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Influence of PbS nanoparticle polymer coating on their aggregation behavior and toxicity to the green algae *Dunaliella salina*

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ABSTRACT

The potential hazards of nanoparticles (NPs) to the environment and to living organisms need to be considered for a safe development of nanotechnology. In the present study, the potential toxic effects of uncoated and gum Arabic-coated lead sulfide nanoparticles (GA-coated PbS NPs) on the growth, lipid peroxidation, reducing capacity and total carotenoid content of the hypersaline unicellular green algae *Dunaliella salina* were investigated. Coatings of PbS NPs with GA, as confirmed by Fourier transform infrared spectroscopy, reduced the toxicity of PbS NPs. Uncoated PbS NP toxicity to *D. salina* was attributed to higher algal cell-NP agglomerate formation, higher lipid peroxidation, lower content of total reducing substances and lower total carotenoid content. Low levels of Pb^{2+} in the growth culture media indicate that PbS NP dissolution does not occur in the culture. Also, the addition of 100 μM Pb^{2+} to the culture media had no significant ($P > 0.05$) effect on algal growth. The shading of light (shading effect) by PbS NPs, when simulated using activated charcoal, did not contribute to the overall toxic effect of PbS NPs which was evident by insignificant ($P > 0.05$) reduction in the growth and antioxidant capacity of the algae. When PbS NP aggregation in culture media (without algal cells) was followed for 60 min, uncoated form aggregated rapidly reaching aggregate sizes with hydrodynamic diameter of over 2500 nm within 60 min. Effective particle–particle interaction was reduced in the GA-coated NPs. Aggregates of about 440 nm hydrodynamic diameter were formed within 35 min. Afterwards the aggregate size remained constant. It is concluded that PbS NPs have a negative effect on aquatic algae and their transformation by GA capping affects NPs aggregation properties and toxicity.

1. Introduction

The nanotechnology field is currently one of the fastest growing branches of science. With the increase in quantities and types of nanomaterials, there is an increasing risk of ecosystem contamination, especially of the aquatic environments, by the nanoscale particles (Leigh et al., 2012; Perreault et al., 2012a,b; Petit et al., 2010; Wang et al., 2008; Youn et al., 2011). In accordance with the great increase in using nanomaterials, the number of publications specifically addressing questions about their potential hazards to the environment and to living organisms has increased rapidly (Peralta-Videa et al., 2011). Nanotoxicology, a branch of nanotechnology, has emerged to study the interactions of nanomaterials with living organisms (Santos et al., 2010). Since nanomaterials undergo various transformations in both the environment and biological systems, nanotoxicology also covers changes in the toxicity of nanomaterials associated with these transformations (Leigh et al., 2012; Lowry et al., 2012; Farkas et al., 2011).

Lead sulfide, PbS, is an important semiconductor with a small band gap (0.41 eV), large Bohr radius (18 nm), high dielectric constant and very high carrier mobility (Kang and Wise, 1997; Scanlon, 1958). These characteristics make PbS nanoparticles (PbS NPs) suitable for a variety of applications such as electroluminescent devices, solar cells, gas sensors and other optoelectronic devices (Bauer and Clemens, 1990; Preier, 1990; Rempel, 2007).

Several physicochemical characteristics of nanomaterials, such as size, aggregation, dissolution, coating and surface properties, can influence nanomaterial toxicity (Griffitt et al., 2008; Johnston et al., 2010; Keller et al., 2010; Perreault et al., 2012a,b). Toxicity of ZnO NPs to the green algae *P. subcapitata* was found to be solely related to Zn^{2+} released by dissolution of ZnO NPs (Aruoja et al., 2009). In another experiment with *P. subcapitata*, toxicities of bulk

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and nano ZnO particles were similar with regard to the toxicity of Zn^{2+} (Aruoja et al., 2009). Thus, the toxicities of these particles were attributed solely to the solubilized Zn^{2+}. In *Lemna gibba*, inhibition of photosynthetic activity was suggested to be mainly due to the solubilization of CuO NPs into copper ions (Perreault et al., 2013).

Particle size influences the toxicity of nanomaterials, with smaller size generally being more toxic to living organisms (Hund-Rinke and Simon, 2006; Petit et al., 2010; Zhang et al., 2007). Nano-sized ZnO particles were more toxic to *Allium cepa* in causing chromosomal aberration and cellular dysfunction compared to bulk ZnO (Kumari et al., 2011). Multi-walled carbon nanotubes were toxic to *Arabidopsis* T87 suspension cells with agglomerates of smaller size inducing stronger toxicity (Lin et al., 2009a,b,c). Bulk TiO_2 and bulk CuO were less toxic to *P. subcapitata* compared to their nano-scale size counterparts (Aruoja et al., 2009).

Aquatic organisms seldom encounter nanomaterials of their primary size (Keller et al., 2010). Nanomaterials usually aggregate to form larger particles and the degree of aggregation is influenced by many factors including pH, ionic strength, natural organic matter of the aqueous media, type of capping agent and NP concentration (Badawy et al., 2010; Keller et al., 2010). Miller et al. (2010) showed that ZnO NPs with a primary size of 15–30 nm aggregated rapidly in seawater forming aggregates of about 450 nm hydrodynamic diameter within 30 min. Median particle size of thioglycolate capped CdTe QDs in algal culture medium increased with time and aggregates of up to 710 nm were detected after 48 h (Wang et al., 2008). Therefore, the toxic effects of NPs on the living organisms observed in some experiments are likely due to their aggregates rather than the original nanoscale particles (Leigh et al., 2012).

Stability of NPs suspensions in aqueous media is, among other factors, affected by capping agents which may reduce or prevent NPs aggregation through electrostatic, steric or electrosteric repulsion (Ju-Nam and Lead, 2008; Keller et al., 2010). When QDs nanocrystals were coated with polyethylene glycol, aggregation was suppressed regardless of the ionic strength of the media (Jiang et al., 2009). Decrease in aggregation of polymer coated CuO NPs relative to uncoated CuO NPs was reported by Perreault et al. (2012a,b). Using near infrared fluorescence spectroscopy, Youn et al. (2011) showed that in the presence of gum Arabic, most of the single walled carbon nanotubes remained suspended and well dispersed in the culture media throughout the algal exposure experiments. In contrast, application of a thin layer of alumina onto the surface of SiO_2 NPs increased particle aggregation in the test medium. The reason for this is likely the low surface charge of the coated particles compared to the uncoated SiO_2 NPs (Van Hoecke et al., 2011).

Toxicological studies have shown that capping plays an important role in determining toxicity of nanomaterials (Griffitt et al., 2008; Van Hoecke et al., 2011). Interactions of NPs with natural organic matter (NOM) have been shown to reduce short term bacterial toxicity for fullerenes and silver nanoparticles (Fabrega et al., 2009; Li et al., 2008). Addition of NOM to SiO_2 NPs suspension decreased their toxicity to *P. subcapitata* (Van Hoecke et al., 2011). Since NOM was able to adsorb onto the NPs surface, decrease in the toxicity was suggested to be due to the shielding of the NPs by NOM. In contrast, poly styrene-co-butyl acrylate-coated CuO NPs and polymer-coated CdSe nanoparticles were more toxic compared to the uncoated forms (Kirchner et al., 2005; Perreault et al., 2012a,b, 2014). Increased penetration into the algal cells, higher precipitation on the cell surface and increased ROS production were correlated with their higher toxicity. It has been shown that some capping agents, such as sodium cholate, Triton X-100 and sodium dodecyl sulfate are themselves toxic to living organisms. In contrast, capping agent like gum Arabic is found to be non-toxic and, indeed, mitigate the toxicity of some NPs (Leigh et al., 2012; Youn et al., 2011; Gao, 2008).

Because surface waters receive contaminants, including NPs, from different sources, understanding the impacts of NPs on the aquatic organisms is crucial to evaluating their potential hazards and toxicity. To the best of authors' knowledge, there are no reports on the potential toxic impact of PbS NPs on living organisms. The objective of this study was thus to evaluate the potential toxic effect of uncoated and gum Arabic coated PbS NPs (GA-coated PbS NPs) to the hypersaline unicellular green algae *Dunaliella salina* under laboratory conditions. Emphasis is placed on determining (1) the effects of coating on the toxicity of PbS NPs; (2) degree of aggregation and dissolution of uncoated and GA-coated PbS NPs; (3) shading effects caused by the PbS NPs; and (4) antioxidant capacity of algal cells as affected by the NPs.

2. Materials and methods

2.1. Chemicals and nanoparticles

All chemicals were purchased from Sigma-Aldrich Chemical Co. (Darmstadt, Germany). Uncoated PbS nanoparticles with the primary size of about 70 nm was synthesized in the Nanotechnology Research Institute, Shiraz University (Shiraz, Iran). Residual Pb^{2+} in the sample was about 2.5 $\mu g\,ml^{-1}$ as determined by Inductive Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES). To study the aggregation of PbS NPs, 100 μM suspensions of uncoated and GA-coated NPs were prepared in algal culture media. Then, the suspensions were sonicated for 10 min and immediately used for hydrodynamic diameter determination by Dynamic Light Scattering (DLS, Horiba LB-550). The aggregation process was followed every 2 min for the first 30 min and then every 5 min up to 60 min.

2.2. Algal culture

Dunaliella salina starin MSI-3 (GenBank accession no. KC477401) which was isolated from Maharlu salt lake in Shiraz, Iran and identified on the bases of morphology and rDNA ITS sequences (Zamani and Moradshahi, 2013) was used in the present investigation. The cells were cultivated in culture medium containing 50 mM $NaHCO_3$, 5 mM $MgSO_4$, 0.75 mM KNO_3, 0.2 mM KH_2PO_4, 0.2 mM $CaCl_2$, 7 μM $MnCl_2$, 5 μM EDTA, 2 μM $FeCl_3$, 1 μM $CuCl_2$, 1 μM $ZnCl_2$ and 2 M NaCl (Ben-Amotz et al., 1989). The culture was exposed to continuous illumination (120 μmol photon $m^{-2}\,s^{-1}$) provided by white fluorescent lamps at 22 ± 2 °C. At the exponential phase of growth, the algal culture was divided using 250 ml Erlenmeyer flasks, each receiving 100 ml of the culture with a cell density of about 10^6 cell ml^{-1}.

2.3. Algal culture exposure experiments

A 24 $mg\,ml^{-1}$ stock dispersion of PbS NPs in deionized water was sonicated for 15 min and immediately used for algal exposure experiments. To the flasks containing 100 ml of algal culture, we added either (1) sonicated suspension of uncoated or GA-coated PbS NPs to give 25, 50, 100 and 200 μM final concentration of NPs; (2) GA at a final concentration of 1%; (3) Pb^{2+} to give 100 μM concentration; or 4) activated charcoal, to evaluate the shading effect of NPs.

The flasks were then kept under the same conditions as described before. For each experiment, samples were taken at indicated time intervals and number of cells was determined using a hemocytometer. In each experiment, control samples were run in parallel with test samples in triplicate.