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Methioninase and selenomethionine but not Se-methylselenocysteine generate methylselenol and superoxide in an *in vitro* chemiluminescent assay: implications for the nutritional carcinostatic activity of selenoamino acids

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Abstract

Methylselenol from selenium metabolism is postulated to be and most experimental evidence now indicates that it is the selenium metabolite responsible for the dietary chemoprevention of cancers. Using the recombinant enzyme methioninase, methylselenol-generating chemiluminesence by superoxide ($O_2^{\bullet -}$) is shown to be catalytically produced from L-selenomethionine and D,L-selenoethionine, but not from methionine or L-Se-methylselenocysteine (SeMC). Methylselenol enzymaticaly generated by methioninase activity from the substrate selenomethionine arises from an initial putative selenium radical as measured by chemiluminesence in the absence of glutathione (GSH). In the presence of GSH, superoxide was generated as measured by chemiluminesence and superoxide dismutase inhibition of chemiluminescence. Ascorbic acid also quenched the chemiluminesence from the activity of methioninase with selenomethionine. Methylselenol and other redox cycling selenium compounds are almost assuredly accountable for inducing cell-cycle arrest and apoptosis in cancer cells *in vitro* and *in vivo*. Methylselenol generated from selenomethionine by methioninase is catalytic alone in oxidizing thiols, i.e. GSH, generating superoxide and inducing oxidative stress in direct proportion to its concentration. Se-methylselenocysteine *in vivo* is very likely carcinostatic in like manner to selenomethionine by generating methylselenol from other enzymatic activity, i.e. beta-lyase or amino acid oxidases.

Keywords: Methylselenol; Selenomethionine; Se-methylselenocysteine; Superoxide; Selenium; Apoptosis; Cancer

1. Introduction

Selenium (Se) is an essential dietary nutrient for all mammals with an RDA of 55 μg per day for human adults being presently observed in the USA. This amount of selenium may fulfill the dietary need for the selenium containing enzymes, several glutathione peroxidases, selenoprotein-P, thioredoxin reductase, 5′-deiodinase and general human health [1]. Most of the human dietary selenium requirement is met by the L-selenomethionine and the lesser amounts of L-selenocysteine from animal protein. Selenium is also thought to protect humans from various cancers when consumed in supplemental amounts of 200 μg Se per day or more. The definitive human study to date is that of Clark *et al.* [2], which revealed that selenium supplements of mostly L-selenomethionine as selenium-yeast reduced the incidence of colon, lung, and prostate cancer by nearly 50%. Similar supplementation with only L-selenomethionine is the focus of the present ongoing National Cancer Institute (NCI) sponsored SELECT Trial for the prevention of prostate cancer in men over 50 years of age (http://www.crab.org/select/).

Studies have shown that many selenium compounds when added to cells in culture or supplemented to animal diets reduces the incidence and severity of the experimental cell growth *in vitro* and cancer *in vivo* [3–5]. The effects of these selenium compounds in reducing the cell growth in cultures or cancers in animals depends upon the dose and

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Table 1
Anticarcinogenic efficacy of different selenium compounds for reduction of mammary tumors in rats and redox cycling

Compound	Dose of selenium for 50% inhibition (ppm Se)	Redox cycling
Se-methylselenocysteine	2	M
Selenobetaine	2	M
Selenobetaine methyl ester	2–3	M
Selenite	3	T
Selenomethionine	4–5	M
Selenocystine	4–5	T
p-XSC	8–10	T
Triphenylselenonium ion	10–12	M
Dimethylselenoxide	>10	M
Triphenylselenonium ion	30	M
Trimethylselenonium ion	No effect at 80	M

M, only by metabolism, T, in the presence of reduced thiol.
Table 1 is modified from Ref. [7].

chemical composition of the selenium compound. What has not been understood is why there is such a qualitative difference in the chemoprevention properties of differing selenium compounds as shown in Table 1 for the mammary cancer studies in rats mostly done by Ip [6] (Table 1 [7]).

Two generalizations have recently emerged from selenium research in the not to distant past. First, it is suggested that selenium compounds that prevent cancers must continuously produce the metabolite, methylselenol (CH_3SeH) [8]. Secondly, several early studies with different selenium compounds suggested that selenium may induce apoptosis as the mechanism of carcinostasis [9–13]. Many studies now have clearly shown that selenium compounds do indeed cause apoptosis in cancer cells including methylselenol [14–22]. One of us (J.E.S.) had postulated that the anticarcinogenic action, as well as the toxicity of selenium compounds, is do to the catalytic nature of the selenide anion (RSe^-), the subsequent generation of superoxide ($O_2^{\bullet -}$) and oxidative stress, which induces the apoptosis [23,24].

Since the presentation of the 1988 abstract at Tubingen, Germany and subsequent publication of Seko *et al.* [25] showing that selenite could generate superoxide in the presence of reduced glutathione (GSH) our laboratory has cataloged many selenium compounds that can and cannot generate superoxide in an *in vitro* chemiluminescent (CL) assay (Table 2). Generalizing from Table 2, selenium compounds that can easily form the selenide anion (RSe^-) generates superoxide *in vitro* via oxidation of GSH and other thiols (RSH). These selenide forming compounds *in vitro* include selenite but not selenate and diselenides but not monoselenides. Also included are methylseleninic acid (Fig. 1A) and dimethyldiselenide (Fig. 1B) which form methylselenol (Fig. 1C) directly upon reduction with GSH and generates superoxide. The trimethylselenonium ion, the urinary metabolite of selenium metabolism, L-Se-methylselenocysteine (SeMC; Fig. 1D) and L-selenomethionine (Fig. 1E) do not generate superoxide *in vitro* nor are they very toxic to cells in culture

Table 2
Selenium compounds that generate and do not generate superoxide *in vitro*

Compounds that generate superoxide	Compounds that do not generate superoxide
Selenite	Elemental selenium
Selenium dioxide	Selenate
Selenocystine	Selenomethionine
Selenocystamine	Se-methylselenocysteine[a]
Diselenodiproprionic acid	Selenobetaine[a]
Dipenyldiselenide	Dimethylselenoxide[a]
Dibenzyldiselenide	Selenopyridine[b]
1,4-Phenyl-bis-(methylene)-selenocyanate[c]	Triphenylselenonium ion[a]
6-Propylselenouricil[d]	K-selenocyanate
Dimethyldiselenide	Selenourea
Methylseleninic acid	Selenoethionine

Assay conditions: borate buffer (pH 9.2) containing GSH (4 mg/mL and lucigenin 20 μg/mL) at 25° or phosphate buffer (pH 7.0) containing GSH (1 mg/mL) at 36°.
Table 2 is modified from Ref. [27].
[a]Courtesy of Dr. Howard Ganther, University of Wisconsin.
[b]Courtesy of Dr. Ahmad Khalil, Yarmouk University, Irbid, Jordan.
[c]Courtesy of Dr. Karam el-Bayoumy, American Health Foundation.
[d]Courtesy of Dr. Alvin Taurog, University of Texas Southwest Medical Center.

or to animals or humans *in vivo*. Nevertheless, in cell culture and *in vivo*, both selenomethionine and SeMC in animal studies have been shown to be carcinostatic [6,7].

It has been recently reported in cell culture that otherwise nontoxic selenomethionine in the presence of methioninase can induce apoptosis in DU-145 human prostate cells at micromolar concentrations [26]. The enzymatic products of methioninase activity are ammonia, α-ketobutyric acid,and methylselenol, the postulated anticarcinogenic metabolite

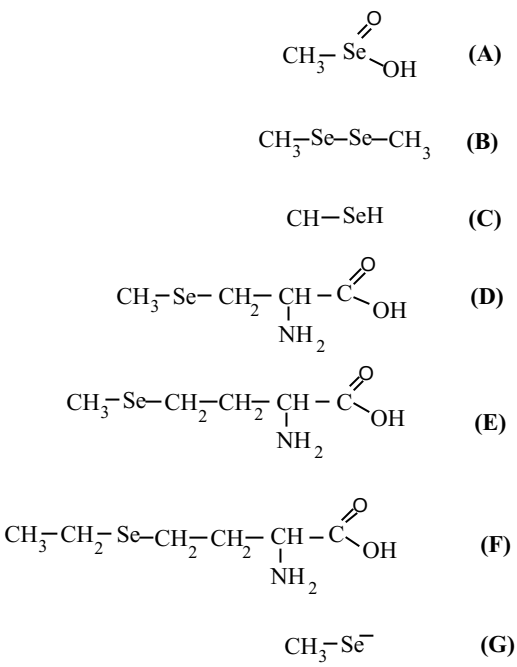

Fig. 1. Chemical composition of methylseleninic acid (A), dimethyldiselenide (B), methlyselenol (C), Se-methylselenocysteine (SeMC; D), selenomethionine (E), selenoethionine (F), and methylselenide (G).

of selenium metabolism. We report here that in our *in vitro* system at physiological pH, methylselenol, a metabolite of methioninase activity and postulated carcinostatic metabolite of selenium metabolism, generates superoxide in the presence of GSH. Unexpectedly, we also report that methioninase catalysis of selenomethionine in the absence of thiol (also) produces an initial putative selenium radical and generates superoxide. The inference of this methioninase CL demonstration is very clear; it is methylselenol or other selenides that initially induce oxidative stress and are the most probable selenium compounds that effect apoptosis and provide for selenium's carcinostatic activity.

2. Materials and methods

L-Selenomethionine and L-Se-methylselenocysteine (SeMC) were from Eburon Organics USA, Inc.(www.eburon-organics.com). Bis-*N*-methylacridinium nitrate (lucigenin), L-methionine, D,L-selenoethionine (Fig. 1F), glutathione (GSH), dimethyldiselenide, and superoxide dismutase were from Sigma or Sigma/Aldrich Chemical Co. (www.sial.com). Ascorbic acid was from United States Biochemical Corporation (www.informagen.com). L-Methionine-alpha-deamino-gamma-mercaptomethane lyase, i.e. methioninase (EC 4.4.1.11) a recombinant enzyme from the Trichomonas vaginalis gene produced from *Escherichia coli* was purchased from Wako Pure Chemical Industries, Ltd. (www.wako.com). Sodium monophosphate used for the buffer was from Fisher Scientific Co. (www1.fishersci.com).

2.1. Chemiluminescent (CL) assay

The control chemiluminescent (CL) assay cocktail without substrates or GSH was made using a 0.05 M sodium phosphate buffer (pH 7.0) and 20 μL lucigenin/mL from a stock solution of 1.0 mg/mL lucigenin in distilled water. The assay cocktail with thiol contained 1.0 mg GSH/mL. To 600 μL test aliquots of the control or thiol containing assay cocktail was added L-selenomethionine or L-Se-methylselenocysteine at 2.0 mg/mL and other substrate concentrations were made by dilution with buffer containing lucigenin. In like manner, D,L-selenoethionine was added at 4.0 mg/mL to the CL cocktail. To the control or substrate containing CL cocktails in the luminometer was added methioninase containing 0.5 U of enzyme activity or graded units of methioninase activity. Methioninase was prepared by adding 1.0 or 2.0 mL of distilled water to 10 U [10] vials of commercial freeze-dried enzyme. The methioninase was reported by Waco technical services to contain no reducing thiol preservative. The enzyme was added in 0.1 mL increments from a 1.0 cm^3 syringe or up to 30 μL from an Eppendorf pipette directly to the chemiluminescent tube in a Los Alamos Diagnostics Model 535 luminometer containing 600 μL of the pH 7.0 cocktail. The CL tube and contents was held at 36° by an attached LKB 2209 multitemp recirculating water bath. Chemiluminescent (CL) data was recorded in 30-s integrated units over a period of up to 20 min. There was a 3-s instrumental delay between integrations. Additional details of this assay including the quenching of chemiluminesence generated by methylselenol from reduced methylseleninic acid and dimethyldiselenide by superoxide dismutase has been previously reported [27]. This CL assay is quantitative (correlation coefficient, $r = 0.99$; $P < 0.001$) in generating CL for small amounts of redox cycling methylselenol. Figure 2 is a standard curve for methylselenol (CH_3SeH) produced CL (relative CL units vs. selenium concentration) from the reduction of dimethyldiselenide by GSH when added directly to the CL cocktail.

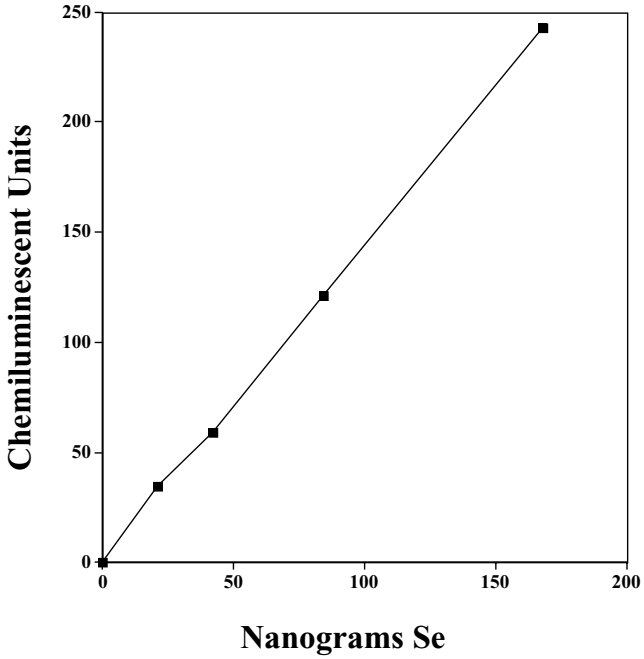

Fig. 2. Mean (N = 5) chemiluminesence generated by methylselenol in 30-s integrations from dimethyldiselenide reduction by GSH (1 mg/mL) in the presence of lucigenin (0.1 mg/mL) in a 0.05 M (pH 9.0) sodium borate buffer (Perason's $r = 0.99$; $P < 0.05$).

3. Results and discussion

Recent cell culture evidence has accumulated showing that many selenium compounds can induce apoptosis with or without DNA fragmentation and/or cell-cycle arrest at G1. The selenium compounds reported to induce apoptosis and cell-cycle arrest have included selenite [28], selenite and selenodiglutathione [29], methylseleninic acid [30], 1,4-phenylene-bis-(methylene)selenocyanate [31], SeMC [18] and selenomethionine [32] as well as others. We have found using lucigenin for the detection of superoxide and superoxide dismutase, which quenches the chemiluminesence confirming the generation of superoxide, that selenite, methylseleninic acid, dimethyldiselenide

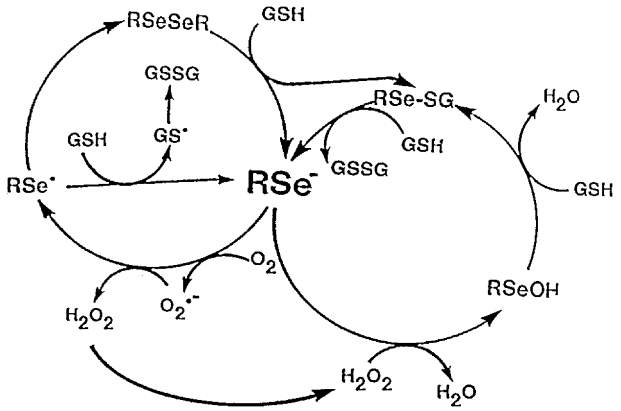

Fig. 3. Redox cycling of methylselenol and other selenides (RSe^-) after Chaudiere et al. [45].

and 1,4-phenylene-bis-(methylene)selenocyanate as well as other selenium compounds (Table 2) all generate superoxide in the presence of reduced but not oxidized glutathione at physiological pH. Superoxide is generated with these and other selenium compounds do to the formation of the selenide anion (RSe^-) from the compound and redox cycling as shown in Fig. 3. Selenomethionine, selenoethionine, and Se-methylselenocysteine being monoselenide ethers ($RSeCH_3$) in the presence of GSH do not generate any detectable superoxide in the lucigenin CL assay, as shown here in Table 3 or as previously reported (Table 2).

Many recent selenium experiments indicate that methylselenol (really methylselenide (CH_3Se^-) at pH 7; Fig. 1G) which metabolically is formed from Se-methylselenocysteine and selenomethionine *in vivo* is the likely selenium metabolite that induces cellular apoptosis. Methylselenol generation *in vivo* likely also accounts for selenium's carcinostatic activity when Se-methylselenocysteine, selenomethionine, and methylseleninic acid are fed to animals at supranutritional levels [8,33]. Very recently, Wang *et al.* have shown that methylselenol induces caspase-mediated apoptosis and G1 cell-cycle arrest of DU-145 human prostate cancer cells in culture [26]. In their cell system, methylselenol was enzymatically generated from the methioninase substrate, selenomethionine, or by the addition of methylseleninic acid, a direct precursor of methylselenol formed upon GSH reduction. As little as 1 μM selenomethionine in the presence of methioninase affected DU-145 cell survival whereas up to 100 μM selenomethionine in the absence of methioninase alone did not adversely affect cell survival. Since methylselenol had recently been shown to generate superoxide from the direct reduction of both dimethyldiselenide and methylseleninic acid [27], we thought it should also be possible to demonstrate that methylselenol is generated enzymatically from selenomethionine using CL. Methioninase generation of methylselenol from selenomethionine and detection of superoxide by CL would explain the apoptosis observed by Wang *et al.* in DU-145 cells [26] and add credence to the carcinostatic action of selenium from selenoamino acids via enzyme-induced methyselenol generation.

Table 3
Chemiluminescence (CL) from methioninase substrate catalysis

Substrate	CL units (blank)	CL units with 0.5 U methioninase added
Methionine	192	212
Selenomethionine	96	15324 ± 10[a]
Selenoethionine	302	20692
Se-methylselenocysteine	185	606

[a]Triplicate mean ± SEM. All others mean of duplicate assays.

The present experiments show that selenomethionine and selenoethionine in the presence of GSH and methioninase in the CL cocktail continuously generates chemiluminescence (Table 3, Column 2) indicative of the presence of superoxide. No CL other than background was observed when just methioninase, methionine, selenomethionine, selenoethionine or Se-methylselenocysteine was present alone in the chemiluminescent cocktail (Table 3, Column 1). When methioninase was added to the CL cocktail no enzymatic chemiluminescence was observed when methionine or Se-methylselenocysteine was present as potential

Table 4
Chemiluminescence (CL) from methioninase substrate catalysis without glutathione (GSH)

Methionine blank	2, 0, 0, 3, 0, 0, 0, 3, 0, 0, 0, 0, 0, 0
+Methioninase	17, 8, 0, 9, 0, 5, 0, 0, 0, 0, 0, 0, 0, 0
Selenomethionine blank	3, 2, 2, 2, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0
+Methioninase	295, 737, 940, 1002, 948, 600, 533, 538, 516, 547, 565, 583, 600, 650
Se-methylselenocysteine blank	33, 10, 19, 8, 13, 15, 15, 3, 10, 16, 18, 8, 10, 7
+Methioninase	251, 344, 342, 352, 333, 313, 274, 288, 272, 243, 250, 240, 225, 221

Assay conditions: 0.05 M sodium phosphate buffer (pH 7.0) and 20 μL lucigenin/mL from a stock solution of 1.0 mg/mL lucigenin in distilled water. The assay cocktail contained no GSH. In 600 μL test aliquots of the assay cocktail was present L-methionine, L-selenomethionine, or L-Se-methylselenocysteine (SeMC; 2.0 mg/mL). To the cocktail was added 0.075 U of methioninase in 15 μL. CL was integrated in 30-s increments over 7 min. Data is presented as the sum total of three separate CL assays of L-methionine, L-selenomethionine, and Se-methylselenocysteine at 14 sequential and continuous 30-s data points. A *t*-test was run for the comparison of the experimental pairs as indicated along with the confidence level. Assuming the null hypothesis, the probability (P) is shown for each comparison: methionine blank vs. methionine + methioninase ($P < 0.103$, not significant); methionine blank vs. selenomethionine blank ($P < 0.821$, not significant); methionine blank vs. SeMC blank ($P < 0.0001$, significant); selenomethionine blank vs. SeMC blank ($P < 0.001$, significant); methionine + methioninase vs. selenomethionine + methioninase ($P < 0.0001$, significant); SeMC + methioninase vs. SeMC blank ($P < 0.001$, significant); selenomethionine + methioninase vs. SeMC + methioninase ($P < 0.001$, significant).

enzyme substrates (Table 3, Column 2). Only when selenomethionine or selenoethionine was present in the CL cocktail did added methioninase cause significant CL indicative of superoxide generation. Quite unexpectedly, we also observed that methioninase catalysis of selenomethionine alone in the absence of any GSH also resulted in the generation of CL indicative of superoxide from a enzymatically generated putative selenide radical (Table 4). Table 4 also shows that the methioninase reaction is limited to selenomethionine and not methionine ($P < 0.0001$). SeMC shows a higher CL background than selenomethionine both with and without methioninase ($P < 0.001$). The kinetics of the methioninase reactions show the initial CL with SeMC declining ultimately to SeMC blank CL levels. However, with selenomethionine as substrate, the CL activity is totally much higher over time (Fig. 3) and increases with time as shown in Table 4 ($P < 0.001$). These simple CL experiments demonstrate that methylselenol which is immediately generated by methioninase catalysis of apparent substrates, L-selenomethionine and D,L-selenoethionine (Fig. 3) produces a putative initial selenium free radical (and) which can redox cycle in the presence of a thiol, GSH, producing additional superoxide [27]. This interpretation of the data is gleaned from reactions in the presence of increasing concentrations of methioninase in the presence of selenomethionine alone (Tables 5 and 6) and in the presence of selenomethionine with superoxide dismutase (SOD) and ascorbic acid.

Addition of 2 mg of ascorbic acid to the selenomethionine cocktail in the presence of GSH reduced CL by 65% when methioninase was added to the cocktail. Similarly, when 1 mg of superoxide dismutase was added to the selenomethionine cocktail prior to the addition of methioninase very little CL was observed (Table 5). The CL generated by methioninase activity upon selenomethionine in the presence of GSH was quenched by 94% in the presence of SOD. These CL results are nearly identical to our previous report showing the generation of superoxide by methylselenol upon reduction of dimethyldiselenide and methylseleninic acid with GSH and CL quenching by SOD [27].

Additional studies (Table 6) found that increasing amounts of SOD would not fully quench CL from the selenomethionine–methioninase catalysis in the absence of GSH indicating the generation of the putative selenium radical which would reduce oxygen producing superoxide and be quenched by SOD. Heated SOD did not restore CL from the selenomethionine–methioninase reaction as expected. Bovine serum albumin (BSA) was then used to replace SOD and actually increased CL. BSA heated in like manner to SOD also quenched the CL. We interpret this data as evidential of the presence of the putative selenium radical detected by CL from the selenomethionine–methioninase reaction and inhibition of that radical by denatured protein. The selenomethionine–methioninase CL could be almost 100% quenched by ascorbic acid.

These experiments, in addition to showing (methylselenol) generation of superoxide-induced CL from selenomethionine alone or in the presence of GSH, also show that methylsulfide does not redox cycle as the addition of the methioninase to methionine generated no detectable CL (Tables 3 and 4; $P < 0.0001$). It has been previously reported that selenomethionine ($K_m = 0.51$ mM) is in fact a better substrate for methioninase than is methionine ($K_m = 1.33$ mM) [26]. The specificity of the methioninase for selenomethionine and apparent greater specificity for selenoethionine than but not SeMC (Table 3) in generating superoxide is notable even though selenoethionine is a substrate, likely generating ethylselenol. SeMC is not a good substrate for methioninase, as is selenomethionine ($P < 0.001$), being one methylene smaller than selenomethionine on the amino acid side of selenium and selenoethionine being one methylene larger on the methyl side of selenium (Fig. 1). The inference from this data is that methioninase present in many animal and human tissues, to include human kidney and liver tissues in particular [34] can metabolize dietary amounts of selenomethionine directly to methyselenol at normally nontoxic concentrations, thereafter methylselenol is methylated to dimethylselenide and the trimethylselenonium ion. That selenoethionine is

Table 5
Methioninase-dependent generated chemiluminesence (CL) from selenomethionine and GSH containing cocktail

Methioninase units	CL units
0.05	7160
0.10	12256
0.15	22048
0.20	20011
0.15 + 2 mg ascorbic acid	7600
0.15 + 1 mg superoxide dismutase	1378

CL data is the sum integration of counts over 12.5 min.

Table 6
Sum total chemiluminesence (CL) from selenomethionine and methioninase catalysis without GSH and with and without superoxide dismutase and bovine serum albumin over 20 min

Selenomethionine blank	110
+methioninase 0.150 U	11324
Superoxide dismutase (SOD) blank	372
SOD 50 μg + methioninase 0.150 U	9593
SOD 100 μg + methioninase 0.150 U	4232
SOD 150 μg + methioninase 0.150 U	4649
SOD 200 μg + methioninase 0.150 U	3676
Selenomethionine blank	110
+Methioninase 0.150 U	6374 ± 1202 (N = 4)
+SOD 200 μg	3676
+SOD heated 100° (5 min)	685
+BSA 200 μg	10575
+BSA heated 100° (5 min)	2614
+Ascorbic acid 1 mg	4730
+Ascorbic acid 10 mg	615

Assays above in 800 μL total volume instead of 600 μL as described in Section 2. SOD and BSA boiled in a microwave oven (5 min) in a closed vial.

a substrate for methioninase but not SeMC indicates that not only methyl and ethylselenols can be generated by the enzyme but also likely amino acid selenopropanols, selenobutanols, etc., may also be generated.

Based upon our observations here showing that SeMC was not a good substrate for methioninase that would generate methylselenol, there must be other enzymes associated with cancer, i.e. beta-lyases, [35] in cancer or precancerous cells that can better generate methylselenol from SeMC as methioninase does from selenomethionine. Such beta-lyase enzymes have been exploited in human kidney whereby various selenocysteine-conjugates have been used as prodrugs [34,35]. From these selenocysteine-conjugates, beta-lyases as well as amino acid oxidases [36] have been used to intentionally generate pharmacologically active selenols from SeMC, Se-allylselenocysteine, Se-phenylselenocysteine, and Se-benzylselenocysteine. Since SeMC does not accumulate in primary protein structure, as does selenomethionine [7], the concentration of potential methylselenol generation will always be higher and perhaps more concentrated in cancer cells on an equivalent selenium basis where beta-lyase or amino acid oxidase activity may be up-regulated in cancer cells. The experimental observations here and the inferences derived may account for the increased effectiveness of SeMC over selenomethionine and other selenium metabolites as dietary carcinostatic selenium supplements in the many animal studies now reported (Table 1).

We have recently utilized similar CL experiments, as described herein, to see if enzymes are present in fish eggs[1] and cancer cells[2] that can catalyze the formation of methylselenol and generate CL from selenomethionine or SeMC. Methylselenol formation can also be monitored by other than CL techniques as described by Rooseboom from animal and human tissues using beta-lyase and amino acid oxidase activity used with the selenocysteine conjugated prodrugs which produce renal apoptosis following activation [34].

Despite its unreactive nature alone in *in vitro* CL assays, selenomethionine is particularly toxic to aquatic birds and their eggs [37]. Environmental exposures to selenium have led to toxic and teratogenic effects in fish [38,39] and aquatic birds [40]. Similar teratogenic results have been induced by selenium compounds as experimentally observed in chickens [37]. While examining the reasons for the differences between *in vitro* cell and *in vivo* exposures to selenomethionine, we have observed that selenomethionine generates superoxide when fish egg homogenates are added to the above CL cocktail (see footnote 1). This CL activity is likely do to the fact that fish eggs will produce a methioninase enzyme for the metabolism of methionine contained within the egg protein during embryonic maturation. The egg methioninase so synthesized during embryogenesis, we speculate, generates significant amounts of methylselenol *in situ* from the elevated selenomethionine present from parental selenomethionine ingestion causing chick or fry teratogenesis via free radical mutations of cellular DNA. This may explain the unusual toxicity of selenomethionine to bird and fish eggs. We had previously shown that the superoxide production by fish egg homogenates from selenomethionine is experimentally observed within a relatively narrow time window of occurrence. The enzyme(s) does (do) not appear until early liver function begins, presumably when methioninase activity arises and before superoxide dismutase production within the fish egg which prevents toxicity in the later developmental stages of the embryo (see footnote 1).

Eggs of aquatic birds and fish can accumulate significant amounts of nontoxic selenomethionine within the egg protein during oogenesis when the adult bird or fish is exposed to high dietary selenomethionine within a contaminated food chain. The presence of an apparent egg methioninase detected during fish embryogenesis at the time of liver formation would appear to generate significant amounts of methylselenol *in situ* from the elevated selenomethionine. The presence of this enzyme causes the unusual toxicity of selenomethionine to the embryonic fish fry in the absence of significant antioxidant protection but not the adult parent. The methioninase activity observed in fish eggs, we now speculate, is also the likely cause of teratogenesis in aquatic birds and may extend to other egg laying reptiles and amphibians, i.e. frog eggs that may have accumulated selenomethionine.

Again using CL, we have preliminarily looked at apparent methioninase and beta-lyase or amino acid oxidase activity in three human prostate cancer cell lines (see footnote 2). We apparently have detected methioninase-like activity in one cancer cell line using selenomethionine as substrate and beta-lyase or amino acid oxidase-like activity in two of the three cancer cell lines examined using SeMC as substrate. We deduce from our observations with fish eggs that biopsies of human cancer tissues might be used to predict the outcome of therapeutic intervention with selenium prodrugs carrying methylselenol, as a synthetic methyl ether, as in selenomethionine or SeMC that could generate methylselenol by predictable and measurable endogenous enzymes.

In summary, methylselenol, really methylselenide, CH_3Se^-, as well as other selenides, redox cycle as showed for the reduction of dimethyldiselenide and methylseleninic acid by GSH and herein for the reaction of methioninase with selenomethionine producing an initial selenium radical. Redox cycling, oxidative stress-induced apoptosis by methyl and other selenides appears to account for the carcinostatic attributes of selenium supplementation to animals and humans. Paradoxically, this redox cycling also likely accounts for the teratogenesis observed in the

[1] Palace VP, Spallholz JE, Holm J, Wautier K, Evans RE, Baron CL. Metabolism of selenomethionine by rainbow trout (*O. mykiss*) embryos can generated oxidative stress. Ecotoxicol Environ Saf, in press.

[2] Spallholz, JE. Unpublished data; 2002.

wild, in birds, in fish and possibly amphibians. Therapeutic redox cycling by methylselenol against human cancers can be delivered by dietary supplements such as selenomethionine or Se-methylselenocysteine [41,42]. In being just one methylene smaller than selenomethionine, this small molecular difference may explain why SeMC, a nonprotein selenoamino acid produced by garlic, broccoli, onions, and other Allium plants is reported to be the most potent natural selenium carcinostatic compound (Table 1 [7]). Selenides other than methylselenol that generate superoxide, ethyl, propyl, or butylselenides may also be delivered as prodrugs or as targeted drugs to treat cancers, bacterial, viral infections and bacterial biofilms. All these applications are possible and some are now becoming a reality by understanding selenium's free radical chemistry in biological systems [43,44].

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