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Light-induced reduction of silver ions to silver nanoparticles in aquatic environments by microbial extracellular polymeric substances (EPS)

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ABSTRACT

Microbial extracellular polymeric substances (EPS) widely exist in natural environments and affect the migration and transformation of pollutants in aquatic environments. Previous works report that EPS have some reducing functional groups and can reduce heavy metals. However, because of the weak reducing capability of EPS, the reduction of heavy metals by EPS without cells is extremely slow, and its effect on heavy metals species is insignificant. In this work, the accelerated reduction of silver ions (Ag^+) by EPS from *Shewanella oneidensis* MR-1 under illumination was investigated. UV–visible spectroscopy, transmission electron microscopy (TEM) coupled with an energy dispersive spectrometer (EDS) and X-ray photoelectron spectroscopy (XPS) were used to confirm the formation of silver nanoparticles (AgNPs) via the reduction of Ag^+ by EPS under light illumination. The Ag^+ reduction by EPS follows pseudo-first-order kinetics under both visible and UV light, and the light irradiation can significantly accelerate AgNPs formation. On the one hand, visible light can excite AgNPs for their surface plasma resonance (SPR) and accelerate the electrons from the EPS to adjacent Ag^+. On the other hand, EPS molecules may be excited by UV light to produce strong reducing species, which enhance Ag^+ reduction. Moreover, pH, dissolved oxygen were found to affect the formation of AgNPs by EPS. This work proves the reducing capability of EPS on the reduction of Ag^+, and this process can be accelerated under light illumination, which may affect the speciation and transformation of heavy metals in natural waters.

1. Introduction

Silver nanoparticles (AgNPs) are the most widely used nanomaterials in our daily lives because of their outstanding bactericidal activity and catalytic activity (Adegboyega et al., 2013). The widely used AgNPs are inevitably released into the environment and threaten the ecosystem (Levard et al., 2012). Thus, there are increasing concerns about the formation and transfer of AgNPs in the environment. Apart from the anthropogenic sources, the AgNPs can also form from both biotic and abiotic processes in natural conditions. For instance, some dissimilatory metal reducing bacteria, like *Geobacter* and *Shewanella*, can export intracellular electrons to extracellular silver ions (Ag^+) to generate AgNPs (Ng et al., 2013; Law et al., 2008). Furthermore, the natural formation of AgNPs by abiotic processes were also observed. Several works have proved that AgNPs can be generated from the reduction of Ag^+ by dissolved organic matters (Yin et al., 2012; Hou et al., 2013; Akaighe et al., 2011; Adegboyega et al., 2014).

Extracellular polymeric substances (EPS), a complex high molecular compound secreted from many organisms in aquatic environments, can be widely found in both fresh water and marine water (Flemming and Wingender, 2010). Particularly for marine environments, microbial EPS can account for up to 40% of the total organic carbon content (Bhaskar and Bhosle, 2005). Many functional groups such as carboxyl, hydroxyl, phosphoryl, sulfhydryl, and phenolic groups are available in microbial EPS to bind heavy metals by electrostatic interaction or complexing bonds (Sheng et al., 2013). Thus, microbial EPS can affect the fate of these heavy metals in the environment. For example, EPS can accumulate cooper and affect its entry into the environmental food chain (Mittelman and Geesey, 1985). In recent years, several groups have reported that EPS can reduce heavy metals (e.g., Cr^{6+}) (Harish et al., 2012), radionuclides (e.g., U^{6+}) (Cao et al., 2011a), and organic pollutants (Kang and Zhu, 2013). The hemiacetal reducing ends in polysaccharides and phenol groups may be responsible for the reducing capacity of EPS (Kang and Zhu, 2013). In addition, some redox proteins in EPS may act as electron donors to reduce some

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pollutants such as U(VI) (Cao et al., 2011a,b). Consequently, more attention should be paid to the roles of EPS on the fate of heavy metals in the environment considering the reducing characteristics of EPS, particularly in natural waters.

Natural sunlight is reported to affect the redox of many metal ions (Fe(III), Hg(II), Cr(VI), Ag(I)) in the presence of humic substances because some redox-active species are generated under light irradiation (Yin et al., 2012; Fukushima and Tatsumi, 1999; Matthiessen, 1998; Gaberell et al., 2003). Many reducing functional groups in humic substances also exist in EPS such as hydroxyls, phenolic-OH, thiols and aldehydes etc (Akaighe et al., 2011; Sheng et al., 2010). Therefore, it is hypothesized that light may stimulate the EPS molecules and affect the Ag^+ reduction to AgNPs in natural environments, which has not been investigated. This investigation will be useful to better understand the effects of EPS on Ag transformation in natural waters.

The objective of this study is to investigate the formation of AgNPs in the presence of microbial EPS under light illumination (290–800 nm). The formation kinetics of AgNPs under visible and UV light irradiation were investigated. To better understand the reduction of Ag^+ by EPS in aquatic environments, the solution chemistry conditions such as pH and dissolved oxygen (DO) were also investigated. With the experimental results, the enhancement mechanisms of AgNPs formation through Ag^+ reduction in the presence of EPS under illumination were proposed. The results from this work are useful to understand the role of EPS in the formation of silver nanoparticles in aquatic environments and broaden our knowledge on the transformation of pollutants in the environments.

2. Materials and methods

2.1. Microbial strain and EPS extraction

Microbial EPS was harvested from *Shewanella oneidensis* MR-1, a bacterial strain widely existing in natural environments. The strain was cultivated in 0.5 mL of LB medium at 30 °C for 10 h and inoculated in 250 mL of fresh LB (1‰, v/v) for additional 12 h. The bacterial cells were collected, washed three times with HEPES buffer, and transferred into 1 L of M1 medium (Nealson and Scott, 2006) containing 30 mM sodium lactate and 30 mM fumarate as the electron donor and acceptor for bacteria growth, respectively (full medium compositions see Table S1). The initial bacterial content was controlled at an OD_{600} of 0.1. After 48 h, the bacterial cells were separated from the medium by centrifugation (5000 × *g*, 5 min) and washed three times with DI water to remove the residual medium. The bacteria pellets were re-suspended for EPS extraction using a Na^+-form cation exchange resin (CER) according to our previous study (Sheng and Yu, 2006) with a slight modification. The CER was added into the bacteria suspended solution with a dosage of 60 g/(g-dry cell weight). Then, the suspensions were stirred at 900 rpm and 4 °C for 5–6 h. The solution was subsequently settled for 3 min to remove the CER. Then, the EPS and bacterial cells were separated by centrifugation at 13 000 × *g* for 10 min. After that, the crude EPS solution was filtered through a 0.22-μm membrane and stored at 4 °C and oxygen free condition for subsequent experiments. The portion of EPS reduced by direct electrochemical reduction on glassy carbon working electrodes at −0.6 V (Aeschbacher et al., 2011) was denoted as reduced EPS. No special statement, the EPS extracted under ambient condition was used as experimental EPS.

2.2. Characterization of EPS

The contents of proteins and carbohydrates in the extracted EPS were measured using the Lowry method with bovine serum albumin (BSA) as a standard and the anthrone method with glucose as a standard, which were 29.97 and 38.53 mg/g-dry cell weight, respectively. The content of humic substances (1.02 mg/g-dry cell weight) in the extracted EPS was measured using the modified Lowry method with humic acids (Sigma aldrich) as the standard. The content of DNA (0.31 mg/g-dry cell weight) in the extracted EPS was measured using diphenylamine colorimetric method with calf thymus DNA as the standard. The low content of DNA in EPS implied that the cell lysis was negligible (Liu and Fang, 2002). The total carbon content (TOC) of the extracted EPS was determined using a TOC analyzer (Multi N/C 2100, Analytik Jena, Germany). In addition, the Fourier transform infrared (FTIR) spectrum and 3-dimensional excitation–emission matrix (EEM) were used to characterize the extracted EPS. The FTIR and EEM were recorded from a VERTEX 70 FTIR (Bruker Co., Germany) and an LS55 fluorescence spectrophotometer (PerkinElmer Co., USA), respectively. The EPS characterization results are provided in the Supporting Information (Fig. S1 and Table S2).

2.3. Formation of AgNPs

To investigate the formation of AgNPs in the presence of EPS under illumination and the effects of the solution chemistry, the experiments were performed in 50-mL transparent vials. Before the experiments, the EPS and $AgNO_3$ solutions were purged with nitrogen gas for 30 min to remove the dissolved oxygen and were subsequently added into the vials in an anaerobic glove box. The vials were wrapped in two layers of aluminum foil to maintain dark conditions before the experiments. Eight general fluorescent lamps (PHILIPS, T8) were used as the light source. The illumination intensity was 500 μW/cm^2 (400–800 nm). The temperature was maintained at 26 °C in all experiments; a 1.2-mL solution was sampled at specific time to monitor the formation of AgNPs.

To explore the effect of light with different wavebands (UV and visible) on the Ag^+ reduction by EPS, a 250/350 W wind-refrigerated Xe lamp with adjustable brightness was used to simulate sunlight, and 50-mL quartz tubes were used. Two filters were used to obtain visible (400–800 nm) and UV light (290–400) nm, (UVA and UVB, no UVC output). The incident intensity of visible and UV light was measured using a solar power photometer (TENMARS, TM-207) and a UV light photometer (LUTRON, UV-340A), respectively. The incident intensity of visible light was 500 μW/cm^2 on the surface of the quartz tubes by using a UV-block filter (>400 nm), and the corresponding UV light intensity under 400 nm was 23 μW/cm^2. By adjusting the distance between the lamp and the samples, the enhanced UV light intensity in our experiments with the vis-block filter (<400 nm) was 70 μW/cm^2. The tubes covered by two layers of aluminum foil were the dark control in the experiment.

To explore the Ag^+ reduction mechanism by EPS under visible-light illumination, the effects of two monochromatic lights at 415 nm and 600 nm were investigated using band-pass filters. The surface plasmon resonance (SPR) peak of AgNPs is near the 415 nm light and far from the 600 nm light. The reaction system was initially irradiated under visible light for 6 h. Then, the reaction solution was divided into two parts under anaerobic and dark conditions, which were then irradiated with 415 nm and 600 nm monochromatic light, respectively.

2.4. Characterization of AgNPs

The UV–vis spectra of the formed AgNPs were recorded at 200–800 nm using a UV-2450 spectrometer (Shimadzu Co., Japan). The morphology of the AgNPs was characterized using