# Recent developments in selenium research

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*Accepted: 5 March 2009*

**ABSTRACT**

Some of the main biochemical features of selenium have emerged only in the last five years, although it has been known to be an essential element for nearly 40 years. The investigations into selenoproteome gene expression and a better understanding of the selenocysteine synthetic pathway have undoubtedly provided the evidence that underpins the biochemical roles of the element. To date, 25 selenium-containing proteins have been identified in humans but the functions of a number of these have yet to be elucidated. The roles of the selenium-containing enyzmes (glutathione peroxidases, thioredoxin reductases and iodothyronine deiodinases) are well established, the first two being linked with antioxidant activity, and the latter involved with thyroid hormone metabolism. Recently, the interaction between sulphur, in the same periodic group and therefore chemically similar, and selenium has been investigated in a bid to understand the role of both elements in disease. There is renewed interest in the anticancer properties of selenium-containing compounds as evidence of their effectiveness in animal models has been demonstrated. Herein, selenium metabolism, gene expression, interaction with sulphur, and role in cancer are reviewed.

KEY WORDS: Antioxidants.
Selenium.
Selenoproteins.
Trace elements.

## Introduction

Selenium (Se) is a naturally occurring metalloid that was not identified as an essential element until 1973, having previously been considered to be toxic. It belongs to the same group of the periodic table as oxygen, sulphur, tellurium and polonium.[1] Whereas the essential biological properties of oxygen and sulphur are well known, those for Se, which appears just below sulphur in the periodic group, have emerged only over the past 10 years. Sulphur and Se share similar chemical properties.[2] Furthermore, their metabolic pathways are intricately linked, and interestingly there are Se analogues for every sulphur compound. However, in contrast to sulphur, which is present at approximately 140 g, the amount of Se in a 70 kg man is about 10 mg.[1,3,4]

Diet is the main source of the sulphur-containing amino acids cysteine and methionine and their Se analogues selenocysteine (SeCys) and selenomethionine (SeMet), respectively. Water-soluble inorganic forms (selenate and selenite) are also present in food and drinking water.[1,3] In this review, the most recent aspects of Se metabolism will be covered and the link with sulphur in human health and disease explored with the aim of providing insights into its biochemistry.

## Diverse biological roles of selenium

Selenium exists in the human body mainly as a component of selenoproteins, of which 25 are known to exist in mammals (Table 1).[5] All selenoproteins that have been identified contain the element in the form of SeCys. The roles of these Se-containing proteins are briefly described below.

### *Glutathione peroxidases*

Glutathione peroxidases (GSH-Pxs) provide protection against oxidative damage to certain biomolecules (e.g., lipids and DNA), as well as aiding in the maintenance of membrane integrity by catalysing the reduction of hydrogen peroxide to water, and lipid hydroperoxides to their corresponding alcohol, using glutathione (GSH) as a reducing substrate.[5,7,8] Four types of GSH-Px have been identified.[5] GSH, the storage form of cysteine, is essential for the reduction of harmful free radicals and reactive oxygen species (ROS) by GSH-Px. Consequently, inadequate intake of cysteine from the diet or increased demand due to disease would have an adverse effect on the ability of the body to protect itself against the harmful effects of oxidative stress.[9] Glutathione is oxidised to glutathione disulphide (GSSG) when $H_2O_2$ or ROOH are reduced to water.

### *Cellular glutathione peroxidase*

Cellular glutathione peroxidase (cGSH-Px) is a homotetramer comprising four identical monomer subunits, each containing a single SeCys residue, and has a molecular mass of approximately 80 kDa.[10–12] It occurs in all cells of the body but its functionality is particularly important in red blood cells and also in the liver, which produces ROS as a product of detoxification.[10,11] Studies have shown that cGSH-Px activity can decrease to less than 1% of its control levels without any adverse effects being observed.[13] This finding has led to the suggestion that cGSH-Px may also function as an Se store, with its antioxidant abilities only being exerted when large amounts of hydroperoxides are present.[13]

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*Plasma glutathione peroxidase*

Plasma glutathione peroxidase (pGSH-Px) also has a tetrameric structure containing one SeCys residue per subunit.[10] It is synthesised mainly in the proximal tubular cells of the kidney and then secreted into the extracellular environment.[7,10] Plasma glutathione peroxidase is believed to be involved in the prevention of hydroperoxide transfer, although its exact role is unknown.[11] However, due to the occurrence of low plasma concentrations of GSH, it has been suggested that the enzyme may have a function other than as a GSH-Px.[13]

*Phospholipid hydroperoxide glutathione peroxidase*

Phospholipid hydroperoxide glutathione peroxidase (phGSH-Px) is a monomer with a molecular size of approximately 20 kDa, which is smaller than that of the other GSH-Pxs.[11,14] The role of phGSH-Px is protection against lipid peroxidation by aiding the reduction of esterified fatty acid hydroperoxides to phospholipids.[7] As a secondary function, it helps to prevent oxidation of low-density lipoproteins (LDL), which would otherwise be absorbed into the endothelial cells of the arterial walls and could lead to cardiovascular disease.[7] The key functional difference between phGSH-Px and the other enzymes of the GSH-Px family is its ability to reduce membrane-bound hydroperoxide derivatives of phospholipids without their prior hydrolysis.[8]

*Gastrointestinal glutathione peroxidase*

Gastrointestinal glutathione peroxidase (gGSH-Px) is a tetramer that contains one SeCys residue per subunit, and its properties and structure are very similar to those of cGSH-Px.[10] The enzyme is expressed in the gastrointestinal tract and also in the liver, where it helps to protect against the toxicity posed by ingested lipid hydroperoxides.[7,11] Support for this proposed function comes from work conducted by Aw *et al*.[15] who demonstrated that GSH reduces the amount of hydroperoxides that are transported from the intestinal lumen to lymph.

In addition to their major role as a component of the GSH-Px family, other selenoproteins include iodothyronine deiodinases (types 1, 2 and 3), thioredoxin reductase, selenophosphate synthetase, selenoprotein P (SeP) and the more recently described selenoproteins W (SeW) and N (SeN).[7]

*Thyroid hormone deiodinases*

The three iodothyronine deiodinases (IDIs) are involved in the formation and regulation of triiodothyronine ($T_3$), the

**Table 1.** Mammalian selenoproteins, their genes and their functions.[6]

| Selenoprotein | Chromosomal location in humans | Function |
|---|---|---|
| 15 kDa (Sep15) | 1p22.3 | Protein folding control within the endoplasmic reticulum |
| DI1 | 1p32.3 | Thyroid hormone maturation |
| DI2 | 14q31.1 | Thyroid hormone maturation |
| DI3 | 14q32 | Thyroid hormone catabolism |
| GSH-Px1 | 3p21.31 | Cytoplasmic – free-radical reduction |
| GSH-Px2 | 14q23.3 | Plasmatic – free-radical reduction |
| GSH-Px3 | 5q33.1 | Gastrointestinal – free-radical reduction |
| GSH-Px4 | 19p13.3 | Reduction of oxidised phospholipids and chromatin condensation during spermatogenesis |
| GSH-Px6 | 6p22.1 | Free-radical reduction. Highly expressed within olfactory epithelium |
| H | 11q12.1 | Unknown |
| I | 2p23.3 | Unknown |
| K | 3p21.31 | Unknown |
| M | 22q12.2 | Unknown |
| N | 1p35.36 | Unknown – associated with muscle disease and localised in the endoplasmic reticulum. Possible function as an antioxidant and in protein processing and calcium homeostasis. |
| O | 22q13.33 | Unknown |
| P | 5p12 | Nitrite reductase and plasma selenium transport |
| R | 16p13.3 | Unknown |
| S | 15q26.3 | Unknown – localised in the endoplasmic reticulum |
| SPS2 | 16p11.2 | Selenocysteine metabolism |
| T | 3q24 | Unknown |
| TR1 | 12q23.3 | Cytoplasmic – involved in multiple biosynthetic pathways and regulation mechanisms |
| TR2 | 3q21.2 | Mitochondrial – involved in multiple biosynthetic pathways and regulation mechanisms |
| TR3 | 22q11.21 | Important for sperm maturation |
| V | 19q13.13 | Unknown – highly expressed in testis |
| W | 19q13.32 | Unknown – associated with cardiac calcification. Possible function in redox catalysis and muscle metabolism. |

DI: iodothyronine deiodinase, GSH-Px: glutathione peroxidase, SPS2: selenophosphate synthetase, TR: thioredoxin reductase.

active thyroid hormone.[16] These enzymes act to catalyse 5′-deiodination of thyroxine ($T_4$) to $T_3$.[7,8] Both $T_4$ and $T_3$ are synthesised and secreted by the follicular cells of the thyroid, but $T_4$ is the major secretory product, which is converted to the active form.[10] Type 3 IDI degrades the active hormone by 5′-deiodination of $T_3$ to an inactive form, diiodothyronine ($T_2$).[10] Type 3 IDI is also capable of converting $T_4$ to reverse $T_3$, which is inactive and therefore may inhibit the activity of $T_3$.[7,10] Thyroid hormones are responsible for the stimulation of a number of specific metabolic activities, including lipid and carbohydrate metabolism, in which the levels of thyroid hormone determine the plasma concentration of fatty acids, entry of glucose into cells and the generation of free glucose by gluconeogenesis and glycogenolysis.[16] This involvement of selenoproteins with thyroid hormones suggests that selenium is important for normal growth and metabolism.

***Thioredoxin reductase***

Thioredoxin reductase (TrxR) is a widely distributed dimeric flavoprotein that contains SeCys and FAD, and catalyses the reduction of thioredoxin using electrons from NADPH.[17–19] This particular redox system is important for cell growth and the transformed phenotype of some human cancers, and the activity of this enzyme is decreased during instances of Se deficiency.[20,21] Therefore, low levels of Se, which result in reduced thioredoxin reductase activity, may be associated with an increased risk of developing cancer, due to the decreased ability of cells to undergo apoptosis.[21] The synthesis of this sulphur-containing protein is dependent on the bioavailabilty of methionine and cysteine.

***Selenophosphate synthetase***

Selenophosphate synthetase is an Se-containing enzyme that catalyses the reaction leading to the production of monoselenophosphate, a reactive Se donor, through utilisation of hydrogen selenide and ATP.[22] Selenophosphate is required for the synthesis of selenocysteyl transfer RNA (tRNA), and is therefore essential for the incorporation of SeCys into selenoproteins.[23]

***Selenoprotein P***

Selenoprotein P (SeP) is an extracellular selenoprotein consisting of a single glycosylated polypeptide and is synthesised mainly in the liver, heart and lungs. It is the most abundant selenoprotein in human plasma, accounting for 60–70% of the plasma Se levels. In humans, the total concentration of SeP depends on the overall Se status in the body. Its levels are reduced to 5–10% of its control levels during Se deficiency.[24] Selenoprotein P is unlike all the other selenoproteins characterised thus far in that it has a higher Se content, containing 10 SeCys residues in comparison to only one Se atom per protein subunit in the other selenoproteins.[25] Although its explicit function remains unknown it has been proposed that, like GSH-Px, SeP acts as an antioxidant.[26] Selenoprotein P is also thought to have a role in the transport of selenium to extrahepatic tissues and has been shown to act as a chelating agent for heavy metals such as mercury.[24,26]

***Selenoprotein W***

Selenoprotein W (SeW) is a single polypeptide containing one molecule of Se and is widely distributed throughout body tissues, but particularly concentrated in skeletal muscle.[27] Little is known about the function of SeW, although a role in redox catalysis has been implied through its isolation from muscle tissues with bound GSH.[27] This close association with GSH may be indicative of an antioxidant role for SeW, but also shows the importance of adequate intake in the normal function of skeletal muscle. Further support for the involvement of SeW in muscle metabolism has been offered through its associations with white muscle disease, a degenerative disorder in which skeletal muscle can become calcified due to Se deficiency, the effects of which can be ameliorated through Se supplementation.[7,28]

***Selenoprotein N***

Selenoprotein N (SeN) is a 70 kDa integral membrane glycoprotein with a cytoplasmic N-terminus. As is characteristic of all selenoproteins, SeN contains Se in the form of a SeCys residue at its centre. Selenoprotein N is primarily expressed in skeletal muscle, brain, lung and placenta and has two isoforms. Isoform 1 refers to the full-length transcript, whereas isoform 2 lacks the third exon and is the more prevalent of the two isoforms.[29] Although SeN has been shown to have a catalytic centre similar to that of the thioredoxin reductase family, its function remains unclear. However, experimental results indicate that SeN is localised in the endoplasmic reticulum. This pattern of localisation indicates a role in the processing of proteins and regulation of ER calcium homeostasis, which is of particular importance to normal muscle function.[29] This, coupled with the recent discovery of its presence, along with SeW, in human muscle tissue, serves to cement the important role that it plays in muscle physiology.

## Selenium incorporation into proteins

Selenium can be incorporated into proteins in two ways. First, it can undergo non-specific incorporation as SeMet into abundant proteins (e.g., albumin).[30] Incorporation of SeMet is directed by the same AUG codon as that for methionine, and, as a result, SeMet is incorporated into proteins in place of methionine because tRNAMet cannot discriminate between methionine and SeMet.[3,30] However, this type of incorporation only occurs at high SeMet intake. It is noteworthy that these Se-containing proteins do not contribute to the essential biological functions of the element.[31] Selenium incorporation occurs specifically in the form of SeCys and a UGA codon, which normally functions as a stop codon, encodes for a single SeCys residue per polypeptide, and discrimination has to be made between UGA meaning either SeCys or stop.[31,32] This recognition depends on a secondary RNA structure known as a selenocysteine insertion element (SECIS), which governs the incorporation of SeCys into selenoproteins.[25,33]

## Selenoproteins and gene expression

In the human genome, all the known selenoproteins are encoded and appear to be conserved in other mammals.[34] Selenoprotein N is a newly discovered selenoprotein that consists of 590 amino acid residues encoded by a 4.5 kb transcript and is produced by the human *SEPN*1 gene,

which comprises 13 exons that span 18.5 kb and is localised on chromosome 1 at position p35.36 (Table 1).[29] This position corresponds to the locus associated with rigid spine muscular dystrophy (RSMD), a rare congenital muscular disease in which early onset hypotonia and axial muscle weakness lead to respiratory insuffciency and scoliosis.[6] The *SEPN*1 gene is contained within this locus and makes a good candidate gene for RSMD.[6] Sequencing of *SEPN*1 in patients affected with RSMD identified a number of mutations that provide a connection between SeN and the genetic disorder.[35]

Mutations in the *SEPN*1 gene have also been linked to two other forms of congenital muscular dystrophy, namely the classical form of multiminicore disease (MmD) and desmin-related myopathies (DRMs), which are diseases characterised by accumulation of desmin and other protein aggregates in the cytoplasm of muscle fibres.[6]

These three forms of congenital muscular dystrophy are different but related and form a myopathy class known as selenoprotein N-related myopathies. The mutations that give rise to these diseases are not specific to each pathological form, with several mutations being shared by RSMD, MmD and DRM disorders, an observation which suggests the three are manifestations of the same disease.[6]

The *SEPW*1 gene, which codes for SeW in humans, has been mapped to chromosome 19q13.3 and comprises six exons spanning approximately 6.3 kb. In a study by Bellingham *et al*. human *SEPW*1 was shown to be ubiquitously expressed and found in all 22 tissues tested, with the highest expression occurring in skeletal muscle and heart, and the lowest expression in the liver.[36] In the rat, levels of SeW and SeW mRNA present in tissue are influenced by selenium status.[26,37] Regulation of *SeW* gene expression in muscle cells is of interest due to the relationship between Se and normal muscle function. Investigation of the expression and regulation of *SEPW*1 in an L8 rat skeletal muscle cell line *in vitro* revealed that the maintenance of SeW mRNA levels depends on the presence of Se rather than cell-cycle differentiation, while protein levels of SeW are more sensitive to a change in Se status than are SeW mRNA levels.[38]

This method of regulation, whereby mRNA levels and selenoprotein content are not paralleled, is similar to the other selenoproteins. However, the transcription rate of *SEPW*1 appears to be independent of Se concentration, with the rate of SeW mRNA synthesis in cells cultured in either low Se or Se-supplemented growth media being the same.[38] These data, taken together, suggest that Se has a stabilising effect on SeW mRNA levels but no effect on transcription.

In humans and mice, SeP is encoded by a single gene (*SEPP*1) that comprises five exons.[39] A study conducted by Schomburg *et al*., in which SeP knockout mice (SEPPKO) were generated, has provided support at the DNA level for the involvement of SeP in Se transport.[39] Research has shown that the SEPPKO mice lacked any obvious phenotype at birth, thus revealing that SeP is not an essential selenoprotein for embryonic developement or for basic cellular function. However, during the third week of life, a distinct phenotype, characterised by severe disruption of Se distribution in the organism and followed shortly by fatality, revealed the essential and biological importance of SeP.

Furthermore, Se deficiency in all tissues except the liver was accompanied by decreased activity of GSH-Px, while selenoprotein synthesis within the liver either remained within the normal range (TrxR) or was markedly increased (GSH-Px). This accumulation of Se in hepatic tissues, accompanied by reduced levels in plasma, brain, testis and kidney in SEPPKO mice, indicates that these tissues depend on the delivery of Se by SeP from the liver.

Another newly discovered selenoprotein is the 15-kDa selenoprotein (Sep15), which is highly expressed in prostate, liver, kidney, testis and brain.[40] This selenoprotein is suspected of being involved in cancer aetiology, and attempts at elucidating the role it plays have led to the characterisation of the *Sep*15 gene in humans. This gene spans 51 kb consisting of five exons and four introns and shows localisation to chromosome 1 at position p31, a genetic locus commonly mutated or deleted in human cancers.[40] Supplementation trials in humans suggest that dietary Se can reduce the incidence of cancer and epidemiological data indicates a significant inverse correlation between Se and prostate cancer.[41,42]

Little is known about the mechanism of cancer prevention by Se and thus far no selenoprotein has been implicated in such protection. However, one study has raised the possibility that Sep15 may possess a functional role in the prevention of cancer, consistent with the observation that the level of this protein was significantly reduced in hepatic tumour cells in mice compared with surrounding non-tumourous hepatic tissue.[40] In addition, expression of Sep15 is also shown to be decreased in a mouse prostate cancer cell line compared to a normal prostate cell line, where it is abundant. Therefore, if lower levels of Sep15 predispose to malignant transformation then determining differences in the function of the SECIS element between two naturally occurring alleles could give an indication as to which individuals are at greater risk of cancer, and those who may benefit from Se supplementation.[40]

## Metabolic pathway

### *Sources of selenium*

Although Se is considered a ubiquitous element, it is unevenly distributed across the earth and this is reflected in the variations in Se soil content around the world.[43,44] The Se content of foodstuffs largely depends on the availability of Se in the soil, and levels vary (~10–500 μg/kg).[43,44] The main sources of dietary Se are organs, such as the liver and kidney (0.5–1.9 μg Se/g), with some seafood products (e.g., lobster, shrimp and oysters) containing relatively high levels (0.4–0.7 μg Se/g).[44,45] Meats contribute significantly to dietary Se (~0.2 μg Se/g), albeit not as much as offal and seafood, and some grain and cereal products can also provide a good source of Se.[44,45] Fruit and vegetables generally contain small amounts of Se (<0.01 μg Se/g), although garlic and mushrooms do contain moderate levels (~0.25 μg Se/g and ~0.13 μg Se/g, respectively), and dairy products also tend to be poor sources of Se (~0.07 μg Se/g).[44,45]

### *Absorption and utilisation*

Following ingestion, the primary site of absorption for Se is throughout the duodenum, with very little absorption occurring in the stomach and the last two segments of the

small intestine.[46] Organic Se is well absorbed from the duodenum via the $Na^+$-dependent neutral amino acid transport system.[38] Of the inorganic forms, selenate is thought to be absorbed by an active transport system, while selenite absorption occurs mainly by passive diffusion.[47] Organic Se, particularly SeMet, has an absorption rate of almost 100%, in comparison to a rate of approximately 50% for the inorganic forms, meaning that all ingested SeMet is absorbed by the body.[46,48] Selenomethionine is also retained to a greater extent than inorganic selenate or selenite, and this can be ascribed to the differing incorporation behaviour between SeMet and inorganic Se.[49] Although organic and inorganic Se are subject to different rates of absorption, both forms are nutritionally available to the body.[1] The utilisation of both organic and inorganic Se is via a common intermediate, hydrogen selenide ($H_2Se$), which plays a central role in Se metabolism, utilisation and excretion.[47]

*Inorganic selenium*

The transformation of the inorganic Se species (selenite and selenate) into selenide occurs by means of a simple reduction.[1,3] Selenite ($SeO_3^{2-}$) first undergoes enzymatic reaction with GSH to form the Se-glutathione conjugate selenodiglutathione.[49] In the presence of excess GSH, selenodiglutathione is further reduced to glutathioselenol, which is then subsequently reduced to hydrogen selenide.[3,40,50] However, the reduction process for selenate ($SeO_4^{2-}$) is more complex and its assimilation follows the sulphate reduction pathway. Selenate and sulphate share the same uptake system, demonstrated through their mutual competition.[51] Analogous to sulphur metabolism, selenate is first thought to be activated by ATP sulphurylase to form adenosine phosphoselenate, which is then reduced to selenite before being further reduced to selenide, as described above.[3,38]

The reduction of selenite to selenide takes place in the red blood cells (RBCs), after which it is effluxed into the plasma, bound to albumin and transferred to the liver.[52] Selenate is taken up directly by hepatocytes via the phosphate transport system and transported directly to the liver, where there is partial excretion in urine.[3] Once in the liver, the selenide derived from selenite or selenate is incorporated in selenoproteins or excreted in urine following methylation.[3,52]

*Organic selenium*

The biotransformation of organic Se species to selenide is complex and involves cleavage of the C-Se bond by β-lyase and γ-lyase reactions for SeCys and SeMet, respectively.[3,53] Selenium in the form of SeCys is transformed directly into selenide through the β-lyase reaction (SeCys $HSe^-$), so-called because the cleavage of the C-Se bond occurs at the β-position.[1,3,48] Selenomethionine can also undergo direct conversion into selenide via the γ-lyase reaction, in which cleavage of the C-Se bond occurs at the γ-position, resulting in the formation of methylselenol, which is subsequently demethylated to form selenide ($HSe^-$).[3,52,54] Alternatively, SeMet can be transformed into SeCys via the transselenation pathway, in which SeMet is initially activated by adenosylation, demethylated and converted into SeCys.[55] This is followed by the β-lyase reaction to form selenide (SeMet SeCys $HSe^-$).[3,54]

It has been suggested that the β-lyase reaction is the main route of Se transformation into selenide, with the γ-lyase reaction only operating following excessive Se intake.[55] Organic selenocompounds can also exist as monomeric Se-methylated selenoaminoacids, such as Se-methylselenocysteine (MeSeCys).[56] Structurally, MeSeCys is similar to SeCys and it has been shown to produce methylselenol (monomethylselenide, MMSe) through the β-lyase reaction. Once in this form, it can be demethylated to form the intermediate selenide, which is subsequently used in the synthesis of selenoproteins for utilisation and selenosugar for excretion.[56] Demethylation of MMSe to selenide occurs efficiently, and it has also been suggested that demethylation may occur during times of Se deficiency in order to transform TMSe to selenide.[57]

*Bioavailability*

The bioavailability of dietary Se largely depends on its chemical form. Of the inorganic Se species, selenite is more bioavailable and also approximately 5–10 times more toxic than selenate.[58] Several studies have shown organic Se to be more bioavailable than inorganic Se. Wang and Lovell demonstrated in channel catfish that Se from organic sources is ~300% more available than inorganic Se for growth, and approximately 148% more bioavailable for liver GSH-Px activity.[59] In cattle, organic Se has been shown to increase the blood, milk and liver Se concentrations two to three times more than inorganic Se.[60,61] Organic Se supplemented to cattle as bioavailable SeMet, in the form of Se yeast (Alkosel 1000), resulted in a 130% increase in milk Se content compared to the control, while inorganic Se only gave a 20% increase.[61] More recently, the work of Zhan *et al.* has further demonstrated the greater bioavailability of organic Se, administered as SeMet, in finisher pigs.[62]

*Excretion*

The primary route of excretion for excess Se is through urine following methylation.[3] The major urinary metabolite is a monomethylated compound identified as Se-methyl-*N*-acetylgalactosamine (SeGal), also known as selenosugar.[63] Selenosugar is the reaction product that results from the transfer of GSH-conjugated selenide to the reactive sugar moiety to form the GSH-conjugated selenosugar GS-seleno-*N*-acetylgalactosamine (selenosugar A). Selenosugar A is then methylated to selenosugar $B_1$, which is the form readily excreted in urine.[1,63,64] A further two compounds that are related to selenosugar $B_1$ have been identified and these are known as selenosugar $B_2$ (Se-methyl-*N*-acetylglucosamine (SeGlu)) and selenosugar C (or selenosugar 3), which is a deacylated analogue of SeGal.[64–67]

However, research has shown that these are only minor metabolites, with SeGlu having a urinary concentration of less than 2% of that of SeGal (the major metabolite), and is only detectable after consumption of large amounts of Se.[63] Toxic doses of Se are known to be excreted in the urine as the trimethylselenonium ion (TMSe; $[CH_3]_3Se^+$), and also in the breath as dimethylselenide (DMSe), following successive methylation steps from the intermediate selenide.[64,65] A recent study has shown that the methylation of selenide to MMSe followed by MMSe to DMSe occurs efficiently, whereas the methylation of DMSe to TMSe is less efficient.[57] The metabolic pathways of the different forms of Se are summarised in Figure 1.

## Selenium and homocysteine

Homocysteine is a sulphur-containing amino acid and is a homologue of cysteine.[68] It is an intermediate in the metabolism of methione, and at elevated levels is a risk factor for a number of diseases.[69] Metabolism of homocysteine can follow one of two pathways: remethylation in which homocysteine is converted to methionine, requiring folate and vitamin $B_{12}$; and transsulphuration, where homocysteine is converted to cysteine through the intermediate cystathionine.[68,70] Cysteine formed through transsulphuration can be incorporated into proteins or can be further transformed into γ-glutamylcysteine (the precursor to GSH).[70]

Investigations have shown a significant decrease in plasma homocysteine and an increase in liver and plasma GSH in rats fed Se-deficient diets.[70–72] Uthus *et al.* showed that plasma homocysteine and cysteine concentrations correlate with dietary Se up to 0.05–0.1 μg/g diet.[72] These findings tend to suggest that Se-deficiency influences the metabolism of methionine. An explanation offered for the observed decrease in plasma homocysteine in Se-deficient animals is that more homocysteine is being used to increase the production of GSH via the transsulphuration pathway.[70]

It has been shown that during periods of oxidative stress the rate-limiting enzyme in the biosynthesis of GSH, γ-glutamylcysteine synthetase, is upregulated.[73,74] An increase in the levels of γ-glutamylcysteine synthetase would favour transsulphuration in the direction of GSH production, leading to the reported decreases in plasma homocysteine.[72]

These animal studies demonstrating that Se-deficiency

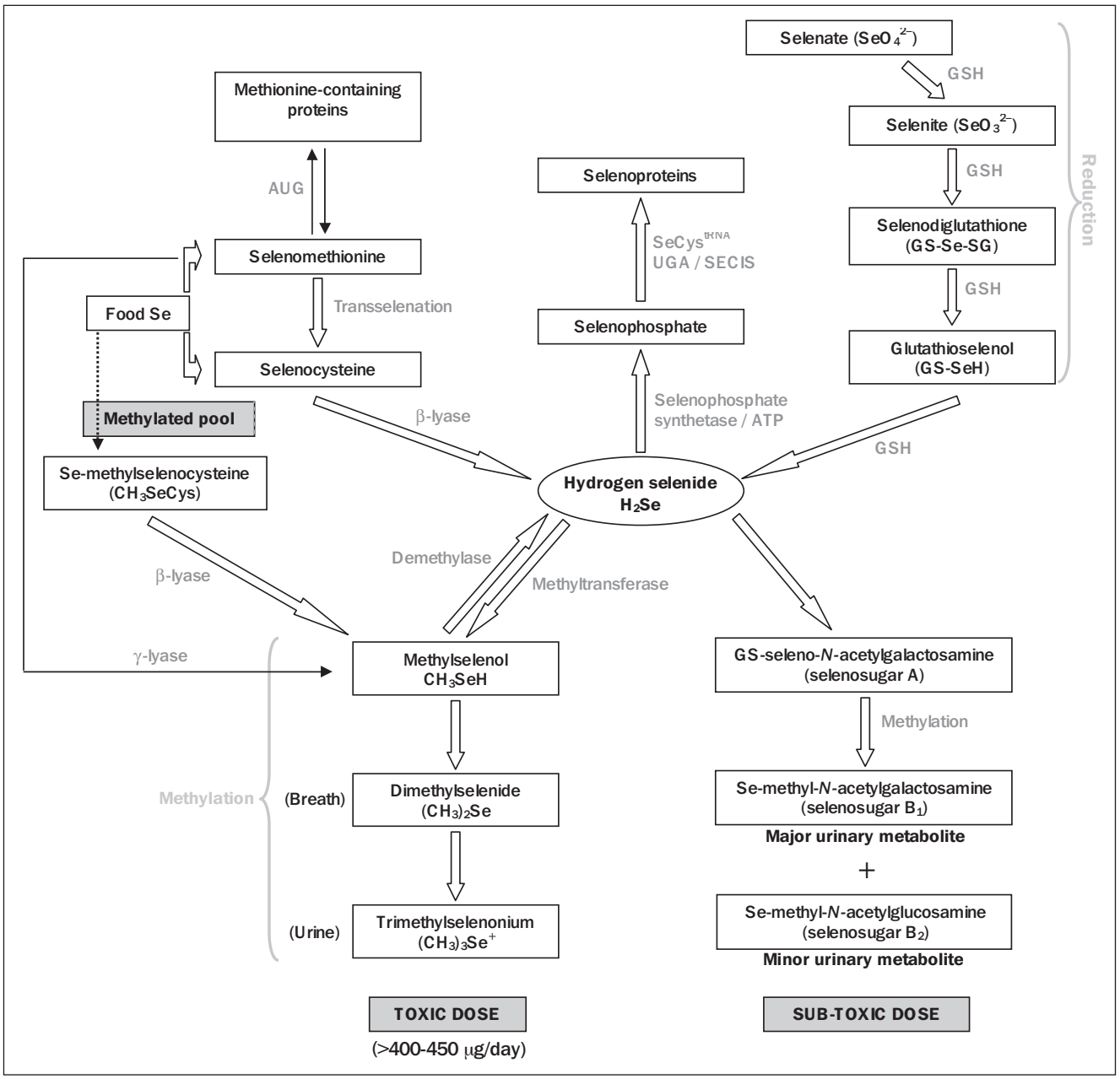


**Fig. 1.** Overview of the metabolic pathways and fates of the different dietary Se species, and showing the central role of hydrogen selenide in selenium metabolism.

results in low plasma total homocysteine, which increases incrementally with supplementation, raises the issue of the relevance of this finding to humans. In humans, elevated levels of homocysteine are a risk factor for a number diseases, while Se deficiency is known to be linked to susceptibility to infectious disease, cardiovascular disease and an increased risk of cancer.[69,75] Research conducted by Venn *et al*.[75] investigated the effect of Se supplementation on plasma homocysteine concentrations in a population with suboptimal Se status, and concluded that Se supplementation, administered at a dose of 200 µg/day over 20 weeks in human subjects, does not influence plasma homocysteine concentration.[75]

## Selenium toxicity

The window between therapeutic and toxic levels of Se is very narrow.[49] It has been suggested that Se toxicity arises as a result of interaction between selenite and GSH, which results in the formation of reactive selenotrisulphides that are capable of producing toxic superoxide ($O_2^-$) and hydrogen peroxide ($H_2O_2$).[76] The recommended daily intake of Se for healthy adults is 55 µg/day, with a minimum requirement of 40 µg/day. Intake of less than 11 µg/day can lead to deficiency.[77] Although consumption of up to 700 µg/day Se has been shown to have no adverse effect, an upper safe limit for daily dietary intake has been set at 400 µg/day.[78,79] Exceeding this upper limit can lead to selenosis, characterised by hair and nail loss and brittleness, gastrointestinal problems, skin rash, garlic breath odour, fatigue and nervous system abnormalities.[80,81]

## Selenium and cancer

During normal cellular metabolism, oxidative processes result in the formation of highly reactive species known as ROS or free radicals. Reactive oxygen species are capable of damaging cellular structures as well as interfering with enzyme functions and denaturing macromolecules.[82] Excessive production of such species creates a state of oxidative stress for cells, resulting in either cell death (apoptosis) or cell repair. Persistent oxidative damage to cells leads to genetic instability, which can result in chronic diseases, including cancer.[79] Antioxidants in the body, such as Se, help to control the levels of ROS by scavenging and deactivating them, and this is implicated in the protection provided by Se against the development of chronic disease.[79,83] Studies have shown that supranutritional supplementation of 200 µg Se/day can provide a chemopreventive effect against several cancers, and particularly prostate cancer.[78–80,84]

The chemopreventive effect of Se is exerted through the inhibition of cell growth and via induction of apoptosis in cells.[78,79,84] The ability of Se to induce apoptosis can be ascribed to the generation of oxidative stress produced by the anticarcinogenic metabolites of Se compounds, (e.g., methylselenol and selenide).[78] Selenium-induced apoptosis is triggered by the direct oxidation of vicinal sulphurdryl groups in cysteine clusters present in the catalytic domains of enzymes such as protein kinase C (PKC), and through the production of methylselenol, which reacts with oxygen to produce ROS.[84] Thus, the role of Se in cancer prevention is as a pro-oxidant rather than as an antioxidant, as had previously been suggested.[84] The chemopreventive properties of sodium selenite and SeMet have been investigated extensively and both have been shown to suppress carcinogenesis in a number of animal models.[84]

From a nutritional perspective, SeMet is more readily available for maintenance of the various Se-dependant systems (e.g., GSH-Px system). However, the same is not true for the anticarcinogenic potencies of the two Se compounds, and it has been shown that SeMet is less active than selenite in inhibiting cancer.[84,85]

Ip and Ganther have proposed that Se compounds, which enter the methylated pool, would be more effective chemopreventive agents than those that are metabolised through the hydrogen selenide pool.[86] Support for this hypothesis is provided through experiments that have shown selenobetaine and Se-methylselenocysteine (compounds that generate monomethylated Se) to be more efficacious as chemopreventive agents compared to selenite and SeMet (which are metabolised to $H_2Se$).[86]

Further interest in the chemopreventive abilities of methylated Se compounds was generated due to the reduced toxicity that results from the methylation of Se, and, as a result, a number of methylated Se compounds have been tested.[87,88] These investigations demonstrate that monomethylated forms of Se are metabolites that can inhibit carcinogenesis, while at the same time lacking some of the toxic effects of inorganic selenite.[86] Moreover, methylated forms of Se (e.g., selenobetaine and Se-methylselenocysteine) act as precursors for the production of methylselenol or methylselenic acid ($CH_3SeOH$) through the action of β- or related lyases.[87]

Thus, Se-methylselenocysteine is capable of providing a source of monomethylated Se from which to maintain the level required to inhibit cell growth. Therefore, a higher dietary intake of Se will lead to increased generation of these critical metabolites, which is why supranutritional levels are required for exertion of anticarcinogenic action.

## Selenium and sulphur

As members of the same group in the periodic table, selenium and sulphur (S) possess similar chemical properties – both share similar atomic size ratios, bond energies, ionisation potentials and electron affinities.[44]

There are two major differences, however, between the two elements. First, Se tends to exist in a reduced form in biological systems, whereas S exists in an oxidised form.[76] Second, the elements also have differing acidic strengths; for example, hydrogen selenide ($H_2Se$) is a stronger acid than hydrogen sulphide ($H_2S$) (pKa of $H_2Se$=3.7; pKa of $H_2S$=6.9). As a result of this higher acidic strength, Se compounds more readily dissociate at physiological pH than do their corresponding S analogues.[76] It is this difference in acidic strength that results in the preferential formation of $H_2Se$ in place of $H_2S$ under physiological conditions, despite the greater abundance of S in the body.

Selenium-containing compounds also tend to be better nucleophiles than their corresponding S-containing compounds.[2] Selenium can be substituted for S in naturally occurring compounds due to the similarity in size between

the radii of $S^{2-}$ (sulphite) and $Se^{2-}$ (selenite) (0.174 nm and 0.191 nm, respectively).[89]

Nutritional interactions exist due to the similarities between the two elements. Selenate directly competes with sulphate for uptake by sulphate membrane transporters and, as previously mentioned, is metabolised by the S assimilation pathway.[89,90] Plant studies demonstrate this metabolic antagonism, with the presence of sulphate inhibiting the uptake of selenate.[89,91] It is possible that S may interfere with the absorption of Se in animals in the same way. This possible effect of sulphate on Se uptake could have practical implications in areas where drinking water is rich in sulphates, or for individuals who have high S content in their diet.

Research by Ivancic and Weiss, who investigated the nutritional relationship between Se and S in ruminants, showed a negative effect of dietary S on the digestibility and absorption of Se.[92] However, S was shown not to affect Se metabolism following absorption in the GI tract. With the chemical similarities between Se- and S-containing compounds and with several organoselenocompounds being shown to possess anticarcinogenic effects, it is important to establish whether this characteristic is specific to Se or that the S compounds are equally effective as chemopreventives. Ip and Ganther showed that Se compounds are far more effective in cancer protection than the corresponding S analogue, with the S compounds requiring 500- to 750-fold higher concentrations to produce comparable tumour suppression.[93]

## In summary

This review focuses on the recent developments in Se research, providing evidence for the essential role of Se in the body and the role of Se as a chemopreventive agent, as well as investigating selenoprotein gene expression and drawing analogies between Se and sulphur.

In addition to the essential role of Se in a number of antioxidant enzyme systems and in regulation of thyroid hormone maturation, investigations have linked Se deficiency with susceptibility to an increased risk of cancer. Moreover, Se deficiency has been shown to result in elevated levels of homocysteine, a risk factor in a number of diseases including cardiovascular disease such as atherosclerosis. This serves to cement the essential role of Se in human health.

Dedication of research into the understanding of Se metabolism has demonstrated the existence of a close relationship between the uptake and assimilation of Se and S. As details of this relationship are elucidated, a better understanding of S metabolism is attained. The preferential uptake and sequestration of Se, despite the greater abundance of S in the body, suggests that there are aspects relating to Se essentiality and toxicity that are still not understood.

The anticarcinogenic properties of Se have long been known; however, it was previously thought to exhibit this function as an antioxidant. In fact, research has shown that Se in cancer prevention acts as a pro-oxidant rather than as an antioxidant, inducing apoptosis through the generation of oxidative stress. Although the organic forms of Se are more bioavailable, selenite is more effective in inhibiting cancer. Evidence for the anticarcinogenic effectiveness of methylated Se compounds is being accumulated, with the methylated forms emerging as the more successful chemopreventive agents compared with other organic and inorganic forms of Se, in part due to their reduced toxicity.

It is noteworthy that the maintenance of selenoprotein mRNA levels depends on the presence of selenium rather than cell-cycle differentiation, while actual selenoprotein levels are more sensitive to changes in Se status. However, the transcription rate of the genes encoding selenoproteins is independent of the levels of the element in the body. Additional work is required to determine the importance of this in relation to the metabolic functions of selenoproteins.

Although there have been significant advances in Se biochemistry, and its importance as an antioxidant in normal cellular metabolism is no longer in doubt, there remain gaps to be filled in our understanding of its role in cell metabolism and in cancer prevention. □

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