



Mutagenicity comparison of nine bioselenocompounds in three *Salmonella typhimurium* strains



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ABSTRACT

Selenium (Se) is an essential element in animals but becomes severely toxic when the amount ingested exceeds the adequate intake level. It is known that the toxicological effects of Se are highly dependent on its chemical form. In this study, we evaluated the mutagenicity of nine naturally occurring Se compounds or the so-called bioselenocompounds, including selenite, selenate, selenocyanate, selenomethionine, selenocystine, *Se*-methylselenocysteine, selenohomolanthionine, *N*-acetylgalactosamine-type selenosugar, and trimethylselenonium ion, by using the Ames test. *Salmonella typhimurium* TA98, TA100, and TA1535 were used for the mutagenicity evaluation in the presence or absence of S9 mix, a metabolic activator. Only selenate showed weak mutagenicity even in the absence of S9 mix. None of the bioselenocompounds except selenate exhibited mutagenicity in all the strains tested in the presence or absence of S9 mix. Selenomethionine and selenocystine reduced the number of colonies in all the strains although no other selenoamino acids exerted the same effect. These results indicate that selenate directly or indirectly injures genome. Among the bioselenocompounds tested, selenomethionine and selenocystine show antibacterial activity, but the mechanism is unclear.

1. Introduction

Selenium (Se) is an essential trace element in animals and exists in selenoproteins as selenocysteine (SeCys). Although human and animals ingest Se in various chemical forms via foods and feeds, Se deficiency has been reported. Keshan disease is reported to originate in Northeast China where soil Se concentration is very low [1]. In the clinical setting, Se deficiency is often reported in patients receiving total parenteral nutrition (TPN) and sick children who are given special milk for specific metabolic diseases [2]. In these cases, Se supplements are frequently prescribed. Se has ambivalent effects, i.e., Se can be highly toxic when the amount ingested exceeds the adequate intake level. In addition, the adequate physiological range between deficient and excess doses is narrow [3], being one order of magnitude (the recommended dietary allowance for Japanese male adult: 30 µg/day; tolerable upper intake level for Japanese male adult: 420 µg/day). The World Health Organization also suggests 40 and 400 µg/day of the lower and upper limits, respectively, for male adults [4]. The toxicity and bioavailability of Se are markedly dependent on the chemical form of Se. Generally, inorganic Se species, such as selenite, are more toxic than organic Se species. The chemical species of Se used in the clinical setting in Se-deficient patients are selenomethionine (SeMet), selenite, and selenate.

Then, other naturally occurring selenoamino acids such as *Se*-Methylselenocysteine (MeSeCys), γ -glutamyl-*Se*-methylselenocysteine [5,6], and selenohomolanthionine (SeHLan) [7] are also feasible species for use in the clinical setting because they are known as plant selenometabolites. 1 β -Methylseleno-*N*-acetyl-D-galactosamine (SeSug1) [8] and trimethylselenonium ion (TMSe⁺) [9], which are human and animal Se metabolites, are also candidates because these Se metabolites are able to be assimilated [10,11]. It was reported that selenocyanate (SeCN⁻) is a Se metabolite in mammalian cells [12]. As mentioned above, it is generally known that inorganic Se species are more toxic than organic ones [13]. However, at present, there is limited information on the toxicity of the possible nutritional and clinical sources of Se.

Se can transit between -II and +VI oxidation states in an organism. Because of its biological characteristics, Se plays ambivalent roles as an antioxidant and a prooxidant in organisms. As an antioxidant, Se forms the active center of antioxidative enzymes, such as glutathione peroxidase [14–17]. It has been shown that Se can induce oxidative stress by producing reactive oxygen species (ROS). These effects strongly depend on the chemical species of Se. According to our previous work [13], the nine Se compounds mentioned above were defined as bioselenocompounds. The bioselenocompounds are composed of several types of compounds, including selenoamino acids, a

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selenosugar, and inorganic species. In this study, the mutagenicity of the bioselenocompounds was compared by the Ames test. Three strains of *Salmonella typhimurium*, TA98, TA100, and TA1535, were used. TA1535 is known to be sensitive to the mutagenicity induced by ROS [18]. Thus, the indirect effect of bioselenocompounds generating ROS on the mutagenicity was also evaluated.

2. Materials and methods

2.1. Selenium compounds

Sodium selenate and potassium selenocyanate (SeCN^-) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sodium selenite and seleno-L-methionine (SeMet) were purchased from Nacalai Tesque (Kyoto, Japan). Se-Methylseleno-L-cysteine (MeSeCys) and L-selenocystine (SeCys_2) were purchased from Acros Organics (Waltham, MA) and Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), respectively. Trimethylselenonium iodide (TMSe^+) was purchased from Tri Chemical (Uenohara, Japan). L-Selenohomolanthionine (SeHLan) and 1 β -methylseleno-N-acetylgalactosamine (SeSug1) were synthesized in our laboratory in accordance with our previous work [7,8]. The chemical structures of the bioselenocompounds used in this study are shown in Fig. 1.

2.2. Preparation of S9 mix, a metabolic activator

Our animal experiment was approved by the Animal Investigation Committee, Chiba University, Japan (No. 28-60), and carried out according to the Guidelines of the Animal Investigation Committee, Chiba University. Specific pathogen free (SPF) male Wistar rats (5 weeks of age; Clea Japan, Tokyo, Japan) were housed in a humidity-controlled room maintained at 22–25 °C with a 12 h light-dark cycle. The rats were fed a commercial diet (MF; Clea Japan) and tap water ad libitum. After a five-day acclimation period, one rat was intraperitoneally injected with phenobarbital (Wako Pure Chemical Industries, Ltd.) at the dose of 60 mg/kg body weight. The animal was sacrificed 24 h after the injection by exsanguination under anesthesia. Then, the liver was excised. An approximately 1.0 g portion of the liver was homogenized with fourfold volume of 150 mM KCl, 50 mM Tri-HCl, pH 7.4, under nitrogen atmosphere. The homogenate was separated by centrifugation at $9000 \times g$ for 20 min at 4 °C to obtain the supernatant. The supernatant was used to prepare S9 mix, a metabolic activator. S9 mix consisted of 8 mM MgCl_2 , 33 mM KCl, 5 mM glucose 6-phosphate, 4 mM NADP,

4 mM NADPH, 100 mM sodium phosphate, pH 7.4, and the supernatant at the concentration of 0.1 mL/mL of mixture.

2.3. Mutagenicity assay – the Ames test

The mutagenicity of the bioselenocompounds was determined by the Ames test. Three *Salmonella typhimurium* strains, TA98, TA100, and TA1535, were obtained from NITE Biological Resource Center (Kisarazu, Japan). All strains were grown by overnight culture in 10 mL of liquid nutrient medium containing 0.8% Difco™ nutrient broth (Wako Pure Chemical Industries, Ltd.) and 0.5% NaCl in a shaker at 37 °C. All tests were performed in the presence or absence of S9 mix. A 2 mL portion of top agar contained 0.6% agar (Nacalai Tesque), 0.6% NaCl, 50 μM D-biotin (Nacalai Tesque), 50 μM L-histidine (Nacalai Tesque), 100 μL of overnight bacterial culture, 100 μL of the bioselenocompound solution at the concentrations of 0.05, 0.5, 5, 10, 20, 50, and 100 μg Se/mL, and 500 μL of S9 mix or 500 μL of phosphate buffered saline (PBS). The sample to which PBS was added instead of a bioselenocompound served as the negative control. The top agar was applied on a minimal nutrient plate containing 0.02% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (Nacalai Tesque), 0.2% citric acid \cdot H_2O (Nacalai Tesque), 1.0% K_2HPO_4 (Nacalai Tesque), 0.19% $(\text{NH}_4)_2\text{H}_2\text{PO}_4$ (Nacalai Tesque), 0.067% NaOH (Nacalai Tesque), 2.0% D-(+)-glucose (Nacalai Tesque), and 1.5% agar (Nacalai Tesque) in the volume of 20 mL per plate. 2-Aminoanthracene (2-AA, Wako Pure Chemical Industries, Ltd.) at the concentration of 10 $\mu\text{g}/\text{mL}$ was used as the positive control for the three strains when S9 mix was added. 2-Nitrofluorene (2-AF, Tokyo Chemical Industry Co., Ltd.) and sodium azide (AZ, Wako Pure Chemical Industries, Ltd.) each at the concentration of 10 $\mu\text{g}/\text{mL}$ served as the positive control for the detection of frameshift (TA98) and point (TA100 and TA1535) mutations, respectively. After 48 h incubation at 37 °C, the revertants were counted and the mutation quotient was calculated as the number of revertants in the sample divided by the number of revertants in the negative control. A bioselenocompound was considered mutagenic when the mutation quotient was higher than 2. Statistical analysis was performed by the Tukey test. The level of significance was set at $p < 0.05$, and values that have a different letter are significantly different.

3. Results and discussion

The mutation quotients of the positive control for all strains were higher than 50. Therefore, the experimental conditions for the

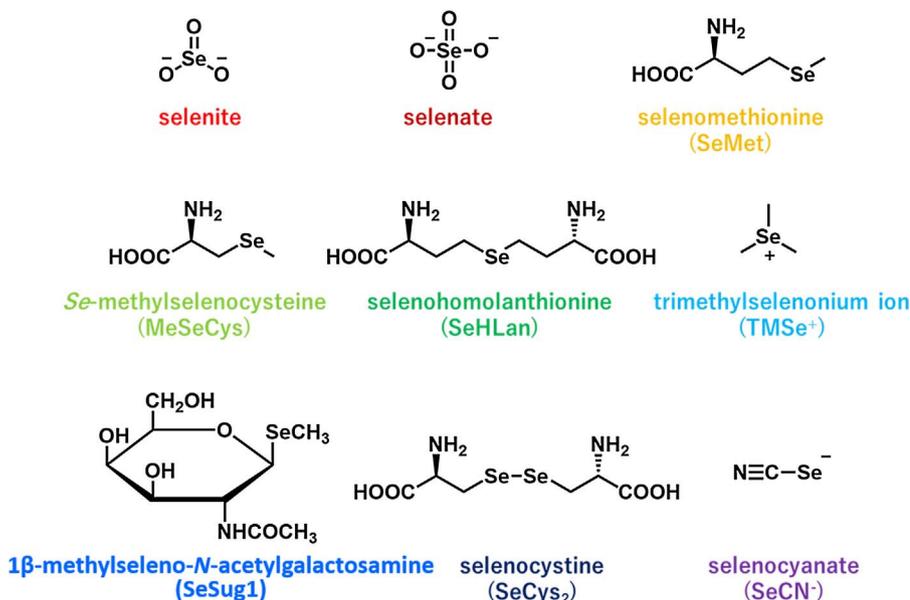


Fig. 1. Structures of bioselenocompounds used in this study. SeCN^- : selenocyanate, SeMet: selenomethionine, MeSeCys: Se-methylselenocysteine, SeHLan: selenohomolanthionine, SeCys_2 : selenocystine, SeSug1: 1 β -methylseleno-N-acetylgalactosamine, TMSe^+ : trimethylselenonium ion.

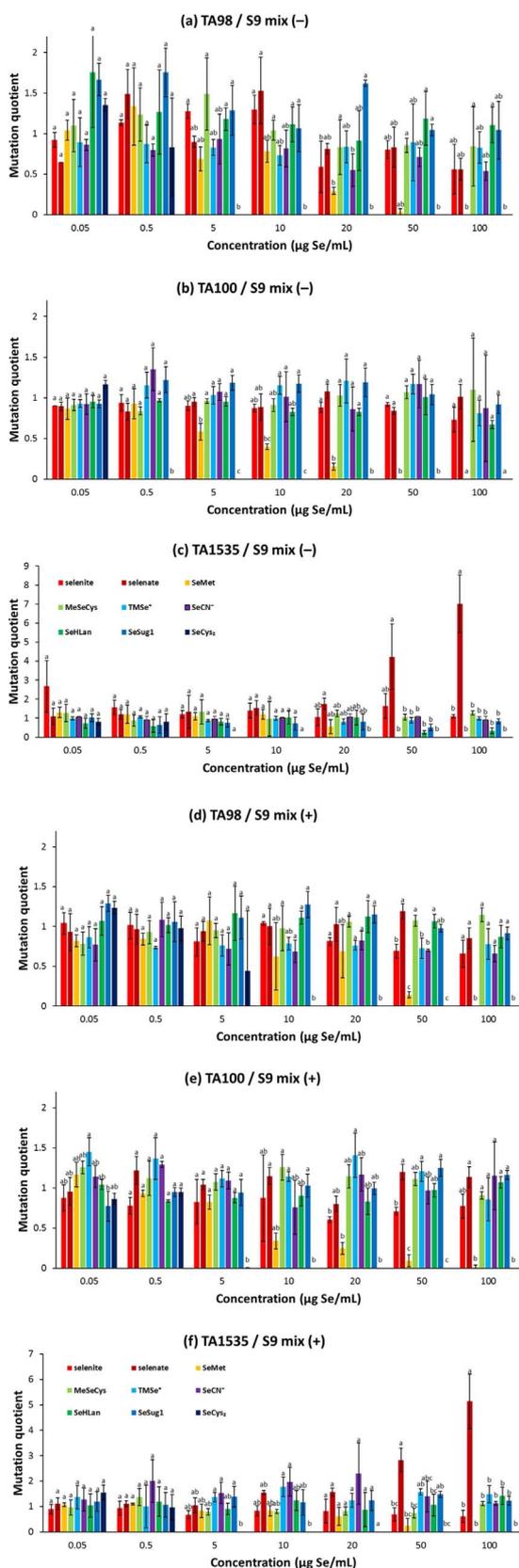


Fig. 2. Mutagenicity of bioselenocompounds determined by the Ames test. Three strains of *Salmonella typhimurium*, TA98 (panels a and d), TA100 (b and e), and TA1535 (c and f), were exposed to selenite, selenate, SeCN⁻, SeMet, MeSeCys, SeHLan, SeCys₂, SeSug1 or TMSe⁺ with (d–f) or without (a–c) S9 mix as the metabolic activator. Statistical analysis was performed by the Tukey test. The level of significance was set at *p* < 0.05, and values that have a different letter are significantly different.

evaluation of mutagenicity were appropriate. The means of the mutation quotients of the bioselenocompounds except SeMet and SeCys₂ at the doses examined were lower than 2 in TA98 and TA100 either with or without S9 mix (Fig. 2a, b, d and e). In addition, there were no significant changes in the mutation quotients of the bioselenocompounds except SeMet and SeCys₂ under the experimental conditions. TA98 can detect frameshift mutation, and TA100 can detect point mutation induced by a chemical substance. The results indicate that the bioselenocompounds except SeMet and SeCys₂ have no direct effects on DNA injury that would result in mutagenicity.

The mutation quotients of SeMet and SeCys₂ were dose-dependently and significantly decreased in TA98 and TA100 either with or without S9 mix. The decrease was due to the reduction in the number of revertants, indicating that SeMet and SeCys₂ exhibited antibacterial activity toward TA98 and TA100. It has already suggested that selenomethionine and its metabolite, selenohomocystine, have an antibiotic activity [19]. Selenohomocystine has the quite similar structure as SeCys₂. Our observations, therefore, are coincident with the literature. Although MeSeCys has a similar structure to SeMet and SeCys₂, it did not show the activity. In addition, methionine, a sulfur analog of SeMet, did not show antibacterial activity up to the dose of 1.27 mM, which corresponded to 100 µg Se/mL (data not shown). It has been reported that SeMet and MeSeCys stimulate the proliferation of mammalian cells [13]. In contrast to mammalian cells, SeMet exerted an inhibitory effect on bacterial cells. On the other hand, SeCys₂ exhibited cytotoxic effects on mammalian cells [13]. It is known that some sulfur-containing amino acids, such as lanthionine derivatives, possess antibacterial activity and are therefore used in the clinical setting as antibiotics meaning antibiotics with lanthionine derivatives [20]. However, no antibacterial activity was noted in SeHLan, a lanthionine derivative. Further studies are needed to unambiguously explain the antibacterial activities of SeMet and SeCys₂.

It has been reported that TA1535 can detect the mutagenicity induced by ROS [21]. Selenate was the only compound that showed a dose-dependent increase in mutation quotient in TA1535 among the bioselenocompounds tested, either with or without S9 mix (Fig. 2c and f). Although the mutation quotient of selenate was low compared to the positive control, the mutagenicity of selenate was obvious in TA1535. These results suggest that selenate indirectly induces mutagenicity by producing ROS. Selenite did not show apparent mutagenicity in TA1535, indicating that the mutagenicity of selenate is not a common property of inorganic Se species but a specific property of selenate. However, vitamin C (VC) and vitamin E (VE) were unable to protect mutagenicity of selenate under our experimental condition (Fig. S1 as a Supplemental material). Thus, it could be speculated that selenate produces DNA adducts in TA1535 although further experiments are needed [22].

SeMet and SeCys₂ exhibited reduced mutation quotients in TA1535 in a dose-dependent manner, suggesting that SeMet and SeCys₂ showed antibacterial activity on this strain as well, as was observed in TA98 and TA100. Selenium-enriched (selenized) yeast is frequently used as a nutritional supplement of Se. Selenized yeast contains almost Se in the form of SeMet. Our observations suggest that Se supplement containing selenized yeast has a possibility to disturb the intestinal flora. Therefore, SeMet seems not to be suitable for use as Se supplement particularly for oral administration. It is speculated that SeCys₂ does not exist in food because SeCys, a reduced form of SeCys₂, is incorporated into selenoproteins. The health risk posed by SeCys₂ seems to be lower than that by SeMet.

In conclusion, bioselenocompounds except selenate showed no apparent mutagenicity. Selenate had low mutagenicity and SeMet possessed antibacterial activity. In addition, selenate showed a strong association with Alzheimer's dementia risk [23,24]. From the perspective of toxicology, both selenate and SeMet are inappropriate for use as Se supplement.

Conflict of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.toxrep.2018.01.005>.

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