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Monodispersed biocompatible silver sulfide nanoparticles: Facile extracellular biosynthesis using the γ-proteobacterium, *Shewanella oneidensis*

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ABSTRACT

Interest in engineered metal and semiconductor nanocrystallites continues to grow due to their unique size- and shape-dependent optoelectronic, physicochemical and biological properties. Therefore identifying novel non-hazardous nanoparticle synthesis routes that address hydrophilicity, size and shape control and production costs has become a priority. In the present article we report for the first time on the efficient generation of extracellular silver sulfide (Ag_2S) nanoparticles by the metal-reducing bacterium *Shewanella oneidensis*. The particles are reasonably monodispersed and homogeneously shaped. They are produced under ambient temperatures and pressures at high yield, 85% theoretical maximum. UV–visible and Fourier transform infrared spectroscopy, dynamic light scattering, X-ray diffraction, transmission electron microscopy and X-ray photoelectron spectroscopy measurements confirmed the formation, optical and surface properties, purity and crystallinity of the synthesized particles. Further characterization revealed that the particles consist of spheres with a mean diameter of 9 ± 3.5 nm, and are capped by a detachable protein/peptide surface coat. Toxicity assessments of these biogenic Ag_2S nanoparticles on Gram-negative (*Escherichia coli* and *S. oneidensis*) and Gram-positive (*Bacillus subtilis*) bacterial systems, as well as eukaryotic cell lines including mouse lung epithelial (C 10) and macrophage (RAW-264.7) cells, showed that the particles were non-inhibitory and non-cytotoxic to any of these systems. Our results provide a facile, eco-friendly and economical route for the fabrication of technologically important semiconducting Ag_2S nanoparticles. These particles are dispersible and biocompatible, thus providing excellent potential for use in optical imaging, electronic devices and solar cell applications.

1. Introduction

The unique size- and shape-dependent linear and nonlinear optoelectronic, physiochemical and biological properties of semiconductor nanoparticles that arise from quantum confinement, high surface-area-to-mass ratios and other mesoscopic phenomena make them powerful tools in various *in vivo* biolabeling and optical imaging, biomedical, sensor, diagnostic, optoelectronic and solar cell applications [1–4]. Ag_2S is one of the most important transition metal sulfide semiconductor nanomaterials due to its direct and narrow band gap ($\varepsilon_g \sim 1$ eV) [5], good chemical stability, high absorption coefficient and excellent optical properties. Ag_2S has been implemented in various nanoscale optical, atomic switching and electronic applications including IR detectors, fuel cells and battery-based superionic conductors, random access memory (RAM) devices, cross bar electronic circuits, H_2S sensing [6], photoconductors, photovoltaic cells [7], resistive switching [8] and solar-selective coatings [9,10]. Due to its thermoelectric properties, it has also been used in photography as a photosensitizer [7]. Considerable effort has been devoted in generating different types of semiconducting nanomaterials utilizing inorganic precursors and various physical and chemical methodologies, as well as molecular beam and lithography techniques [6,7,11–13]. Most of these methods are cumbersome and ecologically unfriendly. They involve the use of toxic and combustible precursors, are accomplished under oxygen and/or a water-free atmosphere requiring high temperatures and pressures, and the resulting nanoparticles can become unstable or agglomerate upon interaction with biological media components or biomolecules. Therefore it is desirable to develop environmentally benign synthesis techniques that can generate particles with the desired properties. An alternate synthesis route

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exploits the biofabrication of nanomaterials and relies on microorganisms for the reduction of metal ions into stable nanocrystallites [14–16]. Biofabrication cannot only exhibit size and shape control over a diverse array of nanomaterials but can also facilitate mass production, high yield and reproducibility. To this end our group and other researchers have shown that numerous microbial systems including fungi [17], yeast [18] and bacteria [19] can be utilized as environmentally benign nano-factories for the production and assembly of various types of semiconductors such as CdSe [17], PbS [20], ZnS [21], CdS [22,23] and CdTe [24], along with metal [14,25] and bimetallic alloy nanoparticles [26]. However, to the best of the authors' knowledge there is no report on the microbial-based biofabrication of Ag_2S nanoparticles to date. Additionally the particles are monodispersed and homogeneous (spherical) in shape.

Shewanella oneidensis is a Gram-negative heterotrophic, facultative bacterium that utilizes a large array of terminal electron acceptors and has been intensively studied for the bioremediation of environmental contaminants [27,28]. Due to this versatility it has been used for the reduction of numerous metals [29–33] and metal oxides [34]. Here we report the first demonstration on the use of the reductive capability of *S. oneidensis* to biofabricate highly stable Ag_2S nanoparticles when seeded with aqueous Ag^{2+} and $S_2O_3^-$ ions under ambient temperatures and pressures. The resulting nanocrystallites are extracellular, spherical, nearly monodispersed, highly stable and soluble in aqueous suspension. The purity, size, shape, surface characteristics and crystallinity of the particles were confirmed by UV–visible and Fourier transform infrared spectra (FTIR), dynamic light scattering (DLS), X-ray diffraction (XRD), transmission electron microscopy (TEM) and X-ray photoelectron spectroscopy (XPS) measurements. Additionally, the antibacterial and cell cytotoxicity measurements of these biogenic Ag_2S nanoparticles were evaluated using Gram-negative *Escherichia coli* and *S. oneidensis* and Gram-positive *Bacillus subtilis* bacterial species and eukaryotic mouse lung epithelial and macrophage cells. The results of our work not only provide a simple, environmentally benign and cost-effective route for the fabrication of pure Ag_2S nanocrystallites but the produced particles are biocompatible and dispersible. These properties should facilitate their use in various biolabeling, bio-imaging, optoelectronic devices and solar cell applications.

2. Materials and methods

The bacterial strains used in our studies were *S. oneidensis* MR-1 (ATCC 700550), *E. coli* (ATCC 700926) and *B. subtilis* (ATCC 6633). All other chemicals and reagents used were from standard commercial sources and of the highest quality available.

2.1. Biofabrication of Ag_2S nanoparticles

S. oneidensis was maintained on Luria-Bertani agar (LBA) at 30 °C. A single bacterial colony from an overnight LBA plate was used to inoculate 100 ml of LB broth in a 500 ml Erlenmeyer flask, followed by incubation at 30 °C, the standard temperature for growth of *S. oneidensis*, on a rotary shaker (200 rpm) for 18 h. The cells (stationary phase) were collected by centrifugation (5000 *g*, 25 °C, 20 min), washed 2–3 times with sterile deionized water, resuspended in 100 ml of 1 mM $AgNO_3$ and 1 mM $Na_2S_2O_3$ aqueous solution in a 500 ml Erlenmeyer flask and incubated at 30 °C with shaking at 200 rpm. Control reactions included 1 mM $AgNO_3$ along with 1 mM $Na_2S_2O_3$ added to 100 ml of the *S. oneidensis* culture supernatant or uninoculated LB media. Biofabrication and growth of Ag_2S nanoparticles were monitored both visually and using UV–visible absorption spectra (200–800 nm), as a function of time. After completion of the reaction process (48 h), the reaction mixture was centrifuged, as mentioned above, to remove bacterial cells, the supernatant was filtered using a sterile Nucleopore 0.2 μm filter and the particles were collected by high speed ultra-centrifugation (100,000 *g*, 1 h). After washing twice with Milli Q water, the Ag_2S nanoparticles were used for further studies. The percentage theoretical yield of the biogenic Ag_2S nanoparticles was determined by dry weight as described previously [30].

2.2. Physical characterization

UV–visible spectra were recorded on a CARY 100 Bio spectrophotometer (Varian Instruments, California) operated at a resolution of 1 nm. FTIR analysis of the samples deposited on a ZnSe window was carried out on a Nicolet Magna-IR 760 spectrophotometer at 4 cm^{-1} resolution. DLS and zeta potential measurements were performed on a Brookhaven 90 Plus/BI-MAS Instrument (Brookhaven Instruments, New York). XRD of dried nanoparticle powder was performed on a Discover D8 X-ray diffractometer with a Xe/Ar gas-filled Hi Star area detector and an XYZ platform, operated at 40 kV and at a current of 40 mA. TEM measurements of samples prepared on carbon coated copper grids were performed on a LIBRA®120 PLUS transmission electron microscope (Carl Zeiss, Germany). Chemical analysis of the drop-coated films of nanoparticles on a conductive Si substrate was performed on a K_α X-ray photoelectron spectrophotometer (Thermo Scientific, USA) at a base pressure of about 10^{-9} Pa. The X-rays used were monochromatic Al K_α photons and photoemitted electrons were analyzed with a hemispherical energy analyzer. Survey scans (0–1400 eV) and the core level spectra were collected at pass energies of 200 and 50 eV respectively. Data analysis was performed using the Avantage software package (v. 4.61) provided by the manufacturers.

2.3. Cell toxicity assessments

Bacterial toxicity evaluation for *E. coli*, *B. subtilis* and *S. oneidensis* was based on minimum inhibitory concentration (MIC) [35], was determined using 100-well bioscreen plates containing 200 μl of the bacterial cultures (~0.097 OD) and varying concentrations of biogenic Ag_2S nanoparticles as described earlier [30].

Cell cytotoxicity assessment was performed using the MTT assay [36] for mouse lung epithelial and macrophage cells seeded at 5×10^3 per well in a 96-well tissue culture plate and grown to 80% confluence in 200 μl of RPMI medium supplemented with 0.2 mM L-glutamine, 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 10% FBS, washed twice with phosphate-buffered saline (PBS) and incubated with different concentrations of biogenic silver sulfide nanoparticles for 4 and 12 h, separately. For statistical data analysis each treatment was performed in octuplets. After exposure the cells were rinsed once with PBS, 200 μl of supplemented RPMI was added and the plate was incubated at 37 °C (the standard temperature for mouse lung epithelial and macrophage cell culture) for 18–24 h. The MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was then added to each well (12.5 μl from 4 mg ml^{-1}) and the cells were reincubated for 4 h. After a final wash with PBS, the PBS was aspirated and 200 μl of DMSO was added to each well, followed by reading the absorbance at 560 nm.

3. Results and discussion

Microorganisms protect themselves against metal ion stress by a process involving reduction and/or precipitation. This is accomplished with the aid of reducing agents that are secreted by the