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Citrate coated silver nanoparticles change heavy metal toxicities and bioaccumulation of *Daphnia magna*

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HIGHLIGHTS

- There were no significant differences in the acute toxicities of As(V) and Cu.
- However, the bioaccumulations of As(V) and Cu were reduced by silver nanoparticle.
- Only Cd acute toxicity and bioaccumulation were enhanced by silver nanoparticle.

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ABSTRACT

Citrate-coated AgNPs (c-AgNPs) have negatively charged surfaces and their surface interactions with heavy metals can affect metal toxicity in aquatic environments. This study used *Daphnia magna* to compare the acute toxicities and bioaccumulation of As(V), Cd, and Cu when they interact with c-AgNPs. The 24-h acute toxicities of As(V) and Cu were not affected by the addition of c-AgNPs, while bioaccumulation significantly decreased in the presence of c-AgNPs. In contrast, both the 24-h acute toxicity and bioaccumulation of Cd increased in the presence of c-AgNPs. These toxicity and bioaccumulation trends can be attributed to the interactions between the AgNP surface and the heavy metals. As(V) and c-AgNPs compete by negative charge, decreasing As(V) toxicity. Copper adheres readily to c-AgNP citrate, decreasing Cu bioavailability, and thus reducing Cu toxicity and bioaccumulation. Citrate complexes with divalent cations such as Ca and Mg reduce the competition between divalent cations and Cd on biotic ligand, increasing toxicity and bioaccumulation of Cd. This study shows that surface properties determine the effect of c-AgNPs on heavy metal toxicities and bioaccumulations; hence, further studies on the effect of nanoparticle by it surface properties are warranted.

1. Introduction

Silver nanoparticles (AgNPs) are widely used in cosmetics, textiles, and household items owing to their antibiotic properties. In 2008, approximately 500 tons/annum of AgNPs were manufactured worldwide and annual usage continues to increase (Mueller and Nowack, 2008). With increased production and usage, AgNPs are expected to be released into the environment through various routes. The predicted environmental concentration (PEC) of AgNPs in surface water, which is the main exposure route, is 0.088–10,000 ng L^{-1}, and the maximum estimated PEC in wastewater treatment plant effluent is 17 $\mu g\ L^{-1}$ (Maurer-Jones et al., 2013). For these reasons, there is a growing interest in the potential risks of AgNPs (Luoma, 2008; Panyala et al., 2008; Quadros and Marr, 2010).

Many studies have demonstrated the toxicity of AgNPs to various organisms (Oberdörster et al., 2005; Moore, 2006; Gubbins et al., 2011; Zhao and Wang, 2012). Generally, three mechanisms are regarded as the main cause of AgNP toxicity: (1) small size, inducing reactive oxygen species; (2) direct cell damage; and (3) release of the Ag ion (Marambio-Jones and Hoek, 2010; Levard et al., 2012; Yu et al., 2013). The unique size and stability of AgNPs have significant effects on their toxicity (Thio et al., 2012; Zhao and Wang, 2012). For example, Ag ions are emitted more efficiently when the AgNPs are smaller because they are more easily dissolved. High ionic strength causes the aggregation of AgNPs by weakening electrostatic repulsion (Marambio-Jones and Hoek, 2010). The aggregation of AgNPs decreases the release and bioaccumulation of Ag ions (Levard et al., 2012) by reducing the surface

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area (Yu et al., 2013). Studies have shown that the dissolution of AgNPs is promoted at low pH (Levard et al., 2012) and that the toxicity of AgNPs decreases with increasing natural organic matter (Gao et al., 2012). AgNP toxicity is considerably affected by its surface properties, which are strongly dependent on water chemistry (Marambio-Jones and Hoek, 2010). Organisms that interact with AgNP exhibit unique physiological responses to AgNP's unique properties (Maurer-Jones et al., 2013). To date, studies on the toxicity of individual AgNPs are robust; however, the effects of AgNPs on heavy metal toxicity are still unclear.

Several studies have reported that heavy metal toxicity is affected by nanoparticles such as carbon nanotubes (CNTs) (Yu and Wang, 2013), nano-CeO_2 (Hu et al., 2012), nano-TiO_2 (Zhang et al., 2007; Fan et al., 2011; Wang et al., 2011b; Hu et al., 2012), nano-Al_2O_3 (Wang et al., 2011a), nano-C_{60} (Baun et al., 2008), and nano-Fe_2O_3 (magnetic) (Hu et al., 2012). CNTs are nanomaterials that are negatively charged in solution and sorb heavy metals on their surfaces by interacting with cationic heavy metals (Srivastava, 2013). Yu and Wang (2013) reported reduced bioaccumulation of Cd and Zn in the presence of CNTs. Likewise, previous studies have shown the influence of nanoparticle-metal complexation on heavy metal toxicity in aquatic environments. The electrostatic force of AgNPs depends strongly on the material coating these particles (Levard et al., 2012). When AgNPs are coated with negatively charged molecules such as citrate, they can sorb cationic heavy metals.

This study investigated the toxicity of coexistent AgNPs and heavy metals to *Daphnia magna*. First, the acute toxicity of heavy metals in the presence of AgNPs was assessed using citrate-coated AgNPs (c-AgNPs). The bioaccumulation of toxicants occurs naturally when aquatic environments are exposed to pollutants. Therefore, to understand the effects of AgNPs on heavy metal toxicity in detail, the bioaccumulation of heavy metals in the presence of AgNPs were also evaluated.

2. Materials and methods

2.1. Reagents and materials

The heavy metals used in this study were $As_2O_5{\cdot}xH_2O$ (Aldrich), $CdCl_2$ (Fluka), and $CuSO_4$ (Aldrich). The c-AgNPs were purchased from ABC NANOTECH (South Korea). Prior to the main experiment, the silver ion was removed from the c-AgNPs stock solution using a dialysis tube (Spectra/Por®Biotech, cellulose ester, molecular weight cutoff: 8kD). The c-AgNPs were used after sonication with a probe sonicator (250/450 Digital Sonifier, BRANSON, USA). To understand the basic properties of the AgNPs used in this study, TEM size, hydrodynamic size, zeta potential, and dissolved fraction were examined. The c-AgNPs were observed by transmission electron microscopy (TEM, 2100 JEOL) and their hydrodynamic size and zeta potential were measured by dynamic light scattering (DLS; Zetasizer Nano, Malvern). To confirm the concentration of dissolved Ag ions from c-AgNPs, the c-AgNPs were centrifuged using an Amicon Ultra (USA) centrifugal filter with a 3-kD Ultracel-YM membrane at 6000 rpm for 20 min. The concentrations of c-AgNPs and heavy metals were analyzed by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500ce, USA).

2.2. Test organism

D. magna, a freshwater invertebrate, has been cultured in the laboratory for more than two years. The culturing conditions followed EPA guidelines (USEPA, 2002). A green alga, *Pseudokirchneriella subcapitata*, and a combination of Yeast, CEROPHYLL®, and Trout chow (YCT) were supplied to *D. magna* every day. EPA synthetic hard water (pH 7.6–8.0, hardness 160–180 mg $CaCO_3$ L^{-1}, alkalinity 110–120 mg $CaCO_3$ L^{-1}) was used for *D. magna* culturing and replaced three times per week.

2.3. Acute toxicity

To measure acute toxicity, 24-h static non-renewal tests were conducted under culturing conditions using neonates (age <24 h) (USEPA, 2002). To observe the changes in heavy metal toxicity due to c-AgNPs, the exposure concentration of c-AgNPs was controlled to 10 μg L^{-1}, which did not show 24-h acute toxicity in *D. magna*. The test concentrations of As(V), Cd, and Cu were 1–50 mg L^{-1}, 0.1–5.0 mg L^{-1}, and 0.005–0.5 mg L^{-1}, respectively, which are the established concentrations for efficiently observing acute toxicity in preliminary tests. The LC_{50} values were estimated by fitting the log–logistic model in the Toxicity Relationship Analysis Program (TRAP), version 1.22 (USEPA, 2013).

2.4. Bioaccumulation

Bioaccumulation experiments were performed to observe the changes in the bioaccumulation of heavy metals in *D. magna* by c-AgNPs. Heavy metal exposure concentrations were LC_{10}; 5 mg L^{-1} for As(V), 0.4 mg L^{-1} for Cd, and 0.07 mg L^{-1} for Cu. The test concentration was low to prevent uptake inhibition caused by heavy metal toxicity. The concentration of c-AgNPs (10 μg L^{-1}) was the same as in the acute toxicity test of heavy metals in the presence of c-AgNPs. To conveniently observe bioaccumulation, these experiments used 7 d-old adults. Ten individual daphnids were exposed to each treatment (one individual per 10 mL) in triplicate. The daphnids were transferred to clean hard water for 6–7 h to allow gut content expulsion and then exposed to the test solution for 0–13 h. After exposure, they were rinsed more than three times using distilled water and dried at 80 °C until they reached a stable weight. Aqua regia was added to the dried daphnids, and samples were completely digested for 1 h at 110 °C using a heating block. The concentrations of Ag and heavy metals in *D. magna* were analyzed by ICP-MS. Bioaccumulation results were interpreted by applying a one-compartment kinetic model (Williams et al., 2000):

$$C_{org} = C_w \frac{k_u}{k_e}(1 - e^{-k_e t}) + C_o e^{-k_e t}$$

C_{org} = metal concentration in daphnia (μg g^{-1})
C_0 = metal concentration in daphnia at t is 0 (μg g^{-1})
C_w = metal concentration in water (μg L^{-1})
k_u = uptake rate constant from water (L dg^{-1})
k_e = elimination rate constant (d^{-1}).

2.5. Sorption of heavy metals by c-AgNPs

The mixed solution of c-AgNPs and heavy metals was prepared with the same exposure concentrations used in the bioaccumulation experiments. After gently shaking for 2 h, samples were centrifuged using an Amicon Ultra Centrifugal filter (3-kD) at 6000 rpm for 20 min to separate the particles and dissolved matter. The filtrate was treated with acid and analyzed by ICP-MS.

3. Results

3.1. Characterization and behavior of c-AgNPs

Table 1 shows the characteristics of c-AgNPs used in this study. The TEM size was 15.5 ± 3.59 nm and the hydrodynamic diameter was 50.0 ± 0.70 nm. At pH 3–10, the zeta potential was negative, indicating that c-AgNPs have negatively charged surfaces.

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