, the enzyme reactions catalyzed by glutathione peroxidase were inhibited, but neither glutathione transferases, nor glutathione reductase were affected by L-penicillamine hydantoin [52].

4.4.2. Noncompetitive inhibitors

4.4.2.1. DL-Buthionine-[S, R]-sulfoximine

DL-Buthionine-[*S, R*]-sulfoximine (BSO) can be used as an inhibitor to estimate the scavenging efficiency of H_2O_2 after GPX inhibition. The inhibitory effect did not act on GPX directly, but through suppress the synthesis of GSH by inhibiting γ-glutamylcysteine synthetase, that cause glutathione decreased sharply in many tissues, especially kidney, liver, and pancreas [53]. BSO has an obvious inhibitory effect, for example in human fibroblasts cells, after 500 μM BSO treated, the GSH levels decreased to154.0 ± 16.9 nmol/mg protein from 418.4 ± 13.1 nmol/mg protein [54].

4.4.2.2. Gold(I) thioglucose

Gold(I) thioglucose in the presence excess of glutathione (GSH) leads to strong and reversible inhibition of selenium-GPXs. Gold(I) could competitively combine in reduced form of selenocysteine in active sites, and gold(I) forms a dead-end complex with glutathione peroxidase resulting in suppression of GPXs. So glutathione peroxidase could be a target of gold drugs that used in the treatment of disease caused by excessive activity of GPXs, such as rheumatoid arthritis [55].

4.5. Activators of GPXs

To our knowledge, most literature studies on enhancing GPXs activity were about how to regulate expression of GPXs, study on the activator by acting the enzyme directly was few and most of them are GPX-mimetic compounds.

4.5.1. Enhance activity of GPX mimetics

For some GPX mimetics, its activity can be enhanced by electron-donating. naphthalene *peri*-diselenide mimeticswas increased by electron-donating methoxy substituents, while a further 100-fold increase was observed with the corresponding ditelluride. This was attributed to the ability of the methoxy group to stabilize the increasing positive charge at the selenium atom during the rate-determining step of the catalytic cycle, which involves the oxidation of Se(II) to Se(IV), thus improved their catalytic activity to levels comparable with their aliphatic counterparts [56]. Others report that 6-bromo-substituted diselenides also enhanced its activity by threefold [57]. Another strategy is to change the aqueous solubility of the mimetics. Diaryl selenides containing *o*-hydroxymethylene substituents function as peroxide-destroying mimetics of the antioxidant selenoenzyme glutathione peroxidase. Several selenide analogues

were attached to polyethylene glycol (PEG) oligomers greatly improved aqueous solubility and catalytic activity (10–100 folds) [58].

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Author details

Xianyong Ma[1,2,3*], Dun Deng[1,2,3] and Weidong Chen

*Address all correspondence to: 407986619@qq.com

1 Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou, China

2 Key Laboratory of Animal Nutrition and Feed Science in South China, Ministry of Agriculture, Guangzhou, China

3 Guangdong Key Laboratory of Animal Breeding and Nutrition, Guangzhou, China

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