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Short communication

Silver nanoparticle toxicity in sea urchin *Paracentrotus lividus*

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ABSTRACT

Silver nanoparticles (AgNPS) are an important model system for studying potential environmental risks posed by the use of nanomaterials. So far there is no consensus as to whether toxicity is due to AgNPs themselves or Ag^+ ions leaching from their surfaces. In sea urchin *Paracentrotus lividus*, AgNPs cause dose dependent developmental defects such as delayed development, bodily asymmetry and shortened or irregular arms, as well as behavioural changes, particularly in swimming patterns, at concentration ~0.3 mg/L AgNPs. It has been observed that AgNPs are more toxic than their equivalent Ag^+ ion dose.

1. Introduction

Ag nanoparticles (AgNPs) are used in health products and are subsequently released in freshwater and marine ecosystems (Wijnhoven et al., 2009). AgNPs flocculate in high ionic strength environments (Buzea et al., 2007; Chen et al., 2007). Hence an accumulation and agglomeration of AgNPs can be expected in sea water (Wijnhoven et al., 2009; Chinnapongse et al., 2011) followed by the release of active silver ions from the nanosilver surface (Liu et al., 2010). There is an urgent need to study the effects of this chemical activity on marine organisms. The toxicity of silver nanoparticles has been observed and proper regulations are required (Auffan et al., 2010).

Sea urchins are model organisms for assessments of sea water quality (Kobayashi, 1991), ecology (Kroeker et al., 2010), developmental biology (Jasny and Prunell, 2006), embryology, and biomineralization processes (Wilt, 2005). Sea urchin embryos are used in a number of toxicological studies such as the effects of UV (Bonaventura et al., 2006) and X-rays (Matranga et al., 2010).

Knowledge of the effects of AgNPs on marine organisms is still insufficient; to date there have been only studies of the organism level impact of AgNPs on blue mussels (Zuykov et al., 2011) and oyster embryos (Ringwood et al., 2010). Here we study whether the same malformations would occur in sea urchin embryos/larvae.

In this work we have studied sea urchin *Paracentrotus lividus* influenced by citrate-stabilized AgNPs and by Ag^+ ions via optical microscopy. The AgNPs were characterized by high resolution transmission electron microscopy (HRTEM) and UV–visible absorption spectroscopy.

2. Materials and methods

Silver nanoparticles were prepared by chemical reduction following the method used by Link et al. (1999). Silver ions were reduced and stabilized by citrate, using silver nitrate and sodium citrate as precursors (see SI). Specimens of AgNPs were prepared for HRTEM measurements on Cu grids. The size distribution of the AgNPs was analysed by HRTEM using a JEOL 2100F FGETEM instrument.

We used UV–Vis spectroscopy (Varian, CARY 100-BIO) in order to follow any flocculation of AgNPs in sea water (the concentration of AgNPs was ~3 mg/L). After mixing, the diluted AgNP solutions were studied immediately and then after 9, 16, 22, 32, 48, 51 h (see SI).

For Ag^+ ions released from AgNPs in sea water, a dialysis membrane (MWCO = 3500; diameter = 11.5 mm) was used. Synthesized AgNP solution (initial concentration = 30 mg/L Ag) was diluted in filtered natural sea water with the dilution ratio of 10 for final concentration of 3 mg/L Ag. The dialysis membrane was filled with 5 mL diluted AgNP solution and then placed in a beaker containing 500 mL DI water. 10 mL DI water was collected from the beaker at 3, 6, 12, 18, 24, 30, 36, 42, 48, 51 h. The DI water was measured for Ag^+ ion concentration by inductively coupled plasma optical emission spectroscopy (Model Unicam 701) (see SI).

Adult sea urchins *Paracentrotus lividus* were collected from different locations in the Adriatic Sea, e.g. Figarola island (N 45° 05.622′, E 13° 37.132′). The sea urchins were kept in sea water aquaria. Gametes were obtained by the injection of 0.5 M KCl

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into the adult species. The collected gametes were pooled and fertilization carried out in 0.22 μm filtered natural sea water (FNSW, collected close to the Ruder Boskovic Institute Rovinj, Croatia). 2 h after fertilization the 4 cell stage was washed in a 40 μm filter using FNSW (salinity about 39‰) and transferred to 6-well multiwell-plates containing FNSW with different AgNP concentrations. These experiments were performed for 4 different fertilizations with at least duplex samples; control development was monitored in NFSW without AgNPs. The influence of AgNPs on embryo development was done with 3, 0.3 and 0.03 mg/L AgNP concentrations. We tested the corresponding Ag^+ ion concentrations; 0.03, 0.003 and 0.0003 mg/L. The embryonic development was at 18–19 °C and the embryos/larvae were documented using an Olympus SZH 10 microscope. For counting and observation of developmental defects, larvae movement was stopped using 5% MgCl or a fixation with 0.1% formaldehyde. Detailed light microscopy pictures were taken with 100% EtOH fixed larvae in an Axiovert 200 M Light microscope. Development was documented after 24 and 48 h for all experimental setups while 100 embryos/larvae per concentration different experimental setups were counted after 24 and 48 h. For statistical analysis 6×100 larvae (4×100 for Ag^+ ion treatment) from different experimental setups were counted for the categories and grouped into normal development/developmental defects. The significance was tested using a one way ANOVA followed by a Bonferroni *t*-test and compared with the control sample. The significance levels of the statistical analysis were set at alpha = 0.05 using the SigmaStat software. After 48 h of development, larvae treated with AgNPs were classified into the following categories: delayed development, deformed pluteus, small pluteus with short arms and small skeleton and normal pluteus.

3. Results and discussion

The average diameter of the AgNPs was found to be in the range of 5–35 nm (Fig. 1B) (see SI). AgNPs with an elongated shape (Fig. 1C) are formed from 2 or more nanoparticles of different sizes. Multiple twinning defects (Fig. 1A) are observed and the particle shape varies between spherical and prolate ellipsoidal. Similar shapes and twinning defects have been detected in studies of AgNPs produced by the citrate method (Henglein and Giersig, 1999). Fig. 1D shows (111), (110), (004) and (101) reflections of the Ag crystal planes.

Fig. 2 displays the 408 nm plasmon absorbance of synthesized AgNPs in (A) DI water and (B) sea water immediately after making the solution (0 h). The intensity of the plasmon absorbance of AgNPs in sea water is smaller than in DI water and is asymmetric towards longer wavelengths. The plasmon absorbance of AgNPs in sea water shows a bi-modal Ag distribution. Previously an additional peak at ~725 nm (Fig. 3) was observed for AgNPs in sea water and attributed to flocculated particles (loosely bound) (Henglein and Giersig, 1999). Recently, the agglomeration of 20 nm AgNPs prepared by the citrate method in 154 mmol/L NaCl solution has been reported, and the particles show similar agglomeration (MacCuspie, 2011). The red-shift of the plasmon peak is due to flocculation arising from the collapse of double layers; the floc behaves as if it were a larger particle.

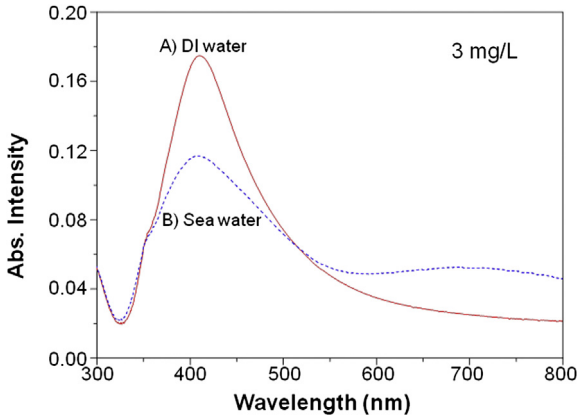

Fig. 2. Plasmon absorbance of AgNPs in (A) DI water and (B) sea water.

According to dialysis experiment for Ag^+ ions released from AgNPs with concentration of 3 mg/L, AgNPs release more Ag^+ ions with increasing time in sea water as shown in Fig. 4. The enhanced release of Ag^+ ions from AgNPs in sea water is due to the effect of

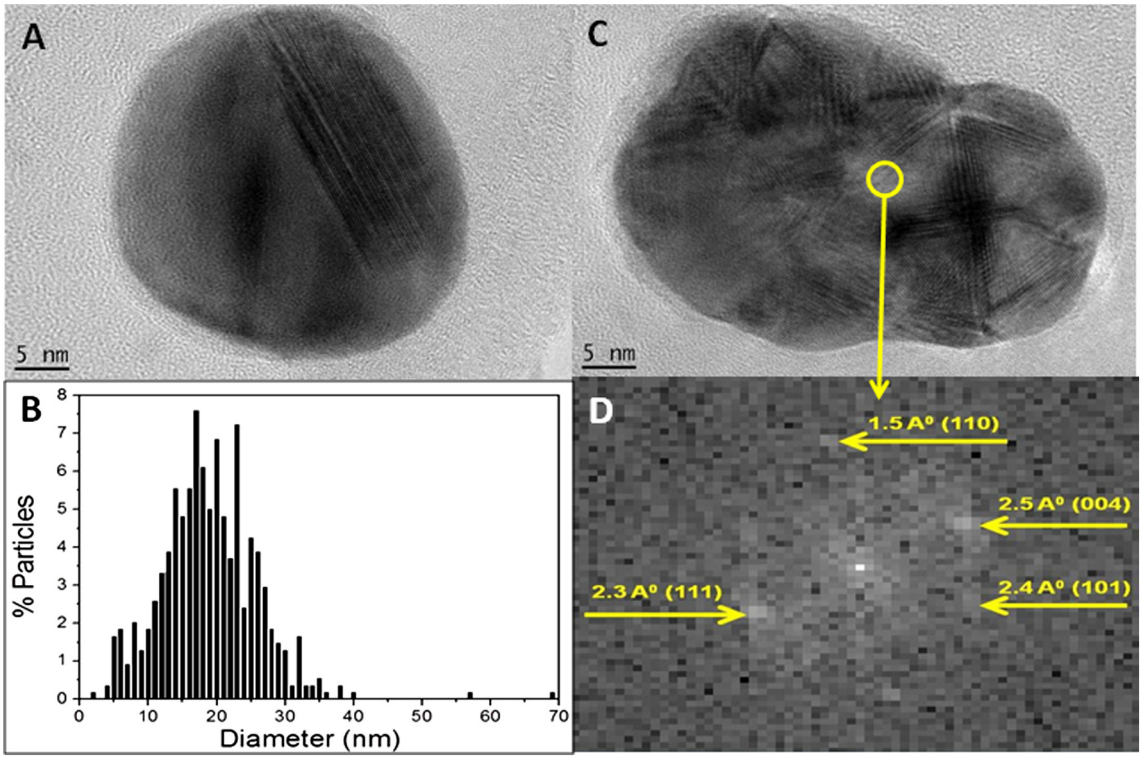

Fig. 1. (A) and (C) HRTEM images showing freshly prepared AgNPs. These are found to have diameters varying from ~5 to 35 nm in (B). (D) shows the Fourier Transform (FT) of the area denoted in (C) with the plane spacing corresponding to the Ag crystal lattice.