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REVIEW PAPER

Antioxidant Activity of Sulfur and Selenium: A Review of Reactive Oxygen Species Scavenging, Glutathione Peroxidase, and Metal-Binding Antioxidant Mechanisms

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Abstract It is well known that oxidation caused by reactive oxygen species (ROS) is a major cause of cellular damage and death and has been implicated in cancer, neurodegenerative, and cardiovascular diseases. Small-molecule antioxidants containing sulfur and selenium can ameliorate oxidative damage, and cells employ multiple antioxidant mechanisms to prevent this cellular damage. However, current research has focused mainly on clinical, epidemiological, and in vivo studies with little emphasis on the antioxidant mechanisms responsible for observed sulfur and selenium antioxidant activities. In addition, the antioxidant properties of sulfur compounds are commonly compared to selenium antioxidant properties; however, sulfur and selenium antioxidant activities can be quite distinct, with each utilizing different antioxidant mechanisms to prevent oxidative cellular damage. In the present review, we discuss the antioxidant activities of sulfur and selenium compounds, focusing on several antioxidant mechanisms, including ROS scavenging, glutathione peroxidase, and metal-binding antioxidant mechanisms. Findings of several recent clinical, epidemiological, and in vivo studies highlight the need for future studies that specifically focus on the chemical mechanisms of sulfur and selenium antioxidant behavior.

Keywords Antioxidant mechanism · Sulfur antioxidants · Selenium antioxidants · Glutathione peroxidase · Reactive oxygen species scavenging · Metal binding

Abbreviations

ROS	Reactive oxygen species
GPx	Glutathione peroxidase
$^{\bullet}OH$	Hydroxyl radical
H_2O_2	Hydrogen peroxide

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Introduction

Reactive oxygen species (ROS) are an inevitable by-product of cellular respiration causing oxidation of lipids, nucleic acids, and proteins, and ROS damage is an underlying cause of disease, including cancer, inflammatory, and neurodegenerative diseases [1–5]. Cells have sophisticated antioxidant regulatory systems to maintain proper balance of ROS; however, disruption in homeostasis can result in oxidative stress and tissue injury [6, 7]. Studies have shown that metals, including iron, copper, chromium, lead, mercury, nickel, and vanadium generate ROS [8]. The contribution of metal ions to ROS generation is most common in Fenton or Fenton-type reactions where endogenous metals, such as Fe^{2+} or Cu^{+}, react with hydrogen peroxide to generate hydroxyl radical ($^{\bullet}OH$) [9].

Antioxidants ameliorate oxidative damage caused by ROS, and research has focused on the role of antioxidants for the treatment and prevention of disease [1, 10]. Antioxidants, including polyphenols, sulfur- and selenium-containing compounds, enzymatic antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), and micronutrients such as vitamins C and E, have been extensively investigated, and numerous studies have demonstrated their antioxidant properties [1, 11–19]. For example, vitamin E supplements decrease the risk of colon and prostate cancers, and also reduce the risk of coronary disease by approximately 40% [20]. Similarly, consumption of fruits and vegetables rich in polyphenols is inversely correlated to the incidence of lung cancer among tobacco smokers [21], and reduces blood pressure and

cholesterol levels, both risk factors associated with cardiovascular disease [22]. SOD and GPx activity is lower in intellectually disabled patients with hypothyroidism, suggesting that premature aging and increased mortality rates among the intellectually disabled could be due to increased ROS generation and imbalance of antioxidant defense mechanisms [23]. Decreased intracellular vitamin E and glutathione concentrations cause lipid peroxidation in mice models mimicking Alzheimer's disease; increased SOD and GPx activities due to elevated levels of oxidative stress are also found [24].

Sulfur and selenium compounds are also studied for their antioxidant properties and their ability to prevent disease. For example, a recent study indicates that aqueous garlic extract protects against arsenic toxicity [25]. Additionally, patients with pulmonary tuberculosis show reduced oxidative stress caused by ROS generation with selenium supplementation [26]. The protective effects of sulfur and selenium compounds against disease are commonly attributed to radical scavenging and enzymatic decomposition of oxygen metabolites [1, 27]. More recently, coordination of sulfur and selenium compounds with metal ions has been proposed as an additional antioxidant mechanism [12, 13, 15, 16]. Collins et al. have reported selenium–copper complexes that utilize both metal binding and ROS scavenging in oxidative stress prevention [28]. Our research has demonstrated that metal coordination is required for inhibition of copper- and iron-mediated DNA damage by sulfur, oxo-sulfur, and selenium compounds [12, 13, 15, 16]. Metal binding as a novel antioxidant mechanism for sulfur and selenium may be complementary to ROS scavenging and GPx activity.

Despite current research investigating the efficacy of selenium and sulfur antioxidants for disease treatment and prevention, little work has focused on the chemical mechanisms responsible for the observed antioxidant properties. Furthermore, it is frequently assumed that chemically similar antioxidants have the same mechanisms of action without sufficient evidence to support these claims. Thus, this review discusses clinical, in vitro, and in vivo studies investigating sulfur and selenium antioxidant activity by several antioxidant mechanisms, including ROS scavenging, GPx activity, and metal-binding interactions. From these studies, we emphasize areas for future research and demonstrate the importance of understanding the mechanisms of antioxidant activity for the treatment and prevention of disease.

Generation and Reactivity of Reactive Oxygen Species

Reactive oxygen species are classically defined as oxygen-containing radicals capable of independent existence with one or more unpaired electrons; however, the term ROS is most often expanded to include reactive oxygen-containing compounds without unpaired electrons, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) [29, 30]. According to this definition, molecular oxygen (O_2) is also a radical species due to the two unpaired electrons in its triplet ground state; fortunately for aerobic organisms, this triplet electronic configuration renders O_2 relatively unreactive [29, 30].

The consumption and utilization of oxygen in physiological processes result in the inevitable generation of ROS. Energy production in mitochondria is dependent on oxygen metabolism, since O_2 is reduced to H_2O. During this complex electron transfer pathway, incomplete reduction of O_2 can result in generation of highly reactive and damaging ROS, including superoxide radical ($O_2^{-\bullet}$), singlet oxygen, hydrogen peroxide, and hydroxyl radical ($^{\bullet}OH$) [31]. Additionally, environmental agents such as ultraviolet radiation, thermal stress, inflammatory cytokines, ozone (O_3), nitrogen dioxide (NO_2), tobacco smoke, carbon tetrachloride (CCl_4), paraquat, and chemotherapeutic drugs contribute to cellular ROS generation and oxidative stress [29].

Superoxide Radical

Generation of superoxide radical occurs upon reduction of O_2, and, in contrast to O_2, is highly reactive (Fig. 1, reaction 1) [29, 32]. Superoxide causes the inactivation of enzymes, including catalase and GPx, and oxidation of intracellular components, such as glutathione, due to its long half-life (0.05 s in the absence of scavengers) [33, 34]. Studies investigating the role of superoxide radical in disease development have implicated $O_2^{-\bullet}$ in cancer [35], inflammatory [36], cardiovascular [37], and neurodegenerative diseases [38]. Superoxide alone, however, is not capable of damaging DNA directly [39, 40].

The toxicity of superoxide radical is greatly diminished by the antioxidant metalloenzyme SOD that catalyzes

(1) $O_2 + e^- \rightarrow O_2^{\bullet-}$

(2) $2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$

(3) $2O_2^{\bullet-} + NO^{\bullet} \rightarrow ONOO^-$

(4) $O_2 + h\nu \rightarrow {}^1O_2$

(5) $2H_2O_2 \rightarrow 2H_2O + O_2$

(6) $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$

(7) $Cu^+ + H_2O_2 \rightarrow Cu^{2+} + {}^{\bullet}OH + OH^-$

Fig. 1 ROS generation and decomposition reactions

reduction of $O_2^{-\bullet}$ to the less reactive H_2O_2 and O_2 (Fig. 1, reaction 2) [33]. Three different isoforms of SOD have been identified in humans and all contain metal ions (copper, zinc, or manganese) in their active sites [41]. The protective effects of SOD have been demonstrated in animal models where SOD significantly protects the heart and brain from ischemic injury [42, 43] and prevents alcohol-induced liver injury [44]. Mutations in SOD have been implicated in amyotrophic lateral sclerosis (ALS), a debilitating neurodegenerative disease characterized by the degradation of motor neurons resulting in paralysis and death [45]. Similarly, mutation in SOD results in increased susceptibility to Type 2 diabetes in humans, cancer, and Alzheimer's disease [46–49]. Overexpression of SOD also results in increased oxidative stress associated with Down syndrome, although the exact mechanism is not well understood [50]. Other antioxidants, including green tea extract [51], are also capable of preventing damage from superoxide radical.

Peroxynitrite

Under inflammatory conditions, cells will generate superoxide and nitric oxide radicals that react to form peroxynitrite ($ONOO^-$; Fig. 1, reaction 3). Peroxynitrite causes DNA damage and lipid oxidation and has been implicated in aging due to damage of guanine repeats in telomeres and joint disease caused by decreased production of collagen [1, 36]. Peroxynitrite generation has also been implicated in cardiovascular disease (vasorestriction) due to decreased availability of nitric oxide [29, 52]. Protection against peroxynitrite damage has been studied with selenium compounds: selenomethionine and selenocystine are reported to prevent single-stranded breaks from $ONOO^-$ in DNA [53], and Klowtz et al. have demonstrated the protective effects of ebselen and GPx against peroxynitrite-mediated damage [54].

Singlet Oxygen

Electronic excitation of molecular oxygen generates singlet oxygen ($^1O_2$; Fig. 1, reaction 4) [55]. Singlet oxygen is not a radical species, but unlike O_2 it is very reactive and has a half-life of 10^{-5} s [55, 56]. Environmental agents such as ultraviolet radiation and ozone can generate singlet oxygen; other processes, including termination of peroxyl radicals, peroxidase-mediated reactions, peroxynitrite reactions, and H_2O_2 reactions, also generate singlet oxygen [56]. Nucleic acids, proteins, lipids, and sterols are the primary biological targets for singlet oxygen damage, and oxidation of these molecules has been implicated in skin cancer. However, antioxidants such as β-carotene and ascorbic acid scavenge singlet oxygen [57].

Interestingly, the deleterious effects of singlet oxygen have been utilized in photodynamic therapy to induce apoptosis in carcinogenic cells. Using this treatment, a light-sensitive agent accumulates in carcinogenic cells, and upon irradiation, generates singlet oxygen and other ROS that cause cytotoxicity and cell death [58, 59]. Thus, singlet oxygen also has beneficial effects in cancer treatment.

Hydrogen Peroxide

Similar to singlet oxygen, H_2O_2 is also not a radical species and is relatively stable [9, 29]. However, interest in H_2O_2 is focused on its ability to generate ROS, in particular the hydroxyl radical ($^{\bullet}OH$). Several biological processes generate H_2O_2: reduction of superoxide by SOD produces H_2O_2 and O_2 (Fig. 1, reaction 2), and other enzymes such as glycolate oxidase, amino acid oxidase, and urate oxidase are also sources of H_2O_2 [31, 33]. Cellular enzymes such as catalase and GPx scavenge H_2O_2 by reducing it to H_2O (Fig. 1, reaction 5) [57].

Hydroxyl Radical

Reduction of H_2O_2 by redox-active metal ions generates the hydroxyl radical, considered to be the most reactive and harmful ROS [9, 60, 61]. The lifetime of $^{\bullet}OH$ is diffusion limited (10^{-9} s); therefore, it reacts with molecules immediately after formation and release. The primary source of cellular hydroxyl radical is from Fenton or Fenton-type reactions with copper(I) and iron(II) (Fig. 1, reactions 6 and 7). Hydroxyl radical formation causes oxidation of lipids, proteins, and nucleic acids; DNA strand breaks, base modifications, and DNA cross linking have also been observed [3, 62]. Due to the dependence of $^{\bullet}OH$ formation on metal ions, disruptions of metal homeostasis and increases in non-protein-bound metal ion concentrations cause significant increases in $^{\bullet}OH$ generation and oxidative stress.

Metal-Mediated Generation of Reactive Oxygen Species

Metal ions such as Cu^+, Fe^{2+}, Mg^{2+}, Zn^{2+}, Ni^{2+}, and Co^{2+} are required for biological processes such as oxygen transport, electron transfer, and catalysis [63], and are found in numerous enzymes and proteins [63]. Without these metal ions, normal physiological function would be impossible. Although transition metal ions are essential, they must also be regulated due to their potential toxic effects. Metal toxicity due to mis-regulation of homeostasis results in increased oxidative stress due to ROS generation and has been implicated in diseases such as hemochromatosis, anemia, Wilson's and Menkes diseases, diabetes,

ALS, cancer, and inflammatory and neurodegenerative diseases [5, 64–70]. Cells employ multiple pathways to maintain metal homeostasis [71]; however, metal-mediated ROS generation and subsequent cellular damage still occur. Under normal conditions, the availability of metal ions to generate ROS is minimal due to sequestration and storage. Iron in the blood is tightly bound by transferrin, an iron-transport protein, and stored in ferritin in cells [72]. Similarly, metallothionein participates in cellular zinc and cadmium binding and storage [73], and copper is sequestered by metallochaperones and proteins, such as ceruloplasmin [74]. However, the availability of metal ions increases when homeostasis is not maintained [6, 7, 40].

By far the greatest research effort has focused on metal-mediated ROS generation by copper and iron. These metals are the most common transition metals found in biological systems and play a key role in the generation of $^{\bullet}OH$. Iron reacts with endogenous H_2O_2 to generate $^{\bullet}OH$ in the Fenton reaction (Fig. 1, reaction 6) [72]. This iron-mediated $^{\bullet}OH$ production is catalytic in vivo if cellular reductants, such as ascorbic acid [62] and NADH [75], are present to reduce Fe^{3+} to Fe^{2+}. Imlay et al. extensively studied cell death in *E. coli* caused by H_2O_2 and non-protein-bound iron and established that iron-mediated DNA damage is the primary cause of cell death [9, 76, 77]. Similarly, DNA damage and cell death in mammalian fibroblasts are attributed to iron-mediated hydroxyl radical formation [78].

Due to its role in ROS generation and resulting oxidative damage and disease, iron levels in cells are tightly regulated [72, 79–82]. The normal concentration of non-protein-bound (or labile) iron in *E. coli* is $\sim$20 μM, but this concentration can increase 4–16 times when homeostasis is not maintained [9, 40, 83]. Non-protein-bound iron promotes tumor growth in rat epithelial cells [84], and elevated levels of labile iron in mice and humans contribute to oxidative stress observed in *Ataxia telangeictasia*, an autosomal recessive disease characterized by pre-mature aging and increased cancer incidence in humans [85]. A recent study of human erythroid cells from blood, bone marrow, and cell cultures found labile iron pools 1.5-fold higher in diseased cells [86]. Patients diagnosed with Type 2 diabetes (for more than 5 years) had serum non-protein-bound iron levels approximately 16 times higher than patients with normal iron levels [87]. Interestingly, Tuomainen et al. demonstrated that even normal body levels of iron in humans generate oxidative stress [88]. Furthermore, the release of redox-active metals due to oxidative stress also contributes to elevated non-protein-bound iron concentrations. Evans et al. have demonstrated that damage to human arterial walls causes the release of iron and copper ions and promotes cardiovascular disease [89]. Hydrogen peroxide also causes the release of iron from hemoglobin, promoting hydroxyl radical formation, and oxidative cellular damage [90]. Metal release due to brain ischemia and reperfusion injuries is also caused by oxidative stress [91].

In addition to iron, copper is an essential metal required for normal biological function and is the third most abundant transition metal ion found in the body after iron and zinc [92]. Copper serves several biological functions, including oxygen transport, electron transfer, and oxidase activity [63]; proteins such as ceruloplasmin and albumin transport copper throughout the body [30].

Similar to iron, copper generates hydroxyl radical in a Fenton-type reaction (Fig. 1, reaction 7); however, hydroxyl radical generation is 50 times faster with copper than iron [62]. Copper homeostasis is closely monitored within a cell to maintain the required amount needed for physiological function while avoiding toxic levels. There has been considerable debate about the concentration of non-protein-bound or labile copper pools within cells [93]. O'Halloran and co-workers estimated the concentration of labile copper to be less than one copper ion per cell (10^{-18} M) in yeast [94]. More recently, Fahrni and co-workers reported the existence of a labile copper pool localized in mitochondria and Golgi apparatus of mouse fibroblast cells using fluorescent sensing [95]. Additionally, Miller et al. also observed labile copper pools in mammalian cells using a fluorescent dye [96]. Unsurprisingly, elevated levels of labile copper have been associated with oxidative stress and disease [93, 97, 98]. Zappasodi and co-workers demonstrated that non-protein-bound copper levels were higher in patients with Alzheimer's disease [99], and elevated levels of copper have been observed in Wilson's disease [69], cancer [100], renal [98], and cardiovascular diseases [70].

Cells have the ability to prevent oxidative damage by chelating redox-active metals that generate ROS. Specific metal-binding sites in metalloproteins, including ceruloplasmin, transferrin, metallothionein, and ferritin, can be used to sequester excess metal ions with high binding affinity [101–103]. Chelating drugs are also used to prevent excess metal accumulation and toxicity. For example, several iron-specific chelating drugs including desferrioxamine B and Deferiprone (L1) are used to treat iron overload associated with hemochromatosis and β-thalassemia [104–106]. For copper, *N*-acetylcysteine amide (NACA), penicillamine, and tetrathiomolybdate have been used to treat Wilson's disease [107]. Sulfur and selenium antioxidant complexes with copper and iron have also been reported and are discussed in detail later in this review [108–112].

The antioxidant activity of sulfur- and selenium-containing compounds has led researchers to focus intensely on developing these compounds to treat or prevent disease. Numerous epidemiological reviews and scientific studies

focusing on the antioxidant properties of sulfur- and selenium-containing compounds are available [101, 102, 113, 114]; however, very little work in the way of antioxidant activity mechanisms of these compounds with metal-mediated oxidative damage has been emphasized. In this review, the antioxidant activity and mechanisms of sulfur and selenium compounds in clinical, in vivo, and in vitro studies are discussed with emphasis on ROS scavenging, GPx activity, and metal binding.

Sulfur Antioxidant Activity

Sulfur is an essential component in normal physiological function and is incorporated into amino acids, proteins, enzymes, and micronutrients [114]. Humans satisfy their nutritional needs for sulfur by consuming plants and animals in their diets, which are found in milk, cheese, garlic, onions, leeks, scallions, chives, shallots [115], eggs, fruits, and cruciferous vegetables (Table 1) [114, 116, 117]. Biological sulfur-containing compounds, including cysteine, methionine, taurine, glutathione (GSH), *N*-acetylcysteine (NAC), and other sulfur compounds (Fig. 2, Table 1) have been extensively studied for their antioxidant properties [113–115].

Methionine is an essential amino acid obtained through diet and is the primary source of sulfur in the body [113, 114]. Methionine is a methyl donor and is required for protein synthesis; radical scavenging activity is also reported for methionine [118, 119]. Cysteine is required for GSH and protein synthesis; biological concentrations are typically in the low micromolar range (100–200 μM in *E. coli*) [9]. Cysteine also plays a critical role in protein structure, forming disulfide crosslinks that stabilize protein conformation [120]. Other amino acid derivatives are also essential for biological function. Taurine is derived from methionine and cysteine metabolism or obtained through the diet [114]. Its primary functions include modulation of calcium levels, detoxification, and bile acid conjugation [113]. *N*-acetylcysteine is an intermediate in the synthesis of glutathione from cysteine. NAC transports cysteine, scavenges ROS, and replenishes GSH levels, and has been widely studied for its antioxidant properties [114, 121–124].

Glutathione is the most abundant non-protein-bound thiol-containing compound found in cells, with intracellular concentrations of 1–15 mM [1, 125]. Glutathione is a major component in cellular antioxidant systems, acting as a detoxifying agent for endogenous radical species and as an essential co-factor for GPx, although glutathione and other sulfur-containing compounds do not have GPx activity [68]. Studies also indicate that non-enzymatic protection against radical species, specifically oxygen radicals, is also a primary function [68]. Additionally, the redox balance of glutathione (GSH/GSSG) and cysteine/cystine in cells has become a biological indicator of oxidative stress and

Table 1 Sources and activities of sulfur compounds discussed in this review

Sulfur compound	Source	Activity	Reference
Methionine	Diet	Antioxidant	[16, 114, 121, 129]
Cystine	Endogenously synthesized	Antioxidant	[16, 114]
Methyl-cysteine	Diet	Antioxidant	[16, 130]
Taurine	Diet/endogenously synthesized	Antioxidant	[114, 131]
Cysteine	Diet/endogenously synthesized	Antioxidant/prooxidant	[16, 114, 132–134]
Homocysteine	Endogenously synthesized	Antioxidant/pro-oxidant	[132, 135–138]
N-acetylcysteine	Diet/endogenously synthesized	Antioxidant/pro-oxidant	[114, 121, 124, 139–146]
N-acetylcysteine amide	Synthetic	Antioxidant	[122, 142]
Dimethyl sulfoxide	Synthetic	Antioxidant	[129]
Diallyl sulfide	Diet (*Allium* vegetables)	Antioxidant	[114, 147, 148]
Diallyl disulfide	Diet (*Allium* vegetables)	Antioxidant	[114, 147, 148]
S-Allyl-L-cysteine	Diet (*Allium* vegetables)	Antioxidant	[147, 148]
Diallyl trisulfide	Diet (*Allium* vegetables)	Antioxidant	[114, 147, 148]
Allitridum	Diet (*Allium* vegetables)	Antioxidant	[149]
Glutathione	Diet/endogenously synthesized	Antioxidant/pro-oxidant	[114, 133, 134, 143–146, 150–156]
Ajoene	Diet (*Allium* vegetables)	Antioxidant	[147]
S-Allyl-L-cysteine sulfoxide	Diet (*Allium* vegetables)	Antioxidant	[98, 147, 148]
Lipoic acid	Diet/endogenously synthesized	Antioxidant	[114, 124, 157, 158]
Meso-2,3-dimercaptosuccinic acid	Synthetic	Antioxidant	[124, 143, 159]
Sodium-2,3-dimercaptopropane sulfonate	Synthetic	Antioxidant	[143, 159]

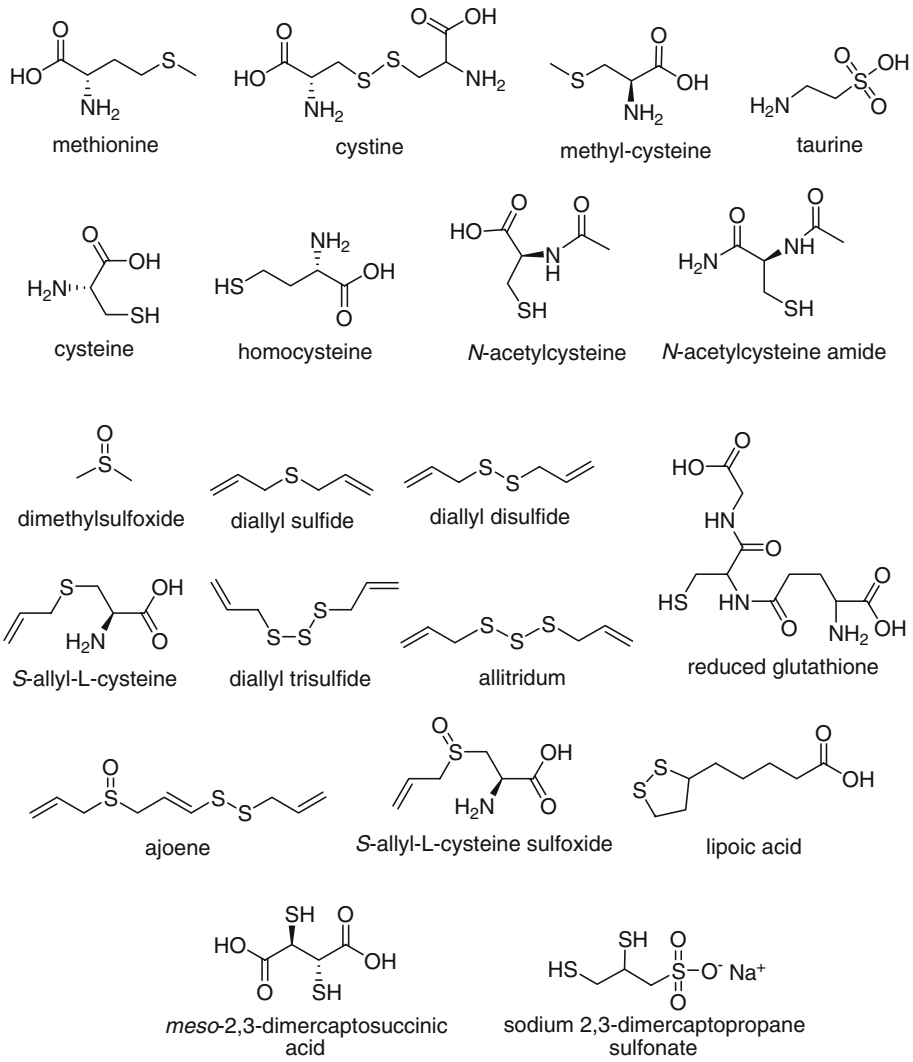

Fig. 2 Antioxidant sulfur compounds discussed in this review

disease progression [126–128]. Jones et al. have extensively studied the redox balance of glutathione and cysteine in cells and found that reduced glutathione and cysteine become increasingly oxidized in response to oxidative stress, aging, and cardiovascular disease [127, 128]. Additionally during aging, cellular concentrations of GSH decrease, a characteristic associated with increasing oxidative damage [126]. In addition to amino acids and proteins, naturally occurring allium derivatives from garlic comprise a large focus of antioxidant research with sulfur compounds [115, 147, 160, 161].

Cellular and In Vivo Studies

Numerous studies, including epidemiological and in vivo studies, focusing on the use of sulfur-containing compounds in the treatment and prevention of disease have established the antioxidant and protective effects of various sulfur compounds. The studies discussed in this review demonstrate the significance of endogenous and dietary sulfur antioxidants and understanding their results is essential to direct future work. These studies particularly highlight the need for future research due to conflicting results, particularly those focusing on the mechanisms of sulfur antioxidant activity. Additionally, experimental conditions can vary widely and the antioxidant properties of sulfur compounds are often oversimplified when compared without taking into account differences in experimental design and methods.

A recent study showed that the sulfur-containing amino acids cysteine and homocysteine inhibit cadmium toxicity in two hepatic cell lines (HepG2 and HTC) by preventing ROS generation through thiol–cadmium coordination [132]. Other studies investigating the role of sulfur-containing amino acids in cadmium-induced carcinogenesis have shown that pre-treatment of K562 chronic myelogenous leukemia cells with NAC reduce ROS concentration; methionine also prevents DNA hypomethylation and cell

proliferation [121]. Abnormal estrogen metabolism can result in DNA adducts and mutations that are implicated in breast cancer; however, a recent study suggests the possible use of NAC supplementation for protection against this estrogen genotoxicity [139]. Venugopal and co-workers tested cell viability of mouse epithelial breast cells (E6) exposed to estrogen-3,4-quinones and NAC. Their studies reveal a significant decrease in adduct formation (63–90% reduction) by NAC, suggesting the possibility for NAC in preventing breast cancer [139]. *N*-acetylcysteine amide prevents the cytotoxic effects of glutamate by preventing lipid peroxidation, scavenging ROS, and maintaining cellular GSH levels in PC12 cells, which are implicated in neurological disorders such as Parkinson's and Alzheimer's diseases [122]. Another study investigating diabetic complications and cardiovascular disease found that NAC prevents insulin resistance and hypertension in rats. For these studies, rats ingested high-dose fructose, causing increased insulin resistance, high blood pressure, and elevated oxidative stress. These symptoms were significantly attenuated when administered NAC, suggesting a protective role for NAC in both diabetes and cardiovascular disease [140].

In addition to studies reported with NAC, Kaufmann et al. have demonstrated that administration of glutamine to rats exposed to the carcinogen 7,12-dimethylbenzanthracene (DMBA) caused increases in GSH concentration, correlating to a 50% reduction in mammary tumorigenesis [150]. Kamada and co-workers have shown that glutathione *S*-transferase prevents H_2O_2-induced DNA damage associated with carcinogenesis in human colonic (HTC8) cells [162]. Research examining dietary supplementation with methyl-cysteine in fruit flies demonstrated increased methionine sulfoxide reductase activity under conditions of oxidative stress, and established this as an underlying cause of Parkinson's disease [130]. Methylmercury-mediated toxicity and neuronal death from ROS generation in chick sympathetic neurons were prevented by cysteine and glutathione, but not methionine [133]. A recent study investigating the cardioprotective effects of taurine found that taurine deficiency in the heart caused by down-regulation of the taurine transporter gene caused extreme cardiac dysfunction (physical defects, reduced endurance, cardiac atrophy, and failure) in mice [131].

In the past, epidemiological studies have indicated that allium derivatives from garlic have chemopreventive effects, most notably with prostate, breast, stomach, and colorectal cancers [115, 160]. These reports prompted a large amount of research aimed at determining the compounds responsible for the observed anticarcinogenic effects. In addition, studies have correlated high consumption of allium-containing vegetables with decreased incidences of stomach, esophageal, and prostate cancers [161]. Very recently, a review by Powolny et al. summarized the chemopreventive effects of some sulfur-containing allium derivatives in human clinical trials [147]. In particular, a clinical trial conducted by Li and co-workers showed a significant decrease in total cancer incidence (22%), particularly with gastric cancer (47% lower incidence) with administration of high-dose allitridum [149]. Beneficial effects of other allium derivatives (aged garlic extract and ajoene) were observed for colorectal and skin cancers [147].

Much of the work investigating the chemopreventive properties of glutathione has demonstrated both beneficial and harmful roles. For example, glutathione levels in patients with breast cancer are lower in blood due to detoxification of oxidative stress [151]. In contrast, high levels of glutathione were observed in breast cancer tissue, suggesting that glutathione may contribute to enhanced cell proliferation and resistance to oxidative stress [151]. Similar effects are observed in other clinical trials, where elevated glutathione levels are associated with drug and radiation resistance [152, 153].

Increases in lipid, protein, and nucleic acid oxidation in the brain from oxidative stress results in the progression of Alzheimer's and Parkinson's diseases [163]. Protective enzymes, including GPx, reduce peroxides using glutathione and ameliorate neurodegeneration [163]. Depletion of glutathione levels leads to ROS generation and is an early predictor for oxidative stress in Parkinson's disease and is extensively reviewed by Zeevalk et al. [154]. Treatment of PC12 cells with *R*-lipoic acid (Fig. 2) prevents depletion of glutathione and prevents oxidative damage associated with Parkinson's disease [157]. As expected, the function of glutathione in Alzheimer's disease is similar [156], and patients with mild-cognitive impairment showed reduced and increasingly oxidized glutathione levels [156]. Glutathione derivatives, including *S*-lauroylglutathione and *S*-palmitoleoylglutathione also reduce ROS concentrations, preventing impairment of radical scavengers and lipid peroxidation, which may make these compounds potentially useful for treatment of Alzheimer's disease [155]. Clinical and in vitro evidence that metal ions such as copper, iron, and zinc contribute to the pathogenesis of neurological diseases is mounting, in some cases, resulting in increased oxidative stress.

Cardiovascular disease is the leading cause of death in the United States, Europe, and Japan [164]. Increased levels of homocysteine are associated with cardiovascular pathology, and reduction of homocysteine levels resulted in a 16% reduction in ischemic heart injury and a 24% reduction in stroke [137]. Additionally, cystine and cysteine enhance homocysteine-mediated oxidation of low density lipoproteins (LDL) in the presence of copper, a process associated with atherosclerosis [135]. Additional investigations related to the harmful effects of homocysteine have

been extensively reviewed [136, 138]. In contrast, improved immune function for patients with HIV infection was observed with NAC supplementation [141]. Lead toxicity, associated with neurological, immunological, reproductive, and circulatory pathologies, has been reduced with the sulfur antioxidants *N*-acetylcysteine and lipoic acid [124]. Several studies investigating sulfur antioxidants in combination with metal-chelating agents such as *meso*-2,3-dimercaptosuccinic acid reveal that chelating agents are less effective when combined with sulfur antioxidants [124].

Extensive review of the literature reveals the primarily protective effects for sulfur-containing compounds in disease prevention. Because of the complex nature of many of these pathologies, however, clinical and in vivo studies may not provide direct evidence for the mechanisms of sulfur antioxidant effects. More work is required to determine how sulfur compounds exert their antioxidant effects mechanistically in order to develop more effective sulfur antioxidants for disease prevention. In addition, the conflicting findings of clinical and in vivo studies suggest that more research is needed to conclusively determine the complex antioxidant properties of sulfur compounds. Future studies should focus on developing standardized methods and conditions that will enable direct comparison of antioxidant activity for various sulfur compounds.

ROS Scavenging Mechanisms of Sulfur Antioxidant Activity

The ability of sulfur compounds to scavenge ROS has been investigated as a possible antioxidant mechanism for these compounds. Allium compounds are known antioxidants, and Kim et al. have examined the radical scavenging activity of five allium compounds (*S*-allyl-L-cysteine (ALI), *S*-allyl-L-cysteine sulfoxide (SAC) [98], diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS) [148]. They determined that only SAC and ALI effectively protect ischemic neuronal cells from damage at 1–100 μM and 10–100 μM concentrations, respectively. These two compounds also effectively scavenge hydroxyl radical in vitro, but have no effect on hydrogen peroxide or superoxide levels. In contrast, DATS and DADS were efficient superoxide scavengers; however, they did not scavenge hydrogen peroxide or prevent neuronal damage. Surprisingly, DATS did not scavenge any radical species. From these results, the authors suggest that certain allium compounds could provide neuroprotective from ROS implicated in neurodegeneration [148].

Chemiluminescence studies have also determined that dimethyl sulfoxide (DMSO) and methionine have radical scavenging activity. Using luminol, hydroxyl radical formation from $FeSO_4$ and O_2 was measured (Fig. 1, reactions 2 and 5). Methionine inhibited ~48% of radical formation at 100 μM and DMSO inhibited ~55% at 100 mM, suggesting that these compounds may be effective hydroxyl radical scavengers in vivo [129]. The H_2O_2 and $^{\bullet}OH$ scavenging activity of NAC and NACA was determined by Ates et al. using UV–vis spectroscopy and compared to scavenging by ascorbic acid. At high concentrations (~0.8 and 1.5 M) NAC had the highest radical scavenging activity for H_2O_2. However, at higher concentrations (~3 M) NACA had ~10% more scavenging activity than NAC. For hydroxyl radical, NAC had the highest radical scavenging activity at all concentrations (~0.3, 0.6, and 1.2 M) with maximum activity (~73%) at 1.2 M. The maximum radical scavenging activity (at ~1.2 M) for NACA was ~57%. In contrast to NAC and NACA, ascorbic acid had the lowest scavenging activity for both hydrogen peroxide and hydroxyl radical [142]. Although these results indicate that NAC and NACA are efficient scavengers in vitro at very high concentrations, lower intracellular concentrations may greatly diminish the scavenging efficacy of these compounds in vivo.

Although these studies indicate the ability of sulfur compounds to scavenge ROS, it is difficult to extrapolate their efficacy in vivo due to the complex antioxidant defense systems. Furthermore, the methods commonly used to determine radical scavenging activity of sulfur compounds may not accurately reflect physiological conditions. For example, Kim et al. investigate the scavenging activity of five allium derivatives; however, the method used to determine hydroxyl radical scavenging was done at low pH and with heating to 100°C [148]. Similarly, Ates and co-workers used acidic conditions for their hydrogen peroxide scavenging experiments [142].

Sulfur compounds have also been investigated for their ability to scavenge peroxynitrite, but more research has focused on peroxynitrite scavenging by selenium compounds. Although not a radical species, peroxynitrite oxidizes thiols such as glutathione to form the corresponding disulfides [165]. Karoui and coworkers determined using the spin trap 5-diethoxyphosphoryl-5-methyl-1-pyrroline-*N*-oxide (DEPMPO) and EPR spectroscopy that glutathione, *N*-acetyl-DL-penicillamine, and sulfite each form sulfur-centered radical species that react with O_2 to yield peroxyl or superoxide anion radicals. They concluded therefore, that sulfur compounds may be of limited use in protecting against peroxynitrite-mediated damage [166]. In addition, selenomethionine and selenocystine were found to be more than twice as effective at preventing $OONO^-$-mediated oxidation or DNA strand breaks as methonine and cystine [53, 167]. Penicillamine, cysteine, and their oxidized disulfides were also reported to increase aconitase inactivation by

peroxynitrite, likely due to production of radical sulfur species [168]. Additionally, high concentrations of sulfite ($\leq$1 mM) reduced neuronal cell viability in combination with peroxynitrite, likely due to the formation of sulfite radicals [169].

In contrast, reduced glutathione and cysteine inhibited myocardial aconitase inactivation by $OONO^-$ with IC_{50} values of 0.43 and 0.80 mM, respectively. This inhibitory effect was attributed to the formation of nitrosylated products such as *S*-nitrosogluathione or the ability of these thiols to keep Fe^{2+} in aconitase in its reduced state [134]. Further evidence for the complex nature of oxidation and nitration by $OONO^-$ was provided by Nakagawa et al. who reported that glutathione and other synthetic sulfur compounds inhibited both oxidation and nitration of tyrosine by peroxynitrite, whereas α-lipoic acid inhibited only nitration while promoting oxidation. The authors concluded that different intermediates were present in both types of damage to tyrosine, and that sulfur compounds interacted differently with each [170]. Similarly, Rezk and coworkers determined that the ability of sulfur compounds to prevent peroxynitrite-mediated damage depended substantially on the method used to detect the oxidized or nitrated products. Lipoic acid, for example, was found to have an IC_{50} value of 0.9 μM when $OONO^-$ damage was measured using the gluthathione-*S*-transferase P1-1 assay, but an IC_{50} value over 1000 times higher for prevention of dihydrorhodamine oxidation [158]. These results also suggest that sulfur compounds interact differently with the intermediates formed in peroxynitrite assays. Lastly, Kim et al. found that the hydrophobic allium-derived sulfur compounds ALI, SAC, DAS, DADS, and DATS (10 μM) all effectively inhibited oxidation of DHR-123 [148]. Clearly, as with other ROS, $OONO^-$ oxidation and nitration reactions are complex, and these complexities are compounded in biological systems. As a result, understanding the structure–activity relationships of sulfur compounds and the mechanisms for $OONO^-$-induced damage are necessary for identifying effective antioxidants to prevent this damage.

Recently, research has investigated the formation of reactive sulfur species (RSS), similar to the formation of ROS [171, 172]. These RSS, such as the thiyl radical ($RS^\bullet$) formed from biological thiols, can damage cellular components and have been implicated in oxidative signal transduction [173–175]. Because study of RSS is a relatively new field, little is known about formation and biological activity of these RSS in vivo, although the tendency of thiols to form RSS has been implicated in the observed prooxidant activity for cysteine and homocysteine [176–178]. Formation of RSS highlights the importance of understanding the chemical reactivity of individual sulfur compounds for development of sulfur antioxidant drugs or supplements to prevent or treat disease.

Metal-Binding Mechanisms for Sulfur Antioxidant Activity

In addition to ROS-scavenging mechanisms for sulfur compounds, metal binding by sulfur antioxidants may also afford significant protection against cellular oxidative damage. Several studies have demonstrated the presence of non-protein-bound (labile) iron and copper pools in cells and have correlated elevated metal ion concentrations with disease or cellular damage [69, 70, 84–86, 97–100]. Sulfur compounds prevent oxidative damage from Cu^+ or Fe^{2+}, and this observed antioxidant activity occurs at biological (low micromolar) concentrations by metal coordination [16], comparable to the levels of labile metal ion pools in cells [9, 40, 83, 93]. Thus, the ability of sulfur-containing compounds to coordinate metals is extremely important in preventing the formation of ROS. Several structures of metal–sulfur coordination compounds have been reported: Miyoshi et al. reported the X-ray crystal structure of a violet glutathione–copper(II) complex [108], and methionine–metal complexes have been reported for Cr^{3+}, Mn^{3+}, Fe^{3+}, Al^{3+}, Bi^{3+}, Rh^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+}, Cd^{2+}, and Ag^{2+} [109, 110]. Metal–sulfur complexes have also been observed for cysteine and methyl–cysteinate with Hg^+, Zn^{2+}, Cd^{2+}, and Pb^{2+} [110, 111], and for methyl–cysteine with Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+}, Cd^{2+}, Pd^{2+}, and Pt^{2+} [111, 112]. From these studies, it is apparent that biological sulfur compounds readily coordinate to metal ions, and that this ability may significantly prevent $^\bullet OH$ generation, oxidative stress, and disease.

Brumaghim et al. recently reported the results of several studies investigating the antioxidant activities and metal-binding properties of sulfur and oxo-sulfur compounds with copper- and iron-mediated DNA damage [13, 16]. Methionine, cysteine, cystine, methyl-cysteine, and reduced and oxidized glutathione significantly inhibited copper-mediated DNA damage with IC_{50} values between 3 and 12 μM. Additional studies revealed that the antioxidant activity of sulfur compounds with copper-mediated DNA damage was due to metal binding [16]. In similar DNA damage assays, these sulfur compounds were found to be much less effective at preventing iron-mediated DNA damage [16]. The chemical mechanisms by which sulfur coordination of metal ions results in the observed antioxidant activity are currently under investigation.

Oxo-sulfur derivatives of these compounds were also examined for their ability to prevent DNA damage with copper or iron and H_2O_2. Methylcysteine sulfoxide and methionine sulfoxide inhibit copper-mediated DNA damage with IC_{50} values in the low micromolar range (8–18 μM). In contrast, oxo-sulfur compounds also show much lower antioxidant activity with iron; methylcysteine sulfoxide and methyl methanethiosulfonate inhibited little

(~20%) iron-mediated DNA damage at high concentrations (1000–5000 μM). The primary antioxidant activity for these oxo-sulfur compounds was attributed to metal coordination; however, a secondary ROS scavenging mechanism was also identified [13].

The ability of sulfur compounds to bind metals and prevent oxidative damage is also very important for the reduction of metal toxicity. It is well known that toxic metals such as cadmium, arsenic, mercury, and lead cause cellular damage and disease, and metallothionein, a cysteine-rich protein that binds to metals through thiol groups [73], protects against this metal toxicity [179]. A study investigating the effects of lead acetate toxicity on metallothionein levels found that severe renal lesions and metastatic renal carcinoma were much more prevalent in mice lacking metallothionein than in healthy mice [180]. Similar findings were also observed with cadmium and arsenic [181]. In addition to examining metal toxicity and the protective effects of metallothionein, studies have also examined DNA damage inhibition by metallothionein. You et al. found that cells expressing human metallothionein-III are more resistant to H_2O_2 challenge and resulting DNA damage and had lower concentrations of ROS. They suggested that the protective role of metallothionein could be due to metal binding, which would prevent the generation of ROS associated with neurological disorders [182]. Presta and co-workers have reported copper binding to metallothionein in rabbit liver, suggesting the protective role of copper–sulfur binding in pathologies associated with copper-mediated oxidative damage [183]. Other proteins that contain metal–sulfur coordination include zinc finger proteins, alcohol dehydrogenase, metallolactamases, and glyceraldehyde 3-phosphate dehydrogenase [184].

In addition to metallothionein and other proteins, exogenous chelating agents have been used to prevent metal toxicity. A review by Rooney discusses thiol-containing chelating agents for the treatment of metal toxicity [143]. Sodium 2,3-dimercaptopropane sulfate (DMPS) and *meso*-2,3-dimercaptosuccinic acid (DMSA) are two dithiol chelating agents that are used to treat mercury, cadmium, arsenic, and lead toxicity with some success; however, these compounds can also bind essential metals such as copper and zinc [143, 159]. In contrast, the use of NAC and glutathione as chelating agents for mercury toxicity is not recommended because these complexes are inefficiently excreted from the body. Furthermore, NAC and glutathione actually contribute to mercury uptake in the kidney and brain [143–146].

The majority of research on sulfur–metal binding as an antioxidant mechanism has primarily focused on metallothionein and metal toxicity. Although the protective effects of metallothionein have been demonstrated, metallothioneins may not provide the first line of defense against metal toxicity. A study by Singhal et al. reports that glutathione provides protection against cadmium toxicity prior to metallothionein synthesis, which they suggest could be due to metal binding [185]. Consequently, antioxidant activity and metal-binding properties of glutathione would be much more significant during the initial stages of metal toxicity.

Numerous studies support the idea that sulfur antioxidants protect against oxidative damage associated with disease development and progression, and have suggested a protective role through multiple antioxidant mechanisms such as ROS scavenging and metal binding. However, not all sulfur compounds demonstrate similar antioxidant activity, showing the need for individual evaluation of these compounds. Furthermore, additional biologically relevant mechanistic studies are needed to support clinical, cellular, and epidemiological studies. It is not clear, for example, how metal binding by sulfur compounds leads to the observed antioxidant effects. A greater understanding of how ROS scavenging and metal-binding antioxidant mechanisms afford oxidative protection will facilitate improved antioxidant therapies for diseases caused by oxidative stress.

Selenium Antioxidant Activity

The body contains complex antioxidant systems that require adequate intake of selenium for normal physiological function; the RDA for selenium is approximately 55 μg/day and selenium can be incorporated into the body by ingesting foods such as carrots, cabbage, garlic, mushrooms, cheese, meats, and grains and selenium-containing supplements [186–188]. Selenium, in the form of selenocysteine, is a constituent of 25 classes of selenoproteins, including GPxs, selenoproteins P, W, and R, and thioredoxins [189–191]. There is evidence that several of these selenoproteins have antioxidant activities; however, the functions of most have not been determined. Recent reviews by Papp et al. and Brown et al. discuss selenoproteins and their role in human health [102, 192]. Early observations linking selenium and pathogenesis started an intense investigation into the role of selenium in antioxidant defense and disease treatment, and many selenium compounds have been investigated for their antioxidant properties (Fig. 3, Table 2).

Cellular and In Vivo Studies

Similar to studies with sulfur compounds, the antioxidant properties of selenium compounds have been investigated in several clinical trials and other in vivo studies for disease prevention and treatment. These studies indicate the essential protective effects of selenium antioxidants but

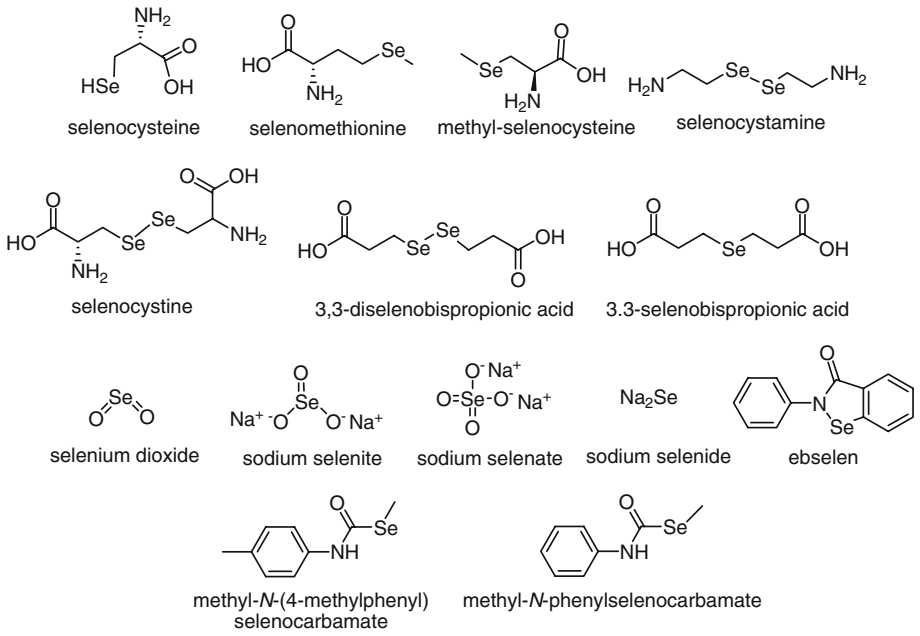

Fig. 3 Antioxidant selenium compounds discussed in this review

Table 2 Sources and activities of selenium compounds discussed in this review

Selenium	Compound source	Activity	Reference
Selenocysteine	Diet	Antioxidant	[116, 194]
Selenomethionine	Diet	Antioxidant	[15, 53, 116, 195–197]
Methyl-selenocysteine	Diet	Antioxidant	[116, 195, 198]
Selenocystamine	Endogenously synthesized	Antioxidant	[98, 198–200]
Selenocystine	Diet/endogenously synthesized	Antioxidant	[198, 201]
3,3-Diselenobispropionic acid	Synthetic	Antioxidant	[15, 202]
3,3-Selenobispropionic acid	Synthetic	Antioxidant	[198]
Selenium dioxide	Synthetic	Antioxidant/pro-oxidant	[13, 203]
Sodium selenite	Environmental/diet	Antioxidant/pro-oxidant	[13, 193, 195, 203–205]
Sodium selenate	Environmental/diet	No effect	[13, 195, 203]
Sodium Selenide	Endogenously synthesized	No effect	[15, 198, 201, 206]
Ebselen	Synthetic	Antioxidant	[54, 207–210]
Methyl-*N*-(4-methylphenyl) Selenocarbamate	Synthetic	Antioxidant	[211]
Methyl-*N*-phenylselenocarbamate	Synthetic	Antioxidant	[211]

demonstrate the need for future studies investigating the mechanism of selenium antioxidant activity. These mechanistic studies suggest that experimental conditions should be standardized to allow direct comparison of various selenium compounds and may provide reasoning for conflicting reports.

A study investigating the chemopreventive effects of sodium selenite (Na_2SeO_3) in Syrian hamsters on *N*-nitrosobis(2-oxopropyl)amine-induced liver tumors determined that low doses of selenium prevented liver cancer [193]. A review by Whanger indicates that of the greater than 100 animal studies of selenium effects on tumor incidence, two-thirds showed selenium anticarcinogenic effects [212]. Several studies have also shown the protective effects of selenium in animal models for cardiovascular and neurodegenerative diseases [204, 205, 207]. Baljinnyam et al. showed that oral supplementation (30 mg/kg or 100 mg/kg) with ebselen (Fig. 3) resulted in cardioprotection and improved function in myocardial infarction of rabbit hearts [207]. Furthermore, selenium supplementation with Na_2SeO_3 protects immature rat hearts from ischemic and cardiac reperfusion injury [204, 205]. The protective effects of selenomethionine were demonstrated in hippocampal neurons in rats exposed to iron/hydrogen peroxide by modulation of GPx radical scavenging activity [213]. In addition, the neuroprotective

effects of ebselen have been demonstrated in rats by reduction of ischemic brain injury associated with stroke [208]. Elevated levels of wild-type α-synuclein are observed in neurological pathologies (Down's syndrome, Alzheimer's, and Parkinson's diseases) and have been linked to neurodegeneration [196]. In addition, Kumar et al. have shown the protective effects of selenomethionine in preventing overexpression of α-synuclein and oxidative stress in murine neuroblastoma clone cells (NBP2), a process believed to be involved in α-synuclein-mediated neurodegeneration [196].

The focus of recent epidemiological and clinical trials with selenium compounds has been mainly on their chemopreventive effects. The Nutritional Prevention of Cancer (NPC) trial was a ground-breaking clinical trial that demonstrated significant chemopreventive effects of selenium in humans. The results indicated that daily supplementation with selenium-enriched yeast (200 μg/day) caused a 63% reduction in prostate cancer, 58% reduction in colorectal cancer, and 46% reduction of lung cancer [214]. However, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) trial investigated the chemopreventive effects of selenium and vitamin E on prostate cancer in 32,400 men and found no effect [158, 215]. The Third National Health and Nutrition Examination Survey conducted by Bleys et al. measured the selenium serum concentration in 13,887 adults and determined that increasing selenium levels were associated with a decrease in deaths due to cancer [216]. Clearly, due to the disparate results of these clinical trials, a more focused approach to understanding the mechanisms of selenium antioxidant and anticancer activity is required.

Several studies have shown the relationship between selenium levels and cancer risk. Combs Jr et al. has extensively reviewed past epidemiological studies on selenium deficiency and carcinogenesis. For most of these studies, an inverse correlation between selenium concentration and cancer incidences was observed [217]. More recently, a review of epidemiological studies by Gromadzinska et al. indicated that low cellular selenium concentrations were also associated with increased risk of lung, prostate, and colorectal cancers; however, demographics of these studies should be considered when assessing the efficacy of selenium in chemoprevention [218]. These epidemiological findings are supported by a recent study in Belgium showing an inverse relationship between selenium levels and bladder cancer incidence; patients with serum selenium concentrations lower than 82 μg/L had a greater risk of bladder cancer [219].

Studies have also shown a correlation between plasma selenium concentrations and leukemia. Zuo et al. measured selenium and copper concentrations and GPx activity in 49 patients with different types of leukemia and found low selenium concentrations and both elevated GPx activity and copper levels in leukemic patients [220]. In 2007, a study investigating the concentration of selenium in the hair of children with leukemia or lymphoma found ∼1.5-fold lower levels of selenium in patients with either leukemia or lymphoma compared to healthy subjects [221]. A study in the Czech Republic measured concentrations of selenium in blood plasma, red blood cells, and toenails from patients with acute pancreatitis or colorectal cancer, and found that selenium concentrations were ∼1.4-fold lower in these patients, and they had lower GPx activity in red blood cells than healthy controls. Furthermore, patients with pancreatitis had lower red blood cell (∼1.2 times) and toenail (∼2 times) selenium levels than patients with colorectal cancer [222]. Not all studies observe a correlation between selenium deficiency and increased cancer incidence. Atomic absorption spectroscopy was used to determine the selenium concentration in blood samples from 45 patients with breast cancer and found no significant deficiency in selenium levels versus healthy controls [223].

Deficient levels of selenium and increased cardiovascular pathology in humans have been observed in China where low soil selenium levels and therefore low selenium intake caused cardiomyopathy in children in the 1970s. The eradication of this disease with selenium supplementation confirms the cardioprotective role of selenium in humans [224, 225]. Other reports investigating the cardioprotective role of selenium show similar results. In a study examining the relationship between serum selenium levels and chronic rheumatic heart disease severity in humans conducted between 2003 and 2004, blood samples showed lower selenium levels in patients with heart disease versus healthy controls. However, no correlation between selenium concentration and disease severity was observed. Interestingly, serum copper concentrations were elevated in diseased subjects, which could have implications for the progression of rheumatic heart disease [226]. A separate study in Belgium from 1985 to 1989 examined the relationship between blood pressure, hypertension, and blood selenium levels. Men with higher selenium levels had a 37% decrease in high blood pressure and hypertension risk; however, these findings were not significant in women, leading researchers to suggest that women have different antioxidant systems than men [227]. Flores-Mateo et al. reviewed 25 studies investigating the effect of selenium levels in blood or toenails on cardiovascular disease. Most studies indicate that selenium levels are inversely related to coronary heart disease, but some presented inconclusive results. Despite these promising findings, researchers do not recommend that selenium supplementation be used to prevent cardiovascular disease because of other studies reporting misleading or invalid results for other antioxidants (β-carotene,

vitamin E, folate) in cardiovascular treatment and prevention [228].

Less is certain about the role of selenium in neurological disorders, but some studies do indicate a protective effect. A study evaluating serum selenium levels and GPx activity in red blood cells of epileptic children found that 81% of these patients had lower selenium levels and 11% had lower GPx activity than healthy controls, suggesting that selenium may have a role in epilepsy progression [229]. Another study in France evaluated selenium levels in 1389 elderly patients (60–71 years) over time and found that short-term decline in selenium levels had no effect on cognitive function but that, with time, selenium deficiency may contribute to reduced neurological cognitive function [230]. Additionally, Chen et al. have reviewed the role of selenium in multiple sclerosis, Alzheimer's and Parkinson's disease [231]. Changes in selenium concentration in diseased brains with Alzheimer's disease and multiple sclerosis were reported, but no change in selenium levels were observed in studies with Parkinson's disease [232–237]. These initial studies suggest that there may be a trend in selenium concentration with certain neurological disorders; however, results from these studies are widely conflicting [231] and further work is needed to confirm the protective role of selenium in neurological disease.

Although the majority of studies suggest that selenium is effective for disease prevention, findings are limited and several have been inconclusive or conflicting. The fact that several studies have conflicting results suggest that additional research is needed to determine the antioxidant properties of selenium compounds in vivo. Similar to the study of sulfur antioxidants, selenium antioxidant studies should focus on standardized assays for accurate comparison of selenium antioxidant behavior to elucidate chemical mechanisms for observed antioxidant activity.

ROS Scavenging Mechanisms for Selenium Antioxidant Activity

Selenium compounds are well known for their ability to scavenge ROS. Kunwar et al. examined the effectiveness of 3,3-diselenobispropionic acid to scavenge peroxyl radical ($CCl_3O_2^{\bullet}$). The reaction between 3,3-diselenobispropionic acid and peroxyl radical forms an intermediate species detectable with UV–vis spectroscopy ($\lambda_{max} =$ 560 nm). Using this method, 3,3-diselenobispropionic acid scavenged radicals at the same rate (2.7×10^{-8} M^{-1} s^{-1}) as other known radical scavengers. Thus, the antioxidant activity of 3,3-diselenobispropionic acid could be attributed to radical scavenging [202].

The ability of six selenocarbamates to scavenge superoxide radical was investigated by Takahashi and coworkers using chemiluminescence. All of the compounds demonstrated superoxide scavenging activity with methyl-*N*-(4-methylphenyl)selenocarbamate and methyl-*N*-phenylselenocarbamate having the highest radical scavenging activity with IC_{50} values for superoxide scavenging of 140 and 162 nM, respectively) [211]. Takahashi et al. also used the same method to investigate the superoxide scavenging activity of selenourea compounds. These compounds have scavenging activity ranging between 52 and 77% at 333 nM, which could have significance in the treatment of pathologies associated with superoxide radical and oxidative stress [238]. In addition, radical scavenging of peroxynitrite ($ONOO^-$) by selenomethionine and ebselen has also been reported [54, 239, 240]. Thus, radical scavenging is a likely mechanism in vivo and may be complementary to other mechanisms of selenium antioxidant activity.

The ability of selenium compounds to prevent peroxynitrite-mediated damage has been extensively reviewed in the past 10 years, and research in this area is more active than for sulfur compounds [54, 241–245]. Glutathione peroxidase can decompose peroxynitirite, and much work has focused on the development of organoselenium compounds capable of similar catalytic reactions [197, 242, 246, 247]. The compounds 4,4′-methoxyphenyl diselenide and the corresponding selenide prevented $OONO^-$-mediated DHR-123 oxidation with IC_{50} values of 0.5 and 2.38 μM, respectively, similar to the IC_{50} value for ebselen (0.2 μM) [246]. In addition, several acyclic ebselen analogs prevented peroxynitrite dye oxidation of Ponceau-4R similar to ebselen itself [247]. De Silva and coworkers examined the ability of selenomethonine and several phenylamino selenoxides to inhibit peroxynitrite-mediated DNA damage, and found that these compounds inhibited 31–40% of DNA damage at 500 μM [197]. Using a similar assay, selenomethionine and selenocystine inhibited similar percentages of DNA damage at double the concentration (25.5 and 41.6%, respectively, at 1000 μM). Interestingly, the sulfur analogs, methionine and cysteine inhibited substantially more DNA damage under the same conditions (56.9 and 85.3%, respectively) [53], although De Silva and coworkers found that the sulfur analogs inhibited roughly half of the DNA damage as the tested selenium compounds [197].

An investigation of combining polyphenol and selenium functionalities in polyphenolic acid esters was reported by Lin et al. These compounds were tested for their ability to scavenge radical species (DPPH assay) and prevent peroxynitrite oxidation (Ponceau-4R assay), and found that addition of a selenium atom in these molecules did not improve their antioxidant activity above the non-selenium-substituted control [248]. Overall, the ability of selenium compounds to catalytically decompose peroxynitrite is promising, but the literature methods for determining peroxynitrite scavenging ability vary, limiting the ability to

compare results. In addition, data collected using similar experimental methods is sometimes contradictory, likely indicating the sensitivity of these assays to slight changes in experimental conditions. Development and use of standardized assays for peroxynitrite scavenging, and increased attempts to determine structure–functional relationships between different classes of selenium compounds would significantly advance this area of research.

Glutathione Peroxidase Activity of Selenium Compounds

Glutathione peroxidases are one of the 25 known classes of selenoproteins; GPx enzymes function as antioxidants by reducing peroxides, such as H_2O_2; Mugesh et al. have discussed the four types of glutathione peroxidases [194, 249]. The sulfur-containing peptide glutathione (GSH) is a necessary cofactor in the reduction of peroxides, and acts as the reducing substrate; however, sulfur compounds themselves do not exhibit GPx activity [27, 250]. The GPx catalytic cycle has been well-studied and involves selenenic acid (PSeOH) reacting with GSH to generate a selenenyl-sulfide adduct (PSeSG). The adduct reacts with an additional GSH to generate the active selenol (PSeH) that reduces peroxide (Fig. 4) [194].

Other important mammalian selenoenzymes, including iodothyronine deiodinases, which catalyze the 5,5′-monodeiodination of the prohormone thyroxine to the active thyroid hormone, and thioredoxin reductases, which catalyze the reduction of thioredoxin have been extensively reviewed by Stadtman and Brown et al. [102, 251].

Due to the antioxidant properties of glutathione peroxidases, researchers have extensively investigated the antioxidant properties of selenium-containing GPx mimics [194, 252, 253]. Ebselen is a well-known, efficient GPx mimic that has been shown to protect biological molecules from oxidative damage. In fact, Li and co-workers have established that ebselen inhibits dopamine/Cu^{2+}/H_2O_2-mediated DNA damage by radical scavenging and reduction of H_2O_2 [209]. Ebselen has also been approved for clinical treatment of stroke in Japan [210]. Due to differences in experimental conditions, the exact mechanism for GPx activity of ebselen is uncertain; however, possible mechanisms for this activity have been reviewed by Mugesh et al. [249].

Since the discovery of GPx-like activity for ebselen, recent research has focused on the development of ebselen analogs that have similar GPx activity. Mugesh et al. have synthesized and investigated the GPx activity of numerous diaryl diselenides (Fig. 5), and determined that selenium compounds lacking selenium-nitrogen interactions in the selenenyl-sulfide adduct (PSeSG) have significant GPx activity [252]. In a later study, compounds having weak selenium–nitrogen interactions were found to have higher GPx activity, which they attributed to faster formation of the active selenol (PSeH) [194].

In an effort to generate GPx mimics with higher activity, Mugesh and co-workers used thiol-containing substituents to overcome strong selenium–nitrogen interactions and enhance GPx activity (Fig. 5) [252, 254]. Further investigation of additional selenium GPx mimics has been extensively reviewed and summarized by Mugesh et al. [249]. A study conducted by Mareque et al. also found that compounds having very weak or lacking selenium–nitrogen interactions had high GPx activity (Fig. 5) [255]. The GPx activity of other selenocompounds without selenium–nitrogen bonds has also been investigated [202, 256]. In one study, selenocystine and selenocystamine both had relatively similar GPx activity; however, 3,3-diselenobispropionic acid had GPx activity 25–29 times lower than

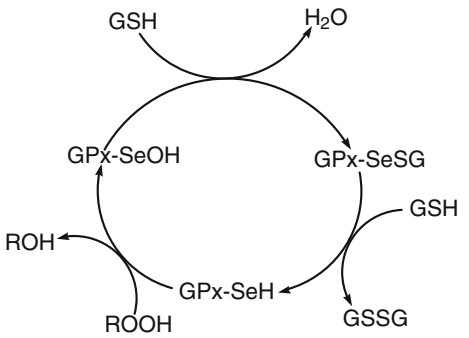

Fig. 4 Catalytic cycle of glutathione peroxidase (GPx); the protein is indicated by P

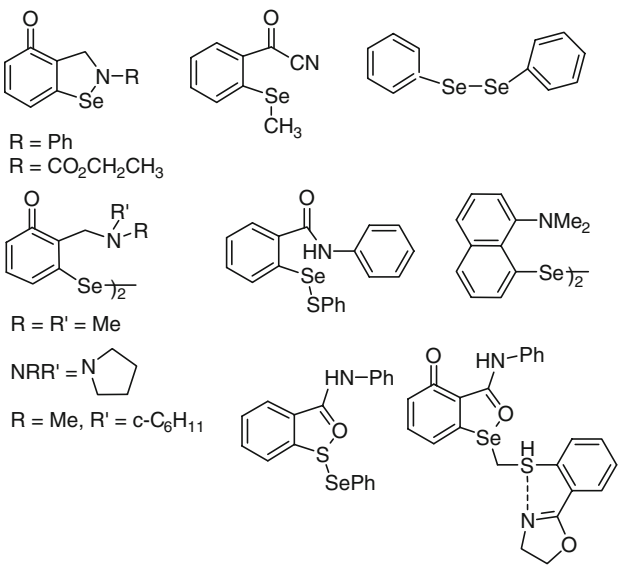

Fig. 5 Selenium compounds examined by Mugesh and Mareque

selenocystine and selenocystamine [256]. Yasuda et al. has also reported similar GPx activity for both selenocystine and selenocystamine with *t*-butyl hydroperoxide decomposition [200].

Although GPx measurements are typically used to determine antioxidant activity of selenium compounds, these measurements may not accurately reflect cellular conditions. GPx activity measurements are often determined under conditions that are not physiologically relevant, such as using non-aqueous solutions or non-biological thiols. For example, a common method for GPx activity determination involves oxidation of benzenethiol (PhSH) to the disulfide (PhSSPh) in methanol [16, 254, 255]. Similarly, small changes in experimental technique can greatly affect GPx measurements. Lastly, H_2O_2 is a relatively non-reactive oxygen metabolite in the absence of metal ions compared to the hydroxyl radical, one of the most highly reactive and deleterious radical species [257]. Directly preventing hydroxyl radical formation would target more oxidative damage than H_2O_2 scavenging. Thus, focusing on development of selenium compounds with high GPx activity and high ROS scavenging ability may result in more effective selenium antioxidants. While the antioxidant activity of selenium compounds is most often determined by their ability to mimic GPx activity and decompose H_2O_2, studies also show that the antioxidant mechanism for metal-mediated DNA damage inhibition of selenium compounds is due to metal binding and not GPx antioxidant mechanism [15, 16, 27].

Metal-Binding Mechanisms for Selenium Antioxidant Activity

Metal-mediated ROS generation has been implicated as a primary cause of many pathological conditions. Because of the importance of selenium-containing compounds in antioxidant defense systems, researchers have studied the metal-binding properties of selenium-containing antioxidants and enzymes. Structures showing selenium–metal coordination in enzymes have been reported: formate dehydrogenase H contains selenocysteine–molybdenum coordination in the active site [258], a selenium–tungsten bond was also identified in formate dehydrogenase [184], and the structure of [NiFeSe] hydrogenase shows nickel–selenium coordination [259]. Additionally, structures of metal–selenium complexes for biologically relevant metal ions $(SeMet)_2Cu$ and $(SeMet)_2Zn$ (SeMet = selenomethionine) have been reported by Zainal et al. Characterization of these complexes by IR and Raman spectroscopy determined that metal coordination was to the nitrogen and oxygen substituents of selenomethionine, not the selenium [260]. Biological selenium concentrations have been measured in the low micromolar range ($\sim$10 μM) [261, 262], in the same range as measured labile iron and copper pools [9, 40, 83, 93].

Evidence that metal–antioxidant coordination leads to antioxidant activity is supported by in vitro studies investigating metal-mediated oxidative stress and disease. Brumaghim et al. have demonstrated the antioxidant activities of numerous selenium compounds with metal-mediated DNA damage caused by copper or iron and hydrogen peroxide. Organic selenium compounds, selenomethionine, selenocystine, methyl-selenocysteine, and other compounds prevent copper-mediated DNA damage with IC_{50} values of 3–26 μM. Iron-mediated DNA damage inhibition is seen for methyl-selenocysteine, selenocystamine, 3,3-diselenobispropionic acid, and 3,3-selenobispropionic acid but to a lesser extent than with copper [198]. The antioxidant activity of these compounds with copper and iron was due to a metal-binding mechanism, a mechanism distinct from GPx activity.

Antioxidant activity of the inorganic selenium compounds sodium selenite, sodium selenate, selenium dioxide, and sodium selenide, were determined in a DNA damage assay with iron and hydrogen peroxide. SeO_2 inhibits iron-mediated DNA damage, $NaSeO_4$ and Na_2Se have no effect on DNA damage, but $NaSeO_3$ shows either antioxidant or pro-oxidant activity depending on the hydrogen peroxide concentration [12]. Similar to organoselenium compounds, the primary mechanism of antioxidant activity for inorganic selenium compounds inhibiting iron-mediated DNA damage was attributed to metal binding [12].

Since the antioxidant mechanism for selenium compounds was attributed to metal-binding, future studies should focus on the coordination environment of these complexes. It appears that the type of metal and specific structural features of the selenium compound greatly affect antioxidant activity. UV–vis absorption bands observed for selenium compounds with Cu^+ may indicate Cu–Se coordination, carboxylate and amino coordination, or both [16, 260]. Presently, it is not clear, however, how selenium–metal binding leads to the observed antioxidant effects.

Additional studies have suggested the importance of metal binding in antioxidant activity of selenium compounds. For example, ebselen, an antioxidant used to treat patients with ischemic stroke, inhibited Fe^{2+} uptake by HEK293T cells overexpressing divalent metal transporter-1 (IC_{50} $\sim$0.22 μM) [263], likely as a result of iron interactions. Using cyclic voltammetry, Collins examined the metal-binding properties of selenium pyridine and aniline derivatives with copper. All of the selenium compounds examined had GPx activity and show positive shifts in the copper reduction potential upon addition of selenium compounds, indicating metal binding. However, 2-aniline disulfide showed significantly larger shifts in the copper

reduction potential than the corresponding diselenide (−225 mV compared to −50 mV, respectively), and these sulfur-containing compounds did not exhibit GPx activity. Taken together, these results suggest a protective role for selenium compounds through multiple antioxidant mechanisms [28].

Oikawa et al. reported the synthesis of the selenium analog of metallothionein and investigated copper binding of metalloselenonein. A broad absorbance is observed between 230 and 400 nm with a shoulder at ~260 nm that they attribute to copper–selenium coordination [264]. Brumaghim et al. observe absorption bands at similar wavelengths (226-241 nm) for selenium antioxidants upon copper addition [15, 16, 198]. Because metallothionein binds and regulates zinc and protects cellular components against metal toxicity through sulfur–metal coordination, metalloselenonein could also have potential use as a protective agent in diseases associated with metal toxicity and oxidative stress [264].

Evidence from numerous clinical and experimental studies has shown the significant protective effects of selenium compounds against oxidative damage in disease treatment and prevention. In spite of this, the antioxidant activities of similar selenium-containing compounds are not identical, suggesting that each compound must be examined individually for its antioxidant behavior. A greater need for studies that focus on the mechanism of antioxidant activity of selenium compounds is also apparent. Such studies would provide a greater understanding of how ROS scavenging and metal-binding antioxidant mechanisms afford oxidative protection as well as facilitate improved antioxidant design for the treatment and prevention of disease.

Conclusions

Reactive oxygen species have been implicated in numerous pathologies, including cancer, neurodegenerative, and cardiovascular diseases. The results of epidemiological, clinical, in vivo, and in vitro studies have undoubtedly shown the protective effects of sulfur and selenium compounds against cellular damage and disease. However, many of these studies have not focused on the underlying chemical mechanisms responsible for the observed activities. The small number of studies that have investigated chemical mechanisms for antioxidant behavior demonstrate that sulfur and selenium compounds utilize multiple, complex antioxidant mechanisms, including ROS scavenging, GPx activity, and metal binding.

Because ROS are implicated in cellular damage and disease, understanding how ROS scavenging, GPx activity, and metal complexation by sulfur and selenium antioxidants prevent oxidative damage is required to fully elucidate and integrate these mechanisms of antioxidant activity. These studies also establish the need for standardized assay development, which would enable the direct comparison of sulfur and selenium antioxidant activity and their chemical mechanisms. In addition to mechanistic studies of sulfur and selenium antioxidants, the efficacy of these compounds should be examined under biologically relevant conditions in order to identify antioxidant therapies for the treatment and prevention of diseases caused by oxidative stress.

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