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Selenomethionine; a unique antioxidant.

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Abbreviations

CZE:	capillary zone electrophoresis
H_2Se:	hydrogen selenide
HPLC-ICPMS:	high performance liquid chromatography
MetSeO:	methionine selenoxide
MMSE:	monomethylselenol
SeAM	selenium adenosyl methionine
Se-Cys:	selenocysteine
Se-Met:	selenomethionine
SeO_3^{2-}:	selenite
SeO_4^{2-}:	selenate

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Abstract:

The scope of this paper is to review the chemistry, metabolism and antioxidant role of Selenomethionine (Se-Met) *in vivo*. Se-Met is one of the major sources of selenium in organic form. Se-Met can either be metabolized *in vivo* to yield selenocysteine (Se-Cys), or be randomly incorporated in place of methionine for protein storage. Se-Met is found in organs such as pancreas, liver, stomach, kidney, erythrocytes and skeletal muscles, with high concentrations found in the brain, even under severe depletion of Se *in vivo*. The presence of selenium in the form of Se-Met has been observed to provide unique antioxidant activity against peroxynitrite and superoxide. Se-Met has also been shown to protect DNA from damage caused by UV radiation. Se-Met can be easily oxidized to MetSeO by prooxidants such as peroxynitrite. MetSeO is just as easily reduced back to Se-Met form by thiols such as glutathione suggesting that the oxidative damage to Se-Met is reversible. Therefore, Se-Met residues on the surface of proteins are being investigated as potential candidates for a protective role for the active sites of proteins from oxidative damage. More studies are under way to determine the full benefits of Se-Met as an antioxidant *in vivo* and its potential application in cancer preventive treatments.

Introduction:

Selenomethionine (Se-Met) is one the most common forms of selenium-containing compounds that exist in organic form [1]. Selenium (Se) was first discovered in Sweden in 1817 by Berzelius and Gahn [2]. Evidence for its toxicity did not appear until the 1840s [2]. Toxic levels of Se can cause impairment of the central nervous system and/or retarded growth, arthritis, loss of hair and possible death [1]. The recommended daily dose of the USDA for Se (taken mainly as Se-Met) is 50 μg [3]. However, in areas where the levels of Se are high, such as the Midwest, levels of 724 μg of ingested Se have been reported, with no toxicity [4]. For a long time Se was believed to be a carcinogen, but in 1957 evidence was brought forth that Se is a vital element for the sustenance of life [2].

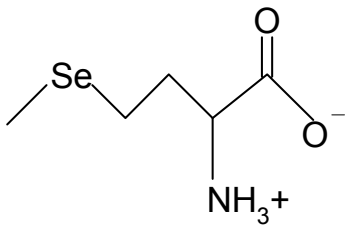
Se is ingested by humans in organic or inorganic forms. Organic forms of Se include selenomethionine (Se-Met) and selenocysteine (Se-Cys) and common inorganic forms are selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) [1]. The most common sources of Se-Met are legumes, soybeans, cereal grains and enriched yeast [2, 4]. Constant ingestion of Se in Se-Met form into the body results in an amplification of Se levels in tissues, however this amplification is not alarming because a steady-state is reached through fast metabolism [4]. This steady state is achieved by the fast metabolism of Se-Met *in vivo*. Once Se-Met is incorporated into the body, it either gets converted to Se-Cys or it gets integrated in proteins replacing methionine, since $tRNA^{Met}$ does not distinguish between methionine or Se-Met [2, 4].

Se uptake *in vivo* is higher for Se-Met than either selenate or selenite. It is thought that the absorption of Se in the body happens through the Na^+-dependent neutral amino acid transport system in the small intestine [4]. Se-Met absorption by brush border membrane vesicles has been observed to be 2.4 nmoles mg^{-1} of protein [2]. Se-Met is present (in varying

concentrations) in organs that have high protein synthesis, such as pancreas, liver, stomach, kidney and skeletal muscles. High levels of Se-Met are also found in the brain (Se concentrations in the cortex and white matter are measured to be about 115-155 and 206-222 ng/g wet wt respectively as determined by graphite furnace absorption spectrometry [5]). High Se levels are retained even under conditions of severe depletion of Se *in vivo*, which points to an important role of Se-Met in the brain [4, 6]. Se-Met is also suspected to be recycled in the body after its average half-life was reported to be 252 d compared to selenite, which was only 102 d [4].

Chemistry:

Selenomethionine ($C_5H_{11}NO_2Se$, molecular weight 196.11 g mol^{-1}) is also known as:



Selenomethionine

butyric acid, 2-amino-4-(methylselenyl); 2-amino-4-(methylselenyl) butyric acid and 2-amino-4 (methylseleno)-butanoic acid[1].

Se-Met is one of two major Se containing amino acids in the body along with Se-Cys. Animals cannot synthesize Se-Met from inorganic forms of Se such as selenate or selenite, thus has to be ingested from plants that produce it. Se-Cys can be synthesized from Se-Met *in vivo* through cystathionase (in rat liver) [7]. Se and sulfur (S) belong to the same group in the periodic table so they are chemically similar. Se and S have similar bonding ability and comparable covalent radii (Se-1.16

Å and S-1.02 Å)[1] and bond energies [7]. Thus, it is believed that they have similar metabolism pathways [7, 8, 9]. Se however, has a lower electronegativity (Pauling values: Se-2.55 and S-2.58)[2] or a higher reduction potential than S (acidic solution: Se = 1.78 eV and S = 0.8 eV)[2], resulting in Se compounds being better *in vivo* electron donors [7, 8]. Se-Met is metabolized in different ways *in vivo* (**Fig.1)**. In bacteria and yeast, Se-Met is converted to Se-adenosyl methionine (SeAM) by the adenosylmethionine synthetase enzymes [7]. This reaction is favored for Se-Met as compared to the methionine analogue. SeAM is also reported to be a better methyl group donor than the sulfur containing analogue [7]. Se-Met is converted into Se-Cys by selenocystathione and selenohomocysteine without the presence of any enzymes. Se-Cys is then degraded to hydrogen selenide (H_2Se) by selenocysteine β-lyase [1, 4]. H_2Se can be harvested for the formation of a specific Se-containing protein or can be degraded further to monomethylselenol (MMSE) and other products.

Figure 1 [1, 4, 10]:

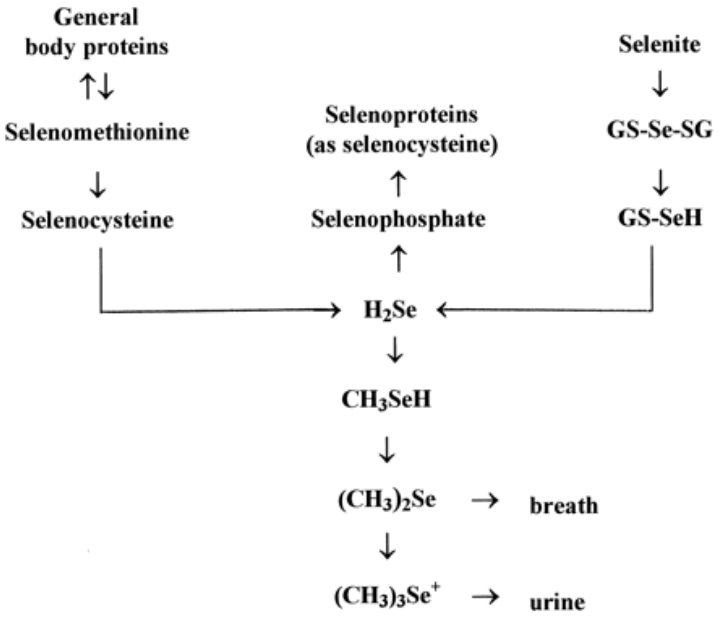


Figure 1. Se-Met can either be incorporated into proteins in place of methionine residues, or it can be metabolized to Se-Cys.
Se-Cys breaks down further to hydrogenselenide and monomethylselenol, which gets excreted as waste. Figure adapted from [10].

[1] From www.webbook.nist.gov accessed on 2/04/05
[2] From http://environmentalchemistry.com/yogi/periodic/Se.html accessed on 2/17/05

MMSE is then excreted or exhaled as waste. Se-Met can also be degraded to MMSE directly by L-methionine γ-lyase (a vitamin B_6 enzyme) in an α-γ-elimination reaction [4, 11]. Other by-products include α-ketobutyrate and NH_3 [4, 11].

More than twenty five selenoproteins have been discovered *in vivo,* which are formed by the metabolism of Se-Met along with Se-Cys [6]. Some common selenoproteins and their functions are listed in **Table 1** [12]:

Table 1. Some selenoproteins and their function (adapted from [12])

Selenoprotein	Known Function
Glutathione peroxidases	Hydroperoxide catabolism
Thioredoxin reductases	Protein thiol redox regulation, vitamin C recycling, DNAsynthesis
Selenoprotein P	Selenium transport

Se-Met has also been reported to react with ferrate ion (in the form of potassium ferrate K_2FeO_4) [13]. The iron in K_2FeO_4 can be in more than one oxidation state. K_2FeO_4 was chosen to simulate Fe activity in biological proteins such as cytochrome P-450 and monooxygenase, which contain Fe in different oxidation states [13]. The overall rate constant for this reaction has been measured to be 5.1 x 10^{13} M^{-2} s^{-1} (measured at a pH range of 8.53-10.13) [13]. This rate constant is approximately 100 times more than that observed for the methionine reduction by ferrate ion (overall rate constant is 1.4 x 10^{11} M^{-2} s^{-1}) [13]. The proposed mechanism is shown in **Reactions 1 and 2** [13]:

$HFeO_4^- \leftrightarrow FeO_4^{2-} + H^+$ **Reaction 1** [13]

$HFeO_4^- + \text{Se-Met} \rightarrow Fe^{IV} + \text{MetSeO}$ **Reaction 2** [13]

Another metabolic pathway for Se-Met has been reported to be its oxidation by peroxynitrite to form methionine selenoxide (MetSeO) according to **Reaction 3** [8, 9, 14, 15, 16]:

$\text{Se-Met} + ONOOH \rightarrow \text{MetSeO} + NO_2^{-1} + H^+$ **Reaction 3** [8]

Reaction 1 is a two-electron reducing process with a second-order rate constant of 20,460 ± 440 M^{-1} s^{-1} at pH 4.6 and 25 ^oC (as compared to the rate constant for its sulfur containing analogue methionine: k = 181 ± 8 M^{-1} s^{-1} at pH 7.4 and 25 ^oC) [8,16]. The enthalpy and entropy of activation at pH 4.6 and 25

^{o}C, for this reaction are measured to be: $\Delta H^{\ddagger} = 2.55 \pm 0.08$ kcal mol^{-1} and $\Delta S^{\ddagger} = -30.5 \pm 0.3$ kcal mol^{-1} K^{-1} making this a favorable reaction both thermodynamically ($\Delta G^{\ddagger} = - 9 \times 10^{3}$ kcal mol^{-1}) and kinetically [8, 16]. It was also found that CO_2 (studies were conducted with 25 mM CO_2) will lower the yield of MetSeO by almost 35% [8]. It is believed that CO_2 reacts with peroxynitrite anion, thus protecting Se-Met from being oxidized by peroxynitrite [8].

The reverse reaction (the formation of Se-Met from MetSeO) is nonenzymatic and it is catalyzed by thiols such as glutathione according to **Reaction 4** [9, 14]:

$$\text{MetSeO} + 2\text{GSH} \rightarrow \text{Se-Met} + \text{GSSG} + H_2O \qquad \textbf{Reaction 4} \qquad [14]$$

This reaction suggests that oxidative damage to Se-Met is reversible [4].

Pulse radiolysis studies have shown that MetSeO is readily reduced by one electron reducing agents such as e_{aq}^-, $CO_2^{\bullet -}$ and $(CH_3)_2C^{\bullet}OH$. A selenium-nitrogen intramolecular positive radical intermediate is formed with an absorption peak at 375 nm and a half life of approximately 70 μs [9]. Second-order rate constants of reactions of MetSeO with these reducing agents are listed in **Table 2** [9]:

Table 2. Second-order rate constants and reduction potentials for the one-electron reduction of MetSeO (adapted from [9])

Reductant	***k* (M^{-1} s^{-1})**	**Reduction Potential (V)**	
e_{aq}^-	1.2×10^{10}	$E^{o'}$ (aq/ e_{aq}^-)	-2.9
$CO_2^{\bullet -}$	5.9×10^{8}	$E^{o'}$ ($CO_2/ CO_2^{\bullet -}$)	-1.8
$(CH_3)_2C^{\bullet}OH$	3.5×10^{7}	$E^{o'}$ (Me_2CO; $H^+/Me_2C^{\bullet}OH$)	-1.5

This mechanism has raised the possibility that Se-Met and other Se-containing compounds play an antioxidant role as free radical scavengers *in vivo* [8, 9, 14].

Studies conducted by Burk *et al.* have excluded Se-Met in the scavenging of peroxynitrite in albumin and immunoglobulin G [15]. Se-Met randomly replaces methionine in proteins and the measured ratio of Se-Met to methionine residues incorporated into a protein is approximately 1 to 8,000 [15]. Even with an increase of Se-Met intake that approaches the upper limit for Se concentration *in vivo* (the maximum safe Se intake, as projected from the Linus Pauling Institute, is

800 μg daily, with 11 μg daily being the lowest dietary intake)[2], the ratio of Se-Met to methionine residues was increased to about 1 to 2800, which is still too small to be significant in quenching peroxynitrite [15]. However, it has been hypothesized, that methionine residues on the surface of proteins act as antioxidants and protect the active site of proteins from being oxidized [17]. It is recognized that Se-Met is more reactive than its analogue methionine, so Se-Met residues that replace the methionine residues on protein surfaces would be even more reactive as radical scavengers, suggesting that Se-Met would provide better protection to active sites of proteins than methionine would [17].

Se-Met has also been observed to have antioxidant properties against the superoxide radical anion ($O_2^{\bullet -}$) [18]. It was discovered that Se-Met and some other Se-containing compounds act as reducing agents, by donating electrons in the dismutation reaction of $O_2^{\bullet -}$ to produce hydrogen peroxide (H_2O_2). Unfortunately they were also found to catalyze the Fenton reaction of H_2O_2 to produce the hydroxyl radical $HO^{\bullet}$ [18]. It is thought that the production of $HO^{\bullet}$ is correlated to Se toxicity, since one of Se toxicity treatments is a metal ion chelating agent [18].

Se-Met has been found to protect DNA against damage caused by UV radiation [3, 4]. Studies have shown that Se-Met triggers a tumor suppressor protein known as p53 and it does so independently of DNA damage. This activation occurs by a redox mechanism that involves Ref1, which is a redox factor [3]. Protein p53 has 10 cysteine residues that can be regulated through redox reactions. After p53 was treated with Se-Met, it was determined that at least two of the ten cysteine residues (Cys-275 and Cys-277) were reduced. Cys-277 is also part of the active site in this protein. Thus Se-Met promotes DNA repair by activating p53 and regulating its redox mechanism [3].

Other studies show however, that low concentrations of Se (released from Se-Met) actually stimulate cancer cell growth, instead of suppressing it. Studies were done with gastric adenocarcinoma cells that were treated with 50 μM Se-Met. It was found that Se-Met actually behaved as a pro-oxidant

[2] From Linus Pauling Institute web page: http://lpi.oregonstate.edu/new/response.html accessed on 2/22/05

by causing slight oxidative stress and triggering DNA replication [19]. Studies done in adipocytes showed that MAPK can be activated by Se which also promoted tyrosyl phosphorylation. Since Se in low concentrations is associated with the stimulation and activation of these processes, it is indirectly responsible for the activation of growth signals in cells [19]. These findings support previous studies that correlate higher cancer rates to regions with low Se concentrations [19].

Detection:

The most common method used for the detection of Se-Met and Se-containing species has been separations through chromatography. Some chromatographic techniques include: high voltage paper electrophoresis, thin layer chromatography, paper chromatography and chromatography on ion exchange resins [2]. These techniques however, due to their low sensitivity and separation problems, are being replaced with more advanced analytical techniques [20]. Some more advanced techniques include: high performance liquid chromatography with inductively coupled plasma-mass spectrometry (HPLC-ICPMS), or capillary zone electrophoresis (CZE) [2, 20]. These techniques provide ample sensitivity and selectivity in determining Se content in biological compounds. Detection limits of HPLC-ICPMS have been reported in the range of ng ml^{-1} [20]. Another advantage of this technique comes from its ability to discriminate between endogenous and exterior Se without the use of radioactive isotopes [20]. The advantage of CZE is the ability to distinguish the purity of the compound and also the analysis of labile species [20]. However these techniques do not provide structural information on unknown compounds of Se. Novel analytical techniques are continuously being developed, because lower detection limits are needed to improve Se determination *in vivo*.

Conclusion:

Se-Met is the main organic form of Se and it is easily metabolized into other forms of Se or it is incorporated into proteins in place of methionine. Se-Met has been observed to have antioxidant properties against peroxynitrite and superoxide anion. Se-Met has also been shown to protect DNA

from damage done by UV radiation. More studies are under way, but the possibilities of therapeutical applications of Se-Met are very promising.

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