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Trophic transfer of gold nanoparticles from *Euglena gracilis* or *Chlamydomonas reinhardtii* to *Daphnia magna*

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ABSTRACT

Understanding the trophic transfer of nanoparticles (NPs) is important because NPs are small enough to easily penetrate into organisms. In this study, we evaluated the trophic transfer of gold NPs (AuNPs) within the aquatic food chain. We observed AuNPs transfer from 2 species of primary producers (*Chlamydomonas reinhardtii* or *Euglena gracilis*) to the primary consumer (*Daphnia magna*). Also, bioaccumulation of AuNPs in *E. gracilis* was higher than that in *C. reinhardtii*. The reasons for the difference in Au accumulation may be the physical structure of these organisms, and the surface area that is available for interaction with NPs. *C. reinhardtii* has a cell wall that may act as a barrier to the penetration of NPs. The size of *E. gracilis* is larger than that of *C. reinhardtii*. This study demonstrates the trophic transfer of AuNPs from a general producer to a consumer in an aquatic environment.

1. Introduction

Gold nanoparticles (AuNPs) have been used in various fields, such as biology (Sperling et al., 2008), automotive and fuel cell industry (Corti et al., 2002), and cosmetics (Guix et al., 2008). AuNPs have been selected as a representative manufactured nanomaterial by the Organisation for Economic Co-operation and Development (OECD) Working Party on Manufactured Nanomaterial (WPMN) (OECD, 2010). AuNPs have catalytic, optical, electronic, and molecular recognition properties. These unique properties make AuNPs applicable in biological labelling, drug delivery, gene transcription, photography, and sensors (Corti et al., 2002). Moreover, they have several advantages because of their easy synthesis (Ghosh et al., 2008) and biocompatibility (Shukla et al., 2005). Some studies on AuNPs have reported that they are non-toxic materials (Connor et al., 2005; Shukla et al., 2005). In contrast, a study on AuNPs has reported that the cytotoxicity caused by AuNPs was size-dependent (Pan et al., 2007). Therefore, further research on the toxicity of AuNPs is required.

NPs are extremely small; therefore, they can easily penetrate into tissues and cells of organisms, accumulate, and then be transferred to high-level organisms via the food chain. Previous studies on the trophic transfer of NPs were reported in an aquatic food chain (Bouldin et al., 2008; Cedervall et al., 2012; Ferry et al., 2009; Holbrook et al., 2008; Renault et al., 2008; Werlin et al., 2011; Zhu et al., 2010; Lee and An, 2014) and a soil food chain (Judy et al., 2011). In an aquatic food chain, Holbrook et al. (2008) studied 3 trophic transfers of carboxylated or biotinylated quantum dots (QDs) by using *Escherichia coli*, *Tetrahymena pyriformis*, and *Brachionus calyciflorus*. They observed trophic transfer of NPs from *T. pyriformis* to *B. calyciflorus*. Bouldin et al. (2008) observed that carboxyl QDs transferred from *Pseudokirchneriella subcapitata* to *Ceriodaphnia dubia*. In a previous study on trophic transfer of QDs, the prey *Pseudomonas aeruginosa* and the predator *Tetrahymena thermophila* were used (Werlin et al., 2011). In that study, the digestion function in *T. thermophila* fed *P. aeruginosa* that was pre-exposed to QD was affected, and biomagnification was also quantified. Zhu et al. (2010) observed that TiO_2 NPs were transferred from *Daphnia magna* to *Danio rerio*. They reported that more TiO_2 NPs accumulated with dietary exposure than with direct exposure. Renault et al. (2008) evaluated trophic transfer by using amine-coated AuNPs from the algae *Scenedesmus subspicatus* to the bivalve *Corbicula fluminea*. Ferry et al. (2009) tested the estuarine food web. They observed that Au nanorods could be passed through the water column to the marine food web. Cedervall et al. (2012) evaluated the transfer of polystyrene NPs in 3 trophic levels (*Scenedesmus* sp., *D. magna*, and *Carassius carassius*). Recently, Lee and

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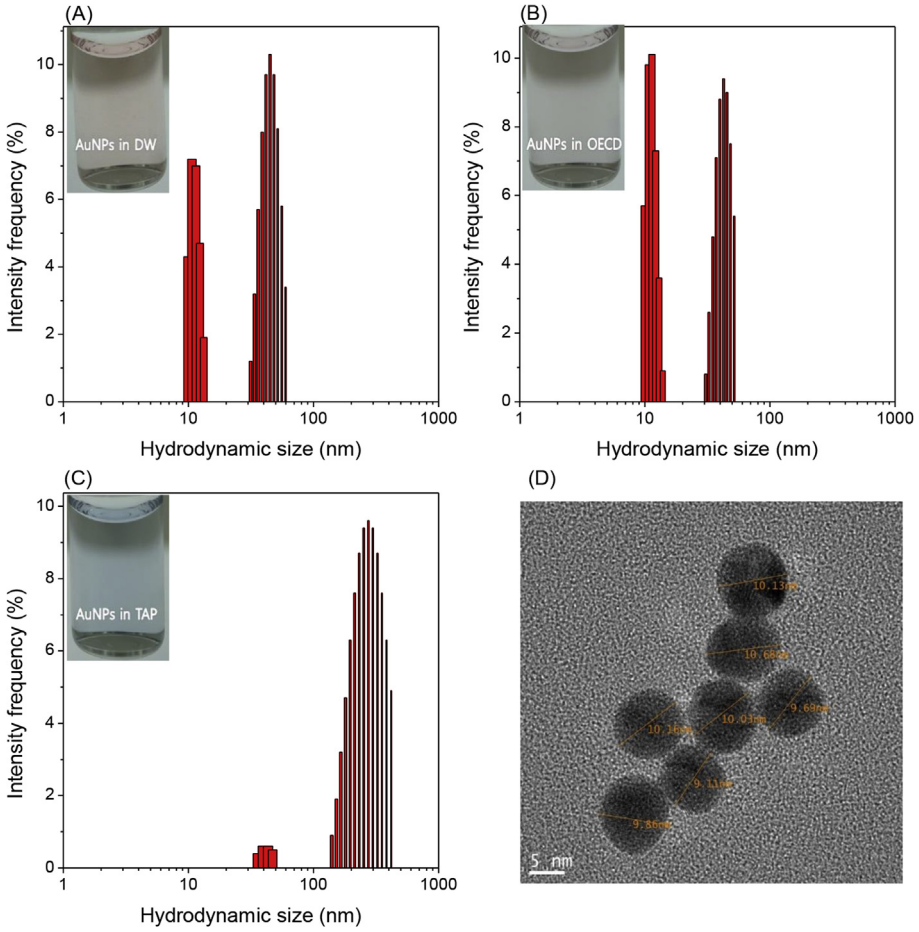

Fig. 1. Hydrodynamic size of AuNPs (10.11 mg L^{-1}) in (A) distilled water, (B) OECD medium, and (C) TAP medium. (D) TEM image of AuNPs. Insert images of (A), (B), and (C) show color change of AuNPs suspension in each media. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

An (2014) have verified that QD transfer from protozoa to zooplankton to fish using bioimaging technique. In a soil food chain, AuNPs were transferred through trophic levels and biomagnified from tobacco (*Nicotiana tabacum*) to tobacco hornworm (*Manduca sexta*) (Judy et al., 2011).

In this study, we performed the trophic transfer of AuNPs from 2 primary producer species, *Chlamydomonas reinhardtii* and *Euglena gracilis*, to the primary consumer *D. magna*. The reason for choosing *C. reinhardtii* and *E. gracilis* as food was that they are general producers in an aquatic environment. *C. reinhardtii* and *E. gracilis* are unicellular microorganisms that have 2 flagella and live in freshwater. *C. reinhardtii* and *E. gracilis* are both autotrophic and heterotrophic (Park et al., 2007; Yamane et al., 2001). The important difference between them is whether they have a cell wall or not. *C. reinhardtii* has a cell wall; however, *E. gracilis* lacks a cell wall and is surrounded by a membrane called the pellicle strip (Margulis and Chapman, 2009; Spellman and Price-Bayer, 2012). This property of the test species can cause a difference in trophic transfer. In this study, we compared direct exposure and dietary exposure by different food sources. First, the effects of direct AuNP exposure on *C. reinhardtii*, *E. gracilis*, and *D. magna* were assessed. Second, the trophic transfer of AuNPs from *C. reinhardtii* and *E. gracilis* to *D. magna* by dietary exposure was evaluated. To the best of our knowledge, this is the first study of the trophic transfer of NPs from *C. reinhardtii* and *E. gracilis* to *D. magna*.

2. Materials and methods

2.1. AuNPs

The AuNPs (Sigma–Aldrich, St. Louis, MO, USA) were supplied in the form of a colloid. AuNPs were suspended in 0.01% $HAuCl_4$, 0.01% tannic acid, 0.04% trisodium citrate, 0.26 mM potassium carbonate, and 0.02% sodium azide. According to the manufacturer, AuNPs ranged in size from 8.5 to 12.0 nm (average, 10 nm) and the concentration was 500×10^{10} particles·mL^{-1}. The size and morphology of AuNPs were characterized using field emission transmission electron microscope (FE-TEM) (JEM-2100F; JEOL, Tokyo, Japan). Hydrodynamic size was measured by an electron light scattering spectrophotometer (ELS-8000; Otsuka Electronics Co., Ltd., Japan).

2.2. Test species

C. reinhardtii and *E. gracilis* were purchased from the University of Texas at Austin (UTEX, USA) and Korea Marine Microalgae Culture Center (KMCC, Korea), respectively. They were cultured in tris-acetate phosphate (TAP) medium (pH 7.1 ± 0.0), as described in Chlamydomonas Connection (2012), and shaken continuously at 100 rpm under continuous fluorescent illumination in a growth chamber (24 ± 1 °C). *D. magna* was obtained from the National Institute of Environmental Research (NIER, Korea). It was cultured in moderately hard water (MHW; pH 7.6 ± 0.3), as described in USEPA (2002), at 21 ± 1 °C with a photoperiod of 16:8 h (light: