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Overview of the biochemistry and biology of selenoneine

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Abstract

Selenoneine (SEN) is one of the major organic selenium (Se) species present in fish and was initially identified in the blood of bluefin tuna. SEN is a selenium analog of ergothioneine (EGT), which is well known as a radical scavenger, with SEN exhibiting greater radical scavenging capacity than EGT. SEN is expected to have beneficial health functions due to its radical scavenging capacity, and elucidation of its biochemical and physiological functions *in vivo* may reveal additionally unknown functions. Herein, we systematically review previous SEN studies and comprehensively discuss SEN concentrations observed in various organisms including humans. Moreover, we describe the chemical, biochemical and biological properties of SEN. The current limitations of the research on SEN are shown to indicate the future studies required on understanding SEN.

Keywords: selenoneine, ergothioneine, Se-methylselenoneine, OCTN1, erythrocyte, melanin, NAFLD
Statements about COI: The authors declare no conflict of interest associated with this manuscript.

Abbreviations:

SEN; selenoneine
MeSEN; Se-methylselenoneine
GPx; glutathione peroxidase
OCTN1; organic cation/carnitine transporter-1
EGT; ergothioneine
SEN-seleninic acid; selenoneine seleninic acid
SelP; selenoprotein P
NAFLD; non-alcoholic fatty liver disease

1. Introduction

Selenium (Se) is an essential trace element in animals, including humans, and plays an important role in the body's redox system. Most animals consume Se in their diet as selenocysteine (SeCys) or selenomethionine (SeMet). In 2010, Yamashita and Yamashita found a novel organic selenium compound in the erythrocytes of bluefin tuna and identified it as selenoneine (SEN, **Fig. 1a, b**) [1]. Even before the identification of SEN, tunas were known to contain high levels of Se. Yamashita and Yamashita found that the blood and dark muscle of bluefin tuna contained high levels of Se, and in 1993 they started purifying the Se compound, which was thought to be an unknown Se species. They separated an extract from the dark muscle of tuna using gel filtration chromatography and the Se compound was not a protein containing Se

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Received: December 05, 2024
Accepted: January 19, 2025
Released online: February 28, 2025

 DOI https://doi.org/10.11299/metallomicsresearch.MR202414

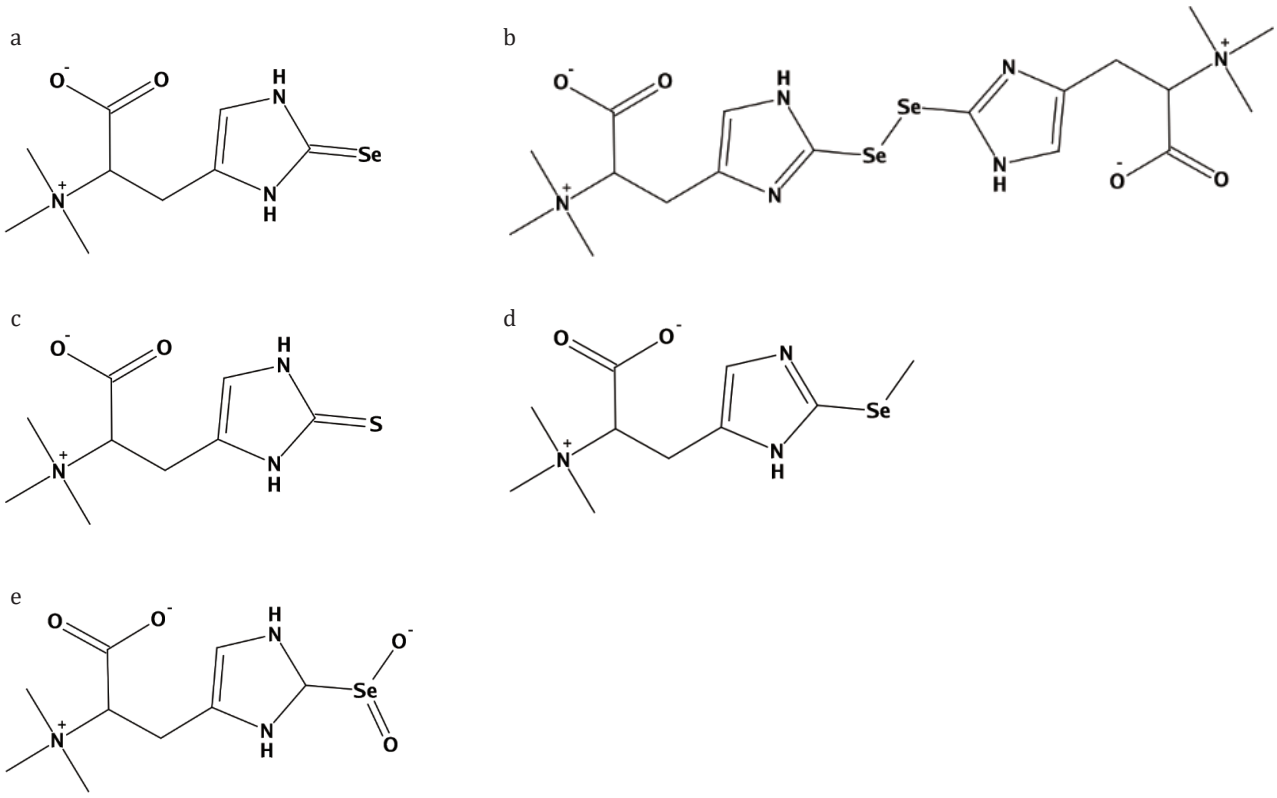

Fig. 1 Selenoneine (SEN) and its derivatives are exhibited;
SEN monomer (a), SEN dimer (b), ergothioneine (EGT) (c), Se-methylselenoneine (MeSEN) (d) and selenoneine seleninic acid (SEN-seleninic acid) (e).

but a low molecular weight compound below 1000 Da. They purified the low molecular weight compound from the blood of bluefin tuna, and they identified it as *m/z* 553 and containing two Se atoms using high resolution mass spectrometer (HRMS) [1]. Its structure was determined using nuclear magnetic resonance (NMR) and they named it as "selenoneine" [1] because it is a selenium analogue of ergothioneine (EGT, **Fig. 1c**) that is known as a radical scavenger in fungi [2].

SEN has a strong radical scavenging activity [1]. SEN is one of the major forms of Se in the dietary intake of humans, especially in those who frequently eat fish, and it is predicted to contribute to redox systems and health maintenance. Since the identification of SEN, many studies aiming to elucidate the functions of SEN have been published in diverse disciplines, including chemistry, biochemistry, biology, nutrition and environmental studies. Moreover, analogous pairs of SEN have been identified, such as Se-methylselenonein (MeSEN, **Fig. 1d**) and selenoneine seleninic acid (SEN-seleninic acid, **Fig. 1e**), and the relationship between their metabolism and function has been actively studied. Herein, we systematically review previous SEN studies across a wide range of disciplines and outline the current status and future prospects of SEN research.

2. Selenoneine contents in organisms

The first report of SEN indicated that it was present in the blood of bluefin tuna, with detectable concentrations also present the blood of other types of fish [1]. SEN is more abundant in the blood of bluefin tuna (430 ± 82 μmol Se/kg, **Table 1**) and Pacific mackerel (437 ± 159 μmol Se/kg, **Table 1**) than in that of wild bluefin tuna (83 ± 29 μmol Se/kg, **Table 1**) [1]. SEN is also detected in the blood of freshwater fish, Tilapia, at 0.9 ± 0.6 μmol Se/kg (**Table 1**) [1]. Notably, SEN is present in the muscles of fishes, with SEN contents of ordinary and dark muscle of bluefin tuna containing 2.4 ± 0.3 μmol Se/kg and 190 ± 8.0 μmol Se/kg, respectively (**Table 1**) [1]. Other types

Table 1. | SEN concentrations in tissues of organisms

Species	Tissues	Concentrations per wet weight SEN μmol Se/kg (mg Se/kg)	Total Se μmol/kg (mg/kg)	Reference
Pacific bluefin tuna (Farm-raised) *Thunnus orientalis*	Whole blood	430 ± 82 (34 ± 7)		[1]
	Spleen	41 ± 16 (3.2 ± 1.3)	266 ± 37 (21 ± 3)	[1], [4]
	Hepatopancreas	39 ± 7.5 (3.1 ± 0.6)	165 ± 38 (13 ± 3)	
	Heart	15 ± 15 (1.2 ± 1.2)	127 ± 13 (10 ± 1)	
	Dark muscle	190 ± 8 (15 ± 1)	215 ± 14 (17 ± 1)	
	Ordinary muscle	2.4 ± 0.3 (0.19 ±0.02)	7.6 ± 1.1 (0.60 ± 0.09)	
Pacific bluefin tuna (Wild) *Thunnus orientalis*	Whole blood	83 ± 29 (6.6 ± 2.3)	152 ± 39 (12 ± 3)	[4]
Yellowfin tuna *Thunnus albacores*	Ordinary muscle	1.6 ± 0.5 (0.13 ± 0.04)	9.4 ± 3.9 (0.74 ± 0.31)	[3]
Albacore *Thunnus alalunga*	Ordinary muscle	1.7 ± 0.3 (0.13 ± 0.03)	19 ± 8 (1.5 ± 0.7)	[3]
Bigeye tuna *Thunnus obesus*	Ordinary muscle	2.6 ± 1.8 (0.2 ± 0.1)	14 ± 7 (1.1 ± 0.5)	[3]
Skipjack *Euthynnus pelamis*	Ordinary muscle	1.0 ± 0.6 (0.05 ± 0.04)	6.2 ± 3.4 (0.49 ± 0.27)	[3]
Swordfish *Xiphias gladius*	Ordinary muscle	2.8 ± 0.6 (0.22 ± 0.05)	6.6 ± 1.5 (0.52 ± 0.12)	[3]
Pacific mackerel *Scomber japonicus*	Whole blood	437 ± 159 (35 ± 13)		[1]
	Ordinary muscle	0.6 ± 0.2 (0.04 ± 0.01)	3.4 ± 0.6 (0.27 ± 0.05)	[3]
Blue mackerel *Scomber australasicus*	Whole Muscle	2.5 ± 0.9 (0.20 ± 0.07)	6.8 ± 1.5 (0.54 ± 0.12)	[4]
Japanese Anchovy *Engraulis japonica*	Ordinary muscle	ND	3.1 ± 1.8 (0.25 ± 0.14)	[3]
Pacific sardine *Sardinops melanostictus*	Ordinary muscle	1.4 ± 0.6 (0.11 ± 0.04)	4.1 ± 0.9 (0.32 ± 0.07)	[3]
Pacific saury *Cololabis saira*	Ordinary muscle	ND	2.6 ± 0.4 (0.21 ± 0.03)	[3]
	Whole Muscle	0.13 ± 0.13 (0.01 ± 0.01)	2.5 ± 0.3 (0.20 ± 0.03)	[4]
Horse mackerel *Trachurus japonicus*	Ordinary muscle	0.5 ± 0.2 (0.04 ± 0.02)	5.0 ± 1.4 (0.40 ± 0.11)	[3]
Japanese barracuda *Sphyraena japonica*	Ordinary muscle	0.1 ± 0.1 (0.008 ± 0.008)	1.8 ± 0.2 (0.14 ± 0.02)	[3]
Japanese conger *Conger myriaster*	Ordinary muscle	ND	2.5 ± 1.1 (0.20 ± 0.09)	[3]
Greeneye *Chlorophthalmus albatrossis*	Ordinary muscle	1.4 ± 0.5 (0.11 ± 0.04)	5.3 ± 0.6 (0.42 ± 0.05)	[3]
Alfonsino *Beryx splendens*	Ordinary muscle	1.3 ± 0.5 (0.10 ± 0.04)	18 ± 13 (1.4 ± 1.0)	[3]
Red sea bream *Pagrus major*	Ordinary muscle	0.4 ± 0.1 (0.03 ± 0.01)	3.7 ± 0.3 (0.29 ± 0.02)	[3]
White croaker *Pennahia argentata*	Ordinary muscle	ND	3.6 ± 1.0 (0.28 ± 0.08)	[3]
Marbled sole *Pleuronectes yokohamae*	Ordinary muscle	ND	1.4 ± 0.0 (0.11 ± 0.00)	[3]
Chum salmon *Oncorhynchus keta*	Ordinary muscle	ND	3.9 ± 0.3 (0.30 ± 0.02)	[3]

Species	Tissues	Concentrations per wet weight SEN μmol Se/kg (mg Se/kg)	Total Se μmol/kg (mg/kg)	Reference
Pacific cod *Gadus macrocephalus*	Muscle	ND	2.9 ± 0.5 (0.23 ± 0.04)	[4]
Slime flounder *MIicrostomus achne*	Muscle	2.3 ± 0.1 (0.18 ± 0.01)	2.3 ± 2.3 (0.18 ± 0.18)	[4]
Tilapia *Oreochromis niloticus*	Whole blood	0.9 ± 0.6 (0.07 ± 0.05)		[1]
Japanese common squid *Todarodes pacificus*	Hepatopancreas	9.3 ± 3.5 (0.73 ± 0.28)		[1]
	Muscle	0.13 ± 0.00 (0.01 ± 0.00)	2.7 ± 0.1 (0.21 ± 0.01)	[4]
Chicken *Gallus gallus domesticus*	Liver	0.3 ± 0.1 (0.02 ± 0.01)		[1]
	Heart	ND		
	Gizzard	ND		
Giant petrel *Macronectes*	Liver	Range: 165–659 (13–52) per dry weight	Range: 241–5395 (19–426) per dry weight	[5]
	Kidney	Range: 329–1114 (26–88) per dry weight	Range: 519–1659 (41–131) per dry weight	
	Muscle	Range: 25.3–317 (2–25) per dry weight	Range: 51–393 (4–31) per dry weight	
	Brain	Range: 63–443 (5–35) per dry weight	Range: 76–507 (6–40) per dry weight	
	Whole blood	Range: 15–304 (1.2–24)	Range: 38–342 (3–27) per dry weight	
Pig *Susscrofa domesticus*	Liver	ND		[1]
	Kidney	0.36 ± 0.1 (0.03 ± 0.01)		
Minke Whale *Balaenoptera acutorostrata*	Muscle	0.63 ± 0.63 (0.050 ± 0.050)	5.1 ± 1.3 (0.40 ± 0.10)	[4]
	Whole blood	1.6 ± 0.3 (0.12 ± 0.02)	11 ± 1.9 (0.88 ± 0.15)	
Striped Dolphin *Stenella coeruleoalba*	Blood cells	56 ± 14 (4.4 ± 1.1)	57 ± 13 (4.6 ± 1.0)	[4]
	Plasma	0.060 ± 0.100 (0.0050 ± 0.0080)	9.6 ± 1.9 (0.76 ± 0.15)	
	Liver	4.8 ± 1.1 (0.38 ± 0.09)	320 ± 330 (26 ± 26)	
	Kidney	35 ± 1 (2.8 ± 1.1)	95 ± 16 (7.5 ± 1.2)	
Risso's Dolphin *Grampus griseus*	Blood cells	47 ± 7 (3.7 ± 0.6)	57 ± 6 (4.5 ± 0.5)	[4]
	Plasma	0.56 ± 0.05 (0.044 ± 0.041)	7.6 ± 1.1 (0.60 ± 0.09)	
	Liver	5.1 ± 2.8 (0.40 ± 0.22)	290 ± 120 (23 ± 9)	
	Kidney	25 ± 10 (2.0 ± 0.8)	61 ± 16 (4.9 ± 1.2)	
Pantropical Spotted Dolphin *Stenella attenuata*	Blood cells	35 ± 15 (2.8 ± 1.2)	37 ± 10 (2.9 ± 0.8)	[4]
	Plasma	1.2 ± 0.4 (0.091 ± 0.031)	13 ± 1 (1.0 ± 0.1)	
	Liver	2.8 ± 1.0 (0.22 ± 0.08)	390 ± 260 (31 ± 21)	
	Kidney	24 ± 8 (1.9 ± 0.6)	95 ± 15 (7.5 ± 1.2)	
Beluga mattaaq *Delphinapterus leucas*	Skin	Median; 23 (1.8) Range; 15 – 94 (1.2 – 7.4)		[19]

Values are mean ± SD. ND < 0.051 μmol Se/kg (0.004 mg Se/kg)

of edible fishes have SEN in their ordinary muscle and their contents are presented in **Table 1** [1,3,4]. Moreover, SEN is present in the spleen (41 ± 16 μmol Se/kg, **Table 1**), hepatopancreas (39 ± 7.5 μmol Se/kg, **Table 1**) and heart (15 ± 15 μmol Se/kg, **Table 1**) [1]. These results indicate that SEN is present more specifically in blood and dark muscle compared to other organs and ordinary muscles. Since SEN is also present at lower levels in ordinary muscle, humans are assumed to routinely consume SEN from eating fish.

Terrestrial organisms are also known to contain SEN. The first report on SEN found that is detected in the liver of chickens (0.3 ± 0.1 μmol Se/kg, **Table 1**) and the kidney of pigs (0.36 ± 0.1 μmol Se/kg, **Table 1**) [1]. Recently, SEN was reported to be present in seabirds, including giant petrels (*Macronectes sp.*) [5]. SEN was detected in the blood and internal tissues, with the highest concentrations found in the kidneys (329–1114 μmol Se/kg in dry weight, **Table 1**) [5]. The study also reports that SEN represents between 78–88% of the total Se in the brain and suggests that it plays a crucial role in the nervous system in the giant petrels [5]. The SEN found in the terrestrial organisms is thought to be derived from their diet because fishmeal is used to some extent in feeds for chickens and pigs, and seabirds consume fish in the ocean. Moreover, an edible mushroom, *Boletus edulis*, is reported to contain SEN at significant levels [6]. More than 80% of the total Se in the mushroom is present as SEN, which reached 1 mg Se/kg in wet weight [6]. This suggests that *Boletus edulis* might also represent a natural supply of SEN for terrestrial organisms.

3. Synthesis and biosynthesis of selenoneine

3-1. Chemical synthesis of SEN

SEN is a low molecular weight Se species and its chemical synthetic and biosynthetic processes have been investigated. In 2019, a total synthesis method for SEN was reported, which described a method to synthesize an oxidized dimer form of SEN (**Fig. 1**) starting with *L*-histidine methyl ester, converting it to 2-selenoimidazole, followed by oxidation to the diselenide racemic form with a yield of 2% [7].

3-2. Biosynthesis studies of SEN

Biosynthetic methods for SEN were developed prior to the total synthesis method. In 2014, a genetically modified fission yeast (*Schizosaccharomyces pombe*) strain was developed (TP1803) for efficiently synthesizing EGT [8]. TP1803 overexpresses ergothioneine biosynthesis protein 1 (egt-1, SPBC1604.1) and synthesizes EGT from hercynylcysteine sulfoxide, which is composed of trimethyl histidine (hercynine) and cysteine [8]. The strain also synthesizes SEN from hercynylselenocysteine, which is composed of hercynine and selenocysteine (SeCys), when the strain is cultured in a medium containing sodium selenate (Na_2SeO_4) as a source of Se [8]. *Aspergillus* sp. like *A. oryzae*, *A. niger* and *A. sojae* also synthesize SEN by induction of ergothioneine biosynthesis genes [9]. They synthesize SEN in both media containing sodium selenite (Na_2SeO_3) and selenocystine, and all *Aspergillus* sp. synthesize more SEN with the addition of selenocystine [9]. SeCys is thought to be a Se source for SEN because microorganisms such as *S. pombe* and *Aspergillus* sp. synthesize SEN from selenocystine in the culture media.

However, Secys is not the only source of Se for SEN synthesis. In 2022, a novel SEN biosynthetic pathway in bacteria was reported where SEN is synthesized from selenosugars [10], which are known metabolites of Se species in the body and are detected in the urine of animals [11, 12]. This report indicates that SEN is not only synthesized by the non-specific incorporation of Se into the ergothioneine pathway published in 2014 [8]. The study compares the SEN production in bacteria with a canonical ergothioneine biosynthetic gene cluster (BGC), with or without the selenometabolite BGC corresponding *selD*, which is a selenophosphate synthase gene necessary for selenoprotein synthesis [10, 13]. Although an actinomycete with a selenometabolite BGC (*Amycolatopsis palatopharyngis*, DSM 444832) and a β-proteobacterium with the BGC (*Variovorax paradoxus*, DSM 30034) synthesize SEN in media containing sodium selenite, a genetically similar actinomycete lacking a selenometabolite BGC (*Streptomyces rimosus*, ATCC 10970) and a similar β-proteobacterium lacking the BGC (*Burkholderia thailandensis*, E264) do not synthesize SEN in the media [10]. These results suggest that selenoneine may in fact be the product of a new cluster termed *sen*, composed of *senA*, *senB* and *senC*; moreover, the authors report that SenA (encoded by *senA*)

transfers Se from a selenosugar to hercynine and synthesizes hercyncyl-SeGlcNAc selenoxide (GlcNAc–SEN=O) as a precursor of SEN [10]. A recent study exhibited that the SenA forms complexes with hercynine and thioglucose (SGlc), a sulfur analogue of selenoglucose (SeGlc) [14] and it is therefore speculated that the enzyme does not discriminate between S and Se. However, a sulfur analogue of selenosugar has not been detected in organisms and only Se compounds are thought to be used as a substrate by SenA consequently.

4. Purification methods

4-1. Purification of SEN dimer

In the initial report, SEN was extracted in acetonitrile with dithiothreitol (DTT) and purified as a SEN dimer using an ODS column (Atlantis dC18; 300 Å, 19 mm × 150 mm, Waters) and a size exclusion chromatography column (Ultrahydrogel 120; 120 Å, 6 μm, 7.8 mm × 300 mm, Waters) [1]. After the development of the genetically modified fission yeast synthesizing SEN [8], methods for purification of SEN from yeast were developed. In a method published in 2018, SEN dimer was purified from the yeast cell lysate, extracted by sonication and centrifugation in cold methanol [15]. The lysate was evaporated to dryness and dissolved in water, and the SEN dimer was isolated by preparative reverse-phase HPLC with an Atlantis dC18 column (Waters Corporation, Milford, USA) [15]. To isolate SEN monomers from EGT, which has similar polarity, dimerization of SEN monomers in methanol enables the separation of SEN from EGT by an ODS column.

4-1. Purification of SEN monomer

An additional method that permits the separation of SEN monomer from EGT in yeast cells involves using HPLC with a pentabromobenzyl (PBr) column [16]. In this method, SEN monomer and EGT are extracted from the yeast cells using boiling water. The supernatant is collected by centrifugation and concentrated by evaporation, and the concentrated solution is cleaned up using HPLC with a reverse phased column, C30 column (Nomura chemical), with 0.1 % acetic acid as a mobile phase by collecting an elution with early retention time that is barely retained on the column [16]. The eluate is then concentrated and injected into an HPLC with a PBr column and SEN monomer, detected at 260 nm wavelength, is collected with water as the mobile phase following its separation from EGT [16]. The PBr column separates substances by dispersion forces, a type of intermolecular forces, and has been reported to separate pnictogen and halogen compounds [17]. Thus, the column is predicted to separate the chalcogen analogs of SEN monomer and EGT.

5. Distribution and metabolism of selenoneine

5-1. Selenoneine concentrations in humans in epidemiological studies

SEN is one of the major Se compounds humans derive from their diet. Its distribution throughout the body has been examined in epidemiological studies using clinical specimens (**Table 2**) and animal experiments using mice. A study in a remote Japanese island revealed that SEN was present in erythrocytes of the island population with concentrations ranging from 6–2380 μg Se/L with a mean of 212 μg Se/L (n = 167) [18]. The concentrations increase depending on the frequency of fish consumption [18]. In a study of Inuit from Nunavik in Canada, erythrocyte SEN concentrations ranged from 3.20–3230 μg Se/L with a median of 413 μg Se/L (n = 210) [19]. Another study reported that SEN concentrations in erythrocytes of Inuit adults ranged from 1–3226 μg Se/L with a mean of 118 μg Se/L (ages: 18–74, n = 885) [20]. The mean was reported to be significantly higher among women (150.3 μg Se/L, n = 488) than men (87.6 μg Se/L, n = 387) [20]. This result differs from that has been reported that there are no sex differences in blood levels of EGT [21]. While no explanation was provided for why SEN concentrations are different between sexes, the authors speculated that the difference may be attributable to a differential response bias in the food frequency questionnaire [20]. The authors also suspect that there may be biological sex differences in the sequestration and function of SEN [20]. The levels of SEN in erythrocytes are highly correlated with total Se in erythrocytes and whole blood, but only weakly correlated with total Se in plasma [20]. The ratio of SEN : total Se in erythrocytes is also positively correlated with total Se in erythrocytes [20]. These results indicate that increases

Table 2. | SEN concentrations in erythrocytes of human

Participants	Tissues	SEN μmol Se/L (μg Se/L)	Reference
People in remote Japanese islands	Erythrocytes	Mean: 2.68 (212)	[18]
(n = 167)		Range: 0.08–30.1 (6–2380)	
Inuit from Nunavik in Canada	Erythrocytes	Median: 5.23 (413)	[19]
(n = 210)		Range: 0.04–40.9 (3.20–3230)	
Inuit adults	Erythrocytes	Mean: 1.49 (118)	[20]
(ages: 18–74, n = 885)		Range: 0.01–40.9 (1–3226)	
Women		Mean: 1.90 (150.3)	
(n = 488)			
Men		Mean: 1.11 (87.6)	
(n = 387)			

in total Se in erythrocytes are found as SEN [20]. However, the association between the ratio of SEN : total Se in erythrocytes is non-linear and the ratio plateaus around 0.8 [20]. The plateaued ratio suggests that there is an upper limit to the accumulation of SEN in erythrocytes and the existence of a SEN clearance mechanism. Notably, methylated SEN, Se-methylselenoneine (MeSEN, **Fig. 1d**), was identified in human urine and blood [22], and is predicted to be the metabolite that is the excreted form of SEN.

5-2. Distribution and metabolism of selenoneine in mice

Animal experiments using mice reveal the distribution of SEN in organs. A study reports that SEN is detected in the liver of mice at a concentration of 8.11 μmol Se/kg after 4 months of ingestion of a diet containing 0.3 mg Se/kg SEN [23]. Another study detected SEN in the kidney and spleen in addition to the liver in ICR mice after 32 days of ingestion of a 2.7 mg Se/kg SEN-containing diet [24]. In this study, SEN was not detected in erythrocytes, and it was suggested that SEN preferentially accumulates in organs such as the liver, kidney and spleen [23]. Concentrations of SEN in the liver (male: 0.411 ± 0.121 μmol Se/kg, female: 0.562 ± 0.203 μmol Se/kg) and spleen (male: 0.939 ± 0.092 μmol Se/kg, female: 1.10 ± 0.35 μmol Se/kg) were not different between sexes, whereas SEN in the kidney of female mice was significantly higher than in male mice (male: 0.323 ± 0.091 μmol Se/kg, female: 0.731 ± 0.151 μmol Se/kg) [24]. The difference between sexes appears to be consistent with the Inuit study in that the blood concentrations of SEN were significantly higher among women than men [20], however, a detailed mechanistic explanation will require further research. An additional study reported SEN accumulation in the liver, kidney and spleen in male Balb/c mice continuously administered 10 μM purified SEN via the drinking water for 6 days [25]. In the study, SEN was detected in erythrocytes at 3.0 ± 0.1 μmol Se/kg, which was lower than in the liver (16.1 ± 3.3 μmol Se/kg) and kidney (13.1 ± 1.9 μmol Se/kg) [25]. The study also showed that MeSEN was a metabolite of dietary SEN by administrating stable isotope-labeled (Se-76) SEN to mice and analyzing their urine [25]. In addition to urine, MeSEN was detected in plasma, liver and kidney, and it was suggested that MeSEN was synthesized in these tissues [25]. Although major Se species in the body, like SeCys and SeMet, are metabolized to selenosugar or trimethylselenonium (TMSe) via selenide (HSe^-) [26], SEN is methylated and retains its original structure (**Fig. 2**). Therefore, SEN is thought to be metabolized by a unique pathway that is distinct from the pathway via HSe^- (**Fig. 2**). However, it is necessary to confirm how SEN is metabolized under selenium-deficient conditions because this study was performed under dietary selenium-replete conditions. Indeed, urinary excretion of the major Se species is not affected by SEN administration [27]. Such features of SEN are important when discussing dietary intake of

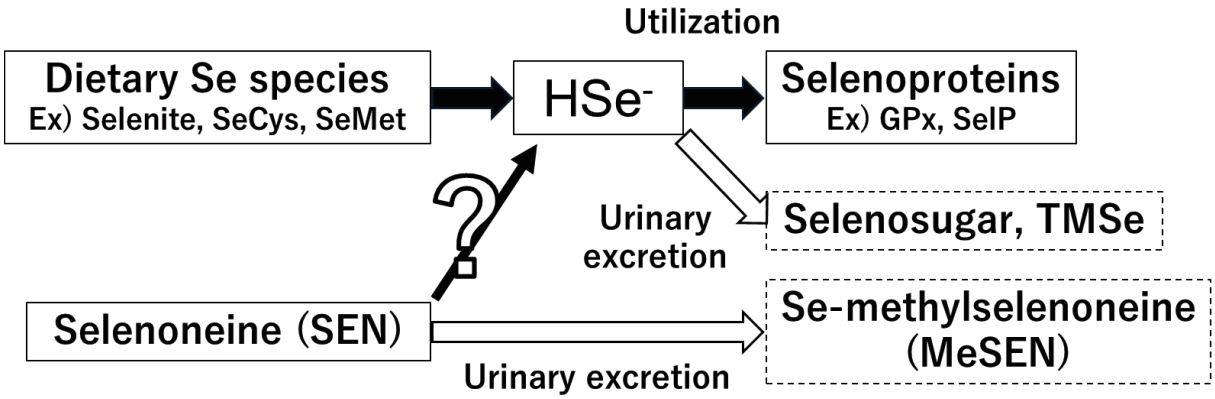

Fig. 2 Dietary Se species metabolism and predicted metabolic scheme of SEN. Dietary Se species such as selenite, SeCys and SeMet are converted to utilization forms in the body, selenoproteins, and urinary excretion forms, selenosugar and TMSe via selenide (HSe^-). Selenoneine (SEN) is converted to Se-methylselenoneine (MeSEN) for the urinary excretion.

SEN as a source of Se because most Se species play a role in the body by being synthesized into selenoproteins via selenocysteine. The methylation mechanism of SEN is not fully understood and further studies of SEN metabolism are required. Notably, a methyltransferase for SEN has not been identified, and it is also not known whether SEN is metabolized enzymatically or non-enzymatically.

5-3. Absorption of SEN by a transporter, OCTN1

SEN is incorporated into cells via the organic cation/carnitine transporter-1, OCTN1 (SLC22A4), which is a known EGT transporter in animals [28, 29]. SEN was incorporated into human embryonic kidney 293 cells (HEK293) over expressing OCTN1 following transient transfection with an OCTN1 vector [28]. The study also demonstrated that SEN concentrations in zebrafish embryo are increased by addition of SEN from the water, and were decreased to control levels by suppression of OCTN1 expression with an antisense morpholino oligo [28]. A transwell assay using the Caco-2 human colon adenocarcinoma cell line as an *in vitro* absorption model of the small intestine showed that SEN and MeSEN are detected on the basolateral side after the addition of SEN to the apical side [30]. OCTN1 is expressed on the apical side of the small intestine of human and mouse [31], and dietary SEN is thought to be absorbed via small intestine epithelial cells expressing OCTN1. The K_m value for SEN uptake was reported to be 13.0 μM [28], which is lower than previously reported for EGT uptake by OCTN1 (21 μM) in OCTN1 transfected HEK293 cells [29]. The amount of SEN uptake [28] is thought to be derived from SEN monomer because SEN dimer is not a substrate of OCTN1 [7]. SEN is distributed in organs in mice continuously administered SEN dimer [25], suggesting that SEN dimer is reduced in the digestive tract and absorbed into the body via small intestinal epithelial cells that express OCTN1. The ratio of SEN distribution to the kidney and spleen relative to the liver does not change [24] compared to the distribution of EGT [32]. This suggests that SEN distributes to organs expressing OCTN1 similar to the trend of EGT and the distribution can be predicted by referring to EGT distribution. EGT distributes to the brain [32] and it has been reported to improve cognitive functions [33, 34]. SEN has been detected in the brains of seabirds [5], but it has not yet been detected in mammals. Notably, SEN has been reported to cross an *in vitro* blood-brain barrier model [35], and SEN is expected to improve cognitive function by its radical scavenging capacity upon distribution to the brain. In section 5-2, it states that SEN in the kidney of female mice was significantly higher than in male mice [24], the difference is suspected to be derived from the difference of the expression of OCTN1. However, mRNA expression of OCTN1 in the kidney of mice does not change between male and female [36]. Therefore, the difference in the concentration of SEN in the kidney of mice [24] is thought

to be caused by mechanisms other than the expression of OCTN1.

6. Functions of selenoneine in organisms

6-1. Antioxidative capacity of SEN

In the first report of SEN, the radical scavenging capacity of SEN was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, and the capacity of SEN was greater than that of EGT and a water-soluble vitamin E, Trolox [1]. SEN is oxidized by hydrogen peroxide (H_2O_2) and converted to selenoneine-seleninic acid (SEN-seleninic acid, **Fig. 1e**) unlike EGT, which is converted to hercynine or sulfonic acid [7]. The difference between SEN and EGT against oxidants is thought to be related to the difference in antioxidative capacity between them. Another study shows that SEN-inspired selenohydantoin derivatives exhibit glutathione peroxidase (GPx)-like activity, reducing hydroperoxides [37].

In a study using *Caenorhabditis elegans* (*C. elegans*), SEN did not diminish ROS induced by *tert*-butyl hydroperoxide immediately after SEN treatment, but an improvement in oxidative stress was observed 48 h after SEN treatment [38]. This study suggests that the protective effect may not be explained by the direct radical scavenging capacity of SEN, but that the delayed protection may rather involve the activation of a molecular signaling cascade corresponding to oxidative stress. Few papers have reported the response of SEN and ROS *in vitro* or *in cellulo*, and further studies are required to understand the antioxidant mechanism of SEN.

6-2. Interactions of SEN with metals

Previous studies have shown that dietary Se intake from fish reduces methylmercury (MeHg) toxicity [39-44], and SEN has been implicated in reducing MeHg toxicity. A study demonstrated that 1.6 μM SEN reduced MeHg toxicity in zebrafish embryos and was associated with the SEN transporter, OCTN1, because the protective effect was eliminated by the inhibition of OCTN1 expression [28]. This study also showed a decrease of MeHg and an increase of inorganic Hg in the presence of SEN in the embryo [28]. The study speculated that SEN forms a SEN-MeHg complex and that the complex can be converted to mercury selenide (HgSe), which is found in the liver of marine mammals and is thought to be a detoxified form of MeHg [45, 46], by translocation into lysosomal secretory vesicles by OCTN1 [28]. In seabirds, SEN is thought to play a role in MeHg detoxication because SEN levels decrease dramatically (from 68 to 3%) with increasing Hg concentrations in the liver [5]. However, a SEN-MeHg complex has not been found in previous studies. Another study showed that SEN did not interact with inorganic Hg and MeHg in the liver homogenate of marine turtles [47]. Further studies are needed to elucidate the MeHg detoxication mechanism of SEN.

Other studies demonstrate interactions between SEN and metalloenzymes. SEN inhibited a zinc-containing metalloprotease, angiotensin converting enzyme (ACE, EC 3.4.15.1), in an *in vitro* assay and the enzyme kinetics indicated that the inhibition is competitive [48]. *In silico* docking simulation suggested that SEN interacts with the catalytic Zn^{2+} located at the active center of ACE; however, the Se atom of SEN does not interact with Zn^{2+}, unlike captopril or selenocaptopril, which inhibit ACE by direct interaction of their thiol or selenol group with Zn^{2+} [49, 50]. SEN also inhibits a copper-containing enzyme tyrosinase (EC 1.14.18.1), which is involved in melanogenesis [51]. In a human 3D epidermal model containing functional melanocytes, melanin synthesis was suppressed by the addition of SEN to the media [51].

6-3. Effect of SEN on disease

SEN is expected to have beneficial health effects due to its strong radical scavenging capacity. In a study using azoxymethane (AOM) and dextran sodium sulfate (DSS)-induced colitis-associated cancer model mice, a diet containing SEN (0.28 mg Se/100 g) from tuna dark muscle extract reduced the pathology of experimental colorectal cancers [52]. The number of macroscopic polyps and tumor diameters were decreased after 74 days of dietary supplementation [52]. The AOM/DSS-induced colitis-associated cancer model corresponds to ROS-induced inflammation and SEN is speculated to improve the pathology through its radical scavenging capacity [52]. However,

an extract from tuna dark muscle was used in this experiment rather than purified SEN. Therefore, the influence of other substances derived from the dark muscle cannot be ignored.

Another study showed an ameliorating effect of SEN against hepatocellular injury and hepatic steatosis in non-alcoholic fatty liver disease (NAFLD) model mice; these mice lack the farnesoid X receptor, which plays an important role in the regulation of lipid and glucose metabolism [23]. The study supplied purified SEN at 0.3 mg Se/kg in the diet for 4 months and SEN-fed mice exhibited reductions of the hepatic damage associated diagnostic markers AST, total bilirubin and total bile acid [23]. Hepatic triacylglycerol levels were suppressed in SEN-fed mice, but the levels did not change in the serum [23]. Interestingly, hepatic mRNA expressions of the selenoproteins, *Gpx1* and *SelP*, were significantly decreased in SEN-fed mice [23]. The production of glutathione peroxidase 1 (GPx1) and selenoprotein P (SelP) were stimulated by excessive consumptions of Se [53-56]. The results in the SEN-fed study suggest that *Gpx1* and *SelP* mRNA expressions are induced by oxidative stress resulting from hepatic steatosis in NAFLD model mice, and SEN supplementation counteracts the induction by reducing ROS and decreasing oxidative stress. However, the mechanism of regulation of selenoprotein expressions by SEN is not clear and should be elucidated to understand SEN function and nutrition in Se metabolism.

7. Future prospects

SEN is a compound with potential health benefits due to its strong radical scavenging capacity. Research on the health functions of EGT is accelerating, and in recent years clinical research has even been conducted on dementia [57]. For SEN, animal studies using purified SEN and clinical studies using supplements or tuna dark muscle containing high concentrations of SEN are required to investigate the effects of SEN against various diseases caused by oxidative stress. These studies will demonstrate the efficacy of SEN on human health and contribute to the growing body of evidence on the relationship between fish diets and health.

Se is known to be a trace element with a narrow range of biologically acceptable levels, which raises concerns about the safety of SEN. SEN is thought to be safe in terms of dietary experience, as some people consume large amounts of SEN by eating marine organisms [18-20]. On the other hand, if SEN derived from extracts from fish processing residues, etc. is taken as a supplement, its safety should be carefully assessed, as it may be highly concentrated. SEN is also believed to be methylated and excreted in the urine [25] and not via the central metabolic pathways of selenium, such as selenoproteins synthesis and selenosugars synthesis, so it needs to be carefully considered whether it should be uniformly considered under conventional selenium intake standards and it is an important research topic in Se nutrition.

8. Conclusion

Since the identification of SEN in 2010, SEN studies have progressed in several areas. Chemical and biological synthetic studies and the development of SEN purification methods allow us to obtain SEN standards easily compared to those obtained from fish, and contribute to studies on SEN functions in organisms, especially mammals. On the other hand, the metabolism and biological functions of SEN in mammals are not sufficiently elucidated because such studies require abundant purified SEN. In the future, the development of such studies will be enabled by the establishment of mass production methods and market availability of SEN standards. Advances in SEN research not only provide new insights into the relationship between fish consumption and human health, but are also leading to the discovery of new redox mechanisms in organisms that accumulate SEN.

Author contributions

Takuya Seko: Conceptualization, Writing-Original draft preparation. Yumiko Yamashita: Visualization, Supervision. Michiaki Yamashita: Supervision, Writing-review and editing.

Acknowledgements

The authors are grateful to the co-authors who were involved in writing our original papers.

References

[1] Yamashita Y, Yamashita M. Identification of a novel selenium-containing compound, selenoneine, as the predominant chemical form of organic selenium in the blood of bluefin tuna. *J Biol Chem*. 2010; 285(24): 18134-18138.

[2] Borodina I, Kenny LC, McCarthy CM, Paramasivan K, Pretorius E, Roberts TJ, van der Hoek SA, Kell DB. The biology of ergothioneine, an antioxidant nutraceutical. *Nutr Res Rev*. 2020; 33(2): 190-217.

[3] Yamashita Y, Amlund H, Suzuki T, Hara T, Hossain MA, Yabu T, Touhata K, Yamashita M. Selenoneine, total selenium, and total mercury content in the muscle of fishes. *Fish Sci*. 2011; 77: 679-686.

[4] Yamashita M, Suzuki T. Yokoyama Y. *Fish Consumption and Health —Biological Effects of Methylmercury Exposure*. Tokyo: Koseisya Koseikaku; 2014.

[5] El Hanafi K, Pedrero Z, Ouerdane L, Marchán Moreno C, Queipo-Abad S, Bueno M, Pannier F, Corns WT, Cherel Y, Bustamante P, Amouroux D. First time identification of selenoneine in seabirds and its potential role in mercury detoxification. *Environ Sci Technol*. 2022; 56(5): 3288-3298.

[6] Peer F, Kuehnelt D. High levels of the health-relevant antioxidant selenoneine identified in the edible mushroom Boletus edulis. *J Trace Elem Med Biol*. 2024; 127536.

[7] Lim D, Gründemann D, Seebeck FP. Total synthesis and functional characterization of selenoneine. *Angew Chem Int*. 2019; 58(42): 15026-15030.

[8] Pluskal T, Ueno M, Yanagida M. Genetic and metabolomic dissection of the ergothioneine and selenoneine biosynthetic pathway in the fission yeast, S. pombe, and construction of an overproduction system. *PloS one*. 2014; 9(5): e97774.

[9] Ichikawa K, Shinohara Y, Hara S, Kurosawa K, Yamashita Y, Yamashita M. Method for producing selenoneine, U. S. Patent Application. 2018; No. 15: 750-792.

[10] Kayrouz CM, Huang J, Hauser N, Seyedsayamdost MR. Biosynthesis of selenium-containing small molecules in diverse microorganisms. *Nature*. 2022; 610(7930): 199-204.

[11] Kobayashi Y, Ogra Y, Ishiwata K, Takayama H, Aimi N, Suzuki KT. Selenosugars are key and urinary metabolites for selenium excretion within the required to low-toxic range. *Proc Natl Acad Sci*. 2002; 99: 15932–15936.

[12] Kuehnelt D, Kienzl N, Traar P, Le NH, Francesconi KA, Ochi T. Selenium metabolites in human urine after ingestion of selenite, L-selenomethionine, or DL-selenomethionine: a quantitative case study by HPLC/ICPMS. *Anal Bioanal Chem*. 2005; 383: 235–246.

[13] Böck A, Rother M. *Selenium metabolism in prokaryotes. Hatfield DL, Berry MJ, Gladyshev VN (eds) : Selenium : its molecular biology and role in human health, Second Edition.* New York: Springer: 2006; 9-28.

[14] Liu M, Yang Y, Huang JW, Dai L, Zheng Y, Cheng S, He H, Chen CC, Guo RT. Structural insights into a novel nonheme iron-dependent oxygenase in selenoneine biosynthesis. *Int J Biol Macromol*. 2024; 256: 128428.

[15] Turrini NG, Kroepfl N, Jensen KB, Reiter TC, Francesconi KA, Schwerdtle T, Kroutil W, Kuehnelt D. Biosynthesis and isolation of selenoneine from genetically modified fission yeast. *Metallomics*. 2018; 10(10): 1532-1538.

[16] Seko T, Uchida H, Yamashita Y, Yamashita M. Novel method for separating selenoneine reduced monomer and ergothioneine from fission yeast extracts. *Sep Purif Technol*. 2021; 254: 117607.

[17] Turowski M, Morimoto T, Kimata K, Monde H, Ikegami T, Hosoya K, Tanaka N. Selectivity of stationary phases in reversed-phase liquid chromatography based on the dispersion interactions. *J Chromatogr A*. 2001; 911(2): 177-190.

[18] Yamashita M, Yamashita Y, Ando T, Wakamiya J, Akiba S. Identification and determination of selenoneine, 2-selenyl-N_{α}, N_{α}, N_{α}-trimethyl-$_{L}$-histidine, as the major organic selenium in blood cells in a fish-eating population on remote Japanese Islands. *Biol Trace Elem Res*. 2013; 156: 36-44.

[19] Achouba A, Dumas P, Ouellet N, Little M, Lemire M, Ayotte P. Selenoneine is a major selenium species in beluga skin and red blood cells of Inuit from Nunavik. *Chemosphere*. 2019; 229: 549-558.

[20] Little M, Achouba A, Dumas P, Ouellet N, Ayotte P, Lemire M. Determinants of selenoneine concentration in red blood cells of Inuit from Nunavik (Northern Québec, Canada). *Environ Int*. 2019; 127: 243-252.

[21] Lee S, Mun S, Lee YR, Lee J, Kang HG. Validation of the metabolite ergothioneine as a forensic marker in bloodstains. *Molecules*. 2022; *27*(24): 8885.

[22] Klein M, Ouerdane L, Bueno M, Pannier F. Identification in human urine and blood of a novel seleniummetabolite, Se-methylselenoneine, a potential biomarker of metabolization in mammals of the naturally occurring selenoneine, by HPLC coupled to electrospray hybrid linear ion trap-orbital ion trap MS. *Metallomics*. 2011; 3(5): 513-520.

[23] Miyata M, Matsushita K, Shindo R, Shimokawa Y, Sugiura Y, Yamashita M. Selenoneine ameliorates hepatocellular injury and hepatic steatosis in a mouse model of NAFLD. *Nutrients*. 2020; 12(6): 1898.

[24] Seko T, Sato Y, Kuniyoshi M, Murata Y, Ishihara K, Yamashita Y, Fujiwara S, Ueda T, Yamashita M. Distribution and Effects of Selenoneine by Ingestion of Extract from Mackerel Processing Residue in Mice. *Mar Biotechnol*. 2023; 25(6): 1020-1030.

[25] Seko T, Uchida H, Sato Y, Imamura S, Ishihara K, Yamashita Y, Yamashita M. Selenoneine is methylated in the bodies of mice and then excreted in urine as Se-methylselenoneine. *Biol Trace Elem Res*. 2024; 202(8): 3672-3685.

[26] Navarro-Alarcon M, Cabrera-Vique C. Selenium in food and the human body: a review. *Sci Total Environ*. 2008; 400(1-3): 115-141.

[27] Seko T. Studies on the biological functions and metabolism of selenoneine. Kyoto University. 2024; Ph. D. thesis, *in publication*

[28] Yamashita M, Yamashita Y, Suzuki T, Kani Y, Mizusawa N, Imamura S, Takemoto K, Hara T, Anwar H, Yabu T, Touhata K. Selenoneine, a novel selenium-containing compound, mediates detoxification mechanisms against methylmercury accumulation and toxicity in zebrafish embryo. *Mar Biotechnol*. 2013; 15: 559–570. https:// doi. org/ 10. 1007/ s10126- 013- 9508-1

[29] Gründemann D, Harlfinger S, Golz S, Geerts A, Lazar A, Berkels R, Jung N, Rubbert A, Schömig E. Discovery of the ergothioneine transporter. *Proc Natl Acad Sci*. 2005; 102: 5256–5261.

[30] Rohn I, Kroepfl N, Bornhorst J, Kuehnelt D, Schwerdtle T. Side-directed transfer and presystemic metabolism of selenoneine in a human intestinal barrier model. *Mol Nutr Food Res*. 2019; 63:1900080.

[31] Sugiura T, Kato S, Shimizu T, Wakayama T, Nakamichi N, Kubo Y, Iwata D, Suzuki K, Soga T, Asano M, Iseki S, Tamai I, Tsuji A, Kato Y. Functional expression of carnitine/organic cation transporter OCTN1/SLC22A4 in mouse small intestine and liver. *Drug Metab Dispos*. 2010; 38: 16651672.

[32] Tang RMY, Cheah IKM, Yew TSK, Halliwell B. Distribution and accumulation of dietary ergothioneine and its metabolites in mouse tissues. *Sci Rep*. 2018; 8: 1–15.

[33] Yang NC, Lin HC, Wu JH, Ou HC, Chai YC, Tseng CY, Liao JW, Song TY. Ergothioneine protects against neuronal injury induced by β-amyloid in mice. *Food Chem Toxicol*. 2012; 50(11): 3902-3911.

[34] Yau YF, Cheah IK, Mahendran R, Tang RM, Chua RY, Goh RES, Feng L, Li J, Kua EH, Chen C, Halliwell B. Investigating the efficacy of ergothioneine to delay cognitive decline in mild cognitively impaired subjects: A pilot study. *J Alzheimers Dis*. 2024; 13872877241291253.
[35] Drobyshev E, Raschke S, Glabonjat RA, Bornhorst J, Ebert F, Kuehnelt D, Schwerdtle T. Capabilities of selenoneine to cross the in vitro blood–brain barrier model. *Metallomics*. 2021; 13(1): mfaa007.
[36] Alnouti Y, Petrick JS, Klaassen CD. Tissue distribution and ontogeny of organic cation transporters in mice. *Drug Metab Dispos*. 2006; 34(3): 477-482.
[37] Alhasan R, Martins GM, de Castro PP, Saleem RSZ, Zaiter A, Fries-Raeth I, Kleinclauss A, Perrin-Sarrado C, Chaimbault P, da Silva Júnior EN, Gaucher C, Jacob C. Selenoneine-inspired selenohydantoins with glutathione peroxidase-like activity. *Bioorg Med Chem*. 2023; 94: 117479.
[38] Rohn I, Kroepfl N, Aschner M, Bornhorst J, Kuehnelt D, Schwerdtle T. Selenoneine ameliorates peroxide-induced oxidative stress in C. elegans. *J Trace Elem Med Biol*. 2019; 55: 78-81.
[39] Friedman MA, Eaton LR, Carter WH. Protective effects of freeze dried swordfish on methylmercury chloride toxicity in rats. *Bull Environ Cont Toxicol*. 1978; 19: 436–443.
[40] Ganther HE, Sunde ML. Effect of tuna fish and selenium on the toxicity of methylmercury. *J Food Sci*. 1974; 39: 1–5.
[41] Ganther H, Goudie C, Sunde M, Kopeckey M, Wagner S, Hoekstra W. Selenium: relation to decreased toxicity of methylmercury added to diets containing tuna. *Science*. 1972; 175: 1122–1124.
[42] Ohi G, Nishigaki S, Seki H, Tamura Y, Maki T, Minowa K, Shimamura Y, Mizoguchi I, Inaba Y, Takizawa Y, Kawanishi Y. The protective potency of marine animal meat against the neurotoxicity of methylmercury: its relationship with the organ distribution of mercury and selenium in the rat. *Food Cosmet Toxicol*. 1980; 18: 139–145.
[43] Raymond LJ, Ralston NV. Mercury:selenium interactions and health implications. *SMDJ Seychelles Med Dent J*. 2004; 7: 52–56.
[44] Raymond LJ, Seale LA, Ralston NV. *Seafood selenium in relation to assessments of methylmercury exposure risks. In: Hatfield DL, Berry MJ, Gladyshev VN (eds) Selenium: its molecular biology and role in human health, Thirdth edn*. New York: Springer; 2012.
[45] Martoja R, Berry JP. Identification of tiemannite as a probable product of demethylation of mercury by selenium in cetaceans. A complement to the scheme of the biological cycle of mercury. *Vie et Milieu/Life Environ*. 1980; 30: 7-10.
[46] Ng PS, Ji H, Matsumoto K, Yamazaki S, Kogure T, Takagi T, Nagasawa H. Striped dolphin detoxificates mercury as insoluble Hg (S, Se) in the liver. *Proc Japan Acad*. 2001; 77: 178–183.
[47] Anan Y, Tanabe S, Ogra Y. Comparison of selenoneine found in marine organisms with selenite in the interaction with mercury compounds in vitro. *J Toxicol Sci*. 2011; 36(6): 725-731.
[48] Seko T, Imamura S, Ishihara K, Yamashita Y, Yamashita M. Inhibition of angiotensin-converting enzyme by selenoneine. *Fish Sci*. 2019; 85: 731-736.
[49] Natesh R, Schwager SL, Evans HR, Sturrock ED, Acharya KR. Structural details on the binding of antihypertensive drugs captopril and enalaprilat to human testicular angiotensin I-converting enzyme. *Biochemistry*. 2004; 43: 8718–8724.
[50] Akif M, Masuyer G, Schwager SL, Bhuyan BJ, Mugesh G, Isaac RE, Sturrock ED, Acharya KR. Structural characterization of angiotensin I-converting enzyme in complex with a selenium analogue of captopril. *FEBS J*. 2011; 278: 3644–3650.
[51] Seko T, Imamura S, Ishihara K, Yamashita Y, Yamashita M. Selenoneine suppresses melanin synthesis by inhibiting tyrosinase in murine B16 melanoma cells and 3D-cultured human melanocytes. *Fish Sci*. 2020; 86: 171-179.
[52] Masuda J, Umemura C, Yokozawa M, Yamauchi K, Seko T, Yamashita M, Yamashita Y. Dietary supplementation of selenoneine-containing tuna dark muscle extract effectively reduces pathology of experimental colorectal cancers in mice. *Nutrients*. 2018; 10(10): 1380.
[53] Qin S, Huang K, Gao J, Huang D, Cai T, Pan C. Comparison of glutathione peroxidase 1 and iodothyronine deiodinase 1 mRNA expression in murine liver after feeding selenite or selenized yeast. *J Trace Elem Med Biol*. 2009; 23(1): 29-35.
[54] Stranges S, Marshall JR, Natarajan R, Donahue RP, Trevisan M, Combs GF, Cappuccio FP, Ceriello A, Reid ME. Effects of long-term selenium supplementation on the incidence of type 2 diabetes: A randomized trial. *Ann Intern Med*. 2007; 147: 217–223.
[55] Steinbrenner H, Speckmann B, Pinto A, Sies H. High selenium intake and increased diabetes risk: Experimental evidence for interplay between selenium and carbohydrate metabolism. *J Clin Biochem Nutr*. 2011; 48: 40–45.
[56] Rayman MP, Stranges S. Epidemiology of selenium and type 2 diabetes: Can we make sense of it? *Free Radic Biol Med*. 2013; 65: 1557–1564.
[57] Yau YF, Cheah IK, Mahendran R, Tang RM, Chua RY, Goh RE, Feng L, Li J, Kua EH, Chen C, Halliwell B. Investigating the efficacy of ergothioneine to delay cognitive decline in mild cognitively impaired subjects: A pilot study. *J Alzheimer's Dis*. 2024; 102(3): 841–854.