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Surface coating-modulated toxic responses to silver nanoparticles in *Wolffia globosa*

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ABSTRACT

With the omnipresence of silver nanoparticles (AgNPs) in our daily consumer products, their release has raised serious concerns. However, the biochemical mechanisms by which plants counteract the toxicity of nanoparticles are largely unknown. This study investigated the exposure of aquatic *Wolffia globosa* to ATP-nAg (AgNPs coated with adenosine triphosphate), cit-nAg (AgNPs coated with citrate), and Ag^+. Hill reaction activity was basically lost in *W. globosa* treated with 10 mg/L ATP-nAg and Ag^+, while the activity was still maintained at 38.7%–38.9% of the respective controls at 10 mg/L cit-nAg. The reduction of amounts of chlorophyll and soluble protein were shown in response to the Ag stresses. This was accompanied by the accumulation of sugar in *W. globosa* treated with cit-nAg. By contrast, the depletion of sugar was recorded after 10 mg/L ATP-nAg and Ag^+ treatments. The superoxide dismutase and peroxidase activities were significantly increased after exposure to 10 mg/L ATP-nAg and Ag^+, which did not occurred in *W. globosa* treated with cit-nAg. The ratio between NADPH/$NADP^+$ was higher after cit-nAg and Ag^+ stresses than the respective controls. The accumulation of Ag was found to increase in a concentration-dependent manner. Ag^+ and ATP-nAg inhibited the uptake of P and K, and promoted the uptake of Fe and Cu. In contrast, cit-nAg only promoted the uptake of Cu. Our results implied that surface coating induced different physiological responses of *W. globosa* to AgNPs. Based on above results, we speculated that after exposure to cit-nAg, citrate possibly could serve as the substrate for the tricarboxylic acid cycle and accumulated sugar may promote pentose phosphate pathways. For ATP-nAg treatments, ATP would act as an exogenous energy source of plant metabolisms. Our findings demonstrate that surface coating regulates the physiological responses of plants to AgNPs through distinct mechanisms.

1. Introduction

Along with the rapid development of nanotechnology, there is a growing interest and an urgent need to understand the biological and environmental consequences of engineered nanomaterials. Nanosilver is among the most commercialized of the nanomaterials in consumer products (Vance et al., 2015), largely because of its excellent antibacterial and photosensitive properties. Like other classes of engineered nanoparticles, nanosilver may eventually be discharged through industrial and research outlets, and then impact living organisms in the environment. Nanosilver is considered a potential threat to aquatic organisms because of the increasing use of silver-based nanomaterials, which release free silver ions (Ag^+) (Kittler et al., 2010).

The functioning of ecosystems relies on a range of organisms, and plants in particular which are located at the bottom of food chain, are a vital part of the ecosystem as they serve as primary producers. As such, any negative effects of nanoparticles on plant growth and metabolism could cause significant changes in the ecosystem, potentially causing irreversible damage (Gubbins et al., 2011). Several lines of evidence have demonstrated detrimental effects of silver nanoparticles (AgNPs) on plants. On exposure to AgNPs, inhibition of seed germination, suppression of root and shoot growth, and reduction of biomass have been demonstrated extensively. In addition to the toxic effects mentioned above, interference of AgNPs with plant metabolism, such as the degradation of photosystem II (PS II) efficiency (Xu et al., 2010), inhibition of chlorophyll biosynthesis and the Calvin cycle (Oukarroum et al., 2012a), accumulation of induction reaction oxygen species (ROS), activation of antioxidative defense systems, and the blocking of ethylene signaling (Syu et al., 2014) have also been well described. Regarding molecular and gene expression levels, transcripts involved in the thalianol biosynthetic pathway (a particular plant defense mechanism) were considerably up-regulated (Kaveh et al., 2013). In addition, gene

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expression involved in cellular events, including cell proliferation, metabolism, and hormone signaling pathways, was activated in AgNP-treated *Arabidopsis thaliana* (Syu et al., 2014). Nanosilver is highly photosensitive, and critical knowledge about light-induced phytotoxic mechanisms is still lacking. A better understanding of the metabolic responses and toxic mechanisms of nanosilver are required to continue the effective use of nanosilver.

AgNPs are typically stabilized with various surface coatings to prevent aggregation or to provide other intended surface functions (Li et al., 2013). Polyvinylpyrrolidone-AgNPs (PVP-AgNPs) and citrate-AgNPs released Ag^+ at a higher concentration than that of uncoated AgNPs, and the aggregation rate was highest for uncoated AgNPs, followed by citrate-AgNPs and PVP-AgNPs (Li et al., 2013). Released Ag^+ is often reported to primarily mediate AgNP toxicity (Piccapietra et al., 2012). The formation of aggregates might reduce the light available to plant cells and thus inhibit their growth, or they may alter the cellular acquisition of essential nutrients by adhering to the cell wall (Oukarroum et al., 2012a). On one hand, such surface modifications, by changing the physico-chemical properties of AgNPs, could change their cellular toxicity and response mechanisms. On the other hand, there surface coatings also exerted additional influences on plants cells.

In this study, we focused on how surface coating altered the toxic mechanisms of AgNPs, while the effects of Ag^+ were also discussed in order to compare the potential differences between nanoparticles and ions. Citrate- and adenosine triphosphate (ATP)-coated AgNPs (cit-nAg and ATP-nAg) were employed because they are most commonly used. In addition, these surface coatings are nontoxic to biological organisms and serve as the metabolic products. The effects of cit-nAg, ATP-nAg, and ionic silver (Ag^+) were compared in the aquatic plant *Wolffia globosa*, which is a widely used model organism, because of its simplified structure, high multiplication rate, and ability to reproduce vegetatively (Zhang et al., 2009). The measured physiological parameters included chlorophyll content, soluble sugar and protein content, Hill reaction activity, superoxide dismutase (SOD) and peroxidase (POD) activity, ATP and nicotinamide adenine dinucleotide phosphate content as well as silver and nutrient uptake. We speculated that these surface coatings would participate in the plant cell metabolisms, which resulted in the different biological responses and toxic mechanisms of *W. globosa* to these two AgNPs.

2. Materials and methods

2.1. Biological materials

Plants of *W. globosa* were obtained from Professor Yongguan Zhu of the Institute of Urban Environment, Chinese Academy of Sciences, and they were grown in an axenic complete modified Hoagland solution (HS_m) (Zou et al., 2016). Before beginning the experiments, plants were grown in a growing chamber for 4 weeks, with a 14 h/10 h light/dark photoperiod. Illumination (6600 Lux) was provided by white fluorescent lamps and the temperature was kept at 20 and 25 °C for night and day periods.

2.2. Synthesis and characterization of AgNPs

In this study, cit-nAg and ATP-nAg were synthesized by the reduction of $AgNO_3$ with $NaBH_4$ in the presence of citrate or ATP, at room temperature. Complete information on the synthesis, and the citrate and ATP coating process of AgNPs has been provided in previous studies (Zou et al., 2016; Zou et al., 2014). The intensity average size and zeta potential of the synthesized cit-nAg were determined to be 18.8 nm and −36.3 mV. In comparison, the ATP-nAg was 20.1 nm and −18.2 mV. The morphology of these synthesized AgNPs was shown in Fig. S1 of the Supporting Information.

2.3. Treatment of W. globosa

Following pre-culture for 4 weeks, *W. globosa* plants were exposed to 0 (used as control groups), 0.1, 1, and 10 mg/L of AgNPs (0, 0.93, 9.27 and 92.7 μM) and 10 mg/L Ag^+ (92.7 μM, in the form of $AgNO_3$) in 100 mL glass flasks. The concentrations refer to the previous reference, with some modifications made by us (Oukarroum et al., 2012b). In order to comparison between nanoparticle and ion effects visually, only 10 mg/L Ag^+ was chosen. In order to avoid the aggregation and precipitation of AgNPs, ultrapure water and 10-fold dilution of HS_m (1/10 strength HS_m) were used in the exposure experiments. Plants were cultured in the growing chamber for 3 days under the same illumination and temperature conditions as pre-culture. The culture conditions were shown in Fig. S2 of Supporting Information.

2.4. AgNP stability experiments

Characterization of AgNP suspensions were performed under the same conditions as plant treatments. Nanoparticle size and shape were observed by transmission electron microscopy (TEM, H-7650, Hitachi, Japan). The stability of AgNP suspensions was measured by the residual total silver concentrations in ultrapure water and 1/10 strength HS_m using an inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 7000DV, PerkinElmer, USA).

2.5. Silver accumulation and nutrient element determination

After the 3-day treatments with AgNPs and Ag^+ as described above, the collected *W. globosa* was rinsed with 1 mM cysteine for 5 min, followed by thoroughly rinsing with ultrapure water. Then, the plants were dried to constant weight by lyophilization. The content of Ag and nutrient elements were analyzed by ICP-OES after acid-digestion in a microwave-accelerated reaction system (CEM Microwave Technology Ltd, Matthews, NC, USA).

2.6. Analysis of soluble sugar content

The soluble total sugar content was determined by a H_2SO_4-anthrone assay using glucose as the standard. Detailed information for this method has been described in previous research (Zou et al., 2016). The relative sugar content (%) was defined as the ratio of the content of the treated-groups to the respective control groups.

2.7. Measurement of chlorophyll content

The analysis of pigment was carried out after 3 days of Ag exposure. For determining chlorophyll and carotenoid content, 100 mg of fresh *W. globosa* was extracted with 80% (v/v) acetone, and the absorbance of the extract at 470, 647 and 663 nm was recorded on a spectrophotometer (Thermo UV-1700 PharmaSpec, Unicam, UK). The concentrations of chlorophyll *a*, *b* and carotenoids were calculated according to Lichtenthaler (Lichtenthaler, 1987).

2.8. Isolation of chloroplasts and Hill reaction activity

The *W. globosa* were ground in a cooled mortar with cooled extract buffer (pH 7.6) containing 0.4 M sucrose, 0.05 M Tris and 0.01 M NaCl. The chloroplast homogenate was filtered and then centrifuged at 500 × *g* for 2 min at 4 °C. The collected supernatant was centrifuged again at 3000 × *g* for 10 min at 4 °C, and the precipitated pellet that contained chloroplasts was suspended in extract buffer at 4 °C to measure the Hill reaction activity. Detailed information for this method has been described in previous research (Zou et al., 2016).