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Investigation of gold nanoparticles uptake and their tissue level distribution in rice plants by laser ablation-inductively coupled-mass spectrometry

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ABSTRACT

The tissue level uptake and spatial distribution of gold nanoparticles (AuNPs) in rice (*Oryza sativa* L.) roots and shoots under hydroponic conditions was investigated using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). Rice plants were hydroponically exposed to positively, neutrally, and negatively charged AuNPs [AuNP**1**(+), AuNP**2**(0), AuNP**3**(−)] with a core diameter of 2 nm. Plants were exposed to AuNPs having 1.6 mg Au/L for 5 days or 0.14 mg Au/L for 3 months to elucidate how the surface charges of the nanoparticles affects their uptake into living plant tissues. The results demonstrate that terminal functional groups greatly affected the AuNP uptake into plant tissues. Au concentration determined by LA-ICP-MS in 5 day treated rice roots followed this order: AuNP**1**(+) > AuNP**2**(0) > AuNP**3**(−) but this order was reversed for rice shoots, indicating preferential translocation of AuNP**3**(−). Bioimages revealed distributions of mesophyll and vascular AuNP dependent on organ or AuNP concentration.

1. Introduction

The use of nanostructures as a mode for bioimaging and sensing in biomedicine is one of the most intensely studied areas in nanotechnology (Dreaden et al., 2012; Loo et al., 2005; Jain et al., 2008). However, the application of nanotechnology to plant and environmental systems has lagged behind. With the increased prevalence of nano products it is vital to monitor the transport, fate, and toxicity of engineered nanoparticles once they are released into the environment and how they behave in biological systems. Gold nanoparticles (NPs) are well suited for examining the bioavailability and toxicity of manufactured nanomaterials in environmentally complex systems, because of their resistance to oxidative dissolution and negligible release of dissolved Au ions as well as the low natural background concentrations of gold that facilitates easily discernible signals. They also have the ability to remain intact in soil and aqueous media, and under the physiological conditions in plants and invertebrates (Unrine et al., 2012). In addition, gold NPs can be functionalized with different surface charge, e.g. positive, neutral, and negative charges. In recent years, a tremendous increase in the applications of gold NPs in biomedical imaging and detection (Copland et al., 2004; Sreenivasan, 2010; Jiang et al., 2006), cancer diagnostics (El-Sayed et al., 2005) and therapy, and biological and sensing of heavy metals (Kim et al., 2001) have been observed. However, there are only a few studies about the detection and fate of gold NPs in plant systems.

Research is progressing on the fate and transport of gold nanoparticles in the environment, their accumulation in the food chain has been documented via amphibian consumption of worms on AuNP treated soil (Unrine et al., 2012), and from producers to primary consumers (e.g. Judy et al., 2011). Biomagnification has been observed for example by up to a factor of 11.6 with 10 nm diameter AuNP in hornworms feeding off tobacco leaves (Judy et al., 2011) raising additional means for concern. Evidence shows that AuNP can be cytotoxic depending on nanoparticle characteristics and can be up taken into various human organs, with positively charged terminal amine groups more cytotoxic than negatively charged carboxyl groups (Lipka et al., 2010), showing potential cause for concern for human health if AuNPs particles enter food crops and livestock. Therefore understanding plant uptake and accumulation of gold nanoparticles is important for developing crop species that can prevent accumulation in above ground organs as well as accumulators for the removal of AuNP from the soil and water.

Unrine et al., 2010 first used laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) to image gold NP distribution in earthworms (*Eisenia fetida*). Recently, the application of gold NPs in plant systems has attracted considerable interest. For

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instance, Judy et al. (2011, 2012) investigated the uptake of 5, 10, and 15 nm diameter gold NPs coated by tannic acid by tobacco *Nicotiana tabacum* L. cv *Xanthi* and the effect of particle size and surface coating on bioavailability of gold NPs by tobacco *Nicotiana tabacum* L. cv *Xanthi* and *Triticum aestivum* (wheat) using inductively coupled plasma mass spectroscopy (ICP-MS), LA-ICP-MS and synchrotron-based X-ray fluorescence (μXRF). In addition, they showed the uptake of AuNPs and subsequent translocation to leaves and demonstrated that the AuNPs are present internally. They found that Au nanoparticles accumulated in the tobacco plant (a dicot) while there was no significant evidence for uptake by monocot plant wheat. They argued the nature of exudates may be responsible for aggregation and subsequent uptake of gold nanoparticles by these plants. Sabo-Attwood et al. (2012) investigated uptake, translocation and toxicity of gold nanoparticles with 3.5 and 18 nm diameter in tobacco *Nicotiana xanthi* seedlings using μXRF and X-ray absorption near-edge microspectroscopy (XANES), and found AuNP entry via roots to the plant vascular system. Judy et al., 2011 used LA-ICP-MS to investigate the spatial distribution of AuNP in tobacco leaves and demonstrated the uptake of AuNP and showed that observed AuNP is not due to surface contamination while Sabo-Attwood et al. (2012) used XANES to characterize elemental speciation of AuNP and X-ray fluorescence microscopy together with high resolution electron microscopy to characterize tissue level distribution of AuNP. Bulk analyses of metal NP concentration in plants and soil media usually entails complete dissolution of sample matrix with strong oxidizing acid such as nitric acid or using mineral acid mixtures (Ferry et al., 2009; Judy et al., 2011) and thereby this analytical approach does not offer spatial distribution of NPs at tissue level. Currently one challenge is how to accurately and easily measure the NP concentration and spatial distribution in the complex media such as plant roots and shoots without destroying the plant samples.

The previous studies did not quantify gold NPs spatial distribution and translocation from below to above ground organs (roots to shoots) as a function of gold NP surface charge. LA-ICP-MS is a highly sensitive elemental analytical method that permits measurement of trace metals at the level of parts-per-billion and hence is a reliable method for analysis of trace metal containing NPs. Sample size and preparation are minimal for LA-ICP-MS minimizing nitric acid waste compared to bulk analysis. XANES can detect metal speciation inside plant tissues while the detection limit is usually higher than 10 μg/g (dry weight). One limitation of the laser ablation approach is the lack of suitable matrix matched solid analyte standards at an appropriate calibration range. Therefore we developed a suite of in house standards for quantitative determination of Au distribution in plant tissues. Our research takes advantage of the ability for the LA-ICP-MS to provide sensitive quantitative measurements of spatial distribution of elements found in NPs in plant tissue.

The applicability of LA-ICP-MS to various sample matrices is well established (Durrant and Ward, 2005) and successfully used for determination of trace elements and their spatial distribution patterns in various sample matrices such as teeth (Cox et al., 1996; Kang et al., 2004; Dolphin et al., 2005; Hare et al., 2011), and in archeological samples such as hair (Byrne et al., 2010; Bartkus et al., 2011), in plants (Becker et al., 2008; Wu et al., 2009; Koelmel and Amarasiriwardena, 2012), gold nanoparticles in plants (Judy et al., 2011) and in earthworms (Unrine et al., 2010). Therefore, LA-ICP-MS approach may offer unique analytical capabilities to investigate the uptake and distribution of nanoparticles within plant tissues. The purpose of this study is to investigate the uptake and spatial distribution of engineered AuNPs with different surface charges in rice roots and shoots using LA-ICP-MS.

2. Materials and methods

2.1. Synthesis and characterization of AuNPs

The surface monolayers and AuNPs (Fig. 1) were synthesized using the methods previously published (Hong et al., 2004; Zhu et al., 2008, 2010). A two-phase liquid–liquid synthesis method was first used to synthesize thiol-derivatised AuNPs with diameters of 1–3 nm (Brust et al., 1994). Later, the ligand-exchange method was used to obtain AuNP**1** (TTMA), AuNP**2** (TEGOH), and AuNP**3** (TEGCOOH) was synthesized by the single phase synthesis method described previously (Kanaras et al., 2002). The TTMA, TEGOH and TEGCOOH [here on referred collectively as AuNP particles: AuNP**1** (+), AuNP**2** (0), AuNP**3** (−), respectively; terminal charge is denoted in parenthesis and see Fig. 1] compounds were kindly provided by Profs. Vincent Rotello and Richard Vachet (Department of Chemistry, University of Massachusetts, Amherst, MA, USA). The core sizes of the synthesized AuNPs were measured on a JEOL 100 S transmission electron microscope (TEM) (Peabody, MA, USA). Dynamic light scattering (DLS) and zeta-potential (ζ-potential) measurements of the AuNPs were obtained with a Zetasizer Nano ZS (Malvern Instrument Ltd., Worcestershire, UK).

2.2. Plant culture and AuNP exposure

The rice seeds [*Oryza sativa* L., (Nipponbare; GSOR 100)] were obtained from USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, Arkansas, USA. The seeds were surface-sterilized in 3% (v/v) H_2O_2 for 30 min and then placed in a water bath at 38 °C for 6 h. Afterward they were thoroughly rinsed with Milli-Q water (18 MΩ cm) and placed in 10 × 1.5 cm Petri dishes (18 seeds per dish). The Petri dishes were placed in an incubator in the dark at 25 °C and the seeds were allowed to germinate for 9 days. The rice seedlings were transferred into a 100 mL jar with 80 mL Milli-Q water for short-term (5-day) treatment experiments. The 50 μM AuNP (containing 2555 mg Au/L) stock suspension was sonicated in a water bath for 3 min before use. Then 50 μL aliquot of the 50 μM (2555 mg Au/L) AuNP**1**, AuNP**2** and AuNP**3**, respectively, was pipetted to the Milli-Q water and the final AuNP treatment concentration was 1.6 mg Au/L. The 9-day-old rice seedlings were also transferred into 80 mL Milli-Q water as the control.

For the long-term (3-months) uptake experiment, the 9-day-old rice seedlings were relocated into a 500 mL jar containing 450 mL of major nutrients (one-fourth-strength of each 1 mM $Ca(NO_3)_2$, 0.5 mM KH_2PO_4, 0.5 mM K_2SO_4, 1 mM $MgSO_4$, and 1.5 mM NH_4NO_3) and micronutrients (full-strength of each 75 μM EDTA-Fe, 46 μM H_3BO_3, 9 μM $MnSO_4$, 0.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$, and 0.8 μM Na_2MoO_4). The pH of the nutrient solution was 5.6. The 25 μM AuNP stock suspension was sonicated in a water bath for 3 min before use. Then 50 μL of the 25 μM (containing about 1278 mg Au/L) AuNP**1**, AuNP**2** and AuNP**3**, respectively, were pipetted to the 450 mL nutrient solution and the final AuNP treatment concentration having 0.14 mg Au/L. The AuNP treatment solution and the nutrient solution control were replaced every week during the long-term uptake experiment. On the third day from the solution replacement, additional nutrient solution was added to each jar to keep the solution level at 450 mL during the short- and long-term uptake experiments.

The 100 mL and 500 mL jars were wrapped with aluminum foil and covered by a net. The short-term and long-term uptake experiments were conducted in a plant growth chamber with a 16 h day length and day/night temperatures of 25 °C/18 °C, a relative humidity between 50% and 60%, and a light intensity of 300 μmol photons m^{-2} s^{-1}. At the termination, rice roots and shoots were separated, washed with tap water for 6 min, rinsed with Milli-Q water for three times, and then freeze-dried for 1 day. Random samples of rice shoots and roots from both long and short-term experiments were used for this study.

2.3. Sample preparation for LA-ICP-MS

Freeze-dried rice shoots and roots samples were washed with distilled deionized water, acetone, and distilled deionized water sequence to remove any surface

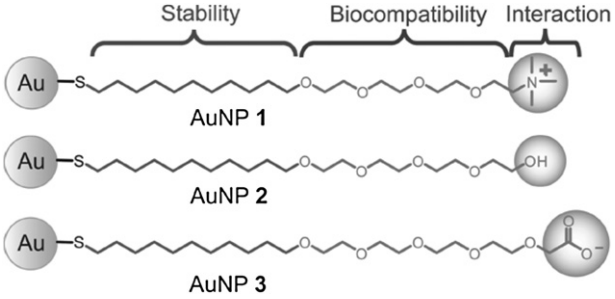

Fig. 1. Structural illustrations of gold nanoparticles with different terminal charges (AuNPs 1–3) (Modified from Zhu et al., 2010, *Small*; with the permission from John Wiley and Sons; [Copyright Clearance License Number 3044880771683]).