Chemical Physics Letters 623 (2015) 108–112

Contents lists available at ScienceDirect

Chemical Physics Letters

journal homepage: www.elsevier.com/locate/cplett

An extracellular enzyme synthesizes narrow-sized silver nanoparticles in both water and methanol

Tripti Rai, Debashis Panda*

Rajiv Gandhi Institute of Petroleum Technology (An Institute of National Importance), Rae Bareli, Uttar Pradesh, India

ARTICLE INFO

Article history:
Received 5 January 2015
In final form 3 February 2015
Available online 9 February 2015

ABSTRACT

Cellulase reduces silver ions in both aqueous and methanolic media yielding stable narrow-sized silver nanoparticles (Ag-NP) at room temperature. The synthesized nanoparticles have been characterized by various spectroscopic, microscopic methods. The redox potentials of tyrosine residues and protein backbone play an instrumental role to reduce the metal ions. The average size of nanoparticles formed in aqueous medium is of 5.04 ± 3.50 nm. Post-synthesis of Ag-NP secondary structure of enzyme is completely lost whereas upon incubation with chemically synthesized Ag-NP a significant gain in secondary structure is observed. Cellulase as a capping ligand stabilizes the silver nanoparticles even in methanol.

1. Introduction

Nanoparticles have been synthesized using various biomolecules such as peptides [1,2], proteins [3–5], and DNA [6] owing to their direct role in biological applications. Intrinsically heterogeneous nanoparticles trigger a wide variety of possible applications in catalysis [7], energy production [8], pollutant removal [9], optical sensing [10], etc. Recently the structure dependent catalytic activities of individual nanoparticles have been resolved using single molecule fluorescence spectroscopy at single turn over resolution [11,12]. Xie et al. [3] first reported that bovine serum albumin (BSA) can act as a reducing agent in alkaline condition and synthesizes metal nanoclusters. On the other hand, proteins are also used as a template for synthesis of metal nanoclusters using sodium borohydride ($NaBH_4$) as extraneous reducing agent [13,14].

The presence of metal ion impurities in cellulose plays a key role in retarding the enzymatic hydrolysis of cellulose and also influences the bio-oil composition [15,16]. The investigation on the metal ion modulated activity of cellulolytic-enzymes is an important aspect to unravel the mechanistic aspects of enzymatic hydrolysis of cellulose obtained from different plants. Cellulases are a class of hydrolase synthesized by both aerobic and anaerobic microbes for degradation of extracellular substrates [17,18]. Their catalytic roles toward degradation of cellulosic materials for biofuel production are of great interest due to global energy scarcity [19]. They also find applications in food processing and treatment for phytobezoars [20]. However, limited reports exist on its interaction with metal ions and how its catalytic efficiency affected in presence of metal ions. Therefore it is intriguing to understand the nature of cellulase–metal ion interactions in detail.

In this study we have investigated the interaction of cellulase with silver metal ions in both aqueous and nonaqueous media in order to understand the solvent dependent response of enzyme to its interaction with metal ions. Silver ions being ubiquitous in nature have been used in the present study and its nanoparticles are used in the medical fields, water-treatments due to its antimicrobial properties [21]. We have also explored the effectiveness of cellulase as encapsulating agent for stabilization of chemically synthesized silver nanoparticles in both the media. The use of enzyme as capping agents which are known to prevent the aggregation of metal nanoparticles is a step toward development of green chemistry protocols [22].

2. Experimental

2.1. Materials

Cellulase from trichoderma ressei ATCC 26921 is purchased from sigma Aldrich and used as received. Silver nitrate (Fisher scientific), sodium borohydride (Merck), dipotassium hydrogen phosphate (SDFCL), potassium dihydrogen phosphate (Rankem, RFCL), sodium hydroxide (Fischer scientific), cetyl trimethyl ammonium bromide (SDFCL), polyvinylpyrrolidone (SRL) and methanol (Fischer scientific) are obtained from respective chemical companies. All chemicals used in the experiments of analytical grade and

* Corresponding author.
E-mail address: dpanda@rgipt.ac.in (D. Panda).

http://dx.doi.org/10.1016/j.cplett.2015.02.003
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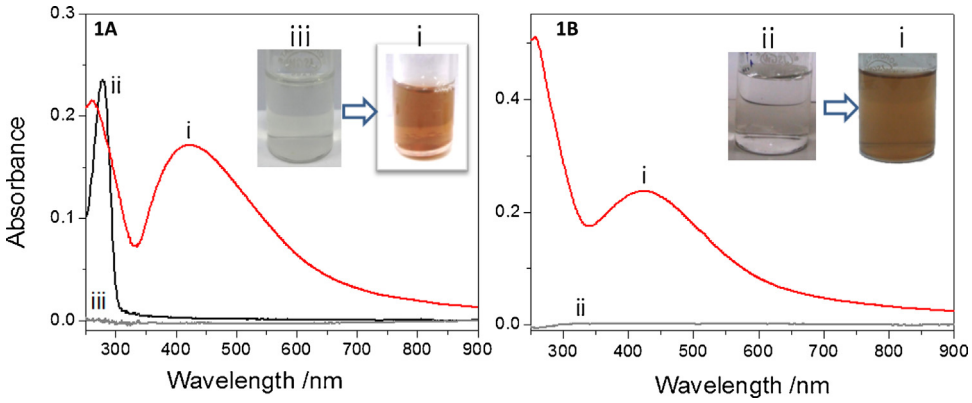

Figure 1. Absorption spectra of silver nanoparticles synthesized by cellulase. (A) (i) 0.5 mM $AgNO_3$ in 5 mg/mL cellulase in phosphate buffer pH 8.5, (ii) 5 mg/mL cellulase alone in phosphate buffer pH 8.5 (iii) control experiment (in absence of enzyme). (B) (i) 0.5 mM $AgNO_3$ in 5 mg/mL cellulase in methanol, (ii) control experiment (in absence of enzyme). Inset: The inset contains the photographs of the Ag-NP synthesized by cellulase under visible light in aqueous (A) and in methanol (B). Reddish brown color observed after 24 h incubation of silver ions with enzyme. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

used without any purification. Water from ELGA LAB Water-purifier (18.2 mΩ) is used throughout the experiments.

2.2. Cellulase-directed synthesis of silver nanoparticle in buffer and methanol

The cellulase–silver ion interaction is carried out using a modified procedure of that used by Xie et al. [3] for synthesis of BSA-directed synthesis of gold nanoclusters. In a typical synthesis, 100 μL of 5 mg/mL cellulase dissolved in 20 mM phosphate buffer (pH 8.5) with 10% glycerol is added to 10 mL of 0.5 mM silver nitrate ($AgNO_3$). After 24 h, color of the solution has changed from colorless to reddish brown suggesting the formation of silver nanoparticles. On the other hand, for the synthesis of Ag NP in methanol, 100 μL of 5 mg/mL cellulase prepared in 80% methanol is mixed with 10 mL of 0.5 mM of $AgNO_3$ solution prepared in 95% methanol. Then to the above solution, 40 μL of 0.01 M NaOH is added to initiate the reaction. After overnight incubation, the color of the solution has changed from colorless to golden brown indicating the formation of silver nanoparticles.

2.3. Cellulase as capping/encapsulating agent

Capping agents or templates are used to prevent the aggregation and to stabilize the synthesized nanoparticles because aggregated nanoparticles are of no use due to complete loss of activities. The effectiveness of cellulase as capping agent or template has been investigated using sodium borohydride ($NaBH_4$) as extraneous reducing agent. In a typical experiment, 40 μL of 1.5 mM $NaBH_4$ solution is added slowly to 5 mL of 0.2 mM silver nitrate ($AgNO_3$) prepared in 95% methanol mixed with the 100 μL of 12 mg/mL cellulase (prepared in 80% methanol). The color of the solution changes immediately from colorless to bright yellow suggesting the formation of stable silver nanoparticles. While in absence of enzyme, the solution turns immediately black from colorless indicating aggregation at room temperature.

2.4. Spectroscopic measurements

The steady state absorption spectra are collected using a LAB India UV–vis 3200 spectrophotometer. The absorption spectra are recorded from 200 to 1100 nm. The Perkin Elmer Spectrum Two FT-IR spectrometer is used to record the FT-IR spectra of the solid samples. The samples are recorded using "attenuated total reflectance" (ATR) mode. The PIKE MIRacle™ single reflection horizontal ATR accessory is used for recording the FT-IR spectra. Steady state PL spectra are recorded on a Varian Cary Eclipse fluorescence spectrophotometer, λ_{ex} = 400 nm, with a bandwidth of 5 nm. The experiments are carried out at room temperature. Time-resolved PL decays are recorded in a time-correlated single photon counting (TCSPC) system, from IBH (United Kingdom), with λ_{ex} = 406 nm. The full width at half-maximum of the instrument response function is 300 ps. The PL decays are collected with an emission polarizer at a magic angle 54.7° and are analyzed by using IBH DAS 8.2 software [23].

2.5. Particle-size determination

The nanoparticles formed are imaged by a transmission electron microscope (TEM) (JEOL 2100F) mounted with field emission gun FEG TEM at 200 kV accelerating voltage.[23] The samples for TEM are prepared on amorphous carbon films supported on a copper grid. The average values are expressed as mean ± standard deviation (SD). DLS measurements are carried out using Brookhaven 90Plus Particle Size Analyzer. Data analysis is performed using Non-Negatively constrained Least Square (NNLS) algorithm coded in BIC Particle sizing software.

2.6. Circular dichromism study

Circular dichromism data are recorded using Jasco J-815 CD spectrophotometer equipped with peltier temperature controller, in the range of 190–400 nm at the rate of 100 nm/s with band width of 1 nm. CD spectra are measured using quartz cuvettes. The spectropolarimeter was purged with N_2 prior to the experiment. Each CD plot is an average of three accumulated plots and also baseline corrected. The molar ellipticity is calculated from the observed ellipticity θ as $100\theta/cl$ where c is the concentration of the protein solution in molarity and l is the path length of the cell in centimeters.

3. Results and discussion

3.1. Cellulase catalyzed synthesis of silver nanoparticles

Cellulase as the sole reducing and nucleating agent unprecendently synthesizes silver nanoparticles in both aqueous solution and methanol at room temperature. Ag-NPs are formed after the addition of cellulase to a solution containing Ag^+ ions either in alkaline medium at pH 8.5 or in methanol. In both the conditions the