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A biofabrication approach for controlled synthesis of silver nanoparticles with high catalytic and antibacterial activities

Cuixian Yang, Sukwon Jung, Hyunmin Yi *

Department of Chemical and Biological Engineering, Tufts University, Medford, MA 02155, United States

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ABSTRACT

We report simple, facile and size-controllable synthesis of uniform Ag nanoparticles with tobacco mosaic virus (TMV) as a biomediator in the absence of external reducing agents. UV–vis and TEM analysis show that Ag nanoparticles with average diameter of 2, 4 and 9 nm were obtained by simply tuning the ratio of TMV/$Ag(NH_3)_2^+$. The Ag formation in the presence of TMV showed autocatalytic growth followed by coalescence. The as-prepared TMV-mediated Ag nanoparticles show substantially higher catalytic and antibacterial activities than previous results. For the 4-nitrophenol hydrogenation reaction, the rate constants per surface area for 2 and 9 nm Ag nanoparticles were determined to be 0.64 and 1.2 L m^{-2} s^{-1} respectively. Both Kirby–Bauer disk diffusion test and tube culture results demonstrate high antibacterial activity of TMV-mediated Ag particles against *Escherichia coli*, with minimal inhibition concentration (MIC) of 2.3 and 2.5 ppm for 2 and 9 nm Ag nanoparticles respectively. We expect that our biomediated Ag synthesis approach can be readily extended to other biomaterials and metal nanoparticle systems.

1. Introduction

Silver (Ag) plays an important role in a wide range of fields, including catalysis [1–3], photonics [4] and biomedicine (e.g. diagnosis or imaging [5], antimicrobial agent [6–9] and cancer therapy [10]). Ag nanoparticles have recently gained much attention due to large surface-to-volume ratio, optical properties by plasmon resonance [11] and convenient surface bioconjugation [12]. Ag nanocatalysts are particularly useful in both ethylene and methanol oxidation [1,2] as well as reduction of nitric oxides (NO_x) or aromatic nitro compounds [3,13–15]. For example, catalytic hydrogenation of 4-nitrophenol is a simple, efficient and environmentally friendly process to produce 4-aminophenol [13–16], a useful pharmaceutical intermediate for the production of several analgesic and antipyretic drugs such as paracetamol, acetanilide and phenacetin [17]. In addition, Ag has been used as an antimicrobial agent for centuries, mainly rising from the adsorption and penetration into the cell wall, thus disturbing cellular respiration and damaging phosphorus or sulfur-containing compounds such as DNA and proteins [7–9]. Ag nanoparticles have been reported to be suitable materials for the antibacterial application mainly due to low Ag amount needed, low toxicity to human cells, and large surface area for improved interaction with bacterial cells [6].

Many methods have been employed for the synthesis of Ag nanoparticles, including chemical reduction [18,19], thermal decomposition [20], laser ablation [21], microwave irradiation [22], and sonochemical synthesis [23]. Among these, chemical reduction is the most commonly used method, involving the reduction of silver salt with a reducing agent (e.g. citrate [18], borohydride [24], ethylene glycol [19]) and additional ligands/capping agents [18,19,21,25] or solid supports [26,27] to produce stable colloidal or supported Ag nanoparticles. More recently, Zheng's group [25] and Bigioni's group [27] reported the synthesis of thiolate-protected single crystal Ag nanoclusters using aryl-thiol ligands. Despite such progress, simple, controllable and environmentally sustainable synthesis of Ag nanoparticles under mild conditions still remains challenging. A few biomimetic approaches utilizing biological molecules (e.g. DNA [28,29], protein or peptide [30–34], bacteria [35], virus [36–39], chitosan [40], and silk [41]) for controlled synthesis of functional nanoparticles [42,43] offer a number of advantages, such as mild aqueous synthesis conditions, controllable size [34,44], genetic engineering to design optimal sequences [45], and absence of external toxic chemicals [46]. Especially, viral-templated nanoparticles have been extensively studied and successfully applied for catalysis [37,39,46], photocatalysis [47], electronic devices [48], imaging [49] and drug delivery [50]. Despite such potential advantages, there exists limited number of biomediated synthesis methods that clearly offer small and uniform size, high catalytic activity and improved antibacterial activity.

* Corresponding author. Tel.: +1 617 627 2195; fax: +1 617 627 3991.
E-mail address: hyunmin.yi@tufts.edu (H. Yi).

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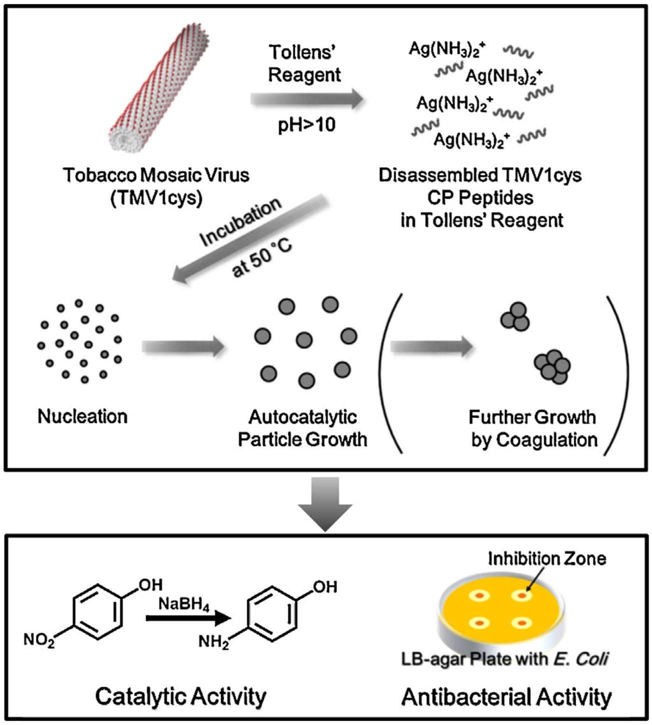

Scheme 1. Synthesis of TMV-mediated Ag nanoparticles with high catalytic and antibacterial activities.

In this work, we utilize tobacco mosaic virus (TMV) as a biomediator to synthesize Ag nanoparticles in a controlled manner under mild aqueous conditions without chemical reducing agents, as shown in Scheme 1. Specifically, simple mixing of Ag precursors in the form of Tollens' reagent ($Ag(NH_3)_2^+$) with TMV under elevated temperature leads to consistent formation of Ag nanoparticles within an hour. UV–vis and TEM analysis show size-controlled synthesis of TMV-mediated Ag nanoparticles (with diameter between 2 and 10 nm), following two-step Finke–Watzky mechanism (i.e. slow nucleation and fast autocatalytic growth) and further growth via secondary coalescence process in some conditions. The as-prepared particles exhibit both higher catalytic activity for 4-nitrophenol hydrogenation and antibacterial activity than previously reported values [16]. Combined these results illustrate the advantage of viral protein-mediated biofabrication approach for controlled synthesis and improved functionalities for catalysis and antibacterial activity.

2. Materials and methods

2.1. Materials

Genetically modified tobacco mosaic virus (TMV1cys) were produced by infecting tobacco plants and extracting from infected tobacco leaves with phosphate extraction buffer, followed by chloroform phase separation, PEG8000 sedimentation and sucrose gradient for TMV purification as previously described.

Silver nitride ($AgNO_3$, Sigma–Aldrich, St. Louis, MO) was used to prepare Tollens' reagent for the silver (Ag) nanoparticle formation. Ammonium hydroxide ($NH_3 \cdot H_2O$) (29.6%, Fisher Scientific, Waltham, MA) and sodium hydroxide (NaOH) (1N, Fisher Scientific, Waltham, MA) were used for the preparation of Tollens' reagent. 4-Nitrophenol ($HO\text{-}C_6H_5\text{-}NO_2$) (99%, Alfa Aesar, Ward Hill, MA) and sodium borohydride ($NaBH_4$) (99%, Fisher Scientific, Waltham, MA) were used for nitrophenol hydrogenation reaction. All the materials were used as received without further purification.

In the antibacterial activity study, Luria–Bertani (LB) broth (tissue culture grade, AMRESCO, Solon, OH) was used for suspended tube culture and LB-agar powder (molecular biology grade, Boston Bioproducts, Ashland, MA) was used for the plate culture.

2.2. Synthesis of Ag nanoparticles with TMV as sacrificial biomediator

Tollens' reagent is commonly used to make silver mirrors, and useful in distinguishing two carbonyl compounds (aldehyde and ketone). In our study, to prepare Tollens' reagent, 1 N NaOH was added to $AgNO_3$ aqueous solution to form Ag_2O brown precipitates. Next, liquid ammonium hydroxide was gradually added to the above mixture and vigorously stirred until the brown precipitates were completely dissolved to form clear Tollens' reagent solution mainly containing $[Ag(NH_3)_2]^+$ complexes.

In order to form Ag nanoparticles, varying volumes of TMV stock solution were added into the freshly prepared Tollens' reagent, then incubated at 50 °C. The mixed solution was monitored with Evolution 300 UV–vis spectrophotometer (Thermo Scientific, Waltham, MA) over 210–700 nm wavelengths for 1 hour to examine the growth kinetics. For the Ag particle growth study, the Ag and TMV mixed solution was also examined by high-resolution transmission electron microscopy (HR-TEM) upon 30 min and 60 min incubation. To study the effect of synthesis condition on the Ag particle formation, various concentrations of Tollens' reagent (1, 5, 10 and 20 mM) and TMV (0.6, 1.2, 1.8 mg/mL) were examined.

For the catalytic activity study, the TMV-mediated Ag solution was centrifuged at 18,000 × *g* for 30 min with a Microfuge 22R centrifuge (Beckman Coulter, Brea, CA). The supernatant was discarded and the collected Ag nanoparticles were rinsed by deionized (DI) water for several times and then resuspended in DI water.

2.3. High-resolution transmission electron microscopy (HR-TEM) characterization

For the HR-TEM characterizations, 10 μL of resuspended Ag nanoparticle solution was placed onto 300 mesh copper grid carbon TEM grids (FCF300-Cu, EMS Sciences, Hatfield, PA) immediately upon synthesis, and left to dry before the examination. The TEM analysis was carried out on a JEOL 2100 TEM at 200 keV at the Center for Nanoscale Systems (CNS) at Harvard University (Cambridge, MA).

2.4. Inductively coupled plasma atomic emission spectroscopy (ICP-OES)

The mass of TMV-mediated Ag particles was determined by ICP-OES. For this, concentrated HNO_3 was added into the collected Ag nanoparticles for acid digestion. After complete dissolution of Ag for about 10 min, the acid-digested solutions were diluted by 5% HNO_3 and analyzed with PerkinElmer 7300 ICP-OES (PerkinElmer Inc., Waltham, MA).

2.5. Catalytic activity examination of TMV-mediated Ag nanoparticles

For Ag nanoparticle-catalyzed 4-nitrophenol (4-NP) hydrogenation reaction, varying volumes of colloidal Ag nanoparticle solution was added to 5 mL reaction solution containing 0.08 mM 4-NP and 40 mM $NaBH_4$. The reaction was monitored by UV–vis spectrophotometer over 210–550 nm wavelength for 1 h.

The characteristic maximum absorbance of 4-NP at 400 nm was used to calculate 4-NP concentration (C_A), using a calibration curve derived from standard solutions in the presence of sodium borohydride. The conversion of 4-NP was obtained from