Sunlight-driven reduction of silver ion to silver nanoparticle by organic matter mitigates the acute toxicity of silver to Daphnia magna

Zhen Zhang1,2, Xiaoya Yang1,2, Mohai Shen2, Yongguang Yin2,⁎, Jingfu Liu2

1. School of the Environment, Jiangsu University, Zhenjiang 212013, China. E-mail: zhangzhen@ujs.edu.cn
2. State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

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ABSTRACT
Due to the unique antibacterial activities, silver nanoparticles (AgNPs) have been extensively used in commercial products. Anthropogenic activities have released considerable AgNPs as well as highly toxic silver ion (Ag⁺) into the aquatic environment. Our recent study revealed that ubiquitous natural organic matter (NOM) could reduce Ag⁺ to AgNP under natural sunlight. However, the toxic effect of this process is not well understood. In this work, we prepared mixture solution of Ag⁺ and AgNPs with varied Ag⁺% through the sunlight-driven reduction of Ag⁺ by NOM and investigated the acute toxicity of the solutions on Daphnia magna. Formation of AgNPs was demonstrated and characterized by comprehensive techniques and the fraction of unconverted Ag⁺ was determined by ultrafiltration-inductively coupled plasma mass spectrometry determination. The formation of AgNPs enhanced significantly with the increasing of solution pH and cumulative photosynthetically active radiation of sunlight. The toxicity of the resulting solution was further investigated by using freshwater crustacean D. magna as a model and an 8 hr-median lethal concentration (LC₅₀) demonstrated that the reduction of Ag⁺ by NOM to AgNPs significantly mitigated the acute toxicity of silver. These results highlight the importance of sunlight and NOM in the fate, transformation and toxicity of Ag⁺ and AgNPs, and further indicate that the acute toxicity of AgNPs should be mainly ascribed to the dissolved Ag⁺ from AgNPs.

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Introduction
Silver nanoparticles (AgNPs) have been widely applied in developing biological nanosensors and optoelectronic nanodevices owing to the unique electronic and chemical properties (Kamat, 2002). More importantly, AgNPs are now also widely incorporated in commercial products such as textiles, food packaging, detergents, wound bandage and personal hygiene products (Benn et al., 2010; Benn and Westerhoff, 2008; Ribeiro et al., 2014) for antibacterial and disinfection purposes (Li et al., 2008). During the manufacturing, use and disposal of these commercial products, the discharging of AgNPs is definitely inevitable (Benn et al., 2010; Benn and Westerhoff, 2008; Geranio et al., 2009). These AgNPs will likely enter the aquatic environment, and then, pose threats to the environment and human health (Choi et al., 2008).
Another silver species, silver ion (Ag⁺), is one of the most toxic metals and has been regulated by the US Environmental Protection Agency (Lam and Wang, 2006). However, high concentration of Ag⁺ could still be observed in industrial wastewater and mine tailings owing to application in electronic, photographic industry and mining (Lasko and Hurst, 1999; Sanudowilhelmy and Flegal, 1992).

Once released into the aquatic environment, AgNPs and Ag⁺ can readily transform, which will affect their transport, fate, bioavailability, and toxicity (Levard et al., 2012). The medium redox potential of silver (EAg⁺/Ag⁻ = 0.80 V) makes both the oxidation of Ag⁺ and reduction of Ag⁺ possible to occur in aqueous systems (Yu et al., 2013). In addition, the high surface area of nanoscale sized AgNPs further promotes their high reactive behavior in environmental compartments (Angel et al., 2013). Oxidation of AgNPs and releasing Ag⁺ can occur in the presence of dissolved dioxygen and protons, and the Ag⁺ ions releasing rate increases with temperature but decreases with pH increase (Liu and Hurt, 2010; Sotiriou et al., 2012). Oxidative dissolution can also be influenced by peroxidation, AgNPs size, initial AgNP concentration, as well as surface coating (Kittler et al., 2010; Liu et al., 2010). On the other hand, ubiquitous natural organic matter (NOM) in aquatic environments could reduce Ag⁺ into AgNPs, and this process could be accelerated by heating or sunlight irradiation (Adegboyega et al., 2012; Hou et al., 2013; Tang et al., 2015; Yin et al., 2012). Our recent study further demonstrated that Ag⁺ released via oxidative dissolution of AgNPs could re-reduce into AgNPs in the presence of NOM under sunlight, which could decrease the dissolved Ag⁺ and account for the morphology change of AgNPs in aquatic environments (Yu et al., 2013).

Although the inter-transformation of AgNPs and Ag⁺ has been demonstrated, how this process influence the toxicity of silver remains unclear. It is widely accepted that both AgNPs and Ag⁺ exert toxicity to organisms (Chernousova and Epple, 2013). However, the toxicity of AgNPs comes from AgNPs itself or released Ag⁺ is still controversial (Misra et al., 2012). Some studies believe that AgNPs has distinguished unique toxicity over Ag⁺ (Qian et al., 2013). Pyrosequencing reveals higher impact of AgNPs than Ag⁺ on the microbial community structure of activated sludge from wastewater treatment process (Yang et al., 2014). Truncated triangular silver nanoplates with a lattice plane as the basal plane displayed stronger biocidal action to gram-negative bacterium Escherichia coli than Ag⁺ (Pal et al., 2007). Sublethal concentrations of Ag⁺ and AgNPs exposure to Daphnia embryos also revealed that there is an AgNP-specific mitotoxicity to Daphnia magna (Stensberg et al., 2014). AgNP-exposed common grass, Lolium multiflorum, failed to develop root hairs, had highly vacuolated and collapsed cortical cells, which was not observed for Ag⁺-exposed group (Yin et al., 2011). However, other studies argue that the toxicity of AgNPs mainly comes from the dissolved Ag⁺. The short term toxicity of AgNPs and Ag⁺ to photosynthesis in Chlamydomonas reinhardtii indicated that the major toxic effect was belonging to Ag⁺ while AgNPs were just acting as the source of Ag⁺ (Navarro et al., 2008). The study by Xi et al. (2012) recently suggests that antimicrobial activity of AgNP are totally from the dissolved Ag⁺ and the particle-specific antibacterial activity of AgNP is negligible. Although there is still debate in the toxicity of AgNPs and Ag⁺, it is generally accepted that the toxicity of AgNPs and Ag⁺ is different. Therefore, the inter-transformation of AgNPs and Ag⁺ should have a great impact on the toxicity of silver (Juganson et al., 2013; Shi et al., 2012).

D. magna, as a small-sized filter-feeding freshwater crustacean, is an ideal model organism to evaluate the short-term toxicity of the mixture of Ag⁺ and AgNPs (Allen et al., 2010; Newton et al., 2013; Zhao and Wang, 2012). In this study, the mixture of Ag⁺ and AgNP with varied Ag⁺ fraction was prepared through reduction of Ag⁺ by NOM under natural sunlight and the acute toxic effect of the mixture of Ag⁺ and AgNP on D. magna was investigated. The formation of AgNPs was demonstrated and characterized by ultraviolet–visible spectroscopy (UV–Vis), high resolution transmission electron microscopy (HR-TEM), energy dispersive spectroscopy (EDS), and selected area electron diffraction (SAED). Furthermore, the fraction of unconverted Ag⁺ was determined by ultrafiltration separation and inductively coupled plasma mass spectrometry (ICP-MS) determination. The acute toxic effect of the mixture of Ag⁺ and AgNP was further investigated by using D. magna as a model. This study is helpful to enrich our knowledge on the environmental transformation of AgNPs and Ag⁺ and the subsequent toxicological effect.

1. Materials and methods

1.1. Reagents and instrumentation

Silver perchlorate (AgClO₄) (with >99.0% purity) were purchased from Beijing Chemicals (Beijing, China). Suwannee River natural organic matter (SRNOM) was obtained from the International Humic Substances Society (IHSS, St. Paul, USA). The NOM solution was stirred homogenously before passing it through 0.2 μm membrane filters (mixed cellulose esters, Millipore Corp., Billerica, USA). The dissolved organic carbon (DOC) of NOM solution was then determined by a Phoenix 8000-persulfate total organic carbon analyzer (Tekmar-Dohrmann, Cincinnati, USA). Nitric acid (65%) was obtained from Merck (Darmstadt, Germany). The other chemicals were purchased from Sinopharm Chemical Reagent Co. (Beijing, China) (analytically pure or better). Ultrapure water (18.3 MΩ) used throughout all the experiments was produced by a Milli-Q Gradient system (Millipore, Bedford, USA). All pH were measured by an ORION 4 STAR pH-ISE benchtop (Thermo Fisher Scientific, Waltham, USA). Before each measurement, the electrode was calibrated by pH standard solution. Silver concentration was measured by ICP-MS (7700, Agilent, Santa Clara, CA, USA) with silver standard solutions prepared by diluting a certified reference material (BW08610, National Institute of Metrology, Beijing, China) with 5% (V/V) HNO₃.

1.2. Formation of AgNPs under natural sunlight

We set three groups of experiment solutions according to the pH (pH 7.4, 8.0, and 9.0). A 100 mL sample (NOM (10 mg/L DOC), AgClO₄ (0.5 mmol/L), borate buffer (2.5 mmol/L)) was added into a 110 mL quartz bottle covered with quartz
stopper. The toxicity test (data not shown) suggested that ClO$_2$ (as NaClO$_2$) at 10 μg/L concentration level did not show any acute toxicity to D. magna, indicating in the toxic test the effect of ClO$_2$ can be neglected. At sunlight exposure intervals, the solution was collected (as shown in Table 1), adjusted to pH 7.4, and stored at 4°C before further chemical analysis and toxic test. Dark control experiment was also performed in quartz bottle and the resulting solution was also stored at 4°C. The intensity of the natural sunlight was measured at 15 min intervals routinely by a LI-192 Quantum Sensor (LICOR Biosciences, Lincoln, USA).

1.3. Characterization of AgNPs

UV–Vis spectrum from 200 to 1000 nm was performed using 1 cm optical path length quartz cuvettes on a Shimadzu UV-3600 (Kyoto, Japan). HR-TEM, EDS and SAED were acquired using a TEANAI G2 200 kV (FEI, Hillsboro, USA) or JEOL 2011 (Herts, England) transmission electron microscope. The sample for HR-TEM was prepared by evaporation of 10 μL solution onto a carbon-coated copper grid at room temperature under vacuum.

1.4. Determination of Ag$^+$ concentration in the irradiated solution

Diluted irradiated solution containing Ag$^+$ and AgNPs was mixed with saturated ethylene diamine tetraacetic acid disodium salt (Na$_2$EDTA) solution to isolate Ag$^+$ from NOM, then ultrafiltration centrifugation tube (Amicon Ultra-15 3 kD, Millipore, Billerica, MA, USA) was used to separate Ag$^+$ and AgNPs. After centrifugation at 9600 r/min (9582 g) for 40 min, the filtration containing Ag$^+$ was collected and diluted with 5% nitric acid for ICP-MS analysis. Then, the concentration of AgNPs can be calculated by subtracting Ag$^+$ concentration from total silver concentration. The recovery of Ag$^+$ in ultrafiltration was over 98%, indicating the adsorption of Ag$^+$ on the filter is negligible.

1.5. Test of toxicity to D. magna

The 8 hr-acute toxicity tests were conducted according to OECD guideline (Daphnia sp. Acute immobilization test) (Asghari et al., 2012; Choi and Hu, 2008; Tejamaya et al., 2012) with some changes. The commonly used ecotoxicology media contained Mg$^{2+}$ and Ca$^{2+}$, which might lead to the aggregation of AgNPs (Mukherjee and Weaver, 2010). To avoid this shortcoming, in this study, fully aerated 0.1 mmol/L NaNO$_3$ was used as ecotoxicology media and the test solutions were prepared by diluting the above obtained irradiated samples in the 0.1 mmol/L NaNO$_3$. Preliminary experiments were conducted to estimate the concentration of silver causing the death of D. magna, and then the effective concentrations were set according to the determined concentration ranges. In the formal tests, ten neonates (<24 hr) were selected randomly and placed in 50 mL exposure solution in glass beaker covered with plastic breathable membrane. Previous study showed that the presence of algae would affect the toxicity of nanoparticles (Allen et al., 2010), and therefore D. magna was not feed during the exposure. Experiments were performed with three replicates. After 8 hr exposure, the numbers of dead D. magna were counted. All statistical data were processed by SPSS version 12.0 (SPSS Inc., Chicago, IL, USA).

2. Results and discussion

2.1. Sunlight-driven reduction of Ag$^+$ to AgNPs by NOM

The sunlight-driven reduction of Ag$^+$ to AgNPs by SRNOM was investigated under pH 7.4, 8.0 and 9.0, respectively. Under sunlight irradiation, the color of the Ag$^+$ solution tints yellow slowly, indicating the reduction of Ag$^+$ to AgNPs. Clearly, the solution with higher pH tints yellow faster and deeper than that with lower pH, suggesting the enhanced formation of AgNPs at higher pH. The solution was sampled and characterized with UV–Vis spectroscopy at different interval or cumulative photosynthetically active radiation (PAR) (Fig. 1). In Fig. 1, absorption peaks centering at approximate 400 nm could be observed, which were characteristic of surface plasmon resonance (SPR) of spherical AgNPs. At pH 7.4 (Fig. 1a), the absorbance intensity first increased then decreased and broadened SPR peak was observed with the accumulation of PAR, which suggested larger sized AgNPs or aggregations were formed. In Fig. 1b–c, the intensity of SPR peak were much higher than that in Fig. 1a, indicating the increased concentration of AgNPs. The enhanced formation of AgNPs at higher pH was ascribed to lower redox potential of NOM at higher pH, which facilitate the formation of AgNPs (Yin et al., 2012). Meanwhile, the maximum absorption wavelength ($\lambda_{max}$) of AgNPs at pH 7.4 red-shifted slightly (Fig. 1a) while the shapes of absorbance spectra of pH 8.0 and 9.0 (Fig. 1b–c) were almost unchanged, indicating that the AgNPs were more stable at higher pH. This might be due to the stronger stabilization effect of NOM as a capping agent at
Photosynthetically active radiation (PAR) was marked in the figure.

Matter (NOM) and 2.5 mmol/L borate buffer under natural sunlight. (a) pH 7.4, (b) pH 8.0, (c) pH 9.0. The cumulative Ag+/min, pH 9.0. All the results revealed that the conversion of Ag+ into AgNPs by NOM under sunlight at various pH (Yin et al., 2012). Fig. 3 shows the cumulative PAR dependent reduction of Ag+ into AgNPs without sunlight irradiation (samples 11 and 16) at pH 8.0 and pH 9.0, respectively, indicating the reduction in the dark at higher pH levels. With the increase of cumulative PAR, the fraction of Ag+ decreased significantly. Fig. 3 also shows that minimum Ag% (Ag%min) followed: Ag%min, pH 7.4 > Ag%min, pH 8.0 > Ag%min, pH 9.0. All the results revealed that the conversion of Ag+ to AgNPs increased with elevated solution pH.

2.2. Fraction of Ag+ under varied sunlight irradiation interval and pH conditions

As the reduction of Ag+ to AgNPs was mainly driven by sunlight and influenced by solution pH, different cumulative PAR (or sunlight irradiation time) and pH conditions could result in different Ag+ or AgNP fractions. Therefore, before toxicity test, the fraction of Ag+ in the mixture of Ag+ and AgNP was determined by ultrafiltration and ICP-MS and the results are shown in Table 1 and Fig. 3. The fraction of Ag+ in total Ag decreased from 100% to 56.6% at pH 7.4, from 96.0% to 35.1% at pH 8.0, and from 53.6% to 11.7% at pH 9.0 with the increase of cumulative PAR. The formation of AgNPs was also detected determined by ultrafiltration and ICP-MS and the results are shown in Table 1 and Fig. 3. The fraction of Ag+ in the mixture of Ag+ and AgNP was determined by ultrafiltration and ICP-MS and the results are shown in Table 1 and Fig. 3. The fraction of Ag+ in total Ag decreased from 100% to 56.6% at pH 7.4, from 96.0% to 35.1% at pH 8.0, and from 53.6% to 11.7% at pH 9.0 with the increase of cumulative PAR. The formation of AgNPs was also detected.

2.3. Toxicity of silver to D. magna

The above obtained mixture of Ag+ and AgNP (samples 1, 2, 4, 7, 15, 17, 19 in Table 1) with varied Ag+ fraction (11.7%–100%) were selected to expose to D. magna (Fig. 4). We prepared 6 different concentrations for each sample, so each curve in Fig. 4 was fitted by 7 points including the blank control (0.1 mmol/L NaNO3). Mortality rate curve for each sample was different while total silver concentration was identical, which showed that the acute toxicity of silver to D. magna does not depend on the concentration of total silver (Newton et al., 2013). Then, 8 hr-LC50 of each sample was obtained from Fig. 4 through SPSS version 12.0 and the resulting Fig. 5 shows that 8 hr-LC50 decreased from 1.252 μg/L to 0.279 μg/L, with the AgNP% increased from 0% to 88.31%. These results clearly demonstrated that sunlight-driven reduction of Ag+ to AgNPs by NOM decreases the acute toxicity of silver to D. magna. In addition, this result indicated that the acute toxicity of silver to D. magna was positively correlated with Ag+, but not AgNPs or total silver.

2.4. Environmental implications

Once released into the environment, both AgNPs and Ag+ undergo various chemical transformations, especially oxidative dissolution and reduction. The oxidative dissolution of AgNPs to Ag+ is usually believed to increase the toxicity of silver to organism (Misra et al., 2012). The reduction of Ag+ by NOM under natural sunlight could decrease the free dissolved Ag+ in the solution, although the potential environmental and toxicological impact of this transformation process is still not well understood. Our study clearly demonstrated that the sunlight-driven reduction of Ag+ to AgNPs by NOM significantly mitigates the acute toxicity of silver to D. magna. This result reveals that the joint effect of sunlight and NOM in surface water is a natural antidote to the toxicity of AgNPs and Ag+. This study also suggested that other environmental processes possibly influence the oxidative dissolution of AgNPs and Ag.
AgNPs and the reduction of Ag⁺ should also have a great impact on the toxicity of silver. At present, it is still a debate that the toxicity of AgNP comes from itself or the released Ag⁺. Our results further indicate that the dissolved Ag⁺ from AgNPs played a major role in the acute toxicity of AgNPs.

3. Conclusions

In this study, we investigated the reduction process of Ag⁺ ions by NOM under natural sunlight, and subsequent toxic impact of this process to D. magna. The formation of AgNPs was demonstrated by combined characterization techniques. The formation of AgNPs enhanced significantly with increasing the solution pH and cumulative PAR of sunlight. The acute toxicity test of the mixture of Ag⁺ and AgNP on D. magna demonstrated that the reduction of Ag⁺ by NOM to AgNPs under sunlight significantly mitigated the acute toxicity of silver to D. magna. These results highlight the importance of sunlight and NOM in the fate, transformation and toxicity mitigation of Ag⁺ and AgNPs, and further indicate that the acute toxicity of AgNPs should be mainly ascribed to the dissolved Ag⁺ from AgNPs.

Fig. 2 – Identification and characterization of AgNPs formed in solution containing 0.5 mmol/L AgClO₄, 10 mg/L NOM and 2.5 mmol/L borate buffer under natural sunlight by high resolution electron transmission microscopy (a–c), energy dispersive spectroscopy (d–f), and selected area electron diffraction (g–i). (a, d, g) pH 7.4, (b, e, h) pH 8.0, (c, f, i) pH 9.0. The accumulative PARs were 306.02, 27.12, and 36.11 E/m² for (a, d, g), (b, e, h) and (c, f, i), respectively.
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