Chapter 40

Soft electrophile inhibition of selenoenzymes in disease pathologies

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List of abbreviations

Cys	cysteine
GPx	glutathione peroxidase
GSH	glutathione
GSSG	oxidized glutathione
Se	selenium
Sec	selenocysteine
Txn(SH)$_2$	reduced thioredoxin
Txn(S-S)	oxidized thioredoxin
TRx	thioredoxin reductase

Introduction

Although most essential trace elements function as cofactors that coordinate with protein ligands to perform their functions,[1] selenium (Se) physiology occurs almost exclusively through the activities of selenocysteine (Sec). A series of biochemical reactions are required to form Sec which is structurally analogous to cysteine (Cys) but is genetically and functionally distinct. Selenocysteine is the 21st DNA encoded amino acid, and the human genome includes 25 genes for selenoproteins which are expressed in tissue-specific occurrence and distributions.[2]

Unlike other amino acids which can be repeatedly used in cycles of protein synthesis, Sec cannot be reused. Although UGA is typically the "opal" stop codon for protein termination, when a specific stem-loop structure in the 3′ untranslated region occurs in coordination with UGA, it codes for Sec insertion. Serine (Ser) is initially aminoacylated onto Sec-tRNA$^{[Ser]Sec}$ by seryl-tRNA synthetase and the aminoacylated serine residue is brought to the ribosome.[3] The selenide released as a result of Sec lyase activity is required for the formation of the high energy selenophosphate produced by selenophosphate synthetase which is itself a selenoprotein.[4] The high energy selenophosphate is used to displace the serine hydroxyl with a Se during de novo synthesis of Sec during its cotranslational insertion into the polypeptide chain.[5]

Although selenoproteins comprise only ~0.1% of the human genome, the high nucleophilic reactivity of Sec's selenolate enables selenoenzymes to catalyze functions that include; preventing/reversing oxidative damage, controlling thyroid hormone status, guiding protein folding, supporting tubulin and actin polymerization, regulating calcium metabolism, Se transport between tissues, and creation of the high energy selenophosphate precursor that is required to synthesize Sec.[6–8] Selenoproteins are expressed in most aerobic life forms and are vitally important in the vertebrate brain, endocrine, and placental tissues.[9] Selenoenzyme functions are required for brain development and physiology[10] and homeostatic mechanisms preferentially transport Se to the brain and the rest of the central nervous system, endocrine organs, and placenta.[11]

Directed Se transport to these tissues occurs through Selenoprotein P and a substantial amount of the body's total Se reservoir cycles through this form each day. While all other selenoproteins have a single Sec, selenoprotein P incorporates 10 Sec residues/molecule. Selective uptake of selenoprotein P by these prioritized tissues is accomplished through the expression of the selenoprotein P receptor (ApoER2) on their cell surfaces.[12] Placental tissues appear to have one or more additional selenoprotein P receptors.[11, 12] Although the brain is protected against Se shortages, deletion of selenoprotein P or ApoER2 genes in mice causes severe Se-deficiencies in brain tissues and oxidative damage in regions of the cerebellum,

Toxicology. https://doi.org/10.1016/B978-0-12-819092-0.00040-6

thalamus, and hippocampus accompanied by impairments in motor functions, sensory abilities, and learning behaviors. Feeding Se-rich diets to these mice can maintain normal brain Se concentrations and support selenoenzyme synthesis at levels sufficient to prevent these consequences.[12, 13]

The Sec obtained from the breakdown of selenoprotein P or endogenous cellular selenoproteins is rapidly degraded by selenocysteine lyase to release the inorganic Se that is required for de novo synthesis of new Sec molecules during each cycle of selenoprotein synthesis.[14] Depending on the form and tissue, selenoprotein half-lives range from minutes to days, but eventually, damage marks them for degradation to their component amino acids. Selenocysteine cannot be reused in subsequent cycles of protein synthesis but must instead be recreated de novo immediately prior to incorporation. Selenocysteine residues are released from dietary as well as endogenous cellular proteins and once Sec lyase releases inorganic Se, the cycle can begin again. Therefore, degradation by Sec lyase is a primary determinant of intracellular availability of Se.[15] Although dietary deficiency may cause somatic tissue Se concentrations to diminish as much as 98%, the uninterrupted supply of Sec to neurological tissues maintains selenoenzyme activities at levels that prevent harm. Only extraordinary circumstances such as genetic knockouts of Selenoprotein P or its receptor protein can disrupt Se homeostasis and induce damaging deficits of selenoenzyme activities in brain, endocrine, or placental tissues.[11,12]

Otherwise, the only means of severely impairing Se supplies and selenoenzyme activities in these tissues is through high exposures to neurotoxic soft electrophiles such as methylmercury (CH_3Hg), cadmium, and certain other metallic and organic electrophiles.[2, 16] The biochemical mechanisms of CH_3Hg toxicity and the beneficial effects of Se and other nutrients obtained from maternal ocean fish consumption on fetal outcomes have been previously reviewed.[2, 16] Therefore, the effects of concomitant exposures to organic and metallic soft electrophiles on development of pathologies in adults are the subject of the current review.

Dietary selenium

Selenium is naturally present in the protein fractions of all foods and occurs in molecular forms that vary in bioavailability for digestive uptake and intracellular incorporation into endogenous cycles of selenoprotein synthesis. Plant Se depends on its availability for uptake from soil or water and the amounts present in livestock reflect the Se contents of the plants they consume. Low Se soils are prevalent in many areas of the world, thus compromising the Se status of food crops, livestock, and populations that eat only locally produced foods. Organ meat, seafood, meat, and grains from regions with abundant soil Se levels are notably rich sources of Se. The American recommended dietary allowance for Se is 55 μg per day for men and women, somewhat less than the United Kingdom reference nutrient intake of 75 μg Se per day for men and lactating women versus 60 μg per day for women.[17] The World Health Organization, Food and Agriculture Organization, and International Atomic Energy Agency (WHO/FAO/IAEA) expert group recommend >40 μg Se per day for men and 30 μg Se per day for women. However, the United Kingdom and other European countries tend to have Se intakes that are ∼half the RNI and in Se-poor regions of China intakes <19 μg Se per day for men and <13 μg Se per day for women are common.

Whole blood Se concentrations observed around the world are often below the level associated with "optimal" blood levels of glutathione peroxidase (GPx), a selenoenzyme commonly measured to assess Se status. Because of centralized food distribution, blood Se contents in the United States are generally rich but can be highly variable due to differences in dietary choices. Direct pathological effects of dietary Se deficiency are rare, even in regions of New Zealand and Finland where intakes are notably low. The molecular forms of Se that predominate in foods are the amino acids selenocysteine (Sec) and selenomethionine (SeMet), a form which is biochemically indistinct from methionine other than being a source of inorganic Se once it is degraded.

Thioenzyme-dependent redox control

Glutathione *S*-transferases (GSTs) are a diverse group of phase II isozymes occurring in cytosolic, mitochondrial, and microsomal compartments that catalyze key reactions in the detoxification of xenobiotic molecules including CH_3Hg.[18] Constituting up to 10% of the cytosolic protein in certain tissues,[19] GSTs catalyze the conjugation of glutathione (GSH)'s sulfhydryl group to the electrophilic centers of substrate molecules to increase their water-solubility.[20] Polymorphic GST variants appear to influence CH_3Hg disposition in humans. Common polymorphisms in the coding region of the human GSTP1, $Ile_{105}Val$ ($GSTP1_{105}$), and $Ala_{114}Val$ ($GSTP1_{114}$) have biochemical effects. The $GSTP1_{105}$ and $GSTP1_{114}$ enzymes are catalytically less active than the wildtype GSTP1wt.[21] Carriers of the $GSTP1_{114}$ allele are more likely to exhibit higher Hg levels as seen in biomarkers of blood or hair.[22, 23] Conflicting results are also reported whereby $GSTP1_{105}$ and $GSTP1_{114}$ carriers showed association with lower Hg retention[24, 25] indicating GSTs may affect

Hg toxicokinetics. This has been supported by epidemiological studies that have shown correlations between predicted GST activities and Hg body burdens.[22, 23, 26, 27] Carriers of the $GSTP1_{114}$ allele was found to be associated with higher erythrocyte Hg contents[22] and with higher hair Hg in individuals with low-level exposures.[23] However, $GSTP1_{105}$ and $GSTP1_{114}$ carriers were associated with lower Hg retention in erythrocytes in a group of individuals with higher levels of ocean fish consumption.[25] Although ocean fish consumption will be accompanied by higher CH_3Hg exposures, ocean fish are among the richest dietary sources of Se.[28] In contrast to the relative stability of selenoenzyme expression in the central nervous system and endocrine organs, somatic tissue expression of selenoenzymes tends to reflect differences in dietary intakes. Thus, there is a need for caution when comparing groups whose ocean fish consumption levels may vary. In contrast to the findings of Gundacker et al.,[23] $GSTP1_{105}$ and $GSTP1_{114}$ has also been reported in association with lower hair Hg in a US dental worker cohort.[24] However, it is important to recognize that the elemental Hg which dental workers are exposed to does not normally distribute into hair, so these GST forms affected by these polymorphisms may interact differently with elemental Hg than with CH_3Hg. In addition to GST's key role in Hg toxicokinetics, it also is important in the metabolism and clearance of xenobiotics.

Glutathione and glutathione peroxidase

Cellular regulation of redox status, the release of oxidizing agents in immune responses to certain pathogens, and prevention or reversal of oxidative damage requires an interlinked network of response elements to preserve dynamic equilibrium. Glutathione is a tripeptide of γ glutamic acid-Cys-glycine which is present at ~5 mM in most cells. It is a key component in the regulation of redox status that acts as a cofactor in certain detoxifying enzymes and limits oxidative stress by scavenging hydroxyl radicals and singlet oxygen while participating in phase 2 metabolic clearance of electrophilic xenobiotics and regenerating vitamin E, vitamin C, and other antioxidants to their active forms. Five of the selenoenzymes expressed by humans are glutathione peroxidases (GPx1–3, 6) that reduce hydrogen peroxide (H_2O_2) while GPx4 reduces lipid peroxides within the cell.

The generalized reactions shown in Eq. (1) reflect the GPx-dependent reduction of lipid peroxides or H_2O_2 to alcohol and water by GSH resulting in the formation of glutathione disulfide (GSSG). Glutathione reductase catalyzes the reduction of GSSG back to 2GSH in the presence of NADPH.[29]

$$\begin{array}{l} \text{R-OOH} + 2\text{GSH} \xrightarrow{\text{Glutathione peroxidase}} \text{R-OH} + \text{H}_2\text{O}) + \text{GSSG} \\ \text{GSSG} + \text{H}^+ + \text{NADPH} \xrightarrow{\text{Glutathione reductase}} 2\text{GSH} + \text{NADP}^+ \end{array} \quad (1)$$

The five GPx selenoenzymes detoxify hydroperoxides using the selenolate (R-Se-) of their active site Sec to acquire a hydroxyl from the hydroperoxide, thus releasing a water molecule, then displaces the hydroxyl with a GSH to release another water molecule. The resulting thiol still bound to Sec is released by interacting with a second GSH which reduces the Sec to selenolate while forming GSSG which is subsequently restored to 2 GSH by glutathione reductase.

The ubiquitous GPx1 is cytosolic and occurs at concentrations that vary depending on nutritional status. GPx1 knockout mice do not show obvious phenotypes but are more vulnerable to pathologies related to increased oxidative stress and virally induced cardiomyopathy. Glutathione peroxidase 2 (GPx2) is present in the liver and gastrointestinal tissues, but not in heart or kidney so it is sometimes referred to gastrointestinal GPx. Genetic knockout mice do not show obvious phenotypes, but GPx1-GPx2 double knockout mice are prone to inflammatory bowel disease, and bacteria-induced tumors. Glutathione peroxidase 3 (GPx3) is the second most abundant selenoprotein in the plasma after selenoprotein P in the plasma and is involved in transporting Se from the digestive tract to the rest of the body. Glutathione peroxidase 4 (GPx4) reduces phospholipid hydroperoxides but exhibits broad substrate specificity and may act as a universal antioxidant in membranes including the nucleus.[30] It is involved in redox signaling and regulatory processes including inhibiting lipoxygenases and apoptosis while preventing oxidation of low-density lipoproteins. GPx4 knockouts are lethal at an early embryonic age, apparently because of disrupted structural compartmentalization. GPx6 is expressed in embryos and olfactory epithelium where it detoxifies H_2O_2.

Cellular GSSG/GSH ratios reflect oxidative balance and maintaining high levels of GSH and a low level of GSSG is a critical index of health. This balance point is maintained by glutathione reductase (GRx) which catalyzes reduction of GSSG to GSH.[31] Glutathione reductase is structurally and functionally related to the three thioredoxin reductase (TRx) selenoenzymes as well as mercuric reductase and a few others that contain a FAD prosthetic group, a NADPH binding domain, and an active site containing a redox-active disulfide bond.[32]

Thioredoxin and thioredoxin reductase

Thioredoxin reductase (TRx) enzymes are the only means of catalyzing reduction of thioredoxin [$Trx(SH)_2$]—a substrate which is itself a unique enzyme. The thioredoxin system consists of TRx acting upon oxidized thioredoxin Trx(S-S) using electrons from NADPH to restore it to the active reduced form $Trx(SH)_2$ which can then perform its role of reducing protein disulfides and other substrates.[33] Thioredoxin-dependent systems maintain the reducing environment required by all living cells and its evolutionary roles are linked to the production of DNA, prevention of oxidative damage due to metabolic by-products of oxygen respiration, and redox signaling using hydrogen peroxide and/or nitric oxide.[33, 34] The primary functions of $Trx(SH)_2$ are reducing oxidized Cys residues and cleaving disulfide bonds, but its thiols are oxidized to Trx(S-S) as a result. Three thioredoxin reductase isozymes: thioredoxin reductase 1 (TRx1, cytosolic), thioredoxin reductase 2 (TRx2, mitochondrial), and thioredoxin reductase 3 (TRx3 which is testis specific) are expressed by humans.[35] Increased $Trx(SH)_2$ decreases cardiac hypertrophy by upregulating transcriptional activity of nuclear respiratory factors 1 and 2 (NRF1 and NRF2) and stimulating peroxisome proliferator-activated receptor γ coactivator 1-α expression.[36, 37]

Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) or free radicals such as hydroxyl radicals or superoxides are normal metabolic products associated with cell signaling pathways, but excessive production of these agents cause oxidative stress and damage.[38] Since peroxides are highly reactive, rapid detoxification is required to prevent the damage they might otherwise cause. Eq. (2) shows the generalized reaction between oxidized protein disulfides and other free radicals being reduced to thiols by the vicinal thiols of thioredoxin ($Trx(SH)_2$) that result in the formation of an intramolecular disulfide (S-S) which is reduced to $(SH)_2$ by TRx in the presence of NADPH.

$$\begin{aligned} &R\text{-}S_2 + Trx\text{-}(SH)_2 \longrightarrow R\text{-}SH_2 + Trx\text{-}(S\text{-}S) \\ &Trx\text{-}(S\text{-}S) + H^+ + NADPH \xrightarrow[\text{Thioredoxin reductase}]{} Trx\text{-}(SH)_2 + NADP^+ \end{aligned} \tag{2}$$

Thioredoxin reductase is named for its first recognized biochemical substrate; oxidized thioredoxin [Trx(S-S)], but the TRx's reduce a broad range of substrates including; hydrogen peroxide, selenite, lipoic acid, ascorbate, ubiquinone, and dietary polyphenols.[39] The functions of TRx are intimately connected with vitamin E since they cooperate in protecting against lipid peroxidation.[40] Once vitamin E has quenched a radical in lipid bilayers, it requires vitamin C (ascorbic acid) to restore it to its active form, thus forming dehydroascorbic acid which is in turn reduced by TRx to the active ascorbic acid form. Since many of TRx's substrates are cellular antioxidants, the activities of TRx are crucial in preserving the intracellular reducing environment required for metabolism. Although less well characterized, selenoprotein F, selenoprotein H, selenoprotein M, selenoprotein O, selenoprotein S, selenoprotein T, selenoprotein V, selenoprotein W, selenoprotein N, and selenoprotein K all appear to have oxidoreductase capabilities (see Table 1) so it is reasonable to expect that they may similarly interact with thiomolecules.

Since the functions of more than half of the selenoenzymes involve detoxification, prevention, and reversal of oxidative damage, disruptions of these pathways may contribute to neurological, endocrine, cardiovascular, and other disease processes.[41, 42] Inherited, acquired, or degenerative disorders that contribute to dysregulation of redox state and increased oxidative damage to tissues may include redisposing genetic differences in selenoenzyme regulation and biosynthetic pathways that affect their vulnerability to soft neurotoxic electrophiles. Progress in this area may reveal novel etiological initiators and pathological pathways, improve diagnosis, enhance pharmaceutical treatments, and improve the prognosis of certain neurological, cardiovascular, and endocrine conditions.

Selenoenzymes and cardiovascular disease

In Se-poor regions of China, New Zealand, and parts of Europe and Russia, people with profoundly low Se intakes tend to develop Keshan disease, a potentially lethal congestive cardiomyopathy characterized by multifocal necrosis, inflammatory infiltrates, and calcification.[43] These pathological consequences appear to be primarily due to Se-deficiency although cardiotoxic Coxsackie B virus contributes to the etiology,[44] possibly as a result of the diminished immunocompetence of Se-deficient hosts.[45] Cardiomyopathy has also been reported in Se-deficient patients receiving total parenteral nutrition with significant inverse correlations between serum Se levels and cardiovascular death in a matched-pair longitudinal study. It has been linked with infections with the Coxsackie B virus that can be lethal, particularly on children and young women.[46] Two Cys residues in histone deacetylase 4 (HDAC4) are reduced by $T(SH)_2$, enabling the oxidized cytosolic form of HDAC4[47] to be imported into the nucleus.[36] Once in the nucleus, reduced HDAC4 downregulates the activity

TABLE 1 Mammalian selenoprotein names, locations, and functions.[a]

Name	Comments, functions, and subcellular locations[b]
Deiodinase 1	Activates/inactivates thyroid hormone (ER)
Deiodinase 2	Provides T_3 during development, activates thyroxine (memb)
Deiodinase 3	Deactivates thyroid hormone, regulating T_3 status in fetus (endo, memb)
GSH peroxidase 1	Detoxifies peroxides: $H_2O_2 + 2\ GSH \rightarrow GSSG + 2H_2O$ (cyto)
GSH peroxidase 2	Detoxifies peroxides: gastrointestinal form (cyto)
GSH peroxidase 3	Secreted into plasma to redistribute Se to/from somatic tissues (plasma)
GSH peroxidase 4	Phospholipid peroxidase: H_2O_2 or fatty acid peroxides (memb, N, cyto)
GSH peroxidase 6	Detoxifies peroxides: loss of function in Huntington's disease (cyto)
Met sulfoxide R reductase	Reduces Met-R-sulfoxides, thus promoting actin polymerization (Nu, cyto)
Selenoprotein F	Oxidoreductase that may assist in disulfide formation and protein folding (ER)
Selenoprotein H	Oxidoreductase that promotes mitochondrial biogenesis (Nu)
Selenoprotein I	Required to synthesize phosphatidylethanolamine (not known)
Selenoprotein K	Oxidoreductase that contributes to T-cell proliferation, regulates calcium (ER)
Selenoprotein M	Oxidoreductase that may participate in disulfide bond formation (ER, Gg)
Selenoprotein N	Oxidoreductase that regulates redox-related calcium homeostasis (ER)
Selenoprotein O	Oxidoreductase; the largest mammalian selenoprotein (mito)
Selenoprotein P	10 Sec/molecule in humans, delivers Se to brain, placenta, etc. (plasma)
Selenoprotein S	Participates in detoxification and may control inflammation (ER)
Selenoprotein T	Oxidoreductase, contributes to Ca^+ release, protects dopaminergic neurons (ER)
Selenoprotein V	Member of the Selenoprotein W family with GPx/TRx activities (not known)
Selenoprotein W	Oxidoreductase that may regulate redox state of 14-3-3 proteins in brain (cyto)
Se phosphate synthetase	Forms high energy selenophosphate precursor required for Sec synthesis (cyto)
Thioredoxin reductase 1	Reduces Trx(S—S), glutaredoxin, etc., actin and tubulin polymerization (cyto)
Thioredoxin reductase 2	Reduces Trx(S—S), glutaredoxin, etc., in mitochondria (mito)
Thioredoxin reductase 3	Reduces Trx(S—S), glutaredoxin, etc., GSSG reductase (N, ER)

[a] *Information regarding functions and subcellular locations are illustrative, rather than exhaustive.*
[b] *Abbreviations:* cyto, *cytoplasm;* ER, *endoplasmic reticulum;* GSH, *glutathione;* Gg, *golgi apparatus;* memb, *cellular membrane;* mito, *mitochondria;* N, *nucleus;* Nu, *nucleolar;* Trx(S-S), *thioredoxin.*

of transcription factors such as NFAT that mediate cardiac hypertrophy.[48] Since $T(SH)_2$ regulates expression of SET and MYND domain-containing protein 1 (SMYD1), a lysine methyltransferase highly expressed in cardiac and other muscle tissues and an important regulator of cardiac development expression, it may be cardioprotective by modulating lysine methylation.[49] Furthermore, $T(SH)_2$ controls micro-RNA levels in the heart and inhibits cardiac hypertrophy by upregulating miR-98/let-7.

Selenoenzymes and neurological disease

Long-term parenteral nutrition has been associated with various signs of Se deficiency including blurred vision, paresthesia in the limbs, dysesthesia and decreased vibration sense in the distal limbs, dysmetria in the limbs, and unstable gait.[50, 51]

These symptoms correspond in several regards with the signs and symptoms associated with methylmercury (CH_3Hg) toxicity, possibly because high exposures to soft electrophiles such as Hg induce conditioned Se-deficiencies.[2] Dietary Se was used to successfully treat a young patient that had been exposed to large amounts of Hg vapor over a period of several weeks.[52] The patient had developed muscular, testicular, and abdominal pain, hypertension, insomnia, delusions, hallucinations, tachycardia, palmar desquamation, diaphoresis, tremor, weight loss (17 kg), and increasingly severe ataxia leading to hospitalization. Examination revealed an elevated blood Hg level of 23 μg/L (~0.11 μM). Chelation with 2,3-dimercaptosuccinic acid (DMSA) was initiated, but the patient's health continued to deteriorate. Dietary Se supplementation with 500 μg Se (~0.1 μmol/kg BW) along with 50 mg of *N*-acetylcysteine per day was initiated to support selenoprotein P, GPx, and GSH synthesis. The patient rapidly improved and by day 11, delusions, delirium, tachycardia, and abdominal pain had resolved and he was released from the hospital but maintained on Se and NAC supplement. After 3 months, all symptoms had resolved except hypertension and after an additional 2 months, he had regained most of the weight that had been lost, hypertension resolved, and he returned to normal activities. Although Se supplementation continued for 8 months, the patient' serum Se levels did not become elevated, suggesting his tissues had a significant Se deficit, possibly due to continued Se sequestration as HgSe that accumulates in cellular lysosomes and exhibits long-term retention, especially following toxic exposures.[53] However, so long as tissue Se availability is sufficient to support brain selenoenzyme activities, high levels of HgSe can accumulate in brain and body tissues without adverse consequences.[54]

Oxidative damage is a focal aspect of the pathologies that accompany Parkinson's disease, senile and drug-induced deafness, schizophrenia, and Alzheimer's.[42, 55] Defective ApoER2 metabolism has been observed in association with neurological disorders including Alzheimer's Disease,[42, 56] antiphospholipid syndrome,[57] major depressive disorder,[58] and selenoprotein P colocalizes with Aβ plaques and neurofibrillary tangles in brains of Alzheimer's disease patients.[59, 60]

Soft electrophiles as selenoenzyme inhibitors

The thiol group of Cys is nucleophilic, meaning its sulfur is an electron donor atom that covalently binds with electrophilic metals such as mercury (Hg), lead (Pb), cadmium (Cd), silver (Ag), or gold (Au). Cysteine residues are abundant in metal-binding proteins (e.g., metallothionein) and peptides (e.g., GSH). Although the physical and chemical properties of oxygen, sulfur, and Se are similar in many respects, reactivity with electrophiles follows the rank order O << S << Se. Selenium is more readily oxidized and kinetically labile, making its biochemical forms more reactive than their sulfur analogues.[7] The pK_a of Cys is ~8.3, meaning it is largely protonated at physiological pH (7.4). In contrast, the pK_a of Sec is ~5.2, indicating it is almost exclusively in the anionic selenolate ($R\text{-}Se^-$) form. The selenolate of Sec is recognized as the most powerful soft nucleophile in the cell.[61] This enables Sec to catalyze reactions that cannot be reproduced by Cys analogues, but the same characteristics that enable it to perform these reactions also make it uniquely vulnerable to binding by a variety of metallic and organic toxicants (Figs. 1 and 2).

In addition to electron-poor metals, organic electrophiles including environmental toxicants (e.g., γ-diketones, quinones, unsaturated aldehydes), industrial pollutants (acrolein, acrylonitrile, methylvinyl ketone), drug metabolites (e.g., acrolein metabolite of cyclophosphamide), dietary contaminants (e.g., acrylamide), and endogenously generated type-2 alkenes (e.g., acrolein, 4-hydroxy-2-nonenal) are known to induce cell damage, quite possibly by interacting with electron-rich nucleophiles.[62, 63] Certain pharmaceuticals employ soft electrophiles such as platinum- and Au-containing compounds to interact with Sec and thus inhibit the thioredoxin system.[64]

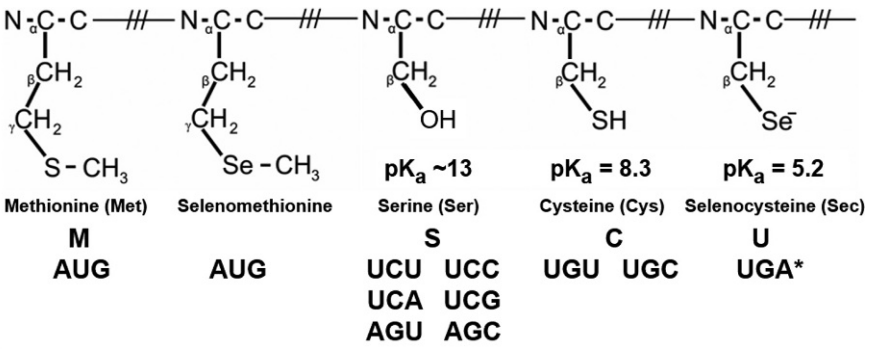

FIG. 1 Comparison of the structures, pK_a's, 3 letter, 1 letter, and genetic codons for the chalcogen amino acids. Other than the release of inorganic Se following degradation of selenomethionine, it is biochemically indistinguishable from methionine. The chalcogens are members of group 16 of the periodic table with amino acid forms that are functionally and biochemically related, but genetically unique.

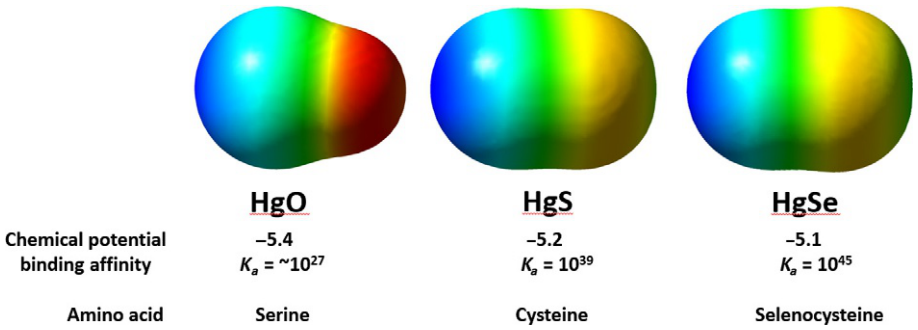

FIG. 2 Depictions of electrostatic potential surfaces of mercury in covalent association with the biologically significant chalcogens, their chemical potentials, and binding affinity constants. The electron cloud depicted in blue indicates a lower e-abundance and a more positive charge, while yellow shading to red indicates an increasingly negative charge. The balance of the HgSe charges stabilizes the molecule, contributing to their remarkably high binding affinities. Images were generated using GaussView software, courtesy of Dr. Alexander Azenkeng, UND.

Once a soft electrophile becomes bound to the thiol of a Cys residue of GSH, $Trx(SH)_2$, or other substrate which directly interacts with the Sec of these selenoenzymes, the thiomolecule serves as a suicide substrate.[2] The selenoenzyme orients the substrate to bring the thiol into close contact with selenolate to accomplish the enzyme reaction, but a thiol which carries a soft electrophile adduct will transfer it to the nucleophilic selenolate. The exchange covalent association to the active site Sec irreversibly inhibits the enzyme. Although CH_3Hg^+ entered the active site linked to a Cys, it will transfer to the Sec of the selenoenzyme and remain bound. Upon degradation of the inactivated enzyme, the CH_3Hg-Sec form is sufficiently stable to persist intact in tissues and may be excreted from the body in that form, but the bound Se is unavailable for participation in Sec synthesis. The rate of selenoenzyme activities in various tissues appears likely to contribute to the transfer of various soft electrophilic metallic or organic electrophiles from thiols to the Sec of selenoenzymes such as GPx, TRx, and/or other oxidoreductase selenoenzymes.

Conclusion

The biochemistry of metallic and organic electrophile interactions with selenoenzymes provide a more comprehensive basis for evaluating risks of dietary and environmental exposures. Concomitant exposures to soft electrophiles are continual throughout life and the aggregate effects of these agents may contribute to the development of degenerative neurological, cardiovascular, and other disease processes. While effects of concomitant exposures to soft electrophiles are additive, exposures to agents that impair selenoenzyme metabolism acting in synchrony with agents that accentuate production of oxidative species would be expected to exert adverse synergies. Improved understanding of the biochemistry and toxicology of organic and metallic soft electrophiles may improve policies intended to protect and improve public health.

Applications to other areas of toxicology

Although acute exposures to poisonous amounts of soft electrophiles will cause brain and endocrine tissues in adults, chronic exposures may instead initiate pathological effects in heart and other somatic tissues. Because the brain and endocrine tissues are preferentially supplied with Se, chronic exposures will be very seldom to impair their selenoenzyme activities. However, such exposures may cause oxidative damage and other forms of harm in tissues that are not preferentially supplied with Se. Such effects would be more likely to occur in exposed populations with low dietary Se intakes.

Most notably, cooperative effects of the Sec-binding capacities of all electrophiles present in the exposed tissues need to be considered in aggregate. Since all soft electrophiles that are present would be contributing, consideration of any individual metallic or organic electrophile would result in mistaken attribution of the shared effect to that single component and erroneous assessment of its relative effects. The toxicokinetics and Se-binding affinities of each individual agent and their various molecular forms will vary. Therefore, each soft electrophile's effects will need to be assessed on a molar basis and additive effects of its exposure to the overall matrix evaluated to distinguish linear versus nonlinear relationships. Careful study will be required to identify potentially synergistic combinations of exposures which may exacerbate clinical consequences in certain populations and subgroups. Improvements in the understanding of the mechanisms of toxicity at the molecular level will enhance interpretation of epidemiological studies and contribute to improved therapeutic interventions in response to environmental, pharmacological, and dietary exposures to metallic and organic soft electrophiles.

Summary points

- Selenium (Se) is a dietarily essential element whose activities occur through its vital role in proteins.
- Selenocysteine (Sec), the 21st proteinogenic amino acid, is expressed in all cells of all human tissues.
- Selenocysteine is required by 25 selenoprotein and selenoenzyme genes expressed by humans.
- The majority of selenoenzymes are responsible for preventing and/or reversing oxidative damage.
- Brain and endocrine tissues are preferentially supplied with Se to preserve selenoenzyme activities.
- Selenocysteine, the most potent intracellular nucleophile readily interacts with soft electrophiles.
- Mercury and other neurotoxic metallic soft electrophiles selectively inhibit selenoenzyme activities.
- Other than genetic ablation, only soft electrophiles can significantly impair brain selenoenzymes.
- High metallic and/or organic soft electrophile exposures may cooperatively inhibit selenoenzymes.
- Soft electrophile toxicokinetics and toxicodynamics will vary in relation to dietary selenium status.

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