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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. 1β-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 1β-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 × *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% $MgSO_4{\cdot}7H_2O$ (Nacalai Tesque), 0.2% citric acid·H_2O (Nacalai Tesque), 1.0% K_2HPO_4 (Nacalai Tesque), 0.19% $(NH_4)H_2PO_4$ (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 μg/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 μg/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

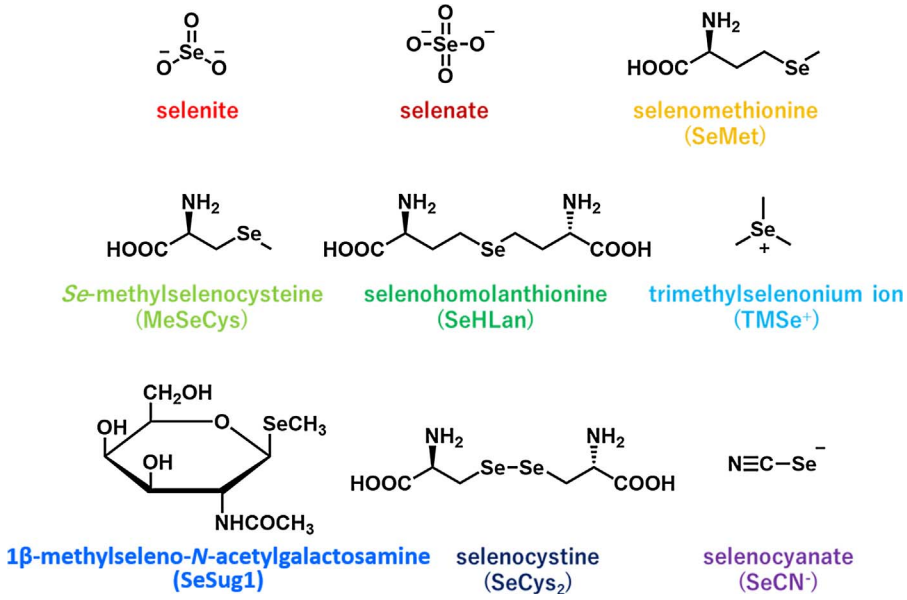

Fig. 1. Structures of bioselenocompounds used in this study. $SeCN^-$: selenocyanate, SeMet: selenomethionine, MeSeCys: *Se*-methylselenocysteine, SeHLan: selenohomolanthionine, $SeCys_2$: selenocystine, SeSug1: 1β-methylseleno-*N*-acetylgalactosamine, $TMSe^+$: trimethylselenonium ion.