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**REVIEW**

# Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase

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**Numerous studies in animal models and more recent studies in humans have demonstrated cancer chemopreventive effects with Se. There is extensive evidence that monomethylated forms of Se are critical metabolites for chemopreventive effects of Se. Induction of apoptosis in transformed cells is an important chemopreventive mechanism. Apoptosis can be triggered by micromolar levels of monomethylated forms of Se independent of DNA damage and in cells having a null *p53* phenotype. Cell cycle protein kinase cdk2 and protein kinase C are strongly inhibited by various forms of Se. Inhibitory mechanisms involving modification of cysteine residues in proteins by Se have been proposed that involve formation of Se adducts of the selenotrisulfide (S-Se-S) or selenenylsulfide (S-Se) type or catalysis of disulfide formation. Selenium may facilitate reactions of protein cysteine residues by the transient formation of more reactive S-Se intermediates. A novel chemopreventive mechanism is proposed involving Se catalysis of reversible cysteine/disulfide transformations that occur in a number of redox-regulated proteins, including transcription factors. A time-limited activation mechanism for such proteins, with deactivation facilitated by Se, would allow normalization of critical cellular processes in the early stages of transformation. There is uncertainty at the present time regarding the role of selenoproteins in chemoprevention model systems where supranutritional levels of Se are employed. Mammalian thioredoxin reductase is one selenoprotein that shows increased activity with Se supplementation in the nutritional to supranutritional range. Enhanced thioredoxin reduction could have beneficial effects in oxidative stress, but possible adverse effects are considered. Other functions of thioredoxin reductase may be relevant to cell signaling pathways. The functional status of the thioredoxin/thioredoxin reductase system during *in vivo* chemoprevention with Se has not been established. Some *in vitro* studies have shown inhibitory effects of Se on the thioredoxin system correlated with growth inhibition by Se. A potential inactivating mechanism for thioredoxin reductase or other selenoenzymes involving formation of a stable diselenide form resistant to reduction is discussed. New aspects of Se biochemistry and possible functions of new selenoproteins in chemoprevention are described.**

## Introduction

Selenium (Se) has been shown to prevent cancer in numerous animal model systems when fed at levels exceeding the nutritional requirement (1–5). A landmark study by Clark *et al*. (6) showed cancer chemopreventive efficacy using a Se supplement in humans. These reports have heightened interest in additional human Se chemoprevention studies to confirm and extend the results in larger populations and have intensified the search for mechanisms by which Se acts to suppress tumorigenesis. Although it is convenient to describe effects of Se in terms of the element, it must always be kept in mind that chemical form and dose are determinants of its biological activities as an essential nutrient, cancer preventive agent or toxicant. For scientific reasons as well as human safety, there is a need to know as much as possible about the origins of such activities, the extent to which they overlap and whether they might be separable.

The purpose of this article is to provide a perspective on possible mechanisms of chemoprevention in relation to the chemical forms of Se that arise in the course of Se metabolism. Low molecular weight metabolites are considered first, then selenoproteins. The thioredoxin reductase/thioredoxin system and Se chemoprevention are discussed in some detail in view of the recent discovery that mammalian thioredoxin reductase is a selenoenzyme and shows increased activity with Se supplementation. Although this discovery offers intriguing possibilities to consider, it seems puzzling taken at face value because chemopreventive levels of Se inhibit cellular growth and proliferation and bring about cell death by apoptosis, whereas thioredoxin and thioredoxin reductase are associated with increased cell growth and proliferation and inhibition of apoptosis.

## Overview of selenium metabolism

The metabolism of selenium is dynamic and can be likened to *in vivo* combinatorial chemistry, in the sense that a wide array of products are formed (7,8). Animals synthesize many different intermediary metabolites in the course of converting inorganic Se to organic forms and vice versa. Hydrogen selenide is a key metabolite, formed from inorganic sodium selenite (oxidation state +4) via selenodiglutathione (GSSeSG) through reduction by thiols and NADPH-dependent reductases (9–11) and released from selenocysteine by lyase action. Methylation is a major pathway for Se metabolism in microbes, plants and animals, but demethylation back to inorganic selenium can occur in animals. Hydrogen selenide provides Se for synthesis of selenoproteins after activation to selenophosphate (12). The known functions of Se as an essential element in animals are attributed to ~12 known mammalian selenoproteins (13), all containing selenocysteine, specifically incorporated through a unique co-translational mechanism (14,15). Non-specific incorporation of Se into proteins occurs through substitution of selenomethionine for methionine and selenomethionine is a major constituent of selenized yeast used in chemopreventive studies (16). Se-tolerant plants of the *Astragalus* genus that accumulate Se to 100- or 1000-fold higher levels than non-accumulator species synthesize large

**Abbreviations:** DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); DTT, dithiothreitol; GSH, reduced glutathione; GSSeH, glutathione selenopersulfide; GSSeSG, selenodiglutathione; GSSG, glutathione disulfide.

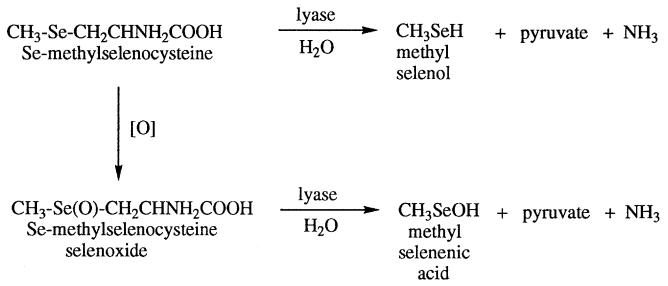


**Fig. l.** Monomethylated forms of Se released from Se-methylselenocysteine or its selenoxide derivative (20). See Ganther (7) and Ganther and Lawrence (8) for additional aspects of Se metabolism and methylated selenide metabolism.

amounts of Se-methylselenocysteine by means of a specific selenocysteine methyltransferase, thereby preventing selenocysteine from competing with cysteine for incorporation into proteins (17). Se-methylselenocysteine is a major constituent of selenized garlic and has several advantages as a chemopreventive form of Se compared with selenomethionine (5,16).

## Chemopreventive metabolites of selenium

A collaborative investigation relating Se metabolism to anticarcinogenic action was initiated some years ago (5). One of the major goals was finding less toxic forms of Se that would retain chemopreventive activity. Methylation of Se produces less toxic forms and for that reason a number of methylated Se compounds were tested (18,19). From that work, monomethylated forms of Se have emerged as a critical class of Se metabolites having powerful effects on carcinogenesis, while lacking some of the toxic effects produced by other forms such as inorganic selenite (5,8). Briefly, stable methylated Se compounds such as selenobetaine or Se-methylselenocysteine serve as precursors, similar to a pro-drug, and release methylselenol or methylselenenic acid through the action of cysteine conjugate β-lyase or related lyases (20; Figure 1). The monomethylated selenium compounds are effective *in vitro* at very low concentrations to give chemopreventive effects (apoptosis and cell cycle arrest) in transformed cells (21–25). Se-methylselenocysteine thus serves as a reservoir that provides a steady stream of monomethylated Se so that a critical level is maintained and cell growth is inhibited. The concept that supranutritional levels of Se would generate increased levels of a critical metabolite proved to be well suited as a rational explanation for Se anticarcinogenic action in the animal model systems.

## Se-induced apoptosis

Apoptosis, by causing deletion of carcinogen-initiated cells and suppression of clonal expansion of a transformed cell population, is an attractive mechanism for chemoprevention (26). Studies from a number of laboratories have shown that it is an important mechanism for the anticancer effects of Se in cultured cancer cells, as discussed by Ip (5). It is important to note that apoptosis can be triggered by Se independent of DNA damage and in cells having a null *p53* phenotype (23). In the initial demonstration of Se-induced apoptosis by Thompson's group with selenite, single-strand breakage indicative of DNA damage was involved (27), but studies with methylated forms of Se showed that apoptotic effects could be dissociated from DNA damage or other signs of toxicity (21). A chemopreventive mechanism based on induction of apoptosis, separate from toxic effects and independent of a functional p53, strengthens the case for Se chemoprevention in the human population (5).

Since Se-induced apoptosis can be dissociated from toxic effects, other mechanisms must be involved. Se-induced alterations in cell cycle proteins, associated with $G_1$/S phase and decreased DNA synthesis, were investigated in two studies with either synchronized or asynchronous mammary epithelial tumor cells (24,25). Growth inhibition caused by Se-methylselenocysteine was coincident with a marked decrease in cdk2 kinase activity and impeded progress through S phase. Recent studies with methylseleninic acid suggest that a monomethylated Se compound is the proximal metabolite responsible for these effects (28).

## Mechanisms of action of low molecular weight Se compounds

Historically, research on the biochemical reactions of Se compounds has been directed toward thiols, especially protein thiols (29). Four different types of reactions by which Se might modify proteins are discussed:

- Type 1, formation of selenotrisulfide bonds (S-Se-S);
- Type 2, formation of selenenylsulfide bonds (S-Se);
- Type 3, catalysis of disulfide bond (S-S) formation or its reversal;
- Type 4, formation of diselenide bonds (Se-Se).

Types 1 and 2 are better known and have been invoked from time to time to explain effects of Se through S-Se bonding. Examples of both types can be found in numerous studies (30–34). Using reduced ribonuclease as a model, stoichiometry of one selenite reacting with four protein thiols was seen, producing one selenotrisulfide and one disulfide (30). The recent work of Gopalakrishna's group emphasizes how the presence of cysteine clusters in proteins such as the catalytic subunit of protein kinase C can lead to inhibition by micromolar levels of selenite (31), whereas proteins lacking such clusters are unaffected. For GSSeSG and other metabolites that are more reduced than selenite, the stoichiometry for the reaction with protein thiols will be different. GSSeSG can have strongly inhibitory effects on thiol proteins, sometimes an order of magnitude greater than selenite (33,35). Proteins having regulatory cysteines can form Se adducts (33,34) and Se inactivation of essential thiol groups of transcription factors controlling numerous cellular processes was proposed as a high amplification mechanism for toxic effects of Se (34). In contrast to the type 1 and type 2 mechanisms, where Se inactivates thiol proteins by bonding to the sulfur to form an adduct, in the type 3 mechanism Se is a catalyst and is not taken up in the protein. Type 3 has been observed with structural cysteines (35) as well as catalytic cysteines (31) and has especially interesting potential with regard to redox-regulated cell signaling. Type 4 is novel and the most speculative, but offers some interesting possibilities with regard to selenoprotein biochemistry and understanding of chemopreventive mechanisms that are presented later.

The type 3 mechanism (Se-catalyzed disulfide bond formation/reversal) is related to thiol/disulfide interchange, long regarded as a potentially useful function for Se (29). One interesting example is oxidative folding of reduced ribonuclease in air in the presence of thioredoxin or protein disulfide

isomerase, where catalytic amounts of sodium selenite (l μM) resulted in rapid disulfide formation and restoration of ribonuclease activity in high yield (36). For reduced thioredoxin, the addition of 0.1 equivalent of GSSeSG resulted in complete oxidation of structural cysteines and inactivation (35). Gopalakrishna's group showed that Se can facilitate intramolecular disulfide bond formation in protein kinase C, leading to inactivation (31). The common theme here is that Se catalyzes disulfide bond formation in the context of reversible processes and the result may be either recovery of the native state or inactivation, depending on the protein. Other interesting examples of intramolecular disulfide formation as an inactivating mechanism are seen with the voltage sensor of the mitochondrial permeability transition pore (37), as well as the well-known redox-regulated transcription factors. Inactivation of key proteins such as protein kinase C is clearly relevant to chemoprevention.

Protein activating reactions involving scission of disulfide or cysteinyl mercaptide linkages in proteins are also potential chemopreventive mechanisms to consider for Se metabolites such as methylselenol. An interesting possibility, particularly with regard to Se-induced apoptosis, might be direct activation of a cysteine protease having the active site cysteine blocked by zinc. The higher affinity of methylselenolate for Zn could result in release of the cysteinylthiolate-bound Zn and activate the enzyme:

$$[\text{caspase}]Zn^{2+} + 2\ MeSe^{-} \rightarrow [\text{caspase}] + (MeSe)_2Zn$$

(inactive) (active)

## Se modulation of redox-regulated transcription factors

Se inactivation of transcription factors such as AP-1 and NF-kB that are known to be modulated by redox control mechanisms was described as a mechanism by which Se compounds inhibit cell growth at micromolar levels (33,34). In a different approach, using cells previously depleted of Se, supplementation of Se at 10–100 nM levels was shown to suppress NF-kB activation (38); in this case the effect was specific for NF-kB and AP-1 was not affected. Restoration of reduced glutathione (GSH) peroxidase and resultant antioxidant activity rather than inhibition of NF-kB by Se was suggested as a mechanism. These studies demonstrate that transcriptional factor modulation by Se may be relevant to gene expression and chemopreventive mechanisms.

One notable characteristic of Se is that its effects are more pronounced in early stages of transformed cancer cells (39,40). On this basis, Se might be considered as an agent inducing normalization of regulatory pathways that are perturbed in early stages of carcinogenesis. A testable hypothesis is that Se could have a role in terminating the activated states of redox-regulated proteins such as transcription factors. Redox-regulated transcription factors can have two states, ON and OFF, that differ by the oxidation state of a cysteine residue. In the case of OxyR and some other factors, this involves the presence or absence of an intramolecular disulfide bond between two cysteine residues (41,42). The formation of a disulfide by oxidation is reversed by cellular disulfide-reducing enzyme systems such as glutaredoxin and thioredoxin. Having a transcription factor poised between the opposing pathways is the basis for a dynamic mechanism that makes activation a transitory process (41,42). As proposed in Figure 2A and B, oxidized or reduced forms of Se react with protein thiols or disulfides and form more reactive intermediates. Se catalysis of the reversible redox changes in redox-regulated proteins (type 3 mechanism) facilitates resetting of the basal state. By shortening the length of time that a transcription factor would be in the ON configuration, less transcriptional activity would be observed. In this mechanism, Se is serving as a redox catalyst that links the cellular redox poise to the critical targets, rather than causing bulk changes in peroxide levels and the cellular redox potential.

## Cancer-preventive mechanisms–what role for selenoproteins?

Experimental evidence began accumulating in the l970s confirming a much earlier report (43) of cancer-preventing effects of Se in animals and studies suggesting that cancer incidence in humans correlated inversely with dietary Se intake attracted attention (reviewed in ref. 5). These studies coincided with the discovery of the first selenoprotein (GSH peroxidase) (44). In view of the role of GSH peroxidase in reactive oxygen metabolism, it was expected by many that Se-dependent GSH peroxidase activity would provide a plausible mechanism for cancer prevention by Se. However, it was found that GSH peroxidase activity was already at maximum levels in tissues of animals fed normal amounts of Se and did not change appreciably as dietary Se was increased to the 10-fold higher levels necessary to see chemopreventive effects in the animal models (45). Other selenoproteins were also shown to reach a maximum level in tissues at nutritional Se levels (45,46) and excessive Se intake may even have decreased activity (46). Because there was no experimental evidence to support a role of known selenoproteins in cancer prevention (40,47), the emphasis shifted to studies of low molecular weight chemopreventive metabolites or unidentified selenoproteins.

## Discovery of 58 kDa selenoproteins and Se thioredoxin reductase

In a search for unknown selenoproteins that might mediate Se chemoprevention, Danielson and Medina (48) used $^{75}Se$ labeling of cultured mouse mammary epithelial cells followed by reductive SDS–PAGE to locate proteins containing Se in forms resistant to extraction, as expected for selenocysteine in a peptide linkage. Labeling of a 58 kDa band containing selenocysteine correlated closely with inhibition of DNA synthesis following selenite treatment. Upon selenite withdrawal, DNA synthesis resumed and Se decreased in the 58 kDa band, but not in bands corresponding to GSH peroxidase (49). The other selenoproteins as well as the 58 kDa band were present constitutively in cells grown in 0.05 μM selenite, but the 58 kDa protein stood out by showing increased labeling coincident with increase in selenite concentration to 5 μM and inhibition of DNA synthesis. It was concluded that the 58 kDa selenoprotein was either an important mediator of selenite-induced DNA synthesis or was a good marker for this effect (49).

Ultimately, two proteins were identified as components of the 58 kDa Se-labeled mouse selenoprotein band. Medina's group isolated the major $^{75}Se$ component and established by sequence analysis that it was l00% identical with protein disulfide isomerase (50). However, the total Se content of the isolated protein was far below a stoichiometric value and varying the level of Se in the culture medium had no influence

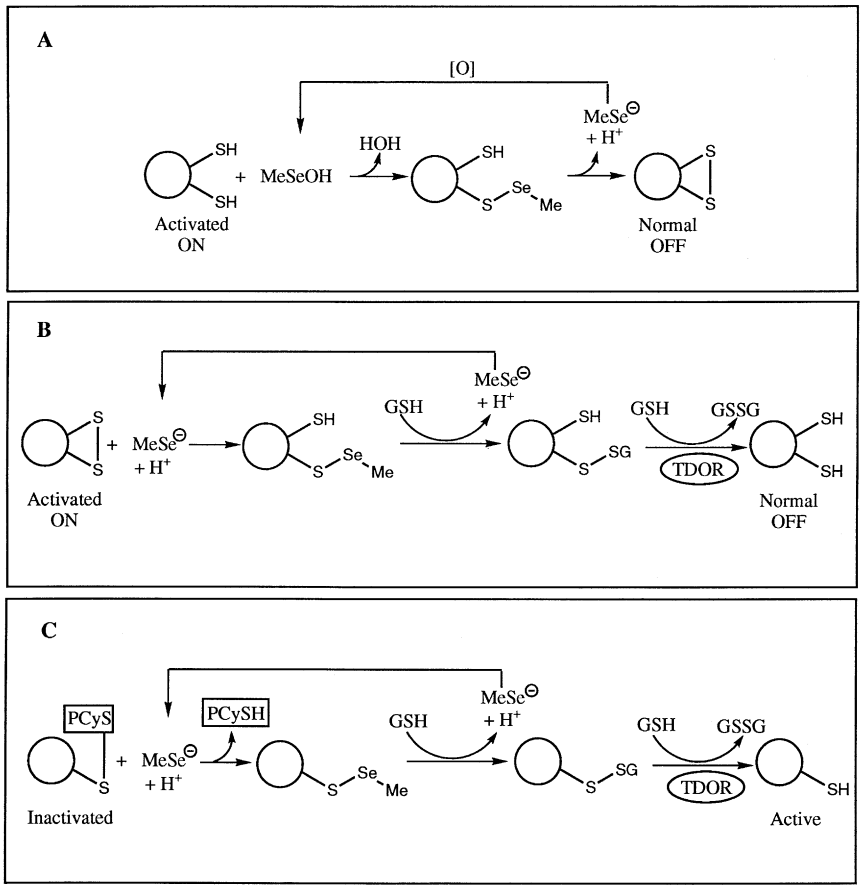


**Fig. 2.** Selenium-catalyzed reactions in a redox-regulated protein such as a transcription factor. Methylselenenic acid (MeSeOH) and methylselenol (Figure 1) are used in the examples but other metabolites such as GSSeSG or GSSeH undergo related reactions. The small size and other physicochemical properties of the methylated Se derivatives may allow greater access to buried sites in proteins compared with larger, more polar compounds such as GSH or glutathione derivatives of Se. (**A**) Example of a redox-regulated protein active in the reduced form. Reaction of an oxidized Se metabolite with a protein thiol forms an activated selenenylsulfide intermediate, which is attacked by a second thiol to form an intramolecular disulfide. Release of the methylselenolate (almost completely ionized at physiological pH) and spontaneous oxidation of the methylselenolate helps drive the coupled reactions. (**B**) Example of a redox-regulated protein active in the oxidized form, such as OxyR (42). The strongly nucleophilic selenolate ion opens the disulfide bond and forms an activated selenenylsulfide intermediate that undergoes facile reaction with GSH to form the glutathione protein mixed disulfide. The glutathione moiety facilitates recognition (81,82) of the protein mixed disulfide by a thiol-disulfide oxidoreductase (TDOR) such as thioltransferase (glutaredoxin) or protein disulfide isomerase, allowing further reduction to protein dithiol with formation of GSSG. NADPH-linked reduction of GSSG by glutathione reductase couples protein disulfide reduction to the cellular reducing systems. (**C**) Example for a transcription factor having a single cysteine sensitive to oxidative inactivation that prevents DNA binding (81). The nature of the oxidized cysteine residue is uncertain, but a bulky adduct such as an intra- or intermolecular disulfide with a second protein cysteine (PCy) is shown in this example. The small size and nucleophilicity of methylselenolate allows it to open the protein disulfide and form a selenenylsulfide derivative that undergoes facile reaction with glutathione, forming the glutathione selenenylsulfide derivative and facilitating reduction of the glutathione-protein mixed disulfide by thioltransferase or protein disulfide isomerase, giving the active (reduced) form of the factor. Protein disulfide isomerase is a relatively abundant cellular protein present in millimolar concentrations in the lumen of the endoplasmic reticulum and has a relatively low catalytic efficiency, but the combined effects of micromolar levels of a Se catalyst and a thiol-disulfide oxidoreductase may have physiological significance for *in vivo* disulfide metabolism (36).

on enzyme activity. Although selenium was tightly bound to the protein, protein disulfide isomerase did not meet the criteria for a true selenoprotein. Recently, following recognition that mammalian thioredoxin reductase was a selenoprotein (51), Gromer *et al*. (52) purified this enzyme from mouse Ehrlich ascites tumor cells and showed that it contained 1 g atom Se per 57 kDa subunit.

Does the incorporation of Se into thioredoxin reductase explain why inhibition of DNA synthesis correlated inversely with Se uptake in the 58 kDa protein band of Medina and co-workers? This is a complex question to be discussed later. Thioredoxin reductase and thioredoxin form a redox system having multiple roles, including redox regulation of transcription factors and provision of reducing equivalents for synthesis of deoxyribonucleotides for DNA synthesis. However, the studies by Medina's group showed that the addition of deoxyribonucleotides to selenite-treated cells did not overcome the selenite inhibition of DNA synthesis (53).

## Selenium in thioredoxin reductase

A radioactive selenoprotein having a subunit mass of 57 kDa was isolated in l996 from a human lung adenocarcinoma cell line grown in medium containing [$^{75}$Se]selenite (51) and identified as a subunit of mammalian thioredoxin reductase. The selenoprotein is a homodimeric flavoprotein using NADPH as electron donor and reduces 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) as well as catalyzing thioredoxin-dependent reduction of insulin, similar to other mammalian thioredoxin reductases (51). The level of thioredoxin reductase in many tissues is very low and extensive purification (several thousand-fold) is necessary to obtain pure enzyme from liver, human

placenta and other normal tissues (54). In contrast, levels in transformed cells are much higher and homogeneous enzyme is obtained after several hundred-fold purification. The enzyme isolated from different sources showed variable immunoreactivity and could be separated into two forms by heparin affinity columns (54).

It has been known for many years that mammalian thioredoxin reductase has properties strikingly different from the *Escherichia coli* enzyme (55). The presence of Se in mammalian thioredoxin reductases has generated much interest in terms of how selenocysteine might participate in the enzyme's mechanism of action. The mammalian enzyme is larger and has an additional redox center at the C-terminus in addition to the two redox-active cysteines in the N-terminal region. This second center is formed by Cys495 and SeCys496, the latter encoded by a termination codon that directs Se-cysteine incorporation if Se is available. The cysteine-selenocysteine center apparently involves a selenenyl sulfide (Se-S) linkage (55) that receives reducing equivalents from NADPH via the conserved cysteine-cysteine dithiol center (56). A number of experimental approaches confirm that the selenocysteine center plays a key role in the novel, wide ranging functions of mammalian thioredoxin reductase (54,55,57–59). A notable characteristic of the thioredoxin reductase selenoprotein is its sensitivity to oxidizing conditions, leading to a change in conformation (57). A conformational change affecting interaction of thioredoxin reductase with other molecules could be important with regard to triggering cell signaling in response to oxidative stress. A role of selenocysteine in thioredoxin reductase as an oxidant sensor controlling cell signaling pathways has recently been suggested (60).

The methodology by which thioredoxin reductase activity is measured must be considered, since there are two redox centers in thioredoxin reductase. If insulin is used as substrate, the activity measured should arise from the selenocysteine-cysteine center (61). If the simple disulfide substrate DTNB is used, either center could catalyze reduction; in that case the selenium center could be non-functional but thioredoxin activity would still be seen. Thioredoxin reductase is two to three orders of magnitude more sensitive to inhibition by organic gold compounds compared with GSH peroxidase (59,62). The use of gold compounds to inhibit the selenium center of thioredoxin reductase may increase specificity so that activity measured using DTNB-based assays will be congruent with insulin-based assays (62), but the potential problem created by choice of assay method must be kept in mind.

### Effect of Se on thioredoxin reductase activity

Prior to the discovery of its selenoprotein nature, mammalian thioredoxin reductase was implicated in Se metabolism by the discovery that selenite and GSSeSG were efficiently reduced to hydrogen selenide by calf thymus thioredoxin reductase or the complete thioredoxin system (11,63). It had already been shown that NADPH-linked reduction of GSSeSG to hydrogen selenide was catalyzed by glutathione reductase (10), a flavoprotein having a mechanism similar to thioredoxin reductase (56). Since hydrogen selenide is rapidly oxidized by oxygen, the Se compounds catalyzed oxygen-dependent oxidation of the thioredoxin system and NADPH. It was suggested that inhibitory effects of selenite on cell growth involved NADPH depletion and competitive inhibition of the thioredoxin system (63); later, oxidation of structural cysteines in thioredoxin to disulfides was shown to be a third possible mechanism (35). These inhibitory effects of Se on the purified thioredoxin system were consistent with the chemopreventive effects of Se seen *in vivo*.

Following the discovery that thioredoxin reductase was a selenoprotein (51), various studies showed that selenite increased the activity of thioredoxin reductase (measured with insulin as substrate) in human cancer cell lines and in rats fed supranutritional levels of selenite. In human colon cancer cells supplemented with 0.1, 1 or 10 μM selenite the increase was dose dependent and 5 day exposure to a high level (10 μM) of selenite gave a 65-fold increase in activity (64). Thioredoxin reductase protein measured with antibody also increased, but to a lesser extent than enzyme activity. Interestingly, Se-methylselenocysteine and selenomethionine had no effect on activity at 0.1 μM and produced only small increases at 1 or 10 μM. Large increases in activity were also observed in a second study with 1 μM selenite in cancer cell lines of epithelial origin, but not in cells of lymphoid origin (65). In a third study (66), using rats fed a moderately high level (1 p.p.m.) of Se as selenite, thioredoxin reductase activity increased ~2-fold in some tissues (lung, liver and kidney) but not in others, in comparison with rats fed a normal Se level (0.1 p.p.m.). In contrast to thioredoxin reductase, GSH peroxidase activity was not increased by the high Se level in any tissue.

Surprisingly, the increased thioredoxin activity was not sustained and in liver the activity returned to control levels by 40 days; the decline was less rapid in kidney and lung (66). Thus, as the authors note, the *in vivo* effects of Se in the nutritional to supranutritional range on thioredoxin reductase activity differ from other selenoenzymes in two ways: (i) increased activity with excess Se; (ii) a decline in activity with continued high level Se administration.

It is important to note the lack of *in vivo* data regarding the functional status of the thioredoxin reductase/thioredoxin system during Se chemoprevention. Although an increased activity of the selenoenzyme in animals and many cancer cell lines, measured *in vitro*, seems to be inconsistent with a chemopreventive mechanism (see below), the apparent inconsistency might be circumvented if there was actually a functional inhibition of the system *in vivo* through effects of Se such as those observed by Holmgren *et al*. (11,35) or perhaps additional mechanisms.

### Consequences of increased thioredoxin reductase activity

If *in vivo* studies show that there is indeed an increased functional activity of thioredoxin reductase with Se administration, there is a need to find out how the Se-induced increase could be a chemopreventive mechanism. Is increased thioredoxin reduction the basis or is some other function of thioredoxin reductase involved? The consequences of alterations in thioredoxin reductase activity are not fully known and possible adverse effects need to be addressed.

Thioredoxin is overexpressed in many forms of cancer, is secreted by tumor cells and stimulates tumor growth while decreasing apoptosis. Cells transfected with thioredoxin cDNA show increased tumor growth and decreased apoptosis *in vivo*. Since it is the reduced form that is active in these effects (67) it is difficult to see how an increase in the reduction of thioredoxin could be a chemopreventive mechanism, when the effects are beneficial to transformed cells. Indeed, a class of

disulfide inhibitors of thioredoxin was developed (68; see below) for use as antitumor agents, on the basis that thioredoxin would be a rational target for drug inhibition (67).

If some other function of thioredoxin reductase could be found that outweighed the potentially adverse effects noted above, a Se-induced increase in activity might still be viewed as a chemopreventive mechanism. Functions to consider include cell signaling and redox regulation of transcription factors or reactivation of oxidatively inactivated proteins. Se is a component of enzymes in two major redox systems of the cell, namely the glutathione and the thioredoxin systems. Gladyshev *et al*. (69) looked at expression of these 'antioxidant selenoproteins' in three types of cancer models compared with normal cells, by measuring $^{75}$Se-labeled proteins along with other methods. Glutathione peroxidase (GPX1) was decreased while thioredoxin reductase was increased in all cancer models compared with normal control tissues or cell lines. If antioxidant functions of these proteins were somehow the basis for an anticancer activity of Se, it might be expected that they would be expressed in parallel.

## Could the thioredoxin system be a target for Se?

Both thioredoxin and thioredoxin reductase offer interesting possibilities for inhibition by antitumor agents. For thioredoxin, Kirkpatrick *et al*. (68) synthesized a series of unsymmetrical 2-imidazolyl disulfides that cause irreversible inhibition. The mechanism proposed involves thioalkylation of a critical cysteine residue that lies outside the conserved redox catalytic site in thioredoxin, giving the Cys-S-S-R derivative. Apparently these disulfide inhibitors target Cys73, causing slow oxidation to a disulfide form that is not a substrate for reduction by thioredoxin reductase. Thioalkylation at the active site also occurred and was much more rapid, but was reversed because the disulfide bond was reduced by thioredoxin reductase and NADPH to regenerate reduced thioredoxin. For similar reasons, the disulfides also failed to give irreversible inhibition of thioredoxin reductase and glutathione reductase (68). Antitumor activity *in vivo* against human MCF-7 breast cancer xenographs growing in SCID mice was demonstrated for several of the disulfides (67).

In view of the effects of thioalkylation on thioredoxin observed with 2-imidazolyl disulfides (68), it might be asked whether the analogous type 2 selenenylation could occur, forming oxidized Cys-S-Se-R derivatives (Figure 3A) that would undergo slow spontaneous conversion to a homodimer that would be resistant to reduction by thioredoxin reductase (see above). In octapeptides having cysteine replaced by selenocysteine, the redox potential of the S-Se bond is more negative than that of a S-S bond in the native peptide, and therefore harder to reduce (70). The possible inactivation of thioredoxin through formation of a cysteinylselenenylsulfide refractory to reduction is readily tested by experiments with pure proteins *in vitro*, although extrapolation to actual cellular or *in vivo* conditions might be more difficult.

In the case of thioredoxin reductase, formation of selenenylated cysteine derivatives may also occur, although their existence could be transitory because Se compounds are substrates for the enzyme (11,63). However, the presence of selenium as selenocysteine in the protein offers a more interesting possibility, the formation of an inhibitory diselenide that would be highly resistant to reduction (Figure 3B). This 'diselenide trap' type of inhibition of a selenoprotein seems to have no precedent

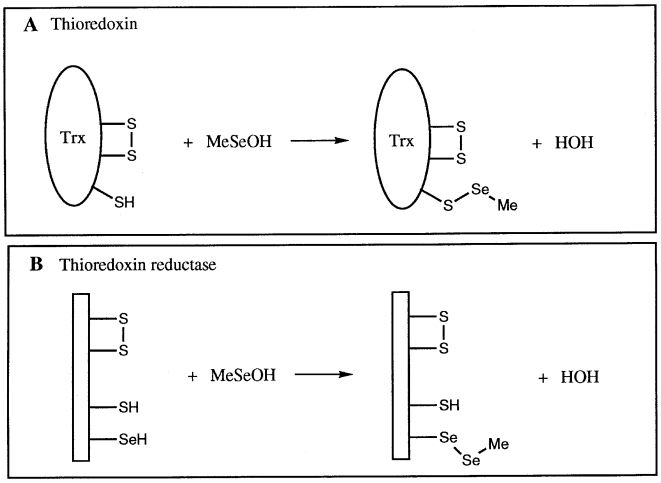


**Fig. 3.** Proposed reactions for the formation of selenium adducts of thioredoxin or thioredoxin reductase. (**A**) An oxidized Se metabolite (methylselenenic acid, MeSeOH, is shown for simplicity, but other forms could also give a similar product) reacts with a non-catalytic site cysteine of thioredoxin to form a selenenylsulfide derivative that cannot be reduced by thioredoxin reductase, analogous to inactivation of thioredoxin by thioalkylation (68). (**B**) An oxidized Se metabolite preferentially reacts with the selenocysteine of mammalian thioredoxin reductase to give a stable diselenide that is inactive and is resistant to reduction by cellular disulfide reductases, allowing accumulation of an inactivated protein having two atoms of Se per molecule. The gradual formation of the inactive diselenide is suggested as a mechanism for declining levels of thioredoxin reductase activity observed over time in animals fed supranutritional levels of Se (see text).

in the literature and therefore is quite speculative. Nevertheless, it provides a means to rationalize several observations regarding the effects of Se on thioredoxin reductase activity. For that reason, evidence supporting stable protein diselenide formation is presented and applications to paradoxical aspects of Se and thioredoxin reductase activity are discussed.

Diselenides are frequently observed in organoselenium chemistry as stable products that arise from more unstable forms of Se. The first clear cut example of a protein having a diselenide bridge formed by oxidation of selenocysteine residues was reported by Muller *et al*. (71) in an *E.coli* cysteine auxotroph grown on selenocysteine in place of cysteine. They obtained 80% replacement of cysteine and observed diselenide (73%) as well as selenenylsulfide (~10%) and disulfide-bridged protein forms by electrospray mass spectrometry. A marked difference between thioredoxin (disulfide) and the diselenide analog in their behavior towards thiol reducing agents was observed. With 1% (0.13 M) 2-mercaptoethanol, reduction of thioredoxin occurred within minutes, whereas the diselenide form was not reduced under these conditions and the mixed selenenylsulfide form was only slightly affected. The diselenide form could be reduced when 2-mercaptoethanol was replaced by an equimolar amount of dithiothreitol (DTT).

Besse *et al*. (70) made more extensive investigations on the redox behavior of peptides containing Se-Se, Se-S or S-S bonds formed from selenocysteine and cysteine residues in a synthetic octapeptide model of glutaredoxin. Their results confirmed the high stability of the diselenide bond toward thiols. Using DTT, they observed that DTT:peptide molar ratios of 0.5, 1.15 and 44 were necessary to obtain 50% reduction of S-S, Se-S and Se-Se- bonds, respectively, after equilibration of the model peptides with the reducing agent at pH 7. The redox potentials of S-Se and Se-Se peptides

were markedly more negative relative to glutathione disulfide (GSSG), whereas the S-S peptide form was slightly more positive. The Se-Se potential was estimated at –381 mV, much lower than DTT (–323 mV), whereas the mixed Se-S bridge was similar to that of DTT. Further work by Pegoraro *et al*. (72) with endothelin derivatives provides additional evidence for the high stability of the diselenide structure under commonly used reducing procedures. Reduction of a disulfide bridge could be accomplished with 1–2 equivalents of DTT, whereas a diselenide linkage in synthetic analogs was unaffected.

These results suggest that a selenocysteine residue in a protein could be inactivated by forming a diselenide bond after reaction with a small Se moiety such as a methylated Se metabolite (Figures 1 and 3B). The stability or resistance of the diselenide bond to cleavage would be expected to vary, depending on such factors as accessibility of the diselenide in the protein and the nature of the second Se moiety. The simple structure of the $CH_3$-Se moiety might escape recognition by protein disulfide reductases that preferentially interact with glutathionyl mixed disulfides. Although the diselenide-inactivating mechanism could be considered for any selenoprotein, only thioredoxin reductase is discussed because of the possible relevance to anticarcinogenic mechanisms of action of Se. It should be emphasized that the diselenide stability observed in well-defined experimental systems *in vitro* requires validation under conditions found *in vivo*; at this time there is no direct evidence that such bonding might occur in cells or in animal tissues, although it might provide a facile explanation of two observations made in these systems: (i) an inverse correlation between $^{75}$Se labeling of a 58 kDa protein band and DNA synthesis; (ii) a decline in thioredoxin reductase activity in animal tissues with continued exposure to Se.

Increased $^{75}$Se labeling of a 58 kDa protein and decreased DNA synthesis (49) could result from reaction of a selenium metabolite with the selenocysteine residue in thioredoxin reductase present in the 58 kDa band, forming a stable, inactive diselenide derivative (PSe-Se-R) by a type 4 mechanism. $^{75}$Se labeling of a selenoprotein could occur in two phases: (i) selenocysteine insertion during polypeptide synthesis; (ii) diselenide formation as a post-translational process. The secondary labeling would increase as the Se level in the cell culture was increased, as a result of increased formation of monomethylated Se, the putative inhibitory Se metabolite. Given the apparent stability of protein diselenides to thiol-reducing agents, Se incorporated in that form might be expected to remain bound to the 58 kDa band during electrophoresis despite treatment with a high level of 2-mercaptoethanol and detergent.

The decline in thioredoxin reductase activity observed with continued exposure to relatively high dietary Se levels (66) could also be explained in terms of biphasic selenocysteine/diselenide incorporation. Sustained exposure of cells to high levels of Se, generating reactive Se intermediates would bring about diselenide formation leading to inhibition of thioredoxin activity over time. Cessation of Se supplementation would diminish metabolite production, allowing an opportunity for removal of the diselenide block and recovery of activity. Testing of this hypothesis would entail measurements of thioredoxin activity by means of appropriate assay procedures in animals exposed to chemopreventive Se levels.

### Se modulation of enzymes *in vivo*

A number of investigators have noted that Se status of animals modulates many enzyme activities besides classic selenoproteins like GSH peroxidase (73). These alterations involve increased activity of some enzymes and decreased activity of others. A series of papers from Wendel's laboratory demonstrated that severe Se deficiency led to reversible and specific Se-dependent enzyme modulations in mouse liver, which could be distinguished from effects on GSH peroxidase activity by differential depletion/repletion kinetics and dose dependency (74–77). Time course studies showed an increase in some activities and decreases in other activities, with a return to control values in all cases upon Se repletion (75). It was emphasized that the loss of GSH peroxidase activity was neither a necessary nor sufficient cause for the profound changes in other enzyme activities (75). It was suggested that a central regulatory mechanism of high amplification involving a Se-dependent mediator might be involved (75); the nature of the mediator was not proposed, but a hormone-mediated mechanism was suggested later in view of interactions of Se with various endocrine glands (76). Since the hydrogen peroxide-metabolizing GSH peroxidase activity did not correlate with the multiple modulations observed by Reiter and Wendel (75), the changes may not have involved a reactive oxygen species of the type often proposed with regard to redox-regulated metabolic control mechanisms. The normalization of enzyme activities observed with Se supplementation may involve effects of Se on redox-regulated transcription factors as discussed earlier.

An example of a selenoenzyme regulating the activity of an enzyme through effects on 'peroxide tone' is seen with 5′-lipoxygenase of leukocytes (77). Dietary Se depletion and repletion in rats combined with measurements of the two GSH peroxidases in isolated leukocytes showed that the phospholipid GSH peroxidase rather than the classic GSH peroxidase was mainly involved in regulating lipoxygenase activity. Interestingly, rats fed a normal diet and dosed with a larger amount of Se by i.p. injection showed a much larger increase in the leukocyte phospholipid GSH peroxidase compared with GSH peroxidase activity.

### New selenoproteins and new biochemical functions

New selenoproteins of unidentified function such as the l5 kDa human selenoprotein found in prostate and other tissues, and expressed at lower levels in some cancers (13), are of great interest. A selenoprotein could have a role in the regulation of signaling pathways through catalysis of thiol/disulfide exchange. Compared with thiol/disulfide interchange reactions, the rate constants observed for analogous reactions of Se compounds are $2.4\times10^5$ higher and such exchanges are $>$10 000 000 times faster when differences in ionization at physiological pH are considered (78). Selenols have been shown to catalyze the interchange reactions of dithiols and disulfides (79,80). Reactivation of oxidatively inactivated transcription factors by thioltransferase and GSH has been demonstrated (81,82) and might be facilitated by Se metabolites, as shown in Figure 3C. A selenoprotein catalyzing thiol/disulfide exchange in the way envisaged for low molecular weight metabolites would combine an appropriate chemistry of Se with the rate-enhancing power of enzymes. An unexplored aspect of Se biochemistry involving the well-established nucleophilic power of selenols might be demethylation of DNA. Epigenetic tumor suppressor gene silencing through methylation of cytosine occurs in many transformed cells. Mammalian cells contain an enzyme that catalyzes demethylation, appar-

ently releasing the methyl group in the form of methanol (83). A possible role for Se in the chemistry of demethylation could involve initiation of the process through nucleophilic attack on a carbon of the cytosine ring or facilitation of the process by serving as a methyl group acceptor. There is some precedent in organoselenium chemistry using selenols in dealkylation reactions (84). An additional role well suited to the chemistry of selenols, already mentioned with regard to caspase activation, is removal of metals from proteins. The ability of Se compounds to release zinc from tightly bound zinc-sulfur clusters in metallothionein has recently been demonstrated and may involve catalysis of redox chemistry as well as thiol/disulfide exchange (85). As noted in this work, the biological chemistry of selenium is neither limited to its reactions with peroxides and GSH nor to the presence of selenocysteine. The potential for Se to release zinc from clusters in zinc fingers of transcription factors or signaling proteins may have importance as a potential chemopreventive mechanism (85).

## Concluding remarks

Summing up what is known about Se and chemopreventive mechanisms, it is likely that low molecular weight Se metabolites are active forms in animal model systems. Evidence is accumulating that monomethylated forms of Se are critical metabolites and this review has focused on the possible chemical mechanisms for their mode of action. Cellular studies implicate Se-induced apoptosis of transformed cells as a likely chemopreventive mechanism. Cell cycle cdk2 or cell signaling protein kinases may be targets of Se metabolites. Some molecular mechanisms of action involving modulation of cysteine residues in proteins by Se are described. In this regard, a novel chemopreventive mechanism is proposed involving Se as a catalyst of the reversible cysteine/disulfide transformations that occur in a number of redox-regulated proteins, including transcription factors, effectively limiting the period of time such proteins are in the activated state. A more provocative concept discussed in this review is that selenoproteins such as thioredoxin reductase could be inactivated by Se as a chemopreventive mechanism through formation of a stable diselenide derivative.

Selenoproteins hold promise for a number of chemopreventive mechanisms, some established, some novel. All of the functions of Se as an essential nutrient seem to involve selenoproteins. Many people feel instinctively that the unique chemistry enabled by the presence of Se in a selenoprotein must be involved somehow in its anticancer effects at near nutritional ranges, if not more generally. Although the classic selenoprotein is GSH peroxidase, it has failed to explain the anticancer effects of Se in the classic experimental system using animal models and supranutritional levels of Se. It remains to be established whether there is a role for the GSH peroxidases at Se levels more within a nutritional range or in less acute processes such as human carcinogenesis. Newly discovered selenoproteins such as mammalian thioredoxin reductase may prove to have a role to play throughout the range of Se-mediated cancer prevention. It would be interesting to know whether or not selenoproteins are obligatory agents of Se anticancer activity. Demonstration of chemopreventive effects with Se compounds that are biologically unavailable as selenoprotein precursors (86) would help answer this somewhat heretical question, at least in the formal sense. A more direct approach to the question would be to determine whether chemopreventive effects of Se are seen in gene knockout models that preclude selenoprotein synthesis.

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## References

1. Ip,C. (1984) Selenium inhibition of chemical carcinogenesis. *Fed. Proc.*, **44**, 2573–2578.
2. Medina,D. and Morrison,D.G. (1988) Current ideas on selenium as a chemopreventive agent. *Pathol. Immunopathol. Res.*, **7**, 187–199.
3. El-Bayoumy,K. (1993) The role of selenium in cancer prevention. In DeVita,V.T.Jr, Hellman,S. and Rosenberg,S.A. (eds) *Cancer: Principles and Practice of Oncology*, 4th Edn. Lippincott, Philadelphia, PA, Vol. 2, pp. 1–15.
4. Combs,G.F.Jr and Gray,W.P. (1998) Chemopreventive agents: selenium. *Pharmacol. Ther.*, **79**, 179–192.
5. Ip,C. (1998) Lessons from basic research in selenium and cancer prevention. *J. Nutr.*, **128**, 1845–1854.
6. Clark,L.C., Combs,G.F.Jr, Turnbull,B.W. *et al*. (1996) Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin: a randomized controlled trial. *J. Am. Med. Assoc.*, **276**, 1957–1963.
7. Ganther,H.E. (1986) Pathways of selenium metabolism including respiratory excretory products. *J. Am. Coll. Toxicol.*, **5**, 1–5.
8. Ganther,H.E. and Lawrence,J.R. (1997) Chemical transformations of selenium in living organisms. Improved forms of selenium for cancer prevention. *Tetrahedron*, **53**, 12229–12310.
9. Ganther,H.E. (1971) Reduction of the selenotrisulfide derivative of glutathione to a persulfide analog by glutathione reductase. *Biochemistry*, **10**, 4089–4098.
10. Hsieh,H.S and Ganther,H.E. (1975) Acid-volatile selenium formation catalyzed by glutathione reductase. *Biochemistry*, **14**, 1632–1636.
11. Bjornstedt,M., Kumar,S. and Holmgren,A. (1992) Selenodiglutathione is a highly efficient oxidant of reduced thioredoxin and a substrate for mammalian thioredoxin reductase. *J. Biol. Chem.*, **267**, 8030–8034.
12. Veres,Z., Kim I.Y., Scholz,T.D. and Stadtman,T.C. (1994) Selenophosphate synthetase. Enzyme properties and catalytic action. *J. Biol. Chem.*, **269**, 10597–10603.
13. Gladyshev,V.N, Jeang,K.-T., Wootton,J.C. and Hatfield,D.L. (1998) A new human selenium-containing protein. *J. Biol. Chem.*, **273**, 8910–8915.
14. Zinoni,F., Birkman,A., Leinfelder,W. and Bock,A. (1987) Cotranslational insertion of selenocysteine into formate dehydrogenase from *Escherichia coli* directed by a UGA codon. *Proc. Natl Acad. Sci. USA*, **84**, 3156–3160.
15. Stadtman,T.C. (1996) Selenocysteine. *Annu. Rev. Biochem.*, **65**, 83–100.
16. Uden,P.C., Bird,S.M., Kotrebai,M., Nolibos,P., Tyson,J.F., Block,E. and Denoyer,E. (1998) Analytical selenoaminoacid studies by chromatography with interfaced atomic mass spectrometry and atomic emission spectral detection. *Fresenius J. Anal. Chem.*, **362**, 447–456.
17. Neuhierl,B. and Bock,A. (1996) On the mechanism of selenium tolerance in selenium-accumulating plants. Purification and characterization of a specific selenocysteine methyltransferase from cultured cells of *Astragulus bisculatus*. *Eur. J. Biochem.*, **239**, 235–238.
18. Ip,C. and Ganther,H. (1990) Activity of methylated forms of selenium in cancer prevention. *Cancer Res.*, **50**, 1206–1211.
19. Ip,C., Hayes,C., Budnick,R.M. and Ganther,H.E. (1991) Chemical forms of selenium, critical metabolites and cancer prevention. *Cancer Res.*, **51**, 595–600.
20. Andreadou,I., Water,B., Commandeur,J.N.M., Worthington,E.A and Vermeulen,N.P.E. (1996) Comparative cytotoxicity of 14 novel selenocysteine Se-conjugates in rat renal proximal tubular cells. *Toxicol. Appl. Pharmacol.*, **141**, 278–287.
21. Wilson,A.C., Thompson,H.J., Schedin,P.J., Gibson,N.W. and Ganther,H.E. (1992) Effect of methylated forms of selenium on cell viability and the induction of DNA strand breakage. *Biochem. Pharmacol.*, **43**, 1137–1141.
22. Lu,J., Jiang,C., Kaeck,M., Ganther,H., Vadhanavikit,S., Ip,C. and Thompson,H. (1995) Dissociation of the genotoxic and growth inhibitory effects of selenium. *Biochem. Pharmacol.*, **50**, 213–219.
23. Kaeck,M., Lu,J., Strange,R., Ip,C., Ganther,H.E. and Thompson,H.J. (1997) Differential induction of growth arrest inducible genes by selenium compounds. *Biochem. Pharmacol.*, **53**, 921–926.
24. Sinha,R., Said,T.K. and Medina,D. (1996) Organic and inorganic selenium

compounds inhibit mouse mammary cell growth *in vitro* by different cellular pathways. *Cancer Lett.*, **107**, 277–284.
25. Sinha,R. and Medina,D. (1997) Inhibition of cdk2 kinase activity by methylselenocysteine in synchronized mouse mammary epithelial tumor cells. *Carcinogenesis*, **18**, 1541–1547.
26. Thompson,H.J, Strange,R. and Schedin,P.J. (1992) Apoptosis in the genesis and prevention of cancer. *Cancer Epidemiol. Biomarkers Prev.*, **1**, 597–602.
27. Lu,J., Kaeck,M., Jiang,C., Wilson,A.C. and Thompson,H.J. (1994) Selenite induction of DNA strand breaks and apoptosis in mouse leukemic L1210 cells. *Biochem. Pharmacol.*, **47**, 1531–1535.
28. Sinha,R., Ganther,H.E. and Medina,D. (1999) Methylseleninic acid, a novel selenium compound inhibits mouse mammary epithelial tumor cells *in vitro*. *Proc. Am. Assoc. Cancer Res.*, **40**, 360.
29. Ganther,H. (1974) Biochemistry of selenium. In Zingaro,R.A and Cooper,W.C. (eds) *Selenium*. Van Nostrand Reinhold, New York, NY, pp. 546–614.
30. Ganther,H. and Corcoran,C. (1969) Selenotrisulfides. II. Cross-linking of reduced pancreatic ribonuclease with selenium. *Biochemistry*, **8**, 2957–2963.
31. Gopalakrishna,R., Gundimeda,U. and Chen,S.-H. (1997) Cancer-preventive selenocompounds induce a specific redox modification of cysteine-rich regions in $Ca^{2+}$-dependent isozymes of protein kinase C. *Arch. Biochem. Biophys.*, **348**, 25–36.
32. Islam,F., Watanabe,Y., Morii,H. and Hayaishi,O. (1991) Inhibition of rat brain prostaglandin D synthase by inorganic selenocompounds. *Arch. Biochem. Biophys.*, **289**, 161–166.
33. Spyrou,G., Bjornstedt,M., Kumar,S. and Holmgren,A. (1995) AP-1 DNA-binding activity is inhibited by selenite and selenodiglutathione. *FEBS Lett.*, **368**, 59–63.
34. Kim,I.Y and Stadtman,T.C. (1997) Inhibition of NF-kB DNA binding and nitric oxide induction in human T cells and lung adenocarcinoma cells by selenite treatment. *Proc. Natl Acad. Sci. USA*, **94**, 12904–12907.
35. Ren,X., Bjornstedt,M., Shen,B., Ericson,M.L. and Holmgren,A. (1993) Mutagenesis of structural half-cystine residues in human thioredoxin and effects on the regulation of activity by selenodiglutathione. *Biochemistry*, **32**, 9701–9708.
36. Lundstrom,J., Krause,G. and Holmgren,A. (1992) A Pro to His mutation in active site of thioredoxin increases its disulfide-isomerase activity 10-fold. New refolding systems for reduced or randomly oxidized ribonuclease. *J. Biol. Chem.*, **267**, 9047–9052.
37. Petronilli,V.P., Costantini,P., Scorrano,L., Colonna,R., Passamonti,S. and Bernardi,P. (1994) The voltage sensor of the mitochondrial permeability transition pore is tuned by the oxidation–reduction state of vicinal thiols. *J. Biol. Chem.*, **269**, 16638–16642.
38. Makropoulos,V., Bruning,T. and Schulze-Osthoff,K. (1996) Selenium-mediated inhibition of transcription factor NF-kB and HIV-1 LTR promoter activity. *Arch. Toxicol.*, **70**, 277–283.
39. Medina,D. and Lane,H.W. (1983) Stage specificity of selenium-mediated inhibition of mouse mammary tumorigenesis. *Biol. Trace Elem. Res.*, **5**, 297–306.
40. Medina,D. (1986) Mechanisms of selenium inhibition of tumorigenesis. *J. Am. Coll. Toxicol.*, **5**, 21–26.
41. Demple,B. (1998) A bridge to control. *Science*, **279**, 1655–1656.
42. Zheng,M., Aslund,F. and Storz,G. (1998) Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science*, **279**, 1718–1721.
43. Clayton,C.C. and Baumann,C.A. (1949) Diet and azo dye tumors: effect of diet during period when the dye is not fed. *Cancer Res.*, **9**, 575–592.
44. Rotruck,J.T., Pope,A.L., Ganther,H.E., Swanson,A.B., Hafeman,D.G. and Hoekstra,W.G. (1973) Selenium: biochemical role as a component of glutathione peroxidase. *Science*, **179**, 588–590.
45. Yang,J.-G., Hill,K.E. and Burk,R.F. (1989) Dietary selenium intake controls rat plasma selenoprotein P concentration. *J. Nutr.*, **119**, 1010–1012.
46. Behne,D., Kyriakoplulos,A., Gessner,H., Walzog,B. and Meinhold,H. (1992) Type I iodothyronine deiodinase activity after high selenium intake and relations between selenium and iodine metabolism in rats. *J. Nutr.*, **122**, 1542–1546.
47. Ip,C. and Ganther,H.E. (1992) Relationship between the chemical form of selenium and anticarcinogenic activity. In Watterberg,L., Lipkin,M., Boone,C.W. and Kelloff,G.J. (eds) *Cancer Chemoprevention*. CRC Press, Boca Raton, FL, pp. 479–488.
48. Danielson,K.G. and Medina,D. (1986) Distribution of selenoproteins in mouse mammary epithelial cells *in vitro* and *in vivo*. *Cancer Res.*, **46**, 4582–4589.
49. Morrison,D.G., Dishart,M.K. and Medina,D. (1988) Intracellular 58-kd selenoprotein levels correlate with inhibition of DNA synthesis in mammary epithelial cells. *Carcinogenesis*, **9**, 1801–1810.
50. Sinha,R., Bansal,M.P., Ganther,H. and Medina,D. (1993) Significance of selenium-labeled proteins for selenium's chemopreventive functions. *Carcinogenesis*, **14**, 1895–1900.
51. Tamura,T. and Stadtman,T.C. (1996) A new selenoprotein from human lung adenocarcinoma cells: purification, properties and thioredoxin reductase activity. *Proc. Natl Acad. Sci. USA*, **93**, 1006–1011.
52. Gromer,S., Schirmer,R.H. and Becker,K. (1997) The 58 kDa mouse selenoprotein is a BCNU-sensitive thioredoxin reductase. *FEBS Lett.*, **412**, 318–320.
53. Morrison,D.G., Berdan,R.C., Pauly,D.F., Turner,D.S., Oborn,C.J. and Medina,D. (1988) Selenium distribution in mammary epithelial cells reveals its possible mechanism of inhibition of cell growth. *Anticancer Res.*, **8**, 51–64.
54. Liu,S.-Y. and Stadtman,T.C. (1997) Heparin-binding properties of selenium-containing thioredoxin reductase from HeLa cells and human lung adenocarcinoma cells. *Proc. Natl Acad. Sci. USA*, **94**, 6138–6141.
55. Nordberg,J., Shong,L., Holmgren,A. and Arner,E.S.J. (1998) Mammalian thioredoxin reductase is irreversibly inhibited by dinitrohalobenzenes by alkylation of both the redox active selenocysteine and its neighboring cysteine residue. *J. Biol. Chem.*, **273**, 10835–10842.
56. Arscott,L.D., Gromer,S., Schirmer,R.H., Becker,K. and Williams,C.H.Jr (1997) The mechanism of thioredoxin reductase from human placenta is similar to the mechanisms of lipoamide dehydrogenase and glutathione reductase and is distinct from the mechanism of thioredoxin reductase from *Escherichia coli*. *Proc. Natl Acad. Sci. USA*, **94**, 3621–3626.
57. Gorlatov,S.N. and Stadtman,T.C. (1998) Human thioredoxin reductase from HeLa cells: selective alkylation of selenocysteine in the protein inhibits enzyme activity and reduction with NADPH influences affinity to heparin. *Proc. Natl Acad. Sci. USA*, **95**, 8520–8525.
58. Zhong,L., Arner,E.S.J., Ljung,J., Aslund,F. and Holmgren,A. (1998) Rat and calf thioredoxin reductase are homologous to glutathione reductase with a carboxy-terminal elongation containing a conserved catalytically active penultimate selenocysteine residue. *J. Biol. Chem.*, **273**, 8581–8591.
59. Gromer,S., Arscott,L.D., Williams,C.H.Jr, Schirmer,R.H. and Becker,K. (1998) Human placenta thioredoxin reductase. Isolation of the selenoenzyme, steady state kinetics and inhibition by therapeutic gold compounds. *J. Biol. Chem.*, **273**, 20096–20101.
60. Gladyshev,V.N., Sun,Q.-A., Wu,Y.L., Zappacosta,F., Jeang,K.-T., Lee,B.J. and Hatfield,D.L. (1999) Redox regulation of cell signaling by selenocysteine in mammalian thioredoxin reductases. *FASEB J.*, **13**, A1579.
61. Hill,K.E., McCollum,G.W. and Burk,R.F. (1997) Determination of thioredoxin reductase activity in rat liver supernatant. *Anal. Biochem.*, **253**, 123–125.
62. Hill,K.E., McCollum,G.W., Boeglin,M.E. and Burk,R.F. (1997) Thioredoxin reductase activity is decreased by selenium deficiency. *Biochem. Biophys Res. Commun.*, **234**, 293–295.
63. Kumar,S., Bjornstedt,M. and Holmgren,A. (1992) Selenite is a substrate for calf thymus thioredoxin reductase and thioredoxin and elicits a large non-stoichiometric oxidation of NADPH in the presence of oxygen. *Eur. J. Biochem.*, **207**, 435–439.
64. Berggren,M., Gallegosm,A., Gasdaskam,J. and Powis,G. (1997) Cellular thioredoxin reductase activity is regulated by selenium. *Anticancer Res.*, **17**, 3377–3380.
65. Gallegos,A., Berggren,M., Gasdaska,J.R. and Powis,G. (1997) Mechanisms of the regulation of thioredoxin reductase activity in cancer cells by the chemopreventive agent selenium. *Cancer Res.*, **57**, 4965–4970.
66. Berggren,M.M., Mangin,J.F., Gasdaska,J.R. and Powis,G. (1998) Effect of selenium on rat thioredoxin reductase activity. *Biochem. Pharmacol.*, **57**, 187–193.
67. Powis,G. (1998) Thioredoxin redox control of cell growth and death and the effects of inhibitors. *Chem. Biol. Interact.*, **111/112**, 23–24.
68. Kirkpatrick,D.L., Kuperus,M., Dowdeswell,M., Potier,N., Donald,L.J., Kunkel,M., Berggren,M., Angulo,M. and Powis,G. (1998) Mechanisms of inhibition of the thioredoxin growth factor system by antitumor 2-imidazolyl disulfides. *Biochem. Pharmacol.*, **55**, 987–994.
69. Gladyshev,V.N., Factor,V.M., Housseau,F. and Hatfield,D.L. (1998) Contrasting patterns of regulation of the antioxidant selenoproteins, thioredoxin reductase and glutathione peroxidase, in cancer cells. *Biochem. Biophys Res. Commun.*, **251**, 488–493.
70. Besse,D., Siedler,F., Dierks,T., Kessler,H. and Moroder,L. (1997) The redox potential of selenocysteine in unconstrained cyclic peptides. *Angew. Chem. Int. Ed. Engl.*, **36**, 883–805.
71. Muller,S., Senn,H., Gsell,B., Vetter,W., Baron,C. and Bock,A. (1994) The formation of diselenide bridges in proteins by incorporation of selenocysteine residues: biosynthesis and characterization of $(Se)_2$-thioredoxin. *Biochemistry*, **33**, 3404–3412.
72. Pegoraro,S., Fiori,S., Rudolph-Bohner,S., Watanabe,T.X. and Moroder,L.

(1998) Isomorphous replacement of cystine with selenocystine in endothelin: oxidative refolding, biological and conformational properties of [$Sec^3$, $Sec^{11}$, $Nle^7$]-endothelin-1. *J. Mol. Biol.*, **284**, 779–792.
73. Burk,R.F. (1983) Biological activity of selenium. *Annu. Rev. Nutr.*, **3**, 53–70.
74. Reiter,R. and Wendel,A. (1983) Selenium and drug metabolism. I. Multiple modulations of mouse liver enzymes. *Biochem. Pharmacol.*, **32**, 3063–3067.
75. Reiter,R. and Wendel,A. (1984) Selenium and drug metabolism. II. Independence of glutathione peroxidase and reversibility of hepatic enzyme modulations in deficient mice. *Biochem. Pharmacol.*, **33**, l923–1928.
76. Wendel,A. and Otter,R. (1987) Alterations in the intermediary metabolism of selenium-deficient mice. *Biochim. Biophys. Acta*, **925**, 94–100.
77. Weitzel,F. and Wendel,A. (1993) Selenoenzymes regulate the activity of leukocyte 5-lipoxygenase via the peroxide tone. *J. Biol. Chem.*, **268**, 6288–6292.
78. Pleasants,J.C., Guo,W. and Rabenstein,D.L. (1989) A comparative study of the kinetics of selenol/diselenide and thiol/disulfide exchange reactions. *J. Am. Chem. Soc.*, **11**, 6553–6557.
79. Singh,R. and Whitesides,G.M. (1991) Selenols catalyze the interchange reactions of dithiols and disulfides in water. *J. Org. Chem.*, **56**, 6931–6933.
80. Singh,R. and Kats,L. (1995) Catalysis of reduction of disulfide by selenol. *Anal. Biochem.*, **232**, 86–91.
81. Bandyopadhyay,S., Starke,D.W., Mieyal,J.J. and Gronostajski,R.M. (1998) Thioltransferase (glutaredoxin) reactivates the DNA-binding activity of oxidation-inactivated nuclear factor I. *J. Biol. Chem.*, **273**, 392–397.
82. Yang,Y., Jao,S.-C., Nanduri,S., Starke,D.W., Mieyal,J.J. and Qin,J. (1998) Reactivity of the human thioltransferase (glutaredoxin) C7S, C25S, C78S, C8S mutant and NMR solution structure of its glutathionyl mixed disulfide intermediate reflect catalytic specificity. *Biochemistry*, **37**, 17145–17156.
83. Bhattacharya,S.K., Ramchandani,S., Cervoni,N. and Szyf,M. (1999) A mammalian protein with specific demethylase activity for mCpG DNA. *Nature*, **397**, 579–583.
84. Reich,H.J. and Cohen,M.L. (1979) Organoselenium chemistry. Dealkylation of amines with benzeneselenol. *J. Org. Chem.*, **44**, 3148–3151.
85. Jacob,C., Maret,W. and Vallee,B.L. (1999) Selenium redox biochemistry of zinc-sulfur coordination sites in proteins and enzymes. *Proc. Natl Acad. Sci. USA*, **96**, 1910–1914.
86. Ip,C., Lisk,D.J. and Ganther,H.E. (1998) Activities of structurally-related lipophilic selenium compounds as cancer chemopreventive agents. *Anticancer Res.*, **18**, 4019–4026.

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