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Sulfidation of silver nanoparticle reduces its toxicity in zebrafish

G. Prathinkra Devi[a], Khan Behlol Ayaz Ahmed[b], M.K.N Sai Varsha[a], B.S. Shrijha[a], K.K. Subin Lal[a], Veerappan Anbazhagan[b,*], R. Thiagarajan[a,c,*]

[a] Department of Bioengineering, School of Chemical & Biotechnology, SASTRA University, Thanjavur 613401, India
[b] Department of Chemistry, School of Chemical & Biotechnology, SASTRA University, Thanjavur 613401, India
[c] Center for Research on Infectious Diseases (CRID), School of Chemical & Biotechnology, SASTRA University, Thanjavur 613401, India

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ABSTRACT

Chemical transformations of metal nanoparticles can be an important way to mitigate nanoparticle toxicity. Sulfidation of silver nanoparticle (AgNPs) is a natural process shown to occur in environment. Very few studies, employing microbes and embryonic stages of zebrafish, have shown reduction in AgNPs toxicity as a direct result of sulfidation. However the feasibility of reducing nanoparticle toxicity by sulfidation of AgNPs has never been studied in adult vertebrates. In this study, we have used adult zebrafish as a model to study the efficacy of sulfidation of AgNPs in reducing nanoparticle toxicity by employing a battery of biomarkers in liver and brain. While AgNPs enhanced liver oxidative stress, altered detoxification enzymes and affected brain acetylcholinesterase activity, sulfidation of AgNPs resulted in significant alleviation of changes in these parameters. Histopathological analyses of liver and sulphydryl levels also support the significance of sulfidated AgNPs in controlling the toxicity of AgNPs. Our study provides the first biochemical data on the importance of sulfidation of AgNPs in reducing biological toxicity in adult vertebrates.

1. Introduction

Due to their nanosize, nanoparticles (NPs) exhibit unique physicochemical properties than their bulk counterparts. These unique properties have been exploited in many applications, including, optoelectronics, catalysis, bioanalytics and biomedicine (Jain et al., 2008). Among metal nanoparticles, silver nanoparticles (AgNPs) are currently being utilized in various commercial products including health care products such as burn dressings, scaffolds, food packing, water purification systems, antimicrobial applications and medical devices (Chaloupka et al., 2010; Chernousova and Epple, 2013; Choi et al., 2012; Li et al., 2008). The increased usage of AgNPs in various fields like textile products (Osório et al., 2012) and surface coatings (Cassagneau and Caruso, 2002) might result in an increased release of nanoparticles into the environment either wittingly or by chance. As a result, they can induce toxicity to the contact organisms and their ecosystems. Toxicology studies have shown that AgNPs are toxic to bacteria, cell lines, plants, vertebrates and mammals (Asha Rani et al., 2009; Kawata et al., 2009; Lee et al., 2007). The antibacterial properties of metal nanostructures are believed to be triggered by the induction of oxidative stress and membrane damage (Nel et al., 2006). Despite the developments in nanotoxicology, there is lack of information on the mechanism of action, in particular, how NPs trigger toxicity in vertebrates including mammals.

Nanoparticles are highly reactive because of their large surface area. In soil or aquatic environment, nanoparticles can react with the abundant electrolytes and can form various complexes or aggregates (Fabrega et al., 2011; Whitley et al., 2013). In aquatic environment AgNPs are reported to undergo chemical transformations in the presence of common ligands such as SO_4^{2-}, S^{2-}, Cl^-, PO_4^{3-} and EDTA (Kaegi et al., 2013). A series of studies from the laboratory of Dr. Wood, have indeed shown that presence of sulphide offers protection against $AgNO_3$ toxicity (Bowles et al., 2002), wherein they subsequently showed that in the presence of sulphide there was active incorporation, rather than mere adsorption, of $AgNO_3$ by *Daphnia* (Bianchini et al., 2002). Subsequently, the authors went on to demonstrate that *Daphnia* (Bianchini et al., 2005; Bianchini and Wood, 2008) and *Oncorhynchus* (Mann et al., 2004), accumulated greater concentrations of Ag in the gills in the presence of reactive sulphide groups in water, which also resulted in rapid clearance of Ag. These results clearly demonstrate that sulphide reduces Ag toxicity and thus could be a potential

* Corresponding authors at: Department of Bioengineering, School of Chemical & Biotechnology, SASTRA University, Thanjavur, Tamil Nadu 613401, India.
Tel.: +91 4362 264101 2359/+91 4362 264101 3689; fax: +91 4362 264120.
E-mail addresses: anbazhagan@scbt.sastra.edu (V. Anbazhagan), raman@biotech.sastra.edu (R. Thiagarajan).

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mechanism for mitigating nanoparticle toxicity. Recently, Kim et al. (2010) identified silver sulfide (Ag_2S) nanoparticles in sewage sludge products. Levard et al. (2011) showed that sulfidation of AgNPs resulted in decreased silver ion mobility and also decreased the dissolution rate. Choi et al. (2009) showed that even a stoichiometric amount of sulfidation can decrease AgNPs toxicity to nitrifying bacteria. Recently, another important report by Levard et al. (2013) showed the feasibility of sulfidation of AgNPs in reducing their toxicity against four diverse organisms; *Danio rerio*, *Fundulus heteroclitus*, *Caenorhabditis elegans*, and the aquatic plant *Lemna minuta*, clearly demonstrating that sulfidation could definitely be a potential mechanism for mitigating nanoparticle toxicity. However, the fate of the newly formed products over time remains enigmatic.

Zebrafish (*D. rerio*) has been widely employed in several toxicology studies and is an important model organism in scientific research. The fish, unlike fruit flies or nematode worms, are vertebrates and are 80% genetically identical to humans. Since the fish share so many common biological pathways with humans, they make excellent test subjects (Spitsbergen and Kent, 2003). The unique features of zebrafish that make them better model organisms include; transparent embryos, small size, easy maintenance, adaptability, short breeding time, and high fecundity (Hill et al., 2005). When exposed to xeno-substances, both zebrafish and mammals demonstrate a similar physiologic response such as the induction of metabolizing enzymes and oxidative stress (Hill et al., 2005). Numerous studies have suggested that exposure to AgNPs reduces the development of zebrafish. Myrzakhanova et al. (2013) reported that AgNPs inhibit the expression of molecules related to cholinergic system and impact early development of zebrafish. Exposure to AgNPs has also been shown to affect the development of zebrafish embryo by altering gene expression, especially of the genes responsible for oxidative phosphorylation and protein synthesis. Exposure of adult zebrafish to AgNPs has also been shown to influence transcriptome activity and bioaccumulation of AgNPs, (Chio et al., 2012; Griffitt et al., 2013; Merrifield et al., 2013) trigger endoplasmic reticulum stress (Christen et al., 2013), and modulate biochemical enzymes (Katuli et al., 2014). Despite these reports, underlying mechanisms responsible for the adverse effects of AgNPs in fully developed organisms remain largely unknown. Therefore, it is important to analyze toxicity in adult organisms because they are the primary source for bioaccumulation leading to biomagnification of xenobiotics in an ecosystem. In addition, the physiological response of adult organisms also differs from larval or embryonic stages. Thus, in this study, we focus on various complementary biochemical assays to assess the toxicity of AgNPs in adult zebrafish. Further, the possible effect of sulfidation in reducing AgNP toxicity was evaluated and the results are discussed.

2. Materials and methods

2.1. Materials

Polyvinyl pyrrolidone (PVP), silver nitrate and sodium sulfide were purchased from Alfa Aesar, India. DTNB, epinephrine, and fast blue B were purchased from Sigma-Aldrich. All other chemicals and reagents were of the highest analytical grade and commercially available.

2.2. Preparation of AgNPs

In a typical procedure to synthesize AgNPs, 50 mg of polyvinyl pyrrolidone (PVP) and 1 mM $AgNO_3$ was dissolved in 50 ml of deionized water. To this reaction mixture, 2 mM of sodium borohydride was injected slowly under constant stirring at room temperature. The immediate change in color from colorless to a bright yellow color indicated the reduction of silver ion to AgNPs. The dispersion was allowed to stir for another 15 min. In order to mimic natural sulfidation, the AgNPs were sulfidized using 2 mM sodium sulfide (Na_2S) aqueous solution. At room temperature, the reaction mixture turns from yellow to blackish yellow, indicating the formation of silver sulfide (Ag_2S). The resulting solution was centrifuged to remove excess sulfide ion and washed with deionized water. Finally, the Ag_2S nanoparticles were resuspended in deionized water and used in subsequent studies.

UV-Vis spectra of the NPs solution were recorded on a Thermo Scientific Evolution 201 spectrophotometer operated at a resolution of 1 nm. The morphology and size were measured by using field emission scanning electron microscope (FESEM) (JEOL-JSM 6701F, Japan) with an accelerating voltage of 30 kV and filament current of 20 mA for 45 s. The samples were stuck onto a double-face conducting carbon tape mounted on a brass stub. Prior to imaging the samples were coated with a thin layer of platinum in an auto fine coater. EDX analysis was conducted with the same instrument to confirm the elemental composition of the sample.

2.3. Animals

Adult zebrafish, *D. rerio*, 4 to 5 cm in length, weighing approximately 300 mg, irrespective of sex, were purchased from a local aquarium. They were maintained in aerated glass tanks containing tap water at 25 ± 2 °C. Fish were fed ad libitum with commercial fish diet and the tanks were cleaned, sterilized and water replaced periodically. Water quality was monitored regularly and water from the same source after filtering was used. The fish were allowed to acclimatize for a week before initiation of nanoparticle exposure.

2.4. Exposure

Optimum dosage for chronic exposure was calculated from previous work (Bilberg et al., 2012). AgNPs were prepared at a concentration of 0.016 g/100 ml, which is 1 mM in stock concentration of AgNPs. Fish were then exposed to 0.1 ppm concentration which equated to 125 μl of PVP and silver and silver sulfide nanoparticles by direct addition to tap water. The study consisted of four groups: control (no treatment, plain tap water), PVP (1 mg/ml), AgNPs & Ag_2S, and each group contained 20 fishes (4 l/tank). The exposure period was for 15 days. Water (from a single source) and the exposure compounds were renewed every day. Water parameters were: DO—8.5 ± 1.3 mg/l; pH—7.58; total hardness (as $CaCO_3$)—145 ± 8.5 mg/l; chlorides—73 ± 3 mg/l; calcium 4.3 ± 0.7 mg/l; magnesium 2.5 ± 0.4 mg/l; alkalinity—352 ± 9.6 mg/l; total dissolved solid—250 ± 5 mg/l. Water temperature was 25 ± 2 °C. The water was always allowed to stand for 24 h before use.

2.5. Tissue preparation

At the end of 15 days, fish were sacrificed (anesthetized by 150 mM MS-222 and euthanized by decapitation), skin was removed and depending on the assay, the liver and brain (for acetylcholinesterase assay) from two fish were pooled and homogenized in ice-cold buffer (Tris–HCl, 0.1 M, pH 7.4). In general, for all the assays, the homogenate was centrifuged at 10,000 × *g* for 10 min at 4 °C, and the supernatants were used for further analyses. From the homogenate prepared from liver or brain pooled from two fishes, duplicates were derived for each assay.