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Effect of ionic strength on bioaccumulation and toxicity of silver nanoparticles in *Caenorhabditis elegans*

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ABSTRACT

The behavior of silver nanoparticles (AgNPs) is influenced by environmental factors which altered their bioaccumulation and toxicity. In this study, we comprehensively investigated the influence of ionic strength on the ecotoxicity of AgNPs to *Caenorhabditis elegans* (*C. elegans*) through the transfer from *Escherichia coli (E. coli)*. Three different exposure media (deionized water, EPA water and KM) were used to pretreat AgNPs. *E. coli* was then exposed to these transformed AgNPs and fed to *C. elegans*. Our results indicated that ionic strength significantly enhanced the reproductive toxicity (germ cell corpses, brood size and lifespan) and neurotoxicity (head trash and body bend) of AgNPs in *C. elegans*. Moreover, ICP-MS analysis showed that higher ionic strength increased bioaccumulation of AgNPs in *E. coli* and the resulting Ag body burden of *E. coli* affected the transfer of AgNPs to *C. elegans*, which might be responsible for the increased toxicity to nematodes. Furthermore, we also found that the reactive oxygen species (ROS) level in *C. elegans* was significantly increased after exposed to *E. coli* contaminated with ionic strength-treated AgNPs, which might play another important role for the enhanced toxicity of AgNPs. Overall, this study showed that the bioavailability and potential ecotoxicity of AgNPs are associated with the environmental factors.

1. Introduction

Silver nanoparticles (AgNPs) are one of the most widely used engineered nanomaterials owing to their antibacterial and physicochemical properties (Ahamed et al., 2010; Cupi et al., 2016). The application of AgNPs in a large number of products will inevitably result in their release into the environment, especially the aquatic environment (Maurer-Jones et al., 2013; Seltenrich, 2013). Previous studies have demonstrated that AgNPs pose potential risks to the environment as well as to different organisms, including bacteria, plants and aquatic organisms (Massarsky et al., 2014; Wijnhoven et al., 2009). Moreover, AgNPs have been demonstrated to be trophic transferred on the food web and distributed in higher trophic levels (Chae et al., 2016; Kubo-Irie et al., 2016; Servin et al., 2017). In addition, our recent study and others showed that the accumulated AgNPs in higher trophic levels through food chain could cause toxicity (Kwak and An, 2016; Luo et al., 2016; McTeer et al., 2014).

It has been showed clearly that environmental factors (such as ionic strength, pH, or ionic type) would transform AgNPs from their original state (Minghetti and Schirmer, 2016; Zhang et al., 2016). And the bioavailability of AgNPs is dependent upon their fate and behavior in the exposure medium. For example, increasing salinity has been shown to increase the bioaccumulation of AgNPs in fish (Salari Joo et al., 2013). The ionic concentration also influenced the cellular accumulation of AgNPs (Minghetti and Schirmer, 2016). Furthermore, the toxicity of AgNPs is sharply changed after transformed in exposure medium (Behra et al., 2013; Chambers et al., 2014; Cupi et al., 2016; Fabrega et al., 2011; Luo et al., 2017). Chambers et al. (2014) reported the agglomeration and toxicity of AgNPs were enhanced in higher ionic strength. Cupi et al. (2016) demonstrated that different pH in exposure media were expected to influence the behavior and the toxicity of AgNPs. These data indicated that the dynamic transformations in turn affected the fate and toxicity of AgNPs in the environment, making it critical to investigate the toxicity of these transformations.

Based on our previous study, AgNPs can transfer from *E. coli* to nematodes and cause serious toxicity to *C. elegans* (Luo et al., 2016).

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Meanwhile, *C. elegans* is suitable for econanotoxicity research due to its important roles in benthic and soil food web (Hanna et al., 2016). In the present study, we chose ionic strength as an environmental factor, to further investigate the bioaccumulation and ecotoxicity of transformed AgNPs to *C. elegans* through food chain. We demonstrated that ionic strength conduced a significant increase on AgNPs bioaccumulation and toxicity, highlighting important roles of environmental factors in assessment of the potential risk of nanomaterials to the environment.

2. Materials and methods

2.1. AgNPs preparation and characterization

Suspensions of PVP-AgNPs (25 nm: PVP25, 75 nm: PVP75) were purchased from NanoComposix, Inc. in the form of stock solutions (5 g/L). The stock AgNPs solution was stored at 4 °C in the dark. Extensive characterization of identical AgNPs in DI water, EPA water or KM has been studied in a parallel study. The particle size and shape of AgNPs were determined with transmission electron microscopy (TEM, JEM-2011, Japan). The hydrodynamic diameter and zeta potentials of PVP25 and PVP75 in solution were measured by dynamic light scattering (DLS) using a Zetasizer system (Malvern Nano series, Malvern, UK). The hydrodynamic diameter of PVP25 and PVP75 is stable at ~ 51 nm and ~ 140 nm, and the zeta potentials are −14.3 mV and −21.0 mV, respectively.

2.2. Exposure of AgNPs in different ionic strength media

The choice of exposure media was based on the commonly-used liquid culture media which are usually used for assessing nanoparticle toxicity in nematodes (Hanna et al., 2016; Huang et al., 2017; Luo et al., 2017). The composition of low ionic strength exposure medium (EPA) and high ionic strength exposure medium (KM) was supplied in Table S1. And deionized water (DI) was used as control group. From stock dispersions, the indicated concentrations of AgNPs in toxicity assays were diluted in different ionic strength media for 5 days. And then the transformed AgNPs was used to treat *E. coli*.

2.3. C. elegans strains and culture

C. elegans were grown in petri dishes on nematode growth medium (NGM) and fed with *E. coli* OP50 on the basis of a standard protocol (Brenner, 1974). For the age-synchronized assay, when nematodes have eggs in their bodies, worm eggs were isolated from mature hermaphrodites using NaClO/NaOH solution. Age-synchronous L1 nematodes were obtained from eggs maintained overnight in M9 medium. Strains N2 (wild-type Bristol), CF1553 (*sod-3::GFP*), SJ4100 (*hsp-6::GFP*), and GA186 (*sod-3* deletion) were purchased from *Caenorhabditis* Genetics Center (CGC) and were maintained at 20 °C. These mutant strains were chosen based on their known mechanisms to certain toxicity. ROS was measured in the transgenic worm strain CF1553, which exhibits oxidative stress induced-expression of GFP-labeled mitochondrial manganese superoxide dismutase SOD-3 (Libina et al., 2003). The unfolded protein response of the mitochondria was measured in the transgenic worm strain SJ4100, which can be monitored by the activation of the *hsp-6* gene (Azzam et al., 2003). To further examine the role of ROS in AgNPs-induced toxicity, we investigated the number of germ cell corpses generated in worm strain GA186 (Han et al., 2017).

2.4. Nanoparticles transfer via feeding C. elegans with AgNPs-exposed E. coli

In order to study the transfer and uptake of AgNPs, we chose the simple food chain in which nematodes as highly level organisms and *E. coli* OP50 as food. Firstly, 400 µL of the recovered bacteria-containing media was added to 20 mL of fresh LB containing ionic strength transformed AgNPs with indicated concentrations (0, 0.1, 1, 5 mg/L). After cultured at 37 °C for 12 h, *E. coli* were centrifuged at 4500 rpm and washed three times with deionized water. Then, the collected *E. coli* was suspended in 2 mL deionized water. Next, 100 µL of collected *E. coli* was placed on the NGM plates as food of *C. elegans*. Finally, synchronized nematodes were added onto the NGM plates containing AgNPs-treated *E. coli* OP50 and were incubated at 20 °C for the toxicity assays.

2.5. Bioaccumulation of silver content in E. coli and C. elegans

The Ag content in *E. coli* and *C. elegans* was calculated by ICP-MS analysis. In brief, *E. coli* was exposed to 5 mg/L of AgNPs preincubated in different ionic strength for 12 h. Then, exposed *E. coli* was collected and washed three times with deionized water. For silver content measurement in *C. elegans*, nematodes were fed with AgNPs-treated *E. coli* for 24 h. After that, L4 stage nematodes were washed with deionized water three times and then transferred to fresh M9 with food to allow the gut to clear for 2 h (verification was done by microscopic analysis of *C. elegans* gut) (Maurer et al., 2016; Yang et al., 2014). Next, *E. coli* or nematodes were freeze-dried for 48 h. The resulting samples were digested using 70% nitric acid (Fisher Scientific, USA) at ~ 90 °C overnight. The digests were diluted with deionized water, and then diluted with mixed acid (0.5% HCl and 2% HNO_3 v/v) before elemental analysis with inductively coupled plasma mass spectrometry (ICP-MS) (Element 2 High Resolution Sector Field ICP-MS, Thermo Finnigan, Germany) (Maurer et al., 2016). The reliability of the measurement was determined using specific water references (M105A, IFA System, Tull, Austria) (Minghetti and Schirmer, 2016). To evaluate the food chain transfer of AgNPs from *E. coli* to *C. elegans*, a bioaccumulation factor (BCF) and a biomagnification factor (BMF) were calculated as follows:

BCF = Ag concentration in *E. coli* or *C. elegans*/ Ag concentration in exposure media

BMF = Ag concentration in *E. coli* / Ag concentration in *C. elegans*

2.6. Assessment of toxicity of transformed AgNPs in C. elegans

Germ cell death in *C. elegans* was measured by Acridine orange (AO, Sigma). L4 stage nematodes were fed with AgNPs-treated *E. coli* for 24 h. Then, the *C. elegans* were stained with AO for 1 h under dark conditions. The nematodes were then transferred to a fresh NGM plate and allowed to recover for 45 min with a food source to repel excessive AO in their intestines. Dead germ cells stained positive for AO were counted with an epifluorescence microscope (Olympus IX71, Japan).

For brood size assay, wild-type L3 stage nematodes were fed with AgNPs-treated *E. coli* for 24 h. Each worm was subsequently moved to a new NGM plate containing untreated *E. coli* every day during the egg-laying period. The number of new hatched larvae was counted after the adult nematodes were removed for 24 h. This process was repeated until the hermaphrodite stopped laying eggs. The number of brood size was the sum of all hatched larvae.

For the life span assay, after exposure to AgNPs-treated *E. coli* for 24 h, nematodes were turned to fresh NGM plates with untreated *E. coli* every day until confirmation of death, i.e., the nematodes remained unresponsive to tapping with a pick.

For the locomotion behavior assay, experimental protocols were adapted from previous studies (Wu et al., 2014; Zhao et al., 2016). L4 stage nematodes were exposed in 24-well sterile tissue culture plates in the presence of AgNPs-treated *E. coli* at 20 °C. After 24 h of treatment, locomotion behavior was assessed using the experimental end points of body bend and head thrash under the dissecting microscope. A change in the direction of the part of nematodes corresponding to the posterior bulb of the pharynx along the Y-axis was counted as a body bend. A change in the direction of bending at the mid body was defined as a head thrash.