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Influence of aspartic acid and lysine on the uptake of gold nanoparticles in rice

Xinxin Ye[a], Hongying Li[b], Qingyun Wang[a], Rushan Chai[a], Chao Ma[a], Hongjian Gao[a,*], Jingdong Mao[c]

[a] Anhui Province Key Laboratory of Farmland Ecological Conservation and Pollution Prevention, School of Resources and Environment, Anhui Agricultural University, Hefei 230036, China
[b] Institute of Soil and Fertilizer, Anhui Academy of Agricultural Sciences, Hefei 230031, China
[c] Department of Chemistry and Biochemistry, Old Dominion University, Norfolk, Virginia 23529, United States

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ABSTRACT

The interactions between plants and nanomaterials (NMs) can shed light on the environmental consequences of nanotechnology. We used the major crop plant rice (*Oryza sativa* L.) to investigate the uptake of gold nanoparticles (GNPs) coated with either negatively or positively charged ligands, over a 5-day period, in the absence or presence of one of two amino acids, aspartic acid (Asp) or lysine (Lys), acting as components of rice root exudates. The presence of Asp or Lys influenced the uptake and distribution of GNPs in rice, which depended on the electrical interaction between the coated GNPs and each amino acid. When the electrical charge of the amino acid was the same as that of the surface ligand coated onto the GNPs, the GNPs could disperse well in nutrient solution, resulting in increased uptake of GNPs into rice tissue. The opposite was true where the charge on the surface ligand was different from that on the amino acid, resulting in agglomeration and reduced Au uptake into rice tissue. The behavior of GNPs in the hydroponic nutrient solution was monitored in terms of agglomeration, particle size distribution, and surface charge in the presence and absence of Asp or Lys, which depended strongly on the electrostatic interaction. Results from this study indicated that the species of root exudates must be taken into account in assessing the bioavailability of nanomaterials to plants.

1. Introduction

Nanomaterials (NMs) are widely used in many commercial products and applications, and it is inevitable that large quantities of NMs will be released into the environment (Maynard et al., 2006; Gao and Jiang, 2010; Gottschalk and Nowack, 2011). The probability of plant exposure to NMs will increase with the increasing quantities of NMs entering soils and water, causing unforeseen environmental and health consequences (Auffan et al., 2009; Stark, 2011; Wang et al., 2012; Zhang et al., 2012). Various factors influence the uptake and translocation of NMs within plants, such as environmental conditions, plant species and NM characteristics (e.g. particle size, shape and surface chemistry) (Remya et al., 2010).

Plant species are extremely important factor influencing the behavior of nanoparticles (NPs) in plants. A few studies have attempted to systematically evaluate the relative importance of plant species on plant bioaccumulation of NMs. Li et al. (2017) observed that Ag nanoparitcles (17 nm) could be assimilated and transferred in different tissues of soybean and rice. Zhu et al. (2012) found that there were significant variations in the uptake and distribution of gold nanoparticles (GNPs) among terrestrial plants, including rice, radish, pumpkin and perennial ryegrass. Different sizes of GNPs (10, 30, and 50 nm) could be taken up by tobacco but not by wheat (Judy et al., 2012). Tomato can take up more GNPs coated with one of three different short ligands than can rice (Li et al., 2016), while CeO_2 NPs could be detected in the shoots of pumpkin but not wheat (Schwabe et al., 2013). However, little is known about the mechanisms for differences in NP uptake between plant species.

The different strategies by which plants obtain nutrients from the soil depend, in part, on fundamental differences in the properties of root exudates between plant species (Marschner, 1995; Gao et al., 2014). The differences in NP accumulation between plant species might be explained by specific alterations in the physico-chemical interactions between NPs and root exudates in the rhizosphere (Rico et al., 2011; Judy et al., 2012). However, few studies have been carried out to investigate the effect of the species on root exudates and the

* Correspondence to: Anhui Province Key Laboratory of Farmland Ecological Conservation and Pollution Prevention, Anhui Agricultural University, 130 Changjiang West Road, Hefei 230036, PR China.
E-mail address: hjgao@ahau.edu.cn (H. Gao).

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bioavailability of NPs.

The presence of natural organic matter (NOM) influences the bioavailability of NPs. Fulvic acid and gum arabic significantly reduced the uptake of CeO_2 NPs in wheat and pumpkin, with the effect of control > fulvic acid > gum arabic (Schwabe et al., 2013). Dissolved organic carbon was found to associate with 4 nm and 18 nm GNPs in suspension and form a NP/organic matter complex that resulted in a decrease in GNP uptake by aquatic plants (Glenn and Klaine, 2013). Greater uptake of CuO NPs by cyanobacterium *Microcystis aeruginosa* was related, to a lesser degree, to NP aggregation by fulvic acid (Wang et al., 2011). Therefore, it is reasonable to hypothesize that differences in type and amount of root exudates between plant species might affect uptake, either by facilitating uptake or by inducing NP aggregation. The influence of concentration and nature of the exudate on the behavior and bioavailability of NPs needs to be investigated further.

Amino acids are an important component of root exudates, and can impact the yields and quality of crops (Aulakh et al., 2011; Zhang et al., 2015). The colloidal stability of dispersed NPs in aqueous medium is strongly affected by various amino acids (Schwaminger et al., 2015). These organic compounds can modify the surface charge properties of NPs entirely or partially, depending on their nature and concentration (Pušnik et al., 2016). In this study, we selected aspartic acid (Asp) and lysine (Lys) because these two molecules are reasonable analogues and are present at high concentrations in root exudates (Inderjit and Weston, 2003). Further, the two amino acids are of opposite charges in the soil environment as Asp reaches its isoelectric point at pH 2.97 and Lys at pH 9.74 (Pušnik et al., 2016).

The objectives of the study were (1) to systematically investigate the effects of Asp and Lys on the physico-chemical properties of the two different GNPs studied, including particle size, structure and surface properties, and on GNP bioaccumulation by rice, and (2) to clarify the processes by which Asp and Lys influence GNP behavior in nutrient media. The results will be important in understanding the possible influence of the surface charge of amino acids on the behavior and bioavailability of NPs under plant growth conditions.

2. Materials and methods

2.1. Preparation of GNPs

The two types of GNPs were synthesized according to the method of Li et al. (2016). Briefly, 2 mL 25 mM $HAuCl_4$ solution was added into 25 mL deionized water in an ice bath with moderate stirring, and then 2 mL 30 mM $NaBH_4$ was added as a reductant to obtain GNPs. To coat the GNPs, 4 mL 25 mM ligand as a capping agent, either thioglycolic acid (TGA) or cysteamine (CA), was added into the reaction solution, resulting in GNPs capped with TGA (GNP–TGA) or CA (GNP-CA). To remove excess reagents ($HAuCl_4$, TGA, and CA), the solution containing GNPs was dialyzed in ultrapure water for five days. The ultrapure water was updated every 12 h and stirred at room temperature.

Sodium borohydride (≥ 98.0%), gold(III) chloride trihydrate (≥ 99.9%, $HAuCl_4{\cdot}3H_2O$), thioglycolic acid (≥ 98.0%), cysteamine (≥ 98.0%), aspartic acid (≥ 99.0%) and lysine (≥ 98.0%) were purchased from Sigma-Aldrich (St Louis, USA).

2.2. The stability of GNPs in a hydroponic system

The two types of coated GNPs were dispersed (1 mg L^{-1}) in a nutrient solution prepared according to Zhu et al. (2012), containing macronutrients of 1 mM $Ca(NO_3)_2$, 0.5 mM KH_2PO_4, 1 mM $MgSO_4$, 0.5 mM K_2SO_4 and 1.5 mM NH_4NO_3 and micronutrients of 46 μM H_3BO_3, 0.8 μM Na_2MoO_4, 9 μM $MnSO_4$, 0.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$ and 75 μM EDTA–Fe. The pH in the nutrient solution was adjusted to 5.6. The GNP suspension in nutrient solution without Asp or Lys was designated the control (CK). The amount of Asp or Lys added to the GNP suspension depended on the concentration of root exudates from rice used in the experiment. The rice culture pots can contain about 1 mg L^{-1} Asp and 0.4 mg L^{-1} Lys during 5 d incubation, and the detailed analysis methods are given in Supporting Information. Different treatments were prepared: 1). Control (CK); 2). 0.25, 4 and 16 mg L^{-1} Asp (L-Asp, M-Asp and H-Asp, where L = low, M = moderate and H = high); 3). 0.1, 1.6 and 6.4 mg L^{-1} Lys (L-Lys, M-Lys and H-Lys), with three replicates for each treatment. The experiment was carried out for 5 d. At the end of the experiment, the solubility, particle size and surface charge of the GNPs was measured in each replicate.

2.3. The solubility of GNPs in a hydroponic system

To determine the solubility of the two types of GNPs in the nutrient solution, the hydroponic nutrient solution was collected after the 5-d incubation period, and the concentration of dissolved Au was determined. The samples were centrifuged and filtered through membrane filters (part number UFC500324, MWCO 3 kDa, Amicon Ultra, Millipore, USA). Only dissolved Au species could get through the filter after the centrifugation step, while the GNPs would remain on the filter. No dissolved Au was adsorbed on the MWCO filters in an adsorption experiment (data not shown). Dissolved Au content in the nutrient solution that got through the filter was then determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, iCAP Q, ThermoFisher, Waltham, MA, USA).

2.4. Plant exposure experiment

The seeds of rice (*Oryza sativa* L. cv. Xinliangyou 343) were gained from the Institute of Rice, Anhui Academy of Agricultural Sciences, Hefei, China. Rice seeds were surface-sterilized in 5% H_2O_2 solution for half hour and then thoroughly rinsed with deionized water. Germinated seeds were cultivated for two weeks in a 5 L box containing a nutrient solution half the strength of the original one described in Section 2.2. The nutrient solution was renewed every 3 days. After two weeks, uniform rice seedlings were collected and transplanted to carry out the experiment on the uptake of GNPs in full-strength nutrient solution. The pH in the nutrient solution was adjusted to 5.6. The rice was grown in a plant growth chamber with the day and night temperatures of 25 °C and 18 °C, respectively, a 16-h photoperiod with a light intensity of 300 μmol photons m^{-2} s^{-1} and a relative humidity of 60–70%. The GNP suspension was added to the hydroponic nutrient solution, and sonicated in a water bath for half hour to ensure a homogeneous distribution at a concentration of 1 mg L^{-1}. The incubated solutions containing GNPs without Asp or Lys were designated as the GNP treatment. 4 mg L^{-1} Asp or 1.6 mg L^{-1} Lys was added to the nutrient solution containing GNPs, which was be defined as GNP + Asp and GNP + Lys treatment. The control (CK) is the treatment without the addition of GNPs, Asp and Lys. Each treatment was replicated three times. The plants were then grown for 5 d, during which time the solution was not replaced. On the third and fifth days, the hydrodynamic diameter and surface charge of the GNPs in the medium were measured with a Zetasizer (Zetasizer3000HSa, Malvern, UK). After 5 d, rice samples were harvested and divided into shoots and roots, which were washed with tap water and then rinsed with deionized water three times.

2.5. Removal of GNPs on root surfaces with KI/I_2

GNPs on the rice roots were removed using a published procedure (Cho et al., 2009; Zhu et al., 2012). In brief, the fresh root samples of rice were dealt with 0.34 mM iodine (I_2) and potassium iodide (KI) in a molar ratio of 1:6 for 5 min. Then, they were washed with tap water, rinsed with deionized water three times, freeze-dried for 1 d and weighed.

The washing efficiency experiment was carried out according to the following methods. The fresh rice root was devitalized under 105 °C for 15 min, in order to exclude the internalization of GNPs into the rice