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Review Article

Pharmacology of organoselenium compounds: Emphasis on puzzling mechanistic switching from their glutathione peroxidase mimic *in vivo*

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ABSTRACT: **Organoselenium compounds are a new class of emerging potent antioxidants. Basically, their rational design and synthesis was aimed at mimicking the native glutathione peroxidase enzyme in their reduction of hydroperoxides at the expense of the ubiquitous antioxidant, glutathione. In this review, emphasis was focused on the seemingly antagonistic mechanisms employed by organoseleniums under *in vitro* and *in vivo* conditions. Summarily, *in vitro* evidences clearly demonstrate that the pharmacological effect of organoseleniums strictly depends on their GPx mimic. However, these selenium based compounds evoke an increase in the level of endogenous thiols suggesting a possible switch in their glutathione peroxidase mimic under *in vivo* conditions. Apparently, this mechanistic switch is puzzling and requires concerted efforts to unravel.**

IN SELENOPROTEINS – THE MAKING OF POTENT NUCLEOPHILES

Selenium, named after the Greek Goddess of the moon, Selene was first discovered in 1817 by the Swedish chemist Jöns Jacob Berzelius (Comasseto 2010; Nogueira and Rocha, 2010). This element shares some chemical properties with sulfur and tellurium. But biologically speaking, selenium shares some properties with sulfur and their sulfhydryl and their selenohydryl and sulfhydryl groups (Figure 1) can be considered two important soft nucleophile center classes in cell (note that sulfhydryl or thiol and selenohydryl or selenol groups are soft analogs of the hard analog hydroxyl group, see Figure 1 for comparisons).

It is a common knowledge in the scientific community that selenium is an essential dietary trace element for mammals (Zwolak and Zaprowska, 2012; Loef et al., 2011; Harthill, 2011; Soni et al., 2010; Brozmanova et al., 2010). Essentially, selenium is a component of selenoproteins found in most living things. For examples, vertebrates have about 2 to 3 dozens of selenoproteins (Lobanov et al. 2007, 2009). In vertebrates, selenium can be considered as a "supersulfur", when present in the form of a selenol (R-SeH; Scheme 1), which is a softer and stronger nucleophile than

Authors' Biography

Ige Joseph Kade *was born in Ondo State, Nigeria. He received his BSc and MSc degrees in Biochemistry in 1998 and 2003 respectively at the Federal University of Technology Akure (FUTA), Nigeria. In 2004, he won a TWAS/CNPq doctoral fellowship to pursue a PhD degree at the Federal University of Santa Maria, RS Brazil, under the direction of Prof. J.B. Teixeira da Rocha. He received a TWAS/CONACYT Postdoctoral Fellowship to work with Prof. V. Granados-Soto at the Department of Pharmacobiology, Centre for Investigation and Advanced Studies, Mexico. He joined the Biochemistry department at FUTA in 1999 where he is currently a Lecturer. His research is focused on the biochemical mechanism of toxic substances as well as therapeutic potentials of organoselenium and isolated natural compounds. He is currently on a research visit to the Departamento de Farmacobiología, Centro de Investigacion y de Estudios Avanzados, México where he is investigating biochemical aspects of pain in diabetic neuropathy.*

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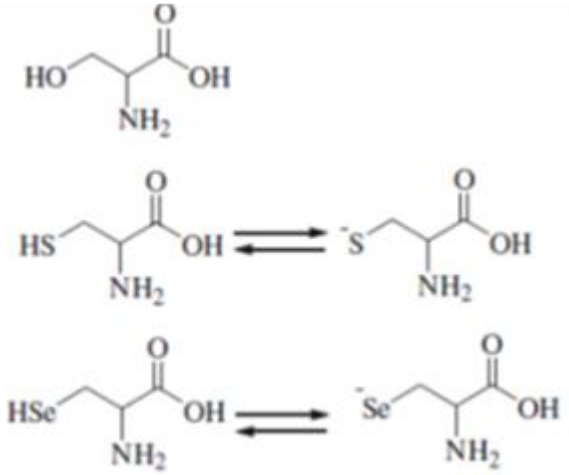
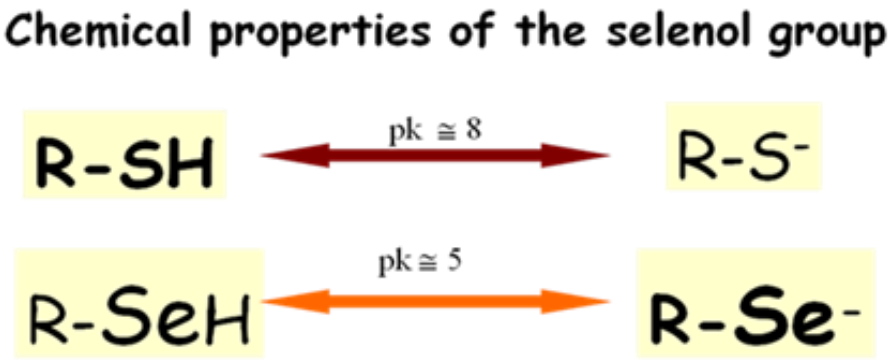


FIGURE 1 Nitration Structures of serine, cysteine (thiol-thiolate form) and selenocysteine (selenol-selenolate form).

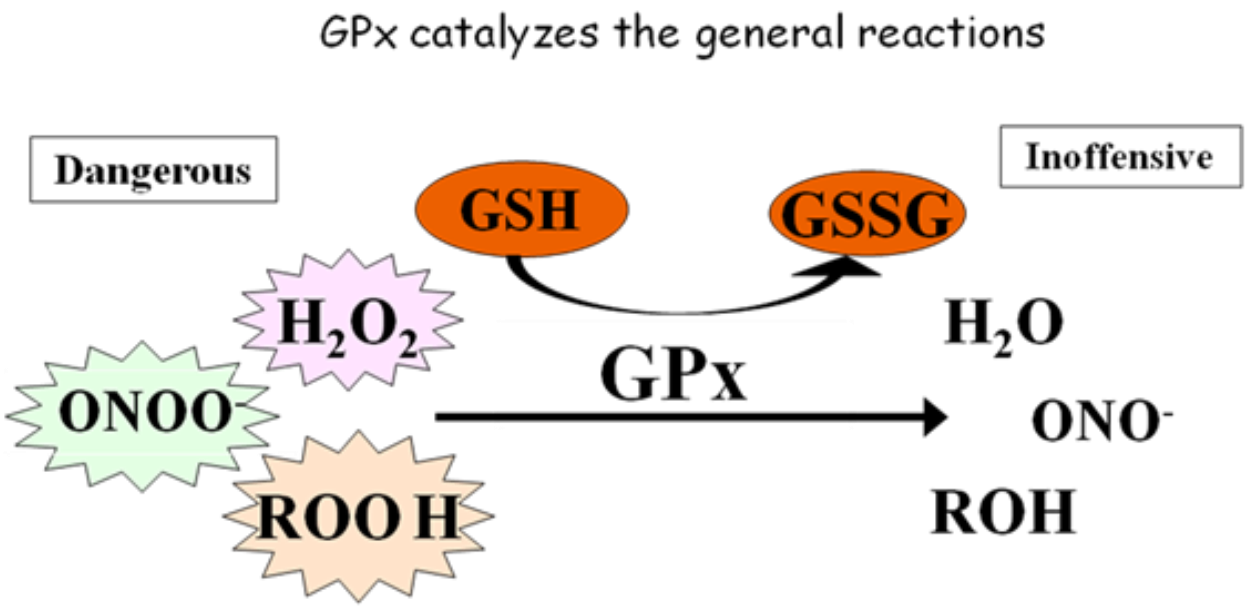


SCHEME 1 Comparison between thiol/thiolate and selenol/selenolate groups. Selenol group is a stronger soft nucleophile than an analog thiol. Selenol groups are also more acidic than the thiol groups by about 3 orders of magnitude (compare the pka of each group).

its thiol analogue (Nogueira and Rocha 2010). Selenol groups are considerably less abundant than thiol groups and are found in a very small number of selenoproteins (Lobanov et al. 2007, 2009; Araie and Shiraiwa 2009). These findings suggest that excess of thiolate over selenolate groups cannot replace selenium in cell physiology.

GLUTATHIONE – AN IMPORTANT UBIQUITOUS ANTIOXIDANT

At this point, it should be noted that the role played by selenium in different classes of prokaryote and eukaryote cannot be viewed as a simple substitution of the sulfur analogue as exemplified in Fig. 1. Generally, in the case of mammals, one of the most important soft nucleophiles found in cells and in the extracellular fluids is the sulfhydryl group (thiol/thiolate; Figure 1), which can be found in the low-molecular-weight compounds such as cysteine and glutathione or in high-molecular-weight proteins. The concentration of low-molecular-weight thiols can be as high as 5–10 mmol/L, depending on the tissue considered (Maciel et al. 2000). Of these low molecular weight thiols, it is worth mentioning that glutathione (GSH, γ-L-glutamyl-L-cysteinylglycine) is a predominant intracellular thiol compound and one of the most important antioxidants in



SCHEME 2 The Generic reactions catalyzed by glutathione peroxidase (GPx). GPx isoforms can decompose different peroxydes (including hydrogen peroxide, organic peroxides and peroxynitrite) forming non-toxic products.

the central nervous system. It is present in cytoplasm, nucleus and mitochondria of neurons and exerts effects on receptor function (such as NMDA receptor), and apoptosis regulation. GSH reacts indirectly with radicals, such as superoxide radical, nitric oxide and hydroxyl radical. Moreover, it is an electron donor in reactions catalyzed by glutathione peroxidase (GPx). Glutathione can also covalently interact with different proteins and can modulate the thiol-containing-proteins functioning via a glutathionylation reaction (For a comprehensive review of the physiological role of glutathione see Drigen, 2000; Garcia-Garcia et al. 2012; Markovic et al. 2010; Naoi et al. 2009; Wu et al. 2004; Cotgrave 2003).

SELENIUM INCORPORATION INTO SELENOPROTEINS

Major milestones along the course of identification that selenium is an element with biological functions were the biochemical confirmation that the mammalian glutathione peroxidase was a selenoprotein (Flohe et al. 1973; Rotruck et al. 1973) and selenium atom is present in selenoproteins in the form of selenocysteine in rat liver glutathione peroxidase (Forstrom et al. 1978). Thereafter, it was determined that all selenoproteins contain at least one selenocysteinyl residue in their structures (Lu and Holmgren 2009; Lobanov et al. 2009). The incorporation of a selenium in selenoproteins is complex and requires different enzymatic steps and complex macromolecular components (including a specific t-RNA charged with serine (t-RNA[Ser]Sec), the selenocysteine insertion sequence (SECIS) element located in the 3′-untranslated region of the mRNA of the selenoprotein, and protein factors such as elongation factor EFSec and the SECIS binding protein 2, SBP2; see Bock et al. 1991).

Indeed, the extremely high chemical reactivity of selenocysteine precludes its existence as free amino acid in aerobic cell environment. Thus, to circumvent this chemical problem, the machinery of synthesis of selenoproteins has evolved as an expansion of the genetic code and the UGA codon, which is a termination code, codifies for a selenocysteine. However, here we must emphasize that this occurs only when UGA is present within the RNA sequences of a given selenoprotein (Allmang et al. 2009). Selenocysteine is formed by the incorporation of Se from selenophosphate in O-phosphoseryl-tRNA([Ser]Sec). Consequently, the serine residue is transformed in selenocysteine and the hard nucleophile group –OH is transformed in the soft nucleophile group -SeH (Allmang et al. 2009) and physiologically there is no a free pool of selenocysteine.

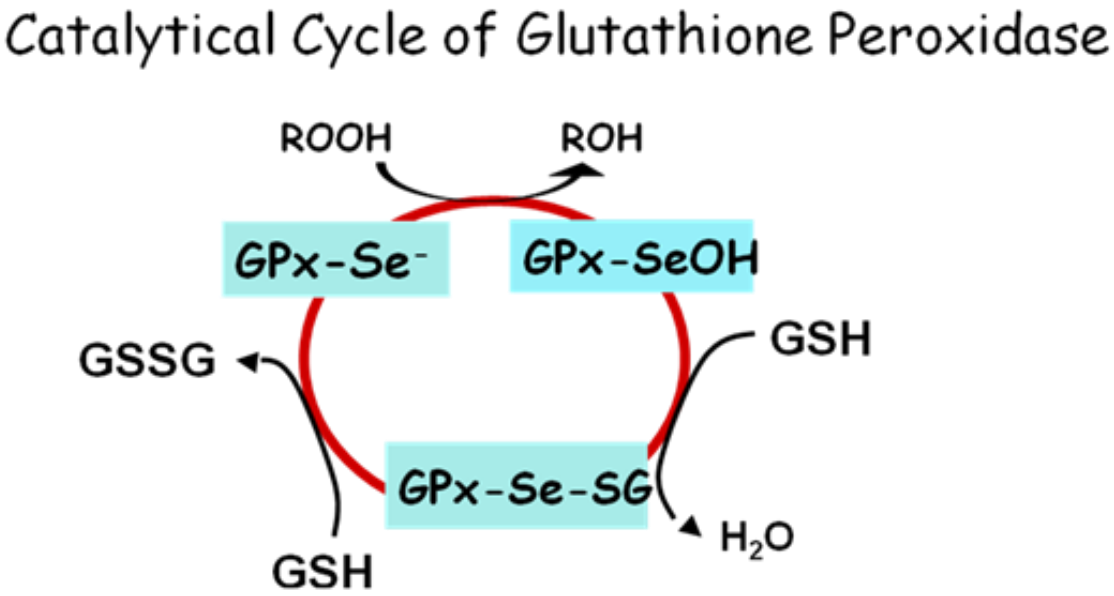


FIGURE 2 The catalytic mechanism of the native glutathione peroxidase. The cycle of the enzyme highlights the role of oxidation of its selenol group (-SeH) to selenenic acid (-SeOH) that is sequentially reduced by GSH to regenerate the native reduced enzyme.

THE SELENOPROTEINS – EMPHASIS ON GLUTATHIONE PEROXIDASE

The glutathione peroxidase (EC 1.11.1.19) is an important selenoprotein that has a direct bearing on our review. This enzyme catalyzes the reduction of a variety of hydroperoxides (ROOH and H_2O_2) using GSH as a reductant. More recently, Sies and collaborators have obtained persuasive experimental points of evidence demonstrating that GPx1 (cytosolic glutathione peroxidase) can decompose peroxynitrite *in vitro* (Scheme 2; Sies and Arteel, 2000; Mugesh et al., 2001). However, the physiological role of GPx1 as a modulator of peroxynitrite level has not yet been demonstrated. In addition, there are at least six seleno-glutathione peroxidase isoenzymes identified in mammals, so far, differing in many properties, including their localization, subunit number, global structure, primary structure, and enzymatic properties. Although, their expression is ubiquitous, the levels of which isoform vary, depending on the tissue type. The classical cellular glutathione peroxidase (GPX1 or cGPX), found in cytosolic space and mitochondria, reduces fatty acid hydroperoxides and H_2O_2. Phospholipid hydroperoxide glutathione peroxidase (GPX4 or PHGPX), found in most tissues and located in both the cytosol and the membrane fraction, can directly reduce the phospholipid hydroperoxides, fatty acid hydroperoxides, and cholesterol hydroperoxides that are produced in peroxidized membranes and oxidized lipoproteins. Finally, cytosolic glutathione peroxidase (GPX2 or GIGPX) and extracellular glutathione peroxidase (GPX3 or eGPX) are rarely detected in most tissues except for the gastrointestinal tract and kidney (Tapiero et al., 2003; Luo et al., 2003). Considering the importance of glutathione peroxidase activity, the enzymatic catalytic cycle was studied by the Flohe and Wendel groups (Flohe et al., 1973; Flohe, 1989; Wendel et al., 1975; Wendel et al., 1984). Thus, they demonstrated that glutathione peroxidase catalyzes the reduction of H_2O_2 at the expense of GSH. As illustrated in Figure 2, the selenol group (-SeH) of a reduced selenocysteine molecule is oxidized by the hydroperoxides to generate a selenenic acid. The tripeptide GSH then reacts with the selenenic acid, resulting in the corresponding water and selenenyl sulfide. A second molecule of GSH attacks the sulfur in the latter species, producing disulfide and regenerating the selenol to complete the catalytic cycle (Flohe, 1989; Wendel et al., 1975).

Since glutathione peroxidase catalyzes the reduction of a wide variety of hydroperoxides and together with GSH constitutes a powerful cellular defense system against so-called oxidative stress, considerable efforts have been made to find compounds capable of imitating the enzymatic

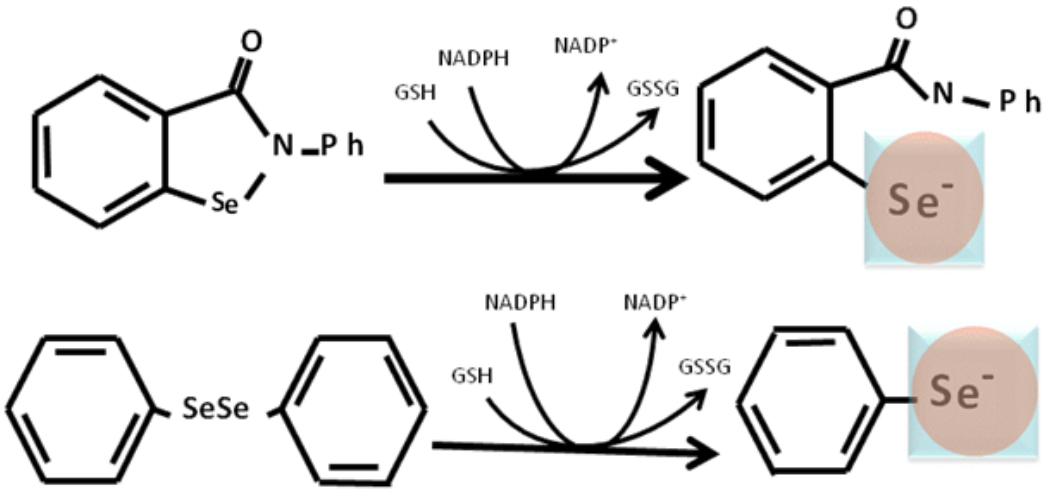


FIGURE 3 Reduction of Ebselen and Diphenyl Diselenide by a direct interaction with Reduced Glutathione or by an indirect Reduction Mediated by Thioredoxin Reductase (TrxR). The mammalian TrxR uses the electron equivalents derived from NADPH to reduce Ebselen and Diselenide. The formation of the selenol intermediates (pink and blue coloured groups) of these compounds is crucial for their thiol-peroxidase like activity that can decompose efficiently different peroxides.

properties of glutathione peroxidase. This becomes crucial considering some shortcomings of the administration of native GPx with therapeutic objectives, including instability and poor availability. Consequently, the high molecular weight of native GPx limits its therapeutic application. Therefore, considerable efforts have been made to find organoselenium compounds capable of imitating the enzymatic properties of glutathione peroxidase and free of these aforementioned shortcomings. In this context, several research groups have developed a number of small molecules, including substituted diselenides, N-Se heterocycles, and other type of organoselenium compounds with glutathione peroxidase-like activity (Bhabak and Mugesh 2010). It is also worth mentioning that semisynthetic enzymes, obtained by enzyme engineering, have been proposed as mimics of glutathione peroxidase (Luo et al., 2003; Ren et al., 2002). Our focus in this review is on the synthetic organoselenium compounds with glutathione peroxidase mimic and we will give emphasis to ebselen and diphenyl diselenide, two compounds that have GPx-like activity (Wilson et al. 1989) and have been found to exert antioxidant and protective effects in different *in vitro* and *in vivo* models of toxicity (Nogueira and Rocha, 2010; 2011). We want to emphasize that for an organoselenium compound to exhibit antioxidant properties, it must show nucleophilicity necessary for glutathione peroxidase-like activity, potential free radical scavenger activity, and low toxicity. To imitate native GPx, organoselenium compounds are thought to be transformed into selenol intermediates either via reduction by thiol or via NADPH-dependent enzymatic reduction by hepatic and cerebral thioredoxin reductase (Figure 3; De Freitas and Rocha 2011) Consequently, the rational synthesis, pharmacological testing and toxicological evaluations of organoselenium compounds have been quite an arduous task (Nogueira and Rocha 2010).

DEVELOPMENT OF SYNTHETIC ORGANOSELENIUM COMPOUNDS WITH GPX MIMICS

The identification of selenocysteine in the active center of hepatic rat glutathione peroxidase in the laboratory of Tappel in 1978 brought to scene the softest nucleophile group found in the biological system, i.e., the selenol group (Tappel, 1978; Pierce and Tappel, 1978). Selenol groups are powerful reducing components of antioxidant enzymes such as glutathione peroxidase and thioredoxin reductase (Lu and Holmgren 2009; Nogueira and Rocha 2010, 2011). Following this, a number of organoselenium compounds have been synthesized and tested as a mimetics of GPx (Muller et al. 1984; Wendel et al. 1984; Parham and Kindt, 1984). Ebselen was the first organoselenium with

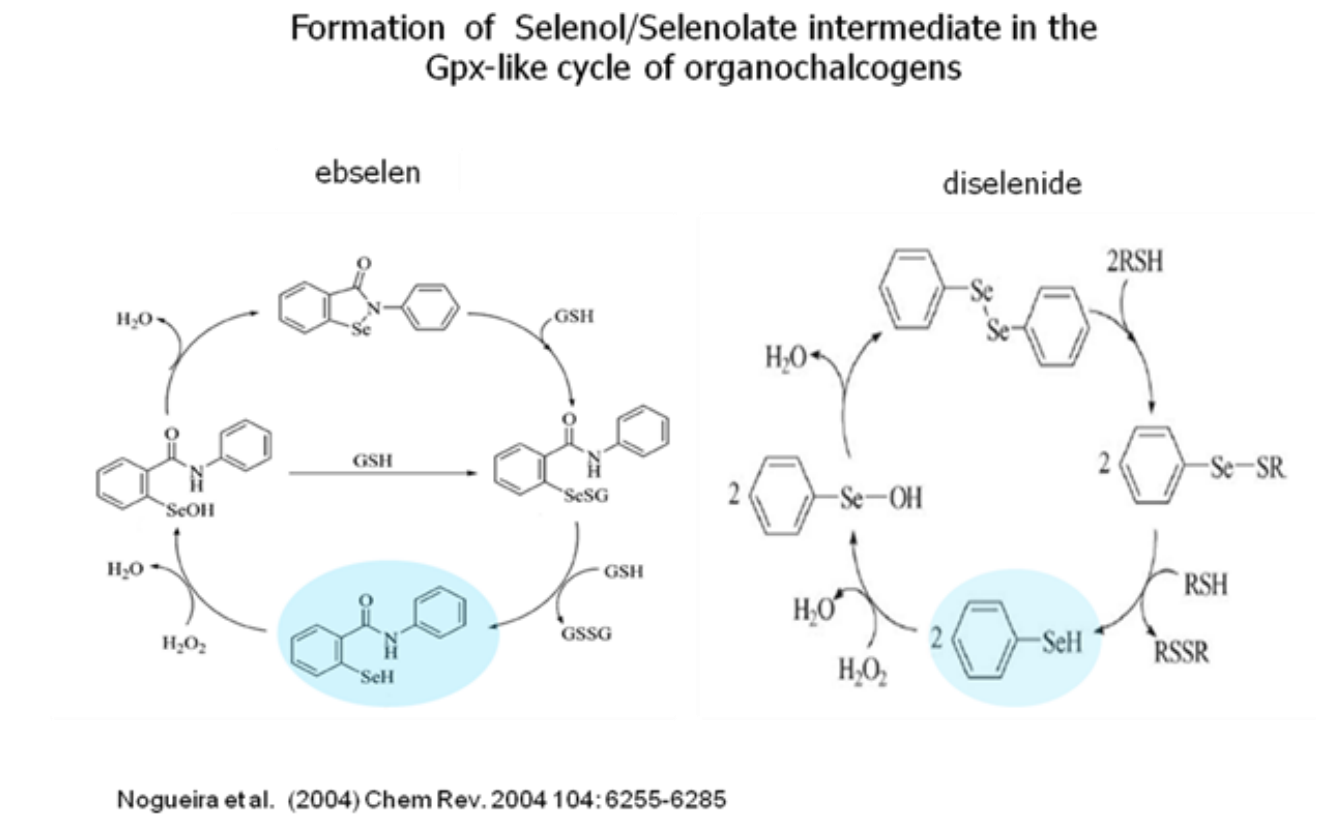


FIGURE 4 **Thiol peroxidase-like Activity of Ebselen (left) and Diphenyl Diselenide (Right). Ebselen and diselenide showing their chemical structure and their derivatives during the catalytic detoxification of H_2O_2 done at the expenses of reducing equivalents derived from GSH (adapted from Nogueira et al. 2004).**

demonstrable GPx-like activity, requiring reduced glutathione or other reduced thiols to catalyze the reduction decomposition of a wide variety of hydroperoxides (Figure 4) (Parnham and Kindt 1984; Wendel et al. 1984; Müller et al. 1984). The mechanism of ebselen mediated decomposition of peroxides have been described to be kinetically similar to that of GPx reaction (compare Figures 2 and 4; Maiorino et al. 1988). First, ebselen reacts with the thiols to generate a selenenyl sulfide. The selenenyl sulfide reacts with a second equivalent of GSH to yield a selenol intermediate. Finally, the selenol reacts with H_2O_2 or organic hydroperoxide to form H_2O or the respective alcohol (ROH) and ebselen selenenic acid. While GSH is an important thiol for ebselen GPx mimic (Maiorino et al. 1988; Haenen et al. 1990), it is important to point out that in contrast to the reaction catalyzed by the enzyme, which contains binding sites conferring substrate specificity, ebselen and other organoselenium compounds can utilize a variety of thiols with varying degree of efficiency (Engman et al. 1992; Iwaoka and Tomoda 1994; Mugesh et al. 2001). In addition to ebselen, another class of organoselenium compounds worth mentioning is the diorganyl diselenides (see Figure 4). Among the diorganyl diselenides, diphenyl diselenide (DPDS) has been well studied. (Nogueira et al., 2004, Nogueira and Rocha, 2010, 2011) and the schematic GPx mimic is presented in Figure 4. The decade of the 1990s was characterized by an enormous development in the field of small synthetic organoselenium compounds that mimic glutathione peroxidase catalytic activity, such as benzoselenazinones (Jacquemin et al. 1992), benzoselenazolinones (Galet et al. 1994), camphor-derived selenenamide (Back and Dick 1997), 2-phenylselenenylnaphthol (Engman et al. 1995), alfa-(phenylseleny) ketones (Engman et al. 1994), and oxygen-containing diselenides (Wirth, 1998). Moreover, a number of attempts have been made to design and synthesize ebselen-related GPx mimics based on substituent effects or isosteric replacements, most of them met with limited success. Ebselen derivatives, such as benzisochalcogenazolones, contain intramolecular interactions as Se-O and consequently increased the catalytic activity of ebselen. For

instance, benzisoselenazolones *N*-alkyl substituted were more active than ebselen. However, benzisoselenazolone *N*-phenyl substituted showed lower peroxidase activity than ebselen, which could be attributed to its poor solubility.

CONSTRAINTS TO GPX MIMIC OF ORGANOSELENIUMS

In Figure 4, we can observe that the GPx mimic activity of ebselen depends on the reduction of the selenenic acid to selenol by thiols. This raises two vital questions: Is the GPx mimic efficiency of a specific organoseleniums strictly dependent on critical thiol type? Is the GPx mimic catalytic efficacy of any organoselenium compound critically dependent on the nature of the peroxides? Elegant findings by Mugesh and his collaborators provided insight to these questions. They observed that any substituent that is capable of enhancing the nucleophilic attack of thiol at sulfur in the selenenyl sulfide intermediate would enhance the antioxidant potency of ebselen and other organoselenium compounds. It was demonstrated that the use of thiol having an intramolecular coordinating group would enhance the biological activity of ebselen. On the other hand, they observed that the nature of the peroxide has little effect on the catalytic efficiencies (Bhabak and Mugesh 2007). However, other authors observed that electronic and steric effects have profound influence on the GPx-like activity of ebselen. The incorporation of a substituent ortho to the selenium atom sterically hinders the attack of a nucleophile at selenium, prevents thiol exchange reactions, and promotes the production of selenol, the GPx-active form, and thus, the GPx-like activity is greatly enhanced. This study further demonstrated that the electronic nature of the substituent groups is less important than their steric effects to the peroxidase-like activity (Pearson and Boyd 2008).

Taking a holistic view of the considerations above, Sarma and Mugesh in 2008 postulated a revised mechanism for the GPx-mimetic activity of ebselen. Considering the complications associated with the catalytic mechanism of ebselen and that none of the intermediates other than the selenenyl sulfides have been confirmed, a reversible cyclization pathway was demonstrated. This study shows the first structural evidence that the seleninic acid, which was never proposed as an intermediate in the catalytic mechanism of ebselen, is the only stable and isolable product in the reaction of ebselen with peroxides.

PHARMACOLOGICAL IMPLICATION OF THE GPX MIMIC OF ORGANOSELENIUMS

From the foregoing, it is apparent that at least some organoselenium compounds are capable of performing the redox cycle of glutathione peroxidase, with the property of imitating the redox physiological chemistry of selenol/selenolate groups. Consequently, specific selenocompounds (such as diphenyl diselenide and ebselen) might supplement natural cellular defenses against the oxidizing agents especially in mammalian systems. Therefore, synthetic organoselenium compounds could represent a novel therapeutic approach to target diseases where oxidative stress plays a role (Arteel and Sies, 2001). For an organoselenium compound to be an antioxidant, it must show nucleophilicity necessary for imitating glutathione peroxidase, potential free radical scavenger activity, and low toxicity. In this way, pharmacological research with organoselenium compounds has provided fascinating challenges in dose–response relationships because of its contrasting behavior that is dose dependent (Nogueira and Rocha, 2010). At this point, we focused on the pharmacological implications of the GPx mimic of synthetic organoseleniums, with emphasis on their antioxidants effects.

In the last four decades, the antioxidant or organoselenium compounds have been reported in different *in vitro* experimental models (Hermenegildo et al. 1990; Christison et al. 1994; Rossato et al. 2002a; Tiano et al. 2003; Andersson et al. 1994; Rossato et al. 2002b; Meotti et al. 2004). A number of newly synthesized analogues of ebselen (2-(5-chloro-2-pyridyl)-7-azabenzisoselenazol-3(2*H*)-one, 2-phenyl-7-azabenzisoselenazol-3(2*H*)-one, 2-(pyridyl)-7-azabenzisoselenazol-3(2*H*)-one, 7-azabenzisoselenazol-3(2*H*)-one, and bis(2-aminophenyl) diselenide) were screened for antioxidant activity in human blood platelets. Among these compounds, only bis(2-aminophenyl) diselenide inhibited lipid peroxidation. Bis(2-aminophenyl) diselenide was also effective in preventing the generation of oxidized low-molecular-weight thiols (GSH, cysteine CSH, cysteinylglycine CGSH) in platelets (Saluk-Juszczak et al. 2006). Ebselen and other organoselenium compounds can potentially react with peroxynitrite, an extremely reactive nitrogen species (NS) and a potent pro-inflammatory agent (Masumoto and Sies 1996; Masumoto et al. 1996). Accordingly, peroxynitrite (ONOO) is a strong electrophile that is produced by the reaction of nitric oxide and superoxide anion. Consequently, uncontrolled generation of peroxynitrite, normally causes exacerbate oxidation of critical biomolecules that is accompanied by an intensive destruction of host cellular constituents. For instance, peroxynitrite causes the nitration of tyrosyl residues of specific proteins (Figure 5) and can disrupt the physiological function of oxidized protein (Figure 5; d'Ischia et al. 2012; Castro et al. 2011; Butterfield et al. 2011).

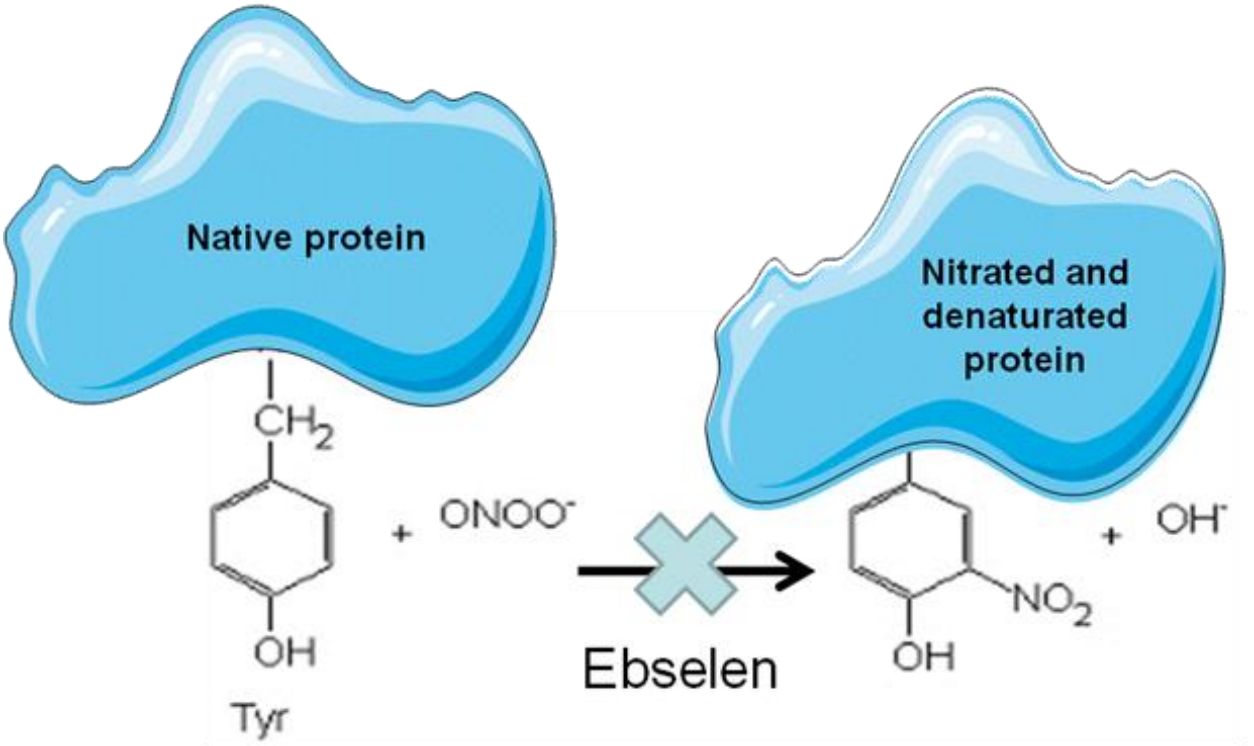


FIGURE 5 Nitration of proteins by peroxynitrite: Inhibition by Ebselen. Protein nitration can cause loss of physiological function of a given protein. Ebselen can inhibit protein nitration via a direct interaction with peroxynitrite.

The reaction of diselenides with peroxynitrite has not been demonstrated. However, diaryl diselenides, diphenyl diselenide and *p*-chlorodiphenyl diselenide (in analogy to ebselen) inhibited lipid peroxidation induced by sodium nitroprusside, which can be a consequence of their reaction with nitric oxide or peroxynitrite formed from its oxidation (Rossato et al. 2002b). However, detailed studies about the reaction of diselenides (and selenolate intermediates formed after reduction by GSH or other reduced thiols) are highly desirable to determine the affinity of these forms towards the cytotoxic peroxynitrite. Of particular pharmacological significance, diphenyl diselenide can reduce the oxidation and nitration found in a model of atherosclerosis in mice (Hort et al. 2011), indicating that diphenyl diselenide can reduce the toxicity of peroxynitrite to the endothelial cell in mice that do not express ApoE receptor.

EVIDENCE OF GPX MECHANISTIC SWITCHING BY ORGANOSELENIUMS *IN VIVO*

Consequent from the GPx mimic described above, it is apparent that in mammals, the pharmacological effect of organoselenium compounds will depend on the ubiquitous antioxidant tripeptide, glutathione to effect their pharmacological actions. In fact several *in vitro* models have consistently shown that the GPx mimic is the critical mechanisms for organsoselenium (For excellent reviews, see Mugesh et al., 2001, Nogueira et al., 2004, Nogueira and Rocha 2010, 2011). In our lab, we have demonstrated that organic moiety of organoselenium compounds can have profound effect on their GPx mimic. In this regard, we compared diphenyl diselenide and dicholesteroyl diselenide, the latter having a bulky organic moiety than the former (Kade *et al*., 2008). In that report, we observed that diphenyl diselenide exhibited marked GPx mimc than dicholesteroyl diselenide. Similarly, diphenyl diselenide exerted strong in vitro antioxidant effect in several models of oxidative stress than dicholesteroyl diselenide. Furthermore, reports have shown that diverse organoseleniums with differential GPx mimic exerted differential pharmacological potencies. In this regard, *p*-chlorodiphenyl diselenide, diphenyl diselenide, and diethyl diselenide were more catalytic than ebselen. Diselenides, *p*-aminodiphenyl diselenide, and dibutyl diselenide had poor GPx-like activity, while *p*-methoxyldiphenyl diselenide and dipropyl diselenide had no effect (Meotti et al. 2004; Wilson et al. 1989). Strict

dependence of organoseleniums on GSH as a substrate for their GPx mimic rather than other thiols is apparent in the light of our observation and other authors wherein the GPx activity of ebselen and related compounds were studied to understand the reason for the relatively poor catalytic activity of these compounds in aromatic thiol assays (Parnham and Graf, 1987; Mugesh *et al*., 2001, Nogueira *et al*., 2004). The reaction of ebselen with thiophenyl (PhSH) does not generate any selenol even when an excess amount of thiol is used. Although the Se-N bond in ebselen is readily cleaved by PhSH to produce the selenenyl sulfide, the reaction of selenenyl disulfide with PhSH does not produce the selenol. This is due to the presence of strong Se · · ·O nonbonded interactions in the selenenyl sulfide, which facilitates an attack of thiol at selenium rather than at sulfur, leading to thiol exchange reaction (Parnham and Graf, 1987). This undesired thiol exchange reaction hampers the formation of selenol. Further studies also indicate that the nature of thiol has a dramatic effect on the catalytic activities of these ebselen analogues. On the other hand, as mentioned above, the nature of peroxides does not appear to have any significant effect on the catalytic efficiencies. Therefore, the discrepancies in the activities of organoselenium compounds in various assays should arise mainly from the variation in thiol used for the reduction of hydroperoxides.

Apparently, the GPx mimic of organoselenium accounts for most of their observed pharmacological effect. At this point, we could speculate that the pharmacological activity of organoseleniums may generate a diminished level of glutathione as well as other thiols levels in physiological systems. Therefore, under *in vivo* conditions, we expect that the antioxidant efficacy of any organoselenium, much like the native GPx, will largely depend on the availability of the endogenous tripeptide, GSH. However, under in vivo conditions and even under various conditions of oxidative stress related disease models where the level of GSH is generally compromised, administration of organoselenium compounds generally elevated the physiological level of the tripeptide, GSH. This is a paradox and a puzzle with respect to the chemistry of organoselenium compounds. This review will now focus on data obtained by several authors vis-à-vis the *in vivo* effects of organoselenium compounds with a view to illustrate this puzzling phenomenon. We want to emphasize that our focus will be tailored towards the influence of organoselenium compounds on the level of the endogenous thiols especially GSH.

At this point, we want to review data obtained by several authors with respect to the relationship of organoseleniums and level of endogenous thiols under *in vivo* conditions. In the study of Pawlas and Maecki, (2007), they observed that under combined conditions of normoxia and ischemia, ebselen (1 - 20 µM) increased the level of intracellular GSH (126.49 ± 9.46% - 144.52 ± 9.84% respectively). However, under ischemic condition alone, ebselen increased GSH level in the range (157.02 ± 18.09 - 119.35 ± 12.00%). It would be observed that under normoxia, higher doses of ebselen exerted more pronounced effects, while in ischemia, the opposite effect was observed. These authors argued that one possible explanation for this observed effect is that the increase in GSH level after ebselen administration could be at least partially explained by the decreased GSH consumption caused by strengthening of antioxidative mechanisms. They concluded that ebselen administration probably replaces ischemia-inactivated GPx activity, thereby contributing to reduction of GSH consumption and diminution of oxidative stress. In another report, Wistar rats were treated with 7,12-dimethylbenz[a]anthracene (DMBA) and the organoselenium compounds [1- isopropyl-3-methylbenzimidazole-2-selenone (Se I) and 1, 3-di-p-methoxybenzylpyrimidine-2-selenone (Se II)] in determined doses. They observed that administration of rats with DMBA significantly resulted in decreased amount of total GSH levels in these rats and that the antioxidant activities and total GSH levels were significantly increased with administration of the organoselenium compounds (Talas *et al*., 2009a,b). In the work of Kiersztan and collaborators, they observed that diabetes induced a decrease in GSH level accompanied by a rise in GSSG content; hence GSH/GSSG ratio in diabetic was 50% lower than the value achieved in control animals. However, this condition was reversed by an organoselenium, methyl-selenocysteine where they observed that blood GSH level in diabetic animals increased by about 40%, following methyl-selenocysteine administration, consequently resulting in an elevation of GSH/GSSG ratio. This effect of methylselenocysteine was attributed to its ability to interfere with the enzyme γ-glutamylcysteine synthetase (Kiersztan *et al*., 2009). In our group, we have also investigated the effect of organoseleniums on diabetes and glutathione homeostasis. For example, we have reported that diet supplementation of diphenyl diselenide positively influenced total hepatic –SH groups in diabetic rats. In fact, diabetes in these rats caused a reduction of about 23% in the hepatic –SH levels. Ingestion of diphenyl diselenide supplemented diet caused a significant increase in hepatic –SH both in diabetic and non-diabetic rats. In addition, level of –SH in erythrocytes was markedly increased by diphenyl diselenide in both diabetic and non-diabetic rats (Barbosa et al., 2008a).

In fact, we have shown that route of administration, vehicle solution can greatly influence the pharmacology of organoselenium compounds (Kade *et al*., 2010). Therefore we again tested subcutaneous administration (pharmacological dose 1 mg/kg body weight) or acute 10 mg/kg body weight (Barbosa *et al*., 2008b) of either ebselen and diphenyl diselenide in diabetic rats (Barbosa *et al*., 2006). We observed that diphenyl diselenide treatment promoted *per se* a significant increase on hepatic, renal and blood GSH levels compared to control group. Similarly, in diabetic rats treated with the organoselenium, diphenyl diselenide, there was an elevated GSH levels in liver and kidney. In addition, oral administration of diphenyl diselenide in soya bean oil greatly increased the otherwise diabetic depleted hepatic GSH levels in rats, and diphenyl diselenide promoted a significant increase in hepatic, renal and spleen GSH levels. Also in the brain, diabetes also caused a noticeable diminution in the level of cerebral GSH and treatment with diphenyl diselenide evoked a significant sparing effect on the levels of GSH in the diabetic rat brain (Kade *et al*., 2009a,b).

From the foregoing, we observed that under in vivo conditions, organoselenium compounds evoked a marked increase in the level of endogenous thiols such as GSH. This is a puzzling in view of the generally acclaimed GPx mimic organoseleniums. In a stride to explain this observed phenomenon, we explored other possible antioxidant mechanisms that may be critical to pharmacology of organoseleniums in different physiological states and in this case, we studied diphenyl diselenides. Although there are few published studies on molecular mechanisms involved in diphenyl diselenide antioxidant property, we further confirmed that diphenyl diselenide generally exert its antioxidant action by mimicking glutathione peroxidase. Therefore, the effect of pH on GPx mimic activity and other possible mechanisms involved in the diphenyl diselenide antioxidant activity were investigated. On the one hand, diphenyl diselenide had neither free radical-scavenging nor Fe^{2+}-chelating ability. However, diphenyl diselenide exhibited increasing ability to reduce Fe^{3+} with increasing pH. It is possible to hypothesize that the formation of stable selenolate ions increases the reducing property of this molecule and its antioxidant property. On the other hand, the GPx-like activity of diphenyl diselenide was maximum at neutral pH diminished in the acidic medium. Furthermore, independent of the pH, diphenyl diselenide decreased deoxyribose degradation mediated by either hydrogen peroxide or Fe^{2+}. This indicates that the antioxidant properties of diphenyl diselenide in the acidic medium may not be related to its GPx-like activity (Ogunmoyole et al. 2009; Hassan et al. 2009a, b, c, d).

FURTHER EVIDENCE OF MECHANISTIC GPX MIMIC SWITCHING BY ORGANOSELENIUM *IN VIVO*

In fact, there are ample indirect evidences that indicate that there is a possible switch in the GPx mimic activity of organoseleniums chemistry under *in vivo* conditions. In this regard, the interaction of these classes of antioxidants with thiol containing proteins is worth mentioning. The sulfhydryl enzymes such as Na^+/K^+-ATPase (also known as the sodium pump), delta aminolevulinic acid dehydratase, lactate dehydrogenase are sensitive to conditions of oxidative stress via the inactivation of their thiols. In our laboratory, we have observed that under *in vitro* conditions, organoselenium such as diphenyl diselenide inhibits sulfhydryl enzymes (Nogueira and Rocha, 2004, 2010, 2011). In fact of note is the fact that the inhibitory effect of these organoselenium compounds is in the micromolar range (Borges et al., 2005, Kade et al., 2008, 2009c). In order to establish the involvement of thiols in the inhibitory effect of organoselenium compounds on the activity of these sulfhydryl enzymes, exogenous thiols such as dithiothreitol have been used to recover the inhibition imposed by organoseleniums (Borges et al., 2005, Kade et al., 2008). Consequently, we can speculate that under *in vivo* conditions organoselenium compounds will have strong inhibitory effect on the activities of this class of enzymes.

However, elegant experimental data have consistently shown that under *in vivo* conditions, organoselenium compounds protects these sulfhydryl enzymes especially under conditions of oxidative stress. In this regard, we reported that when mice were injected subcutaneously with diphenyl diselenide or dicholesteroyl diselenide previously dissolved in soya bean oil at chronic doses of 0.5 mmol kg^{-1} body weight for four consecutive days, the activities of cerebral Na^+/K^+-ATPase were not markedly inhibited by both diselenides, suggesting that this cerebral enzyme may not be a molecular target of organodiselenides toxicity. We equally observed in this study that the administration of these organoseleniums in mice is accompanied by elevated levels of GSH. Generally when these enzymes were evaluated in models of oxidative stress related diseases such as diabetes wherein their levels were consistently decreased, we equally consistently observed that organoselenium interventions improves the activities of these enzymes with concomitant increase in the level of endogenous thiols evaluated as glutathione (Kade et al., 2009, 2009a, 2010; Barbosa et al., 2006, 2008). From these data and others (Nogueira and Rocha, 2011), we observed that the increase in the levels of these endogenous thiols necessitated by organoselenium intervention is suggestive and possibly

indicate that organoseleniums do not employ the classical *in vitro* GPx mimic activity to effect their pharmacological action under *in vivo* conditions.

GPX MIMIC OF ORGANOSELENIUMS: PRE OR POST METABOLISM *IN VIVO*?

In the light of the above and data obtained by other authors, we further argue that there is a strong but complex dynamics associated with the chemistry of organoselenium compounds under in vitro and in vivo conditions. Data obtained from studies conducted on lower organisms with organoseleniums suggests that in these organisms, they probably exhibited strong GPx-mimic. For example, the effect of diphenyl diselenide on GSH antioxidant capacity in a total radical trapping antioxidant parameter (TRAP) assay was studied by Zafarullah and his collaborators in yeast strains and they found that diphenyl diselenide attenuated the GSH antioxidant TRAP pattern in a time-dependent manner when an equimolar concentration of diphenyl diselenide and GSH is used, suggesting that it may react with GSH, forming an inactive adduct. They equally observed that when diphenyl diselenide was incubated with N-acetyl cysteine, the latter was able to neutralize and revert the pro-oxidant effect of diphenyl diselenide. They reasoned that diphenyl diselenide may either stimulate the increase of endogenous GSH biosynthesis or by a direct reaction with the drug (Zafarullah *et al*., 2003). We equally want to emphasize that at higher doses, organoselenium compounds can be toxic and this effect can be accompanied by decreased level of glutathione in animal tissues. A study by Rosa and collaborators (Rosa *et al*., 2005, 2007) may illustrate this point. In their work, they investigated the possible genotoxic effect of diphenyl diselenide in multiple organs (brain, kidney, liver, spleen, testes and urinary bladder) and tissues (bone marrow, lymphocytes) of mice using in vivo comet assay. They observed that the genotoxicity of diphenyl diselenide is accompanied with decreased glutathione in tissues tested. Equally, pre-treatment of animals with *N*-acetyl-cysteine completely prevented diphenyl diselenide induced oxidative damage by the maintenance of cellular GSH levels, reinforcing the positive relationship of diphenyl diselenide induced GSH depletion and DNA damage.

Consequently, we conclude that the pharmacological mechanism of organoseleniums whether *in vitro* or *in vivo* can be complex and is dependent on so many factors that are far from being fully understood. In this review, we have observed that although the utilization of glutathione in the antioxidant action of organoseleniums appears to be their major antioxidant mechanism, we speculate that the glutathione peroxidase mimic activity of organoseleniums shift depending on multifactorial considerations. It is noteworthy however, that the antioxidant action of organoseleniums *in vivo* may involve the activity of the non-organic moiety of these organoseleniums. Consequently, there may be a sequential biotransformation of organoseleniums generating diverse intermediates and with each intermediate product utilizing diverse, but potent antioxidant mechanism which would apparently exclude glutathione peroxidase mimic that require the utilization of endogenous glutathione or other thiols. Equally, these metabolic intermediates may have some forms of positive interaction with the pathway leading to glutathione synthesis *in vivo*.

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