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Mutual detoxification of mercury and selenium in unicellular *Tetrahymena*

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ABSTRACT

Selenium (Se) is commonly recognized as a protective element with an antagonistic effect against mercury (Hg) toxicity. However, the mechanisms of this Hg–Se antagonism are complex and remain controversial. To gain insight into the Hg–Se antagonism, a type of unicellular eukaryotic protozoa (*Tetrahymena malaccensis*, *T. malaccensis*) was selected and individually or jointly exposed to two Hg and three Se species. We found that Se species showed different toxic effects on the proliferation of *T. malaccensis* with the toxicity following the order: selenite (Se(IV)) > selenomethionine (SeMeth) > selenate (Se(VI)). The Hg–Se antagonism in *Tetrahymena* was observed because the joint toxicity significantly decreased under co-exposure to highly toxic dosages of Hg and Se *versus* individual toxicity. Unlike Se(IV) and Se(VI), non-toxic dosage of SeMeth significantly decreased the Hg toxicity, revealing the influence of the Se species and dosages on the Hg–Se antagonism. Unexpectedly, inorganic divalent Hg (Hg^{2+}) and monomethylmercury (MeHg) also displayed detoxification towards extremely highly toxic dosages of Se, although their detoxifying efficiency was discrepant. These results suggested mutual Hg–Se detoxification in *T. malaccensis*, which was highly dependent on the dosages and species of both elements. As compared to other species, SeMeth and MeHg promoted the Hg–Se joint effects to a higher degree. Additionally, the Hg contents decreased for all the Hg–Se co-exposed groups, revealing a sequestering effect of Se towards Hg in *T. malaccensis*.

Introduction

Mercury (Hg) is recognized as a global toxic pollutant (Jiang et al., 2006; Driscoll et al., 2013). The toxicity of Hg depends on both its concentration and species (Fitzgerald et al., 2007; Du et al., 2015; Shao et al., 2016). In aquatic systems, inorganic divalent mercury (Hg^{2+}) and monomethylmercury (MeHg) are the main species and their toxicity has been paid great attention (Fitzgerald et al., 2007; Chen et al., 2013; Peng et al., 2015). Considering the environmental risks, it is essential to explore potential pathways for mitigating the toxicity of Hg.

Selenium (Se) is commonly regarded as a protective element with an antagonistic effect against Hg. As in the case of Hg, Se occurs naturally on Earth. Unlike Hg, Se is an essential trace element for human body since it is incorporated into the activities of antioxidant selenoenzymes (Stadtman, 1991; Wyatt et al., 2016). With regard to *Tetrahymena*, the selenocysteine tRNA has been identified in *Tetrahymena thermophila* (Shrimali

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et al., 2005), indicating the need for Se of this species and the potential similarity for other *Tetrahymena* species.

Tetrahymena is a type of unicellular eukaryotic protozoa located at the bottom of food chain. Owing to its rapid proliferation, unique nuclear dualism, extensive membrane structure and fast reaction upon external exposure, *Tetrahymena* has been used as a useful model organism for evaluating the toxicity and environment risks of chemicals. For example, *Tetrahymena* has been used to evaluate the toxicity of 33 organic compounds with different structures and to explore the carrier effect of TiO_2 nanoparticles on Cd bioaccumulation (Schramm et al., 2011; Yang et al., 2014). Given the wide distribution and low trophic level of *Tetrahymena* in freshwater ecosystems, the Hg uptake of *Tetrahymena* is an original and essential pathway for Hg entering food chains that can affect the transportation and transformation of Hg in the environment.

Although the existence of Hg–Se antagonism has been confirmed, the joint effects of Hg and Se are very complex and the antagonistic mechanisms are still unclear (Ganther et al., 1972; Sumino et al., 1977; Wang et al., 2016; Tang et al., 2017). The antagonistic effect has been previously proposed to involve the formation of Hg–Se complexes. These complexes are considered to decrease the bioaccumulation of Hg by reducing the uptake or promoting the removal of Hg in organisms (Sormo et al., 2011; Zhang et al., 2012; Zhao et al., 2014). Recent studies have shown that the Hg–Se antagonism in marine fish or *Caenorhabditis elegans* is highly dependent on the chemical species involved (Dang and Wang, 2011; Wyatt et al., 2016), although inconsistent results were also obtained for the joint effects of Se and MeHg. In addition, the influences of the Hg species and the dosages of Hg and Se remain uncertain. While Se has been typically regarded as a beneficial element for organisms at trace dosages, its role (*i.e.*, protective or toxic agent) under high dosages has been controversial for a long time (Hilton et al., 1980; Hodson and Hilton, 1983; Spallholz, 1994; Lemly, 2002; Hoffman, 2002; Hamilton, 2004; Branco et al., 2014; Aborode et al., 2016; Friesen et al., 2017). Thus, further detailed studies dealing with the Se toxicity and the effects of the species and dosages on the Hg–Se antagonistic mechanisms should be carried out.

This work was aimed to explore the joint effects of different species of Se and Hg at varying dosages by using a novel unicellular model organism. Thus, a type of eukaryotic protozoa, *Tetrahymena malaccensis* (*T. malaccensis*), was selected and subsequently exposed to two Hg and three Se species under various dosages. The cell numbers and total contents of Hg and Se in cell bodies were analyzed after individual or joint exposure. The effects of the different species and dosages of Se and Hg on the Hg–Se antagonism were discussed in detail. We revealed herein, for the first time, the detoxification of Hg towards highly toxic dosages of Se.

1. Materials and methods

1.1. *Tetrahymena* species and culture methods

T. malaccensis was kindly provided by Dr. Wei Miao from the Institute of Hydrobiology of the Chinese Academy of Sciences (Wuhan, China). The *T. malaccensis* used herein was grown axenically at 28°C in a medium rich in proteose peptone (Morin and Cech, 1988). The culture medium was comprised of 2% (*w*/*v*) proteose peptone (Becton, Dickinson and Company, USA), 0.2% (*w*/*v*) glucose (Sigma, USA), 0.1% (*w*/*v*) yeast extract (OXOID, Thermo Fisher Scientific, USA), and 0.003% (*w*/*v*) ferric citrate (Sigma, USA) dissolved in 1000 mL of ultra-pure water (Millipore, Darmstadt, Germany) containing a 1% (*v*/*v*) penicillin–streptomycin solution (10,000 units/mL penicillin and 10,000 mg/L streptomycin, HyClone, GE Healthcare Life Sciences, USA) (Liu et al., 2017).

1.2. Exposure to gradient dosages of the three Se species

The selected three Se species, sodium selenite (Se(IV)), sodium selenate (Se(VI)), and selenomethionine (SeMeth), were all obtained from Sigma-Aldrich (USA). The exposure was carried out at the early logarithmic growth phase of *T. malaccensis* with same dosage ranges (*i.e.*, 0, 0.1, 1, 10, 100, 1000, and 10,000 μM), following a previous procedure (Wyatt et al., 2016). After exposure for 24 hr, 500 μL of the cell suspension were mixed with the same volume of a phosphate buffered saline (PBS, GE Healthcare Life Sciences, USA) solution for all groups, and the mixtures were counted by flow cytometry (Accuri C6, BD, USA). The effect of Se on *T. malaccensis* was calculated by the ratio of the cell numbers in the Se-treated groups to those in the control group. In order to observe the toxicity of Se species, *T. malaccensis* cells were photographed with a laser scanning confocal fluorescence microscope (Leica, TSC SP5, USA) after exposure to individual Se species. Three parallel experiments were carried out for each group.

1.3. Co-exposure to multiple dosage combinations of two Hg species and three Se species

Based on the results of growth inhibition induced by individual Se species, the dosages of Se species used for the co-exposure experiments were 0, 10 (low dosage, "L") and 1000 (high dosage, "H") μM. This range covered highly toxic (1000 μM Se(IV) and SeMeth) and non-toxic (the remaining) dosages. For Hg species, the following dosages were selected according to our previous work: 0; 5 μM Hg^{2+} and 4 μM MeHg (representing high dosages producing inhibitions larger than 20%, "H"); 1 μM Hg^{2+} and 0.5 μM MeHg (non-toxic low dosages, "L") (Liu et al., 2017). Either individual or combined solution was added to the medium at the early logarithmic growth phase. The individual Hg and Se solutions were used as the control, while the pure medium exclusively containing *T. malaccensis* cells (no Hg or Se addition) was used as the blank. After 24 hr exposure, the cell numbers of all groups were counted by flow cytometry. All the experiments were repeated three times.

1.4. Analysis of the total Hg and Se contents in *T. malaccensis* cells

After counting, the *T. malaccensis* cell samples were cleaned for three times (Liu et al., 2017). Then, the cell suspensions were centrifuged, collected and digested in a microwave digestion system (MASTER-40, Shanghai Sineo Microwave Chemistry Technology, China). In detail, 8 mL of concentrated HNO_3 (65%, *v*/*v*) and 2 mL of H_2O_2 (30%, *v*/*v*) were added to Teflon®

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