Silver nanoparticle dissolution in the presence of ligands and of hydrogen peroxide

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Abstract

Dissolution of silver nanoparticles (AgNP with carbonate or citrate coating, total Ag 1 – 5 μM) was examined in the presence of the ligands cysteine, chloride and fulvic acids and of the oxidant hydrogen peroxide (H_2O_2) at low concentrations at pH 7.5. Dissolved Ag was separated from AgNP by ultrafiltration. Cysteine in the concentration range 0.2 – 5 μM resulted in an initial increase of dissolved Ag within few hours. Chloride (up to 0.1 mM) and fulvic acids (up to 15 mg L^{-1}) had little effect on the dissolution of AgNP within hours to days. In contrast, very rapid dissolution within 1 – 2 h of both carbonate and citrate coated AgNP was observed in the presence of H_2O_2 in the concentration range 0.1 – 10 μM , under dark or light conditions. The high efficiency of H_2O_2 in dissolving AgNP is likely to be of importance in toxic effects of AgNP to algae, as H_2O_2 is produced and released into solution by algae.

Capsule

Dissolution of silver nanoparticles in the presence of cysteine, chloride and fulvic acids and of hydrogen peroxide is an important process influencing toxicity to aquatic organisms.

Keywords: silver nanoparticles, dissolution, cysteine, chloride, fulvic acids, hydrogen peroxide

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Highlights

- Dissolution of silver nanoparticles under relevant conditions for natural waters and for toxicity experiments;
- Effects of cysteine on AgNP dissolution;
- Weak effects of chloride and fulvic acids on AgNP dissolution;
- Rapid dissolution of AgNP with hydrogen peroxide;
- Relevance of dissolution with H_2O_2 for toxicity of AgNP to algae.

Graphical abstract

Introduction

Dissolution of silver nanoparticles (AgNP) is an important process determining both their effects to organisms and their fate in aqueous environments (Levard et al., 2012; Lowry et al., 2012; Behra et al., 2013; Sigg et al., 2014). Dissolved Ag^+ ions are known to be toxic to various aquatic organisms, including bacteria, algae and fish (Bury et al., 1999; Bury and Wood, 1999; Hiriart-Baer et al., 2006; Navarro et al., 2008; Xiu et al., 2011). For toxicity assessment of silver nanoparticles, it is thus essential to take into account the dissolution reactions in order to distinguish between ion and nanoparticle effects. Toxic effects of silver nanoparticles have already been shown in various organisms to be mostly due to the effects of silver ions which are released into solution from AgNP as a result of interactions with exposure media and with the organisms (Navarro et al., 2008; Xiu et al., 2011; Yang et al., 2012; Navarro et al., 2015). The processes influencing dissolution of silver nanoparticles need thus to be investigated in natural waters as well as in experimental media.

Dissolution of AgNP, which includes an oxidation step of $\text{Ag}(0)$ to $\text{Ag}(I)$, is expected to depend on the presence of oxygen and other oxidants, on pH and on available ligands for $\text{Ag}(I)$ in solution (Liu and Hurt, 2010; Ma et al., 2011; Liu et al., 2010; Gondikas et al., 2012). AgNP dissolution has been shown to be more efficient and faster at lower pH (Liu et al., 2010). Strongly binding ligands for $\text{Ag}(I)$ include mostly sulfide and thiol ligands (e.g. cysteine), whereas chloride forms only weaker complexes. Binding of silver to humic or fulvic acids is expected to be stronger to the few thiol groups than to the more abundant carboxyl, hydroxyl and amino groups (Chen et al., 2012). Thiol ligands may accelerate the dissolution process of AgNP, as shown for high cysteine concentrations (Gondikas et al., 2012). However, binding of inorganic sulfide to AgNP has been shown to lead to the rapid formation of a solid silver sulfide solid phase with very low solubility (Kaegi et al., 2011). Effects of chloride have to be carefully considered, as depending on the concentration, formation of solid $\text{AgCl}(s)$ or of dissolved complexes with $\text{Ag}(I)$ may occur. Furthermore, dissolution of AgNP is also dependent on the various coatings used for colloidal stability and on the nanoparticle size (Ma et al., 2011). Dissolution of AgNP by H_2O_2 has been examined in the context of conditions of washing of textiles containing AgNP (Geranio et al., 2009), in which H_2O_2 was shown to efficiently increase Ag^+ release. Reactions of H_2O_2 with AgNP have also been studied in more detail with respect to the reaction kinetics and mechanisms (He et al., 2011; He et al., 2012).

The aim of this study is to examine dissolution of silver nanoparticles under conditions which are relevant for experimental media used in toxicity studies of AgNP to algae and other

aquatic organisms, as well as for natural freshwaters. Two often used AgNP coatings are considered, namely carbonate and citrate. The effect of some relevant ligands for Ag(I) is examined, namely chloride, cysteine and fulvic acids, as well as the effect of the oxidant hydrogen peroxide (H_2O_2) at low concentrations. Hydrogen peroxide is used here as a reactive oxidant at low concentrations, which may also be produced by algae (He et al., 2012a; Pospisil, 2012). The implications of the observed dissolution reactions and of the dissolved Ag concentrations for toxicity experiments are discussed.

Materials and methods

Materials

Silver nanoparticles with carbonate or citrate (carb-AgNP and cit-AgNP) coating were obtained from NanoSys GmbH (Wolfhalden, Switzerland) as aqueous suspensions with a nominal concentration of 1 g Ag L^{-1} . The same types of nanoparticles have previously been used in several studies in our laboratory (Navarro et al., 2008; Piccapietra et al., 2012a; Piccapietra et al., 2012b). The stock nanoparticle suspensions contained a small fraction of dissolved Ag, which originates from the AgNP production. The average size of the carb-AgNP in the stock suspension was 29 nm and the size of the cit-AgNP 17 nm (Piccapietra et al., 2012a; Navarro et al. 2015).

MOPS (3-morpholino propanesulfonic acid), NaCl, L-cysteine, NaOH were purchased from Sigma-Aldrich, H_2O_2 (30%) from Merck (suprapure chemicals). Fulvic acids (Standard Suwannee River I) were obtained in dry form from the International Humic Substances Society.

All solutions were prepared using nanopure water (Barnstead Nanopure Skan AG). Fulvic acid stock solutions were prepared by adding 5 mg fulvic acid to 100 mL of MOPS buffer (10 mM). Cysteine solutions were freshly prepared on ice on the day of the experiment. H_2O_2 was diluted to the experimental concentrations immediately before start of the experiment.

Experimental design

AgNP (carbonate or citrate coated) were added to the appropriate medium from stock solutions containing 0.1 g L^{-1} AgNP to reach a total Ag concentration of $5 \text{ }\mu\text{M}$ or $1 \text{ }\mu\text{M}$. Dissolved Ag was measured after various time intervals (minutes to several days) after separation of the nanoparticles by ultrafiltration. All experiments were carried out in solutions

containing 10 mM MOPS as buffer, which were adjusted to pH 7.5. Ligands (chloride (0.02 - 0.1 mM), cysteine (0.2 – 5 μ M), fulvic acids (8 or 15 mg L⁻¹), or H₂O₂ (0.1 – 10 μ M) were added to this buffer. After addition of AgNP, the experimental solutions were thoroughly mixed and kept on a shaker for the duration of the experiment. For experiments under dark conditions, the samples were wrapped in aluminum foil. For experiments under light conditions, the samples were exposed to daylight in the laboratory.

A dissolution experiment was also carried out in a conditioned culture medium for algae, which means a medium in which algae had been growing for 24 h and were removed by centrifugation. The experiment was started in this medium immediately after removing the algae.

Samples were taken from the experimental solutions at various time intervals. The first sample (time 0) was taken immediately after mixing and was further treated as quickly as possible. Dissolved Ag was then separated from AgNP by centrifugal ultrafiltration (Amicon Ultra Tubes, cut off 3 kD). Centrifugation was carried out over 30 or 10 min. The ultrafiltrate was acidified to 0.01 M HNO₃. To check for total concentrations, suspensions were digested using HNO₃ (65 %) and H₂O₂ (30%) in a high-performance microwave unit (mks 1200 Mega).

Ag measurements

Ag in ultrafiltered solutions or in digested solutions was measured by HR-ICP-MS (Thermo Finnigan Element 2) using the ¹⁰⁹Ag isotope. Reference solutions (NIST Standard Reference Material 1640) were used to check for accuracy of the measurements.

Results

Dissolution of AgNP in the presence of the ligands cysteine, chloride, fulvic acids and in algae media

Dissolution of Ag from carb-AgNP (total Ag = 5 μ M) in the presence of various concentrations of cysteine is shown as a function of time in Figure 1. The initial dissolved Ag concentration in this medium in the absence of cysteine was 0.1 μ M. An initial increase in dissolved Ag was observed within few hours at the higher cysteine concentrations (2 – 5 μ M), but not at the lower added cysteine concentrations (0.2; 0.5 μ M). At all cysteine concentrations, dissolved Ag was decreasing after 24 h to values below the initial concentration. At the highest used cysteine concentration (5 μ M), dissolved Ag initially increased to 0.95 μ M (19% of total Ag) after 4 h, before decreasing to less than 0.1 μ M after 24 h. In the case of citrate coated cit-AgNP, all used cysteine concentrations resulted in lower dissolved Ag concentrations than in the absence of cysteine. The dissolved Ag concentrations decreased in the presence of cysteine as a function of time over 24 h (Figure 2).

Fig. 1: Dissolved Ag from carbonate AgNP (total Ag = 5 μM) in the presence of various cysteine concentrations (0.2 – 5 μM). a) all data over time up to 168 h; b) lower concentrations over time up to 24 h. (error bars based on duplicate samples are in most cases within the symbol size).

Fig. 2: Dissolved Ag from citrate AgNP (total Ag = 5 μM) in the presence of various cysteine concentrations (0.5 – 5 μM) as a function of time over 24 h. (error bars based on duplicate samples are in most cases within the symbol size).

Addition of chloride up to 0.1 mM only had a slight effect on carb-AgNP dissolution (Figure 3). No significant differences among experimental samples with or without chloride were detected within 24 h, whereas at the highest used Cl^- concentration (0.1 mM) an increase in dissolved Ag was observed after 168 h. The highest dissolved Ag concentration was in this case 0.4 μM (8% of total Ag). This concentration is below the solubility limit of AgCl(s) ($\log K_{s0} = -9.75$) in the presence of 0.1 mM Cl^- .

Addition of fulvic acids (8 or 15 mg L^{-1}) did not significantly affect dissolution of carb-AgNP over 24 h (Figure 4). The highest dissolved Ag concentrations reached after 24 h were about 0.4 μM and were similar in the presence or absence of fulvic acids.

No dissolution of carb-AgNP was observed in conditioned culture media, both under light and dark conditions (SI Figure SI 1). The initial dissolved Ag concentration slightly decreased over 24 h in these media.

Agglomeration of carb-AgNP and of cit-AgNP has been examined under various conditions in other studies (Navarro et al., 2015; Piccapietra et al., 2012b; Topuz et al., 2014). Carb-AgNP had average size of 50 – 53 nm in the medium 10 mM MOPS at pH 7.5, and the addition of NaCl in the concentration range 0.02 – 0.1 mM was not expected to induce any agglomeration, in a similar way as NaNO_3 (Piccapietra et al., 2012b). FA in the used concentration range did also not induce agglomeration. Cit-AgNP had also a stable size around 45 nm at the same ionic strength and pH 7.5. No size increase was observed upon addition of low Cl^- and FA concentrations (Topuz et al., 2014). Addition of cysteine in a similar concentration range did not affect the size of carb-AgNP within 2 h (Navarro et al., 2008), but led to some increase of the size of cit-AgNP (Navarro et al., 2015).

Fig. 3: Dissolved Ag from carbonate AgNP (total Ag = 5 μM) in the presence of chloride (0.02; 0.1 mM) as a function of time over 672 h (28 d).

Fig. 4: Dissolved Ag from carbonate AgNP (total Ag = 5 μM) in the presence of fulvic acids (8 and 15 mg L^{-1}) under dark conditions as a function of time over 24 h.

Dissolution of AgNP with H_2O_2

Rapid dissolution of carb-AgNP was observed upon addition of H_2O_2 in the concentration range 0.1- 10 μM , both under dark and light conditions (Figure 5). With an excess of H_2O_2 (10 μM), carb-AgNP were completely dissolved within about 30 minutes, both under dark and light conditions. The initial measured dissolved Ag value reflected in this case the time required for sampling and separating the suspension and corresponded to dissolved Ag after about 10 – 15 minutes, which was relevant for the very fast reaction under these conditions. At the lower H_2O_2 concentrations, the dissolved Ag-concentration continuously increased over 2 h and reached values of 1.1 μM Ag with 0.1 μM H_2O_2 and 3.4 μM Ag with 1 μM H_2O_2 under light conditions, whereas under dark conditions somewhat lower dissolved Ag were measured. If the dissolved concentrations in absence of H_2O_2 were subtracted from the final concentrations, the increase in dissolved Ag with H_2O_2 under dark conditions was 0.26 μM with 0.1 μM H_2O_2 and 2.4 μM with 1 μM H_2O_2 . Even the addition of a low concentration of 0.01 μM H_2O_2 resulted in a detectable increase in dissolved Ag, especially under light conditions (supporting information Figure SI 2).

Using cit-AgNP, similar results for dissolved Ag were obtained upon addition of H_2O_2 , with a rapid dissolution within 4 h and close to complete dissolution of 5 μM AgNP with 1 μM H_2O_2 after 4 h, both under light and dark conditions (Figure 6). The difference between light and dark conditions was in this case smaller than this difference for carb-AgNP.

Fig. 5: Dissolved Ag from carbonate AgNP (total Ag = 5 μM) with H_2O_2 (0.1/0.5/1/10 μM) under dark (a) and light conditions (b) as a function of time over 120 min (error bars based on duplicate samples are in most cases within the symbol size).

Fig. 6: Dissolved Ag from citrate-AgNP (total Ag = 5 μM) with H_2O_2 (0.1/1 μM) under dark and light conditions as a function of time over 240 min (error bars based on duplicate samples are in most cases within the symbol size).

Dissolution rates were calculated by linearization from the first part of the curves of dissolved Ag versus time for the various H_2O_2 concentrations (Figures 5 and 6). In the case of the highest H_2O_2 concentration (10 μM), a linear dissolution rate could not be calculated because of the very rapid dissolution. These dissolution rates are plotted as a function of the initial H_2O_2 concentrations in Figure 7 and illustrate the dependence on H_2O_2 concentrations. In the case of carb-AgNP, the dissolution rates were higher in the presence of light, whereas for cit-AgNP they were nearly identical in the presence or absence of light. The dissolution rates are comparable for carb-AgNP and cit-AgNP, with $r = 0.02 - 0.03 \mu\text{M Ag min}^{-1}$ for 1 μM H_2O_2 and 5 μM AgNP. These dissolution rates can also be related to the surface area of the nanoparticles. Using nanoparticle diameters of 40 – 50 nm, as obtained for these AgNP in similar media, the surface area of one nanoparticle is $5.03 - 7.85 \times 10^{-15} \text{ m}^2$, and the total surface area in a suspension of 5 μM AgNP is $7.7 \times 10^{-3} \text{ m}^2 \text{ L}^{-1}$ for $d = 40 \text{ nm}$ and $6.1 \times 10^{-3} \text{ m}^2$

L^{-1} for $d = 50$ nm. The resulting dissolution rates are then for $r = 0.02 \mu M Ag min^{-1}$ $2.6 \times 10^{-6} - 3.3 \times 10^{-6} mol Ag m^{-2} min^{-1}$ and for $r = 0.03 \mu M Ag min^{-1}$ $3.9 \times 10^{-6} - 4.9 \times 10^{-6} mol Ag m^{-2} min^{-1}$.

Fig. 7. Dissolution rates ($\mu M Ag min^{-1}$) for carb-AgNP and cit-AgNP as a function of H_2O_2 (μM).

Discussion

Dissolution in the presence of ligands

Dissolution of AgNP under the influence of the ligands used here was not strongly induced at these relatively low concentrations, which are realistic with respect to media for toxicity experiments or to natural waters. Cysteine as a strong binding ligand for Ag^+ had the most pronounced effect on dissolution, with increased dissolution at the highest concentrations used here and over a few hours. However, reactions of Ag^+ with cysteine appear not only to lead to increased dissolution, but also to decreased dissolved concentrations after longer reaction times. Possible reactions after longer times (> 24 h) include reduction reactions of Ag^+ and formation of polymeric species (Andersson, 1972; Gondikas et al., 2012). Those species formed after longer reaction times may be retained by the ultrafiltration membranes used here. Furthermore, some agglomeration effects in the presence of cysteine may also decrease dissolution reactions at longer times. In contrast to our present results, higher cysteine concentrations ($400 \mu M$) and higher ratios of cysteine to total silver appear to lead to more extensive dissolution of AgNP, without evidence for reduction or formation of non-filterable species for times up to 48 h (Gondikas et al., 2012). Other ligands with sulfide functional groups may have similar effects. Rapid dissolution of AgNP by reaction with a

copper binding protein involving a thiol group has been shown, which highlights the high reactivity of thiols in biological ligands for silver binding (Martinolich et al., 2012).

In contrast to the high reactivity of thiol ligands, the effects of chloride and of fulvic acids to dissolution of AgNP were very limited. No agglomeration effects were expected under these conditions with low added chloride or fulvic acid concentrations. The Ag^+ - complexes with chloride have only a relatively low stability ($\text{AgCl}^0(\text{aq})$: $\log K = 3.3$; AgCl_2^- : $\log \beta_2 = 5.2$). At the highest Cl^- concentration used here (0.1 mM), only the formation of the $\text{AgCl}^0(\text{aq})$ complex is expected to occur according to equilibrium calculations. The formation of this complex appears to be of only slight effect in accelerating the dissolution of AgNP. At higher chloride concentrations, dissolution reactions may be faster, but are also complicated by precipitation of $\text{AgCl}(\text{s})$ (Yue et al., 2014). Complexes of Ag^+ with fulvic acids are also of limited stability, in particular if mostly carboxylic or phenolic functional groups are involved in binding (Chen et al., 2012). Those complexes appear also to have little effect in dissolving AgNP at realistic concentrations (Figure 4). Furthermore, reduction processes by fulvic acids may also occur, which may lead to a decrease of dissolved Ag^+ by renewed formation of AgNP. Formation of AgNP from dissolved Ag^+ was observed under the influence of extracellular polymeric substances extracted from periphyton biofilms (Kroll et al., 2014).

In conditioned media, it is expected that some ligands released by the algae are available, which may also include thiol ligands. However, there was no evidence for increased dissolution of AgNP in these media. The concentrations of produced ligands by algae may be too low to be effective. The decreasing dissolved Ag concentrations may indicate some reduction reactions, possibly due to organic algae products, or to other components of the culture media, or binding to higher molecular weight organic compounds. It is possible that $\text{Fe}(\text{II})$ was released from the FeEDTA complexes under light conditions and that $\text{Fe}(\text{II})$ then reduced Ag^+ .

Dissolution with H_2O_2 as oxidant

Dissolution of AgNP with both carbonate and citrate coatings by H_2O_2 appears to be very efficient, even at low H_2O_2 concentrations. Complete dissolution of AgNP was achieved within less than 1 hour in the presence of an excess of H_2O_2 . H_2O_2 is a reactive oxidant, which is expected to react more readily with AgNP than oxygen, which was present in all experiments. The medium for the reactions with H_2O_2 was MOPS buffer, in which both carb- AgNP and cit-AgNP were stable with respect to NP size.

302 Ag(0) is expected to be dissolved by H₂O₂ with the overall stoichiometry:

304 Ag(0) was thus dissolved in excess of this stoichiometry, especially under light conditions,
305 probably due to catalytic reactions involving produced radicals and oxygen, as oxygen was
306 not excluded under these experimental conditions.

307 The obtained kinetic characteristics of the reaction of H₂O₂ with AgNP may be compared to
308 literature values. The reaction of AgNP with H₂O₂ was examined in detail in (He et al.,
309 2012b; He et al., 2011) and was written with the following general reactions:

312 The intermediates may include the formation of hydroxyl radicals (OH[·]) or of bound hydroxyl
313 radicals (Ag-O(H)-OH), with very short life-times. The decomposition of H₂O₂ upon contact
314 with AgNP was followed over time in this study (He et al., 2011), which showed that H₂O₂
315 was consumed within about 10 minutes with an excess of AgNP (in the concentration range 1
316 – 10 µM) at pH 9.5. Strong pH-dependence of this reaction was observed in (He et al.,
317 2012b), with very rapid decomposition at alkaline pH (9 – 14), and slower reaction rates at
318 acidic pH. The produced superoxide anion O₂^{·-} (reaction 3) can then further react with AgNP
319 and Ag⁺ to form again AgNP, or may recombine to form H₂O₂. The pH-dependence of these
320 reactions indicated that at higher pH (> 9) the new formation of AgNP was important, and
321 therefore after an initial decrease of AgNP concentration a new increase of AgNP was
322 observed after longer times (He et al., 2012b). However, at intermediate pH 7, dissolution of
323 AgNP appeared to be predominant.

324 No obvious indication of reformation of AgNP was found in our experiments at pH 7.5, but
325 rather complete dissolution of AgNP in cases with an excess of H₂O₂, or formation of
326 dissolved Ag⁺ in proportion to added H₂O₂. However, the nearly identical dissolution rates
327 with and without light in the case of cit-AgNP may indicate some re-reduction of dissolved
328 Ag⁺ under light conditions with citrate. This reaction did not appear to be important in the
329 case of carb-AgNP. The observed kinetics in our experiments were in line with the fast
330 kinetics of H₂O₂ decomposition in the studies by He et al. (2011, 2012). Using the second-
331 order rate constant for the reaction of cit-AgNP with H₂O₂ given in He et al. (2012), k₂ = 40
332 M⁻¹s⁻¹, an initial dissolution rate r = 0.012 µM Ag min⁻¹ is obtained for 1 µM H₂O₂ and AgNP
333 = 5 µM, which is comparable to the values obtained here. The small differences in dissolution

rates between cit-AgNP and carb-AgNP may be due to the smaller size of the cit-AgNP which would result in somewhat higher surface area.

Ag speciation and implications for toxicity experiments

The speciation of Ag(I) in dissolved form is of utmost importance for its toxicity effects to aquatic organisms. The free Ag^+ ion is expected to be directly available for uptake to algae or bacteria, and may also be determining for uptake in higher organisms (Navarro et al., 2008; Yang et al., 2012; Piccapietra et al., 2012a; Xiu et al., 2012). The reactions described here may lead to either dissolved free Ag^+ -ions or to complexes with the available ligands.

Dissolution of AgNP in the presence of cysteine would mostly lead to Ag-cysteine complexes (AgCysH and $\text{Ag}(\text{CysH})_2$) with high stability (Adams and Kramer, 1999; Navarro et al., 2008), as well as to possible polymeric species (Andersson, 1972). In the presence of an excess of cysteine over dissolved Ag(I), the free Ag^+ -ions are expected to be in a very low concentration range ($10^{-12} - 10^{-11}$ M) for the typical concentrations used here, based on equilibrium calculations as described in Navarro et al., 2008. In contrast, in the presence of chloride with $\text{Cl}^-(\text{tot}) = 0.1$ mM, binding of Ag(I) in chloride complexes is incomplete and a large fraction of free Ag^+ would still be present. In a similar way, binding of Ag(I) by fulvic acids appears to be relatively weak (Chen et al., 2012), and a large fraction of Ag^+ may be expected in the presence of realistic concentrations of fulvic acids as used here. With regard to toxicity, increased dissolution of AgNP in the presence of cysteine would thus lead to the formation of cysteine-complexed Ag(I), which has been shown to be unavailable to algae and to exert no toxic effects to algae and to other organisms (Navarro et al., 2008; Xiu et al., 2012; Yang et al., 2012; Leclerc and Wilkinson, 2014; Navarro et al., 2015). In contrast, the Ag-chloride complexes may play a role in toxicity, as shown for fish cells (Yue et al., 2015).

The high efficiency of H_2O_2 in dissolving AgNP is likely to be of importance in toxic effects of AgNP to algae. H_2O_2 may be produced and released into solution by algae, in varying concentrations depending on the algae species and conditions (Palenik et al., 1987; Pospisil, 2012; He et al., 2012a; Suarez et al., 2013). The release of H_2O_2 by algae in contact with AgNP would lead to a rapid release of Ag^+ , as shown by the high reactivity reported here. These reactions would result in increasing Ag^+ availability close to algae cells and thus in increased Ag^+ -uptake. The produced H_2O_2 may be available in the nearest layer around a cell and react with any available AgNP in this layer. Such reactions may result in a higher Ag^+ uptake from AgNP than expected from the measured dissolved concentrations in the absence

367 of algae and thus contribute to AgNP toxicity, as previously inferred from results on toxicity
368 of AgNP to algae (Navarro et al., 2008; Navarro et al., 2015; Piccapietra et al., 2012a).

369

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Supplementary Information

Fig. SI 1: Dissolved Ag from carbonate AgNP (total Ag = 5 μM) in conditioned algae culture media under dark and light conditions as a function of time over 24 h.

Fig. SI 2: Dissolved Ag from carbonate AgNP (total Ag = 1 μM) with 0.01 μM H_2O_2 under dark and light conditions as a function of time over 4 h.