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Silver nanoparticles or free silver ions work? An enantioselective phytotoxicity study with a chiral tool

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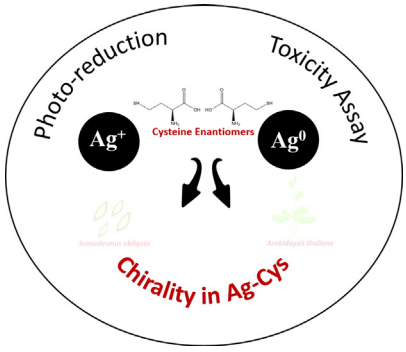
HIGHLIGHTS

- Chiral Ag-cysteine complex was made to elucidate role of Ag^+ in AgNP toxicity.
- Ag^+ was partly photo-reduced to AgNP by cysteine and toxicity was assayed.
- Ag-L/D-Cys differ in Ag^+/AgNP quantification and show different toxic effects.
- Ag-D-Cys was more toxic to *S. obliquus* and Ag-L-Cys was more toxic to *A. thaliana*.
- AgNP toxicity to *S. obliquus* and *A. thaliana* was due to Ag^+ and AgNP, respectively.

GRAPHICAL ABSTRACT



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ABSTRACT

Nowadays, silver nanoparticles (AgNP) have been widely used and there are raising concerns about their potential adverse effects on organism. As for the exact toxicity mechanism of AgNP, opinions still vary and whether the released silver ions (Ag^+) or AgNP themselves are responsible for the toxicity remains debatable. In the present study, we have designed two exposure systems where Ag^+ and AgNP coexisted but differed in quantification by using photo-reduced method with cysteine enantiomers, and their toxicities to freshwater microalgae *Scenedesmus obliquus* and model plant *Arabidopsis thaliana* were determined. In the results, Ag^+ was in suit photo-reduced by cysteine enantiomers, and the UV–Vis and circular dichroism spectrum evidence confirmed the quantification difference between Ag-L-cysteine (Ag-L-Cys) and Ag-D-cysteine (Ag-D-Cys), where there was more AgNP and less Ag^+ in Ag-L-Cys. Furthermore, the toxicity assay data revealed that Ag-D-Cys was more toxic to *S. obliquus* but *A. thaliana* was more susceptible to Ag-L-Cys. The metal element distribution in *Arabidopsis* leaves was also influenced in an enantioselective manner, which was related to the oxidative stress. Considering the quantification difference between Ag-L-Cys and Ag-D-Cys, it can be concluded that AgNP exhibited their toxicity to *S. obliquus* by the action of Ag^+, but toxicity brought to *A. thaliana* was attributed to AgNP themselves rather than Ag^+. The results of the present study help to better clarify the role of Ag^+ in AgNP toxicity and offer a chiral tool and a new sight to investigate the toxicity mechanism of AgNP.

1. Introduction

The high pace of nanotechnology developments have led to increasing production and utilization of engineering nanoparticles (ENPs). Even though the values in material fields and medical applications

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have long been recognized (Lanone and Boczkowski, 2006; Xu et al., 2014; Li et al., 2015; Liu et al., 2016), ENPs still pose risk to the ecosystem and raising concerns over their potential adverse impacts have also been reported (Colvin, 2003). As one of the most widely commercialized ENPs, silver nanoparticles (AgNP) have received special attentions due to their potential harmful effects on bacteria (Beddow et al., 2017), aquatic and terrestrial animals (Conine and Frost, 2017), and plants (Cox et al., 2016; Wang et al., 2016). In particular, adverse influences to plants may finally threaten our human beings along the food chains (Wijnhoven et al., 2009; Ahamed et al., 2010; Marambio-Jones and Hoek, 2010).

Even though AgNP have been extensively investigated, there still remain hot debates about the exact action mechanism. Because AgNP can release Ag^+, whether Ag^+ or AgNP cause the toxic effects remain controversial. On the one hand, pioneering researches have compared the toxicity of Ag^+ and AgNP at same concentrations and found that Ag^+ was more toxic than AgNP, and revealed that the toxicity of AgNP was mainly attributed to the release of Ag^+ (Xiu et al., 2011; Priester et al., 2014). Therefore, the toxicity of AgNP was controlled primarily by the extent of nanoparticle dissolution (Gunsolus et al., 2015). On the other hand, some investigations also claimed that Ag^+ did not account for all of the observed toxicity (Kwok et al., 2016). Evidence was also provided when compared as a function of the Ag^+ concentration, toxicity of AgNP appeared to be much higher than that of Ag^+, indicating that the culprits of AgNP toxicity can be tracked back to more than the released Ag^+ but the nanoparticles themselves (Navarro et al., 2008; Yin et al., 2011).

To compare the toxicity of Ag^+ and AgNP, some investigations were performed by using the commercial coated AgNP and $AgNO_3$, making sure about the same Ag concentrations before the exposure experiments (Navarro et al., 2015). However, there standing an unavoidable defect is that the effects of anionic such as NO_3^- cannot be ignored, because NO_3^- is one of the most important nitrogen nutrients, which may cause bias in the toxic effects assessments. In addition, to better and more efficiently distinguish the source of the toxicity, AgNP coated with natural organism matters (NOMs) or the Ag^+ binding agents were further used in the toxicity assays (Gunsolus et al., 2015). Among the various Ag^+ binding agents, cysteine has been frequently applied as a strong Ag^+ ligand, which can abolish the inhibitory effects caused by Ag^+ (Navarro et al., 2008). However, it also takes a risk to conclude that AgNP exhibited its toxicity by releasing Ag^+ even though cysteine alleviates the toxicity. Because the effects of cysteine should be taken into consideration and more importantly, due to the binding ability, the complex Ag-cysteine may also exhibit its own influences. Unfortunately, no investigation has been performed to explore the effects of Ag-cysteine complex so far.

As for the Ag-cysteine complex, former research has found that enantiomers of cysteine, L- and D-cysteine can interact with Ag^+ in an enantioselective manner due to the chiral carbon center (Liu et al., 2012), and the chiral recognition of cysteine by Ag^+ has also been observed (Zhang and Ye, 2011). Furthermore, it has been reported that Ag^+ can be photo-reduced into AgNP by cysteine without any other reducing agents (Liu et al., 2012) and the enantioselectivity was also observed between the AgNP, which enlightens us that AgNP reduced from Ag^+ by cysteine enantiomers may be a good tool to investigate the toxic effects of AgNP.

Therefore, as shown in Scheme 1, the aims of the present study is to establish two exposure systems containing AgNP and Ag^+ at the same time but differing in quantification, by photo-reducing Ag^+ with cysteine enantiomers. Furthermore, freshwater microalgae *Scenedesmus obliquus* and model plant *Arabidopsis thaliana* were selected as model organisms for their representative role in aquatic toxicology study and short life cycle, respectively. We aimed to (1) compare the toxic effects of Ag-cysteine complexes in an enantiomeric level and (2) to determine the effect of Ag^+/AgNP quantification on the toxicity to both type plants. In addition, the phenotypic effects and potential mechanism such as oxidative stress were also investigated. By comparing the toxicity of Ag-cysteine complexes, we believe the results obtained in the present study may help to better distinguish the role of AgNP and Ag^+ in AgNP toxicity and offer a useful tool as well as new sight to investigate the toxic mechanism of AgNP.

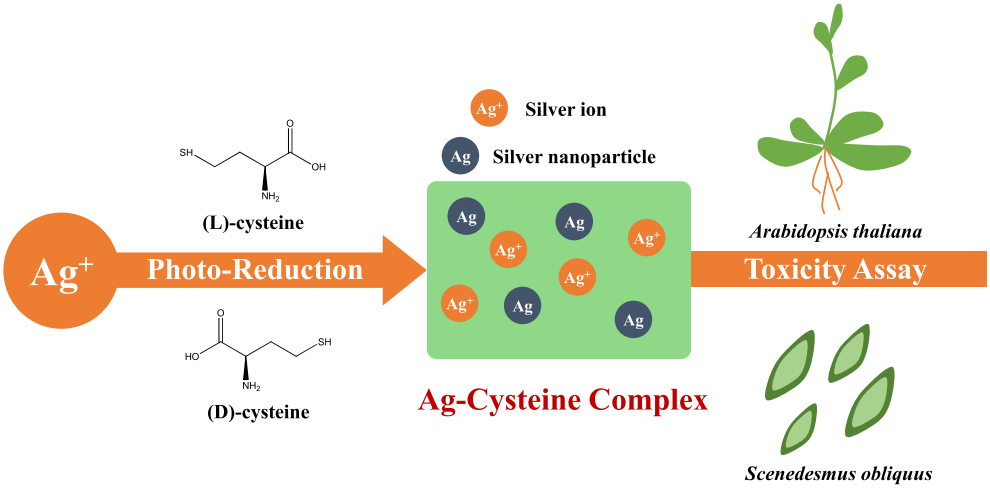
2. Materials and methods

2.1. Chemicals and materials

$AgNO_3$ (analytically pure, 99.85%) and cysteine (analytically pure, ≥97%) with L- and D-enantiomers were purchased from Sigma-Aldridge (St. Louis, MO, USA). Freshwater microalga *Scenedesmus obliquus* was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). Doubly distilled water (ddH_2O) was used throughout the experiments. All other chemical reagents were analytical grade and all glassware was sterilized in an autoclave.

2.2. Preparation and characterization of Ag-L/D-cysteine complex

The mixed reaction between Ag^+ and L/D-cysteine was performed according to previous study (Liu et al., 2012) with slight modification.



Scheme 1. Synthesis and toxicity assay of Ag-cysteine complex.