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Phytoproteins in green leaves as building blocks for photosynthesis of gold nanoparticles: An efficient electrocatalyst towards the oxidation of ascorbic acid and the reduction of hydrogen peroxide

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ABSTRACT

Herein, we present a simple and green method for the synthesis of gold nanoparticles (AuNPs) using the phytoproteins of spinach leaves. Under ambient sunlight irradiation, the isolated phytoprotein complex from spinach leaves reduces the gold chloride aqueous solution and stabilizes the formed AuNPs. As prepared nanoparticles were characterized by UV–visible spectroscopy, Fourier transform infra-red (FTIR) spectroscopy, zeta potential, transmission electron microscopy (TEM) and energy dispersive X-ray analysis (EDS). The surface plasmon resonance (SPR) maximum for AuNPs was observed at 520 nm. The zeta potential value estimated for the AuNPs is −27.0 mV, indicating that the NPs are well separated. Transmission electron micrographs revealed that the particles are spherical in nature with the size range from 10 to 15 nm. AuNPs act as a catalyst in the degradation of an azo dye, methyl orange in an aqueous environment. The reduction rate was determined to be *pseudo*-first order. Electrocatalytic efficiency of the synthesized AuNPs via this green approach was studied by chronoamperometry using ascorbic acid and hydrogen peroxide as a model compound for oxidation and reduction, respectively. Electrocatalytic studies indicate that the gold nanoparticles can be used to detect ascorbic acid and hydrogen peroxide in micromolar concentrations with response time less than 3 s.

1. Introduction

Gold is generally considered to be an inert metal and widely used as ornamental [1]. However, in nanoscale, gold exhibits unique optoelectronic properties and found applications in catalysis, sensor, electronics, biomedical diagnostics, drug delivery and medicine [2]. Owing to the huge application, many methods have been developed to synthesize gold nanoparticles (AuNPs) with control size, shape and morphology [3,4]. Among them, plant extracts mediated syntheses are of special interest due to their non-toxicity and ease of preparation [5,6]. Plant extracts contain a diverse array of phytoconstituent including proteins, reducing enzymes, cofactors, flavonoids, polyphenols, polysaccharides, proteins, triterpenes, sterols, saponins, ascorbic acid, amino acids, glucose, and fructose. It was proposed that these biomolecules are involved in the synthesis and stabilization of metal nanoparticles [7]. Despite the advantages, the complex phytoconstituents preclude to propose a proper mechanism for the plant extract based synthesis of nanoparticles. In *Cinnamomum camphora* leaf extract, polyols and the water-soluble heterocyclic molecules are suggested to play a reducing and stabilization role during the formation of AuNPs [8]. The proteins present in *Capsicum annuum* extract are mainly responsible for the synthesis of stable silver nanoparticles [9]. In the case of *Azadirachta indica*, terpenoids and reducing sugar are suggested to be involved in the formation of NPs [10]. In order to understand the molecular level of formation of NPs, it is imperative to identifying the phytomolecule involved in the synthesis of NPs.

Sunlight has been exploited in the green synthesis of NPs because it is renewable, nontoxic, nonpolluting and traceless in chemical processes [11–13]. However, the molecules mediate the reduction under sunlight irradiation are rarely identified. Plants, algae and some types of bacteria use sunlight energy to synthesize organic compounds by a process known as photosynthesis. In this process, a set of complex proteins undergoes photoinduced electrochemical reaction to drive the synthesis of organic compound [14–16]. Hypothetically, the complex set of proteins involved in the photosynthesis could play an important part in the sunlight mediated green synthesis of NPs. Recently, our team delineated the involvement of redox protein in the sunlight-mediated synthesis of silver nanoparticles [17]. To date, there is no report on the preparation of AuNPs by employing phytoproteins of spinach, which is an edible plant with rich in protein content. In the present work, we demonstrated a facile and green synthesis of AuNPs using the phytoprotein complex from spinach leaves as capping and reducing agents under sunlight irradiation. As-prepared AuNPs were characterized

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by UV–visible spectroscopy, transmission electron microscopy, zeta potential analyzer.

AuNPs stabilized with various monolayers are known for their excellent electrocatalytic properties [18]. In this paper, we described the electrooxidation capabilities of the AuNPs using ascorbic acid (AA), one of the most reported electroactive species. Moreover, AA has been chosen due to its simple electrochemical response and its involvement in many other biosensors, e.g., interference in glucose, uric acid and dopamine [19]. Hydrogen peroxide (H_2O_2) is chosen for electroreduction reactions because it has been widely used as a marker in various biosensors such as glucose, lactate, etc. [20]. The obtained results showed that the protein capped AuNPs is highly efficient in doing electrooxidation of AA and electroreduction of H_2O_2. In addition, it can be used to detect AA and H_2O_2 in micromolar concentrations with response time less than 3 s.

Azo-dyes are the major class of synthetic organic dyes has been widely used in various industries including paper, leather, plastic, cosmetic, food and pharmaceutical industries [21]. Besides the benefits, the release of an azo dye into the environment causes serious threats to aquatic life and human health [22,23]. Therefore, it should be properly destroyed or degraded before releasing into the environment. Many of the current procedures are either complicated or expensive [24]. In search of new methods, researchers started to explore the metal nanoparticles for degrading hazardous organic dyes [25]. Recently, we reported the sodium borohydride mediated reduction of organic dyes using biogenic silver nanoparticles [26]. In this work, we demonstrated that the biocapped AuNPs catalyze the sodium borohydride ($NaBH_4$) mediated reduction of an azo dye, methyl orange.

2. Material and Methods

2.1. Materials

Fresh spinach leaves were obtained from the local vegetable market. Gold(III) chloride, ammonium sulfate, sodium chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium bicarbonate, sodium potassium tartrate, copper sulfate, Follin–Ciocalteu reagent, sodium hydroxide, sodium borohydride and hydrogen peroxide were purchased from Merck. Acrylamide, bis-acrylamide, TEMED, ammonium persulfate, sodium dodecyl sulfate and ascorbic acid were purchased from Sigma, India. All other reagents are of analytical grade obtained from the local supplier. Double distilled water was used throughout the study.

2.2. Preparation of Protein Rich Extract

Protein rich extract was prepared by conventional protein isolation procedure with a slight modification of the procedure reported in Ref. [27]. Briefly, about 75 g of fresh spinach leaves was mixed with 100 ml of 10 mM phosphate buffer, pH 8 (PBS buffer) and homogenized. The non-soluble debris was removed by filtering through the double cheese cloth. The resulting green solution was mixed with 35% of precooled acetone (*v*/*v*) to remove any lipophilic molecules and centrifuged at 6000 rpm for 15 min at 4 °C. The obtained precipitate was discarded and the supernatant was mixed with 50% of ammonium sulfate (*w*/*v*). After 1 h of gentle stirring at 4 °C, the mixture was centrifuged at 6000 rpm for 15 min at 4 °C. The resulting supernatant was discarded and the pellet was collected and dried using the suction pump to remove residual acetone. The obtained precipitate was dissolved in 10 ml of PBS buffer and dialyzed six times against 2 l of PBS buffer. The presence of protein in the extract was judged by 10% SDS-PAGE [28] and quantified by Lowry assay using bovine serum albumin as standard [29].

2.3. Preparation and Characterization of Gold Nanoparticles

In a typical procedure, 0.5 ml (1 mg/ml) of protein extract (pH 6.8) was mixed with 4.5 ml of $AuCl_3$ (1 mM) aqueous solution. The aqueous solution of $AuCl_3$ and protein extract was exposed to direct sunlight. The color of the solution gradually turns from yellow to pink in 60 min, indicating the formation of AuNPs.

Absorbance spectra of as-prepared AuNPs were measured by UV–Vis spectrophotometer (Thermo Scientific Evolution 201). The morphology, size and crystallinity of AuNPs were measured by transmission electron microscope (TEM) (JEM, 2100F, JEOL, Japan). Samples for TEM were prepared by placing a drop of AuNPs solution on the graphite grid and drying it in the vacuum. Zeta potential of the synthesized nanoparticles was determined by a Malvern Zetasizer version 6.20.

2.4. Electrocatalytic Activity

For all the electrochemical analysis, a three-electrode system was used with bare platinum or gold nanoparticle-modified platinum as working electrode, saturated calomel electrode as reference electrode and platinum wire counter electrode. Protein stabilized AuNPs were immobilized on the platinum electrode using drop-casting method after dissolving it in de-ionized water. Briefly, 3 times 5 μl of aqueous AuNPs (1 mM) was dropped on the platinum electrode and allow it for drying at room temperature for 2 h and used as it is. Chronoamperometric (CA) studies were performed to obtain the efficiency of the nanoparticles as electrocatalyst. For this, CA were carried out at a more positive potential than the oxidation potential of AA or at a more negative potential than the reduction potential for H_2O_2 with constant stirring conditions. Required amount of analyte species was added to the electrolyte solution in an interval of 120 s and the resulting faradaic current was measured. All the electrocatalytic studies were performed at room temperature and ambient air condition in aqueous phosphate buffer solution (pH 7) using CHI660E electrochemical workstation (CH Instruments, USA).

2.5. Dye Degradation

The catalytic activity of the biogenic AuNPs in degrading methyl orange (MO) was studied with colorimetric and absorbance spectroscopy. In a typical experiment, 3 mg of $NaBH_4$ and 100 μl of AuNPs (1 mM) were added to 3 ml of organic dye (15 μM). The change in color was monitored by naked eye. The disappearances of the color suggest the degradation of dyes. Due to degradation, the UV–Vis spectra of MO undergo drastic change with concomitant decrease in their absorbance

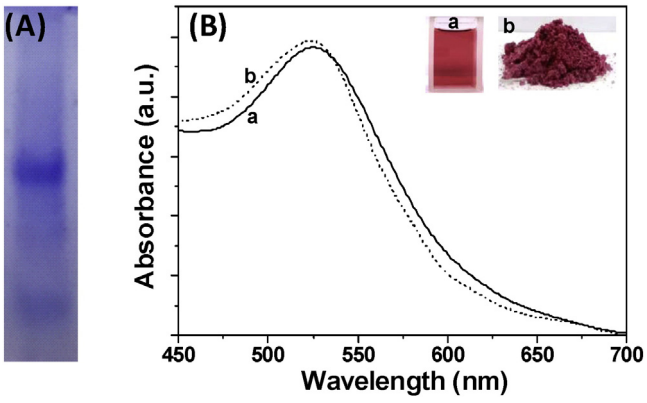

Fig. 1. (A) SDS-PAGE. Dark blue bands indicate the presence of phytoproteins. (B) UV–Vis spectra of phytoprotein stabilized AuNPs. (a) Aqueous suspension of AuNPs (b) lyophilized AuNPs powder resuspended in water. Inset corresponds to the photographs of (a) aqueous AuNPs solution and (b) lyophilized powder. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)